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for your dedication, effort, and cooperation.
Together, we have achieved many successes, overcome
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We thank you for your contribution to the
overall success of the Biotechnical Faculty.

As we celebrate the festive season, we wish each and every one of you
a happy and joyous Christmas and a prosperous New Year.
May this time of year bring you warmth, love, and
laughter that will carry over into the coming year.

As we move into the new year, we hope that your paths are illuminated
with success, new opportunities, and personal satisfaction.

Best wishes from the Biotechnical Faculty, University of Ljubljana.

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Skupaj smo dosegli mnogo uspehov,
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Hvala vam za vaš prispevek
k skupnemu uspehu Biotehniške fakultete.

V teh prazničnih dneh želimo vsakemu od vas
voščiti radostno božično praznovanje in srečno novo leto.
Naj bodo prazniki polni ljubezni, topline in smeha,
ki naj se prenesejo tudi v novo leto.

Naj bodo vaše poti v letu, ki prihaja, obsijane z uspehi,
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Using pyrimidinecarboxylic acids as growth stimulants for *Rhododendron ledebourii* Pojark and *Rhododendron smirnowii* Trautv.

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Using pyrimidinecarboxylic acids as growth stimulants for *Rhododendron ledebourii* Pojark and *Rhododendron smirnowii* Trautv.

Abstract: Synthesised organic compounds of pyrimidinecarboxylic acids are characterised by high biological activity, even when their concentrations are low. These compounds, when applied to the seeds of *Rhododendron*, a genus of woody plants, with concentrations of 0.01, 0.05, and 0.1 %, stimulate the growth of the plants. The effect is more obvious 3 months after the start of the experiment, rather than 7 months after. Thus, *Rhododendron ledebourii* Pojark. seedlings grew by 13.3-33.5 %, and *Rhododendron smirnowii* Trautv. seedlings grew by 29.6-48.1 %. *Rhododendron ledebourii* and *Rhododendron smirnowii* seedlings demonstrated similar direct correlations: when the concentration of 2-benzylamino-4-methyl-pyrimidine-5-carboxylic acid and 4-methyl-2-morpholin-4-pyrimidine-5-carboxylic acid (for *Rhododendron ledebourii* seeds only) rose from 0.01 to 0.1 %, the height of the seedlings increased. It is suggested using 4-methyl-2-piperidin-1-yl-pyrimidine-5-carboxylic acid, 2-benzylamino-4-methyl-pyrimidine-5-carboxylic acid, and 4-methyl-2-morpholin-4-pyrimidine-5-carboxylic acid at concentrations of 0.01, 0.05, and 0.1 % as growth stimulants for the species of *Rhododendron* genus.

Key words: growth stimulants, synthesized organic compounds, pyrimidine carboxylic acids, *Rhododendron*

Uporaba pirimidin karboksilne kisline kot rastnega vzpodbujevalca za vrsti rododendrona, *Rhododendron ledebourii* Pojark in *Rhododendron smirnowii* Trautv.

Izvleček: Za sintetične organske spojine pirimidin karboksilnih kislin je značilna velika biološka aktivnost, tudi pri majhnih koncentracijah. Tretiranje semen rododendrona s temi spojinami v koncentracijah 0,01; 0,05 in 0,1 %, stimulira rast rastlin. Ta učinek je jasnejši 3 mesece po začetku poskusa kot po 7 mesecih. Sejanke vrste *Rhododendron ledebourii* Pojark. so rastle pri koncentracijah 13,3-33,5 %, sejanke vrste *Rhododendron smirnowii* Trautv. pa pri koncentracijah 29,6-48,1 % stimulanta. Sejanke obeh vrst so se na tretiranje odzvale podobno in sicer: kadar se je koncentracija 2-benzilamino-4-metil-pirimidin-5-karboksilne kisline in 4-metil-2-morfolin-4-pirimidin-5-karboksilne kisline (le za semena vrste *Rhododendron ledebourii*) dvignila iz 0,01 na 0,1 %, se je višina sejank povečala. Priporočamo uporabo 4-metil-2-piperidin-1-il-pirimidin-5-karboksilne kisline, 2-benzilamino-4-metil-pirimidin-5-karboksilne kisline in 4-metil-2-morfolin-4-pirimidin-5-karboksilne kisline v koncentracijah 0,01; 0,05 in 0,1 % kot rastne vzpodbujevalce za vrste iz rodu *Rhododendron.*

Ključne besede: rastni vzpodbujevalci, sintetične organske spojine, pirimidin karboksilne kisline, *Rhododendron*

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1 INTRODUCTION

Over the past few years, the cultivation of plants has been facing a number of challenges, including a dramatic increase in temperatures, lack of soil water, and the need for more resistant and hardy plants, which grow even under the harshest conditions. It is therefore vital to minimise the time required for the growth of planting material, which can be done using growth and germination stimulants, including synthesised chemical compounds Pentelkina, 2003; Vasin et al., 2008, 2009; Ostroshenko and Ostroshenko, 2011; Schuchka, 2006; Baranova, 2013a; Khodaei-Joghan et al., 2018; Nesterkina et al., 2019) and other original modes (Shibaeva et al., 2018).

It has been noted high biological activity of pyridines and pyrimidines. For example, 1, 4-dihydropyridines have received large attention because of their fundamental role in different biological processes (Goldmann and Stoltefuss, 1991; Litvinov, 1998; Balalaie et al., 2008). It has been reported about the wide biological activity of dihydropyridine derivatives (Ghoneim and Assy, 2015). But a promising area of the study, related to the growing need for the development of effective and safe drugs, is the synthesis of new heterocyclic systems, containing a pyrimidine fragment. Pyrimidine fragments and pyrimidine base are included in DNA. So pyrimidine derivatives show different biological activity. There is another mode for applying chemical compounds, containing pyrimidine fragments, for instance, as mutagens.

The composition of diethyleneimide-2-amidopyrimidyl phosphoric acid (phosphazine, phosphomid, syn. phosphemide - phospemidum) includes two ethylene imine groups connected to phosphorus and a pyrimidine base. Ethyleneimine causes mutations, the pyrimidine base is included in the chromosome during DNA synthesis, determining the specificity of the effect (Weisfeld, 2015). Laboratory seed germination and morphometric parameters of seedlings (length of roots and shoots, number of leaves) were studied after seed treatment with heterocyclic sulfur-containing compounds on the example of woody plants (Vostrikova et al., 2020) and agricultural crop (Vostrikova et al., 2021). As the composition of the phosphemide mutagen, pyrimidine-carboxylic acids contain a pyrimidine base. Sulfur (in the composition of alkylating compounds: diethyl- and dimethylsulfonate, ethylmethanesulfonate) enhances biological activity. It is known that mutagens in low concentration have stimulating effect for plant objects. A necessary working step is the determination of concentration ranges (Bome et al., 2017).

Over the last years, attempts have been made to synthesise new organic compounds, derivatives of pyrimidines (Dlugosz and Dus, 1996; Elkholy and Morsy, 2006; El-Gazzar et al., 2009; Marjani et al., 2011; Tkachenko et al., 2013, 2018; Azizian et al., 2014), which can be used as growth regulators. Such compounds should be more effective than the existing commercial formulations because of their different concentrations that may have either stimulating or inhibiting activity (Dlugosz and Dus, 1996; Gavrilov et al., 1988; Litvinov, 1998; Brown et al., 2004; Balalaie et al., 2008; Moiseeva et al., 2012a).

The effect of pyrimidine carboxylic acids on seed germination and plant height was studied using another annual flower – spreading marigold (*Tagetes patula* L.) (Vostrikova et al., 2012; Kalaev et al., 2013a). However, the effect of pyrimidinecarboxylic acids on the growth of other plants has not been studied yet.

Up to the present time, there have also been no studies considering the results of application of synthesised chemical compounds of pyrimidinecarboxylic acids to the seeds of woody plants, which grow much slower than annual plants. It is therefore of great importance to conduct a longitudinal study and measure the height of ornamental woody plants over long time intervals (eg. 3 and 7 months after the application of the growth regulator) in order to determine, whether the growth stimulating effect lasts or deteriorates over time.

Two ornamental woody plants, namely *Rhododendron ledebourii* Pojark. and *Rhododendron smirnowii* Trautv. were used. The long history of studying these species (Moiseeva et al., 2012b; Baranova, 2013b) at the B.M. Kozo-Polyansky Botanical Garden of Voronezh State University has demonstrated, that *Rhododendron ledebourii* is a winter-hardy, drought-resistant, and fruit-bearing shrub. *Rhododendron smirnowii* is also quite winter-hardy, though less drought-resistant. It also grows slower than *Rhododendron ledebourii* (Alexandrova, 2003; Vostrikova, 2011). It has been reported about the antioxidant activity in *Rhododendron* leaves, connections between this characteristic in maternal plant and the cytogenetic structures in the seedlings of the *Rhododendron* species (Vostrikova et al., 2022). Cytogenetic polymorphism of seed progeny of introduced plants has been studied on the example of *Rhododendron ledebourii* (Burmenko et al., 2018). It has been revealed the high seeds quality. So the representatives of the *Rhododendron* genus are valuable resource plant.

The aim of our research was to study the effect from pre-sowing seed treatment of the following woody plants: *Rhododendron ledebourii* and *Rhododendron smirnowii* by pyrimidine carboxylic acids.

2 MATERIALS AND METHODS

The research was conducted at the B.M. Kozo-Polyansky Botanical Garden of Voronezh State University (geographic coordinates: 39°22' N, 51°40' E; 168.2 metres above sea level).

It has been focused on the effect of synthesised organic compounds of pyrimidinecarboxylic acids on the height of seedlings of *Rh. ledebourii,* and *Rh. smirnowii.* The following compounds were used: 4-methyl-2-piperidin-1-yl-pyrimidine-5-carboxylic acid, 2-benzylamino-4-methyl-pyrimidine-5-carboxylic acid, and 4-methyl-2-morpholin-4-pyrimidine-5-carboxylic acid synthesised at the Department of Organic Chemistry of Voronezh State University.

Prior to the sprouting process, the seeds of *Rh. ledebourii* and *Rh. smirnowii* were soaked in a water suspension of the above listed compounds with concentrations of 0.01 %, 0.05 %, and 0.1 % for 18 hours. The control group consisted of the same type of seeds soaked in tap water solution of a commonly used growth stimulator, Epibrassinolide (commercial fraction Epin Extra produced by NNPP NEST M, Russia), with the concentration of 0.05 % (in accordance with the instruction). In case of each of the studied concentrations of the acids, as well as the control group, the experiment was conducted three times using a set of 100 seeds. After soaking, the rhododendron seeds were placed in Petri dishes containing blotting paper, and germinated in the laboratory conditions at a constant temperature 22 °C. On the 21st day, the sprouts were planted in containers filled with high-moor peat and then kept in a greenhouse. The height of the seedlings of *Rhododendron ledebourii* was measured with a ruler, 3 and 7 months after the start of the experiment. Since *Rhododendron smirnowii* grows slower, the height of its seedlings was measured 7 months after the start of the experiment.

Sprouts are formed during the early stage of plant ontogenesis, which starts after the germination stage, i.e. when the seed coat develops, and finishes, when the first leaf of the hypocotyledonous stem (the shoot rising from the plumule) develops (Korovkin, 2007). After the first true leaves appear, young plants are considered seedlings (Korovkin, 2007).

The results were statistically processed using the STADIA software package. The procedures of data grouping and processing were described by A. P. Kulaichev (2006). The mean values were compared using Student's t-test. Coefficient of variation (Cv) was counted according to G. F. Lakin (1990). If Cv was below 10 %, it meant that the degree of variation was low, with Cv between 10 and 25 % it was medium, and when Cv was over 25 % - the degree of variation was high (Lakin, 1990). Average values of seedlings (plants) height were compared using Student's *t*-test. The seed germination in control and experimental variants were compared using Z-test for equality of frequencies. To estimate the influence of various concentrations of the chemical compounds on the height of the plants, one-way analysis of variance was used. The power of influence was calculated according to Snedecor (in %).

3 RESULTS AND DISCUSSION

The standard growth stimulant hasn't shown influence of the seed treatment on the height of *Rhododendron ledebourii* seedlings. It hasn't been revealed the significant difference between Control group and Epin group. That means special stimulant impact for sowing qualities in *Rhododendron* seeds or specific seeds reaction for the treatment. The seeds germination wasn't strongly increased (Table 1-3). But there were significant differences between control and experimental groups in this parameter (Table 1-3).

Suspensions of 2-benzylamino-4-methyl-pyrimidine-5-carboxylic acid at concentrations between 0.01 and 0.1 % proved to be more effective than other growth stimulators (Table 1). When suspensions of 4-methyl-2-morpholin-4-pyrimidine-5-carboxylic acid at concentrations of 0.05 and 0.1 % were used, the seedlings of *Rhododendron ledebourii* grew up to 55.6–116.7 % higher (Table 1).

Within 7 month after the start of the experiment all the studied compounds at any concentration proved to be effective for *Rhododendron ledebourii* (Table 2).

Within 7 month after the start of the experiment, the height of the *Rhododendron smirnowii* seedlings also increased (Table 5). The parameter "height of the seedlings" varied greatly for the control group of *Rhododendron ledebourii* 3 months after the start of the experiment. This is indicated by Cv = 44 %. For the experimental groups, Cv was medium (Table 1), which indicated that the reactions of individual seedlings to the compounds used were similar. 7 month after the start of the experiment, the degree of variation was medium in the control group, and even low in the experimental group. This might mean that older seedlings adapt better to experimental conditions. Changes of the seedlings growth are illustrated in Fig. 1-3.

For the seedlings of *Rhododendron smirnowii*, a medium coefficient of variation was observed (Table 3). Because of the slow growth and the small size, differences between experimental and control groups of *Rhododendron smirnowii* are not significant 3 month after the start of the experiment.

Table 1. The seedlings height of *Rhododendron ledebourii* seedlings 3 months after the seed were treated with the studied synthesized organic compounds

Concentration, %	Average height of the plants, cm	Min - max, cm	Cv, %	The seeds germination, %	Increase in the height of the plants, %
Control group	1.8 ± 0.2	1.0–3.0	44	72	–
Epin group	1.9 ± 0.1	1.5–2.5	15.8	75	–
4-methyl-2-piperidin-1-yl-pyrimidine-5-carboxylic acid					
0,01 %	3.3 ± 0.1***3	3.0–3.5	9.1	78*	83.3
0,05 %	2.8 ± 0.1**2	2.5–3.0	10.7	78*	55.6
0,1 %	2.2 ± 0.1¹	2.0–2.5	13.6	79*	–
2-benzylamino-4-methyl-pyrimidine-5-carboxylic acid					
0,01 %	2.9 ± 0.2**2	2.5–3.0	12.5	78*	61.1
0,05 %	3.4 ± 0.2***3	3.0–3.5	13.5	80*	88.9
0,1 %	3.8 ± 0.2***3	3.5–4.0	11.9	82**	111.1
4-methyl-2-morpholin-4-pyrimidine-5-carboxylic acid					
0,01 %	2.3 ± 0.1¹	2.0–2.5	13.0	74	–
0,05 %	2.8 ± 0.1**2	2.5–3.0	10.7	75	55.6
0,1 %	3.9 ± 0.2**2	3.0–4.5	15.4	78*	116.7

Designations:
Cv – variation coefficient
* – differences with the control group are significant ($p < 0.05$)
** – differences with the control group are significant ($p < 0.01$)
*** – differences with the control group are significant ($p < 0.001$)
1 – differences with the Epin group are significant ($p < 0.05$)
2 – differences with the Epin group are significant ($p < 0.01$)
3 – differences with the Epin group are significant ($p < 0.01$)

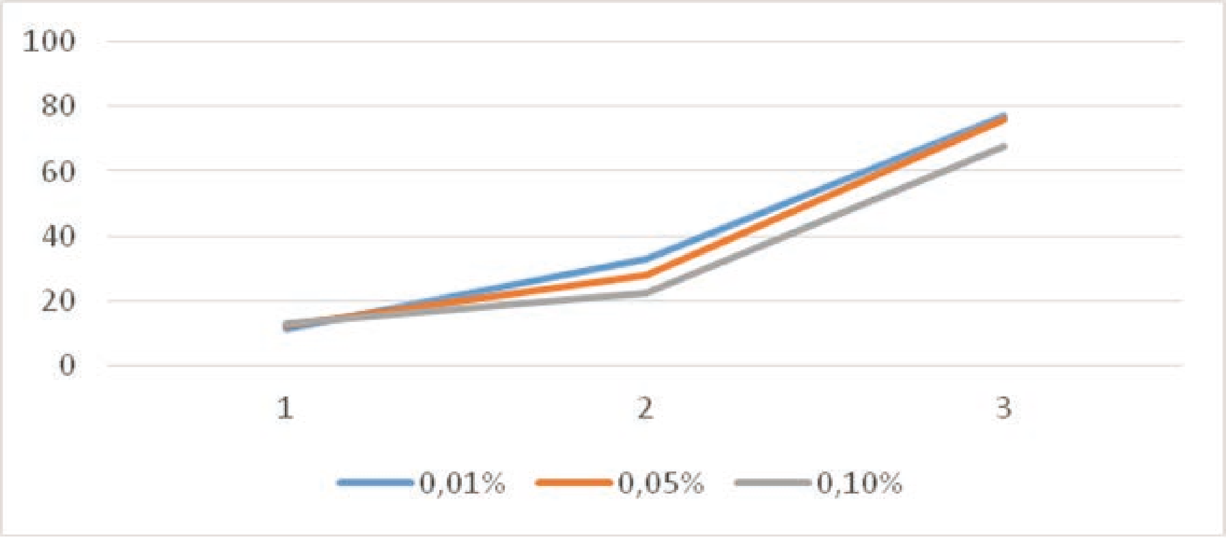

Fig. 1: The height of the seedling of *Rhododendron ledebourii* (in mm) seedlings after the seed were treated with 4-methyl-2-piperidin-1-yl-pyrimidine-5-carboxylic acid; 1 – seedlings (21 days), 2 – plants 3 months, 3 – plants 7 months

Table 2: The height of *Rh. ledebourii* seedlings 7 months after the seed were treated with the studied synthesized organic compounds

Concentration, %	Average height of the plants, cm	Min - max, cm	Cv, %	The seeds germination, %	Increase in the height of the plants, %
Control group	5.6 ± 0.2	4.5–6.5	11.5	72	–
Epin group	5.7 ± 0.2	5.0–6.5	9.3	75	–
4-methyl-2-piperidin-1-yl-pyrimidine-5-carboxylic acid					
0,01%	7.7 ± 0.2***3	7.0–8.5	7.6	78*	22.9
0,05%	7.6 ± 0.2***3	6.5–8.5	8.7	78*	27.5
0,1%	6.8 ± 0.2***3	6.0–7.5	7.9	79*	33.5
2-benzylamino-4-methyl-pyrimidine-5-carboxylic acid					
0,01%	7.2 ± 0.2***3	6.5–8.0	7.5	78*	22.9
0,05%	7.7 ± 0.2***3	7.0–8.5	7.5	80*	27.0
0,1%	8.4 ± 0.2***3	7.5–9.0	6.9	82**	17.8
4-methyl-2-morpholin-4-pyrimidine-5-carboxylic acid					
0,01%	6.4 ± 0.2*1	5.5–7.5	11.5	74	13.3
0,05%	6.5 ± 0.2**2	5.5–7.5	11.5	75	14.6
0,1%	6.9 ± 0.2***3	6.0–7.5	6.9	78*	19.0

Designations:
Cv – variation coefficient
* – differences with the control group are significant ($p < 0.05$)
** – differences with the control group are significant ($p < 0.01$)
*** – differences with the control group are significant ($p < 0.001$)
1 – differences with the Epin group are significant ($p < 0.05$)
2 – differences with the Epin group are significant ($p < 0.01$)
3 – differences with the Epin group are significant ($p < 0.01$)

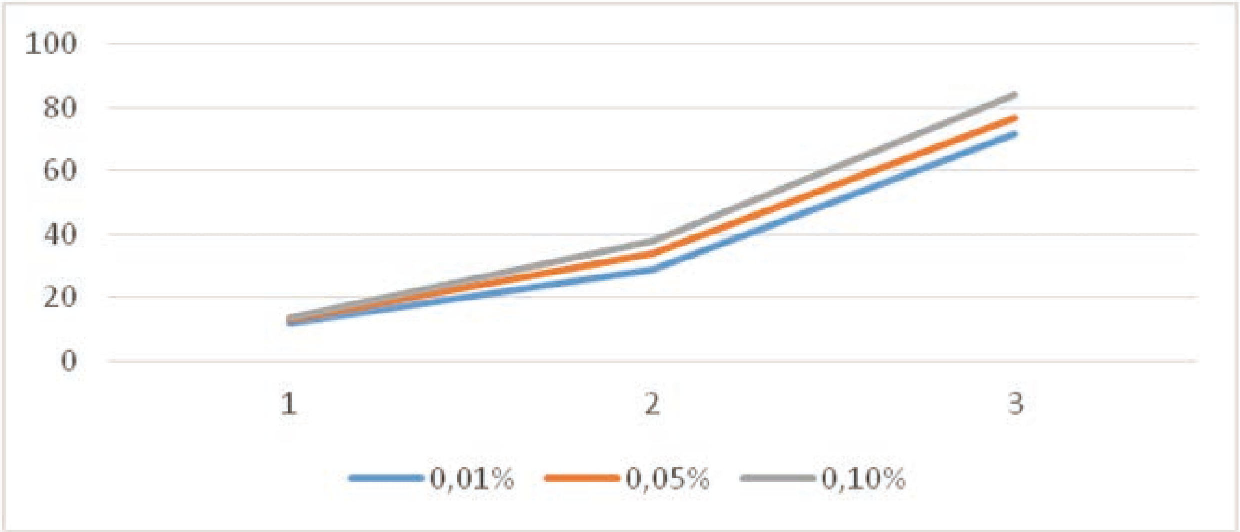

Fig. 2: The height of the seedling of *Rhododendron ledebourii* (in mm) seedlings after the seed were treated with 4-methyl-2-morpholin-4-pyrimidine-5-carboxylic acid, 1 – seedlings (21 days), 2 – plants 3 months, 3 – plants 7 months

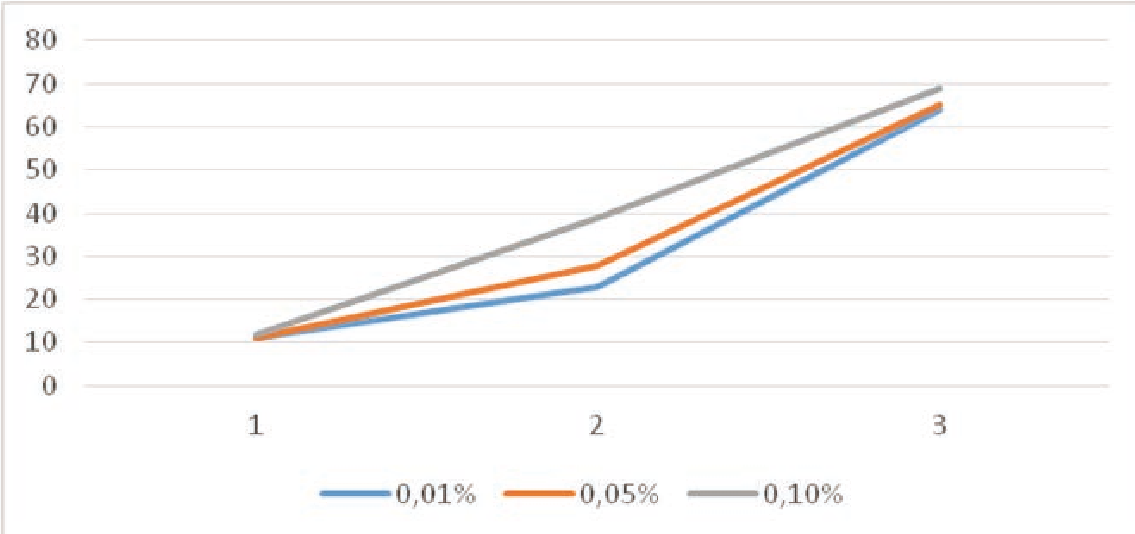

Fig. 3: The height of the seedling of *Rhododendron ledebourii* (in mm) seedlings after the seed were treated with 2-benzylamino-4-methyl-pyrimidine-5-carboxylic acid1, 1 – seedlings (21 days), 2 – plants 3 months, 3 – plants 7 months

Table 3: The height of *Rh. smirnowii* seedlings 7 months after the seed were treated with the studied synthesized organic compounds

Concentration, %	Average height of the plants, cm	Min - max, cm	Cv, %	The seeds germination, %	Increase in the height of the plants, %
Control group	1.4 ± 0.1	1.0–2.0	24.1	58	–
Epin group	1.4 ± 0.1	1.0–2.0	28.1	60	–
2-benzylamino-4-methyl-pyrimidine-5-carboxylic acid					
0,01 %	1.8 ± 0.1*1	1.5–2.0	14.7	64*	29.6
0,05 %	1.9 ± 0.1**1	1.5–2.0	12.7	68*	37.0
0,1 %	2.0 ± 0.1**2	1.5–2.5	20.4	72**	48.1

Designations:
Cv – variation coefficient
* – differences with the control group are significant ($p < 0.05$)
** – differences with the control group are significant ($p < 0.01$)
*** – differences with the control group are significant ($p < 0.001$)
1 – differences with the Epin group are significant ($p < 0.05$)
2 – differences with the Epin group are significant ($p < 0.01$)
3 – differences with the Epin group are significant ($p < 0.01$)

The growth ratio of *Rhododendron ledebourii* seedlings (in the experimental group as compared to the control group) after the seeds were treated with organic compounds of pyrimidinecarboxylic acids, demonstrates that the stimulating effect is most obvious 3 month after the start of the experiment. However, some treatment do not effect growth (Table 1). 7 month after the start of the experiment, the stimulating effect deteriorates, but all the compounds at any concentration studied in this paper still have a positive effect (Table 2).

It was shown significant influence of the treatment of seeds with the synthesised organic compounds (as a factor) on the height of the *Rhododendron* seedlings. The power of influence of the treatment of seeds with the synthesised organic compounds on the height of the seedlings was evaluated using the one-way analysis of variance (Table 4-6).

4-methyl-2-piperidin-1-yl-pyrimidine-5-carboxylic acid, 2-benzylamino-4-methyl-pyrimidine-5-carboxylic acid, and 4-methyl-2-morpholin-4-pyrimidine-5-carboxylic acid with concentrations of 0.01, 0.05, and 0.1 % resulted in the increase in the height of

Table 4: The power of influence (in %) of the seed treatment on the height of *Rhododendron ledebourii* seedlings on the 3 month after the start of the experiment

Stimulator	as compared to the control group	as compared to the Epin group	as is
4-methyl-2-piperidin-1-yl-pyrimidine-5-carboxylic acid	5.0***	8.8***	8***
2-benzylamino-4-methyl-pyrimidine-5-carboxylic acid	7.4***	7.9***	8.3***
4-methyl-2-morpholin-4-pyrimidine-5-carboxylic acid	7.2***	8.9***	8.2***

Designations: *** - the influence of the factor is significant ($p < 0.001$)

Table 5: The power of influence (in %) of the seed treatment on the height of *Rhododendron ledebourii* seedlings on the 7 month after the start of the experiment

Stimulator	as compared to the control group	as compared to the Epin group	as is
4-methyl-2-piperidin-1-yl-pyrimidine-5-carboxylic acid	6.7***	7.5***	6.7***
2-benzylamino-4-methyl-pyrimidine-5-carboxylic acid	8.4***	8.9***	8.5***
4-methyl-2-morpholin-4-pyrimidine-5-carboxylic acid	4.9***	3.3**	5.6**

Designations: *** - the influence of the factor is significant ($p < 0.001$)
*** - the influence of the factor is significant (p < 0.001)

Table 6: The power of influence (in %) of the seed treatment on the height of *Rh. smirnowii* seedlings on the 7 month after the start of the experiment

Stimulator	as compared to the control group	as compared to the Epin group	as is
2-benzylamino-4-methyl-pyrimidine-5-carboxylic acid	4.3***	4.2***	6.2***

Designations: *** - the influence of the factor is significant ($p < 0.001$)

Rhododendron ledebourii seedlings. A direct correlation can be observed: the higher the concentration of 2-benzylamino-4-methyl-pyrimidine-5-carboxylic acid (within the range between 0.01 and 0.1 %), the higher the seedlings of *Rhododendron ledebourii* and *Rhododendron smirnowii* are. The same correlation is observed for *Rhododendron ledebourii* seedlings, when 4-methyl-2-morpholin-4-pyrimidine-5-carboxylic acid is applied. When *Rhododendron ledebourii* seeds were treated with 4-methyl-2-piperidin-1-yl-pyrimidine-5-carboxylic acid at the concentration between 0.01 and 0.1 %, a negative correlation was observed: the lower the concentration, the higher the seedlings.

We can thus say, that the studied synthesised organic compounds of pyrimidinecarboxylic acids are characterised by high biological activity and stimulate the growth of ornamental woody plants when their concentrations are low. The stimulating effect is most obvious 3 months after the seed treatment (the seedlings are 55.6–116.7 % higher than the seedlings in the control group). 7 month after the start of the experiment the stimulating effect starts deteriorating. Compounds of pyrimidinecarboxylic acids increase the height of *Rhododendron ledebourii* seedlings by 13.3-33.5 %, and the height of *Rhododendron smirnowii* seedlings by 29.6-48.1 %. Apparently, pyrimidinecarboxylic acids are more biologically active than other chemical substances, as their chemical structure is similar to the structure of a natural growth stimulator - indole acetic acid (heteroauxin). It can be assumed that auxin activity was kept better within 3 month, than during 7 month after the start of the experiment.

Earlier research studied the stimulating effect of 0.01–0.05 % 4-methyl-2-piperidin-1-yl-pyrimidine-5-carboxylic acid on the germination and growth of the seedlings of spreading marigold: with the concentration of 0.03-0.05 %, the height of the plants increased (differences with the control group are reliable, $p < 0.001$). However, other compounds of pyrimidine-5-carboxylic acids at the studied concentrations demonstrated stronger stimulating effects (Vostrikova et al., 2012; Kalaev et al., 2013 a).

Obtained results are consistent with earlier studies by R. G. Gafurov and co-workers on carbon N- and O-benzyl-containing compounds that have bright auxin activity, which is ensured by the presence of a benzyl group at the nitrogen or oxygen atom (Gafurov and Makhmutova, 2003, 2005). These compounds contain effector fragments that together determine the stress protective activity, namely, the quaternary ammonium and benzyl groups and the hydroxyethyl group - an analog of the benzoxyethyl group (Budykina et al., 2005; Timeyko et al., 2005). Tested substance (2-benzylamino-4-methyl-pyrimidine-5-carboxylic acid) contains similar fragments, so it also shows bright auxin activity. Based on the literature data (Budykina et al., 2005; Timeyko et al., 2005) and the results of our research, it can be assumed that used compound has the stress-protective activity for valuable ornamental plants of *Rhododendron* species.

4 CONCLUSIONS

In this paper, it has been demonstrated that the effect of the same concentrations of synthesised organic compounds of pyrimidine carboxylic acids on the seedlings of woody plants after the pre-sowing seed treatment doesn't differ. *Rhododendron ledebourii* and *Rhododendron smirnowii* seedlings demonstrated similar direct correlations: when the concentration of 2-benzylamino-4-methyl-pyrimidine-5-carboxylic acid and 4-methyl-2-morpholin-4-pyrimidine-5-carboxylic acid (for *Rhododendron ledebourii* seeds only) rose from 0.01 to 0.1 %, the height of the seedlings increased. Therefore, 4-methyl-2-piperidin-1-yl-pyrimidine-5-carboxylic acid, 2-benzylamino-4-methyl-pyrimidine-5-carboxylic acid, and 4-methyl-2-morpholin-4-pyrimidine-5-carboxylic acid at concentrations of 0.01, 0.05, and 0.1 % can be used as growth stimulators for species of *Rhododendron* genus. 2-benzylamino-4-methyl-pyrimidine-5-carboxylic acid proved to be more effective at the initial development stages of *Rhododendron* seedlings. Within 3 month after the seeds of *Rhododendron ledebourii* were soaked in water suspensions of 2-benzylamino-4-methyl-pyrimidine-5-carboxylic acid at concentrations of 0.01-0.1 %, the height of the seedlings increased by 61.1-111.1 %. Thus, investigated pyrimidine carboxylic acids at concentrations of 0.01-0.1 % can be recommended as effective growth stimulants for *Rhododendron.*

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Application of fluorescence spectroscopy as a field method in the determination of varietal differences after tomato harvesting

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Application of fluorescence spectroscopy as a field method in the determination of varietal differences after tomato harvesting

Abstract: The study's purpose is to establish the application based on fluorescence spectroscopy as a field method in the determination of varietal differences after tomato harvesting. The tomato fruits will be compared to determine the spectral distribution due to the varietal differences of a particular genotype. This will allow the approach to be practiced non-invasively in the quality control of tomato production in unspecified rooms and outdoors.

The experimental studies have been conducted locally at the Institute of Plant Genetic Resources "K. Malkov" - Sadovo for three varieties.

The spectral installation for the generation of emission fluorescence spectra is mobile. In its adjustment (optical adjustment), a system engineering approach based on the classical principles of modern optoelectronics was applied. The results of the experiment can be used to optimize the time for the analysis of the varietal difference of tomato genotypes after harvesting, under uncontrolled conditions. This will support the process of determining the belonging of a specific accession to a given variety (even for accessions of unknown origin) when it is necessary to qualify a score of samples in a short time.

Key words: tomato fruits, uncontrolled conditions, field method, fluorescence spectroscopy

Uporaba fluorescentne spektroskopije kot metode za ugotavljanje razlik med sortami paradižnika na polju po obiranju

Izvleček: Namen raziskave je bil razviti poljsko metodo za ugotavljanje razlik med sortami paradižnika po obiranju z uporabo fluorescenčne spektroskopije. Razlike med sortami so določene na osnovi razlik med porazdelitavmi spektrov, ki so odvisni od genotipov. To bo omogočilo praktičen neinvazivni nadzor kakovosti pri pridelavi paradižnika na prostem in v nespecializiranih skladiščih. Poskusi so bili izvedeni na ustanovi Institute of Plant Genetic Resources "K. Malkov – Sadovo, Bolgarija, na treh sortah.

Naprava za generiranje emisijskega fluorescentnega spektra je mobilna. Za njeno nastavitev (optična nastavitev) je bil uporabljen klasični princip moderne optične elektronike. Rezultati te raziskave bi se lahko uporabili za optimiziranje časa analize razlik med genotipi različnih sort paradižnika po obiranju v nenadzorovanih razmerah. To bo podpora pri določanju akcesij, ki pripadajo določeni sorti paradižnika, tudi tistih neznanega izvora, kadar je potrebno pregledati kakovost vzorcev v kratkem času.

Ključne besede: plodovi paradižnika, nenadzorovane razmere, poljska metoda, fluorescentna spektroskopija

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1 INTRODUCTION

The established function of fluorescence spectroscopy as a field method in the assurance of varietal differences after tomato harvesting is the aim of the present study. Various techniques have been investigated for non-invasive spectrometric analysis of tomatoes. Near-infrared spectroscopy is used to determine the content of soluble solids (Slaughter et al., 1996) and detect carotenoids (Pedro and Ferreira, 2005) has been successfully applied. Also, reflectance approaches (Polder et al., 2004) and fluorescence spectroscopy (Lai et al., 2007) have been well enforced to assess surface pigmentation. Raman spectroscopy is a proven technique in carotenoid research (Schulz et al., 2005; Pudney et al., 2011).

In the assessment of the ripeness and firmness of tomatoes (Qin and Lu, 2008), the absorption and scattering properties are applied. Advances in fiber optic applied science attempt to provide outstanding conveniences for the development of an ample range of highly deftly fiber optic sensors in many modern application fields. Fiber optic insides have been successfully becoming assemblies with micro-optic pieces such as lenses, mirrors, prisms, gratings, and others (Dakin & Brown, 2006; Mitchke, 2010).

In many analytical areas of science, fluorescence spectroscopy is an important research tool. It is currently the dominant methodology and is widely recycled in biotechnology, flow cytometry, medical diagnostics, DNA sequencing, agriculture, and genetic investigations, as well as in many other application areas. Methods using this light phenomenon are highly sensitive and rapid and do not require the expense and difficulty of using radioactive tracers (Becker et al., 2003; Albani, 2006).

For quality control of vegetable crops, including tomatoes, the effects of light apply to spectral analysis such as fluorescence, transmission, and diffuse reflectance. Also, they can serve as a field method in the determination of varietal differences after tomato harvesting, since fluorescence emission is visualized in the visible spectral range and from ultraviolet rays. The spectral distribution of the emission signal in tomato fruits consists mainly of two maxima in the visible range. The intensity and shape of the fluorescence emission spectrum at room temperature depend mainly on the concentration of the fluorophores and to a lesser extent on the structure, photosynthetic activity, and arrangement of the cells in the tissue (National Research Council, 1968; Leo et al., 2007).

In connection with the demands of consumers for high food quality, the conducted research can serve as a basis for the creation of mobile detecting devices with which to carry out instant analysis of warehouse production of tomatoes in uncontrolled conditions, both in processing plants and in food retail outlets.

The present study aims to establish the function of fluorescence spectroscopy in the act of field method in the determination of varietal differences after tomato harvesting. They will be compared to determine the spectral distribution due to the varietal differences of a particular genotype. The specimens were grown under uncontrolled field conditions. This will permit the technique to be applied non-invasively in the quality control of tomato production in unspecified rooms and outdoors.

2 MATERIALS AND METHODS

The fruits that are the subject of the research are the varieties Local Dwarf (determinate), Pikador (determinate), and Ideal (indeterminate). The seeds that were sown to grow the accessions were taken from GenBank at the Institute of Plant Genetic Resources, Sadovo.

The local Dwarf tomato was dense, planted up to 40–50 cm tall, had potato-type leaves, bright red fruits with a flat round shape, and a mass of 100–120 g.

The determinate cultivar Pikador is a highly efficient variety, making fruit with fiercely red flesh that is considerable for preserving. Admirably, lightly stretched tomatoes that parallel pears in shape are firm and hard, with a mass of 50–60 g. The fruits of tomatoes of this variety are located in the form of a bunch on the plant. The variety is strongly resistant to mold.

The indeterminate Ideal was a medium-early tomato variety with considerable fruits, 130–180 g, flat-round to arced, kind of ridged, multi-chambered, orange-red in color, and an amiable taste.

The indeterminate Ideal was a medium-early tomato variety with considerable fruits, 130–180 g, flat-round to arced, kind of ridged, multi-chambered, orange-red in color, and an amiable taste.

The experiment was conducted at the Institute of Plant Genetic Resources „Konstantin Malkov" - Sadovo. The seeds are set in seedling plates filled with peat-pearlitic substrate and launched at a temperature of 25–28 degrees C for impregnation. Tomato seedlings are transplanted once they are 15–25 cm in height and have 3–5 true leaves.

Indeterminate 'Ideal' is planted at an area amid rows of 75-80 cm and 35-40 cm - betwixt plants in the row. The branches are attached to a supporting structure.

Determinate 'Pikador' and 'Local Dwarf were planted 25–30 cm between tomato plants, and space rows 60–90 cm aside.

The field experiment was block design with three repetitions, with ten (10) plants in repetition.

The agrotechnical measures were carried out in the excellent terms for the crop.

Fluorescence spectroscopy was applied to determine the varietal differences after tomato harvesting under uncontrolled conditions. The spectral analysis was performed locally at the Institute of Plant Genetic Resources „Konstantin Malkov“ - Sadovo.

The analysis was carried out with a fiber-optic spectrometer, which enables the formation of emission fluorescence signals from 200 nm to 1200 nm.

The unproved setup adds a laser diode (emission wavelength 285 nm, optical power 16 mW, DC) and a compact spectrometer (exemplary AvaSpec-ULS2048CL-EVO). The accessions are placed one by one on a duralumin plate. The experimental setup allows emission fluorescence signals to be detected through a Y-shaped optical fiber at 180 degrees to the sample and at a distance of 1.7 cm from it. The fruits are placed and arranged on a duralumin plate, which allows the encounter of an emission signal in perpendicular geometry at 180 degrees and at a distance of 1.7 cm by Y-shaped optical fiber. This curtails aberrations and allows the formation of an exceptional emission fluorescence signal (Fig. 1). The resolution of the spectrometer can range from 0.06 - 20 nm, with that of the setup recycled for our experiment being 0.09 nm. The useful fluorescence signal is generated in a direction that is 180° from the excitation radiation, so as not to saturate the receiver. A laser diode (LED) was used as a source because its spectral width was too small. The source is high-power and more sensitive than other commercial LEDs. It generates a continuous or pulsed light signal. It has an SMA-905 connector for connecting optical fibers and works with a 5V or 1.6A power supply. LED has a relatively wide spectral width of emission (30–40 nm) and an angular distribution of emission in the range of +/−30°. The sensitivity of the spectrometer is in the range of 200 nm to 1200 nm. The mode of operation of the AvaSpec-ULS2048CL-EVO enables both the emission spectrum and the excitation source spectrum to be recorded. The emission signal represents the spectral distribution of the signal emitted by the accession after fluorescence has occurred. The excitation spectrum represents the dependence of the emission intensity measured for one scanning wavelength against the excitation wavelength of the LED. This spectrum is represented as a function of the wavelength of the light signal incident on the photodetector in the spectrometer. Complementary metal-oxide semiconductor (CMOS) instead of conventional charge-coupled device (CCD) technology, this spectrometer owes its key advantage over others with a similar configuration to the dominant position of the CMOS detector in its design. For this particular circuit, the photodetector is of the CMOS model S9132 type. Its sensitivity is in the range of 200 nm to 1200 nm. Its resolution is $\delta\lambda = 5$ nm. S9132 was chosen because it could detect emission radiation from fruit with analogous cell morphology, biology, and chemical composition.

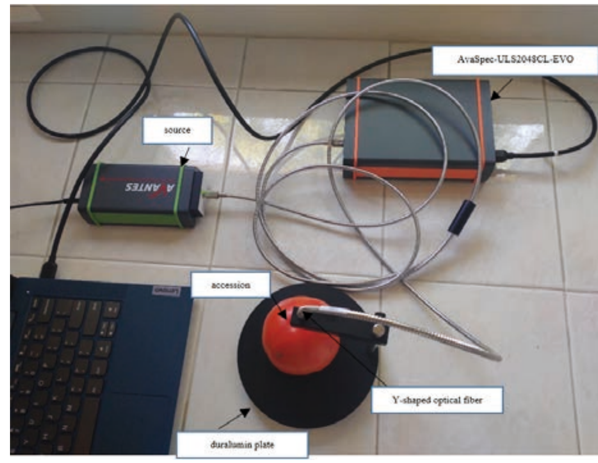

Figure 1: Accustomed aspect of the experimental installation used by fluorescence spectroscopy

The laser radiation is deflected against the source and hits the sample. Afterward, the sample fluoresces, and the emission signal is conducted on a U-shaped optical fiber with a core diameter of 200 μm, a step-index of refraction, and a numerical aperture of 0.22. It sends a signal to the detector. In the spectrometer, the light signal is converted to an electrical-digital signal using a USB 2.0 wire, downloaded to a computer with AvaSoft8 software, and exported to Excel. This allows analysis, processing, and visualization of the results of the conducted research.

3 RESULTS AND DISCUSSION

The optical properties of tomato fruits are determined by their energy structure, which includes both the occupied and free energy levels as well as the energy levels of the atomic vibrations of the molecules in the crystal lattice. The achievable transitions between these energy levels, as a function of photon energy, are specific to the tomato, resulting in spectra and optical properties unique to it. Tomato fruits contain particles with sizes smaller than the wavelength of visible light. Particles in the turbid medium (such as tomato fruits) act as independent light sources, emitting incoherently, causing the samples to visibly fluoresce.

Therefore, fluorescence spectroscopy finds application for analysis in this vegetable crop. The optical parameters and spectral properties also change as a function of temperature, pressure, external electric and magnetic fields, etc. This allows obtaining essential information about changes in the chemical and cellular morphological composition of the tomato.

The spectral distributions of tomato fruits of the Local Dwarf and Pikador varieties are presented in Fig. 2. A certain correlation is observed between them (the emission wavelength of Local Dwarf t is 425 nm; the emission wavelength of Pikador is 421 nm). Their emission fluorescence signals are close in terms of peak wavelength and signal intensity level. The emission wavelengths are due to the content of certain fluorophoric compounds in tomatoes, for example, nucleocapsid (N) proteins, lectin, some carboxyl compounds, and others. This is because both varieties are determinant tomatoes. They are close in biological and cellular morphological composition. The method of fluorescence spectroscopy is applied in this study to distinguish the fruits of these two varieties since the correlation in the spectral distribution is sufficiently distinct and distinguishable. This fact is used in this study to determine the tomato fruit belonging to a given variety.

Fig. 3 shows the spectral distributions of tomato fruits of the Local Dwarf and Ideal varieties; a significant correlation is observed between them (emission wavelength for Local Dwarf is 425 nm; emission wavelength for Ideal is 410 nm). Their fluorescence emission signals are not close and have a significant offset in wavelength localization and signal intensity level. The method of fluorescence spectroscopy can be applied to distinguish the fruits of these two cultivars because the correlation in the spectral distribution is of considerable distinctness and distinction. The method of fluorescence spectroscopy may practically be used to qualitatively resolve the belonging of fruits to a given variety.

Fig. 4 shows the spectral distributions of tomato fruits of the Pikador and Ideal varieties. A significant difference is observed between them (the emission wavelength for Pikador is 421 nm; the emission wavelength for Ideal is 410 nm). Their fluorescence emission signals are not close and have a significant offset in wavelength localization and signal intensity level. Since the differences in the spectral distribution are available for these two cultivars, the method of fluorescence spectroscopy can be applied to distinguish their fruits. The method of fluorescence spectroscopy can practically be used to qualitatively determine the belonging of fruits to a given variety.

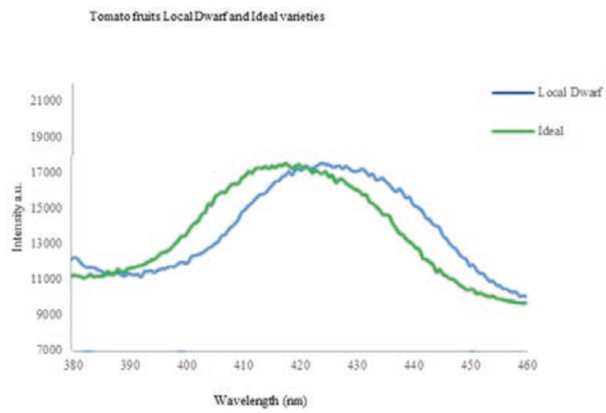

Figure 3: Emission wavelengths of tomato fruits Local Dwarf and Ideal varieties

A literature survey was conducted using similar methods. It turned out that, until now, the described experimental approach for the field method in the determination of varietal differences after tomato harvesting has not been applied internationally. This gives us reason to claim that for the first time, fluorescence spectroscopy was used in the application of fluorescence spectroscopy as a field method in the determination of varietal differences after tomato harvesting under uncontrolled conditions. The method is successfully applied to distinguish

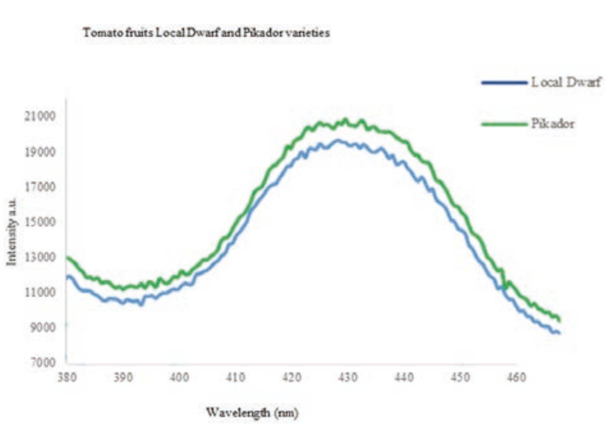

Figure 2: Emission wavelengths of tomato fruits Local Dwarf and Pikador varieties

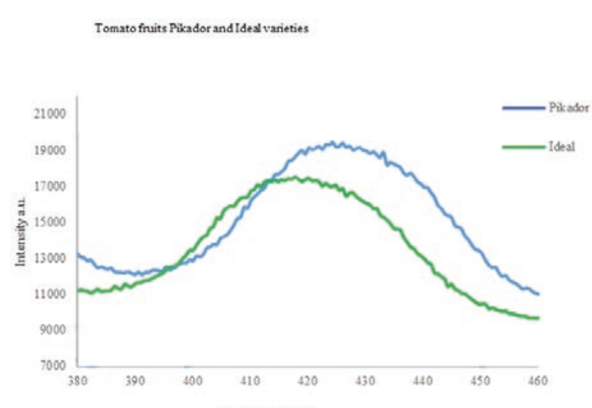

Figure 4: Emission wavelengths of tomato fruits Pikador and Ideal varieties

fruit tomatoes from different varieties. Fluorescence spectroscopy can be applied to analyze the tomato fruit of unknown cultivars and establish its origin with a sufficiently well-structured data library. Because it can be applied topically to test specimens. It eliminates sample damage during transport and provides a highly sensitive assay.

4 CONCLUSIONS

The fluorescence spectroscopy method is fast-acting in application as a field method in the determination of varietal differences after tomatoes harvesting locally under uncontrolled conditions.

It has been proven that fluorescence spectroscopy will successfully apply as a rapid tool to establish the origin of unknown tomato fruits in the presence of a rich library of spectra. The developed method can be used successfully in tomato breeding programs. The stability of the breeding line and its common blacks with an established cultivar of the same species can be observed by following the difference in the spectral distribution.

The differentiation of related varieties is a laborious and time-consuming task. For these reasons, the development of techniques that *could* assist in an early, quick, and accurate differentiation of related varieties is of utmost importance.

A systematic engineering approach to the setup (optical setup) of a mobile fiber optic plant for fluorescence spectroscopy research was found to be applicable in determining varietal differences in tomato cultivation.

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Investigation of the medium for barley (*Hordeum vulgare* L.) immature embryo culture

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Investigation of the medium for barley (*Hordeum vulgare* L.) immature embryo culture

Abstract: Germination of immature embryos of interspecific crosses *in vitro* is the most important problem in barley breeding programs. The effect of four media types (MS, ½ MS, B-5, ½ B-5 on immature embryo culture of nine barley varieties (Sameer, Buraq, Aksad, Shuaa, Arevat, Alwarkaa, Alhadher, Amel, and Rihan) was studied by using completely randomized design (C.R.D.) with six replicates. Analysis of variance showed highly significant effects due to varieties, media, and interaction for all studied characteristics, indicated high variation among varieties and media. Aksad variety was significantly superior to all varieties for all studied traits except root number (RN). Medium of ½ B-5 was significantly superior to all media for all studied traits. Aksad interactions with media except B-5 media gave full germination with highly significant superiority over all interactions. Aksad x ½ B-5 interaction showed a highly significant superiority for shoot length (SL) and RL. As a result, ½ B-5 medium was the efficient medium for germination of immature embryos of most varieties. These varieties could be used in breeding program of interspecific crosses with wild species by using ½ B-5 medium.

Key words: barley varieties, MS medium, Gamborg's B-5 medium, final germination percentage, shoot length, root length, root number

Preučevanje gojišč za gojenje nezrelih embrijev ječmena (*Hordeum vulgare* L.)

Izvleček: Kalitev nezrelih embrijev *in vitro* je pri medvrstnih križanjih največji problem pri žlahtnjenju ječmena. Preučevan je bil učinek štirih vrst gojišč (MS, ½ MS, B-5, ½ B-5) na kulture nezrelih embrijev devetih sort ječmena (Sameer, Buraq, Aksad, Shuaa, Arevat, Alwarkaa, Alhadher, Amel, in Rihan) v popolnem naključnem poskusu s šestimi ponovitvami. Analiza variance je pokazala visoko značilne učinke sort, gojišč in njihovih interakcij za vse preučevane lastnosti, kar kaže na velike razlike med sortami in gojišči. Sorta Aksad je bila značilno superiorna napram vsem ostalim sortam za vse preučevane lastnosti, razen za število korenin. Gojišče ½ B-5 je bilo značilno najboljše za vse preučevane lastnosti. Sorta Aksad je imela popolno kalitev na vseh gojiščih, razen na gojišču B-5. Sorta Aksad je imela na gojišču ½ B-5 značilno najdaljše poganjke in korenine. Izkazalo se je, da je gojišče ½ B-5 najbolj učinkovito za kalitev nezrelih embrijev večine obravnavanih sort. Te sorte bi lahko bile uporabljene v žlahtniteljskih programih za medvrstna križanja z divjimi vrstami ječmena z uporabo gijišča ½ B-5.

Ključne besede: sorte ječmena, MS gojišče, Gamborg's B-5 gojišče, odstotek končne kalitve, dolžina poganjka, dolžina korenine, število korenin

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1 INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops in the world. It is ranking as the fourth major crop in production and area next to wheat rice and maize (Goedeke et al., 2007, Blake et al., 2011). Barley is one of the earliest, the most domesticated food crop that humans have known since prehistoric times. It was the main source for making bread, soup and porridge dishes in the countries of the ancient world. Currently, barley has many uses, whether in human nutrition or animal feed. Barley flour is used alone or mixed with wheat flour in the industry of various types of pastries, especially bread. Barley is also included in many food industries, such as baby food, breakfast cereals, biscuits, beer, other alcoholic and non-alcoholic beverages, malt syrup, malted milk, and chemical industries. Barley is also a rich source of vitamins, minerals and dietary fiber. Its high content of soluble dietary fiber has reinforced the importance of barley and its place as an important food ingredient. Many studies have shown that regular consumption of barley reduces the risk of certain diseases such as colon cancer, gallstones, high blood pressure and chronic heart disease (Idehen et al., 2017, Biel et al., 2020). In India, most of the barley produced (90 %) is used for human consumption. Barley is also used as animal feed. Its grain (rich in carbohydrates) and the resulting straw are used to feed animals. It is also grown in some countries for use as green fodder or as pasture for animals. Some countries, including Iraq, use almost 80 % of grown barley as fodder (FAO, 2009). The use of barley as feed has a special value by giving desirable parts of visceral fat (Kumar et al., 2012). Barley is also grown as a cover crop to prevent soil erosion (water and air erosion) and curb weeds (Mohammed, 2018). Abiotic and biotic stresses lead to a decrease in crop production and nutritional value of crop grains over the world. Drought, salinity, and disease are among the most important stresses from an economic point of view and the most important in biology (Hussain, 2006; Mohammed et al., 2021). Due to the limited genetic variance in the cultivated varieties of barley, the use of large genetic variance that available in the wild species is necessary in the breeding programs (Ellis et al., 2000, Sreenivasulu et al., 2008). Therefore, interspecific crosses between domestic barley and wild species such as *H. bulbosum* L. and *H. murinum* L. are a major source of genetic differences and gives plant breeders a great opportunity to recombine the chromosomes set in order to take advantage of the positive aspects found in several individuals and collect them in one organism (Araus et al., 2003, Nevo and Chen, 2010). Many researchers have pointed that the widespread of wild species of barley indicates the high potential for great genetic diversity and adaptation to biotic and abiotic stresses that serve as an important genetic resource for breeding programs. Where the different genotypes of wild species constitute an important source for plant breeders in improving the adaptive traits that contribute to increasing and stabilizing production under conditions of biotic and abiotic stresses, and can be an entry point for improving varieties (Suprunova et al., 2007; Alassaf, 2018).However, interspecific crosses between domestic and wild species of barley has many sexual barriers such as very low seed set and abort endosperm due to sexual incompatibilities. Therefore, plant tissue culture plays an important role in solving these problems that faced by plant breeders in breeding programs to improve barley varieties (Houben et al., 2011). Immature embryo rescue is one of the most important applications of tissue culture to solve the problem of sexual incompatibilities. Embryo culture technology also has several uses such as producing rare species, haploid plants that use to produce homozygous diploid plants, testing seed viability, and breaking seed dormancy. In addition, embryo culture is used to produce large number of plants from one embryo by the callus method. However, mature embryos can grow in a simple nutrient medium, but immature embryos need a more complex nutrient medium. As a result, immature embryos need to be grown in efficient culture media to mature and give a complete and healthy natural plant (Burun and Poyrazoglu, 2002; Pickering and Johnston, 2005; Chahal and Gosal, 2006). Crosses also differ significantly in the type of medium that suitable for each cross (Mohammed et al., 2020). Therefore, there is a need to determine the most suitable culture media for the largest number of genotypes that provide the regular growth of embryo in order to obtain successful breeding programs to improve barley varieties.

2 MATERIALS AND METHODS

Experiments were carried out in the field and laboratory of College of Agriculture/ Tikrit University in order to study the effect of different types of media on immature seed germination of nine varieties of barley. Seed of varieties used in this study were obtained from Seed Technology Center/ Office of Agricultural Research/ Iraqi Ministry of Science and Technology. Barley varieties were: (Sameer, Buraq, Aksad, Shuaa, Arevat, Alwarkaa, Alhadher, Amel, Rihan). Seed of each of the nine varieties were planted in the field during the winter session of 2020/2021 to obtain immature embryos for use in the media assessment experiment. All crop service operations such as irrigation, fertilization and weed control were carried out according to researchers' recommendations for the barley crop. Spikes from each variety were harvested 12-14 days after pollination which embryo length was approximately 1.5 mm. The harvested spikes (with peduncle) were placed vertically in a beaker containing a little amount of water which cover spike peduncle and then placed in the refrigerator until dissection (Devaux 2003). Seed were removed from spikes of each of the nine varieties then were sterilized in 70 % ethanol for a minute. After that seeds were disinfested with 1 % sodium hypochlorite solution (NaClO) for 6 minutes then rinsed three times with sterile distilled water then dried on sterile paper napkin. After that, immature embryos of each variety were dissected from their caryopses and placed in 100 ml jars (five embryos per jar) containing either full concentration of Murashige and Skoog medium (Sigma-Aldrich Co., M-5519, St. Louis, MO), half concentration of MS medium, full concentration of Gamborg B-5 medium (Sigma-Aldrich Co., G-5893, St. Louis, M), or half concentration of Gamborg B-5 medium. All types of media were without plant growth regulators. Planted jars were labeled and wrapped with ParafilmM® PM-996 (Bemis Company Inc., Neenah, WI). Process was conducted under a laminar flow hood (*ENVIRCO*, *Environmental Air Control, Inc*., Hatfield, PA) to control contamination. All types of media were supplemented with 0.8 % agar (Sigma-Aldrich Co., A-1296, St. Louis, MO); 3 % and 2 % sucrose (Fisher Scientific, S5-500, Fair Lawn, NJ) for MS and Gamborg B-5 media, respectively; pH of MS and Gamborg B-5 media was adjusted to 5.7 and 5.5, respectively by using 0.1 N NaOH (Burun and Poyrazoglu, 2002; Houben et al., 2011). Germination process was carried out in the growth chamber (Percival, Mod. 135LLVL, Controlled Environments. Boone, IA) in the darkness at a temperature of 25 °C ± 2 for ten days. The number of normal germinated embryos was only counted daily to determine final germination percentage by using the following equation described by Scott et al. (1984):

$$FG\ \% = \frac{\text{Final number of germinated embryos}}{\text{Total number of embryos planted}} \times 100$$

Comparisons were made among the types of media for final germination percentage, shoot length (cm), root length (cm), and root numberfor the nine varieties of barley. Experiment was arranged as a completely randomized design (C. R. D.) with six replications (jars).

Data were statistically analyzed as a CRD by using PROC MEANS and PROC GLM in SAS (Version 9.4, SAS Institute, 2011, Cary, NC). Significant mean separation among types of media, varieties, and the interactions was determined using Fisher'sleast significant difference (LSD) at $\alpha = 0.05$ *and* 0.01. Data were graphed usingSigmaPlot version 13 (Systat Software Inc., San Jose, CA).

3 RESULTS AND DISCUSSION

Analysis of variance showed highly significant differences among barley varieties, media types, and barley varieties x media types of interaction for all studied characteristics (Table 1).

The significant effects among barley varieties, and among media types indicated that there was a high genetic variation among varieties, and high variation among media. Furthermore, the significant interaction between varieties x media indicated that each variety responded to each medium type independently. Previous studies have also found significant differences among varieties, media types, and barley varieties x media interaction (Burun and Poyrazoglu, 2002; Hayes et al. 2003; Han et al. 2011; Houben et al. 2011; Kahani et al. 2012; Mohammed, 2018; Mohammed et al. 2020).

Germination of immature embryos is the most important problem that plant breeder faces in breeding program of interspecific crosses between domestic barley and wild barley to improve barley varieties. The final germination percentage (FGP) of immature embryos of nine barley varieties were evaluated based on four types of media (Table 2). The results showed a high significant differences among barley varieties for FGP trait. The

Table 1: Analysis of variance of nine varieties of barley (Sameer, Buraq, Aksad, Shuaa, Arevat, Alwarkaa, Alhadher, Amel, Rihan), four types of media (MS, ½ MS, B-5, ½ B-5), and their interaction for final germination percentage (FGP), shoot length (SL), root length (RL), and root number (RN)

Source of variation	Degrees of freedom	FGP	SL	RL	RN
Varieties (V)	8	***	***	***	***
Media (M)	3	***	***	***	***
V x M	24	***	***	***	***
Error	180				

*** Significant at the 0.001 probability level
NS, significance level $p > 0.05$

mean of FGP of immature embryos of barley varieties ranged from 36.11 to 99.17 % at four types of media. Aksad variety gave the highest FGP value (99.17 %) and was highly significant superior to all varieties; it fallowed by Arevat variety with 86.67 % of FGP. While Rihan variety had the lowest value of FGP (36.11 %). Table 2 also showed highly significant differences among media types for this trait and ranged from 64.33 to 77.69 % of immature embryos. The half concentration of Gamborg's B-5 medium (1/2 B-5) showed the highest value of FGP (77.69 %) and was significant superior on all media types; while the half concentration of Murashige and Skoog (1/2 MS) medium gave the lowest value (64.33 %).

Interaction between barley varieties x media types revealed highly significant difference for FGP trait. The value of FGP of combinations ranged from 23.33 to 100.00 % (Table 2). The interactions between Aksad variety with MS, ½ MS, and ½ B-5 media were superior by giving the full and highest value of FGP (100.00 %). While the interaction between (Rihan x ½ MS), (Rihan x B-5), and (Shuaa x ½ MS) showed the lowest average of FGP which amounted to 23.33 %, 26.67 %, and 28.33 %, respectively. These results confirmed the high significant differences among varieties as well as media types. Also, these data confirm that individual varieties of barley responded differently.to each type of media. For example, Shuaa, Alwarkaa, Alhadher, and Rihan varieties gave the highest value of FGP of immature embryos when they cultured on ½ B-5 medium. While Buraq, Amel, and Arevat varieties had the highest value in ½ MS, B-5, and ½ MS & B-5 media, respectively. On the other hand, Arevat variety was superior with complete FGP in MS, ½ MS, and ½ B-5 media. In conclusion, Aksad variety and half concentration of B-5 medium were the best for final germination trait. Many researchers have found that MS, ½ MS, B-5 and other media can be used for culturing immature and mature embryo. These results were confirmed by previous studies. Mihailescu and Giura (1996) reported that germination of barley embryo was better in B-5 medium than MS. While Burun and Poyrazoglu (2002) found that MS and ½ MS media were better than B-5 medium when they evaluated the FGP of Kaya 7794 variety on MS, ½ MS, B-5, and RC (Randolph and Cox) media. Han et al. (2011) reported that FGP of barley varieties in B-5 medium was higher by 10 % than MS medium. Also, Chen et al. (2011) reported that is difficult to draw a conclusion about the most suitable medium for different barley genotypes. Chen's result confirmed by Mohammed (2018) and Mohammed et al. (2020) found that each genotype of barley responded to each culture medium independently of germinated immature embryo of many crosses that obtained from interspecific crosses between domestic and wild barley. This result may be due to component of media, genotypes, embryo size, sucrose rate, and pH. As a result, these varieties could be used in breeding program of interspecific crosses with wild barley species by using the ½ B-5 media to improve some quality and quantity traits of barley varieties.

Table 3 showed the shoot length (SL) of germinated immature embryos of nine barley varieties that cultured at four types of media. The results showed highly significant differences among barley varieties for SL

Table 2: Mean of final germination percentage (FGP) of nine barley varieties in four types of media

	Types of Media				
Varieties of Barley	MS	½ MS	B-5	½ B-5	Mean (%)
Sameer	67.67	62.22	74.17	82.22	71.57
Buraq	62.98	74.60	56.11	70.63	66.08
Aksad	100.00	100.00	96.67	100.00	99.17
Shuaa	58.89	28.33	52.67	73.33	53.31
Arevat	87.78	93.33	93.33	72.22	86.67
Alwarkaa	68.89	63.81	67.78	84.78	71.31
Alhadher	59.33	56.67	60.00	76.00	63.00
Amel	58.89	73.33	93.33	86.67	78.06
Rihan	41.11	26.67	23.33	53.33	36.11
L.S.D. (0.05)	8.212				4.106
L.S.D. (0.01)	10.900				5.450
Mean (%)	67.28	64.33	68.60	77.69	
L.S.D. (0.05)	2.737				
L.S.D. (0.01)	3.633				

Table 3: Mean of shoot length (SL) of nine barley varieties in four types of media

Varieties of Barley	Types of Media				Mean (cm)
	MS	½ MS	B-5	½ B-5	
Sameer	5.73	1.29	1.97	3.94	3.23
Buraq	5.07	4.58	4.92	5.30	4.96
Aksad	3.50	2.23	8.97	11.98	6.67
Shuaa	2.35	1.38	2.43	4.18	2.59
Arevat	6.97	6.97	3.06	5.58	5.65
Alwarkaa	2.06	2.95	2.99	3.17	2.79
Alhadher	1.87	1.53	5.18	5.18	3.44
Amel	2.43	5.38	7.50	8.00	5.83
Rihan	4.07	1.80	1.98	5.98	3.46
L.S.D. (0.05)	1.670				0.835
L.S.D. (0.01)	2.217				1.108
Mean (cm)	3.78	3.12	4.33	5.92	
L.S.D. (0.05)	0.556				
L.S.D. (0.01)	0.739				

trait. The mean of SL of germinated immature embryos of barley varieties ranged from 2.59 to 6.67 cm at four types of media.

Aksad variety gave the highest SL value (6.67 cm) and was highly significant superior to all varieties; it fallowed by Amel and Arevat varieties with values of 5.83 and 5.65 cm of SL, respectively. Whilst, Shuaa and Alwarkaa varieties gave the lowest value 2.59 and 2.79 cm of SL. Table 3 also revealed highly significant differences among media types for SL trait and ranged from 3.12 to 5.92 cm. The type ½ B-5 medium gave the highest value of SL (5.92 cm) and was significant superior on all media types; while the type 1/2 MS medium had the lowest value of SL (3.12 cm). The barley varieties x media types interaction showed highly significant difference for the trait of SL.The shoot length value of combinations ranged from 1.29 to 11.98 cm (Table 3).The interaction of Aksad variety x ½ B-5 medium was a high significant superior by giving the highest value of SL which amounted to 11.98 cm; it followed by Aksad x B-5 and Amel x ½ G-5 with 8.97 and 8.00 cm of SL, respectively.Whereas the interactions of Sameer, Shuaa, Alhadher, and Rihan varieties with ½ MS media gave the lowest values of SL. These results confirmed the high significant differences among varieties and among media types for the SL trait. These data confirm that individual varieties of barley responded differently to each type of media for this trait. For instance, MS medium was suitable for Sameer and Arevat varieties as well as ½ MS medium for Arevat by giving the highest value of SL of germinated immature embryos. While ½ B-5 medium was suitable medium for Aksad, Shuaa, Rihan, Alhadher, and Amel varieties as well as B-5 medium for 'Alhadher' and 'Amel'. On the other hand, Buraq and Alearkaa varieties did not show significant differences for SL trait in the four types of media. For this trait, Aksad variety was also the best variety when its immature embryos cultured on 1/2 B-5 medium. The same result was found by Mohammed (2018) that some crosses were superior when cultured in MS, while other crosses were superior in B-5 medium for SL trait These data are consistent with previous findings which reported that genotypes responded to culture medium independently (Burun and Poyrazoglu, 2002; Russowski et al., 2006; Chen et al., 2011; Mohammed et al., 2020).

The root length (RL) of immature embryos of nine barley varieties were evaluated based on four types of media (Table 4).

The results showed a highly significant difference among barley varieties for RL trait. The mean of RL of immature embryos of barley varieties ranged from 1.45 to 4.68 cm at four types of media. Aksad variety had the highest value (4.68 cm) of RL and was highly significant superior to all studied varieties of barley for RL trait; Arevat and Amel varieties followed Aksad variety in terms of superiority of RL trait (4.02 and 4.01, respectively). While Shuaa and Sameer varieties had the lowest RL values (1.45 and 1.79, respectively). Table 4 also showed highly significant differences among media types for RL trait and ranged from 2.34 to 3.66 cm of immature embryos of barley varieties. The medium of 1/2 B-5 had the highest value (3.66) of RL trait and was significantly superior on all types of media. Whilst the 1/2 MS medium showed

Table 4: Mean of root length (RL) of nine barley varieties in four types of media

	Types of Media				
Varieties of Barley	MS	½ MS	B-5	½ B-5	Mean (cm)
Sameer	3.07	0.38	1.40	2.30	1.79
Buraq	4.37	2.96	4.00	3.63	3.74
Aksad	3.62	1.66	5.48	7.97	4.68
Shuaa	1.75	1.30	1.00	1.76	1.45
Arevat	4.79	4.83	2.76	3.72	4.02
Alwarkaa	1.62	3.05	2.76	2.29	2.43
Alhadher	2.06	1.77	3.95	4.12	2.97
Amel	1.71	3.90	5.61	4.83	4.01
Rihan	4.25	1.22	2.27	2.37	2.53
L.S.D. (0.05)	1.197				0.598
L.S.D. (0.01)	1.590				0.795
Mean (cm)	3.03	2.34	3.25	3.66	
L.S.D. (0.05)	0.399				
L.S.D. (0.01)	0.530				

the lowest RL value (2.34 cm). The combination between barley varieties x media types showed highly significant difference for RL trait. The value of RL of interactions ranged from 0.38 to 7.97 cm (Table 4). The combination between Aksad variety x 1/2 B-5 media were superior by giving the highest RL value (7.97 cm); it followed by the interaction of Amel and Aksad with B-5 medium which amounted to 5.61 and 5.48 cm, respectively. While Sameer variety x ½ MS interaction gave the lowest average of RL trait (0.38 cm). These results confirmed the high significant differences among varieties and among media types. These data also confirm that individual barley varieties responded differently.to each type of media. For example, Sameer, Rihan, and Buraq varieties were superior in RL when cultured on MS medium as well as B-5 medium for Buraq variety. While Alwarkaa and Arevat varieties had the highest value in ½ MS medium as well as MS medium for Arevat variety. On the other hand, ½ B-5 was suitable medium for Aksad variety as well as B-5 medium for Alhadher and Amel varieties. Whilst four types of media had no significant effect on RL of Shuaa variety. For this trait, Aksad variety was also the best variety when its immature embryos cultured on 1/2 B-5 medium. These results were confirmed by previous studies (Burun and Poyrazoglu, 2002; Russowski et al., 2006; Han et al., 2011; Mohammed et al., 2020) that is difficult to draw a conclusion about the most suitable medium for different barley genotypes.

Table 5 revealed the root number (RN) of germinated immature embryos of nine barley varieties that cultured at four types of media. The results showed a highly significant differences among varieties of barley for RN trait. The mean of RN of germinated immature embryos of varieties of barley ranged from 2.88 to 4.32 at four media types. Aksad, Amel, Arevat, and Buraq varieties had the highest values of RN and were highly significant superior to all other studied of barley varieties with values of 4.32, 4.29, 3.98, and 3.94 of RN trait, respectively. While the other five varieties gave the lowest values of RN. Table 5 showed highly significant differences among types of media for RN trait and ranged from 2.99 to 4.12 roots. The medium type of ½ B-5 gave the highest value of RN which amounted to 4.12 roots and was significant superior on all media types; whereas the medium type of 1/2 MS had the lowest RN value (2.99). The interaction between barley varieties x media types showed highly significant difference for the trait of RN. The root number value of combinations ranged from 1.67 to 5.58 roots (Table 5). The interaction of Aksad and Amel varieties with ½ B-5 medium, Arevat x ½ MS medium, and Aksad x B-5 medium had the highest values of RN which amounted to 5.58, 5.27, 5.20, and 5.11 roots, respectively. While the interactions of Rihan and Sameer varieties with ½ MS medium, 'Alhadher' x MS medium, and 'Shuaa' x B-5 medium gave the lowest values of RN. These results confirmed the high significant differences among varieties and among media types for the RN trait. Also, these data confirm that individual varieties of barley responded differently.to each type of media for this trait.

For instance, MS medium was suitable for Sameer variety by giving the highest value of SL of germinated immature embryos. While ½ MS medium was suitable

Table 5: Mean of root number (RN) of nine barley varieties in four types of media

	Types of Media				
Varieties of Barley	MS	½ MS	B-5	½ B-5	Mean (cm)
Sameer	4.67	1.69	2.44	3.78	3.15
Buraq	4.06	3.33	3.81	4.55	3.94
Aksad	4.17	2.42	5.11	5.58	4.32
Shuaa	3.33	2.84	1.94	4.03	3.04
Arevat	3.78	5.20	3.21	3.72	3.98
Alwarkaa	3.07	2.44	3.50	2.50	2.88
Alhadher	1.89	3.00	3.94	3.44	3.07
Amel	3.11	4.33	4.45	5.27	4.29
Rihan	3.58	1.67	2.33	4.17	2.94
L.S.D. (0.05)	1.045				0.522
L.S.D. (0.01)	1.387				0.693
Mean (cm)	3.52	2.99	3.42	4.12	
L.S.D. (0.05)	0.348				
L.S.D. (0.01)	0.462				

medium for Arevat variety. Aksad, Buraq, Shuaa, Amel, and Rihan varieties were superior in RN when cultured on ½ B-5 medium, while Alwarkaa and Alhadher varieties gave the highest value in B-5 medium. For this trait, Aksad variety was also the best variety when its immature embryos cultured on 1/2 B-5 medium. These results are consistent with previous studies which reported that genotypes responded to culture medium independently (Burun and Poyrazoglu, 2002; Russowski et al., 2006; Chen et al., 2011; Han et al., 2011; Mohammed et al., 2020).

4 CONCLUSION

There were highly significant differences among barley varieties, media types, and interactions for all studied characteristics except the leaf number, which indicated that there was a high genetic variation among varieties, and high variation among media. Aksad variety, ½ B-5 medium, and Aksad x ½ B-5 gave the highest values for all studied traits. Varieties of barley responded to each type of media independently. As the FGP is the most important trait, ½ B-5 medium was suitable for most varieties, while some varieties were superior either in B-5, MS, or ½ MS media. As a result, it is difficult to draw a conclusion about the efficient medium for all varieties. These varieties could be used in breeding program of interspecific crosses with wild barley species by using the efficient type of media for each variety to improve some quality and quantity traits of barley varieties.

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Physical and aerodynamic properties of date palm pollen grains

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Physical and aerodynamic properties of date palm pollen grains

Abstract: The present study was designed to determine the effect of four moisture content levels (4, 5, 6, and 7 %) on the physical and aerodynamic properties of date palm pollen grain (DPP). The physical properties of DPP included pollen length (*L*), width (*w*), thickness (*T*), projected area (A_p), geometric mean diameter (d_g), mass (m), sphericity (*S*), and bulk density (ρ_p). It was observed that the moisture content did not significantly influence the physical properties of the DPP. The aerodynamic properties of DPP included the terminal velocity (V_t), drag coefficient (D_c), drag force (*Df*), and Reynolds number (*Re*). The pollen Reynolds number (*Re*) is significant at different pollen grain moisture content, and regression models were developed in the form of polynomial and exponential relationships. Also, the 3rd order polynomial relationship was found between *Re* and D_c. The results showed that the average values of V_t, D_c, *Df*, and *Re* were about 0.6 m s^{-1}, (0.38 to 0.45), 1.09E-11 N, and (0.29 to 0.42), respectively. The results of this study will be helpful in the performance of date palm pollination machines.

Key words: aerodynamic, date palm, pollen grain, properties, terminal velocity

Fizikalne in aerodinamične lastnosti pelodnih zrn dateljeve palme

Izvleček: Namen raziskave je bil določiti učinek štirih vsebnosti vode (4, 5, 6, in 7 %) na fizikalne in aerodinamične lastnosti pelodnih zrn dateljeve palme (DPP). Fizikalne lastnosti so obsegale dolžino peloda (*L*), širino (*w*), debelino (*T*), projekcijsko površino (A_p), poprečni geometrični premer (d_g), maso (m), sferičnost (*S*), in gostoto (ρ_p). Ugotovljeno je bilo, da vsebnost vode ni značilno vplivala na fizikalne lastnosti peloda. Aerodinamične lastnosti peloda so obsegale končno hitrost (V_t), koeficient upora (D_c), moč upora (*Df*) in Reynoldovo število (*Re*). Reynoldovo število peloda (*Re*) je bilo značilno različno pri različnih vsebnostih vode, razvit je bil regresijski model na osnovi polinomnih in ekspotencialnih razmerij. Med *Re* in D_c je bilo ugotovljeno polinomno razmerje tretjega reda. Rezultati so pokazali, da so bile poprečne vrednosti parametrov V_t, D_c, *Df*, in *Re* okrog 0,6 m s^{-1}, (0,38 do 0,45), 1,09E-11 N in (0,29 do 0,42). Rezultati raziskave bodo pripomogli k boljšemu delovanju opraševalnih naprav.

Ključne besede: aerodinamika, dateljeva palma, pelodna zrna, lastnosti, končna hitrost

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1 INTRODUCTION

Date palm is the source of a wide range of products and services, including many necessities of life. The primary product of the date palm is fruit, which is rich in protein, vitamins, and mineral salts. The total date palm production in Egypt is about 1.64 million tons annually (FAOSTAT, 2019). Date palm pollination has been identified as a major factor for successful date production since quality and fruit yield depend on the correct application of pollen grains. It has conclusively been shown that the benefits of the pollination process lie in obtaining large-sized, high-quality fruits, avoiding the presence of small fruits (Salomón-Torres et al., 2017).

In Egypt, farmers select male palm trees and exchange palm pollen between farms even over long distances from the country (Bekheet and El-Sharabasy, 2015) Notably, the production of pollen from male palms differs significantly according to changing geographic regions. Generally, any male palm variety's pollen can be used to pollinate any female variety. Either, to establish a perfect pollination and achieve the highest yield with good fruit quality, it was advantageous to mix pollen grains with different carriers, essential minerals and ascorbic acid. It also improves the effectiveness of pollination (Radwan et al., 2022). However, as most male palms are seedlings, there are significant variances in the pollen quality of these plants (Salomón-Torres et al., 2021). In addition, pollen's physical and aerodynamic properties are one of the most important properties that affect pollination. The characteristics of pollen can affect its transmission from date palm pollinating machines to female flowers. Alharbi and Mousa (2021) studied some physical properties of palm pollen in the Qassim region, Saudi Arabia; they reported that date palm pollen grains come in a wide variety of shapes, sizes, and surface marking characteristics. The palm pollen sizes range from 3.3 to 704 µm. The pollen shape found in this study was spherical. The aerodynamic properties are the most important in describing the movement of grains in the air. For designers and operators of agricultural machinery, knowledge of the aerodynamic properties of the grain (floating velocity, final velocity, aerodynamic coefficient of resistance) is important and essential (Polyák and Csizmazia, 2010). These properties are terminal velocity, drag force, drag coefficient, and Reynolds number (Khoshtaghaza and Mehdizadeh, 2006). Terminal velocity is the steady air velocity value at which an object or a material is suspended in the vertical air stream. In other words, the maximum or terminal speed attained in free fall before air resistance will keep it from falling faster (Mohsenin, 2020). The morphology of pollen grains may also affect the aerodynamic properties. The relationship between terminal velocity and moisture content of pistachio nut was studied by Unal et al. (2008), 14.2 % increasing percentage in the terminal velocity was found depending on the change in moisture content from 5.83 to 30.73 % (*wb*). Morever, Matouk et al. (2008) mentioned that coffee cherries ('Catual') drag coefficient decreased from 0.05 to 0.03 as moisture content increased from 10.7 to 53.9 % (*d.b*). According to Eşref, and Nazmi (2016), as the moisture content of yellow lentil seeds increased the terminal velocity increased linearly from 1.5 to 2.09 m s^{-1} due to the increasing seed mass per unit frontal that faces the air stream. Abubakar et al. (2019) fabricated a measuring device to determine the terminal velocity of paddy rice, sorghum, and bean grains. Terminal velocities results in that study were 6.95 ± 0.37, 4.71 ± 0.24, and 10.98 ± 0.27 m s^{-1} for paddy rice, sorghum, and beans, respectively. In addition, the device efficiency was found to be 70.06 %. Thus, the main objective of this article is to determine the physical and aerodynamic properties of date palm pollen grain because these properties significantly impact the performance of date palm pollination machines.

2 MATERIALS AND METHODS

2.1 DATE PALM POLLEN GRAINS (DPP) PREPARATION

DPP collected from three resources of a local market in Siwa Oasis, Matrouh Governorate, Egypt, free of foreign matters, immature, and broken grains were selected for this study. The date palm pollen variety used in this research is not specified because commercial companies selling pollen have collected from more than one source and mixed them to improve the properties of the resulting crop.

Traditionally, pollen grains for some varieties are mixed and then used for pollination. Moisture content is one of the most important factors affecting the quality of the pollination process. The moisture content of the pollen grains was determined using the oven method at 103 ± 2 °C until reaching a constant mass (AOAC, 2000) and as described in many investigational studies. Results of minimum and maximum moisture content values of DPP were 3.6 and 8.2 % respectively. Considering the significance of moisture content in pollination process. Four levels of moisture content (4 %, 5%, 6 %, and 7 % *db.*) were used to determine the aerodynamic properties of the DPP. So far, each moisture content level was prepared using a pollen sample taken and placed in the oven at a temperature of 50 °C, then the sample mass was measured, and moisture content was calculated every 15 minutes' interval, in order to find out the time required to remove moisture to reach the different required moisture contents (4 %, 5 %, 6 %, and 7 % *dry basis*). After that, each sample was placed inside a desiccator in order to acclimatize it to the ambient environment (Coşkun et al., 2005). Conversely, the levels of chosen moisture content cover the range often seen in stored DPP and was checked before each experiment.

2.2 PHYSICAL PROPERTIES OF DATE PALM POLLEN GRAIN

2.2.1 Date palm pollen grain mass (*m*)

The mass (*m*) of the date palm pollen grain was calculated by dividing the 1 g of pollen mass by the number of pollen per one gram. Using the hemocytometer method, it is possible to count the number of pollens in a specific volume of water (suspensions solution: water + pollen) (Mahmoud-Aly et al., 2018). A hemocytometer method consists of microscope (QUANTA FEG250, Japan) and a thick glass microscope slide (dimension 3×3 mm). The slide is divided into nine squares (dimension 1 × 1 mm), as shown in (Fig. 1). The volume covered by one square is calculated by multiplying the area of one square (1 mm x 1 mm = 1 mm^2) by the depth of the square (0.1 mm). Hence, the final volume of each square is 0.0001 ml. The 2.5 g of pollen grains were added to 500 ml of distilled water and mixed for 5 min using magnetic stiller, so the dilution factor is 200 ml of water 1 g^{-1} of pollen (500 ml of water 2.5 g^{-1} of pollen). The sample from suspension solution was loaded into slide by using a micropipette. Then the hemocytometer slide was placed under the microscope, so it could determine the pollens number. The average pollen per small square was calculated (448 pollen). The number of pollen grains per one gram was calculated from equation (1).

$$N_{pg} = \frac{P_{ss} \times Du}{vss} \quad (1)$$

Where; N_{pg} is the number of pollen grains per one gram, P_{ss} is the average number of pollens per small square (448 pollen), *Du* is the dilution factor (equal to 200 ml g^{-1}), and *vss* is the volume of suspension upper small square (equal to 0.0001 ml).

2.2.2 Date palm pollen grain dimensions and projected area

The DPP dimensions were measured at different moisture content levels (4, 5, 6, and 7 %) using scanning electron microscopy (SEM, JSM-5200, Jeol Japan). Twenty date palm pollen were randomly selected from each moisture content level to determine grain dimensions and projected area (*Ap*). Three major dimensions of the pollen grain, namely length (*L*, µm), width (*w*, µm), and thickness (*T*, µm) were measured according to the biggest and smallest surface of the pollen grain (Obi and Offorha, 2015). The pollen samples were placed on a copper holder and coated with a fine gold layer using a fine coat (JFC-1100 E, Ion sputtering device, JEOL, Japan) before the observation to avoid electrostatic charging during observation. Then, the sample was observed under a high vacuum with acceleration voltage (25 kV) and at 500, 2000, and 5000-fold magnifications. The projected area of the pollen was determined by using the Image J program.

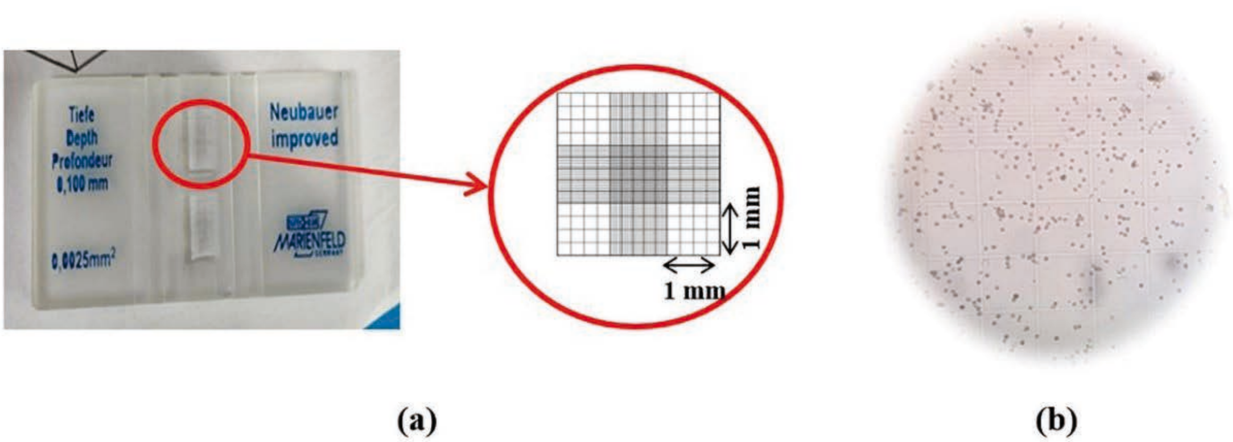


Figure 1: Pollen number determination using hemocytometer methods: (a) hemocytometer slide, and (b) pollen under microscope

2.2.3 The bulk density (ρ_p)

The bulk density (ρ_p) defined as the mass per unit volume of a particle, was determined for date palm pollen grain at different moisture content levels according to Abdelhady et al. (2023).

2.2.4 The geometric mean diameter (d_g)

The geometric mean diameter (d_g, µm) was calculated from equation (2) according to Mohsenin (2020)

$$d_g = (L.w.T)^{1/3} \quad (2)$$

Where; L is the pollen grain length (µm), w is the pollen grain width (µm), and T is the pollen grain thickness (µm).

2.2.5 Sphericity (S)

The sphericity expresses the shape character of the pollen relative to that of a sphere of the same volume. Assuming that the diameter of the circumscribed sphere is equal to the longest intercept L of the ellipsoid and that the volume of the pollen grain is equal to the volume of a triaxle ellipsoid with intercepts *L, w,* and *T.* Also, the degree of sphericity (S) was calculated from equation (3), according to Mohsenin (2020).

$$S = \frac{d_g}{L} \quad (3)$$

2.3 AERODYNAMIC PROPERTIES OF DATE PALM POLLEN GRAIN

2.3.1 Terminal velocity (V_t)

To evaluate the performance of pollination operations and options involving the presence of air flow, it is necessary to determine the terminal velocity of the date palm pollen grain. It will be used in studying the pollen grain in the airflow. When the pollen grain is immersed into an ascendant air flow, the pollen is subjected to the action of two kinds of forces: gravitational force and resisting drag force as shown in Fig. (2).

When these vector magnitudes are balanced (Fig. 2), the pollen grain begins a movement at a constant speed so-called terminal velocity that is depending on pollen mass (m), acceleration due to gravity (g), pollen grain density (ρ_p), air flow density (ρ_a), drag coefficient (D_c), and pollen grain projected area (A_p).

The experimental DPP terminal velocity (V_t) was measured at different moisture content levels using a device illustrated in Fig. (3). It consists of a centrifugal fan connected with an electric motor (Dayton- 1/70 HP). The air velocity was controlled by a digital control switch/regulator inverter connected to the blower motor; it is allowed to change the speed of the motor (Obaia and Ibrahim, 2015). The next unit was a cylindrical tube, it is divided to three parts. The first part is a PVC pipe with a length of 200 mm and a diameter of 50 mm, connected at its upper end to a grid to homogenize airflow. The second part is a PVC pipe with a length of 700 mm and a diameter of 50 mm connected at its upper end to a perforated screen to carry pollen on it. This tube has two holes in the middle with a diameter of 5 mm to connect the sensors to measure the speed of the air stream. The third part of the tube is a transparent glass tube (acrylic) with a length of 900 mm and a diameter of 50 mm to watch the pollen grains being carried by the air stream. The terminal velocity of the studied DPP could be obtained by measuring the air velocity required to suspend the particles

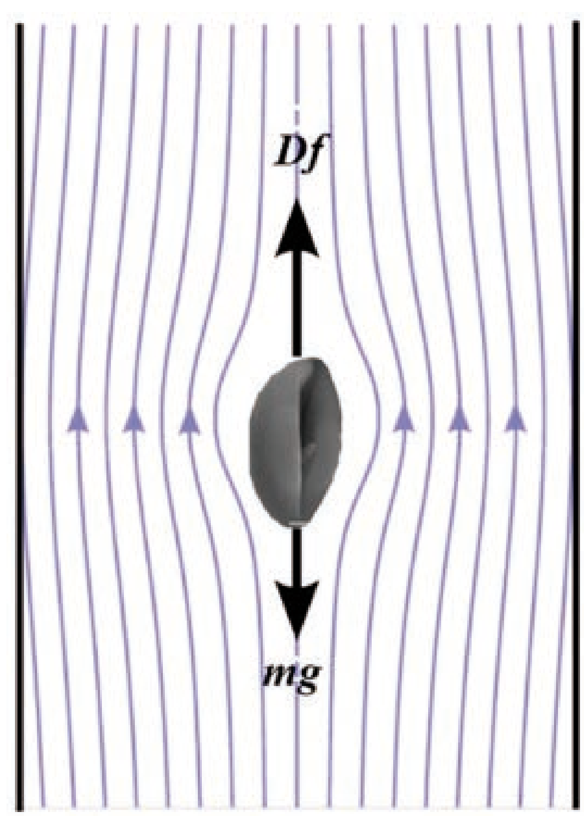
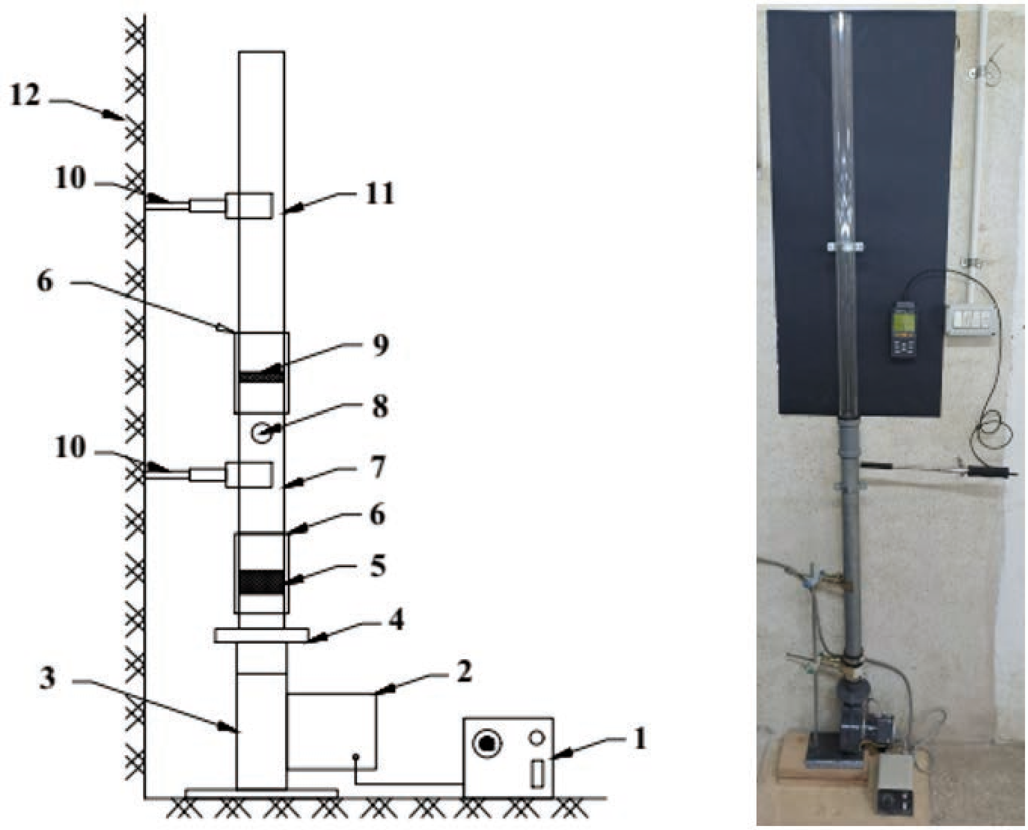


Figure 2: Pollen weight and drag force acting on the date palm pollen grain



1- Electrical controller. 2- Electrical motor.
3- Centrifugal fan. 4- First part.
5- Straighteners. 6- Collar.
7 -The second part. 8- A hole for air speed measurement.
9- Screen. 10- Clamp.
11- Cylindrical transparent glass tube. 12- The wall.

Figure 3: Experimental set-up used for measuring the terminal velocity

in the vertical air stream. The air velocity was measured by using the hot wire air velocity meter connected with a velocity probe (TENMARS TM-4001-the air velocity: 0.01 - 25 m s^{-1}).

2.3.2 The drag coefficient (D_c)

The drag coefficient (D_c) of date palm pollen grains was calculated from equation (4) according to Mohsenin (2020).

$$D_c = \frac{2m.g.(\rho_p - \rho_a)}{\rho_p.\rho_a.A_p.V_t^2} \quad (4)$$

Where D_c is the drag coefficient (dimensionless), m is the date palm pollen grain mass (kg), g is the gravitational acceleration (9.81 m s^{-2}), ρ_p is the particle bulk density (kg m^{-3}), ρ_a is the air bulk density which equals to 1.206 kg m^{-3} at room temperature, A_p is projected area of date palm pollen grain (m^2), and V_t is the pollen grain terminal velocity (m s^{-1}).

2.3.3 The drag force (Df)

When air flow occurs around the date palm pollen grain, the action of the forces involved can be illustrated by Fig. (4).

The pressure on the upper side of the pollen grain is less than the pressure P in the undisturbed air stream and that on the lower side is greater than the pressure P in the undisturbed air stream. The results in a decrease of pressure, -P, on the upper side indicated by arrows drawn

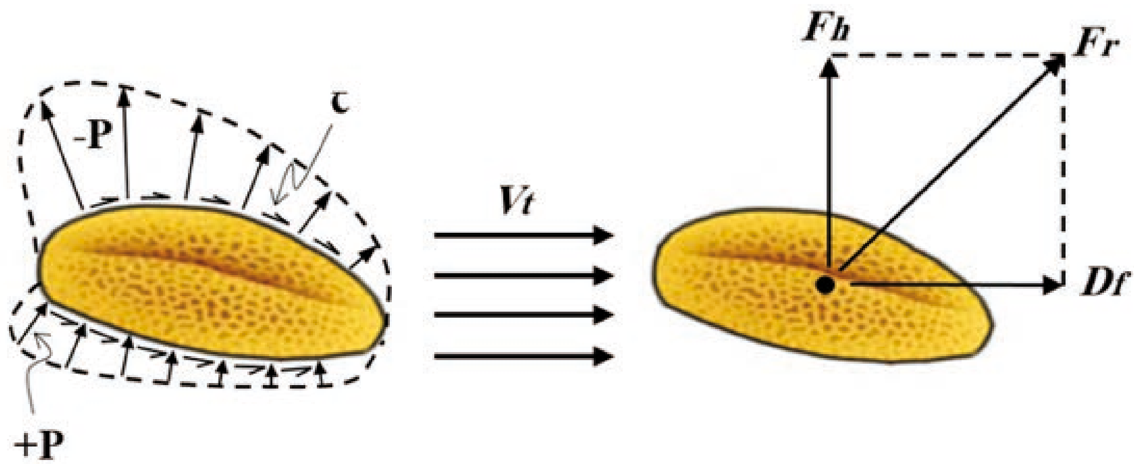


Figure 4: Air flow rate and force analysis acting the pollen grain

away from the surface, and an increase of pressure, *+P*, shown by arrows drawn toward the object. In addition, these forces normal to the surface of the pollen, and there are shear stresses, τ, acting tangential to the surface in the direction of airflow and resulting from frictional effects. The resultant force *Fr*, may be resolved into components of drag force (*Df)* and lift force (*Fh*). The equations for calculating *Df* and *Fh* have been derived by dimensional analysis assuming the smooth pollen grain having a projected area (*Ap*), moving through air flow density (ρ_a), drag coefficient (D_c), and air velocity (V_t). Therefore, the *Df* will be as the following equation,

$$Df = f(\rho_a,\ V_t, Ap, Dc) \tag{5}$$

To derive the drag force using the Kirchhoff and Gogh-Mann dimensional analysis method, the following steps are followed:

1. Determine the number of properties involved in the relation, n = 5

Table 1: Symbols and dimensions of the properties affecting the drag force of date palm pollen grain

		Dimension $M^{\mu} L^{\lambda} T^{\tau}$		
Property	Symbol	μ	λ	τ
Drag force	*Df*	1	1	-2
Air density	ρ_a	1	-3	0
Air velocity	V_t	0	1	-1
Projected area	*Ap*	0	2	0
Drag coefficient	D_c	0	0	0

2. Write the dimensions of all the properties involved in the relationship are shown in table (1).

3. Choose three base quantities, in this case it is (ρ_a, V_t, *Ap*), which must satisfy the following condition:

$$\Delta = \begin{vmatrix} \mu_1 & \lambda_1 & \tau_1 \\ \mu_2 & \lambda_2 & \tau_2 \\ \mu_3 & \lambda_3 & \tau_3 \end{vmatrix} = \begin{vmatrix} 1 & -3 & 0 \\ 0 & 1 & -1 \\ 0 & 2 & 0 \end{vmatrix} \neq 0$$

4. Determine the number of criteria (k)
K = n-3 = 5-3 = 2

5. The relation (5) can be writen as follows:

$$\frac{Df}{{\rho_a}^{\mu_1}\ {V_t}^{\lambda_1}\ \ {A_p}^{\tau_1}} = f(1,\ 1{,}1, \frac{Dc}{{\rho_a}^{\mu_2}\ {V_t}^{\lambda_2}\ \ {A_p}^{\tau_2}}) \tag{6}$$

6. On the basis of π theory, it can be writen:

$$\pi_1 = \frac{Df}{{\rho_a}^{\mu_1}\ {V_t}^{\lambda_1}\ \ {A_p}^{\tau_1}} \quad , \pi_2 = \frac{Dc}{{\rho_a}^{\mu_2}\ {V_t}^{\lambda_2}\ \ {A_p}^{\tau_2}} \tag{7}$$

7. Estimating the values of μ, λ and τ for each criterion using dimensional analysis and solving equations algebraically and by substituting in the original exponential functional relationship, we get that:

$$Df = \frac{D_c . A_p . \rho_a . {V_t}^2}{2} \tag{8}$$

Where *Df* is the drag force (N), A_p is the date palm pollen grain projected area (m^2), ρ_a is the air density which is equal to 1.206 kg m^{-3} at room temperature(Gupta, 2013), and V_t is the pollen grain terminal velocity (m s^{-1}).

2.3.4 Reynolds number (*Re*)

Reynolds number (*Re*) is an essential aerodynamic attribute that represents the ratio of inertial effects to viscous effects on the particle, in similar words, it is the ratio between the product of the particle's velocity and length scale to viscosity of the medium/fluid in which the particle is moving in this case, air (Zare et al., 2013). In most recent methodology, the Reynolds number (*Re*) was calculated from equation (9) (Grega et al., 2013):

$$Re = \frac{\rho_a . V_t . d_g}{\mu} \quad (9)$$

where ρ_a is the air density, which is equal to 1.206 kg m^{-3} at room temperature, V_t is the pollen grain terminal velocity (m s^{-1}), d_g is the geometric mean diameter of the seed (m), and μ is air viscosity (1.816×10^{-5} N s m^{-2} at room temperature).

The tested values of moisture content (*MC*) were 4, 5, 6, and 7 % *dry basis*.

2.4 STATISTICAL PROCEDURE

Measured data with all variables were statistically analyzed by a software program (CoStat ver. 6.400, 2008), applying a complete randomized design via analysis of variance. The means of the treatments were obtained, and Student-Newman-Keuls differences were tested at a 5 % level of probability.

3 RESULTS AND DISCUSSION

3.1 PHYSICAL PROPERTIES OF DATE PALM POLLEN GRAIN

The average values of the date palm pollen grain length (*L*), width (*w*), thickness (*T*), geometric mean diameter (d_g), projected area (A_p), mass (*m*), sphericity (*S*) and bulk density (ρ_p) at four levels of moisture content 4, 5, 6, and 7 % are listed in Table (2) and Fig. (5). It is clear that the pollen grain dimensions *L, w, T,* d_g, *S*, *m*, and *ρs* were insignificant at different pollen grain moisture content. The pollen grain length (*L*) increased by 1.0 and 2.03 % when the moisture content increased from 4 to 5, and 6 %, respectively while the *L* decreased by 1.5 % when the moisture content increased from 6 to 7 %. Also, the *L* increased by 1.0 % when the *MC* increased from 5 % to 6 %. The same trend was found for *w*, and *T*. The geometric mean diameter (d_g) remained nearly constant (10.4 µm), with the *MC* increased from 4 to 7 %. The pollen mass (*m*), and sphericity (*S*) remained nearly constant at 1.1E-12 kg, and 0.52, respectively, with the *MC* increased from 4 to 7 %. The pollen grain projected area (*Ap*) increased by 1.4, 1.9, and 2.5 % when the moisture content increased from 4 to 5, 6, and 7 %, respectively. Also, the *Ap* increased by 0.5 and 1.1 % when the pollen grain moisture content increased from 5 % to 6 and 7 %, respectively. The bulk density (ρ_p) increased by 0.7, 1.4, and 2.6 % when the moisture content increased from 4 to 5, 6, and 7 %, respectively. Also, the ρ_p increased by 0.7 and 1.9 % when the pollen grain moisture content increased from 5 % to 6 and 7 %, respectively. The lack of increase in the dimensions and projected area of date pollen grain with the increase of moisture content is due to the hardness of the pollen cover and its lack of water absorption (Pope, 2010; Bunderson and Levetin, 2015).

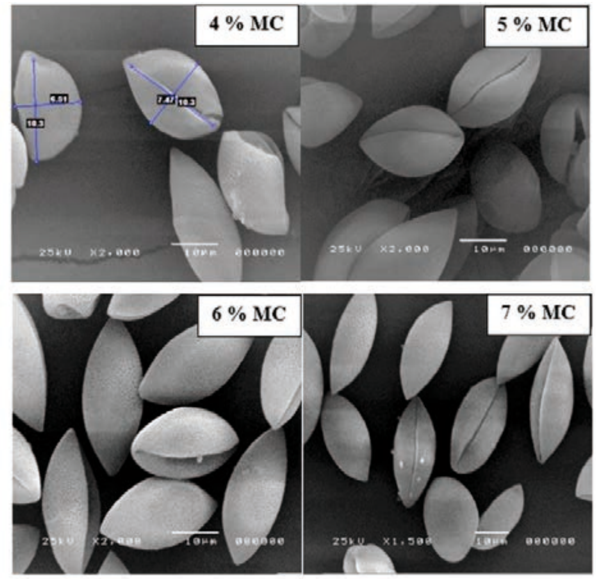


Figure 5: Scanning electron microscopy of date palm pollen grain at different levels of moisture content

3.2 AERODYNAMIC PROPERTIES OF DATE PALM POLLEN GRAIN

The average values of aerodynamic properties included terminal velocity (V_t), drag coefficient (D_C), drag force (*Df*), and Reynolds number (*Re*) of the date palm pollen grain (DPP) at different moisture content (4, 5, 6, and 7 %) are listed in Table (3). It's clear that the V_t, D_C, and *Df* were insignificant at different pollen grain moisture content. However, the pollen Reynolds number *Re* are significant at different pollen grain moisture content.

Table 2: Physical properties of date palm pollen grain at different moisture contents

MC,% dry basis	Pollen grain dimensions *L*, µm	*w*, µm	*T*, µm	d_g, µm	*S*	*m*, kg	*Ap*, µm^2	ρ_p, kg m^{-3}
4	19.7 ± 8.6 a	8.6 ± 0.9 a	6.8 ± 2.6 a	10.4 ± 1.8 a	0.52 ± 0.1 a	1.1E-12 ± 6.2E-28 a	133.33 ± 58.0 a	692.9 ± 21.9 a
5	19.9 ± 4.1 a	8.8 ± 1.8 a	6.8 ± 1.7 a	10.4 ± 1.4 a	0.52 ± 0.1 a	1.1E-12 ± 6.2E-28 a	135.21 ± 41.9 a	697.6 ± 22.4 a
6	20.1 ± 4.2 a	8.8 ± 1.4 a	6.8 ± 1.4 a	10.5 ± 1.2 a	0.52 ± 0.1 a	1.1E-12 ± 6.2E-28 a	135.92 ±3 7.6 a	702.4 ±2 3.7 a
7	19.9 ± 4.3 a	8.9 ± 1.4 a	6.9 ± 1.4 a	10.4 ± 1.4 a	0.52 ± 0.1 a	1.1E-12 ± 6.2E-28 a	136.70 ± 38.1 a	711.2 ± 25.2 a
Significant	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

MC = Pollen moisture content, *L* = pollen length, *w* = pollen width, *T* = pollen thickness, d_g = pollen geometric mean diameter, *S* = pollen sphericity, *m* = pollen mass, *Ap* = pollen projected area, ρ_p = pollen bulk density
n.s. Not significant at the 0.05 and 0.01 probability levels

Table 3: Aerodynamic properties of date palm pollen grain at different moisture content

MC *(% dry basis)*	V_t, m s^{-1}	D_c	*Df*, N	*Re*
4	0.59 ± 0.03 a	0.45 ± 0.16 a	1.09E-11 ± 7.1E-16 a	0.29 ± 0.014 b
5	0.60 ± 0.02 a	0.41 ± 0.13 a	1.09E-11 ± 3.1E-16 a	0.31 ± 0.015 b
6	0.60 ± 0.03 a	0.39 ± 0.12 a	1.09E-11 ± 5.1E-16 a	0.39 ± 0.011 a
7	0.60 ± 0.03 a	0.38 ± 0.10 a	1.09E-11 ± 7.2E-16 a	0.42 ± 0.039 a
Significant	n. s	n. s	n. s	*

MC = Moisture content, V_t = Terminal velocity, D_c = drag coefficient, *Df* = drag force, *Re* = Reynolds number
* Significant at the 0.05 probability level.
n.s. Not significant at the 0.05 and 0.01 probability level

For terminal velocity (V_t), the average values of V_t were about 0.59 and 0.6 m s^{-1}. The terminal velocity (V_t) of pollen grains was thoroughly examined as it plays an important role in the performance of pollination machines. The frequency distribution of the measured values of V_t are shown in Figure (6). It is clear that the 25, 35, 30, and 30 % of the V_t measured values ranged from 0.59 to 0.6 m s^{-1} for 4, 5, 6, and 7 % (*dry basis*) of moisture content, respectively.

Also, from Table (3), the average values of drag coefficient (D_c) were 0.45 ± 0.16, 0.41 ± 0.13, 0.39 ± 0.12, and 0.38 ± 0.10 for 4, 5, 6, and 7 % (*dry basis*) of moisture content respectively. The results confirmed an inverse relationship between D_c and pollen moisture content, as the moisture content of pollen grains increased from 4 to 7 %. (dry basis) the drag coefficient decreased from 0.54 to 0.38. The pollen grain D_c decreased by 8.0, 13.3, and 15.5 % when the moisture content increased from 4 to 5, 6 and 7 % (*dry basis*) respectively. While, the D_c decreased by 4.9, and 7.3 % when the moisture content increased from 5 to 6 and 7 % (*dry basis*) respectively. In addition, the D_c decreased by 2.6 % when the moisture content increased from 6 to 7 % (*dry basis*). For drag force (*Df*), the *Df* remained nearly constant, 1.09E-11 N, with the *MC* increased from 4 to 7 % (*dry basis*). This means that the DPP mass is not affected by the increase in moisture content (From Table 3), and therefore, it does not need an increase in the drag force.

The Reynolds number (*Re*) of date palm pollen grain increased by increasing moisture content. A direct relationship was observed for the *Re* as it increased from 0.29 to 0.42 as the moisture content increased from 4 to 7 % (*dry basis*) and *Re* increased by 6.9, 34.5, and 44.8 % when the moisture content increased from 4 to 5, 6, and 7 % (*dry basis*), respectively. While the *Re* increased by 25.8, and 35.5 % when the moisture content increased from 5 to 6 and 7 % (*dry basis*) respectively. In addition, the *Re* increased by 7.7 % when the moisture content increased from 6 to 7 % (*dry basis*). The maximum value of drag coefficient was found at moisture content level 4% (dry basis), while the highest value of V_t and *Re* were performed at 7 % (*dry basis*) moisture content level.

The relation between Reynolds number (*Re*) and drag coefficient (D_c) is shown in Fig. (7). It's clear that there is an inverse relationship between *Re* and D_c. The data of *Re* and D_c was analyzed to give the best fit relation of the 3rd order polynomial function:

$$D_c = a\,Re^3 + b\,Re^2 + c\,Re + d \quad (10)$$

The range of the Reynolds number (*Re*) into equation (10) were 0.42 ≥ *Re* ≥ 0.29 while, the values of the

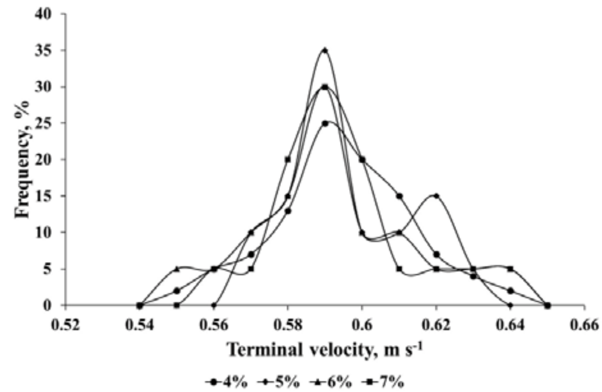


Figure 6: The frequency distribution of the pollen grain terminal velocity

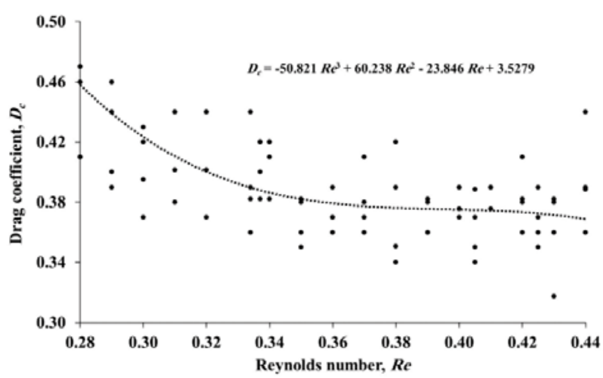


Figure 7: Drag coefficient (D_c) versus Reynolds number (*Re*) at four levels of moisture content

constants *a, b, c, and d* were (-) 50.821, 60.238, (-) 23.846, and 3.527 respectively.

3.3 REGRESSION MODELS

Data from the research of the date palm pollen grain's (DPP) aerodynamic characteristics at various *MC* levels were fitted and the best fit was chosen. The models created for the DPP's terminal velocity (V_t), drag coefficient (D_c), and Reynolds number (*Re*) as functions of *MC* are shown in Table 4. The R^2 varied between 0.93 and 1.00. The suitable models for predicting the aerodynamic characteristics of DPP as a function of moisture content were found to be polynomial regression models. As a function of moisture content, Pradhan et al. (2010) initiated a polynomial model for the terminal velocity of mung bean seeds, whereas linear models were created for the drag coefficient and Reynolds number. Additionally, Nalbandi et al. (2010) initiated a polynomial model for predicting Makhobeli seed terminal velocity as a function of moisture content. A linear relationship was reported by Khodabakhshian et al. (2012) between the V_t of Tef grain and its *MC*, while for the terminal velocity of coffee cherries and beans as a function of their moisture content and true density, a non-linear equation was derived by Matouk et al. (2008).

Table 4: Regression models of aerodynamic properties of date palm pollen grain

Regression models	R^2
$V_t = -0.0025\,MC^2 + 0.0155\,MC + 0.5775$	0.93
$D_c = 0.0075\,MC^2 - 0.0605\,MC + 0.5025$	1.00
Df = Constant = 1.09E-11	
$Re = 0.249\,e^{0.134\,MC}$	0.95

4 DISCUSSION

The present study evaluated and modeled the physical and aerodynamic properties of date palm pollen grain (DPP) as a function of moisture content. In summary, the physical properties of DPP included pollen length (*L*), width (*w*), thickness (*T*), projected area (A_p), geometric mean diameter (*dg*), mass (*m*), sphericity (*S*), and bulk density (ρ_p). It was observed that the physical properties of the DPP were not significantly influenced by the moisture content. The aerodynamic properties of DPP included the terminal velocity (V_t), drag coefficient (D_c), drag force (*Df*), and Reynolds number (*Re*). The results indicated that the terminal velocity increased slightly from 0.59 to 0.6 m s^{-1} with an increase in moisture content from 4 to 7 % (*dry basis*). As it was noted by Nalbandi et al. (2010) the slight increase in the terminal velocity of the DPP with an increase in the moisture content may be attributed to the formation of the pollen grain cover is a solid layer that does not absorb water, so there is no increase in the pollen grain mass thus the critical speed of pollen is not affected. Furthermore, Sharma et al. (2012) reported an increase in the terminal velocity of mung bean from 4.86 to 5.29 m s^{-1} as the moisture content increased from 7.28 to 17.77 % (*db*). Strong evidence was found according to Galedar et al. (2010) study, the terminal velocity (m s^{-1}) of sunflower, soybean, and canola seeds increased by 10.67 %, 2.16 %, and 4.31 %, as the moisture content of the seeds increased from 7.35 to 23.7, 9.52 to 24.64, and 7.11 to 25.72 % (wb), respectively, these results agree with the present study findings. Also results showed that the D_c od DPP decreased from 0.45 to 0.38 with the increase in moisture content from 4 to 7 % (*dry basis*). The results of this investigation concur with the bulk of other studies conducted by various researchers, which found an inverse relationship between the drag coefficient and the moisture content of seeds. The inverse connection could be explained by variations in the seeds' surface characteristics, actual densities, morphologies, and sizes as the moisture content increased. Also, it was mentioned by Nalbandi et al. (2010) that Makhobeli, triticale, and wheat seeds' drag coefficient decreased by increasing seeds' moisture content.; while Mohsenin (2020) reported that as the *MC* increased from 6.2 to 14.4 % (*dry basis*) for the cultivars NSFH-36, PSFH-118, GKSFH-2002, and SH-3322, respectively, the drag coefficient of four different cultivars of unshelled sunflower seeds decreased from 0.23 to 0.18, 0.31 to 0.20, 0.27 to 0.16, and 0.36 to 0.12. The current study's findings are consistent with those of Pradhan et al. (2010) for mung bean seed; the drag coefficient decreased as moisture content increased. The trend observed in the Reynolds number of DPP investigated in this study as the moisture content increased from 0.29 to 0.42 was approximately similar to that reported by Schwendemann et al. (2007) for saccate pollen grains. It was reported that the Reynolds number of saccate pollen grains increased from 2.3E-2 to 6.5E-2 with increased moisture content. The *Df* of DPP remained constant (1.09E-11 N) at different moisture content levels.

5 CONCLUSION

The physical and aerodynamic properties of date palm pollen grain (DPP) as a function of moisture content were evaluated. The physical properties of DPP included pollen grain dimensions (length *L*, width *w*, thickness *T*), projected area (A_p), geometric mean diameter (*dg*), mass (*m*), sphericity (*S*), and bulk density (ρ_p). The results indicated that, the pollen grain dimensions' *L, w, T,* and *dg* were insignificant at different pollen grain moisture content. Also**,** the pollen mass (*m*), and sphericity (*S*) remained nearly constant 1.1E-12 kg and 0.52, respectively, with the moisture content increased from 4 to 7 %. While, the pollen grain projected area (*Ap*) increased by 1.4, 1.9, and 2.5 % when the moisture content increased from 4 to 5, 6, and 7 % respectively. The pollen grain bulk density increased from 692.9 to 711.2 when the moisture content increased from 4 % to 7 %. The aerodynamic properties of DPP included the terminal velocity (V_t), drag coefficient (D_c), drag force (*Df*), and Reynolds number (*Re*). The results of aerodynamic properties indicated that the pollen grain V_t, *Df*, and *Df* were insignificant at different pollen grain moisture content. However, the pollen grain Reynolds number is significant at different pollen grain moisture content. There is an inverse relationship between the *Re* and D_c. The optimal models for predicting the aerodynamic properties of DPP as a function of moisture content were found to be polynomial regression models. Finally, the results of this study will be helpful in the performance of date palm pollination machines.

6 ACKNOWLEDGMENTS

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Phytochemical profile and allelopathic potential of *Haloxylon scoparium* Pomel (Chenopodiaceae) from Algerian Sahara

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Phytochemical profile and allelopathic potential of *Haloxylon scoparium* Pomel (Chenopodiaceae) from Algerian Sahara

Abstract: The aim of the present work is to study the chemical composition, to estimate the phenolic compounds content and to evaluate the potential allelopathic effects of the *Haloxylon scoparium* Pomel. Phytochemical tests revealed that *Haloxylon scoparium* contains tannins, saponins, coumarins, alkaloids, flavonoids and steroids. Furthermore, it contains high levels of total phenolic (588.33 mg GAE 100 g^{-1}) and flavonoids (95.45 mg QE 100 g^{-1}) contents. Moreover, LC-MS-MS analysis allowed us to determine their chemical composition. The results of this characterization confirm the presence of vanillin, naringenin, folic acid, maleic acid, benzoic acid, myricetin, qwuercetin, beta-carotene, butylhydroxyanisole (BHA), butylated hydroxytoluene (BHT), rutin, cafeic acid, hydroxy-4-coumarine, ascorbic acid, and gallic acid. The allelopathic effect was studied on seed germination and seedling growth of four weed species. The bioassays were performed using different concentrations (1 %, 2.5 %, 5 % and 10 %) against a negative control. The seed germination, shoot and root length of weed species were completely inhibited at the highest concentrations (10 %, 5 %). However, the lower concentrations exhibited lesser inhibition percentages on the germination and the seedling growth. The phytochemical results and the significant allelopathic effects of the plant extract suggest that this species may offer new substances for the biocontrol of weeds.

Key words: phytochemical profile, allelopathic potential, *Haloxylon scoparium*, LC-MS-MS analysis, allelochemicals, Algerian Sahara

Fitokemični profil in alelopatski potencial vrste *Haloxylon scoparium* Pomel (Chenopodiaceae) iz alžirske Sahare

Izvleček: Namen raziskave je preučiti kemijsko sestavo in vsebnost fenolnih spojin za ovrednotenje alelopatskega potenciala vrste *Haloxylon scoparium* Pomel. V raziskavi je bilo ugotovljeno, da vrsta vsebuje različne tanine, saponine, kumarine, flavonoide, alkaloide in steroide. Vsebuje velike količine celokupnih fenolov (588,33 mg GAE 100 g^{-1}) in flavonoidov (95,45 mgQE 100 g^{-1}). Podrobnejša kemijska LC-MS-MS analiza je pokazala prisotnost vanilina, naringenina, folne, jabolčne in benzoične kisline, mircetina, kvercetina, beta-karotena, butil hidroksianizola (BHA), butiliranega hidroksitoluena (BHT), rutina, kavne kisline, hidroksi-4-kumarina, askorbinske in galne kisline. Alelopatski učinek je bil preučevan na kalitvi in rasti kalic štirih vrst plevelov. Preizkušene so bile različne koncentracije alelopatskih snovi (1 %; 2,5 %, 5 % in 10 %) napram kontroli. Kalitev semen in dolžinska rast korenin in poganjkov plevelov je bila popolnoma zavrta pri največjih koncentracijah alelopatskih snovi (10 %, 5 %). Manjše koncentracije alelopatskih snovi so pokazale manjši odstotek zaviranja kalitve in rasti sejank plevelov. Alelopatski učinek izvlečkov te rastline nakazuje, da bi ta rastlina lahko bila vir novih učinkovin pri biokontroli plevelov.

Ključne besede: fitokemični profil, alelopatski potencial, *Haloxylon scoparium*, LC-MS-MS analiza, alelokemikalije, alžirska Sahara

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1 INTRODUCTION

Weed interference in agricultural fields reduces the quantity and quality of crops, causing enormous economic losses for farmers (Sarić-Krsmanović et al., 2019). Control strategies of weed have relied mainly on the application of synthetic herbicides. However, the continuous and excessive application of these treatments cause environmental pollution, negative effects on human health and unsafe agricultural products. Moreover, this practice increases herbicide resistance in weeds (Batish et al., 2007). Therefore, in order to resolve this problem, and minimize the dependency on chemical herbicides for weed control, great efforts have been given to develop natural and eco-friendly alternatives (Bhadoria, 2011).

Allelopathy refers to any process that involves allelochemicals produced by plants. Some plants may inhibit seed germination, emergence and growth of other plants by exuding toxic substances. These substances are called allelopathic chemicals or allelochemicals (Monem, 2012). Biochemical compounds offer a great potential for the discovery of new environmentally safe herbicides, referred to as "bioherbicides". Thus, the usage of plant secondary metabolites proved to be a promising solution in biological control (Weih et al., 2008).

The Algerian Sahara, known for its richness in spontaneous plants, harbors about 500 species of higher plants, some of which are used as medicinal plants. The *Haloxylon scoparium* Pomel plant, belonging to the Chenopodiaceae family, and locally named "*remth*", is used in traditional medicine to treat eye disorders (Chehma, 2006; Salah et al., 2002).

Several studies have been carried out on this species extract; mostly targeting its' polyphenol contents or its' biological activities. This plant has been reported to possess antidiabetic potential (Benkherara et al., 2021), antimicrobial and antiradical properties (Drioiche et al., 2019), antibacterial and antioxidant activities (Bouaziz et al., 2016), antidiabetic, antiseptic and anti-inflammatory effects (Ziyyat et al., 2014), anticancer activity (Bourogaa et al., 2014), anti-leukemic agent (Bourogaa et al., 2011), molluscicidal activity (Mezghani- Jarraya et al., 2009), anti-cancer and anti-plasmodial activities (Sathiyamoorthy et al., 1999), larvicidal activity (Sathiyamoorthy et al., 1997).

The allelopathic potential of *Haloxylon scoparium* on various weeds and crops have rarely been investigated (Salhi, 2011). Therefore, this study aims to evaluate the allelopathic effects of *Haloxylon scoparium* leaf extract on seed germination and seedlings growth. As well as to determine the chemical composition we carried out extraction of phenolic compounds, then their qualitative and quantitative characterization. The analytical techniques used are phytochemical tests based on precipitation or coloration of extract by specific reagents; Determination of total polyphenol and total flavonoids contents was determined by spectrophotometry and identification of phenolic compounds by Liquid Chromatography- Mass Spectrometry (LC-MS-MS).

2 MATERIAL AND METHODS

2.1 EXTRACT PREPARATION

The leaves of *Haloxylon scoparium* were harvested from the plant in its' natural habitat, located in the northeastern region of the Algerian Sahara, during its' vegetative stage. The leaves were subsequently washed with tap water then shade dried. The dried leaves were ground using an electric blender and stored in glass jars until the extraction process. The plant extract was obtained using reflux extraction. In a flask, 50 g of plant material were added to a hydro-methanolic solution. Using a flask heater, the mixture was heated at 60 °C for six hours. Filtration was then carried out using Whatman No. 1 filter paper. The collected filtrate underwent treatment under reduced pressure in a rotary evaporator in order to eliminate the methanol. The recovered aqueous extract was subsequently stored at 4 °C in a refrigerator until used for the biological testing (Kemassi et al., 2019).

To evaluate the dose dependent effect on the germination and seedling growth of weed, different concentrations were prepared from the stock solution, diluted with distilled water (10 %, 5 %, 2.5 % and 1 %). Distilled water served as control.

2.2 PHYTOCHEMICAL TESTS

The leaf extract was examined for the presence of the following phytochemical classes, using the numerous standard methods of evaluation described by various authors in the scientific literature

2.2.1 Tannins

A solution of $FeCl_3$ (5 %) was added to the crude extract. The presence of tannins was indicated by the appearance of a black or a blue-green color (Lerato et al., 2017).

2.2.2 Anthocyanins

Two milliliters of leaf extract, two milliliters of HCl (2 N) and ammonia (2 ml) were mixed. The appearance of a pink red coloration that turned blue violet confirmed the presence of anthocyanins (Lerato et al., 2017).

2.2.3 Saponins

Distilled water was added to the crude leaf extract in a test tube, followed by vigorous stirring. The formation of a persistent froth confirmed the presence of saponins in the extract (Lerato et al., 2017).

2.2.4 Coumarins

A NaOH solution (10 %) was added to the leaf extract. The formation of a yellow color confirmed the presence of coumarins (Lerato et al., 2017).

2.2.5 Alkaloids

The presence of alkaloids was assessed by adding three milliliters of HCl (1 %) to three milliliters of crude extract. The mixture was heated for twenty minutes. Subsequently, Mayer's reagent was added in drips to the mixture. The formation of a cream precipitate or the occurrence of a green coloration indicates the presence of alkaloids (Lerato et al., 2017).

2.2.6 Flavonoids

The plant extract was treated with a NaOH solution (10 %). The appearance of an intense yellow color of the solution indicated the presence of flavonoids (Lerato et al., 2017).

2.2.7 Steroids

Chloroform and H_2O_4 were added to the leaf extract. The presence of steroids was indicated by a color change, from violet to blue or green, or the occurrence of a blue-green ring (Lerato et al., 2017).

2.2.8 Carbohydrates

A boiled mixture of Fehling solutions A and B, with equal volumes, was added to the leaf extract. A red colored precipitate indicated the presence of reducing sugars (Jaradat et al., 2015).

2.3 DETERMINATION OF POLYPHENOLS COMPOUNDS CONTENT

2.3.1 Determination of total polyphenol content

Total polyphenol content (TPC) was measured according to the colorimetric method (Singleton and Rossi, 1965), with some modification (using a UV spectrophotometer). 200 µl of leaf extract were added to 1 ml of Folin-Ciocalteu reagent (diluted 10 times with distilled water) and 800 µl of Na_2CO_3 (7.5 %). The mixture was then incubated at 50 ºC for 30 minutes. Subsequently, the absorbance of the solution was measured at 765 nm. Gallic acid served as a control for the creation of a calibration curve to estimate the TPC (Cliffe et al., 1994).

2.3.2 Determination of total flavonoid content

The total flavonoid content (TFC) was measured according to the Aluminium Chloride colorimetric method (Djeridane et al., 2006), with some modification. In a test tube, 25 µl of the plant extract was mixed with 300 µl of $NaNO_2$ and 300 µl of $AlCl_3$ (10 %) and left for five minutes. 100 µl of NaOH (2 %) were added. The absorbance of this mixture was measured at 510 nm. Calibration curve of standard quercetin solution was prepared to calculate TFC (Kim et al., 2003).

2.4 EXTRACTION OF PHENOLIC COMPOUNDS FOR LC-MS-MS ANALYSIS

The crude extract was fractionated (liquid-liquid) by the addition of ethyl acetate to obtain ethyl acetate fraction. Extract fraction was dried and dissolved in 5 ml of methanol then stored at 4 °C until analysis.

2.5 LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS CONDITIONS (LC-MS-MS)

The analysis of the *Haloxylon scoparium* leaf extract was carried out in the Technical Platform of Physico-Chemical Analysis (PTAPC-CRAPC)-Ouargla-Algeria, using a UPLC-ESI-MS-MS Shimadzu 8040 Ultra-High sensitivity with UFMS technology was employed and equipped with binary bump Nexera XR LC-20AD identified the extracted phenolic compounds. For optimization of polyphones standards, we used direct injection without column. All standards were prepared in methanol with a 500 µg l^{-1} concentration. The ion trap mass spectrometer was used in both positive and negative ions with MRM mode (multiple reaction monitoring). The mobile phase was made of water, 0.1 % formic acid and 70 % methanol. The injection volume was six µl and the flow rate was 0.3 ml min^{-1}. The samples were separated using an ultra-force C18 column (I.D. 2.5 mm × 100 mm, 1.8 µm particle size; Restek), the oven temperature was 25 °C. Isocratic elution was applied with 0.1 % formic acid and methanol. The injection volume was 10 ml and the flow rate was 0.30 ml min^{-1} (Ben amor et al., 2022).

2.6 BIOASSAY EXPERIMENT

The bioassay experiments were arranged in a completely randomized design, with four replications of each treatment. Seeds of *Bromus ruben*s L., *Phalaris minor* L., *Plantago lagopus* L. and *Ammi visnaga* L. were placed on filter paper in sterile Petri dishes and treated with three ml of different concentrations of plant extract. The control was treated with three ml of distilled water. The petri dishes were kept under laboratory conditions with day temperature ranging from 19-24 °C and night temperature from 12-15 °C. The germination assessment was evaluated daily, for ten days, by counting the number of germinated seeds, measuring the shoot and the root lengths and determining fresh mass at the end of the experiment (Otmani et al., 2022).

2.7 EVALUATION OF ALLELOPATHIC EFFECTS

The allelopathic effects can be defined as the inhibition or the retardation of seed germination and reduction or stimulation of root and shoot length and mass.

2.7.1 Calculation of inhibition percentage

The inhibition percentage was calculated according to the equation proposed by Côme (1970). This parameter explains the ability of a plant extract to inhibit seed germination. In the equation, mentioned below, N is the number of germinated seeds, and A is the total number of the sown seeds.

$$Inhibition\ (\%) = ((A-N)/A)*100 \quad (1)$$

2.7.2 Average germination time

The average germination time (AGT) was determined through daily counting of germinated seed to the tenth day and calculated with the equation proposed by Labouriau (1983), being the expressed results in days.

$$AGT = \Sigma ni*ti/\Sigma ni = (n_{1*}\ t_1+n_2*t_2+ \dots +n_n*t_n)/n_1+n_2+\dots\dots+n_n \quad (2)$$

$n_{1:}$ number of germinated seeds at time $t_{1.}$
n_2: number of germinated seeds at time $t_{2.}$
n_n: number of germinated seeds at time $t_{n.}$

2.7.3 Effects of extract on seedling's growth

After the germination test, the shoot and root lengths of the weed species were measured. Afterwards, the seedlings were separated into shoot and root parts in order to measure the fresh mass.

2.8 STATISTICAL ANALYSIS

The results obtained from the various experimental tests were analyzed by one-way ANOVA with the "XL-STAT version 2014" software. Results were evaluated by the Fisher LSD test

($p = 0.05$), and presented as mean ± SD (Standard deviation).

3 RESULTS AND DISCUSSION

3.1 PHYTOCHEMICAL TESTS

The results of the phytochemical screening, presented in Table 1, clearly indicate the presence of different secondary metabolites in *Haloxylon scoparium* leaf extract. These tests revealed the presence of phenols (tannins, saponins, coumarins, flavonoids), alkaloids and steroids. However, the absence of carbohydrates, anthocyanin and betacyanin is noted.

The phytochemical tests carried out on the leaf extract of *Haloxylon scoparium* allowed us to highlight the presence of several phytochemicals. These research results are in agreement with those obtained in previous studies that indicated the richness of *Haloxylon scoparium* in secondary metabolites (Haida et al., 2020). Ben kherara et al. (2021) confirmed the presence of six major compounds (alkaloids, flavonoids, saponins tan-

Table 1: Results of phytochemical tests of *Haloxylon scoparium* Pomel. leaf extract

Constituents	Leaf extract
Tannins	+++
Anthocyanin and Betacyanin	-
Saponins	+
Coumarins	+++
Alkaloids	+++
Flavonoids	+++
Steroids	+++
Carbohydrates	-

+++: Strong positive result, ++: Moderate positive result, +: Weak positive result, - : Negative result

nins, anthocyanins, terpenes and sterols) and the absence of two other important compounds (leucoanthocyanins and cardinolids). A study done by Lachkar et al. (2021) showed that the aqueous and organic extracts of the aerial part of *Haloxylon scoparium* collected in Taza (Marroco) contains catechic tannins, flavonoids, saponins, alkaloids, anthracenosides, and free quinones. However, gallic tannins, sterols and anthraquinones were absent. Furthermore, Bourogaa and collaborators (2014) revealed the presence of flavonoids and alkaloids, while quinones and sterols are absent from the aqueous extract. In contrast, Zerriouh (2015) showed that the aqueous extract of the aerial part of *Hammada scoparia* collected in Algeria is devoid of flavonoids but contains the alkaloids and saponins.

These secondary metabolites possess allelopathic effects on the seed germination and seedling growth of weed species. These phytochemicals present in *Haloxylon scoparium* leaf extract might be controlling the observed allelopathic activity of the plant extract. alkaloids, flavonoids, terpenoids, curcurbitacins, glycosides, coumarins, saponins and tannins are the plant components identified as allelochemicals in the allelopathic effects of several plant extracts on weeds and crops (Mseddi et al., 2018; Naz and Bano, 2013).

3.2 TOTAL POLYPHENOL AND FLAVONOIDS CONTENT

The total polyphenol and total flavonoids contents obtained for leaf extract of *Haloxylon scoparium* are presented in Table 2. The total polyphenols content of the plant extract was determined in comparison to the standard gallic acid, expressed as mg GAE 100 g^{-1} of dry

Table 2: Quantitative results of *Haloxylon scoparium* Pomel. leaf extract

Constituents	Leaf extract
Total Polyphenols Content (mg GAE/100 g DM) ± SD	588.33 ± 1.87
Total Flavonoids Content (mg QE / 100 g DM) ± SD	95.45 ± 1.21

GAE: Gallic acid equivalent; QE: Quercetin equivalent; DM: dry mass

plant sample, whereas the total flavonoids content was measured in comparison to the standard quercetin, and expressed as mg QE 100 g^{-1} of dry plant sample.

These interesting results of colorimetric analysis show a very high content of total polyphenols (588.33 ± 1.87 mg GAE g^{-1} DM) and total flavonoids (95.45 ± 1.21 mg QE 100 g^{-1} DM) in the *Haloxylon scoparium* leaf extract. These amounts were significantly better than those found by Allaoui et al. (2016) who obtained a high content of total polyphenol (397.743 mg GAE g^{-1} of extract) and flavonoid (82.835 mg QE g^{-1}). The obtained results were three time higher than those quantified in the same studied plant species (Zeghada, 2009; Lachekar et al., 2021; Ben kherara et al., 2021)

The extraction yields depend on the plant species, part of plant used, period of harvesting, climate and geographical position, drying conditions, plant material, nature and polarity of the solvent and the method and modality of extractions.

The qualitative and quantitative analysis results show the superior biochemical quality of *Haloxylon scoparium*.

3.3 LC-MS-MS ANALYSIS RESULTS

The analysis results of *Haloxylon scoparium* leaf extract by LC-MS-MS are shown in Table 3. This analysis revealed the presence of several secondary metabolites. Twenty-three phenolic compounds were detected based on the LC-MS-MS in which fifteen were identified by comparison with standards. The results of this characterization confirm the presence of vanillin, naringenin, folic acid, maleic acid, benzoic acid, myricetin, quercetin, beta-carotene, butylhydroxy anisole (BHA), butylated hydroxytoluene (BHT), rutin, cafeic acid, hydroxy-4-coumarine, ascorbic acid, and gallic acid. However, keampferol, coumaric acid, picric acid, cinnamic acid, chlorogénic acid, chrysin, esculin, hesperetin were absent.

The phytochemical composition of *Haloxylon sco-*

Table 3: LC-MS-MS-determined phenolic compounds of *Haloxylon scoparium* Pomel. leaf extract

N°	Compound Name	Charge + / -	Precursor m/z	Product m/z	Haloxylon scoparium
01	Keampferol	$[MH]^+$	287.1	255.25	-
02	Vanillin	$[MH]^+$	153.10	71.15	+
03	Naringenin	$[MH]^+$	273.10	147.15	+
04	Coumaric Acid	$[MH]^+$	165.10	59.10	-
05	Picric Acid	$[MH]^-$	227.8	198.05	-
06	Cinnamic Acid	$[MH]^+$	149.1	77.2	-
07	Folic Acid	$[MH]^+$	442.90	323.45	+
08	Maleic Acid	$[MH]^+$	117.10	85.20	+
09	Benzoïc Acid	$[MH]^+$	123.10	91.20	+
10	Chlorogénic Acid	$[MH]^+$	355	73.15	-
11	Myricetin	$[MH]^+$	336.25	72.15	+
12	Quercetin	$[MH]^+$	303.10	85.05	+
13	Chrysin	$[MH]^+$	255.10	223.30	-
14	Esculin	$[MH]^+$	341.30	309.40	-
15	Hesperetin	$[MH]^-$	300.9	255.25	-
16	Beta-carotene	$[MH]^+$	537.20	199.25	+
17	Butylhydroxyanisole (BHA)	$[MH]^+$	181.10	140.15	+
18	Butylated hydroxytoluene (BHT)	$[MH]^+$	221	161.30	+
19	Rutin	$[MH]^+$	611.20	73.20	+
20	Cafeic Acid	$[MH]^-$	178.80	135.10	+
21	Hydroxy-4-Coumarine	$[MH]^-$	160.80	117.10	+
22	Ascorbic Acid	$[MH]^-$	174.90	131.10	+
23	Gallic Acid	$[MH]^-$	168.80	125.10	+

+ : present, - : not present

parium plant has not been the subject of many publications. Few researchers have investigated its phenolic composition. Chemical characterization of *Hammada scoparia* essential oils confirmed the presence of carvacrol, p-cymene, γ-terpinene and z-caryophyllene (Drioiche et al., 2019). In addition, Chao et al. (2013) showed the presence of some phenol acids such as Coumaric acid, Cinnamic acid and Caffeoylquinic acid, simple phenols (catechol and a chrysoeriol). However, Benkrief et al. (1990) identified isosalsoline dehydrosalsolidine, isosalsolidine (tetrahydroisoquinolines), N-methylcorydaldine (isoquinolone), tryptamine and N-methyltryptamine (tryptamines) as minor alkaloids. Other studies have isolated and identified two principal alkaloids: carnegine and N-methylisosalsoline from *Hammada scoparia* leaf extract (Jarraya et al., 2008; Bouaziz et al., 2016). A new flavonol triglycoside has been isolated from the leaves of *Hammada scoparia* (Salah et al., 2002).

3.4 PERCENTAGE OF GERMINATION INHIBITION

The allelopathic effect is expressed as the percentage of inhibition. The germination of target species, treated with *Haloxylon scoparium* leaf extract, decreased compared to the control. The degree of inhibition varies depending on the concentrations (Fig 1, 2, 3, and 4). A high inhibitory effect on germination was observed on all tested seeds, and the inhibition percentage increased with increasing concentrations of leaf extract. As illustrated in the graphs, at the 1 % and 2.5 % extract concentrations, the inhibition percentage values were, respectively, of 71.66-76.66 % for *Bromus rubens* , 63.33-93.33 % for *Phalaris minor* , 50.0-66.66 % for *Plantago lagopus* , 30.0-43.33 % for *Ammi visnaga* The 5 % and 10 % extract concentrations significantly inhibited the germination of weed seeds, which corresponds to a 100% inhibition percentage.

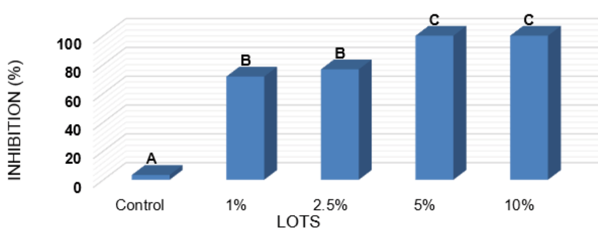

Figure 1: Inhibition percentage observed in control and treated lots by various concentration of leaf extract of *Haloxylon scoparium* Pomel. on *Bromus rubens* L. For each concentration, means (mean ± SD) followed by different letter (A, B, C) are significantly different at $p < 0.05$ level according to Tukey's LSD test

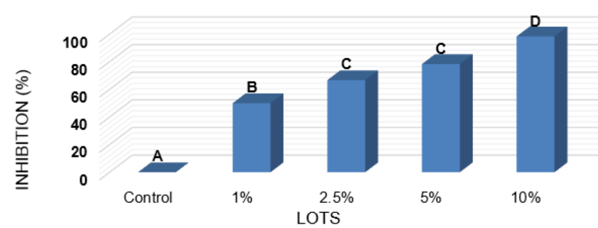

Figure 3: Inhibition percentage observed in control and treated lots by various concentration of leaf extract of *Haloxylon scoparium* Pomel. on *Plantago lagopus* L. For each concentration, means (mean ± SD) followed by different letter (A, B, C, D) are significantly different at $p < 0.05$ level according to Tukey's LSD test

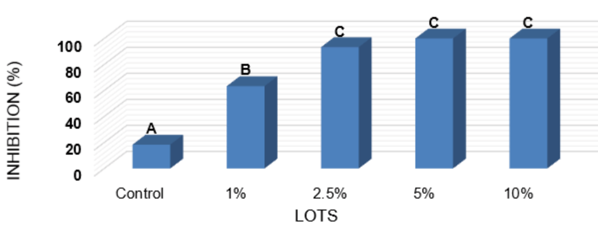

Figure 2: Inhibition percentage observed in control and treated lots by various concentration of leaf extract of *Haloxylon scoparium* Pomel. on *Phalaris minor* L. . For each concentration, means (mean ± SD) followed by different letter (A, B, C) are significantly different at $p < 0.05$ level according to Tukey's LSD test

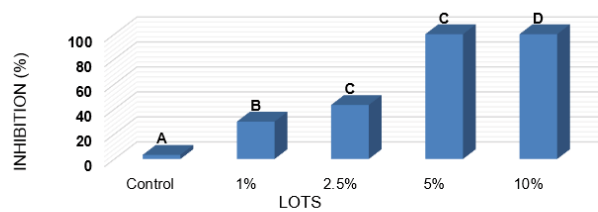

Figure 4: Inhibition percentage observed in control and treated lots by various concentration of leaf extract of *Haloxylon scoparium* Pomel. on *Ammi visnaga* L. For each concentration, means (mean ± SD) followed by different letter (A, B, C, D) are significantly different at $p < 0.05$ level according to Tukey's LSD test

According to the present research, *Haloxylon scoparium* leaf extract presents an allelopathic effect on the seed germination of weed species (*Bromus rubens* , *Phalaris minor* , *Plantago lagopus* , *Ammi visnaga*). These results are in agreement with those obtained by Karous et al. (2020); they demonstrate that the tested aqueous extract possessed an effective inhibitory activity against two weed species. Numerous studies have suggested that the presence of allelochemicals may cause a total or partial suppression of germination and a reduction in seedling growth (Qasem, 2002; Naz and Bano, 2013; Saadaoui et al., 2015; Mseddi et al., 2018). The presence of these secondary metabolites suggests that the plant might be of allelopathic and bioherbicidal importance.

3.5 AVERAGE GERMINATION TIME

In the present study, it was observed that the average germination time of weed species treated with leaf extract of *Haloxylon scoparium* increased in all treatments, from the lowest to the highest concentrations. The values show slower times compared to those of the control lot (Table 4). These results showed that the average germination time varies between 6.14 and 8.75 days.

The results of the current study show that *Haloxylon scoparium* leaf extract had an impact on average germination time. Da Silva et al. (2016) reported that *Ricinus communis* leaf extract significantly affected the average germination time, which increased with concentrations. Allelochemicals can increase cell membrane permeability, which prevents plants from absorbing nutrients from their environment and affects their normal growth (Li et al., 2010).

3.6 EFFECT OF EXTRACT ON SEEDLING GROWTH

The effect of *Haloxylon scoparium* leaf extract on the shoot and root growth of treated weed species (*Bromus rubens* , *Phalaris minor* , *Plantago lagopus* , *Ammi visnaga*) are shown in tables 5, 6, 7 and 8 respectively. In laboratory bioassay, all concentrations of leaf extract of *Haloxylon scoparium* decreased the seedling growth of weed species. They significantly decreased the shoot

Table 4: Effect of *Haloxylon scoparium* Pomel. leaf extract on average germination time of *Bromus rubens* L., *Phalaris minor* L., *Plantago lagopus* L. and *Ammi visnaga* L

	Average Germination Time (AGT) (Days)							
Extract conc. (%)	Bromus rubens		Phalaris minor		Plantago lagopus		Ammi visnaga	
	Mean ± SD	Group	Mean ± SD	Group	Mean ± SD	Group	Mean ± SD	Group
Control	6.33 ± 0.11	A	7.31 ± 0.08	A	6.19 ± 0.05	A	6.53 ± 0.03	A
1 %	6.14 ± 0.16	A	7.91 ± 0.16	A	6.68 ± 0.36	AB	7.33 ± 0.05	B
2.5 %	6.54 ± 0.66	A	8.75 ± 1.44	A	7.13 ± 0.25	B	7.64 ± 0.29	C
5 %	-	-	-	-	7.85 ± 0.76	C	-	-
10 %	-	-	-	-	8,63 ± 0.47	D	-	-
LSD	0.72		1.51		0.72		0.30	

-:100 % inhibition percentage

Table 5: Effect of *Haloxylon scoparium* Pomel. leaf extract on shoot and root lengths and fresh mass of *Bromus rubens* L

	Bromus rubens							
Extract conc. (%)	Shoot length (cm)		Root length (cm)		Shoot mass (g)		Root mass (g)	
	Mean ± SD	Group	Mean ± SD	Group	Mean ± SD	Group	Mean ± SD	Group
Control	12.61 ± 2.27	C	14.33 ± 1.49	C	0.0336 ± 0.0036	B	0.0262 ± 0.0078	B
1 %	5.45 ± 1.49	B	6.57 ± 1.05	B	0.0257 ± 0.0049	A	0.0096 ± 0.0024	A
2.5 %	2.12 ± 1.35	A	2.01 ± 1.05	A	0.0177 ± 0.0059	A	0.0058 ± 0.0020	A
LSD	3.16		2.47		0.0085		0.0043	

Table 6: Effect of *Haloxylon scoparium* Pomel. leaf extract on shoot and root lengths and fresh mass of *Phalaris minor* L

	Phalaris minor							
Extract conc. (%)	Shoot length (cm)		Root length (cm)		Shoot mass (g)		Root mass (g)	
	Mean ± SD	Group	Mean ± SD	Group	Mean ± SD	Group	Mean ± SD	Group
Control	9.08 ± 0.65	B	9.26 ± 0.92	C	0.0155 ± 0.0019	B	0.0051 ± 0.0007	C
1 %	6.69 ± 0.87	A	3.97 ± 0.98	B	0.0096 ± 0.0018	AB	0.0018 ± 0.0009	B
2.5 %	5.45 ± 1.51	A	2.05 ± 1.41	A	0.0082 ± 0.0052	A	0.0005 ± 0.0001	A
LSD	1.93		1.92		0.0061		0.0011	

Table 7: Effect of *Haloxylon scoparium* Pomel. leaf extract on shoot and root lengths and fresh mass of *Plantago lagopus* L

	Plantago lagopus							
Extract conc. (%)	Shoot length (cm)		Root length (cm)		Shoot mass (g)		Root mass (g)	
	Mean ± SD	Group	Mean ± SD	Group	Mean ± SD	Group	Mean ± SD	Group
Control	3.38 ± 0.49	D	4.03 ± 0.29	C	0.0084 ± 0.0015	C	0.0038 ± 0.0005	C
1 %	2.85 ± 0.29	C	1.53 ± 0.46	B	0.0058 ± 0.0024	B	0.0014 ± 0.0010	B
2.5 %	1.38 ± 0.25	B	0.28 ± 0.16	A	0.0026 ± 0.0021	A	0.0005 ± 0.0004	A
5 %	± 0.06	A	0.08 ± 0.03	A	0.0007 ± 0.0004	A	0.0001 ± 0.00005	A
10 %	0,35 ± 0.05	A	0,12 ± 0.05	A	0,0003 ± 0.0001	A	0,0001 ± 0.00005	A
LSD	0.38		0.41		0.0026		0.0009	

Table 8: Effect of *Haloxylon scoparium* Pomel. leaf extract on shoot and root lengths and fresh mass of *Ammi visnaga* L

	Ammi visnaga							
Extract conc. (%)	Shoot length (cm)		Root length (cm)		Shoot mass (g)		Root mass (g)	
	Mean ± SD	Group	Mean ± SD	Group	Mean ± SD	Group	Mean ± SD	Group
Control	8.25 ± 1.03	B	6.49 ± 0.63	C	0.0251 ± 0.0047	A	0.0073 ± 0.0016	A
1 %	3.91 ± 0.79	A	1.73 ± 0.26	B	0.0083 ± 0.0012	A	0.0013 ± 0.0004	B
2.5 %	2.94 ± 0.45	A	0.59 ± 0.30	A	0.0063 ± 0.0026	A	0.0006 ± 0.0004	C
LSD	1.44		0.78		0.005		0.001	

length, root length, shoot fresh mass and root fresh mass of the test species compared to those of the control. This inhibitory effect on the root and shoot growth increased with increase of the concentrations.

The present study indicate that *Haloxylon scoparium* leaf extract presents allelopathic effect and contains allelochemicals responsible for the inhibitory activities on the germination and the seedling growth of test weed species. Other authors in their studies on weeds and crops observed similar findings (Scavo et al., 2018; Bhowmik and Doll, 1984). Allelochemicals affect plant germination and growth (Salhi et al., 2013). The capacity to inhibit seed germination and seedling growth are a complex process, and several hypotheses about allelochemicals of plant extracts have been formulated. These hypotheses suggest that these compounds might affect enzymes responsible for plant hormone synthesis, to inhibit the action of the amylase or inhibition of their tissue actions (Feeny, 1976). The alteration of the synthesis or activities of gibberellic acid in the seed could be due to the presence of phenolic compounds (Olofsdotter, 2001). Cell division and elongation are susceptible to the presence of allelopathic compounds (Muller 1965), resulting in the reduction of root and shoot growth (Qasem, 2002).

4 CONCLUSION

The results of the present research confirmed the strong allelopathic effects of *Haloxylon scoparium* leaf extract, on the germination, shoot and root growth of tested weed species (*Bromus rubens*, *Phalaris minor*, *Plantago lagopus*, *Ammi visnaga*). The phenolic compounds present a great interest for the researchers due to their various biological activities. These findings encourages future research for identifying and characterizing germination and growth inhibitors; it could be the source of these species' significant allelopathic potential. These allelochemicals maight be used in the research and development of weed-controlling environmental herbicides**.**

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Effects of particle size on determination of the contents of grain and legume dietary fibre and resistant starch

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Effects of particle size on determination of the contents of grain and legume dietary fibre and resistant starch

Abstract: Dietary fibre comprises non-digestible carbohydrates, including resistant starch, and lignin, and it is an important constituent of a healthy diet. The aim was to define the influence of particle size on contents determined for dietary fibre and resistant starch in unprocessed grain and canned legumes. Five samples of unprocessed and processed grains were analysed, as oatmeal, buckwheat, dehulled barley, wheat and spelt, and three canned legumes, as beans, chickpeas and peas, with and without their brine. Samples were initially milled unscreened, and then again through 500 µm or 350 µm screens. For unprocessed grain samples, there was generally no influence of particle size, except for the 350-µm milling of dehulled barley, with significantly decreased contents determined for insoluble dietary fibre and resistant starch presumably due to damaging of starch granules and disrupting crystalline formation of starch. For canned legumes with and without their brine, particle size had little effect on contents determined for dietary fibre and resistant starch.

Key words: dietary fibre, resistant starch, particle size, canned legumes, milling, grains

Vpliv velikosti delcev na določitev vsebnosti prehranske vlaknine in rezistentnega škroba v žitih in stročnicah

Izvleček: Prehranska vlaknina je sestavljena iz neprebavljivih ogljikovih hidratov, vključno z rezistentnim škrobom, in lignina ter je pomemben del uravnotežene prehrane. Cilj naše raziskave je bil preveriti vpliv velikosti delcev vzorca na določanje vsebnosti prehranske vlaknine in rezistentnega škroba v vzorcih žit in procesiranih žit ter konzerviranih stročnic. Analizirali smo pet vzorcev žit, in sicer ovsene kosmiče, ajdo, ješprenj, pšenico in piro ter tri vzorce konzerviranih stročnic, fižol, čičeriko in grah. Vzorci stročnic so bili analizirani z nalivom in brez naliva. Vzorci so bili mleti na nedefinirano velikost ter na 500 µm ali 350 µm. V skupini žit ni bilo zaznati vpliva velikosti delcev, razen pri vzorcu ješprenja, mletega na velikost 350 µm, kjer sta bili določeni značilno manjši vsebnosti netopne prehranske vlaknine in rezistentnega škroba. Predvidevamo, da je to posledica poškodb škrobnih zrn in porušenja kristalinične formacije škroba. V vzorcih konzerviranih stročnic z ali brez naliva je imela velikost delcev zanemarljiv učinek na določeno vsebnost prehranske vlaknine in rezistentnega škroba.

Ključne besede: prehranska vlaknina, rezistentni škrob, velikost delcev, konzervirane stročnice, mletje, žita

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1 INTRODUCTION

Dietary fibre is recognized as an important component of the human diet, due to its beneficial effects on the gastrointestinal tract and its positive effects on reducing the risk of developing non-communicable diseases. Dietary fibre consists of carbohydrates that are resistant to hydrolysis by the human endogenous enzymes and that are not readily absorbed in the small intestine, as also for resistant starch. However, the fermentation processes by microbiota in the colon can partially use the dietary fibre for energy production, while metabolising it to bioactive compounds, such as short-chain fatty acids (Fuller et al., 2016; Kendall et al., 2010). The definition of dietary fibre describes it as carbohydrate polymers with ten or more monomeric units that are not hydrolysed by the endogenous enzymes in the small intestine of humans, and that belong to following four categories:

(i) Edible carbohydrate polymers that occur naturally in the food consumed;

(ii) Carbohydrate polymers that have been obtained from raw food material by physical, enzymatic or chemical means, and that have been shown to have beneficial physiological effects on health, as demonstrated by the generally accepted scientific evidence available to competent authorities;

(iii) Synthetic carbohydrate polymers that have been shown to have beneficial physiological effects on health, as demonstrated by generally accepted scientific evidence available to competent authorities.

(iv) Carbohydrate polymers with three to nine monomeric units that belong to one of the previous categories, if the national authorities decide to include them in these definitions (De Menezes et al., 2013).

Dietary fibre can be classified based on its solubility in water. Insoluble dietary fibre (IDF) consists of mainly cellulose, lignin and some hemicelluloses, while (water)-soluble dietary fibre (SDF) consists of mainly pectin, gum and mucilage. The solubility of dietary fibre governs its physiochemical properties, as IDF has strong hygroscopic properties and can thus swell and absorb water. SDF, on the other hand, can form a gel network or a dense network under some physiochemical conditions, and can thus bind water in this way (Thebaudin et al., 1997).

Increased intake of dietary fibre promotes more frequent defecation and lowers the risk of diabetes mellitus, obesity, coronary heart disease and various cancers; this increased intake also lowers cholesterol levels in the blood (Dahl & Stewart, 2015; Fernstrand et al., 2017; Perry & Wang, 2012; Tarcea et al., 2017). In terms of the physiological effects on blood sugar levels and lipid levels, SDF shows potential for great benefits through its regulatory effects (Kapoor et al., 2016). The particle size of the dietary fibre itself can have an influence on the physiological properties of the colon. Smaller particles of bran have been shown to increase the microbiological production of short-chain fatty acids, which appears to be due to the increased surface area of the smaller particles. Particle size of corn bran can also influence its swelling, with a consequent influence on faecal wet mass and faecal bulking, with increases in liver cholesterol and butyrate levels (Ebihara & Nakamoto, 2001; Stewart & Slavin, 2009).

The dietary fibre content of food is generally determined by enzymatic–gravimetric methods, where the macro nutrients are digested *in vitro* or removed, and the remaining portion of the sample represents the dietary fibre. The most common of these methods are AOAC 985.29 and AOAC 991.43 (Westenbrink et al., 2013). Recently, new integrated methods that comply with the new definition of dietary fibre that also includes low molecular weight SDF are gaining value in food composition analyses (Zielinski & Rozema, 2013; Macagnan et al., 2016). The enzymatic breakdown of starch and protein is very important for accurate determination of the dietary fibre content of a food. In the protocol for methods AOAC 985.29 and AOAC 991.43, sample milling is defined, for particles to pass through a 500 µm screen (AOAC 991.43).

Effects of sample particle size on the determination of dietary fibre content have been reported for animal feed samples, where it was shown that the particle size of the feed has effects on hemicellulose, cellulose and lignin determination. With decreasing particle size, the fibre contents determined also decreased. This was consistent regardless of forage cultivar, season of the animal feed preparation and annual variations in the animal feed (Ehle, 1984). Differences in dietary fibre content have also been reported for wheat bran that was milled to particle sizes of 50, 160, 400 and 750 µm, although here these differences were seen for SDF, while total dietary fibre (TDF) remained unchanged (Coda et al., 2014). The main concern about the accuracy of methods AOAC 985.29 and 991.43 is that these only quantify part of the resistant starch, while the proportion that is included in the dietary fibre determination is not known (Champ et al., 2003).

The particle size of a sample can have effects on the enzyme activity of α-amylase. For particles > 500 µm in size, starch granules can potentially remain entrapped and will not be reached by the enzyme. Then, for particles < 350 µm in size, the α-amylase activity approaches a constant value, which shows influence of particle size on releasing starch granules from plant cells, making them more susceptible to hydrolisation with α-amylase (Al-Rabadi et al., 2009). Starch digestibility is related to particle size, due to the increase in surface area with the decrease in particle size. Here, this larger surface area of the finer ground particle allows for more rapid digestion of the starch and greater penetration of the amylase into the starch granules, therefore improving the starch digestibility (Mahasukhonthachat et al., 2010).

As well as particle size, the food matrix and technological processing are also important factors in enzymatic breakdown of starch (Singh et al., 2010). Lipids and proteins can also have an influence on starch digestibility. In combination with lipids, starch can form amorphous structures that are similar to slowly digestible starch. A similar effect has been reported for starch interactions with protein, where the protein can form a structural 'cage' around starch granules, and thus prevent enzymatic digestion (Zhang et al., 2009). Changes in particle size, for dietary fibre analysis, can lead to disruption of cell wall, therefore freeing starch granules from the cell, also milling of the samples on smaller particle size can disrupt starch – protein complex or damage starch granules and not least milling can destroy crystalline formation of starch (Li et al., 2014).

However, to the best of our knowledge, there are no studies on the influence of particle size of whole food samples on the dietary fibre content determined using enzymatic–gravimetric methods. The main aims of our study were thus to determine whether the particle size of five grains (i.e., oatmeal, buckwheat, dehulled barley, wheat, spelt) and three canned legumes (i.e., canned beans, chickpea, pea; with and without their brine) have an influence on the contents determined for dietary fibre, and resistant starch. Grain is frequently used as a model food for dietary fibre determination, while canned legumes are precooked food and therefore represent a different analytical matrix compared to grain. Furthermore, the effects of the brine present in the cans on the legume content of dietary fibre, and resistant starch were determined.

2 MATERIALS AND METHODS

2.1 SAMPLES AND SAMPLE PREPARATION

For evaluation of the effects of particle size on the contents determined for dietary fibre and resistant and digestible starch, the grain (oatmeal, buckwheat, dehulled barley, wheat, spelt) and canned legumes (beans, chickpea, pea; both with and without their brine) were air dried at 40 °C for 48 h. Their moisture contents were then determined gravimetrically from the difference in mass before and after drying at 105 °C for 5 h, along with the dry matter.

Each sample was initially milled in a cyclone mill (AR100G31; Moulinex, Ecully, France) for 30 s, with no screening for particle size separation (i.e., unscreened). For 500 µm or 350 µm particle size the samples were milled as described, then passing of the samples through the mesh of desired size was ensured. If part of the sample was unable to pass the mesh, the milling was continued until all of the sample passed selected mesh. These milled samples were stored in plastic containers at –20 °C until analysis.

2.2 DETERMINATION OF DIETARY FIBRE

The dietary fibre was determined according to enzymatic–gravimetric method AOAC 991.43 (AOAC 991.43), in quadruplicate. The method was modified slightly: for the enzyme digestion, 50 ml centrifuge tubes were used instead of Erlenmeyer flasks, according to our previous study (Ferjančič et al., 2018). This method is based on three enzymes used under different conditions: heat stable α-amylase, a protease, and an amyloglucosidase (all enzymes Cat N° 112979; Merck KGaA, Darmstadt, Germany).

The dietary fibre fractions were obtained as indigestible residues after this enzymatic digestion of the non-dietary fibre components. The IDF was obtained by filtration, and the SDF was precipitated from the filtrate with 96 % ethanol. Determination of the residual ash content (ashed at 525 °C in a muffle furnace for 5 h, and weighed) and residual protein content from the nitrogen content after Kjeldahl, was carried out on the residues, for the corresponding data correction. The TDF was defined as the sum of IDF and SDF. All of these data are presented as fresh mass (FM).

2.3 DETERMINATION OF RESISTANT STARCH

The resistant starch was determined according to AOAC 2002.02 method (McCleary et al., 2002), in quadruplicate, and with modifications for the sample milling. The proposed particle size in the original method is given as <1 mm for dry samples, and < 4.5 mm for fresh samples. The samples in the present study were milled as described above.

Resistant starch assay kit was used for resistant starch determination (K-RSTAR; Megazyme, Bray, Ireland). Samples were incubated with pancreatic α–amylase and amyloglucosidase for 16 h at 37 ° C, for hydrolysation of digestible starch to glucose. Resistant starch is recovered in form of a pellet, obtained by centrifugation of the sample. First centrifugation is followed by two suspensions of the pellet in 50 % ethanol (v/v) and centrifugation. Resistant starch in the remaining pellet is dissolved in 2 M KOH and hydrolysed to glucose with amyloglucosidase. D-glucose is measured with glucose oxidase/peroxidase reagent (GOPOD), which develops a pink colour in presence of glucose that can be measured by spectrophotometer. By obtaining concentration of glucose, content of resistant starch can be calculated.

For the contents determined for resistant starch, the light absorbance was measured using a UV–Vis spectrophotometer (Carry 8458; Agilent, Victoria, Australia) at 505 nm (instead of 510 nm), following identification of the absorbance peak maximum at this wavelength. All of these data are presented as FM.

2.4 STATISTICAL ANALYSIS

Statistical analyses were performed using the R commander software (version 3.3.3). To ensure the appropriateness of ANOVA, variances between the treatments were determined using Levene's tests ($\alpha > 0.05$). Further ANOVA was performed with *post-hoc* Tukey's tests. The threshold for statistical significance was $p \le 0.05$. For comparisons of the influence of the brine, F-tests were performed to ensure the homogeneity of variance, followed by Student's t-tests. The threshold for statistical significance was $p \le 0.05$.

3 RESULTS AND DISCUSSION

3.1 EFFECTS OF MILLING ON THE CONTENTS DETERMINED FOR DIETARY FIBRE AND RESISTANT STARCH OF THE GRAIN

The data for the dietary fibre and resistant starch contents determined in grains are presented in Table 1. Across these five grain samples (i.e., oatmeal, buckwheat, dehulled barley, wheat, spelt) that were milled to the three different particle sizes (unscreened, or 500 μm, 350 μm screening), the highest TDF was determined for dehulled barley (10.15-13.40 g 100 g^{-1} FM), followed by oatmeal (9.13-10.01 g 100 g^{-1} FM) and wheat (8.96-12.36 g 100 g^{-1} FM). These determined contents for TDF in the oatmeal grain are similar to those reported for nordic countries (10.8-12.3 g 100 g^{-1} FM) (Rainakari et al., 2016). Similarly, Škrabanja et al. (2004) reported TDF of buckwheat as 2.7 to 21.3 g 100 g^{-1} FM across different milling fractions, which corresponds to TDF determined for buckwheat in the present study (3.92-4.59 g 100 g^{-1} FM). TDF determined for the dehulled barley was also similar to a previous determination (10.8-12.3 g 100 g^{-1} FM) (Yalcin et al., 2006), as also for wheat (9.2-20.0 g 100 g^{-1} FM) (Ciudad-Mulero et al., 2019) and spelt (8.8-14.9 g 100 g^{-1} dry mass [DM]) (Shewry & Hay, 2015). For dehulled barley, there was a significant decrease across the two specific particle sizes from 500 μm to 350 μm screening, respectively, for IDF and TDF determination.

Similarly, for resistant starch determination, significant decreases across the unscreened to fine milling (i.e., 350 μm screening) of the samples were seen for dehulled barley, and also for wheat (Table 1). Such influences of the particle size on the contents determined for dietary fibre and resistant starch should be related to the enzyme kinetics of the α-amylase. Larger particles would be expected to be less digested due to the slower penetration for larger particles, and therefore here it is possible that some starch in the samples with larger particle sizes remained undigested (Al-Rabadi et al., 2009). As well as consideration of the enzyme penetration for digestion of the starch in these samples, milling can also cause mechanical damage to starch granules, which can result in conversion of resistant starch to digestible starch (De La Hera et al., 2013). Differences in DF and resistant starch determination can be explained by influence of milling on starch digestion kinetics. Dhital et al. (2011) reported influence of cryo-milling of starch granules on molecular structure of starch itself. Their results suggest influence of milling on disruption of helical and crystalline structures of the starch, without breaking the covalent bond of starch molecules due to mechanical force. Furthermore, resistance of starch to hydrolysis is not purely molecular level effect but foremost inability of enzyme to digest starch due to mechanical obstacles, mainly absence of pores on starch granules, which are commonly present in rapid digested starch. Also, internal starch granules in the plant cell are commonly resistant to hydrolysis due to inaccessibility for enzymes. Milling however, can cause starch granule damage therefore facilitating starch hydrolysis (Dhital et al., 2010a). Also, an important note to starch digestion is resistance of starch to hydrolysis. Mechanisms behind the resistant starch are physical in nature (inaccessibility of starch to enzymes, recrystallization, physical entrapment and complexes with other macronutrients), therefore it should be possible for mechanical manipulation of sample particle size to have an effect on starch digestion kinetics (Dhital et al., 2017). The absence of significant differences in other grain samples can be explained by differences in structural features (Dhital et al., 2010b).

Table 1: Unprocessed grain dry matter, dietary fibre content, and resistant starch content according to milled screen setting for particle size

Sample	Milling screen setting (µm)	Dry matter (g 100 g^{-1})	Dietary fibre (g 100 g^{-1} FM) Insoluble	Soluble	Total	Resistant starch (g 100 g^{-1} FM)
Oatmeal	Unscreened	96.4	4.35 ± 0.49 ^{a}	4.80 ± 0.31 ^{a}	9.15 ± 0.33 ^{a}	0.24 ± 0.01 ^{b}
	500	96.4	4.92 ± 0.81 ^{a}	5.09 ± 0.46 ^{a}	10.01 ± 1.23 ^{a}	0.15 ± 0.03 ^{a}
	350	96.4	4.42 ± 0.35 ^{a}	4.70 ± 0.32 ^{a}	9.13 ± 0.33 ^{a}	0.23 ± 0.03 ^{b}
Buckwheat	Unscreened	96.8	2.75 ± 0.20 ^{a}	1.17 ± 0.14 ^{a}	3.92 ± 0.25 ^{a}	0.72 ± 0.20 ^{a}
	500	96.8	3.32 ± 0.35 ^{b}	1.28 ± 0.29 ^{a}	4.59 ± 0.32 ^{b}	0.84 ± 0.02 ^{a}
	350	96.8	3.06 ± 0.18 ^{ab}	1.22 ± 0.25 ^{a}	4.28 ± 0.35 ^{ab}	0.62 ± 0.16 ^{a}
Dehulled barley	Unscreened	93.9	8.23 ± 0.44 ^{c}	5.17 ± 0.15 ^{a}	13.40 ± 0.33 ^{c}	2.41 ± 0.49 ^{b}
	500	93.9	5.88 ± 0.13 ^{b}	4.95 ± 0.44 ^{a}	10.83 ± 0.38 ^{b}	1.52 ± 1.64 ^{ab}
	350	93.9	4.94 ± 0.32 ^{a}	5.21 ± 0.13 ^{a}	10.15 ± 0.22 ^{a}	0.19 ± 0.12 ^{a}
Wheat	Unscreened	88.6	7.98 ± 0.47 ^{a}	1.25 ± 0.27 ^{a}	9.23 ± 0.56 ^{a}	11.94 ± 0.84 ^{b}
	500	88.6	7.64 ± 0.42 ^{a}	1.46 ± 0.30 ^{a}	8.96 ± 0.35 ^{a}	10.95 ± 0.73 ^{b}
	350	88.6	10.64 ±0.10 ^{b}	3.53 ± 0.42 ^{b}	12.36 ± 0.28 ^{b}	6.71± 0.68 ^{a}
Spelt	Unscreened	89.2	8.02 ± 0.78 ^{b}	1.60 ± 0.31 ^{a}	9.62 ± 0.99 ^{b}	12.06 ± 0.47 ^{a}
	500	89.2	7.80 ± 0.13 ^{b}	1.70 ± 0.14 ^{a}	9.52 ± 0.06 ^{b}	11.60 ± 0.37 ^{a}
	350	89.2	4.16 ± 0.36 ^{a}	1.39 ± 0.11 ^{a}	5.55 ± 0.20 ^{a}	12.27 ± 1.25 ^{a}

FM, fresh mass
Data are means ± SD (n = 4). Means with different letters within each sample are significantly different across the particle sizes ($p \leq 0.05$; ANOVA with *post-hoc* Tukey's tests)

3.2 EFFECTS OF MILLING ON THE CONTENTS DETERMINED FOR DIETARY FIBRE AND RESISTANT STARCH OF THE LEGUMES

The data for dietary fibre and resistant starch for the legume samples (i.e., canned beans, chickpeas, peas; with and without their brine) are presented in Table 2. Across these three legume samples that were milled to the three different particle sizes (unscreened, or 500 µm, 350 µm screening), the highest TDF was determined for chickpeas (5.90-6.74 g 100 g^{-1} FM) and beans (6.09-6.65 g 100 g^{-1} FM), both without the brine. These relative levels for TDF were generally paralleled for IDF and SDF, with the exception of the legume samples without the brine.

The dietary fibre data for these legumes are in agreement with other studies, where IDF and SDF were determined for raw beans (11.4-19.9 g 100 g^{-1} DM; 2.42-3.40 g 100 g^{-1} DM; respectively) and raw peas (20.3 g 100 g^{-1} DM; 1.73 g 100 g^{-1} DM), and IDF for raw chickpeas (13.9 g 100 g^{-1} DM) (Li et al., 2002; De Almeida et al., 2006; Kleintop et al., 2013).

Significant differences across the two specific particle sizes from 500 µm to 350 µm screening were seen for TDF and IDF determined, as increases for beans without brine, and decreases for chickpeas with brine. Significant increases were also seen according to decreased particle size for SDF determined for beans with brine and chickpeas without brine.

For resistant starch determination, again from 500 µm to 350 µm screening, significant difference was only seen as a decrease for peas without brine.

Considering these data, generally the effects of the different milling processes of these foods for the determination of the dietary fibre and resistant starch contents were not uniform. For example, for beans, this was seen for SDF determined with brine and for IDF, SDF and TDF determined without brine (lowest as 500 µm milling), and for chickpeas, numerically (but not significantly) for IDF and SDF determined without brine (lowest as 500 µm milling). On the other hand, across the unscreened to fine milling (i.e., 350 µm screening) of the samples, for chickpeas with brine this showed a significant increase in SDF and decrease in IDF, thus corresponding to decreased particle size. For the beans, chickpeas and peas, the uniform resistant starch determined would be a consequence of the food preparation, which transformed the resistant raw starch granules into digestible starch (Brouns et al., 2002). For the samples of grains and processed grains (previous chapter), in terms of the relatively uniform contents determined for resistant starch despite the different particle sizes might relate to starch gelatinisation. Starch gelatinisation occurs when starch granules receive enough energy to break their intermolecular bonds, thus undergoing irreversible loss of the native structure. As a result of this gelatinisation, the starch granules become more readily digestible (Rooney &Pflugfelder, 1986). All legume samples were cooked beforehand, due to the fact that they were canned, therefore starch in said samples should be gelatinised. Similar effect could be induced in oatmeal, however the amount of water present in thermal treatment is different, thus not allowing for full gelatinisation of starch.

The changes for the legume samples without the brine, as the canned beans, chickpeas and peas, were also examined for the dietary fibre and resistant starch contents determined (Table 2). Significant differences were observed without the brine compared to the samples with brine for TDF, IDF, SDF and resistant starch determined. The TDF and IDF determined were significantly higher in all of these samples without the brine, while the SDF determined was significantly lower only for the beans and chickpeas without the brine. These data for TDF, IDF and resistant starch can be explained in terms of the differences in the dry matter contents. The samples without the brine had higher dry matter contents in comparison to the corresponding samples with the brine (Table 2), and therefore there was an effect of dilution. At the same time, the differences in the SDF determined for these legume samples can be explained in terms of the solubility of this dietary fibre in the brine. A large proportion of SDF in the legumes in brine was dissolved in the brine, and this was thus lost when the brine was discarded prior to the analyses (i.e., some 50 %–80 % of SDF lost in the brine). Also, the canned legumes used in the present study had been cooked and sterilised, with cooking previously shown to lower SDF (Martin-Cabrejas et al., 2006; Wang et al., 2008). At the same time, Shin et al. (2003) reported no changes in resistant starch contents in their samples with regard to the presence of brine, due to the low solubility of resistant starch in water.

4 CONCLUSIONS

The particle sizes of the grain samples generally had non-uniform effects on the determined dietary fibre and resistant and digestible starch. Some particle size effects were seen for dehulled barley, where decreasing the particle size from unscreened to 500 µm to 350 µm screening, the IDF determined significantly decreased, as also for the TDF determined in these samples. With the exception of oatmeal, all of the grain showed some susceptibility to these changes in particle size according to the IDF and TDF determined; however, overall, only the smallest particle size had any effect on dietary fibre determina-

Table 2: Legume dry matter, dietary fibre content, and resistant starch content according to milled screen setting for particle size

Sample	Brine	Milling screen setting (µm)	Dry matter (g 100 g^{-1})	Dietary fibre (g 100 g^{-1} FM) Insoluble	Dietary fibre Soluble	Dietary fibre Total	Resistant starch (g 100 g^{-1} FM)
Beans	With	Unscreened	17.4	3.02 ± 0.18 ^{a}	1.28 ± 0.03 ^{b}	4.30 ± 0.21 ^{a}	0.51 ± 0.02 ^{a}
		500	17.1	2.95 ± 0.19 ^{a}	1.15 ± 0.04 ^{a}	4.10 ± 0.15 ^{a}	0.47 ± 0.07 ^{a}
		350	17.6	3.06 ± 0.12 ^{a}	1.28 ± 0.07 ^{b}	4.34 ± 0.15 ^{a}	0.52 ± 0.01 ^{a}
	Without	Unscreened	27.8	5.98 ± 0.14 ^{b,***}	0.67 ± 0.05 ^{a,***}	6.65 ± 0.10 ^{b,***}	1.34 ± 0.03 ^{a,***}
		500	27.7	5.39 ± 0.30 ^{a,***}	0.70 ± 0.04 ^{a,***}	6.09 ± 0.27 ^{a,*}	1.32 ± 0.05 ^{a,***}
		350	28.5	5.83 ± 0.09 ^{b,***}	0.68 ± 0.09 ^{a,***}	6.51 ± 0.06 ^{b,***}	1.32 ± 0.04 ^{a,***}
Chickpeas	With	Unscreened	8.8	1.92 ± 0.05 ^{b}	0.38 ± 0.02 ^{a}	2.30 ± 0.04 ^{b}	0.40 ± 0.03 ^{a}
		500	9.2	1.86 ± 0.06 ^{b}	0.41 ± 0.02 ^{ab}	2.26 ± 0.04 ^{b}	0.40 ± 0.06 ^{a}
		350	9.6	1.50 ± 0.18 ^{a}	0.45 ± 0.03 ^{b}	1.95 ± 0.15 ^{a}	0.36 ± 0.01 ^{a}
	Without	Unscreened	31.4	6.49 ± 0.22 ^{a,**}	0.32 ± 0.14 ^{a,*}	5.90 ± 0.69 ^{a,*}	1.69 ± 0.06 ^{a,***}
		500	30.9	5.70 ± 0.72 ^{a,***}	0.20 ± 0.06 ^{a*}	6.74 ± 0.19 ^{a,***}	1.68 ± 0.06 ^{a,***}
		350	31.5	6.26 ± 0.16 ^{a,***}	0.40 ± 0.05 ^{b,}	6.65 ± 0.16 ^{a,***}	1.62 ± 0.11 ^{a,***}
Peas	With	Unscreened	13.2	3.44 ± 0.14 ^{a}	0.49 ± 0.02 ^{a}	3.92 ± 0.13 ^{a}	1.06 ± 0.04 ^{b}
		500	13.0	3.42 ± 0.14 ^{a}	0.48 ± 0.04 ^{a,}	3.89 ± 0.12 ^{a}	1.00 ± 0.02 ^{a}
		350	13.5	3.44 ± 0.09 ^{a}	0.48 ± 0.02 ^{a}	3.93 ± 0.08 ^{a}	1.01 ± 0.04 ^{ab}
	Without	Unscreened	20.2	5.15 ± 0.14 ^{a,*}	0.39 ± 0.03 ^{a}	5.53 ± 0.12 ^{a,***}	1.21 ± 0.03 ^{b,***}
		500	20.5	4.83 ± 0.12 ^{a,***}	0.46 ± 0.11 ^{a,}	5.29 ± 0.07 ^{a,***}	1.22 ± 0.01 ^{b,***}
		350	20.7	4.87 ± 0.30 ^{a,***}	0.55 ± 0.12 ^{a}	5.42 ± 0.20 ^{a,***}	1.16 ± 0.02 ^{a,***}

FM, fresh mass
Data are means ±SD (n = 4). Means with different letters within each sample are significantly different across the particle sizes ($p \leq 0.05$; ANOVA with *post-hoc* Tukey's tests)
Student's t-test * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, significant differences between sample without *versus* with brine sample, within each particle size

tion. The particle size in the present study had little or no systematic effect on resistant starch determined.

This possibility of manipulation of dietary fibre determined was generally shown when these grain samples were milled according to the 350 µm screening. Therefore, for dietary fibre determined for grain samples, it is advisable to maintain the particle size at the level of the 500 µm screening, rather than for the 350 µm screening. In these canned (pre-cooked) legumes, particle size had little effect on the contents determined for dietary fibre, and also for resistant starch. However, overall, particle size can have some influence on the contents determined for dietary fibre and resistant determination, in terms of possible sources of error in such analyses, especially for grains.

The additional part of the present study showed that consumption of these canned legumes with the brine increased the SDF intake, although due to the dilution effects seen in the DW analysis, less TDF would be consumed for the same quantity of food.

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6 CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Razvoj kmetijske rabe na območju fotovoltaične elektrarne D3 ob pretočni akumulaciji HE Brežice

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The development of agricultural use in the area of the photovoltaic power plant D3 next to the flow accumulation of the HE Brežice

Abstract: Agrovoltaics as a method of dual use of agricultural land is the subject of many researches and projects of scientific research institutions and investors. The company Hidroeletrarne na Spodnji Savi, d.o.o. wants to develop the concept of agricultural use of agricultural land between and under the existing panels of the solar power plant. The solar power plant is located in the municipality of Brežice, the plots of land are in the cadastral municipality of Krška vas. The total area suitable for the development of agricultural use, on which the solar power plant is located, is 8.8 hectares. Through a case study, we reviewed the theoretical starting points for planning agricultural production in the area of the existing solar power plant. Through our study, we selected suitable species and varieties of plants that would be suitable for cultivation in this area. For the long-term financial sustainability of agricultural production, the study also checked the expected costs and revenues for the agricultural land use plan. Based on the case study, we conclude that this area could also be used for plant production, such as the production of asparagus, currants and raspberries. At the end of the research project, we proposed that in the future photovoltaics should be planned together with agricultural use, since there are significantly more possibilities for the dual use of agricultural land with the simultaneous planning of energy and agriculture.

Key words: agrovoltaics, dual use of agricultural land, flow accumulation of HE Brežice, photosynthesis light saturation point

Razvoj kmetijske rabe na območju fotovoltaične elektrarne D3 ob pretočni akumulaciji HE Brežice

Izvleček: Agrovoltaika kot način dvojne rabe kmetijskih zemljišč je predmet številnih raziskav in projektov znanstveno raziskovalnih inštitucij in investitorjev. Podjetje Hidroelektrarne na Spodnji Savi, d.o.o. želi na obstoječi deponiji s postavljeno sončno elektrarno ob pretočni akumulaciji hidroelektrarne v Brežicah razviti koncept kmetijske rabe kmetijskih zemljišč med in pod obstoječimi paneli fotovoltaične elektrarne. Sončna elektrarna je postavljena v občini Brežice, parcele so v k.o. Krška vas. Celotna površina, primerna za razvoj kmetijske rabe, na kateri v večjem delu stoji sončna elektrarna, je 8,8 hektarjev. S študijem primera smo pregledali teoretična izhodišča za načrtovanje kmetijske pridelave na območju obstoječe sončne elektrarne. S študijo smo izbrali primerne vrste in sorte rastlin, ki bi bile primerne za pridelavo na tem območju. Za dolgoročno finančno vzdržnost kmetijske pridelave smo v študiji preverili tudi pričakovane stroške in prihodke za načrtovano kmetijsko rabo zemljišč. Na podlagi študije primera ugotavljamo, da bi to površino lahko uporabili tudi za rastlinsko pridelavo, kot je pridelovanje špargljev, ribeza in malin. Ob zaključku raziskovalne naloge smo predlagali, da se v bodoče načrtuje fotovoltaika skupaj s kmetijsko rabo, saj je možnosti za dvojno rabo kmetijskih zemljišč ob sočasnem načrtovanju energetike s kmetijstvom bistveno več.

Ključne besede: agrovoltaika, dvojna raba kmetijskih zemljišč, pretočna akumulacija HE Brežice, svetlobna saturacijska točka fotosinteze

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1 UVOD

Republika Slovenija se je v strateških dokumentih zavezala povečati delež obnovljivih virov energije v strukturi pridobljene energije. Obnovljive vire energije podpirata dve ključni strategiji, kot sta Nacionalni energetski in podnebni načrt (NEPN) (Ministrstvo za infrastrukturo, 2022) in Strateški načrt Skupne kmetijske politike (SN SKP) (Ministrstvo za kmetijstvo, gozdarstvo in prehrano, 2022). NEPN med drugim predvideva vsaj 27 % obnovljivih virov energije v bruto končni rabi energije. Prav tako predvideva do leta 2030 zmanjšanje emisij toplogrednih plinov v sektorju kmetijstvo za 1 % glede na leto 2005. Enako tudi SN SKP naslavlja obnovljive vire energije kot pomemben korak k zmanjšanju emisij toplogrednih plinov na področju kmetijstva in gozdarstva. Poleg vezave CO_2 intenzivno naslavlja investicije za pridobivanje energije iz obnovljivih virov, med drugimi tudi iz sonca. V tem delu se sklicuje na NEPN in v ključnih delih, povezanih z energetiko, povzema njegovo strategijo. Po drugi strani pa SN SKP izrazito poudarja zaščito proizvodnega potenciala kmetijskih zemljišč za pridelovanje hrane.

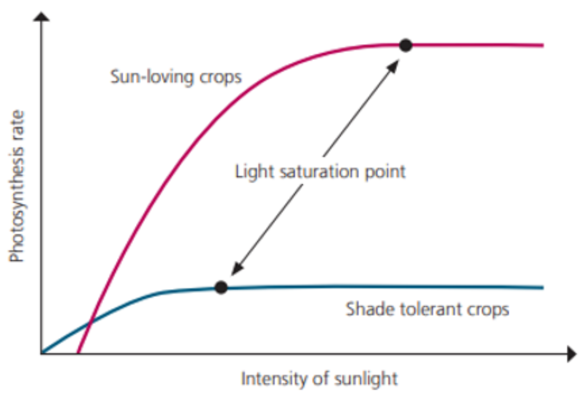
Številne raziskovalne inštitucije v Evropi in Svetu so pričele z intenzivnim raziskovanjem kombinirane rabe kmetijskih zemljišč za namene pridelovanja hrane in pridobivanja električne energije iz sonca. Nastaja nova skupna raba kmetijskih zemljišč, agrovoltaika. Številni raziskovalci že raziskujejo to novo tehnološko področje in spremljajo možnosti za razvoj agrovoltaike na kmetijskih zemljiščih. Predvsem iščejo priložnosti in prednosti za oba načina rabe kmetijskih zemljišč. Tako [1]Gorjian s sod. (2022) ugotavlja, da implementacija agrovoltaike izjemno hitro narašča. Trenutno se za proizvodnjo električne energije še vedno postavljajo klasični neprosojni paneli. Takšni so postavljeni tudi na proučevani fotovoltaični elektrarni D3 ob pretočni akumulaciji HE Brežice. Pri tem tipu agrovoltaike je največja, kritična omejitev za rastlinsko pridelavo pod paneli senčenje, kar v svojih raziskavah navajajo tudi drugi raziskovalci (Trommsdorff s sod., 2022; Takashi in Nagashima , 2019; Tani s sod., 2014; Herbert, 2018). Zato se v raziskavah vse bolj uporabljajo delno prosojni paneli, zasnovani na podlagi kristalnega silicija, tankoplastnih panelov, luminisenčnih solarnih koncentratorjev in panelov z na sončno sevanje občutljivim barvilom. Rezultati poskusov kažejo, da so delno prosojni paneli, zasnovani na podlagi kristalnega silicija, najbolj razširjeni v raziskovalnih projektih. Ti moduli imajo namreč v primerjavi z ostalimi številne prednosti, kot so nizki stroški, velika stabilnost in odpornost na vremenske vplive ter velika učinkovitost. Z razvojem novih tehnologij zlasti pri tankoplastnih panelih, ki uporabljajo tehnologijo organskih panelov ali panelov z na svetlobo občutljivim barvilom, se povečujejo možnosti agrovoltaike, saj ti paneli prepuščajo fotosintetsko aktivno sončno sevanje, ostali spekter svetlobe pa se uporabi za proizvodnjo električne energije. Nekatere študije dokazujejo povečanje ekonomske vrednosti kmetij z uvajanjem agrovoltaike.

Harshavardhan s sod. (2016) ocenjuje, da postavitev sončnih elektrarn v kombinaciji z rastlinsko proizvodnjo z na senčenje tolerantnimi vrstami poveča ekonomsko vrednost kmetij za preko 30 %. Še več, nekateri raziskovalci ([2]Gorjian s sod., 2022) vidijo priložnosti agrovoltaike tudi v lastni proizvodnji elektrike za potrebe električnih kmetijskih strojev in sodobne opreme, vključno z roboti v kmetijstvu. Kot navajajo raziskovalci, sta pred nami dva glavna izziva, ki trenutno zavirata široko uporabo sodobnih električnih kmetijskih strojev. To sta visoki začetni stroški v povezavi s postavitvijo sončne elektrarne in pomanjkljivosti v tehnologijah za shranjevanje električne energije.

Poleg pridobivanja električne energije pravilno načrtovana in rastlinski pridelavi prilagojena agrovoltaika tudi izboljšuje nekatere razmere za rast gojenih rastlin v tej kombinaciji. Trommsdorff s sod. (2022) navaja prednosti agrovoltaike za gojene rastline, kot so zmanjšanje potreb po namakanju za 20 % v sušnih obdobjih, možnost zbiranja vode za potrebe namakanja, zmanjševanje vetrne erozije, uporaba konstrukcije fotovoltaike za zaščitne mreže ali folijo in optimizacija razpoložljive svetlobe za gojene rastline. Raziskovalci navajajo tudi prednosti za proizvodnjo električne energije zaradi povečanja učinkovitosti fotovoltaičnih modulov zaradi konvekcijskega hlajenja in povečanje učinkovitosti bifacialnih panelov, ki uporabljajo osvetlitev za proizvodnjo elektrike na obeh straneh, saj je večja razdalja med paneli, tlemi in sosednjimi vrstami panelov.

Za določitev vrst rastlin, ki bi uspešno uspevale v tej kombinaciji dvojne rabe kmetijskih zemljišč, je potrebno upoštevati svetlobno saturacijsko točko fotosinteze posameznih rastlin. Optimizacijo fotosinteze in nadzorni model je možno vzpostaviti ravno na podlagi te lastnosti rastlin (Xin s sod., 2019). Svetlobna saturacijska točka fotosinteze kaže na vpliv spreminjanja jakosti fotosintetsko aktivnega sevanja na učinkovitost fotosinteze. Nekatere rastlinske vrste namreč dosežejo za njih značilen največji učinek fotosinteze pri manjši jakosti svetlobe kot druge rastlinske vrste (Thrommsdorff s sod., 2022). Princip svetlobne saturacijske fotosinteze točke pri rastlinah, ki so ali niso tolerantne na senčenje, je prikazan na sliki 1

Nekatere rastlinske vrste imajo svetlobno saturacijsko točko blizu 1.000 µmol s^{-1} m^{-2} ali celo pod 1.000 µmol s^{-1} m^{-2}, spet druge bistveno višje. In višja, kot je svetlobna saturacijska točka, večjo jakost fotosintetsko aktivnega



Slika 1: Svetlobna saturacijska točka fotosinteze pri različni intenzivnosti sončnega sevanja in stopnja fotosinteze (Thrommsdorff s sod., 2022)
Figure 1: Light saturation point of photosynthesis at different intensities of solar radiation and photosynthesis rate (Thrommsdorff et al., 2022)

sevanja potrebujejo te vrste. Saturacijske točke fotosinteze nekaterih rastlinskih vrst so navedene v preglednici 1.

Številni raziskovalci (Trommsdorff s sod., 2022; Takashi in Nagashima , 2019; Tani s sod., 2014; Herbert, 2018) navajajo vpliv agrovoltaike na kmetijsko proizvodnjo. Sončni paneli senčijo površino pod sabo. Intenzivnost senčenja je v veliki meri odvisna od načina postavitve panelov. Pravilno načrtovana agrovoltaika je zasnovana tako, da kolikor je mogoče čim manj ovira kmetijsko pridelavo, zato so običajno sončnimi paneli visoko nad rastlinami. Pri takšnih postavitvah pride do razpršenega difuznega sevanja fotosintetsko aktivnega sevanja, kar omogoča rastlinsko pridelavo neposredno pod sončnimi paneli. Glede na postavitev sončnih panelov je potrebno za rastlinsko pridelavo načrtovati tudi ustrezno namakanje. Ne glede na to so v običajnih letih brez temperaturnih ekstremov pridelki gojenih rastlin pod sončnimi paneli nekoliko manjši kot pri vzgoji brez kombinacije s sončnimi paneli. Pri 30 do 35 % senčenju zaradi sončnih panelov so pridelki manjši za okoli 10 % pri solati, 30 % pri brokoliju, 20 % pri ozimni pšenici, 15 % pri krompirju, 20 % pri zeleni. V letih, ko je bilo poletje nadpovprečno toplo s temperaturnimi ekstremi blizu 40 ^{o}C pa so bili pridelki pri pridelavi pod sončnimi paneli celo večji. Tako so bili pridelki večji pri zeleni za okoli 12 %, krompirju za okoli 11 %, vinski trti za okoli 25 %, koruzi okoli 5 %, brokoliju okoli 40 %, papriki 40 %. Stallknecht E. J. s sod. (2023) navaja, da je za kvaliteten pridelek tržne pridelave bazilike zadosten že indeks dnevne svetlobe malo nad 12 mol m^{-2} dan^{-1}, kar se doseže z uporabo selektivnih modulov fotovoltaične elektrarne. Podatki teh raziskovalcev kažejo tudi, da pa je pridelek paradižnika manjši že pri minimalnem zmanjšanju fotosintetsko aktivnega sevanja.

2 MATERIALI IN METODE

Agrovoltaika postaja zanimiva tudi za nekatere investitorje v Sloveniji. Eden od resnih investitorjev se pojavlja podjetje Hidroelektrarne na Spodnji Savi, d.o.o. z vizijo po razvoju kmetijske rabe na območju fotovoltaične elektrarne D3 ob pretočni akumulaciji HE Brežice. Za preverjanje potenciala kmetijske rabe smo uporabili metodo študije primera. Tako smo na konkretnem primeru preverili potencial in investicijsko vrednotili razvoj kmetijske rabe.

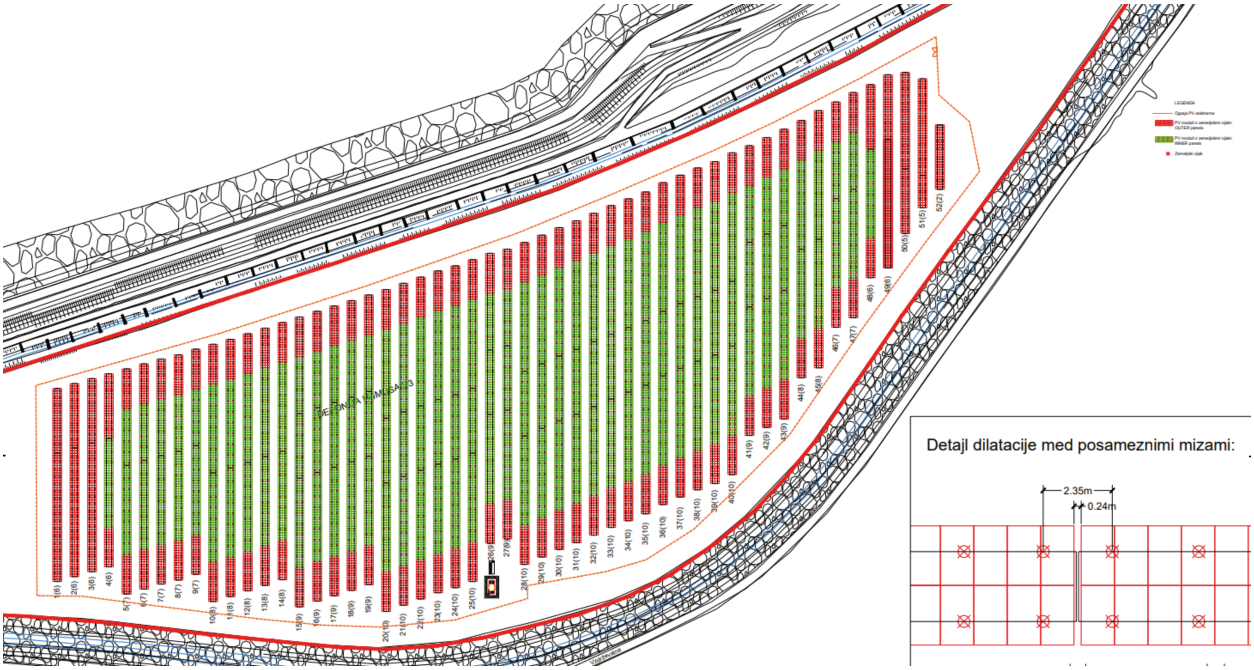
2.1 OBMOČJE RAZISKAVE

Fotovoltaična elektrarna D3 ob pretočni akumulaciji HE Brežice leži v občini Brežice, k.o. Krška vas na parcelah 6741, 6676/2, 6746, 6749, 6784, 6779, 6321/2, 6775, 6790, 6770, 6331/2, 6680, 6752, 6766, 6763, 6755. Velikost celotne parcele je okoli 88.000 m^2. Na tej parceli so v smeri vzhod-zahod postavljeni klasični neprosojni fotovoltaični moduli. Postavitev fotovoltaične elektrarne D3 je predstavljena na sliki 2.

Kmetijsko pridelavo naj bi umestili med posamezne linije fotovoltaičnih panelov glede na natančen načrt postavitve modulov, razmakov med njimi in višinami fotovoltaičnih panelov. Prav tako smo upoštevali višino

Preglednica 1: Svetlobne saturacijske točke fotosinteze nekaterih rastlinskih vrst (Thrommsdorff s sod., 2022; Hidaka s sod., 2023)
Table 1: Light saturation points of photosynthesis of certain crop species (Thrommsdorff et al., 2022; Hidaka et al., 2023)

Rastlinska vrsta	Svetlobna saturacijska točka (µmol s^{-1} m$^{-2)}$
Paradižnik	1985
Kumare	1421
Jajčevci	1682
Zelje	1324
Cvetača	1095
Solata	857
Špinača	889
Jagode	800 – 1200
Borovnice	600 - 800
Jagodičevje	700 – 1000
Šparglji	900 – 1100



Slika 2: Postavitev fotovoltaičnih modulov na fotovoltaični elektrarni D3 ob pretočni akumulaciji HE Brežice (HESS, 2023)
Figure 2: Installation of photovoltaic modules on the photovoltaic power plant D3 next to flow accumulation of HE Brežice (HESS, 2023)

rastlin predlagane rastlinske pridelave. Rastlinsko pridelavo smo umeščali z vidika minimalnega senčenja fotovoltaičnih panelov glede na razvojno fazo rastlin in višino sonca v posameznih mesecih leta.

2.2 KONCEPT POSTAVLJENE FOTOVOLTAIČNE ELEKTRARNE D3

Fotovoltaični moduli fotovoltaične elektrarne D3 ob pretočni akumulaciji HE Brežice so postavljeni v 52 vrst v smeri vzhod – zahod, s čimer se doseže največja učinkovitost pridobivanja sončne energije iz sonca, saj so posamezni paneli orientirani proti jugu. Dolžine posameznih vrst fotovoltaičnih modulov so od 30 m do 150 m. Postavitev modulov glede na osončenost je predstavljena na sliki 3.

Paneli fotovoltaične elektrarne so postavljeni na predhodno pripravljeno konstrukcijo. Konstrukcija je zasnovana po nizih, ki se lahko poljubno sestavljajo v različne dolžine posamezne linije, glede na prostorske možnosti. Konstrukcija za 1 niz je zasnovana tako, da se nanjo lahko postavi 17 panelov v dveh vrstah, skupno 34 panelov. Konstrukcija niza je sestavljena iz 5 okvirjev na razdalji 4,15 m, medsebojno povezanih z vzdolžnimi nosilci, na katere se pritrjujejo moduli. Konstrukcija je postavljena pod naklonom 30 °. Višina okvirjev je določena tako, da je spodnji rob panelov 1 m od temelja oziroma 1,1 m od tal. Podrobnejši podatki konstrukcije so predstavljeni na sliki 4.

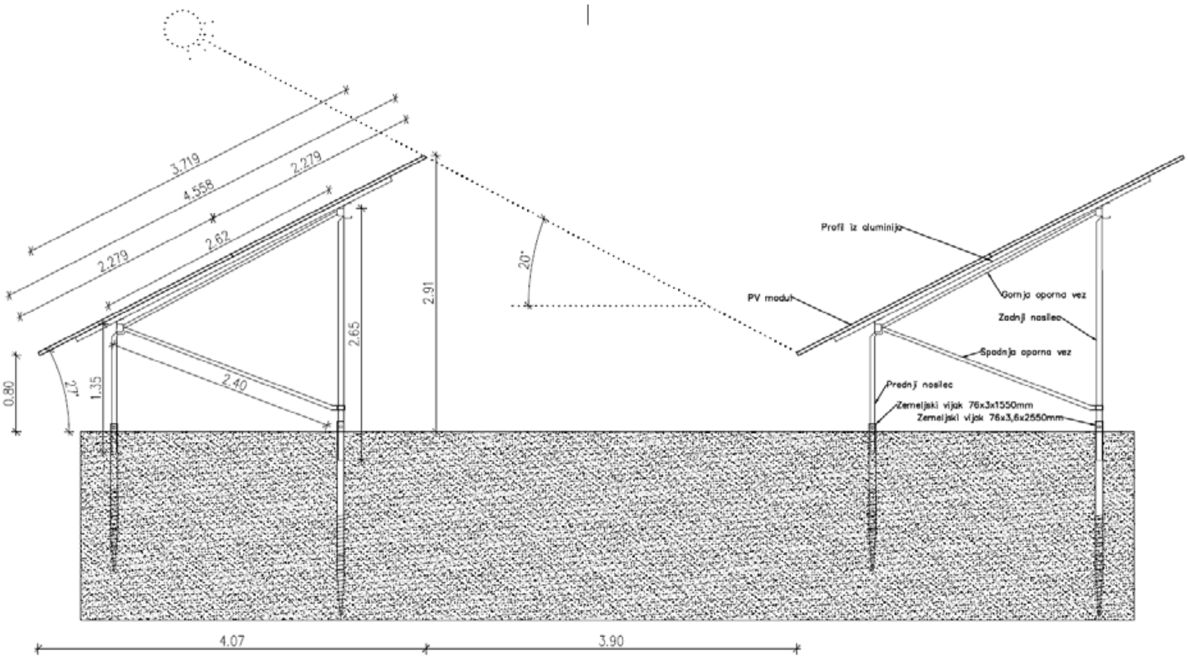
V študiji smo pripravili načrt kmetijske rabe prostora med posameznimi vrstami panelov.

2.3 METODA IZBIRE PRIMERNE KMETIJSKE RABE

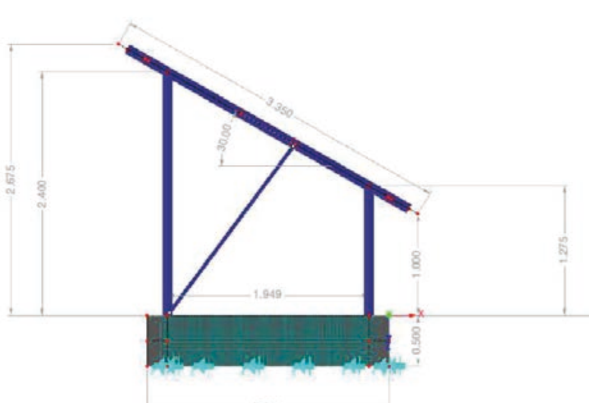
Poseben izziv v raziskavi je bila izbira primerne kmetijske rabe na lokaciji že postavljene fotovoltaične elektrarne D3 ob pretočni akumulaciji HE Brežice. Investitorji na že postavljeni in delujoči elektrarni želijo vzpostaviti kmetijsko rabo zemljišč predvsem okoli samih panelov, v primeru reje manjših živali pa tudi pod paneli. Glede na dejstvo, da je elektrarna že postavljena, smo v študiji primera morali kmetijsko rabo povsem prilagoditi obstoječemu stanju. Zato smo pri načrtovanju kmetijske rabe sladili usmeritvi investitorja, da kmetijska raba ne sme senčiti ali kako drugače ovirati delovanja fotovoltaične elektrarne. Prav tako smo upoštevali dejstvo, da investitor želi izvajati ekološko pridelavo hrane in da želi kombinirati rejo živali z rastlinsko pridelavo.

Izbor rastlin smo naredili na podlagi sledečih parametrov:

- primernost rastlin za gojenje pri manj močni svet lobi (nižja svetlobna saturacijska točka fotosin teze),



Slika 3: Postavitev fotovoltaičnih modulov na fotovoltaični elektrarni D3 ob pretočni akumulaciji HE Brežice (HESS, 2023)
Figure 3: Installation of photovoltaic modules on the photovoltaic power plant D3 next to flow accumulation of HE Brežice (HESS, 2023)



Slika 4: Podrobnejša predstavitev načrta konstrukcije za montažo panelov fotovoltaične elektrarne D3 (HESS, 2023)
Figure 4: Detailed presentation of the construction plan for the installation of D3 photovoltaic power plant panels (HESS, 2023)

- habitus rastline v polni rodnosti, ki ne sme senčiti fotovoltaične elektrarne,
- možnost ekološke pridelave,
- želje lastnika fotovoltaične elektrarne.

Pri izboru primernih živalskih vrst pa smo upoštevali sledeče parametre:

- višina živali,
- agresivnost živali,
- sposobnost živali za pašo,
- uporabnost gnoja za gnojenje ekološke pridelave rastlin,
- želje lastnika fotovoltaične elektrarne.

V nadaljevanju smo naredili podrobnejšo analizo po principu študije primera zgolj za predlagano rastlinsko pridelavo, rejo živali v analizo nismo vključili.

2.4 METODA EKONOMSKE ANALIZE

Za investitorja smo pripravili tudi kratko ekonomsko analizo načrtovane rastlinske pridelave. Osredotočili smo se predvsem na investicijski strošek, izračunali predviden letni donos in potrebo po delovni sili za vzdrževanje v enem letu polne rodnosti zasnovane rastlinske pridelave. Investicijske stroške smo ocenili glede na potrebne materiale za postavitev rastlinske pridelave in aktualne cene na trgu, na podlagi prejetih ponudb v letu 2023. Predviden donos smo izračunali glede na pričakovan pridelek in aktualne cene na trgu v sezoni 2023. Podatke o potrebnem času za vzdrževanje rastlinske pridelave pa smo črpali iz Kataloga kalkulacij za načrtovanje gospodarjenja na kmetijah v Sloveniji (Jerič s sod., 2001).

3 REZULTATI IN DISKUSIJA

Na podlagi parametrov izbire rastlinskih vrst smo se odločili za sledeče rastlinske vrste:

- maline (*Rubus idaeus* L.),
- ribez (*Ribes*),
- špargljі (*Asparagus officinalis* L.).

Na obstoječi fotovoltaični elektrarni D3 smo predlagali sledečo razporeditev posameznih rastlinskih vrst, kar prikazuje tudi slika 5:

- od vrste 15 do vrste 28: pridelovanje malin,
- od vrste 28 do vrste 40: pridelovanje ribeza,
- od vrste 40 do vrste 52: pridelovanje špargljev.

Na površini fotovoltaične elektrarne D3 od vrste 1 do vrste 15 smo predvideli rejo kokoši nesnic oziroma drobnice z namenom pridobivanja ekološkega gnoja za gnojenje predlagane rastlinske pridelave. Rejo živali v študijo nismo vključili, zato je tudi nismo podrobneje analizirali.

3.1 POTREBNA OPREMA ZA VZPOSTAVITEV KMETIJSKE RABE ZEMLJIŠČ

3.1.1 Maline

Za pridelovanje malin smo predlagali površino od vrste 15 do vrste 28 fotovoltaičnih panelov. Glede na načrtovano višino malin v času obiranja smo predlagali eno vrsto malin v medvrstni prostor fotovoltaičnih modulov s sajenjem čim bližje najvišjemu delu fotovoltaičnih modulov. Priporočena sadilna razdalja v vrsti je pri malinah 40 cm, zato je za predlagano površino potrebnih 6.300 sadik.

Za pridelovanje malin predlagamo sorte, ki omogočajo ekološko pridelavo in imajo manjši habitus, torej ne senčijo panelov fotovoltaične elektrarne. Predlagali smo sledeče sorte: Clarita, Enrosadira in Polka.

Za namakanje malin smo predlagali kapljično namakanje. Za postavitev kapljičnega namakanja predlagane površine malin so potrebni sledeči materiali:

- kapljična cev: 1.900 m,
- gibka cev za sekundarni razvod namakanja: 105 m,
- priključne spojke: 13 kosov.

Maline se sadijo na grebene, ki jih je potrebno prekriti s tkanino proti razvoju plevela. Predlagali smo tkanino teže 100 g m^{-2}, širine 1,25 m. Potrebno je okoli 1.900 dolžinskih metrov te tkanine.

Za pripravo opore predlaganega nasada malin se potrebujejo sledeči materiali:

- betonski stebri 7 x 7,5 cm, višine 2,8 m: 450 kosov
- kovinska sidra, 0,85 m x 130 mm: 80 kosov
- napenjalna žica 2,5 mm: 100 kg.

Po postavitvi nasada malin bo potrebno postaviti še oporo za prekrivanje malin s folijo v času rodnosti, pri čemer se priporoča uporaba obstoječe konstrukcije elektrarne kot pomemben konstrukcijski element.

3.1.2 Ribez

Za pridelovanje ribeza smo predlagali površino od vrste 28 do vrste 40 fotovoltaičnih panelov. Glede na načrtovano višino grmov smo predlagali eno vrsto ribeza v medvrstni prostor fotovoltaičnih panelov. Priporočena sadilna razdalja v vrsti je 1 m, zato je za predlagano površino potrebnih 1.800 sadik.

Izbor sort ribeza je temeljil na možnosti ekološke pridelave in višini posameznih grmov z vidika morebitnega senčenja panelov fotovoltaične elektrarne. Predlagamo sledeče sorte ribeza: Jonkher van Tets in Rovada (rdeči riberz) ter Ben Nevis in Titania (črni ribez).

Tudi ribez se namaka kapljično. Za postavitev kapljičnega namakanja predlagane površine ribeza so potrebni sledeči materiali:

- kapljična cev: 1.900 m,
- gibka cev za sekundarni razvod namakanja: 105 m,
- priključne spojke: 12 kosov.

Ribez se sadi v vrste, ki jih je potrebno prekriti s tkanino proti razvoju plevela. Predlagali smo tkanino teže 100 g m^{-2}, širine 1,25 m. Potrebno je okoli 1.800 dolžinskih metrov te tkanine.

3.1.3 Šparglji

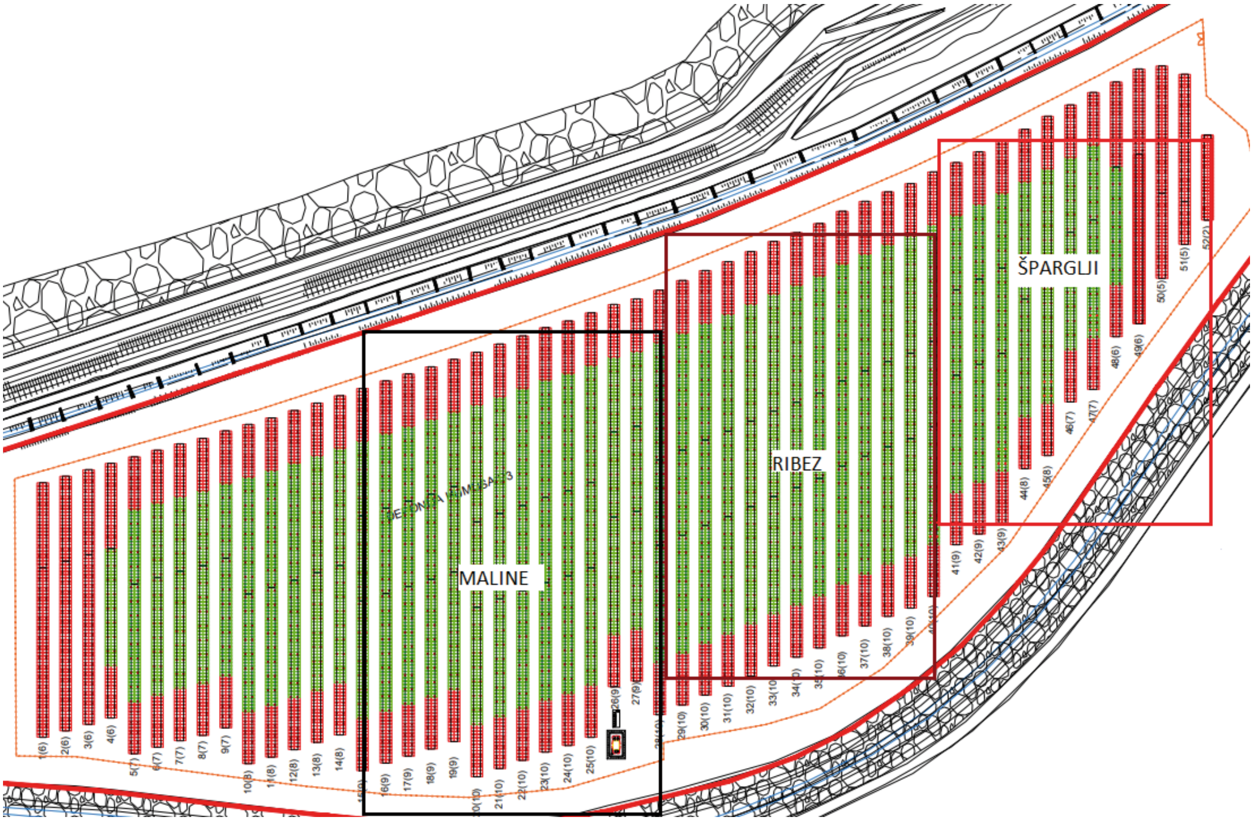
Za pridelovanje špargljev smo predlagali površino od vrste 40 do vrste 52 fotovoltaičnih panelov. Priporočena sadilna razdalja v vrsti je 30 cm, za načrtovano površino je potrebnih 4.350 sadik. Glede na načrtovano višino zelenja po končanju pobiranja špargljev predlagamo eno vrsto špargljev v medvrstni prostor fotovoltaičnih panelov.

Pri izboru sort špargljev smo sledili izhodišču, da bo pridelava potekala na ekološki način in prilagojenosti sort na težja zemljišča. Predlagamo sledeče sorte: Eros, Marte in Giove.

Šparglje je priporočljivo namakati. Za pripravo kapljičnega namakanja predlagane površine špargljev so potrebni sledeči materiali:

- kapljična cev: 1.500 m,
- gibka cev za sekundarni razvod namakanja: 115 m,
- priključne spojke: 12 kosov.

Material, ki je potreben za zasnovo predlagane rastlinske pridelave po posamezni rastlinski vrsti predstavljamo v preglednici 2.



Slika 5: Razporeditev izbranih rastlinskih vrst na obstoječi fotovoltaični elektrarni D3
Figure 5: Distribution of selected crop species at the existing photovoltaic power plant D3

3.2 OKVIRNI STROŠKI VZPOSTAVITVE IN POTREBA PO DELOVNI SILI

3.2.1 Maline

Ocenjena vrednost sadik in materiala za postavitev nasada malin iz preglednice 3 je po prejetih ponudbah iz marca 2023 okoli 14.000 EUR (brez DDV).

Za napravo predlaganega nasada malin se ocenjuje porabo okoli 400 delovnih ur. Za spravilo pridelka in vzdrževanje nasada pa okoli 2.500 ur letno. Izrazita delovna konica je v času spravila pridelka. Samo za spravilo pridelka se predvideva porabo okoli 2.200 ur časa, kar pomeni okoli 3,1 FTE v času od sredine junija do konca oktobra (Jerič s sod., 2001). Pri bruto urni postavki 6,92 EUR (Odredba o uskladitvi najnižje bruto urne postavke za opravljeno začasno ali občasno delo v kmetijstvu, 2023) je ocenjen strošek dela 20.068 EUR.

Pričakovan pridelek malin na predlagani površini je okoli 14.000 kg. Pri prodajni ceni 5,00 EUR kg^{-1} je pričakovan prihodek 70.000 EUR.

3.2.2 Ribez

Ocenjena vrednost sadik in materiala za postavitev nasada ribeza iz preglednice 3 je po prejetih ponudbah iz marca 2023 okoli 6.500 EUR (brez DDV).

Za napravo predlaganega nasada ribeza se ocenjuje porabo okoli 100 delovnih ur. Za spravilo pridelka in vzdrževanje nasada pa okoli 1.200 ur letno. Izrazita delovna konica je v času spravila pridelka (konec junija do začetka avgusta) (Jerič s sod. 2001). Samo za spravilo pridelka ocenjujemo porabo okoli 1.000 ur časa, kar pomeni okoli 3,8 FTE v času od konca junija do začetka avgusta. Pri bruto urni postavki 6,92 EUR (Odredba o uskladitvi najnižje bruto urne postavke za opravljeno začasno ali

Preglednica 2: Vrste in količine potrebnega materiala za zasnovo predlagane rastlinske pridelave na fotovoltaični elektrarni D3
Table 2: Types and quantities of material needed for the design of the proposed crop production at the D3 photovoltaic power plant

	Rastlinska vrsta		
Material	Maline	Ribez	Špargljі
število sadik (kos)	6.300	1.800	4.350
kapljična cev (m)	1.900	1.900	1.500
gibka cev - sekundarni razvod namakanja (m)	105	105	115
priključne spojke (kos)	13	12	12
tkanina (m)	1.900	1.800	
betonski stebri (kos)	450		
kovinska sidra (kos)	80		
napenjalna žica (kg)	100		

občasno delo v kmetijstvu, 2023) je ocenjen strošek dela 8.996 EUR.

Pričakovan pridelek ribeza na predlagani površini je okoli 7.000 kg. Pri prodajni ceni 4,00 EUR kg^{-1} je pričakovan prihodek 28.000 EUR.

3.2.3 Šparglji

Ocenjena vrednost sadik in materiala za postavitev nasada špargljev iz preglednice 3 je po prejetih ponudbah iz marca 2023 okoli 3.600 EUR (brez DDV).

Za napravo predlaganega nasada špargljev se ocenjuje porabo okoli 120 delovnih ur. Za spravilo pridelka in vzdrževanje nasada pa okoli 600 ur letno (Jerič s sod., 2001). Izrazita delovna konica je v času spravila pridelka, ki v času polne rodnosti traja 8 tednov (prvo leto spravila pobiramo poganjke 3 tedne in drugo leto 5 tednov). Za spravilo pridelka ocenjujemo porabo okoli 550 ur časa, kar pomeni 1,5 FTE v času obiranja. Pri bruto urni postavki 6,92 EUR (Odredba o uskladitvi najnižje bruto urne postavke za opravljeno začasno ali občasno delo v kmetijstvu, 2023) je ocenjen strošek dela 4.982 EUR.

Pričakovan pridelek špargljev v času polne rodnosti je na predlagani površini okoli 2.400 kg špargljev. Pri prodajni ceni 5,00 EUR kg^{-1} je pričakovan prihodek 12.000 EUR.

Predvideni stroški zasnove predlagane rastlinske pridelave na fotovoltaični elektrarni D3 in stroški enega leta vzdrževanja nasada, vključno z obiranjem, ter predviden pridelek in prihodek v polnem letu rodnosti so po posameznih rastlinskih vrstah predstavljeni v preglednici 3.

Preglednica 3: Predvideni stroški in prihodki za postavitev in vzdrževanja nasada v enem letu polne rodnosti po posameznih rastlinskih vrstah predlagane rastlinske pridelave na fotovoltaični elektrarni D3
Table 3: Estimated costs and revenues for the installation and maintenance of the plantation in one year of full fertility by crop species of the proposed crop production at photovoltaic power plant D3

	Rastlinska vrsta		
Predvideni stroški	Maline	Ribez	Šparglji
material iz preglednice 2 (EUR)	14.000	6.500	3.600
delo za postavitev nasada (ure)	400	100	120
delo za postavitev nasada (EUR)	2.768	692	830
delo za vzdrževanje in obiranje (ure)	2.500	1.200	600
delo za vzdrževanje in obiranje (EUR)	17.300	8.304	4.152
Predviden prihodek			
pričakovan pridelek (kg)	14.000	7.000	2.400
pričakovana prodajna cena (EUR/kg)	5,00 €	4,00 €	5,00 €
pričakovan prihodek	70.000,00 €	28.000,00 €	12.000,00 €

3.3 DISKUSIJA

Študija primera, ki smo jo izvedli na konkretnem primeru, kaže na potencialno možnost rastlinske pridelave na območju Fotovoltaične elektrarne D3 ob pretočni akumulaciji Brežice. Pri načrtovanju rastlinske pridelave smo se povsem prilagodili obstoječi fotovoltaični elektrarni, ki je že postavljena. Paneli so postavljeni na primernih razdaljah in se ne senčijo med sabo. Prav tako razdalje med paneli omogočajo nekatere vrste rastlinske pridelave. Pri tem gojene rastline ne senčijo panelov.

Zasnova nasada malin in ribeza je predvidena z zastirno folijo, ki preprečuje rast in razvoj plevelov in hkrati omogoča vsakoletno obnavljanje nasada z novimi poganjki. Zastirna folija je široka 1,25 m, kar pomeni, da bo pokrivala le grebene, kjer bodo rastle predlagane kulture. Ostali del površine bo zatravljen (trenutno je celotna površina zatravljena). Zato bo potrebno stalno vzdrževanje travne površine. Glede na prostorsko stisko glede na sajene rastline med dvema vrstama panelov bo traktorsko mulčenje trave onemogočeno, zato bo potrebna košnja z manjšo samohodno kosilnico.

Načrtovana rastlinska pridelava okoli panelov fotovoltaične elektrarne bo omogočala stalen dostop do elektrarne. Rastlinska pridelava ne bo posegala v komunikacijske in logistične poti, ki so potrebne za vzdrževanje in spremljanje fotovoltaične elektrarne. Prav tako bo v vsakem trenutku omogočen dostop do katerega koli panela oziroma električne napeljave v primeru potrebe po odpravi okvar na katerem koli delu fotovoltaične elektrarne.

Investitor fotovoltaične elektrarne D3 ob pretočni akumulaciji HE Brežice je študijo naročil z namenom, da se po metodi študije primera ugotovijo realne možnosti za kmetijsko pridelavo na lokaciji fotovoltaične elektrarne. Investitorji naj se ne bi ukvarjali s kmetijsko pridelavo, temveč bodo študijo uporabili za potencialnega najemnika zemljišč. Zainteresiran pridelovalec bo lahko na podlagi študije dokaj predvidljivo zasnoval rastlinsko pridelavo na podlagi pogodbenega razmerja. Glede na ekonomske izračune, ki sicer ne vključujejo potrebne strojne opreme in stroškov za pripravo pridelka za trg (pakirnice, hladilnice, embalaža ipd.) predlagamo, da se pogodbeno razmerje sklepa za čas amortizacijske dobe posamezne rastlinske vrste in konstrukcije pri postavitvi nasada malin, vsekakor pa ne krajši od 10 let.

4 SKLEPI

Študija primera na primeru fotovoltaične elektrarne D3 je pokazala, da je rastlinska pridelava mogoča in ekonomsko zanimiva. Stroški zasnove rastlinske pridelave so ocenjeni na 24.100 EUR. Za zasnovo rastlinske pridelave je potrebnih 620 delovnih ur, zato je strošek dela za zasnovo rastlinske pridelave ocenjen na 4.290 EUR. Torej je skupen strošek zasnove rastlinske pridelave ocenjen na 28.390 EUR.

Rastlinska pridelava zahteva vsakoletno oskrbo in spravilo pridelka. Ocenjujemo, da je v enem letu polne rodnosti zasnovane rastlinske pridelave za ta dela skupaj potrebnih 4.300 delovnih ur, kar predstavlja strošek dela v višini 29.756 EUR. Največ dela je potrebnega za spravilo pridelka. Za nemoteno opravljanje dela in redno oskrbo rastlinske pridelave je letno potrebnih okoli 550 delovnih ur, medtem ko je za spravila pridelka letno potrebnih okoli 3.750 ur. Predlagamo, da se za pokrivanje tega izrazito sezonskega dela najame sezonska delovna sila za opravljanje začasnega dela v kmetijstvu.

Predvideni prihodki od predlagane rastlinske pridelave so v skupni vrednosti 110.000 EUR. Zato ocenjujemo, da je vrednost rastlinske pridelave ekonomsko zanimiva. Prav tako ocenjujemo, da razlika med ocenjenimi stroški in prihodki omogoča izvajanje vseh tehnoloških ukrepov, ki jih ekološko pridelovanje zahteva, ter opreme in embalažo za spravilo pridelka.

Za še učinkovitejšo rabo zemljišč za kmetijsko rabo je potrebno skupno načrtovanje fotovoltaike s kmetijsko pridelavo. Pri tem je potrebno uskladiti višino, na kateri se postavljajo paneli fotovoltaične elektrarne glede načrtovane kmetijske rabe zemljišč in tip panelov, ki zagotavljajo zadostno prepustnost fotosintetsko aktivnega sevanja za zmanjševanje vpliva senčenja na višino in kvaliteto pridelka.

Raziskava po metodi študije primera, ki smo jo izvedli na konkretni lokaciji fotovoltaične elektrarne D3 ob pretočni akumulaciji HE Brežice je ena prvih takšnih raziskav v Sloveniji. Rezultati raziskave, ki nakazujejo na možnost rastlinske pridelave na obstoječi fotovoltaični elektrarni, bodo v nadaljevanju lahko podlaga za načrtovanje novih fotovoltaičnih elektrarn, kjer bi se z boljšim načrtovanjem prostor še bolje izkoristil za rastlinsko pridelavo. Kot smo že omenili, študija nakazuje na priložnosti pri sočasnem načrtovanju rastlinske pridelave in fotovoltaične elektrarne, pri čemer se vidik kmetijske rabe in vidik energetske rabe prostora prilagajata drug drugemu s ciljem optimizacije obeh načinov rabe.

Študija nakazuje priložnost za optimizacijo rabe kmetijskih površin. Z vidika razvoja dvojne rabe prostora bo potrebno uskladiti zakonodajo na način, da bodo dvojne rabe kmetijskih zemljišč dopustne in izvedljive. Trenutna zakonodaja omejuje investicije v dvojno rabo kmetijskih zemljišč, ki so po namenski rabi kmetijska zemljišča.

Navedena dejstva nam nakazujejo, da bodo za bodoče urejanje agrovoltaike v Sloveniji potrebne dodatne študije in raziskave z namenom optimizacije dvojne rabe kmetijskih zemljišč. Na podlagi pridobljenih rezultatov bo zakonodajalec lahko pripravil zakonodajni okvir, ki bo omogočal investicije. Z vidika kmetijskih gospodarstev je agrovoltaika lahko priložnost za povečanje prihodkov in s tem stabilizacijo delovnih mest, kar bo utrdilo kmetijska gospodarstva in jim povečalo odpornost ob vremenskih, tržnih ali drugih odmikih v kmetijski pridelavi.

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Influence of mulches on soil moisture and water infiltration in the tomato crop

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Influence of mulches on soil moisture and water infiltration in the tomato crop

Abstract: Soil moisture is a key parameter of soil monitoring for observation of vegetation growth, predicting crop production, and improving water resource management. In this study, the objective is to compare the evolution of soil moisture in different mulches to determine the best mulch and its characteristics of infiltration in the soil. The experiment was conducted during the summer season in July-September 2022 on four different mulches (wood chips, sawdust, straw, mixture), and control at the experimental plot of Blida. The results showed that silt is the main matrix of the soil. The analysis of infiltration data identified modified Kostiakov as the best model of the study site, whose period of plant growth represents the phase during which we have a better infiltration under the mixture. The application of the mulch changes the moisture mainly at 15 cm and the conductivity at 10 cm. In addition, the mixture is the best mulch to conserve moisture in the soil while reducing the frequency of irrigation. The correlation between soil moisture and conductivity was overall very good. This was due to the effect of mulch, soil texture, plant root development, and capillary rise.

Key words: tomatoes, mulching, soil moisture, infiltration, soil electrical conductivity

Vpliv mulčenja na vlažnost tal in infiltracijo vode v nasadu paradižnika

Izvleček: Vlažnost tal je glavni parameter pri spremljanju lastnosti tal pri opazovanju rasti, napovedovanju pridelka in pri izboljševanju upravljanja z vodnimi viri. Predmet te raziskave je bil primerjati razvoj vlažnosti tal pri različnih načinih mulčenja z namenom določiti najboljši način mulčenja za infiltracijo vode v tla. Poskus je potekal v poletni sezoni od julija do septembra 2022 s štirimi načini mulčenja (lesni sekanci, žagovina, slama, mešanica) in kontrolo na poskusnem polju v Blidi, Alžirija. Rezultati so pokazali, da je bil kremenčev drobir glavna sestavina anorganskega dela tal. Analiza podatkov o infiltraciji vode je pokazala, da se je izkazal spremenjen Kostiakov model kot najboljši za preučevanje tal, v katerem predstavlja obdobje rasti rastlin fazo, v kateri je infiltracija vode v tla najboljša pri mulčenju z mešanico. Uporaba mulčenja spreminja vlažnost tal v glavnem na globini 15 cm in prevodnost tal na globini 10 cm. Dodatno je mešanica materialov za mulčenje najboljša, ker ohranja vlažnost tal in hkrati zmanjšuje pogostost namakanja. Korelacija med vlažnostjo in prevodnostjo tal je bila nasplošno zelo dobra kar je bilo posledica mulčenja, teksture tal, razvoja korenin in kapilarnega dviga.

Ključne besede: paradižnik, mulčenje, vlažnost tal, infiltracija, električna prevodnost tal

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1 INTRODUCTION

One of the fundamental soil parameters affecting the life of plants, animals, and microorganisms is soil moisture (Safari et al., 2021). Commonly defined as the amount of water present in the unsaturated zone, it is a key element for predicting agricultural production and improving water resource management. Its measurement in precision agriculture is an essential agronomic component for monitoring crop growth (Goel et al., 2020). Due to the importance of the role played by soil moisture, some scientists have developed different instrumentation observation methods, including the dialectical method by time domain reflectometry (TDR). Indeed, it is a method that cannot be ignored for the quality, ease of use, and accuracy of its measurements (Freire et al., 2020). Other researchers have instead focused on the issue of water conservation in the soil, establishing different techniques, including mulching (Stelli et al., 2018). An old practice (Jacks et al., 1955), consists of covering the soil surface with other materials, called "mulch" (Almetwally et al., 2019; Telkar et al., 2017). They can be applied to perennial or seasonal crops to: conserve water (Zegada-Lizarazu and Berliner, 2011), improve crop performance and wind control (Sharma et al., 2023; Simsek et al., 2017), increase plant health and vitality (Stelli et al., 2018), improve the action of microorganisms in the soil, and increase soil organic matter (Sharma et al., 2023; Simsek et al., 2017). According to an experiment conducted on different organic and inorganic mulches (Safari et al., 2021), the ideal depth is 15 cm with an increase of 2 to 5 % compared to bare soil (control). Organic materials are the most recommended as they can actively promote soil desalination and assist in the degradation of pesticides and other pollutants (Telkar et al., 2017). Several studies have been conducted in semi-arid and arid areas on the effects of mulching in conserving soil moisture (Almetwally et al., 2019; Mkhabela et al., 2019; Stelli et al., 2018; Simsek et al., 2017; Telkar et al., 2017) with interesting results. Some have also tested the effects of mulching on specific crops, such as tomatoes. It's a crop that is widely cultivated for its fruits that are consumed fresh or processed (Chaux and Foury, 1994). Currently, it represents the most cultivated and processed crop in the world (Sharma et al., 2023). Tomatoes play a major role in Algeria's agricultural economy. According to the Ministry of Agriculture and Rural Development (MADR), nearly 33,000 hectares of land are devoted to this crop (horticultural and industrial), with an average production of 11 million quintals and average yields of around 311 Qx ha^{-1} (MADR, 2009 in Tarchag, 2020, MADR, 2011 in Amichi et al., 2015). However, according to Sharma et al. (2023), water is one of the elements that directly affects tomato productivity. In addition to efficient use of available soil moisture, weed control, spacing, timing of planting, and judicious application of manure and fertilizer are all aspects that influence the success of tomato production (Lamont, 2005). The Mitidja, which is heavily dominated by vegetable crops and arboriculture, is one of the most fertile plains in Algeria (Meddi et al., 2013). However, in recent years, this plain is facing a decline in the water table of at least 40 m in some areas. This phenomenon is mainly due to poor water management, overexploitation of the water table by various industries, and drought episodes combined with the importance of irrigated areas (Djouda-Hallah, 2014). Another important process to consider in agriculture is infiltration, as it is one of the important components of the soil water balance in semi-arid areas (Liao et al., 2021). Furthermore, researchers (Oku and Aiyelari, 2011) have shown that soil infiltration properties can be quantified by fitting field infiltration data to model infiltration. Similarly, detailed knowledge of soil infiltration rates and characteristics can increase irrigation water use efficiency and reduce water losses (Haghiabi et al., 2011; Xing et al., 2017). To this end, scientists (Liao et al., 2021; Farid et al., 2019; Vand et al., 2018, Furman et al., 2006; Mishra et al., 2003) have developed several models to determine the infiltration rate and its characteristics.

The most commonly used are Philips, Kostiakov, modified Kostiakov, and Horton because of their performance and efficiency. However, despite all the advantages mentioned, mulching in general and organic mulching in particular is not a common practice in agricultural crops in Algeria. However, this technique can be a design solution to the great theme that is the conservation of moisture to the maximum and with efficiency in the soil, without destructuring and impoverishing it. That is why we studied the effects of mulching on soil moisture and infiltration of the soil in the cultivation of tomatoes. The specific objectives were to (1) characterize the soil of the site where tomato plants were planted; (2) determine the best infiltration model that best fits the study area; (3) study the effects of mulching on soil electrical conductivity, soil moisture retention and identify the best mulch among those studied as well as its ideal depth.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL SITE

The experiments were carried out in the Mitidja plain, at the experimental site of the National Higher School of Hydraulics (ENSH) of Blida, Algeria (36°30'31" N, 2°53'15" E, 110 m) (Fig. 1), during the period from

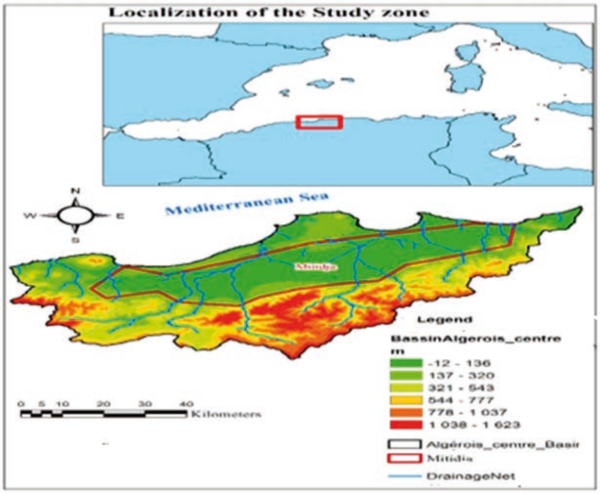

Figure 1: Location map of Mitidja plain

July to September 2022. According to Laribi et al. (2023), this plain extends today over four Wilayas (Blida, Tipaza, Bourmedès and Algiers), with an average annual temperature of 18 °C and a rainfall between 600 mm and 900 mm/year. According to this author, this plain is located in the bioclimatic Mediterranean subhumid area with mild winters. An agro-meteorological station was installed in the plot to obtain data on rainfall, air and soil surface temperature, wind speed, solar radiation and humidity. The data processing for the period July-September 2022 shows that the range of air temperatures varies from 25.25 °C (September) to 50.77 °C (August). On the other hand, on the surface of the ground, the range temperature is about 28.00 °C (September)–54.45 °C (August). The average relative humidity is 64.71 % with a minimum of 33.13 % and a maximum of 75.41 %; solar radiation is 247.70 W m^{-2}, wind speed is 2.54 m s^{-1} and evapotranspiration is 129.95 mm/day. There was no precipitation during this period.

2.2 SITE DESIGN

At this site, five plots of 4 m^2 were built (Fig. 2) (i.e. a total area of 40 m^2 with the respective additions of coarse (wood chips) and fine (sawdust) white wood bark mulch, wheat straw (cut to about 5 cm), the mixture of the first three mulches (with the same quantity of each mulch), and bare soil (here referred to as the control). The choice of mulches was based on the availability of inexpensive, abundant, and effective local materials. To monitor moisture until fruiting, 3-week-old 'Bahadja F1' hybrid tomato plants were planted in these plots. The distance between the ramps was 50 cm and 40 cm for the tomato plants and drippers, respectively. Each dripper was placed at the base of each plant and was self-regulating. In total, there were 12 tomato plants and 12 drippers per plot. The plants were irrigated with the surface drip system according to their needs at one-hour (1h) intervals in the evening. For the irrigation control, in addition to the

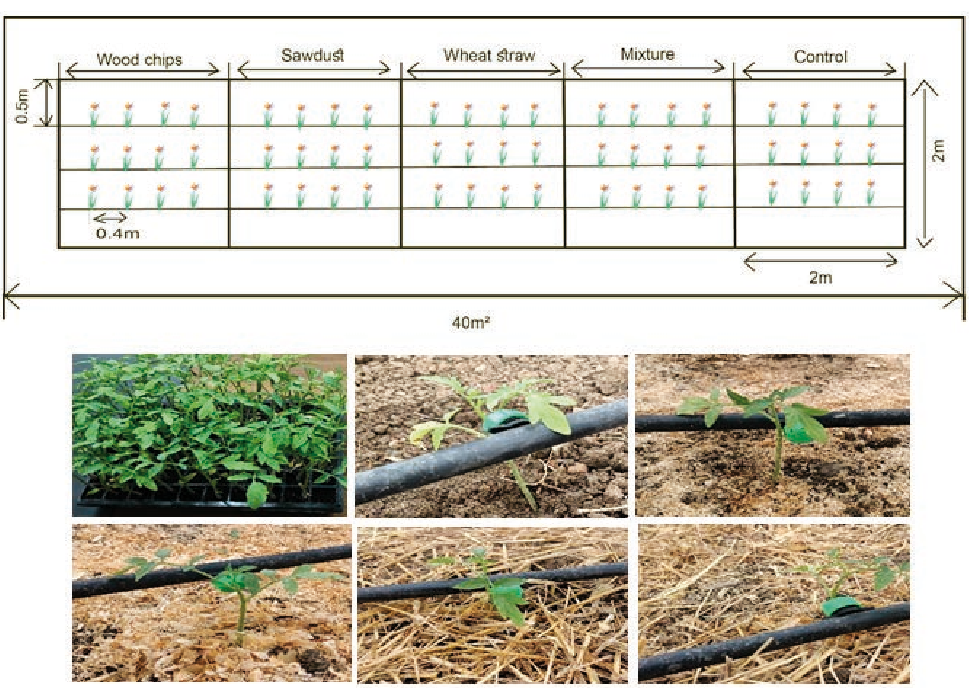

Figure 2: Design of the experimental setup and installation of mulch on tomato plants

literature, we used the template provided by the company responsible for the TDR 150 device, which visualizes the delineation of the moisture at the wilting point (WP) and the field capacity (FC) for each soil type. During this irrigation period, the average flow rate used was 7.72 l h^{-1}.

2.3 SOIL SAMPLING

The soils on which the tomato plants were planted were first physically analyzed to determine soil texture and bulk density. Five samples of disturbed soil, one per plot, were taken with a hand auger from 0 to 30 cm deep. Then five more undisturbed samples (always at the same points and depth) were taken with special samplers (cylinders). The entire sample collection was sent to the Agroecology Laboratory of the National Higher School of Hydraulics (ENSH) for analysis. The international method of Robinson (1949) was used to determine the different proportions of sands, clays, and silts of the reworked samples. Also, the texture of each soil was determined through its texture diagram. To determine the bulk density of each sample, a balance was used for wet and dry weighing, and an oven at 105 °C was used to dry the samples.

2.4 INFILTRATION MEASUREMENT

The double ring infiltrometer with an outer diameter of 32 cm and an inner diameter of 11 cm was installed in the ground to measure the infiltration rate in the field. During our fieldwork, this device was driven into the ground at a depth of 5 cm on a flat surface. The float was placed inside the inner ring to read the amount of water infiltrating into the soil. Measurements were taken at one-minute intervals. Infiltration measurements were taken before sowing tomato plants on bare soil in each plot, during the period of tomato plant growth under mulch, and at the end of crop growth at each soil sampling point. The rest of the experiment continued until the equilibrium infiltration rate was reached. After reaching this equilibrium infiltration rate (mm min^{-1}) in the soil, the experiment was stopped. This experiment was then repeated three times per plot.

2.5 DETERMINATION OF PARAMETERS OF DIFFERENT INFILTRATION MODELS

Philip, Kostiakov, and modified Kostiakov are the three models used in the study due to their popularity, effectiveness, and the type of soil present in the study area (Kostiakov, 1932; Philip, 1957). Experimental field data were used to evaluate these infiltration models and to obtain numerical values of the model hydraulic parameters. The hydraulic parameters of each model were determined using EXCEL software. The equations used for each model are described below.

- Philips model

This model is one of the physical models commonly used to estimate infiltration. Based on Darcy's law and the law of conservation of mass (Vand et al., 2018, Philips, 1957), it has the equation:

$$f(t) = \frac{1}{2}St^{-0.5} + K \tag{2.1}$$

Where: $f(t)$ is the infiltration rate at time t, S is the soil sorptivity, K is the hydraulic conductivity of the soil at saturation, and t is the time since infiltration began. In this study, to estimate the values of the parameters S and K, the infiltration rate data (mm min^{-1}) were fitted as a function of time transformed by least squares regression for all data obtained before sowing, during growth and at the end of fruiting at the five points.

- Kostiakov model

Kostiakov is a model based on the collection of experimental data obtained in the field, as well as in the laboratory. As an empirical model, it allows estimating the infiltration rate according to the equation:

$$f(t) = At^{-B} \tag{2.2}$$

Where $f(t)$ is the infiltration rate at time t; A and B are the parameters of the unknown equation representing the infiltration characteristics of the soil, with A the initial measurement of infiltration rate and soil structural condition, and B the stability index of the soil structure, t the time. To determine its parameters A and B, the logarithms (*ln*) of the infiltration measurements $f(t)$ and time (t) were taken.

- Modified Kostiakov model

Also known as Kostiakov-Lewis or Mezencev it is commonly used in the infiltration function for surface irrigation applications (Haverkamp et al., 1988, Furman et al., 2006). Its equation is as follows:

$$F(t) = At^{-B} + f_c \tag{2.3}$$

Where $F(t)$ is the cumulative infiltration rate as a function of time; A and B are the hydraulic parameters of the equation, t is time, and fc is the stable infiltration rate. As with Kostiakov, the logarithms (*ln*) of the infiltration $f(t)$ and time (t) measurements were taken to determine the A and B parameters.

2.6 SELECTION OF THE INFILTRATION MODEL ACCORDING TO PERFORMANCE CRITERIA

The comparison between the simulated and measured field data was performed according to the selected performance criteria to determine the best - fitting model in the range. The different criteria selected were based on their popularity and efficiency. These include, among others :

- Determination Coefficient (R^2)

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(x_i - y_i)}{\sum_{i=1}^{n}(x_i - \bar{x})} \quad (2.4)$$

- The Mean Square Error (RMSE)

$$RMSE = \sqrt{\frac{1}{n}\left(\sum_{i=1}^{n}(x_i - y_i)^2\right)} \quad (2.5)$$

- Nash and Sutcliffe efficiency coefficient (NASH)

$$NASH = 1 - \frac{\sum_{i=1}^{n}(x_i - y_i)^2}{\sum_{i=1}^{n}(x_i - \bar{x})^2} \quad (2.6)$$

- Error Index (PBIAS)

$$PBIAS = \frac{\sum_{i=1}^{n}(x_i - y_i) * 100}{\sum_{i=1}^{n}(x_i)} \quad (2.7)$$

where n is the number of observations, x_i is the observed infiltration depth, y_i is the simulated infiltration depth and $\bar{x}$ is the mean of observed data

The best-fitting model was selected based on the maximum of the coefficient of determination R^2, Nash; the minimum of the root mean square error (RMSE) and the prediction error index (PBIAS), as established by Moriasi et al. (2007).

2.7 MEASUREMENT OF MOISTURE AND ELECTRICAL CONDUCTIVITY IN THE SOIL

The measurement of the amount of water in the soil was carried out throughout the period from the sowing of the tomato plants to the fruiting stage in each of the plots. It was carried out from July to September 2022. In our study, the TDR 150 was used because of its affordability, reliability, accuracy and ease of use. What's special about this device is that it can measure soil moisture, soil electrical conductivity and soil temperature at the same depth and on the same time scale. For this study, we considered only the first two parameters, because of their importance in irrigation and nutrient management. In the field, its spikes were inserted at 5 cm, 10 cm, 15 cm and 20 cm depths next to the tomato plants in each plot. Measurements were taken daily, three repetitions per plot between 4–8 p.m. and reported on data sheets. The data obtained was then processed in Excel software to produce graphs of the evolution of humidity and electrical conductivity at different depths.

3 RESULTS

3.1 PHYSICAL CHARACTERIZATION OF SOIL

The granulometric analysis by sedimentometry carried out according to the method of Robinson (1949) on five (05) samples, made it possible to obtain the percentages of sand (% S), silt (% L), and clay (% A) particles at a depth of 30 cm (Tab. 1). Observation of this table shows that silt is the main matrix of these soils, whose main texture is fine silty. The values of apparent density are between 1.24 and 1.67 g cm^{-3} with an average of 1.33 g cm^{-3}. The water holding capacity varies from 32 to 38 % depending on soil type, wilting point varies from 14 to 17 %, and field capacity varies from 32 to 38 %.

Table 1: Physical analysis of soil samples at 30 cm depth

Samples	Sand %	Clay %	Silt %	Textural Class	Bulk density	WP (%)	FC (%)	SWC (%)
Wood chips	41.7	10.3	49	Loam-Silty Loam-Silty sand	1.24	14	35	35
Sawdust	19.9	8.5	71.5	Silty loam	1.3	17	38	38
Wheat straw	38.1	11	52	Silty loam-loam	1.24	15	36	36
Mixture	21.9	7	70.5	Silty loam	1.18	17	38	38
Control	17.5	6	76.3	Silty loam	1.67	17	38	38

Note: *WP* = wilting point, *FC* = field capacity and *SWC* = Soil water capacity. The value of *WP*, *FC* and *SWC* were given by the company responsible for the TDR 150 device and the granulometric analysis was performed at the ENSH pedology laboratory

3.2 COMPARISON OF INFILTRATION RATES

Figure 3 shows the evolution of the infiltration rate observed in the field in each mulch and in the control before sowing, during the growth and after the ripening of the tomato plants. The general appreciation of these graphs highlights the rapidity of water infiltration into the soil during plant growth in all mulches and bare soil (control).

However, a closer look at the sawdust graph shows that the infiltration rate is slightly higher after the fruiting phase. In short, it evolves through the different phases. Based on this observation, we can deduce that this mulch improves the humidity of the soil over time, unlike other mulches. In fact, after the growth and fruiting phase, the soil water infiltration behavior of other mulches tends to be similar to that obtained during the pre-sowing phase of tomato plants. This behavior is best illustrated in the mixture. With this in mind, factors such as soil plowing, mulch decomposition and root system development need to be taken into consideration to explain the evolution of the humidity.

3.3 SELECTION OF THE BEST MODEL FOR THE STUDY SITE

The identification of the best infiltration model (Table 2) followed the determination of the hydraulic parameters (Table 2a) and the evaluation of the performance criteria (Table 2b).

3.3.1 Determination of the hydraulic parameters of each model in each mulch before sowing, during growth and after fruiting of tomato plants

Table 2a shows the hydraulic parameters of each model for each mulch before sowing, during growth, and after maturation of the tomato crop. From the annotation of these dashboards, it can be seen that high simulated hydraulic conductivity values are recorded for each model in all three phases during the plant development phase. The mixture also records very high values during this phase.

3.3.2 Evaluation of performance criteria for Philip, Kostiakov and modified Kostiakov models

Table 2b shows the different performance criteria used to determine the best model under each mulch in the study area. Looking at the values obtained from the different performance criteria for different models in each mulch, we note that the modified Kostiakov is the best model in the study area. We also note that the Philip model has the lowest PBIAS value, but unlike Kostiakov, very high RMSE values were found. Overall, these three models are applicable in the area, although modified Kostiakov is the best of the three.

3.4 EFFECT OF MULCH ON SOIL ELECTRICAL CONDUCTIVITY

Daily, soil electrical conductivity data were recorded in each mulch at different depths (Fig. 4). From the general shape of these curves, it can be seen that the highest conductivity values are recorded at 10 cm depth and lower at 5 cm depth at each mulch. However, a difference is observed first in wood chip and sawdust between 08/22/2022 - 01/09/2022, then in control between 16/09/2022 - 26/09/2022, where there is a high peak at 5 cm depth, unlike the rest of the depths. The individual observation of each figure shows that high conductivity rates are recorded within:

- treatments (wood chips, sawdust, straw, and mixture) between 08/22/2022-01/09/2022 and between 11/09/2022-21/09/2022
- bare soil (control) between 27/08/2022-06/09/2022 and between 16/09/2022-26/09/2022

The comparative study of the variation of the electrical conductivity of the soil shows overall very high rates of conductivity between 17/08/2022-06/09/2022.

The overview of Table 3 notices a slightly more pronounced increase in conductivity at 10 cm depth (0.44 μS cm^{-1}). The following classification can be made: 10 cm (0.44 μS cm^{-1}) > 15 cm (0.42 μS cm^{-1}) > 20 cm (0.39 μS cm^{-1}) > 5 cm (0.27 μS cm^{-1}. Also, a more pronounced increase in conductivity at the level of wood chips (0.41 μS cm^{-1}), followed by sawdust (0.37 μS cm^{-1}), straw (0.37 μS cm^{-1}), control (0.38 μS cm^{-1}), mixture (0.36 μS cm^{-1}). The difference between the mulches and the control varied from 0.1 to 0.3 μS cm^{-1}. The standard deviation values for the different mulches at each depth are less than 3 %. The very low deviations indicate that the data points are close to the mean. Showing that the data are homogeneous.

3.5 EFFECT OF MULCH ON SOIL MOISTURE

An overview of the various graphs at 5 cm, 10 cm, 15 cm and 20 cm depth (Fig.5) in each mulch shows that sawdust has a high water content at almost all soil depths,

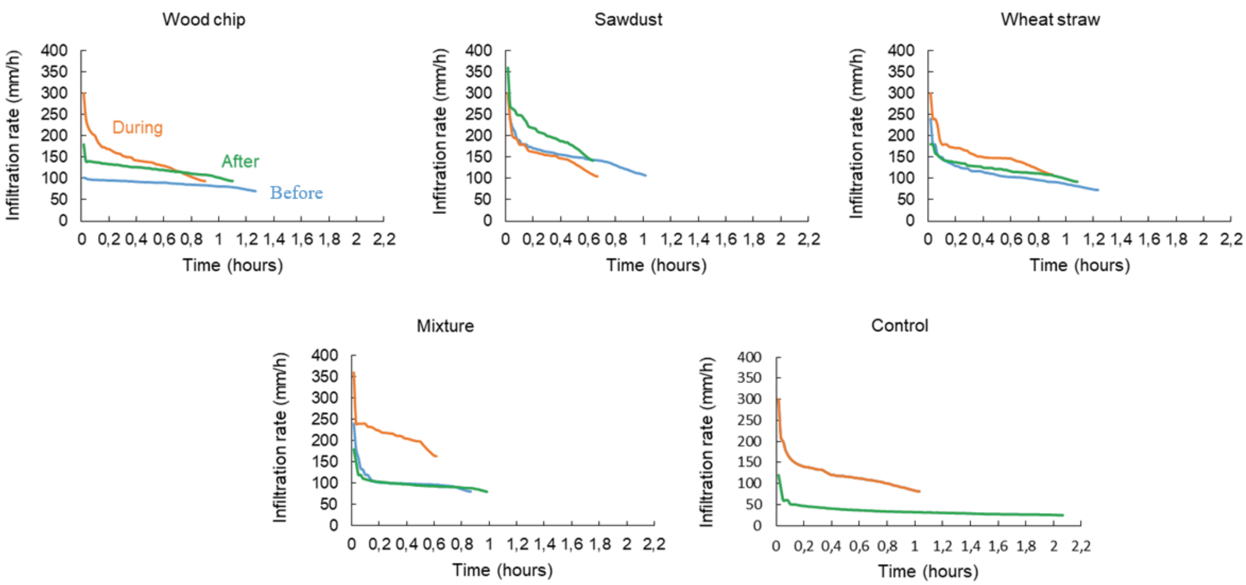

Figure 3: Comparison of infiltration rate (mm h^{-1}) observed before seeding tomato plants (blue color), during plant growth (orange color) and after fruiting tomatoes (green color) in the wood chip, sawdust, wheat straw, mixture and control

Table 2a: Hydraulic parameters of different models before sowing tomato plants, during tomato plant growth and after maturation of tomato plants

Plot	Period	P			K			KM		
		S (mm h^{-1})	K (mm h^{-1})	M	A (mm h^{-1})	B	M	A (mm h^{-1})	B	M
Wood chips	Before	9.27	80.07	87.65	82.47	0.07	87.65	0.63	0.85	1.46
	During	61.73	87.12	146.03	106.87	0.26	146.03	4.19	0.64	2.43
	After	21.35	102.16	120.77	108.34	0.11	120.77	1.41	0.75	2.01
Sawdust	Before	53.13	105.47	153.45	124.22	0.2	153.45	3.68	0.64	2.55
	During	53.54	96.93	155.34	111.34	0.23	155.34	3.59	0.63	2.58
	After	60.81	136.7	204.57	148.65	0.21	204.57	3.98	0.66	3.4
Wheat straw	Before	47.9	68.78	108.4	87.94	0.24	108.4	3.39	0.62	1.8
	During	54.62	103.57	155.69	122.01	0.21	155.69	3.81	0.62	2.59
	After	28.73	97.16	122.38	105.96	0.15	122.38	1.93	0.7	2.03
Mixture	Before	41.03	64.75	104.57	82.83	0.19	104.57	3.18	0.48	1.74
	During	44.02	164.69	214.4	173.55	0.14	214.4	2.95	0.67	3.57
	After	25.05	75.54	98.52	85.52	0.13	98.52	1.84	0.57	1.64
Control	Before	59.07	69.5	122.46	93.11	0.26	122.46	4.23	0.58	2.04
	During	59.07	69.5	122.46	93.11	0.26	122.46	4.23	0.58	2.04
	After	24.83	18.38	34.56	31.05	0.27	34.56	2.11	0.5	0.57
Min		0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Max		0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Mean		42.94	89.35	130.08	103.80	0.20	130.08	3.01	0.63	2.16

Note. *S*= Sorptivity (mm h^{-1}); *K*= parameter related to saturated hydraulic conductivity (mm h^{-1}); *A* and *B* = hydraulics parameters with *A* measurement initial infiltration (mm h^{-1}) and *B* is index evaluated stability; *P*= Philip model; *K*= Kostiakov model; *KM*= Modified Kostiakov model

Table 2b: Performance criteria and selection of the best model in each mulch (chip, sawdust, wheat straw, mixture) and control

Plot	Soil Texture	Period	R^2			NASH			RMSE			PBIAIS			Best model
			P	K	KM	P	K	KM	P	K	KM	P	K	KM	
Wood chips	L-Ls	Before	0.48	0.70	0.83	0.48	0.69	0.99	5.27	4.03	1.03	0.00	8.33	-13.33	Modified Kostiakov
		During	0.90	0.92	0.96	0.90	0.94	0.99	12.27	8.96	2.21	0.00	3.92	-19.35	
		After	0.70	0.81	0.94	0.70	0.82	0.99	7.82	6.07	1.24	0.00	12.47	-9.57	
Sawdust	lf	Before	0.90	0.91	0.98	0.90	0.94	0.99	9.83	7.82	1.61	0.00	15.42	-12.91	Modified Kostiakov
		During	0.91	0.90	0.98	0.91	0.93	0.99	10.55	9.36	1.11	0.00	23.28	-7.23	
		After	0.87	0.91	0.96	0.87	0.92	0.99	15.05	11.63	1.75	0.00	17.53	-13.29	
Wheat straw	lf	Before	0.91	0.94	0.97	0.91	0.96	0.99	7.88	5.39	1.93	0.00	3.36	-17.24	Modified Kostiakov
		During	0.92	0.92	0.98	0.92	0.94	0.99	9.61	7.85	1.37	0.00	17.35	-12.69	
		After	0.81	0.91	0.94	0.81	0.92	0.99	7.95	5.01	1.45	0.00	5.80	-16.70	
Mixture	lf	Before	0.94	0.86	0.99	0.94	0.84	0.99	5.72	10.13	0.28	0.00	45.45	-2.01	Modified Kostiakov
		During	0.79	0.79	0.98	0.79	0.79	0.99	14.42	14.51	0.82	0.00	42.23	-0.30	
		After	0.95	0.90	0.99	0.95	0.87	0.99	3.24	5.27	0.30	0.00	12.66	-2.04	
Control	lf	Before	0.95	0.95	0.98	0.95	0.96	0.99	7.69	6.96	1.63	0.00	12.22	-12.06	Modified Kostiakov
		During	0.95	0.95	0.98	0.95	0.96	0.99	7.69	6.96	1.63	0.00	12.22	-12.06	
		After	0.96	0.97	0.98	0.96	0.94	0.99	2.17	2.88	1.06	0.00	7.27	-6.60	
Mean			0.86	0.89	0.96	0.86	0.89	0.99	8.48	7.52	1.30	0.00	15.97	-10.49	
Min			0.48	0.70	0.83	0.48	0.69	0.99	2.17	2.88	0.28	0.00	3.36	-19.35	
Max			0.96	0.97	0.99	0.96	0.96	0.99	15.05	14.51	2.21	0.00	45.45	-0.30	

Note. *P* = Philip model; *K* = Kostiakov model; *KM* = Modified Kostiakov model

Table 3: Averages and standard deviations of soil conductivity retained for different mulches (wood chips, sawdust, wheat straw, mixture) and control at 5 cm, 10 cm, 15 cm and 20 cm depths

Mulches	Averages and standard deviations of soil conductivity (µS cm^{-1})					
	5 cm	10 cm	15 cm	20 cm	Mean (%)	SD (%)
Wood chips	0.24	0.5	0.46	0.43	0.41	0.10
Sawdust	0.32	0.41	0.38	0.37	0.37	0.03
Wheat straw	0.26	0.48	0.41	0.34	0.37	0.08
Mixture	0.2	0.39	0.43	0.43	0.36	0.10
Control	0.31	0.44	0.44	0.35	0.38	0.06
Mean (%)	0.27	0.44	0.42	0.39		
SD (%)	0.04	0.04	0.03	0.04		

Note: *SD* = standard deviation, *Mean* = average of soil conductivity in different mulches and depths

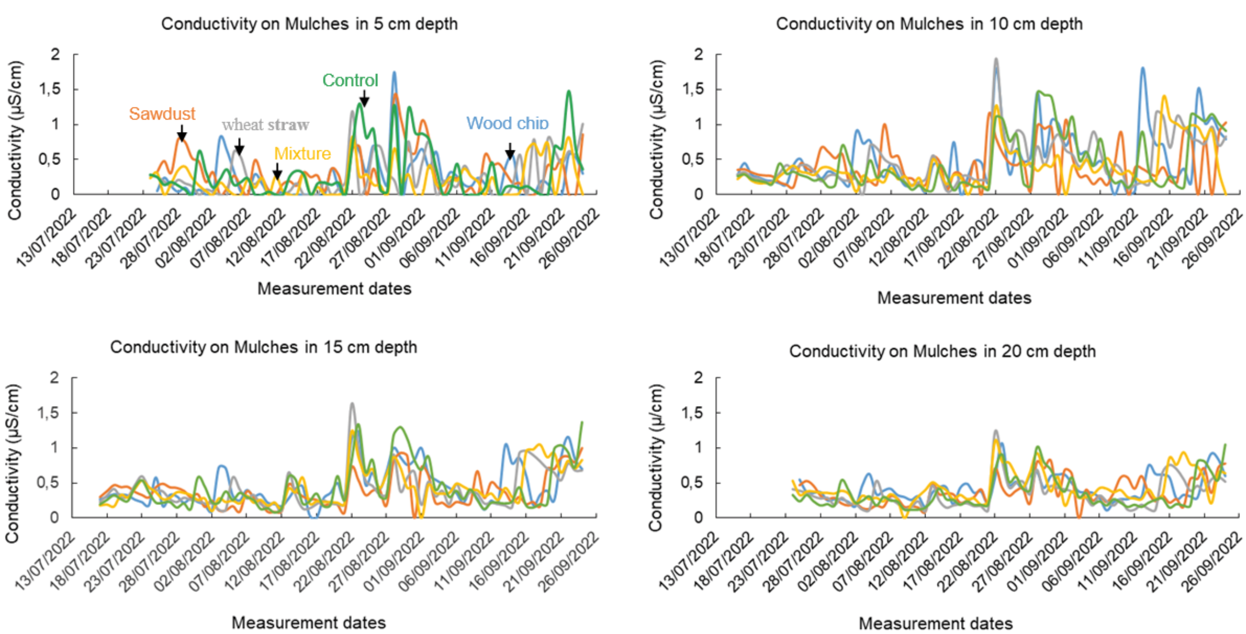

Figure 4: Variation in soil conductivity (µS cm^{-1}) each daily of wood chips (blue color), sawdust (orange color), wheat straw (gray color), mixture (yellow color) and control (green color) at 5 cm, 10 cm, 15 cm and 20 cm depth

while wood chips have a lower water content. However, at a depth of 20 cm, the opposite phenomenon is observed. Wood chips retain more moisture than sawdust.

The moisture data (Fig. 5) obtained on each mulch before and after irrigation allowed us to establish an existing relationship between the parameters: irrigation, moisture, mulch and actual evapotranspiration. The evapotranspiration calculated with the Pennan-Montheih formula was corrected with the crop coefficients of tomato to have plot - specific values. After irrigation under different mulches, the observation is mentioned in the level:

- of the wood chips of great moisture conservation in the soil between the end of August - beginning of September (25/08/2022-09/09/2022), that is 15 days of maximum retention. The observation of the graph shows a particular retention of only three days, justified by a strong increase in real evapotranspiration during this period. In addition, nine irrigation cycles were carried out during the crop.

- of sawdust of a great retention of moisture in the soil of maximum 13 days at the beginning of the growth and flowering stage of the plant (11/07/2022-04/08/2022). Observation of this graph also identifies eight rounds of irrigation during the development of the plant.

- of the straw, the great retention was identified

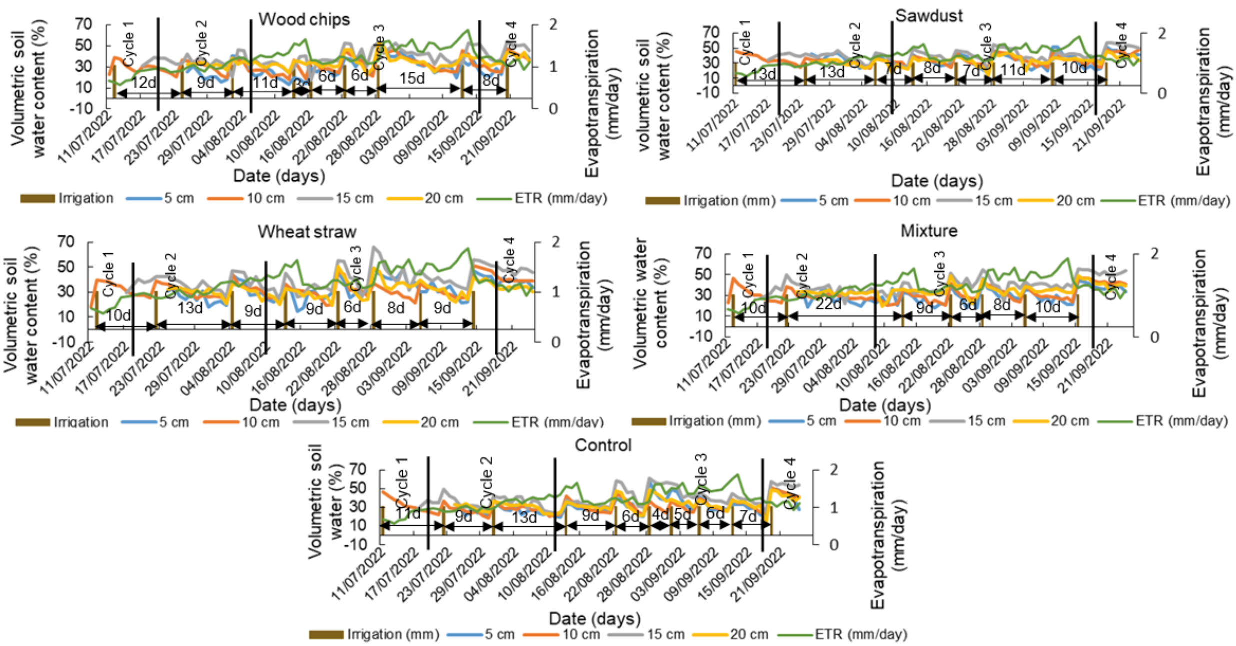

Figure 5: Effects of wood chips, sawdust, wheat straw, mixture, control on moisture at 5 cm; 10 cm; 15 cm and 20 cm depth each daily; relationship between soil moisture (%), irrigation (mm) and reel evapotranspiration (ETR) in mm/day to different mulches and control. The vertical bars represent plant growth stages; the horizontal bars represent the number of days (*d*) of moisture retention in the soil after each irrigation round and the small histograms represents the number of irrigated turns for each treatment

in the second cycle of the plant between 23/07/2022-01/08/2022 which is a maximum of 13 days of conservation. During the growth of the plant, the observation of the graph shows eight rounds of irrigation as the sawdust.

- of the mixture, conservation of humidity in the soil between 20/07/2022-10/08/2022 that is to say a maximum of 22 days. On the graph, we can also see that during the period of growth of the plant, seven rounds of irrigation were carried out.

- of the control, a maximum of 13 days between 07/29/2022-10/08/2022. Also on the graph, we see that during the growth the plants were irrigated 10 times.

Considering the average moisture content data in Table 4, the following classification by the average moisture content obtained in percentages is sawdust (35.6 %) > straw (34.3 %) > mixture (33.9 %) > control (33.8 %) > wood chips (33.7 %). We also note that 15 cm (39.7 %) is the depth at which we record a very high rate of water storage in the soil. In the same table, we observe a slightly significant difference in low water retention in wheat

Table 4: Averages, and standard deviations soil moisture retained for different treatments (wood chips, sawdust, wheat straw, mixture and control) at 5cm, 10cm, 15cm and 20cm depths

Mulches	Averages and standard deviation of soil moisture (%)					
Depths	5 cm	10 cm	15 cm	20 cm	Mean (%)	SD (%)
Wood chips	29.6	31.6	39	34.5	33.7	3.5
Sawdust	34.3	34.5	40.4	33.1	35.6	2.8
Wheat straw	32.4	32.5	40.3	32.1	34.3	3.5
Mixture	29.3	31.5	39.6	34.9	33.9	3.9
Control	31.6	31.4	39.4	33	33.8	3.3
Mean (%)	31.4	32.3	39.7	33.5		
SD (%)	1.8	1.2	0.5	1.0		

Note: *SD* = standard deviation, *Mean* = average of soil moisture in different mulches and depths

Table 5: Correlation between average soil electrical conductivity and soil moisture values at 5cm, 10 cm, 15 cm, 20 cm depths for different treatments

	5 cm	10 cm	15 cm	20 cm
Average humidity (%)	31.40	32.30	39.70	33.50
Average conductivity ($\mu S\ cm^{-1}$)	0.27	0.44	0.42	0.39
R^2 Conductivity - Humidity	0.70	0.73	0.91	0.95

Note: R^2 = coefficient of determination

straw and the control; being of the order of 0.3 % (between 20 cm and 5 cm depth) and 0.2 % (between 10 cm and 5 cm depth), respectively. Similarly, indicating the homogeneity of the data, the standard deviation values are low (less than 8 %).

If we make a correlation between the averages of the values of electrical conductivity of the soil and humidity at different depths in different mulches (Tab.5), we obtain good results of coefficient of determination going from 0.70 to 0.95 in almost all the depths.

4 DISCUSSION

The results of the particle size analysis showed that silt is the main matrix of the soils of the different plots, whose main texture is fine silt. These results are consistent with those obtained by Ecrement and Seghir (1971) in the region. According to Skhiri (2019), the average bulk density within the silty soils is 1.3 g cm^{-3}. This result is consistent with that obtained in the study area, which is 1.33 g cm^{-3}. The general observation of the graphs showing the evolution of the infiltration rate observed in the field in each mulch and bare soil, before sowing, during the growth and after the ripening of the tomato plants, allows us to see that the infiltration of water into the soil is more rapid during the period of plant growth. This phenomenon is observed in all the mulches and in the bare soil (control). This can be explained, on the one hand, by the mulching of the soil surface and, on the other hand, by the plowing of the soil before sowing, in addition to the root development of the plant during this period. Several researchers (Liao et al., 2021, Farid et al., 2019) indicate that mulching would facilitate infiltration after irrigation or very heavy rainfall. The hydraulic parameters of each model observed in each mulch before sowing, during growth and after ripening of the tomato crop show that high values of simulated hydraulic conductivity are recorded for each model during the three phases of plant development. The mixture, which is the mulch, records the highest values. Some researchers attribute this increase to the mulching of the soil surface. They consider that mulching improves, if not increases, the rate of hydraulic conductivity in the soil (Mkhabela et al., 2019; Stelli et al., 2018, Simsek et al., 2017). As for the high values of the mixture, according to Bear (1972), they are justified by the very high ability of the soil under this mulch to let water through, unlike other mulches. Based on the evaluation of the selected performance criteria, the modified Kostiakov emerged as the best model in the study area. According to several researchers (Niyazi et al., 2022; Vand et al., 2018; Yuemei et al., 2008), the best model is the one with minimum RMSE and PBIAS, maximum R^2 and NASH. In our study, modified Kostiakov is the best because it has: R^2 equal to 0.996; Nash equal to 0.99997; RMSE equal to 0.279 and PBIAS equal to -19.35. Overall, these three models are applicable in the field, with the best being the modified Kostiakov. This result is similar to that obtained by Smerdon et al. (1988). These researchers have shown that modified Kostiakov is one of the best models to apply in surface irrigation. Thus, from these performance criteria, we can establish the following ranking: modified Kostiakov > Kostiakov≥ Philip. From the research conducted by Zolfaghari et al. (2012) on seven infiltration models, it is found that modified Kostiakov and Kostiakov are the two models with better ranking among all models. Mirzaee et al., (2014) on the one hand state that modified Kostiakov is the best model for fine silty loam soils and on the other hand it is among the best models suitable for loam, silty clay loam and clay loam soils. About the graphs illustrated concerning the simulation of the infiltration rate and the cumulative infiltration, we note that the best simulation is within the soil mulched with the mixture. Likewise, following the values of NASH and R^2 obtained at the level of the mixture, after the stage of maturation of the tomato, we identify very good values and therefore very good simulations, contrary to the first two stages. The plausible explanation is the presence of the mulch, because according to some researchers (Simsek et al., 2017; Zhang et al., 2014), mulching improves infiltration into the soil. As well, as the colored presence of roots in the soil coupled with the soil texture.

The average electrical conductivity values obtained in the soil are, on the whole, very low. Let us remember that electrical conductivity is a very important parameter in agriculture. It is mainly used to determine soil salinity, but it can also be used to estimate other soil properties (soil water content, soil temperature, soil pH, soil texture, organic matter, solution ion concentration, etc.) at the non-saline soil level (Cornwin and Lesh, 2005). According to the range suggested by the NRCS Soil Survey Handbook, we begin to have saline soil when the conductivity value is higher than 2 dS m^{-1} and non-saline soil when the conductivity value is lower than 2 dS m^{-1}. The values obtained in our study are well below the range suggested by the latter for non-saline soils. This is an assurance that the soil quality is ideal for agriculture. Also, a more pronounced increase in conductivity was observed at the level of wood chips (0.41 µS cm^{-1}), followed by sawdust (0.37 µS cm^{-1}), straw (0.37 µS cm^{-1}), control (0.38 µS cm^{-1}), mixture (0.36 µS cm^{-1}). Some authors (Simsek et al., 2017) have shown that mulching does not affect pH, electrical conductivity, bulk density and carbonate content in the soil. On the other hand, others have shown that all types of mulches have a much more positive effect because they maximally reduce the electrical conductivity within the soil properties (Kumar et al., 2012). According to our results, we notice that the electrical conductivity increases in almost all mulches, except at the level of the mixture, where it decreases compared to the control. The low value recorded at the level of the mixture compared to the control is indeed consistent with the results of the authors mentioned above. On the other hand, the opposite phenomenon (increase in conductivity) observed in the other mulches may be due, according to some researchers (Sadek et al., 2019, Pakdel et al., 2013, Chalker-Scott, 2007), to the decomposition of the organic mulch under the effect of appropriate nutrients released in the soil, which become available to plants. But also to the texture and other properties of the soil, which unfortunately we could not study. For example, the level of phosphorus in the soil, as pointed out by Donogemma et al. (2008). The peaks in conductivity observed between 17/08/2022-06/09/2022 could be due to soil moisture. According to the work of (Costa et al., 2014), electrical conductivity is strongly influenced by soil moisture. This is similar to the result obtained in our study during this period. We found during the same period, an increase in soil moisture. The increase in conductivity that is slightly more pronounced at 10 cm depth may be due to the root development of the plants and the texture of the soil.

Synthesizing the moisture data on graphs of each mulch at different depths, it is very clear that the mixture retains more moisture than the rest of the mulch and that the ideal retention depth is 15 cm. According to Dinushika et al. (2019) and Adams (1996), in arid or semi-arid zones, mulching would increase water retention in the soil from 0-40 cm depth. However, Stagnari et al. (2014) state that in a Mediterranean environment, mulching in general and straw in particular would increase the water retention in the soil from 5-15cm depth. According to an experiment conducted on different organic and inorganic mulches (Safari et al., 2021), the ideal depth is 15 cm with an increase varying from 2 to 5 % compared to the control on bare soil. The ideal depth chosen in our study is within the range suggested by the above authors. However, the rate recorded by Safari et al. (2021) is mainly consistent with sawdust. Kumar and Dey, (2011) remind us that the use of mulches on the soil surface would increase water diffusion under the vapor gradient during the growing season. They added by saying that this factor would increase the maximum water absorption under mulch. Next, we note that the mixture was irrigated less than the other mulches. This justifies its low percentage difference in soil water storage (2.3 %) compared to sawdust. Finally, we see that the mixture conserved a lot of soil moisture in the first two cycles. Over time, however, it tends to have the same characteristics as straw in particular. The plausible explanation for this is that the wood chips and sawdust mulch decomposed faster over time than the straw. The work of Kaboneka et al. (2021) and Boyer (2021) emphasizes the fact that these mulches have in common a very slow decomposition. In fact, according to the studies carried out in Burundi by Kaboneka et al. (2021) on the decomposition of wheat straw, it appears that this mulch is characterized by a predominance of substances resistant to decomposition (cellulose, hemicellulose, lignin) and by a very high ratio of carbon to nitrogen (C/N) (76.4), that is, three times higher than the standard (25) ideal for the mineralization of nitrogen and the rapid release of nutrients contained in organic materials. Likewise, the study by Boyer (2021), conducted in Quebec on the bibliographic research of the potential use of organic mulches, showed that the C/N ratio of wood chips is about 39.2; that is, slightly higher than the standard set. Furthermore, Nicolardot et al. (2001) point out that this ratio is considered to be the simplest biochemical indicator of the quality of organic matter, its decomposability and nutrient release potential. According to Kaboneka et al. (2021), its value indicates whether an organic substrate is rapidly or slowly decomposable. Thus, comparing the ratios of the values obtained by their authors shows that wheat straw does indeed take longer to start decomposing compared to wood. This result supports the one obtained in the study area. Furthermore, the straw was the mulch that retained the least amount of water in the soil during the period 13/08/2022-16/08/2022. This can be explained by the peak of water demand observed during the same period. Thus, at the level of mulches, the effective classification is as follows: mixture (07), sawdust (08), straw (08), wood chips (09) and control (10). The fact that the number of irrigated turns is lower for the mulch than for the bare soil control allows us to say that the mulch used allows to reduce the irrigation frequency. This result is in line with the researches carried out by some scientists (Ahmad et al., 2020, Chalker-Scott, 2007, Rasmussen, 1999). The latter emphasize the fact that the application of mulch can significantly reduce the frequency of irrigation and even eliminate the need for irrigation. The choice of mulch is crucial in that some will reduce irrigation more than others, as we observed with the mulches used in the study. During the period from 10/08/2022 to 12/09/2022, when real evapotranspiration was high (marked by two peaks, one on 13/08/2022 and the other on 09/09/2022), we find that the mulched soils were more resistant than the bare soil control because they were less irrigated. This result is consistent with that of Duchaufour et al. (2017), who mention that under the effect of mulching, soil evapotranspiration is reduced. Overall, it is noted that the mulches used retain more moisture in the soil than the control. This result is consistent with the research of Mkhabela et al. (2019) and Telkar et al. (2017), who indicated that soil moisture is retained under mulch. They also indicated that the percentage of water stored in the soil is higher under mulch than under bare soil. However, in our study, this result is not entirely true for the wood chips, which is an exception to the rule. In fact, the difference between the control and the wood chips is slightly higher than that of the wood chips, i.e. 0.1 %. This difference can be explained by the short duration of the study, but also by the nature of the soil. In fact, the wood chips, which are more enriched with sand, retain less moisture due to their texture. Also, since the difference between the two soils is very small, we can take into account the time of the study. Considering the differences that exist between the wood chips and the sawdust in terms of moisture retention and the number of watering cycles, we can explain them by the grinding of the wood. The finer the wood is ground, the more water it retains in the soil.

About the availability of the mulches used in the study, it should be noted that wood waste is widely available in Algeria. Indeed, (Irinslimane, 2007), points out that its recycled waste amounts to 7 to 8 million tons per year, and comes mainly from forestry operations and sawmills. As for (Chachoua, 2015), cereal growing accounts for more than 50 % of useful agricultural areas. The figures given by these researchers reassure us that sufficient water is being used for large areas of irrigation fields in Algeria.

The correlation between the average values of soil electrical conductivity and soil moisture at different depths in different mulches gave good results with coefficients of determination ranging from 0.70 to 0.95 in almost all depths. The strong correlation observed reflects the influence of soil moisture on conductivity. This result is in agreement with the one obtained by Costa et al. (2014) in Brazil.

5 CONCLUSION

In addition to climate change, the overuse of water, chemical fertilizers and pesticides in the cultivation of tomatoes in Algeria has become almost commonplace, in the quest for extreme profitability. Our study is intended to be useful in the sense that, in addition to conserving soil moisture, it can contribute to soil restructuring, as well as enrich the soil with the organic matter and nutrients plants need to grow and produce good yields. In addition, knowledge of the soil's infiltration rate and characteristics enables better irrigation management.

For scientists and researchers, this study contributes to a better understanding of organic mulch and soil moisture in crops. As for legislators, we encourage them to set up a policy to raise awareness among individuals, farmers and water managers of the advantages of organic mulch in agriculture.

Through this study, which aims to be simple in its application, economical and save time and energy, we encourage the above stakeholders to increasingly practice the use of organic mulch in their various crops. This limits weeds, preserves the environment, reduces irrigation requirements, improves water infiltration and conserves soil moisture. What's more, once decomposed, they form compost, ideal for plant growth. This reduces the need for nitrogen fertilizers.

However, if the organic mulch chosen has a positive effect on the various aspects mentioned above, it should be noted that the questions of application density, effect on the growth of tomato crops, and agricultural yield depending on the mulch chosen remain unknown. As is the question of soil temperature and organic matter content. We are currently working on these questions to gain a better understanding them.

6 ACKNOWLEDGMENTS

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Effect of the alcohol content on sensory perception of the fruit spirits

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Effect of the alcohol content on sensory perception of the fruit spirits

Abstract: Fruit spirits must have an aroma of the raw material, which is balanced by ethanol. Since many aroma compounds are more soluble in ethanol than in water, ethanol is the most important carrier of aroma compounds. The alcohol concentration seems to be crucial for the sensory profile of spirits. Alcohol content of 40% vol is the standard alcoholic strength of fruit spirits. Regulations specify a minimum alcohol content of 37.5% vol. However, ethanol reduction can result in change in sensory profile of spirits. The aim of this research is to determine whether lowering the alcohol content of spirits may make them less acceptable to customers. On this occasion, 5 pairs of fruit spirits were sensory tested: pear, plum, apple, raspberry, and grape spirits, each with a commercial and reduced alcohol concentration to 37.5% vol. The results showed that customers can recognize the difference in alcohol content of fruit spirits and dilution to lower alcohol content led to decreasing aroma for all tastes fruit spirits. However, typicality and intensity of fruit odour and the overall note of the spirits, were very similar perceived for Williams, plum and grape spirits whereas apple and raspberry spirits showed better characteristic at higher alcohol content.

Key words: fruit spirits, alcohol content, aroma, sensory perception

Učinek vsebnosti alkohola na senzorično zaznavanje žganih pijač

Izvleček: Čeprav sta voda in etanol glavni sestavini sadnih žganih pijač, ne določata njihove narave in zvrsti, niti njihove senzorične kakovosti. Vzrok za to je prisotnost številnih aromatskih sestavin. Sadne žgane pijače morajo imeti aromo njihovih izvirnih surovin, ki je uravnavana z etanolom. Glede na to, da je veliko aromatičnih sestavin bolj topnih v etanolu kot v vodi, je etanol najbolj pomemben nosilec aromatskih spojin. Koncentracija alkohola je odločilna za senzorični profil žganih pijač. Za konzumiranje je standardna alkoholna moč žganih sadnih pijač 40 volumskih odstotkov. Predpisi določajo minimalno vsebnost etanola kot 37,5 volumskih odstotkov, kar uporabljajo mnoge distilerije pri proizvodnji žganih pijač. Namen te raziskave je bil ugotoviti, če lahko takšno zmanjšanje v vsebnosti alkohola potencialno zmanjša sprejemljivost žganih pijač pri potrošnikih. V ta namen je bilo preiskušenih 5 parov žganih sadnih pijač in sicer hruškovo, slivovo, jabolčno, malinovo in grozdno žganje, v vseh primerih s komercialno in na 37,5 % vol. zmanjšano koncentracijo alkohola. Rezultati so pokazali, da uživalci zaznavajo razliko v vsebnosti alkohola v žganih sadnih pijačah, ker razredčenje na manjšo vsebnost alkohola zmanjša aromo in okus vseh preiskušenih žganih pijač. Kljub temu so značilen vonj, okus kot splošne značilnosti bili podobno zaznani pri žganju iz hrušk viljamovk, sliv in grozdja, med tem, ko sta žganji iz jabolk in malin imeli boljše lastnosti pri večjih vsebnostih alkohola.

Ključne besede: sadne žgane pijače, vsebnost alkohola, aroma, senzorično zaznavanje

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1 INTRODUCTION

Spirits are alcoholic beverages produced by three successive processes: fermentation, distillation and alternative ageing in wood barrel. During the production of fruit spirits, numerous volatile substances are produced. Fruits are the source of the so-called primary aroma of spirits, which are the components that give the beverage identity and uniqueness for a particular fruit species or variety (Januszek et al., 2020; Spaho et al., 2023). Most volatiles are produced during alcoholic fermentation (Stacner et al., 2023), creating a fermentative or secondary aroma of spirits. Distillation is a process that controls the alcohol concentration and the composition of volatile compounds in distillates. It is enabled by fraction cutting (Spaho et al., 2013; Xiang et al., 2020), thermal energy input and reflux rates (Heller & Einfalt, 2022), and is strongly influenced by the type and design of distillation apparatus used (Balcerek et al., 2017; Rodríguez-Solana et al., 2018; Hodel et al., 2021). During distillation, alcohol and water are the actual carriers of hundreds of volatile compounds contained in the initial fermented mash. The quantity and quality of these volatile compounds in the vapor depend on their boiling point, their better solubility in water or ethanol, and the variation of ethanol content during distillation. The ethanol is enhanced and refined during the distillation process. Balcerek et al. (2017) and Xiang et al. (2020) demonstrated that increasing the final alcohol concentration in the heart fraction resulted in lower amounts of main volatile components in the distillates. Wie et al. (2018) showed that ester species and amounts increased significantly with increasing alcohol concentration in the heart fraction, while acidity decreased. During distillation heart fraction separate from head and tail fractions because these fractions are responsible for negative aroma attributes. With head fraction the majority of acetaldehyde, ethyl acetate, acetone are removed. Those compounds give sharp and unpleasant flavour. The tail fraction contains, acetic acids and fusel oils, such as propyl, butyl and amyl alcohols and their isomers, that are associated with unpleasant aroma attributes (Bohn et al., 2022).The maximum alcohol content in the middle (heart) cut of fruit distillates after distillation might be 86% vol, although in practice it is usually around 65-75% vol, depending on the distillation apparatus used (Durr, 2010; Lukić et al., 2011; Esteban-Decloux, et al., 2021; Tian-Tian et al., 2022; Lončarić et al., 2022). After distillation is completed, the fresh high-proof distillate has to storage for a period of time to harmonize. The concentration of alcohol in the heart fraction is especially important for the aging process, since the extraction of wood components, the clarity of the distillates, and the volatile compounds strongly depend on the alcohol content of the distillates (Różański et al., 2020; Valcarcel-Munoz et al., 2022; Butron et al., 2023).

A spirit straight from the still is not palatable, so it must rest for at least three months. The fresh distillates have a high alcohol concentration, and the sharpness of the alcohol affects the sensory perception of the usual fruit aromas. They also contain a large number of aldehydes, even if they have been properly separated beforehand, which, due to their stale and pungent smell and taste, lead to an inharmonious, unripe overall impression. The aroma-determining esters, which mostly form during storage of the distillate, are also missing (Scholtten, 1999).

Consequently, the distillate must be diluted until bottling. The greater the dilution of the alcohol with distilled or demineralized water, the fewer odour components a spirit has. Today, alcohol concentrations of 40 to 45% vol. are common. This is the alcohol content to which consumers are accustomed. However, the EU Regulation (No. 2019/787) for fruit and wine spirits stipulates a minimum alcohol content of 37.5% vol. These regulations allow distilleries to offer spirits with a lower alcohol content than usual. For the industry, this means an increase in sales value, as the addition of water to dilute spirits is commonly regarded as a means of stretching production volumes. If finished spirit is diluted from 40 to 37.5% alcohol by volume, this means that 6.7 litres of distilling water were added to 100 litres of 40% vol alcohol distillates. It means 6.7 more litres of beverages for the industry.

This is added value for industry, but the question is: Is it acceptable to consumer? Do consumers perceive the alcohol reduction in fruit spirits and do they welcome the sensory changes caused by this reduction? Currently, there is limited data on the effects of alcohol reduction on the perceived sensory quality of these spirits and their appeal to consumer. Therefore, this study examined the impact of alcohol reduction in fruit spirits on consumer′ perceptions and potentially reduction of their acceptability of spirits with low alcohol content.

2 MATERIALS AND METHODS

2.1 MATERIALS

This study evaluates five spirits produced from pear Williams, plum, apple, raspberry, and grape spirits. The spirits were purchased from various producers on the market. With the exception of raspberry spirits, these spirits were selected for their distinct aroma and popularity among customer from the West Balkan (Mrvcic et al., 2021). Each original bottled spirit with declared alcohol content served as a control, and the corresponding sample was prepared by reducing the alcohol concentration to 37.5% vol. Table 1 shows the alcohol content of the samples.

2.2 METHODS

2.2.1 Sensory analysis

Sensory analysis was performed by a consumer panel. Consumer panel members were recruited through online and in-person surveys. After 72 people were surveyed, a group of 30 individuals was selected based on their past experience with consuming spirits. They claimed to be moderate drinkers who believed they understand the range of quality of spirits. The panel consisted of 80% men and 20% women between the ages of 20 and 60.

All samples were sensory analysed using three sensory tests: paired comparison difference test, paired preference test and descriptive test (Stone and Sidel, 2004). Sensory analysis was performed in two separate sessions. In the first session, assessors are used the paired comparison difference test and the paired preference test, and in the second session, the descriptive test.

The two coded products of each fruit spirit (control and reduced alc. sample) are served for the test of differences. In the directional test, the two presentation orders are AA, BB, AB, BA, where A is the control and B is the sample with reduced alcohol concentration. The paired samples are served simultaneously, and the individual is asked if "there is a difference." Each assessors received a set of five pairs of samples (Fig. 1). They have taken a break between evaluation of each single paired. The order of the spirit series was randomised. The assessors were asked if "there is a difference". If they notice a difference, they must choose more desired (preferred) samples.

After a one-hour break, the assessors evaluate the samples by a descriptive test. Prior to the analysis, the assessors received a brief training in the evaluation of spirits as well as insight into the sensory attributes of spirits.

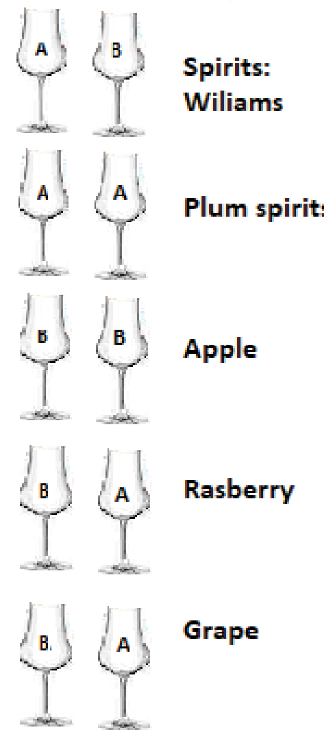

Figure 1: Series of five pairs of spirit samples that served to assessor, where A is the commercial alcohol content and B is the reduced alcohol content

The five sensory attributes were evaluated: typicality of odour and intensity of fruit odour, aroma, mouthfeel and overall note. Typicality of odour and intensity of fruit odour were evaluated by ortonazal while the aroma and mouthfeel were evaluated by retronazal. The overall sensation was evaluated as general impression of the spirit quality. Each sensory attribute was evaluated using a 5-point intensity scale (1-very weak, 5-very strong).

2.2.2 Statistical analysis

Analysis of paired comparison difference test based on the binominal distribution of answers. The binomial

Table1: Alcohol content of commercial fruit spirits and their reduced value

Spirits from	Declared alcohol content in %vol	Reduced alcohol content in %vol
Wiliams	40	37.5
Plum	42	37.5
Apple	40	37.5
Raspberry	43	37.5
Grape	43	37.5

test is used to determine the probability of selecting the correct answer. Based on the total number of traces, the number of correct choices is taken from the table of binomial numbers to determine significance at the 0.05 probability level (O′ Mahony, 1986). The Chi-square test was used to test whether the testers showed a significant preference for one of the samples (Meyners, 2007). The mean scores for the sensory atribbutes of the spirits were tested with a t-test using the Microsoft Excel software program.

3 RESULTS AND DISCUSSION

The assessor evaluated each pair of spirits and asked, „Are the samples different?" The paired condense responses for all samples tested are shown in Table 2.

Sensory analysis of all spirits revealed significant differences between commercial and reduced alcohol content (Table 2). The assessors found a significant difference in the alcohol content of the tested spirit samples. This means that the sensory perception of beverages is significantly influenced by the alcohol concentration. This was evident in all fruit species (varieties) tested in this experiment. Raspberry spirits had the fewest incorrect responses in the evaluation, possibly due to the significant difference in alcohol content between the commercial and reduced versions of the spirits. Although the difference in alcohol content between the commercial (40 % vol) and light (37.5 % vol) versions of apple spirits was not as great, most incorrect responses were observed. However, the distribution of responses shows that respondents perceived a difference between apple spirits with high and reduced alcohol content.

In the statistical analysis of the preference test, only the responses of the assessors who correctly identified the differences were considered. The results of the preference test are shown in Figure 2.

Regarding the preference test, many of the assessors indicated that they preferred the beverages with higher alcohol content. However, a statistically significant difference is observed between pairs of the commercial and light versions of Williams pear, apple, and raspberry spirits. Although more assessors indicated that a stronger sample of grape and plum spirits was more acceptable to them, there was no statistically significant difference in the distribution of responses between these pairs.

In a descriptive test, assessors were asked to rate the sensory attributes of each pair of spirits. Figure 3 shows the average scores for each sensory attribute of the spirits along with the results of the t-test.

Sensory perception of fruit spirits has been shown to be influenced by ethanol concentration, consistent with the findings of Ickes and Cadwallader (2017; 2018).

Spirits with higher alcohol content were mostly evaluated favourably by the assessors compared to their "light" versions. According to average ratings, Williams pear spirits with 40 % vol were rated significantly better in aroma than Williams pear spirits with 37.5 % vol. All other sensory attributes of Williams pear spirits were rated about the same. The difference in alcohol content was not sufficient to clearly distinguish all individual sensory properties except aroma. Nikičević (2005) states that a higher alcohol content is the ultimate for Williams pear spirits, as flavour and pleasure aroma are favoured at an alcohol content of more than 40 % vol.

Similar to Williams pear spirits, plum spirit with 42 % vol of alcohol was perceived significantly superior in aroma, while differences in smell attributes between stronger and lighter versions of spirits were not perceived. Mouthfeel (warming sensation) was also more intense for stronger plum spirits than for lighter version of plum spirits. This is not surprising, as ethanol causes a warming sensation in the mouth (Demiglio and Pickering, 2008; Longo et al., 2017; Ickes and Cadwallader, 2017).

However, mouthfeel is rated significantly better for apple spirits with lower alcohol concentration. Other studies have found that increasing the alcohol percentage causes a higher assessment of hotness or a burning mouthfeel experience (Le Berre et al.,2007; Jones et al 2008).

The dilution of the samples, with distilled water coused significant changes to the sensory profiles of apple and raspberry spirits. All sensory attributes of

Table 2: Number of responses of the assessors in the test of paired differences - commercial and reduced alcohol concentrations in the tested spirits where * indicates significance

Samples of spirits	Correctly noted difference	Incorrectly noted difference	Significance at $p < 0.05$
Williams pear	27	3	*
Plum	26	4	*
Apple	23	7	*
Raspberry	28	2	*
Grape	26	4	*

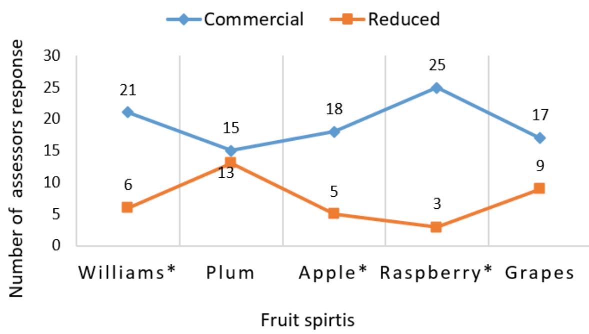

Figure 2: The results of the test preferences between fruit spirits with commercial alcohol concentration (higher % vol) and reduced alcohol concentration of alcohol (37.5 %vol), where * indicates significance at $p < 0.05$ for the *Chi-square* test

stronger apple and raspberry spirits are significantly better scored. In the study of Wei et al. (2018) it was shown that the concentration of esters, terpenes, and alkanes increased with increasing alcohol concentration. These authors also reported that spirtis with higher content of alcohol were more fragrant than the spirits with lower alcohol. Also, Durr et al. (2010) pointed out that in finished spirits, some primary aroma compounds become more prominent at higher alcohol concentrations. The most important primary aroma compounds are terpenes, phenol compounds, aromatic ethyl esters of short-chain fatty, but also aldehyde compounds and alcohols (Spaho et al., 2021; Wang et al., 2022).

Because apple and raspberry spirits in this study, are significantly better scored in aroma and overall note in stronger versions of spirts it appears that these spirits are more characterized by primary aroma components where higher concentration of alcohol affects the release of apple and raspberry aromas. Many factors, as stated by Lyu et al. (2021), can influence the results of this sensory analysis: physical and chemical properties of volatile aroma components, low detection threshold, so that every dilution of spirits leads to a decrease in the aromatic value of aroma components, or physiological factors during tasting. More exact claims cannot be made unless the aromatic components in the tested spirits are identified and quantified analytically.

The influence of alcohol content was not as pronounced in distinguishing the sensory characteristics of grape-derived spirits. Grape spirit with 43 % vol alcohol was evaluated better in terms of aroma perception and less intense mouthfeel, while there were no differences between grape spirits with 43 and 37.5 % vol alcohol in the perception of other sensory properties. Although grape spirits with higher alcohol content (between 43 and 45 % vol) are frequently offered on the Balkan market, this study found that consumers did not perceive any

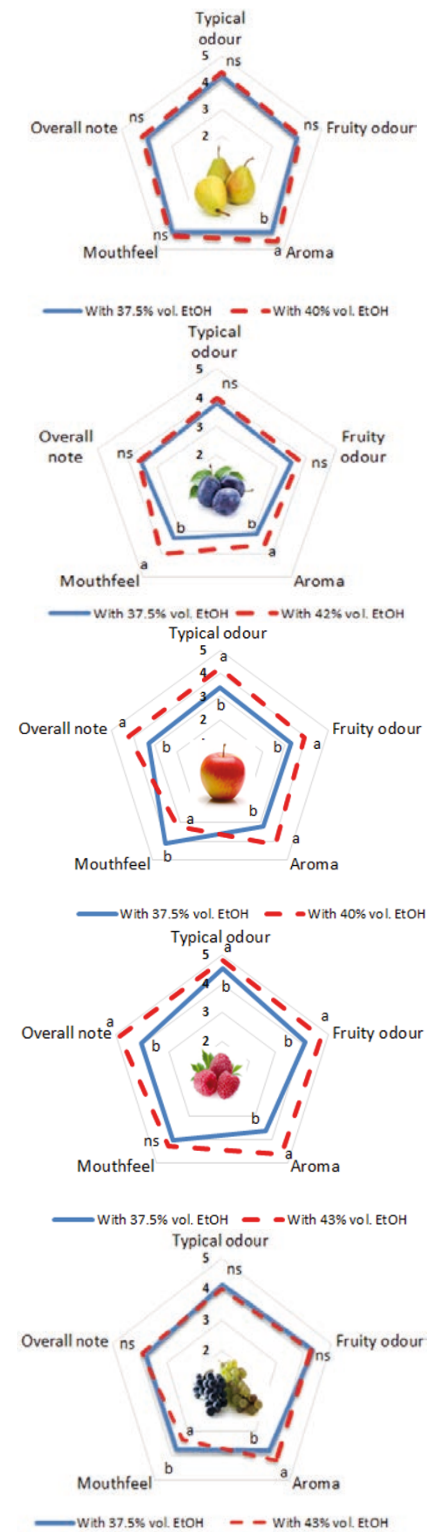

Figure 3: Sensory evaluation of Williams's pear, plum, apple, raspberry and grape spirits with higher alcohol content (40 % vol, 42 % vol, 40 %vol, 43 %vol and 43 % vol, respectively) and their „light" version with 37.5 % vol of alcohol. The attributes with different letter are statistically different according to the results of t-test and $p < 0.05$; ns-no significance

changes in sensory quality when alcohol was diluted to 37.5 % vol. Our results are consistent with the findings of Scholten (1999), according to which the lowest possible alcohol content is preferred for spirits with sensitive, fine aromas, e.g., grape spirits, so that the fruit-typical odour and aroma can be better perceived.

4 CONCLUSIONS

According to the results of the paired comparison difference test, and the descriptive test, the consumers noticed the difference between the »strong and laight« version of spirits much more easily, although it was much more difficult to determine what this difference was manifested in. In other words, they know what they like but are unsure why. Consumer panels are susceptible to various biases, including response bias, and the sample size of the consumer panel used in this study may limit the generalizability of the results. Nonetheless, the outcomes of this study have repeatedly shown that consumers can detect a difference in the alcohol content of fruit spirits and, as a result, prefer Williams pear, apple, and raspberry spirit with alcohol content higher than 37.5 % vol.

The aroma of all fruit spirits with a higher alcohol content than 37.5% vol significantly better. In other sensory attributes our findings showed that the fruit spirits with commercial alcohol content and their dilutions version were more similar to one another. Reduction in ethanol concentration can affect consumers' perception of grape, plum and Wiliams pear spirits in terms of aroma and mouthfeel but in terms of fruit odour and intensity and overall note cannot. The apple and raspberry spirits, had better sensory quality in "stronger" versions of the spirits and these results indicate that it is better to bottle apple and raspberry spirits with high alcohol concentration.

The results of this study are a signal for the industry, as they show that customer preferences for alcohol content depend on the type of fruit spirit, as different fruit spirits have different requirements for alcohol content in bottled beverages.

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A rapid and efficient DNA extraction method from high oily content seeds: *Ricinus communis* L.- apt for PCR based assay

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A rapid and efficient DNA extraction method from high oily content seeds: *Ricinus communis* L.- apt for PCR based assay

Abstract: *Ricinus communis* seeds harbor high oil and polyphenolics contents that hinder DNA extraction. Here, a rapid and efficient protocol for isolating total DNA from *R. communis* seeds was developed. The current method implies the use of repeated cycles of freeze/heat shock for the seed tissue to lyse the cells. DNA isolated with this protocol was successfully used as a template for PCR amplification of the internal transcribed spacer (ITS) region of the rRNA encoding gene that widely used for molecular identification of different plant species. As far to our knowledge, this study is the first one that report the efficient use of freeze/heat shock repeated cycles for isolation of a high-quality DNA from plant cells. The current protocol would support the subsequent analysis for seed lot purity analysis.

Key words: castor bean seed, cell lysis, heat shock, ITS, PCR, sequencing

Hitra in učinkovita metoda DNK ekstrakcije iz semen kloščevca (*Ricinus communis* L.), bogatih na oljih, primerna za PCR analizo

Izvleček: Semena kloščevca so bogata na oljih in polifenolih kar ovira ekstrakcijo DNK. V tej raziskavi je bil razvit hiter in učinkovit protokol za izolacijo celokupne DNK iz semen kloščevca. Metoda uporablja ponavljajoče se cikle zmrzovanja in segrevanja, kar povzroči lizijo celic v tkivu semena. DNK izolirana po tem protokolu je bila uspešno uporabljena kot osnova za PCR namnoževanje ITS regij v rRNK, ki kodirajo gene, na širokouporabljene pri molekularni identifikaciji različnih rastlinskih vrst. Kolikor nam je znano, je to prva raziskava, ki poroča o učinkoviti rabi ponavljajočih se cikličnih šokov zmrzovanja in segrevanja za izolacijo visoko kakovostne DNK iz rastlinskih celic. Ta protokol bo pripomogel k hitri analizi čistosti semen.

Ključne besede: kloščevec, lizija celic, vročinski šok, ITS, PCR, sekvenciranje

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1 INTRODUCTION

Castor bean (*Ricinus communis* L.) seed is one of the most common oil seed plants that is widely used economically in biodiesel production, cosmetics industry, lubricants, biomedical applications and as a rich animal fodder (Patel et al., 2016; Sturtevant et al., 2019; Sánchez et al., 2019; Awais et al., 2020). The seeds are externally protected by hard, brittle, mottled brown and shinning testa. The outermost layer of the seeds coat is the waxy cuticle, which represents the first barrier to water imbibition. The mature desiccated seed coat is rich in the oxidative products of polyphenolic compounds including phenolic acids, tannins, and flavonoids which are the source of the brown color of the mature seed coat, and play a significant role in plant disease resistance. Also, peroxidases and other antioxidant scavenging enzymes are commonly found in seeds coats (Moïse et al., 2005).

Employing genomics and molecular technologies promise to accelerate our knowledge of seeds and thus open new potentials for uses and conservation. The low concentration of DNA present in seed tissues makes it difficult to isolate intact DNA. Additionally, the DNA in seeds can be tightly bound to various proteins, polyphenols, and sugars. These biochemical interactions further complicate the process of extracting high quality DNA from seeds (Roy Davies, 1977; Sliwinska, 2006; Lee et al., 2020). The isolation of a good quality DNA is a prerequisite for any molecular biology work because proteins, polyphenols and polysaccharides impurities that co-precipitate with the DNA may hinder any enzymatic action, such Taq DNA polymerase in Polymerase Chain Reaction (PCR) and subsequent sequencing (Lakay et al., 2007; Wnuk et al., 2020) and endonucleases in genotyping and blotting techniques (Brown, 2001; Harju et al., 2004; Shiraishi and Iwai, 2020).

PCR based technique is successfully used to detect *R. communis* candidate genomic loci that are associated with important agronomic traits (Fan et al., 2019). PCR is essential for specific gene detection that prevailed in molecular identification and characterization (Manjunath and Sannappa, 2014). Enhancing the current seed traits to add value or to overcome an existing problem may be achieved by the generation of genetically modified plants. This wouldn't be achieved without isolating a good quality DNA that would serve as a template for a PCR reaction. The production of transgenic *R. communis* plants expressing the *Bacillus thuringiensis cry1Aa* gene help in lepidopteran insect pest management that were responsible for 30–50 % of yield losses (Muddanuru et al., 2019).

CTAB (Cetyl trimethylammonium bromide) DNA based extraction protocol and tailored modification versions of it, is widely used for isolating DNA from different plant tissues. However efficient, the CTAB is harsh and toxic in nature for human health and should be followed by phenol purification to ensure sufficient DNA purity. The phenol is volatile and can burn the skin (Doyle and Doyle, 1990; Shukla et al., 2018; Aboul-Maaty and Oraby, 2019). The used CTAB based protocol is time consuming as it requires long incubation time reached up to 60 min at 65 ^{0}C (Novaes et al., 2009). Alternatively, the commercial DNA extraction kits is an efficient substitute, despite the associated cost burden. So, a rapid efficient isolation method is of demand to facilitate any genomics and molecular biology work.

Therefore, the current study was designed for developing a rapid and efficient seed DNA isolation method that implies repeated cycles of freeze/heat shock to lyse the seed cells. Subsequent removal of the coagulated proteins and lipids by chloroform extraction was made and finally, high quality DNA was precipitated by ethanol.

2 MATERIALS AND METHODS

2.1 PLANT MATERIALS

Ten different samples of *R. communis* seeds were collected from their wild habitat in Egypt. Their DNA was extracted according to the newly developed protocol and used as a template for PCR amplification to amplify the ITS region of the corresponding rRNA encoding gene (Cheng et al., 2016).

2.2 MATERIALS AND EQUIPMENTS

- 1.5 ml Eppendorf tubes
- Mortar and pestle
- Tips
- Cooling centrifuge (Hettich MIKRO 22)
- Crushed ice
- UV-transilluminator (Vilber Lourmat-Germany)
- Digital balance (RADWAG Wagi Elektroniczne, AS 220/C/2)
- Micropipettes
- Vortex (VELP SCIENTIFICA)
- Water bath (MLW W21)
- Gel electrophoresis unit
- Thermal cycler (Biometra, Germany)

2.3 REAGENTS

- Lysis buffer: 2 % Triton X-100 (ADVENT), 100

mM NaCl (POWER CHEMAL), 1 % SDS (Sodium Dodecyl Sulfate), 10 mM Tris-HCl (pH 8.0) (OX FORD) and 1 mM EDTA (pH 8.0) (Hoffman and Winston, 1987, Harju et al., 2004)
- Ice cold 99 % ethanol (POWER CHEMICAL) or isopropyl alcohol
- 70 % ethanol
- 1 × TAE buffer
- Ethidium bromide (ALPHA CHEMIKA)
- Chloroform (SIGMA-ALDRICH)
- 3 M sodium acetate pH 5.2
- TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0, autoclaved)
- Agarose (molecular grade, Cleaver Scientific Ltdm CAS 9012-36-6)

2.4 THE DETAILED PROTOCOL

1. In 1.5 ml Eppendorf tubes, 0.1 g crushed decoated *R. communis* seeds were combined with 400 µl of the DNA lysis buffer. And then vortexed vigorously in a bench top vortex for up to one minute, followed by incubation on ice.

a. TIP: Avoid using a large amount of seed sample because the polysaccharides, polyphenols, and their derivatives in the seed would increase concurrently. These substances could interfere negatively with proper DNA isolation, reducing the isolated amount.

b. TIP: Rather than using a mortar and pestle, the sample could be crushed inside a round end 2 ml Eppendorf tube using a glass rod.

c. TIP: Grinding in liquid nitrogen would facilitiate the grinding process and increase DNA integrity.

2. After two minutes on crushed ice, the tubes were immersed abruptly in a 95 °C water bath for 5 minutes with interval vortexing.

3. After repeating the freeze/heat shock procedure exactly as described above, the tubes were vigorously vortexed for a continuous 30 seconds.

TIP: The solution takes on the appearance of a milky emulsion.

4. 400 µl of chloroform was added and vigorously vortexed for one minute, followed by centrifugation at 4 °C, 5 min, 10,000 rpm (RCF: 17,507).

TIP: Chloroform and isoamyl-alcohol can be mixed in a ratio of (24:1 v/v).

5. The aqueous layer was transferred to a new tube and the chloroform purification was repeated twice.

TIP: The chloroform purification should be repeated whenever necessary till the aqueous fraction is clear.

6. To allow DNA precipitation, the clear aqueous layer was transferred to a new tube containing 1 ml of ice-cold 99 % absolute ethanol and 40 µl of 3 M sodium acetate pH 5.2. After 15 minutes on ice, the tubes were centrifuged for 10 minutes at 4 °C and 10,000 rpm.

TIP: Instead of absolute ethanol, isopropyl alcohol may be used.

7. Following the removal of supernatants, DNA pellets were washed with 0.5 ml of 70 % ethanol and quickly centrifuged at 4 °C for 2 minutes at 10,000 rpm. The pellet was then air dried for 5 minutes.

TIP: To expedite the drying of the DNA pellet, invert the Eppendorf on a piece of clean. tissue.

8. Resuspend the DNA in 30 µl TE buffer [10 mM Tris, 1 mM EDTA (pH 8.0)]. The samples were stored at -20 °C until they were used.

TIP: It is recommended to dissolve the DNA pellet in a small volume of TE buffer to ensure that the DNA is adequately concentrated. If the sample was dissolved in a larger volume, it resulted in a low concentration of DNA. Reprecipitate your DNA and resuspend it in a reduced volume of TE buffer.

2.5 QUANTIFICATION AND VISUALIZATION OF DNA

The concentration of isolated DNA was determined using a NanoDrop® ND-1000 UV-Vis Spectrophotometer (Thermo Scientific-USA). The optical density (OD) at A260 and A280 was used to determine the purity. Electrophoresis of samples was performed on a 1 % agarose gel in 1× TAE (Tris-Acetate- Ethylenediaminetetraacetic acid) buffer containing 0.5 µg ml^{-1} ethidium bromide. The DNA was visualized and photographed with the aid of a UV-transilluminator embedded in Gel Documentation system (Virballurmate-Germany).

2.6 PCR AMPLIFICATION AND SEQUENCING

The PCR was carried out according to the manufacturer's instructions in a 50 µl reaction volume using (BIOLINE cat # BIO-21108) Mytaq Red DNA polymerase master mix. ITS1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') primers were used. The reaction mixture contains 1 × mytaq Red DNA polymerase master mix, 2.0 µl of each primer at a concentration of 10 pm l^{-1}, 1.0 µl of DNA template, and 0.25 µl of MyTaq™ DNA polymerase (5U µl^{-1}). The following PCR cycles were run in a Thermal Cycler (Biometra, Germany): Initial denaturation was carried out for 3 minutes at 95 °C, followed by 35 cycles of 20 seconds at 95 °C (denaturation), 20 seconds at 55 °C (annealing), 30 seconds at 72 °C (extension), and then a final extension for 10 minutes at 72 °C. On a 1 % agarose gel, the PCR products were separated.

The amplified fragments were excised and gel purified according to the manufacturer's protocol using the PCR-M clean up system (VIOGENE cat# PF1001). Following that, their sequences were determined at GATC Company using an ABI 3730xl DNA sequencer and an ITS4 primer. The obtained nucleotide sequences were validated against the NCBI database using the MEGA blast tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Geneious 11.1.5 software was used to align all sequences. The following sequences were deposited in the NCBI GenBank: MN880879, MN880880, MN880881, MN880882, MN880885, MN880886, MN880887, MN880888, MN880889, and MN880890.

3 RESULTS

The following is a description of a rapid DNA isolation protocol called the "ES DNA isolation method" (Fig. 1). The method is based on the use of repeated freeze-and-heat shock cycles to disrupt the cell wall and release genomic DNA from crushed de-coated *R. communis* seeds in lysis buffer. Following that, two rounds of chloroform purification were performed to remove co-bound proteins and lipids to the DNA following cell lysis. After transferring the aqueous phase to a clean tube, the DNA is easily precipitated using absolute (Abs.) ethanol and 3 M sodium acetate (pH 5.2). Following a 70 % ethanol wash, the air-dried pellet was dissolved in TE buffer to ensure the extracted DNA was preserved for the long term.

The isolated DNA's purity and lack of degradation are demonstrated by gel electrophoresis (Fig. 2).

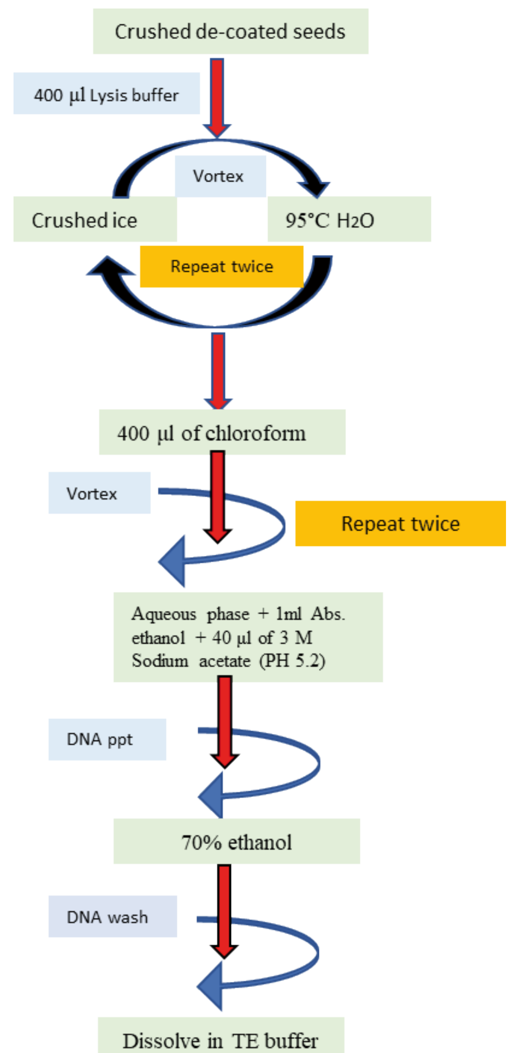

Figure 1: Flow chart representation of the current *R. communis* seed DNA isolation protocol termed "ES DNA isolation method"

Table 1: Quantitative estimates of DNA purity and concentration revealed by Nanodrop Spectrophotometer

Sample ID	Concentration ng µl^{-1}	A260/A280 readings (DNA Purity)
Rc1	95.1	2
Rc 2	81.4	2.57
Rc 3	49.5	2.3
Rc 4	55.6	2.28
Rc 5	79.7	1.99
Rc 6	52.8	2.11
Rc 7	56.9	2.3
Rc 8	60.1	2.5
Rc 9	66.3	1.99
Rc 10	55.7	2.44

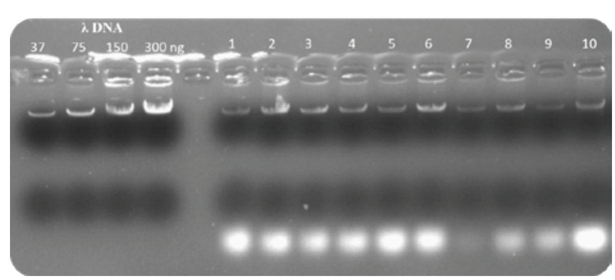

Figure 2: Gel electrophoresis of DNA isolated from the de-coated *R. communis* seeds used in the current protocol; "ES DNA isolation method". Lanes 1 to 10 refers to the ten samples used. λ DNA refers to serial dilution of undigested λ DNA for quality control and quantification of isolated DNA. The flourecent bands towards the bottom of the gel likely represent degraded RNA, which commonly co-purifies during DNA extraction protocols

The A260/280 ratio, which ranges between 1.99 and 2.57 for all *R. communis* seed DNA samples examined, indicates the quality of the isolated DNA (Table 1). The isolated DNA concentration ranges between 49.5 and 95.1 ng µl^{-1}. The yield and purity of the isolated DNA were sufficient for performing polymerase chain reaction amplification (Fig. 3). All samples were amplified to the expected product size of 700 bp.

All PCR products were sequenced and the nucleotide sequences verified using the National Center for Biotechnology Information's (NCBI) website. Alignment of the ITS sequences was performed to demonstrate their homology (Fig.4). The matched sequences revealed that all samples shared a significant degree of homology. The results demonstrates that the sequenceable PCR products obtained are of a high grade.

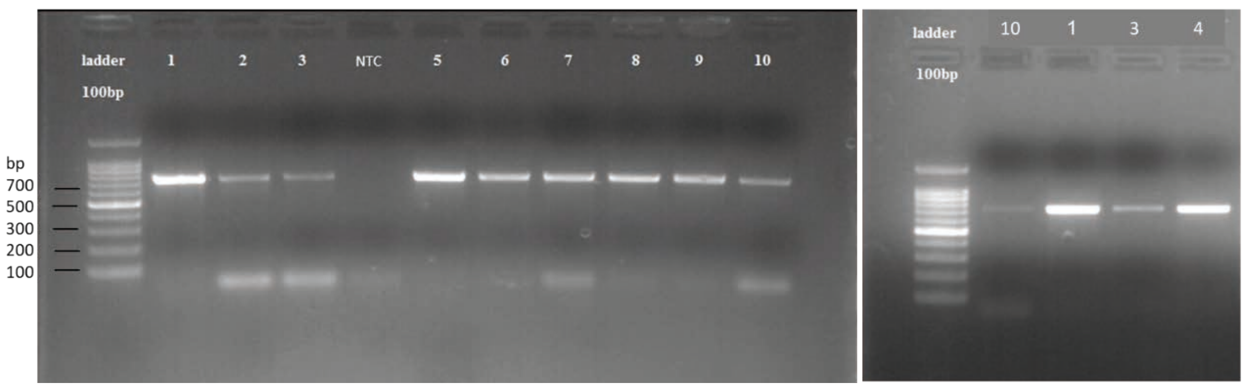

Figure 3: PCR-amplification of the ITS-rRNA encoding gene of the different *R. communis* seeds using the DNA extracted by the current method "ES DNA isolation method" as a template. Lanes 1 to 10 represent the ten *R. communis* samples. 100 bp ladder refers to the DNA ladder. NTC refers to non-DNA template of the PCR, no amplification means no PCR contamination

```
         1        10        20        30        40        50        60
         |        |         |         |         |         |         |
 Rc 6    AACCCCGGCGCAGGACGCGCCAAGGAAAATTAAATGAAAAGAGCACGCTGCTGTAGGCCT
 Rc 7    AACCCCGGCGCAGGACGCGCCAAGGAAAATTAAATGAAAAGAGCACGCTGCTGTAGGCCT
 Rc 2    AACCCCGGCGCAGGACGCGCCAAGGAAAATTAAATGAAAAGAGCACGCTGCTGTAGGCCT
 Rc 9    AACCCCGGCGCAGGACGCGCCAAGGAAAATTAAATGAAAAGAGCACGCTGCTGTAGGCCT
 Rc 3    AACCCCGGCGCAGGACGCGCCAAGGAAAATTAAATGAAAAGAGCACGCTGCTGTAGGCCT
 Rc 1    AACCCCGGCGCAGGACGCGCCAAGGAAAATTAAATGAAAAGAGCACGCTGCTGTAGGCCT
 Rc 4    AACCCCGGCGCAGGACGCGCCAAGGAAAATTAAATGAAAAGAGCACGCTGCTGTAGGCCT
 Rc 8    AACCCCGGCGCAGGACGCGCCAAGGAAAATTAAATGAAAAGAGCACGCTGCTGTAGGCCT
 Rc 5    AACCCCGGCGCAGGACGCGCCAAGGAAAATTAAATGAAAAGAGCACGCTGCTGTAGGCCT
Rc 10    AACCCCGGCGCAGGACGCGCCAAGGAAAATTAAATGAAAAGAGCACGCTGCTGTAGGCCT

 Rc 6    CGGAAACGATGCGCCTTAGGCACGCGTCGCCCTCTTTCGAGAACCATAACGACTCTCGGC
 Rc 7    CGGAAACGATGCGCCTTAGGCACGCGTCGCCCTCTTTCGAGAACCATAACGACTCTCGGC
 Rc 2    CGGAAACGATGCGCCTTAGGCACGCGTCGCCCTCTTTCGAGAACCATAACGACTCTCGGC
 Rc 9    CGGAAACGATGCGCCTTAGGCACGCGTCGCCCTCTTTCGAGAACCATAACGACTCTCGGC
 Rc 3    CGGAAACGATGCGCCTTAGGCACGCGTCGCCCTCTTTCGAGAACCATAACGACTCTCGGC
 Rc 1    CGGAAACGATGCGCCTTAGGCACGCGTCGCCCTCTTTCGAGAACCATAACGACTCTCGGC
 Rc 4    CGGAAACGATGCGCCTTAGGCACGCGTCGCCCTCTTTCGAGAACCATAACGACTCTCGGC
 Rc 8    CGGAAACGATGCGCCTTAGGCACGCGTCGCCCTCTTTCGAGAACCATAACGACTCTCGGC
 Rc 5    CGGAAACGATGCGCCTTAGGCACGCGTCGCCCTCTTTCGAGAACCATAACGACTCTCGGC
Rc 10    CGGAAACGATGCGCCTTAGGCACGCGTCGCCCTCTTTCGAGAACCATAACGACTCTCGGC

 Rc 6    AACGGATATCTCGGCTCTCGCATCGATGAAGAACGCAGCAAAATGCGATACTTGGTGTGA
 Rc 7    AACGGATATCTCGGCTCTCGCATCGATGAAGAACGCAGCAAAATGCGATACTTGGTGTGA
 Rc 2    AACGGATATCTCGGCTCTCGCATCGATGAAGAACGCAGCAAAATGCGATACTTGGTGTGA
 Rc 9    AACGGATATCTCGGCTCTCGCATCGATGAAGAACGCAGCAAAATGCGATACTTGGTGTGA
 Rc 3    AACGGATATCTCGGCTCTCGCATCGATGAAGAACGCAGCAAAATGCGATACTTGGTGTGA
 Rc 1    AACGGATATCTCGGCTCTCGCATCGATGAAGAACGCAGCAAAATGCGATACTTGGTGTGA
 Rc 4    AACGGATATCTCGGCTCTCGCATCGATGAAGAACGCAGCAAAATGCGATACTTGGTGTGA
 Rc 8    AACGGATATCTCGGCTCTCGCATCGATGAAGAACGCAGCAAAATGCGATACTTGGTGTGA
 Rc 5    AACGGATATCTCGGCTCTCGCATCGATGAAGAACGCAGCAAAATGCGATACTTGGTGTGA
Rc 10    AACGGATATCTCGGCTCTCGCATCGATGAAGAACGCAGCAAAATGCGATACTTGGTGTGA

 Rc 6    ATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCTTTCGG
 Rc 7    ATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCTTTCGG
 Rc 2    ATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCTTTCGG
 Rc 9    ATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCTTTCGG
 Rc 3    ATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCTTTCGG
 Rc 1    ATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCTTTCGG
 Rc 4    ATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCTTTCGG
 Rc 8    ATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCTTTCGG
 Rc 5    ATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCTTTCGG
Rc 10    ATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCTTTCGG

 Rc 6    CCGAGGGCACGCCTGCCTGGGTGTCACGCAATTGTCGCCCCCAACCCTTTCGATACATCG
 Rc 7    CCGAGGGCACGCCTGCCTGGGTGTCACGCAATTGTCGCCCCCAACCCTTTCGATACATCG
 Rc 2    CCGAGGGCACGCCTGCCTGGGTGTCACGCAATTGTCGCCCCCAACCCTTTCGATACATCG
 Rc 9    CCGAGGGCACGCCTGCCTGGGTGTCACGCAATTGTCGCCCCCAACCCTTTCGATACATCG
 Rc 3    CCGAGGGCACGCCTGCCTGGGTGTCACGCAATTGTCGCCCCCAACCCTTTCGATACATCG
 Rc 1    CCGAGGGCACGCCTGCCTGGGTGTCACGCAATTGTCGCCCCCAACCCTTTCGATACATCG
 Rc 4    CCGAGGGCACGCCTGCCTGGGTGTCACGCAATCGTCGCCCCCAACCCTTTCGATACATCG
 Rc 8    CCGAGGGCACGCCTGCCTGGGTGTCACGCAATCGTCGCCCCCAACCCTTTCGATACATCG
 Rc 5    CCGAGGGCACGCCTGCCTGGGTGTCACGCAATCGTCGCCCCCAACCCTTTCGATACATCG
Rc 10    CCGAGGGCACGCCTGCCTGGGTGTCACGCAATCGTCGCCCCCAACCCTTTCGATACATCG
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Figure 4: Partial sequence alignments of the ITS-rRNA encoding gene sequences obtained from the 10 *R. communis* seeds using Geneious 11.1.5 software. The data confirms the quality of the obtained PCR products that could be sequenced

4 DISCUSSION

While a PCR-based assay requires adequate quality genomic DNA, a rapid isolation methodology is required to permit the processing of large numbers of samples. Because the majority of polyphenolic chemicals in seeds are localized in their seed coats, de-coating the seeds reduces their interference with DNA isolation (Moïse et al., 2005). Repeated freeze-heat shock cycles are employed to shatter the cell wall and release genomic DNA from crushed de-coated *R. communis* seeds in lysis buffer, obviating the necessity for enzymatic or mechanical degradation (Harju et al., 2004). The lysis buffer contains mild detergents such as Triton X-100 and SDS and is frequently used to lyse cells, extract proteins, extract oils, and permeabilize live cell membranes by dissolving protein-lipid and lipid-lipid interactions without denaturing proteins (Johnson, 2013). However, freezing and heat shock may affect cell wall permeability and denature proteins, thereby expediting their removal, without the need for hazardous polyvinyl pyrrolidone (PVP), phenol, or β-mercaptoethanol (John, 1992; Shukla et al., 2018).

Seed polysaccharides, polyphenols, and their derivatives may impair the integrity of isolated DNA from *R. communis* seed (Porebski et al., 1997). When cells are lysed, these compounds covalently link to DNA, impairing DNA integrity and impeding PCR amplification (Wnuk et al., 2020). Therefore, the 60 °C CTAB incubation may be particularly important to dissociate polysaccharides from DNA, allows efficient and thorough lysis of cells and breakdown of proteolytic enzymes that could otherwise degrade DNA. This contributes to high DNA yields with CTAB and improve purity of the extracted DNA. The current lysis protocol using repeated freeze-heat shock cycles may achieve comparable degree of enzymatic degradation and release of DNA from cellular components without prolonged optimum heated incubation (Carey et al., 2023). Chloroform can be used to remove these compounds during DNA extraction. Additional to removing denatured proteins, it aids in the removal of other colouring chemicals such as pigments and dyes. Chloroform facilitates the separation of lipids, proteins, and cellular detritus into the organic phase, while the recoverable DNA is dissolved in the aqueous phase and then easily precipitated with absolute (Abs.) ethanol. Because the low seed DNA content necessitates efficient DNA precipitation, 3 M sodium acetate (pH 5.2) was added to the absolute ethanol during the precipitation process (Júnior et al., 2016; Heikrujam et al., 2020).

Gel electrophoresis was used to determine the feasibility of the current DNA isolation approach. No degradation was seen on the gel. Additionally, this was also observed with the nanodrop measurements. The A260/280 ratio of isolated DNA varied between 1.99 to 2.57 for all *R. communis* seed DNA samples examined, indicating that the DNA was of acceptable quality (Nzilibili et al., 2018). The isolated DNA concentration ranged between 49.5 and 95.1 ng μl^{-1}, which was sufficient for downstream applications such as PCR. Although the A260/280 ratio for high-quality DNA should be between 1.8 and 2, values greater than 2 have been observed previously for DNA samples isolated from various plant tissues using a modified CTAB-based approach (Aboul-Maaty and Oraby, 2019). This could be attributed to ionic strength and altered pH of the solutions used in the extraction process (Wilfinger et al., 1997; Boesenberg-Smith et al., 2012). Despite the fact that the DNA isolated using the current approach had a slightly higher A260/280 ratio, the DNA isolated was effectively employed for the downstream application, PCR. The extracted DNA was used as a template for PCR amplification of the ITS region of the rRNA encoding gene, which is commonly used for molecular identification and assessment of the molecular diversity of eukaryotic cells (Cheng et al., 2016; Yang et al., 2018; Ghareb et al., 2020; Soliman and A., 2021). PCR amplification of the extracted DNA samples yielded the expected product size of approximately 700 bp, as shown in Figure 3. The amplified PCR products were successfully sequenced, and the obtained sequences were deposited in the NCBI database (Fig. 4) (Soliman and A., 2021). These results demonstrate that the isolated DNA was of suitable quality for PCR amplification and downstream sequencing applications.

5 CONCLUSION

This rapid DNA extraction protocol demonstrates the ability to isolate DNA from seed tissues even without relying on specific chemical reagents typically used for extraction, such as CTAB. By utilizing simple, readily accessible lysis buffer components and efficient physical disruption of cells through freeze-heat cycles. This method provides an alternative approach to extract PCR-suitable DNA without requiring specialized chemicals. While CTAB is considered a staple reagent for high-quality DNA purification, this work shows that effective extraction is still achievable using very basic buffers and lysing techniques. The simplicity and accessibility of the reagents needed could make this rapid protocol easily adoptable, especially in resource-limited settings where procuring chemicals like CTAB may be difficult or cost-prohibitive. The proposed approach would facilitate the molecular investigation of seed lot quality and the characterization of germplasm.

5.1 DECLARATIONS

5.1.1 List of abbreviations

PVP, polyvinyl pyrrolidone. ITS-rRNA, Internal transcribed spacer of ribosomal RNA encoding gene.

5.1.2 Ethics approval and consent to participate

"Not applicable"

5.1.3 Consent for publication

"Not applicable"

5.1.4 Availability of data and material

All data generated or analyzed during this study are included in this article.

5.1.5 Competing interests

"The author declare that she has no competing interests"

5.1.6 Funding

This study received no funding grant

5.1.7 Authors' contributions

ERSS: generates the idea, performs all experimental work, analyze the data, submit the sequences to the database, wrote the manuscript, revised it, and corresponding the publication.

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Effect of sowing time and fertilizer on the protein content, seed- and protein yield of dry beans (*Phaseolus vulgaris* L.)

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Effect of sowing time and fertilizer on the protein content, seed- and protein yield of dry beans (*Phaseolus vulgaris* L.)

Abstract: The beans are an important protein source. In our three-year experiment, we examined the effect of sowing times and fertilizer doses on the protein content of the seed and the protein yield per hectare. We set up the trial on sandy soil, without irrigation, in four replications with 300,000 plant per hectare in Nyíregyháza (Hungary). 10 m^2 plots were in randomized blocks, with three sowing times (in normal time, earlier and later) and three fertilizer doses (0 %; 100 % and 150 %). We measured the nitrogen content of the seed with a Vario-Max CNS analyzer. The protein contents were counted from that value. The data were evaluated with Excel and SPSS 22.0. In examined 3 years the largest protein contents were in the third sowing time with 150 % fertilizer dose. In 2016-17 the yield and protein yield of the third sowing time were larger than the value of earlier sowing times for all three fertilizer doses. This was because of favourable weather. The significant effect of increased fertilizer doses was not proved at a given sowing time. If the weather was favourable, then the significant relationship among the sowing time, protein content, yield, and protein yield was positive and strong.

Key words: bean, protein, yield, sowing time, fertilizer

Učinek časa setve in gnojenja na vsebnost beljakovin v semenu in pridelku beljakovin v suhem fižolu (*Phaseolus vulgaris* L.)

Izvleček: Fižol je pomben vir beljakovin. V triletnem poskusu smo preučevali učinek časa setve in odmerka gnojil na vsebnost beljakovin v semenu in na pridelek beljakovin na hektar. Poskus je bil zasnovan na peščenih tleh, brez namakanja, v štirih ponovitvah, s 300,000 semeni na hektar, v Nyíregyházi (Madžarska). 10 m^2 velike ploskve so bile razporejene v naključnih blokih, s tremi časovi setve (normalen čas, zgodnja in pozna setev) in s tremi odmerki gnojil (0 %; 100 % in 150 %). Vsebnost dušika v semenih je bila izmerjena z Vario-Max CNS analizatorjem. Vsebnosti beljakovin so bile izračunane iz teh vrednosti. Podatki so bili ovrednoteni z Excelom in SPSS 22.0. V treh letih poskusa je bila ugotovljena največja vsebnost beljakovin v tretjem času setve in pri odmerku gnojil 150 %. V letih 2016-17 sta bila pridelek in pridelek beljakovin v tretjem obdobju setve večja kot pri zgodnji setvi, pri vseh odmerkih gnojil. Vzrok za to je bilo ugodno vreme. Značilen učinek povečanja odmerka gnojil ni bil ugotovljen pri nobenem času setve. V primeru ugodnega vremena je bila značilna in močna povezava med časom setve, vsebnostjo beljakovin, pridelkom in pridelkom beljakovin.

Ključne besede: fižol, beljakovine, pridelek, čas setve, gnojila

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1 INTRODUCTION

Ensuring the protein requirement is an important aspect of both human nutrition and animal feeding. Legumes have 2-3 x more protein than cereals (Siddiq et al., 2010). The protein content of beans seed is 24-28 % (Chávez-Mendoza et al., 2019; Kahraman & Onder, 2013; Bildirici & Oral 2020). The ratio of protein and fiber is also important (Brick et al., 2022). Bean flour is excellent for supplementing lysine-poor wheat flours, improving the ratio of essential amino acids in bread while reducing carbohydrate content, thus improving the nutritional value of the bread produced (Hoxha, 2020; Singh, 2017). Its flour can also play a major role in the production of gluten-free products (Siddiq, 2010). In addition, the ethical question of the use of animal proteins and their effect on health is gaining more and more space. Bean protein is also used in the production of vegetable meats, muffins, mayonnaise and yogurt (Ferreira, 2022).

Grown of dry beans are strongly affected by climate change. In our country this appears with extremely high summer temperatures occurring, as well as the rather extreme distribution and intensity of precipitation.

In terms of consumption, it is important how much protein a unit of food contains, and in terms of production, how much protein we can produce from a unit of area. This gives one aspect of the economy.

Based on the role of beans in nutrition, we considered it important to investigate the effect of fertilizer dose and sowing time on the protein content of seed beans and the protein yield per hectare. Beans are an ecologically sensitive crop. With this experiment, we get an answer, to how we can reduce the effects of the extreme weather conditions experienced nowadays with the technological elements mentioned above.

In this publication, we present the response of seed protein content and protein yield of the Start pearl bean variety to sowing time and fertilizer treatments.

1.1 LITERATURE REVIEW

Factors affecting the protein content are the variety and the weather, soil, irrigation and through these, fertilization and sowing time (Singh, 2017; Bildirici & Oral, 2020). Barampama & Simards (1993) in their experiment statistically confirmed that the variety and locality affect the nutrient content of the plant. The effect of sowing time and fertilization can be lead back to favourable cultivation conditions of the variety.

The beneficial effect of fertilization can be only enforced under favourable weather conditions, especially the amount of precipitation (Ermolaev & Radkov, 1975; Unk, 1984; Kádár, 2005; Togay & Anlarsal, 2008; Bellaloui et al., 2011b, Bellaloui et al., 2013; Kawaka et al., 2018; Celmeli et al., 2018).

Islam et al. (2016) and Shehata et al. (2011) proved in their experiment that the amount of protein was affected to a different degree by the use of different types of manure. Nitrogen is involved in protein formation (Marschner, 1995). Several researchers have shown the effect of nitrogen (Kádár, 2005; Morshed et al., 2008; Balláné Kovács, 2011; Gulmezoglu & Kayan, 2011; Soratto et al., 2017; Varfolomeyeva et al., 2021), phosphorus (Yin et al., 2016; Kahraman & Onder, 2013; Bildirici & Oral, 2020) and potassium (Bellaloui et al., 2013) fertilizers to increase the protein content. Miya & Modi (2015) also statistically confirmed the effect of NPK fertilization on increasing total crude protein. When organic manure is applied with NPK fertilizer, it increases the protein content more than if they were applied separately (Dikshit & Khatik, 2002; Jagannath et al., 2002; Hegazi et al., 2011; Chaturvedi et al. 2012; Tomar et al., 2016; Saikia, 2018). In Singh's (2002) experiment different organic fertilizers and pesticides were used to influence yield and protein content. However, there is also literature where N fertilization did not increase the NPK content of the seed (Nascente, 2017; Ovacikli & Tolay, 2020).

The effect of the vintage is reflected in the higher protein content of the dryer year (Somos, 1983; Nemeskéri & Nagy, 2003; Asemanrafat & Honar, 2017), respectively the control had a higher protein concentration in non-irrigated conditions than the fertilized one (Bellaloui et al., 2011b). According to Celmeli et al. (2018), the protein content of cereal crops decreased as the crop grows. Asemanrafat & Honar (2017) confirmed this in the case of beans.

The effect of sowing time on the protein content of soybeans was shown by Bellaloui et al. (2011a). Based on their studies, they found that early-sowed soybeans had a higher protein content. Singh et al. (2012) investigated the protein content of wheat semolina at different sowing times under irrigated and non-irrigated cultivation conditions. It was established that the temperature during the period of grain saturation had an effect on the amount of protein quantity. In non-irrigated conditions, the early-sown wheat had a higher protein content due to the lower temperature. However, in irrigated cultivation, the protein content was higher in the late sowing period.

The role of weather is important because strongly influences utilization of fertiliser and crop yield. Beans are a water-intensive plant, one of the yield limiting factors is the lack of rainfall (Nagy, 2006). Its water requirement during vegetation is 300-400 mm, the most critical is the period of flowerbud formation, when it is very sensitive to drought. Its water-demanding period lasts from budding to 12-16 days after the initial set. During this period, a single irrigation of 30 mm results in a 50 % yield increase of green pods (Tóth, 1979; Hadnagy, 1981). It needs a relative humidity above 65 % for pod set (Géczi, 2003).

The effect of temperatures above 25-30 °C during flowering and atmospheric drought is unfavourable for the yield (Kádár, 2005). The yield and element content were more influenced by weather conditions than row spacing and fertilizer treatments (Russo, 2006). At the legumes, the increase in yield often entails a decrease in the protein content of the seed (Varfolomeyeva et al., 2021).

There are differences in protein content between varieties (Már & Juhász, 2003; Köse et al., 2019). Cultivation of the short growing season (60-90 days) varieties is safer in drought-prone climates (CGIAR, 2016). The examined Start variety is also like this, in our country it can be safely sown until May 20, because it can ripen its crop.

2 MATERIAL AND METHODS

We set up the experiment with the institute's three dry bean *(Phaseolus vulgaris* L.*)* cultivars ('Start', 'Hópehely' and 'Diana') in Nyíregyháza (Hungary) in 2015-17. We examined the effects of sowing times, plant densities and nutrient supply on yield and yield characteristics. We describe in this publication the protein content test of the *Phaseolus vulgaris* 'Start' at a plant density of 300,000 plant ha^{-1} was examined over a 3-year period. *Phaseolus vulgaris* 'Start' is a white, pearl-shaped and small-seeded dry bean cultivar.

The experiment was set up on sandy soil (Table 1.) without irrigated conditions with randomized blocks in 4 replications on 10 m^2 plots. The first sowing was when the soil temperature has permanently risen to 14 °C. The second sowing was at the generally accepted beginning of May (~ May 8-10), and the third sowing happened until May 20. The plant densities were set 200 000; 300 000 and 400 000 germs ha^{-1}. Treatment of nutrient replenishment included the control (0 %), 100 % and 150 % NPK dose based on Antal (1983) and Velich (1994) recommended 95 kg N, 40 kg P and 80 kg K to achieve 1 ton grain yield. Used fertilizers: ammónium-nitrát (N 34 %), szuperfosz fát (P 18 %) and kálium-szulfát (K 50 %).

We calculated the protein content by the nitrogen values of the sample which was taken from 50 g minced dry seed crop. We measured the nitrogen values with a Vario-Max CNS analyzer and multiplied them with a conversion factor of 6.25. The study was in 3 replicates which were taken from the crop of field replicates. We used the SPSS software package for the evaluation. At the one-way analysis of variance, the homogeneous sample was tested by Tukey's-b, and the non-homogeneous sample was tested by the Games-Howel test at a 5 % SD level. Spearman's and Pearson's correlation analysis were used for the quantify relationship between the factors. The weather factors were analyzed with an Excel program.

The weather is discussed in more depth because the rainfall and temperature conditions during flowering have a significant impact on the yield of beans *(*Table 2*)*.

In 2015 in the third sowing time, the proportion of hours with relative humidity above 65 % was the lowest and the temperature also was very high strongly decreasing the yield. During the flowering of the 2nd sowing season, temperatures above 30 °C did not occur and the relative humidity was also favourable. 1st sowing time received the most rainfall during its flowering period.

In 2016 more rainfall fell (24 mm) during the flowering of the 1st sowing time than during the flowering of the other two sowing times. The number of hours of favourable relative humidity, which is necessary for generative processes, was the highest here. However, the number of hours of critical temperature values was the highest in this sowing time, and the yield was the lowest.

Table 1: Soil characteristics and GPS position of the experiment

	2015	2016	2017
GPS position	47.978401, 21.675888	47.974961, 21.691528	47.975930, 21.697846
pH $_{(KCl)}$	6,00	5,58	7,12
Plasticity index according to Arany	27	35	38
Water-soluble total salt (m m^{-1}) %	0,02	0,04	0,04
$CaCO_3$ (m m^{-1}) %	< 0,100	< 0,100	4,34
Organic carbon in humus (m m^{-1}) %	0,842	1,98	2,07
AL-soluble P_2O_5 (mg kg^{-1})	96,4	123	142
AL-soluble K_2O (mg kg^{-1})	247	211	328
KCl-soluble NO_3- + NO_2- - N (mg kg^{-1})	10,1	51,6	35,8

Table 2: Weather characteristics during flowering

	2015			2016			2017		
Sowing time	1	2	3	1	2	3	1	2	3
Length of flowering (day)	20	10	14	17	15	13	20	17	15
65 % < relative humidity (h)	379	195	220	334	272	235	397	336	317
Average of affected days (h)	19	20	16	20	18	18	20	20	21
In percentage of flowering hours	79	81	65	82	76	75	83	82	88
30 °C < number of hours	17	0	71	61	46	20	18	16	5
Average of affected days (h)	9	0	8	8	8	7	3	3	5
In percentage of flowering hours	4	0	21	15	13	6	4	4	1
25 °C < number of hours	66	42	152	156	129	87	144	122	58
Average of affected days (h)	7	6	12	10	11	9	10	9	7
In percentage of flowering hours	14	18	45	38	36	28	30	30	16
Rainfall amount	24	14	13	24	9	15	50	50	44

During the flowering of the 1st sowing season, the temperature was above 30 °C twice, through several days (5 and 3 days). In the 2nd sowing season, the temperature was above 30 °C through 3 days on two occasions. In the 3rd sowing season it happened only once, which lasted for 3 days.

In the 3rd sowing season, which had the largest yield, 15 mm of rainfall fell during flowering. This favoured crop formation. The number of hours of critical temperature values and the values of relative humidity were the lowest in the 3rd sowing times among the 3 sowing times. Lower temperatures also supported crop formation.

The percent of critical values within the flowering period is an important indicator. The relative humidity did not vary greatly between sowing times. However, in the first two sowing seasons, the proportion of hours above 30 and 25 °C during the flowering period was very high, which resulted in a low yield.

In 2017, there was no big difference in the flowering weather of the 1-2. sowing times. The rainfall amounts also developed similarly. However, the 3rd sowing time had a much lower proportion of values above the critical temperature during the flowering time, which was shown in a significant increase in yield. The value of the 3rd sowing time was also more favourable in the proportion of hours with relative humidity above 65 %.

The big differences between crop results could also be attributed to the weather.

3 RESULTS AND DISCUSSION

3.1 DIFFERENCES BETWEEN SOWING TIMES IN THE AVERAGE OF FERTILIZER TREATMENTS IN THE CASE OF THE *Phaseolus vulgaris* 'Start'

In 2015-16-17, the seed yield of the 3rd sowing time had the highest seed protein content. Its significant difference was different from the other two sowing times in a given year. In 2016 we did not prove any difference between them using the GH method. The protein content of the 1st sowing time was significantly lower than that of the 3rd sowing time in 2015 and 2017. We verified a statistical difference between the seed protein content of the 1st and 2nd sowing times in 2015. In contrast to the other two years, the value of the 2nd sowing time was significantly lower than the protein content of the 1st sowing time (Table 3).

There was a significant difference between seed yields in all three years between each sowing time. In 2016-17, when the soil and weather conditions were more ideal, the yield increased as the sowing times were postponed. In 2015 acidic soil with little humus content, extremely high temperatures during the flowering period and a growing season with poor rainfall were unfavourable for beans, so the 3rd sowing time produced the least and the 2nd sowing time the most yield.

Table 3: Protein content, seed yield, and protein yield data of 3 years per sowing times (2015-2017)

	Protein content (%)			Seed yield (kg ha^{-1})			Protein yield (kg ha^{-1})		
	2015	2016	2017	2015	2016	2017	2015	2016	2017
1. sowing time	27.9 a	23.4	21.6 a	200.9 a	98.8 a	248.5 a	55.8 a	22.3 a	53.9 a
2. sowing time	26.1 b	25.6	22.8 a	336.3 b	224.2 b	712.0 b	87.5 b	56.4 b	162.1 b
3. sowing time	32.1 c	26.4	28.8 b	19.7 c	751.7 c	1490.3 c	6.3 c	197.2 c	428.8 c

We also confirmed significant differences in protein yield between all three sowing times. In the average of the treatments, the 3rd sowing time had significantly the highest protein yield and the 1st sowing time had the lowest in 2016-17. In 2015, due to the extreme weather, different results were obtained. Despite significantly the highest protein content of the 3rd sowing time, its protein yield was the lowest because of the very low yield.

3.2 DIFFERENCES BETWEEN THE RESULTS OF FERTILIZER DOSES IN THE AVERAGE OF THE SOWING TIMES IN THE CASE OF THE *Phaseolus vulgaris* 'Start'

We verified a significantly difference in protein content in 2016. The value of the treatment without fertilizers was statistically proven to be lower than the treatments with fertilizers (Figure 1-2). In 2015 and 2017, it was observed that the value of the treatment without fertilizers was the smallest and the value of the treatment with the most fertilizers was the highest.

In yield per hectare and protein yield, we did not show any statistically proven differences between the treatments and the differences were also small.

3.3 DIFFERENCES BETWEEN FERTILIZER TREATMENTS AT A GIVEN SOWING TIME IN THE CASE OF THE *Phaseolus vulgaris* 'Start'

3.3.1 Seed protein content

The unfertilized treatment had the lowest seed protein content in all 3 sowing times and during all 3 years. The significantly deviation of this was different per year and per sowing time.

In 2016, we significantly verified that the protein content of the seeds of fertilized plots was higher than that of non-fertilized plots (Table 4). We established the same thing in 2017, with the clarification that in the 2nd sowing season, the protein content of the two fertilized treatments was also significantly different from each other: the treatment receiving 150 % had the highest protein

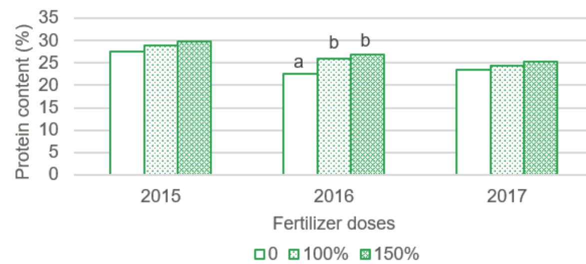

Figure 1: Effects of fertilizer doses in the average of the sowing times on protein content

content. This year, in the 3rd sowing time, the difference between the protein contents was minimal, we did not verify a significant difference between the fertilizer treatments.

In 2015, the protein content of the treatment with a 150 % fertilizer dose was significantly higher than that of the non-fertilized treatment in the first two sowing seasons. In the 3rd sowing season, the protein content of the 100 % fertilizer dose differs significantly from that protein of the unfertilized.

3.3.2 Yield (kg ha^{-1})

In 2015-16, when the fertilizer treatments were examined by sowing time, we did not prove a significant

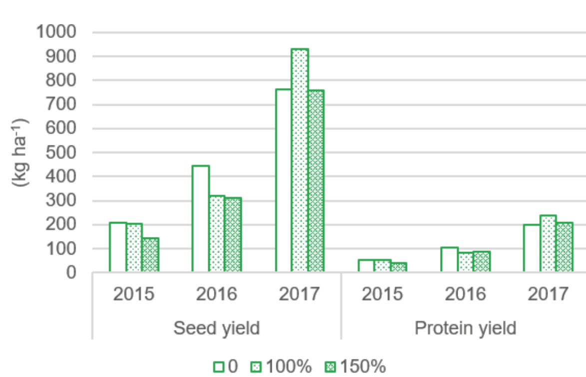

Figure 2: Effects of fertilizer doses in the average of the sowing times on seed yield and protein yield

Table 4: Effect of treatment combinations on protein content, yield and protein yield

	2015			2016			2017		
Fertilizer dose	1. sowing time	2. sowing time	3. sowing time	1. sowing time	2. sowing time	3. sowing time	1. sowing time	2. sowing time	3. sowing time
				Protein content (%)					
0	27,2 A a	24,9 A a	30,8 A b	19,0 A a	23,6 A b	25,1 A c	20,6 A a	20,8 A a	28,7 b
100%	27,6 A a	25,7 AB b	33,1 B c	25,1B	26,3B	26,7 B	22,3 B a	22,7 B a	28,1 b
150%	28,9 B a	27,7 B a	32,4 AB b	26,2B	26,9B	27,4B	22,0 B a	24,8 C b	29,5 c
				Yield (kg ha$^{-1)}$					
0	219,1 AB a	372,4 b	30,1 c	134,0 a	302,1 a	895,6 A b	274,4 a	531,1 A b	1482,0 c
100%	280,3 B a	312,6 a	23,0 b	110,1 a	171,3 a	647,8 B b	279,2 a	1039,8 B b	1473,0 c
150%	103,4 A a	323,9 b	6,1 c	52,2 a	199,1 a	711,8 AB b	191,8 a	565,2 A b	1516,0 c
				Protein yield (kg ha^{-1})					
0	59,6 A a	92,4 A b	9,4 A c	25,3 a	71,4 b	224,3 c	56,9 a	110,8 A b	424,6 c
100%	77,8 B a	80,2 B a	7,7 A b	27,8 a	44,9 ab	172 b	62,4 a	236,1 B b	415,5 c
150%	29,9 C a	89,8 A b	1,9 B c	13,8 a	53 a	195,2 b	42,4 a	139,2 A b	446,2 c

Note on the figures:
Capital letter: indicates a significant effect of fertilizer doses within the same sowing time and the same year
Lower case: indicates a significant effect of sowing times within the same fertilizer dose and same year

difference using the Tukey-b method between the fertilizer treatments. In 83 % of cases, the without fertilizers had the most yield. In 2015, at the 1st sowing time, at the LSD 5 % level, the 150 % dose fertilizer treatment had yield significantly less than the treatment that received the 100 % dose.

In 2016, in the case of the 3rd sowing time, at the LSD 5 % level, we verified a significant difference between the without fertilizer treatment with the highest yield and the treatment that received the 100 % dose.

In 2017, the difference in the yield of the earlier and later sowing times was minimal, so we did not detect a significant difference. In the 2nd sowing season, the yield per hectare of the treatment with a 100 % fertilizer dose was significantly higher than the yield of the treatment without fertilizer and treatment with a dose of 150 %.

In the 2nd sowing season of 2017, we statistically verified that the protein yield of the treatment with a 100 % dose of fertilizer was significantly higher than the yield of the treatment without fertilizer and with a dose of 150 %. This can be explained by its significant surplus of yield, which was able to compensate for its protein content, which was located in the average protein content of the other two fertilizer doses.

In 2015-16, except for the 1st sowing time, the treatment without fertilizers always had the highest protein yield, despite the lowest protein content. However, this difference in protein yield was not significant. We showed a significant difference at the LSD 5 % level between the lowest and the highest protein yield in 2015. In 2015, in the 1st and 3rd sowing times, the protein yield of the treatment with the highest fertilizer dose was significantly the lowest, but in the 2nd sowing time, the protein yield of the treatment with 100 % fertilizer dose was the lowest. In the 1st sowing seasons, the protein yield of treatments with the highest fertilizer dose was the lowest, which was also statistically confirmed in 2015. This trend was also observed in 2017. It was likely that the early sowing was unfavourable for the small-eyed, white-seeded Start variety, which was only amplified by the higher fertilizer dose.

In the case of a lower seed protein content, the protein yield may be higher due to the higher yield. For example: in 2017, in the case of 100 % treatment of the 2nd sowing time, and in 2015-16, regardless of the sowing times, in the treatments without fertilizers.

3.4 DIFFERENCES BETWEEN THE RESULTS OF SOWING TIMES AT A GIVEN FERTILIZER TREATMENT IN THE CASE OF THE *Phaseolus vulgaris* 'Start'

3.4.1 Protein content

In 2015 and 2017, regardless of the fertilizer dose, the seed protein content of the 3rd sowing time had significantly higher than that of the previous sowing times

(Table 4). The 1st and 2nd sowing times were significantly different at the 100 % fertilizer treatment in 2015 and in the 150 % dose treatment in 2017.

In 2016, on the protein content of the fertilized treatments had no significant effect by sowing times. In the treatment without artificial fertilizers, the protein content of the 3rd sowing time was significantly higher than the that of 1st sowing time.

3.4.2 Yield (kg ha^{-1})

In 2017, regardless of the fertilizer doses, the yield increased significantly in the 2nd and 3rd sowing times. In 2016, the 3rd sowing time also had significantly the highest yield, however, the yields of the 1st and 2nd sowing times did not differ significantly from each other.

In 2015, regardless of the fertilizer doses, due to the extreme weather, the 2nd sowing time had significantly the highest yield. This was also significantly different from the result of the 3rd sowing time, except for the treatment with a 100 % fertilizer dose, where the yield of the 1st sowing season was not significantly lower.

3.4.3 Protein yield (kg ha^{-1})

The differences in protein yield (kg ha^{-1}) formed in accordance with the yield in 2015 and 2017. We observed a deviation from this in 2016, where the protein yield of the 3rd sowing time was significantly higher in all fertilizer treatments. In the case of those without artificial fertilizers, the protein yield of each sowing time differed significantly. In the treatment with a 100 % fertilizer dose, despite the large difference, the 2nd sowing time did not differ significantly from the protein content of the 3rd sowing time, even though the 2nd sowing time had 74 % less protein yield compared to the 3rd sowing time.

3.5 RESULTS OF TREATMENT COMBINATIONS IN THE CASE OF THE *Phaseolus vulgaris* 'Start'

3.5.1 Protein content

In 2015 and 2017, the highest seed protein content was measured in the 3rd sowing time treatment combinations. In 2016, the protein content of without fertilizer treatments were the least. The largest protein content was measured in 150 % fertilizer dose of 2nd and 3rd sowing times.

3.5.2 Yield and protein yield (kg ha^{-1})

In 2015, treatments of 3rd sowing time produced significantly less than other treatments. The highest fertilizer dose treatment of 1st sowing time also produced little, so did not differ significantly from the yield of 3rd sowing times. The treatment without fertilizer of 2nd sowing time yielded the highest that did not significantly differ from the yield of treatment with fertilizer of 2nd sowing time and from the yield of treatment with 100 % dose of 1st sowing time.

In 2016-2017, the treatment combinations of 3rd sowing time were significantly more productive than treatments of earlier-sowing times. Accordingly, its protein yields also were significantly higher than those of earlier sowing times. The treatments with fertilizer were not significantly more productive than the control.

3.6 CORRELATIONS BETWEEN THE EXAMINED ELEMENTS IN THE CASE OF THE *Phaseolus vulgaris* 'Start'

The yield volume, protein content and protein yield were correlated with each other at the 1 % significance level. In all three years, we showed a very strong, positive relationship between yield and protein yield (Table 5). Only the significant relationships are shown in the table.

Between the protein content and protein yield, we verified a very strong, positive relationship in 2017. The high protein content resulted in a high protein yield (also due to the increased yield per sowing time). However, we showed a strong, negative relationship between them in 2015. The reason for this was that the highest protein content of the 3rd sowing time was combined with the lowest protein yield because of the low yield. In 2017, the increasing yield was coupled with higher protein content, and in 2015, a lower yield was coupled with higher protein content.

In all three years, in most cases, the sowing time was related with protein content, yield per hectare and protein yield at the 1 % significance level. In 2016-17, we verified a very strong positive relationship with the protein yield, in 2017 we showed a very strong relationship with the yield per hectare, and in 2016 a strong significant relationship. We verified a medium significant relationship with the protein content in all three years.

In 2015, the sowing time had a negative, medium relationship with yield per hectare and protein yield because of the extreme growing conditions.

Table 5: Significant correlation values of 3 years between the examined factors

	Protein content (%)			Seed yield (kg ha^{-1})			Protein yield (kg ha^{-1})		
	2015	2016	2017	2015	2016	2017	2015	2016	2017
Protein content (%)	1	1	1	-,845**		,862**	-,811**		,909**
Seed yield (kg ha^{-1})				1	1	1	,995**	,996**	,992**
Sowing time	,536**	,425*	,798**	-,518**	,885**	,938**	-,530**	,920**	,938**
Fertilizer		,780**	,387*						

3.7 DISCUSSION IN THE CASE OF THE *Phaseolus vulgaris* 'Start'

The yield of beans is strongly defined by the weather. This was also reflected in the yield as Kádár (2005) and Russo (2006) established during their experiment that the temperature during flowering and the lack of precipitation greatly influence the yield. In 2016-17 the fertilizer was utilized by the effect of favourable weather, thereby the protein content of seed, yield, and yield protein were increased.

3.7.1 Effect of sowing times in the average of fertilizer treatments

The seed protein content of the 3rd sowing season was the highest in each of the 3 investigated years. In the case of Start beans variety, this was different from what was found in the literature in the case of soy and wheat. Bellaloui et al. (2011a) found that early-sowed soybeans had a higher protein content. Singh et al. (2012) determined that in non-irrigated conditions, the early-sown wheat had a higher protein content due to the lower temperature. Early sowing of the Start bean variety is unfavourable due to the small, white seed.

In case of favourable weather, the 2nd and 3rd yield of sowing times was greater than that of the 1st (early) sowing and their protein yield also increased.

3.7.2 The effect of fertilizer treatments at a given sowing time

The non-fertilized treatment had the lowest seed protein content in all 3 sowing times during 3 years, which is understandable, since many literatures support the protein-increasing effect of fertilization. The protein yield of the treatment that received a 100 % fertilizer dose in the 2nd sowing season of 2017 was significantly higher. In the early sowing times, the protein yield of treatments with the highest fertilizer dose was the lowest, that difference was significant in 2015. The reason for this was that the Start variety produced very little in the early sowings, because early sowing with a 150 % fertilizer dose was unfavourable for it.

3.7.3 Effect of fertilizer treatments in the average of sowing times

In 2016 the protein content of the treatment without fertilizer was significantly lower than that of the treatment with fertilizer. We did not verify a significant difference in protein yield per hectare between the fertilizer treatments.

3.7.4 The effect of sowing times in a given fertilizer treatment

In 2015 and 2017 independently of the fertilizer dose, the seed protein content of the later sowing was significantly higher than the values of the earlier sowing times. In a favourable weather, the later sowing times had significantly the highest yield and protein yield.

3.7.5 Results of treatment combinations

In examined 3 years the largest protein contents were in the third sowing time with 150 % fertilizer dose. In 2016-17 the yield and protein yield of the third sowing time were larger than the value of earlier sowing times. This was because of favourable weather. The significant effect of increased fertilizer doses was not proved.

If the weather was favourable, then the significant relationship among the sowing time, protein content, yield, and protein yield was positive and strong.

4 CONCLUSIONS

The observations of Ermolaev & Radkov (1975) and

Unk (1984) are still valid today, the yield-increased effect of fertilizers only takes effect in case of favourable weather. We verified significantly different on one time of 9 variations. Examining the effect of fertilizers by sowing times, we already showed significantly different in more cases. The protein content of treatments with fertilizer was higher than that of without fertilizer.

In 2015, we verified significantly that the protein yield of treatment with 150 % fertilizer dose was least at the 1st and 3rd sowing times, because its yield was very little by the unfavourable weather. In 2017, at the 2nd sowing time, the protein yield of treatment with 100 % fertilizer dose was significantly more than that of other fertilizer treatments.

Examining the sowing times, with the favourable weather, the 3rd sowing time was the more favourable at the protein content, yield and protein yield for the growing of the white and small-seed Start variety.

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Effectiveness of local biopesticides in the control of Diamondback Moth (*Plutella xylostella* L.) in cabbage production in Zanzibar, Tanzania

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Effectiveness of local biopesticides in the control of Diamondback Moth (*Plutella xylostella* L.) in cabbage production in Zanzibar, Tanzania

Abstract: This experiment was conducted to determine the effectiveness of different types of local biopesticides to control diamondback moth (DBM) in cabbage production in Zanzibar. The experiment was conducted in horticulture farms at Zanzibar Agricultural Research Institute. The experiment was conducted in a randomized complete block design, with 6 treatments and a control, each of which was replicated 3 times. The following plants were used as biopesticide which are garlic (T1), hot pepper (chilli) (T2), clove (T3), mixture of garlic and pepper (T4), mixture of garlic and clove (T5), synthetic insecticide (T6) and a control (T0). The parameters were evaluated by conducting initial data collection (before treatment) and final data collection (after treatment). The average number of leaves affected by DBM and yield in terms of average mass (kg) of cabbage were recorded. Results showed that T6 was significantly associated with a lowest average number of affected leaves and higher yield followed by T4 and T5. Overall, garlic-treated plots had recorded higher yield compared to the non-garlic treated plots. Therefore, garlic extract as local biopesticide can effectively repel DBM. This study recommends that garlic can be used as an alternative to using synthetic chemicals to control DBM in cabbage crop.

Key words: cabbage, biopesticide, agriculture, diamondback moth, Zanzibar, Tanzania

Učinkovitost lokalnih biopesticidov za uravnavanje kapusne sovke (*Plutella xylostella* L.) pri gojenju zelja v Zanzibarju, Tanzanija

Izvleček: Poskus je bil izveden za določitev učinkovitosti različnih lokalnih biopesticidov za nadzor kapusne sovke pri gojenju zelja v Zanzibarju. Poskus je potekal na vrtnarskih kmetijah v Zanzibarju, na Zanzibar Agricultural Research Institute. Poskus je bil izpeljan kot popolni naključni bločni poskus s šestimi obravnavanji v treh ponovitvah in kontrolo. Obravnavanja so bila sledeča: česen (T1), čili (T2), klinčki (T3), mešanica česna in paprike (T4), mešanica česna in klinčkov (T5), sintetični insekticid (T6) in kontrola (T0). Obravnavanja so bila ovrednotena z zbiranjem podatkov pred in po obravnavanjih. Pridobljeni podatki so obsegali število po sovki napadenih listov in pridelek zelja, izražen kot poprečna masa zelja (kg). Rezultati so pokazali, da je imelo obravnavanje T6 značilno najmanjše število napadenih listov in večji pridelek, temu sta sledili obravnavanji T4 in T5. Nasplošno je bil pridelk na ploskvah, kjer so sovko zatirali s česnom večji, v primerjavi s ploskvami brez česna. Iz tega lahko sklepamo, da bi lahko bili izvlečki česna uporabljeni kot učinkovit lokalni biopesticid za zatiranje kapusne sovke. Izsledki raziskave kažejo, da bi lahko uporabili česen kot alternativo sintetičnim kemičnim pripravkom za uravnavanje zeljne sovke pri pridelavi zelja.

Ključne besede: zelje, biopesticidi, kmetijstvo, kapusna sovka, Zanzibar, Tanzanija

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1 INTRODUCTION

The agriculture sector contributed an average of 25 % of the total GDP and employing the majority of the country's worksforce in Zanzibar. On average, 70 % of the population depends directly or indirectly in the agriculture sector for their livelihood. However, diseases and pests such as insect infestation in agricultural production is among a key causes of quantitative damage of the crops which finally leads to degraded in quality and quantity of food (Stathas et al., 2023).

Cabbage, *Brassica oleracea* L. ssp. *oleracea* convar. *capitata* (L.) Alef. is a green leafy vegetable with very high nutritional value and it is infested by varieties of insects. This crop is cultivated mainly by small-scale farmers in Zanzibar. The Diamondback Moth, *Plutella xylostella* (L., 1758) is one of the greatest threats to cabbage production in many parts of the world (Hill & Foster, 2000). It is a major cabbage pest that can cause a 100 % loss in yield if not well controlled and managed. In recent years, crop production in various tropical and sub-tropical countries has been largely affected by developed resistance to a wide range of synthetic chemical to control infestations. The introduction of these chemical products into the natural environment resulted in the disruption of biological balance, and poses a great threat to human, animals and environmental health (Magierowiczet al., 2020). In addition, they are known to cause cancers and abnormalities, and they remain in the environment for many years (Scholtz et al., 2002).

On the other hand, biopesticides are naturally occuring compounds or agents that are obtained from animals, plants, or microorganisms such as bacteria, and are used to control wide range of agricultural pests and pathogens (Feniboet al., 2021). The use of biopesticides to protect crops against pests has a long history in insect pest management (Moshi & Matoju, 2017). Previous literature shows that the use of biopesticides is more advantageous than the use of their conventional chemical pesticides, as they are eco-friendly and poses little threat to food safety (Khursheed et al., 2022). These opportunities coupled with increasing costs of synthetic pesticides as well as increased consumer demand of organic products have created the impetus to search for potential biopesticides.

In spite of the large number of researches conducted on effectiveness of biopesticides (Rusdi & Rusaldy, 2023), hardly any evidence, especially in Zanzibar have been conducted and published. Zanzibar has always been renowned for producing exceptionally good spices and herbs such as cloves, cinnamon, cardamon and blackpepper (Mahenya et al., 2014). Most of these products are good sources of bioactive compounds which may provide inhibitory activity against pests. In the absence of effective management options to tackle pests, there would be extensive dependence on synthetic pesticides for their management on crop, with significant negative impacts human, animal and the environment (Akutseet al., 2020). Therefore, there was a need for a research to identify specific biopesticides in the control of pests such as diamondback moth in Zanzibar. The main objective of this study was to determine the effectiveness of local biopesticides in the control of diamondback moth in cabbage production. The information obtained from this research is expected to contribute in finding solutions for for a better cabbage production among small holder farmers, especially in reducing the level of pests attack.

2 MATERIALS AND METHODS

2.1 STUDY SITE

The research was conducted in Zanzibar, Tanzania. Zanzibar is a one of the two partner states that form the United Republic of Tanzania, comprised of two main islands – Unguja and Pemba. The experiment was conducted at the horticulture farms of Zanzibar Agricultural Research Institute (ZARI) which is located at Kizimbani area. The area is about 5 kilometers from Zanzibar Town. The institute is situated at latitude 60 south, longitude 390 east and 20 m above sea level. The area receives an average rainfall of 1564 mm/annum and annual average temperature of 25.7 ^{0}C. The experiment was conducted from June to September 2021.

2.2 CABBAGE PLANTING

The chinese cabbage seeds (Michihili type) were bought from local agro-dealers directly to the field of 1000 m^2. The land was ploughed followed by harrowing on 24th June, 2021. Field measured and laid out was conducted using measuring tape, rope, a hammer and pegs to mark the planting area. On 06th July; total 63 raised planting beds of 6 x 1.5 m (length x width) were made. Each bed was separated from one another by 50 cm apart. Seeds were sown directly to the prepared seed beds on 23rd July; at a spacing of 60 cm x 30 cm. Before sowing, soils were mixed and incorporated with about 45 kg of cow dung manure per bed, whereby each plant was estimated to take 0.75 kg of manure, this was done on 14th July, 2021. Transplanting and gap filled was done on after weeding which was done three days before.

2.3 TREATMENT APPLICATIONS

There were 6 treatments which are garlic (local cultivar) water extract (T1), hot pepper (Habaner*o* type) water extract (T2), clove (local type) water extract (T3), mixture of garlic and pepper water extract (T4), mixture of garlic and clove water extract (T5), synthetic insecticide (T6) and a control (T0). These treatments were allocated randomly in an experimental area of 33m^2 each. The number of treatments were allocated in a Randomized Complete Blocked Design (RCBD) in three replications (Rusdi & Rusaldy, 2023). 1 litre of each botanical spice water extracts was applied in 3 beds of a respective treatment for all replications in the morning before 10 o'clock using a 7 litre sprayer. Garlic, chilli pepper and cloves were separately blended and grounded respectively then mixed with water and soap to make stock solutions as previously explained (Hardiansyah & Al Ridho, 2020). In summary, 1 kg of plant materials (pepper, cloves, garlic) were diluted in 5 litres of water. For mixed biopesticides (e. g garlic and cloves/ pepper), the ratio was 1:1. During application, 1 litre of stock solution was then diluted in 5 litres of water. Biopesticide application was done after 48 hours from the time bio-pesticides prepared and were applied once for both biopesticides and synthetic. Data collection was done before application of pesticides and after application on 20th August and 30th August respectively.

2.4 DATA COLLECTION

Presence of diamondback moth attack and yields were collected before and after treatments. The data collection continued untill at harvest stage (maturity). At harvest, marketable yield (mass in kg) data were recorded for each cabbage plant. The mass (kg) of cabbage was measured obtained from a randomly selected plants in each plots. The parameters for data collection were number of total leaves, number of affected leaves per plant and yield (mass) of harvested cabbage. The level of effectiveness of the studied biopesticide treatments was calculated using the formula as follows:-

$$EI = \frac{Ca - Ta}{Ca} \times 100\%$$

Description:

EI = Insecticidal (biopesticide) efficacy (%)

Ca = Number of infested cabbage leaves in the control (without treatment biopesticide)

Ta = Number of infested cabbage leaves in the biopesticide treatment.

2.5 DATA ANALYSIS

All statistical results were considered significant if $p < 0.05$. One-Way analysis of variance (ANOVA) was performed to determine the significant differences in number of affected leaves. All analyses were performed using STATA software version 16.

3 RESULTS AND DISCUSSION

3.1 DBM INFESTATION AND EFFECTIVENESS OF BIOPESTICIDE

The intensity of DMB attacks is presented in Table 1 and Figure 1 below. This study shows that T6 (synthetic insecticide) was significantly associated with the lowest average number of affected leaves among all set of treatments followed by T4 (mixture of garlic and pepper) and T5 (Mixture of garlic and clove). With reference to the control, T3 (clove only) was significantly associated with greater average number of affected leaves in cabbage plants. As T4 and T5 are local biopesticides associated with a lower attack intensity of DBM, the significant reduction of the DBM attack on cabbage after their application was indicative of the potency of garlic extract in controlling this pests. This significant finding highlights that garlic extract can be used to control DBM and other plant pests affecting cabbages such as red flour beetle, aphids, and whiteflies (Batool Syeda & Butt, 2021).

It can be seen that all treatments provided very low level of effectiveness in controlling DBM. In comparison between treatments, T4, T5 and T6 had relatively higher efficiency compared to the rest. Meanwhile, T2 and T3 exhibited negative efficiency in controlling DMB which may implies that they are not very effective. The results reported in this study are in line with the findings reported previously using garlic extract to control plant pests (Batool Syeda & Butt, 2021; Hardiansyah & Al Ridho, 2020). For-example, garlic was intercropped with cabbage and found to have a strong repellant property against DMB (Karavina et al., 2014). Treatment of garlic is known to be a potential mean than other chemical because it provide pungent smell which plant pests do not like (Batool Syeda & Butt, 2021). Garlic belongs to same family as onion and it is known to contain similar repellent properties to insects (Elmadawyet al., 2023). Also, garlic is known to contain sulphur compounds which deter insects from feeding on plants. The use of garlic to control pests was reported to be cheaper, safer and environmentally friendly. Futher research needs to examine how much and how long the level of effectiveness of

Table 1: Average number of affected leaves per plants before and after treatments and efficiency of biopesticides in cabbage

Types of treatments	Average number of leaves			Average number of affected leaves				Efficacy (%)
	Before	After	New leaves after treatment	Before	After	Net affected leaves	*P*-value	
T_0	10.03	14.87	4.83	2.67	4.13	1.47	Reff	Reff
T_1	10.47	15.20	4.73	2.47	3.70	1.23	0.382	15.9
T_2	9.87	15.00	5.13	2.00	3.77	1.77	0.154	-20.5
T_3	8.63	13.60	4.97	1.47	3.03	1.57	0.002	-6.8
T_4	9.70	14.57	4.87	1.77	2.87	1.10	0.003	25.0
T_5	9.10	14.37	5.27	1.30	2.60	1.30	< 0.001	11.4
T_6	9.47	14.93	5.47	1.73	2.57	0.83	0.001	43.2

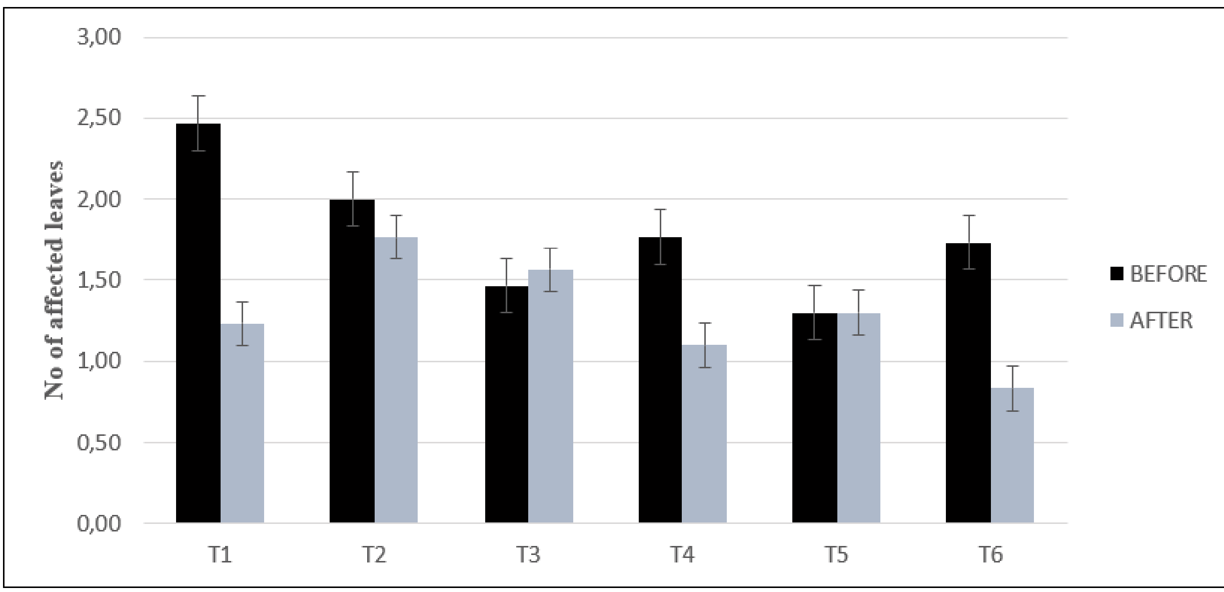

Figure 1: Average number of affected leaves per plant before and after treatment

garlic is related to the function of DBM and other pests during dry and rainy season.

3.2 YIELD OF CABBAGE

The findings concerning average mass (kg) of harvested cabbage is presented in Table 2. Result shows that the highest yield was found in T6 followed by T1 and T4. Even though the garlic extracts reduced DBM attacks, their yield performance was not comparable to the synthetic insecticide. This may be due to the fact that the active ingredient of garlic, allicin is known to degrade very fast compared to chemicals (Baidoo & Mochiah, 2016). This study showed that there was no statistically significant variations in yield among treatments ($p > 0.05$). As shown earlier, T1, T4 and T6 had significantly lower intesity of DBM attack, which may influence good growth and development of cabbages. However, data shows that the lowest yield were in T0 and T5. Further analysis revealed that there was significant difference in yield between T0 with T1 and T6, which may be attributed by the differences in the intensity of DBM infestation (Table 1). In comparison, Baidoo and Mochiah (2016) found that yield of plots sprayed with garlic were significantly higher compared to the control (Baidoo & Mochiah, 2016).

4 CONCLUSION

It can be concluded that using garlic extract as local biopesticide can effectively repel diamondback moth. Therefore, this study recommends that it can be used as an alternative to using synthetic chemicals to control

Table 2: Average mass of cabbage after harvest

Types of treatments	Mass (Kg)
T_0	0.46
T_1	0.63
T_2	0.54
T3	0.51
T4	0.58
T_5	0.47
T_6	0.64

diamondback moth in cabbage crop. Further studies are needed to confirm the effectiveness of biopesticides to control plant pests for other horticultural products in different planting seasons.

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4.2 DATA AVAILABILITY

The data to support this findings are available from authors upon special request.

4.3 CONSENT FOR PUBLICATION

Not applicable.

4.4 COMPETING INTERESTS

The authors declare no conflict of interest.

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Herbicides weed management in changing environmental conditions

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Herbicides weed management in changing environmental conditions

Abstract: Elevate CO_2 levels in the atmosphere might have prominent effects on weed phenology, consequently changing herbicide performance on weeds. Increased atmospheric CO_2 concentration increase leaf thickness and reduce stomatal number and conductance potentially reducing the absorption of POST–emergence applied herbicides. From the other side, higher temperature stimulates stomata conductance, reduce the viscosity of epicuticle waxes, thus increasing the penetration and diffusion of herbicides as a result of changes in the composition and the permeability of the cuticle. However, in some circumstances higher temperatures might cause hastened metabolism, which consequently decreases herbicide activity on target plants. In conditions of higher RH, cuticle hydration and stomatal conductance increases, consequently increases the permeability and translocation particularly of hydrophilic herbicides into the leaves. Similar, under higher irradiance, stomata stay open, photosynthetic rate increases consequently increasing absorption, penetration and subsequent phloem translocation of POST–em systemic herbicides in weed tissue. Drought might cause increased cuticle thickness and increased leaf pubescence, with consequent reductions in herbicide absorption into the leaves. Rainfall after POST–emergence herbicides application might reduce their efficiency through washing out. Increased frequency and intensity of precipitation will have a negative effect on absorption, translocation, and activity of PRE–emergence herbicides.

Key words: environmental conditions, weeds, control, herbicides

Uravnavanje plevelov s herbicidi v razmerah spreminajočega se okolja

Izvleček: Povečane koncentracije CO_2 v ozračju bi lahko imele znatne učinke na fenologijo plevelov kar bi posledično lahko spremenilo učinkovanje herbicidov nanje. Povečane koncentracije CO_2 v ozračju povečujejo debelino listov in zmanjšujejo število rež, kar potencialno zmanšuje njihovo prevodnost in potencialno zmanjšuje absorbcijo POST–em nanešenih herbicidov. Po drugi strani višje temperature pospešujejo prevodnost rež in zmanjšujejo viskoznost epikutikularnih voskov in s tem povečujejo penetracijo in difuzijo herbicidov kot posledico sprememb v sestavi in prevodnosti kutikule. V nekaterih razmerah lahko višje temperature pospešijo presnovo, ki posledično lahko zmanjša aktivnost herbicidov na tarčnih rastlinah. V razmerah večje relativne zračne vlažnosti se povečata hidratacija kutikule in stomatarna prevodnost kar posledično poveča permeabilnost in translokacijo, še posebej hidrofilnih herbicidov v liste. Podobno v razmerah večjega obsevanja ostajajo reže dalj časa odprte, povečana fotosinteza posledično poveča absorbcijo, penetracijo in translokacijo sistemskih POST-em herbicidov po floemu v tkiva plevelov. Suša lahko povzroči povečanje debeline kutikule in dlakavosti listov kar posledično zmanjša absorbcijo herbicidov vanje. Dež lahko po nanosu POST–em herbicidov zmanjša njihovo učinkovitost zaradi izpiranja. Povečana pogostost in jakost padavin imata negativni učinek na absorbcijo, translokacijo in aktivnost PRE–em herbicidov.

Ključne besede: okoljske razmere, pleveli, nadzor, herbicidi

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1 INTRODUCTION

Agriculture production in terms of quantity and quality, as well as agronomic practices, including weed management, may be affected significantly in conditions of climate change (Varanasi et al., 2016). Elevating CO_2 levels associated with changes in temperature and precipitation are important concerns for upcoming weed management and crop production. Taking into account the greater physiological flexibility (Ziska et al., 2010; Davidson et al., 2011; Billore, 2019) and their greater intra specific genetic variation (Dukes & Mooney, 1999), weeds are expected to show greater competitiveness and better accommodation regarding increasing CO_2 concentrations and temperature in comparison with crops (Singh et al., 2011; Varanasi et al., 2016). Considering its positive effect on weed growth, shifting environmental conditions will impact directly or indirectly on the weed control methods by reducing their effectiveness on weeds and making them a considerable issue for sustainable agriculture production as well as costlier in same time (Ziska et al., 1999; Karl et al., 2009). Climatic variation factors are estimated to have significant effects on the growth and physiological processes of weedy plants, like growing rate, stomatal conductance, and photosynthetic efficiency (Fuhrer, 2003; Manisankar & Ramesh, 2019). Elevate CO_2 and temperature, sunlight intensity, relative humidity, rainfall, and drought influence the coverage, penetration, translocation, persistence and activity of herbicides (Muzik, 1976; Hatzios & Penner, 1982; Bailey, 2003; Bailey, 2004; Malarkodi et al., 2017). Additionally, interactions among these environmental factors may have uncertain consequences on herbicide efficacy (Sutherland et al., 2017). Numerous studies confirmed that shifting climate conditions might also decrease the susceptibility of weeds to some herbicides (Varanasi et al., 2016; Ziska, 2016; Fernando et al., 2016; Matzrafi et al., 2018). For example, elevated CO_2 reduced the efficacy of glyphosate and glufosinate against *Cirsium arvense* (L.) Scop., and *Elytrigia repens* (L.) Desv.ex Nevski (Ziska & Teasdale, 2000). Similarly, Manea et al. (2011) reported that glyphosate efficacy at increased CO_2 concentrations is diminished in C_4 weeds such as *Eragostis curvula* (Schrad.) Nees, *Paspalum dilatatum* Poir., and *Chloris gayana* Kunth, as a result of increased leaf area and total plant biomass. Higher temperatures may worse the consistency of cuticular lipids, thereby increasing the absorptivity and penetration of herbicides through the cuticle (Price, 1983; Patterson et al., 1999); for example, uptake and translocation of ^{14}C-glyphosate was found to be higher at 22 °C than at 16 °C in *Desmodium tortuosum* (Sv.) DC. (Sharma & Singh, 2001). Although tendency of elevated air temperatures is to increase absorption and translocation of most POST-em applied herbicides (Patterson et al., 1999), in some cases higher temperatures also might encourage rapid metabolism, which consequently decreases herbicide efficacy on target plants (Kells et al., 1984; Madafiglio et al., 2000; Medd et al., 2001; Johnson & Young, 2002). Increased CO_2 and temperature might change weed growth phenology, with shortened the period spent in the seedling stage, i.e. the stage of greatest POST-em herbicide efficacy (Ziska et al., 1999). Also, changes in these factors caused alteration in leaf morphology, leaf surface characteristics or variation in root-to-shoot ratio which affect herbicide absorption, distribution and efficacy (Olesen & Bindi, 2002; Poorter & Navas, 2003; Ziska et al., 2004; Dukes et al., 2009). Additionally, enhance in tuber and rhizome growth, joined with enhance in biomass, particular in perennial weeds (Oechel & Strain, 1985), would induce a dilution effect on any herbicide treatment (Patterson, 1995), making their control more complicated (Patterson et al., 1999). Modifications in environmental factors, such as drought spells or prolonged rainy periods, might restrict the field conditions necessary for optimal herbicide applications (Amare, 2016). Generally, dry soil conditions decrease the activityof PRE-em herbicides, affect their behavior in the soil and the herbicide effectiveness “windows” due to strong herbicide adsorption (Bailey, 2004; Howden et al., 2007), whereas severe or frequent rainfall after the application may cause herbicide leaching (Soukup et al., 2004; Pacanoski & Mehmeti, 2021) and dilution (Kanampiu et al., 2003).

2 INTERACTION CO_2 – HERBICIDE EFFICACY

The importance of interaction CO_2 concentration - herbicide efficacy has occupied research attention in recent decades as a result of the constant increase in concentrations of atmospheric CO_2. Elevate CO_2 levels in the atmosphere might have prominent effects on weed phenology (Anwar et al., 2021), consequently altering herbicide effectiveness on weeds (Ziska, et al., 1999; Ziska & Teasdale, 2000; Ziska et al., 2004; Ziska & Runion, 2007). One of the most pronounced effects of increased CO_2 concentrations is the minimizing of stomatal conductance, which could increase up to 50 % in some weeds (Bunce, 1993). Minimized number and stomatal conductance with increasing CO_2 could decrease transpiration resulting in decreased herbicide absorption and efficacy, particularly of POST-em applied herbicides (Bunce & Ziska, 2000; Ziska & McClung, 2008; Ziska, 2008). Additionally, Nowak et al. (2004) and Ainsworth & Long (2005) indicated that C_3 and C_4 weeds grown in condition of increased CO_2 concentrations have increased leaf pubescence and developed thicker cuticle. Apart from increasing leaf thickness, increased CO_2 concentrations might also generate partially stomatal closure (Ziska, 2008; Jackson et al., 2011). These characteristics might minimize up take and efficacy of POST-em applied herbicides. Manea et al. (2011) found that in three of four C_4 grass species tolerance to glyphosate in conditions of raised CO_2 is significantly increased. Similar results were obtained by Ziska & Goins (2006). The explanations for the minimized efficacy of the herbicides could be that elevating CO_2 increase leaf consistency and reduce stomatal number and their conductivity potentially reducing the absorption of POST-em applied herbicides. Furthermore, an increase in the apparent photosynthesis rates as a result of increased CO_2 concentrations, mainly in C_3 weeds, might cause rapid seedling growth, the most susceptible stage for optimal weed control, which could modify the efficacy of POST-em herbicides. For example, *Chenopodium album* L., a C_3 weed, demonstrated higher tolerance to glyphosate as a result of increased growth and plant biomass at raised CO_2 concentration (Ziska et al., 1999). In addition, perennial weeds may become even more troublesome, if vegetative growth is stimulated as a result of increased photosynthesis in relation to elevated CO_2. This could be due to less herbicide translocation as the root system becomes more vigorous. In this context, *Elymus repens (L.) Gould* (Ziska & Teasdale, 2000) and *Cirsium arvense* (Ziska et al., 2004) showed prominent tolerance to glyphosate due to elevated CO_2 levels, which caused large stimulation of belowground growth. Elevate CO_2 levels increases concentration of starch in leaf tissue (Patterson, 1995), particularly in C_3 weeds (Wong, 1990), but reduce protein concentration (Bowes, 1996; Taub et al., 2008; Loladze, 2014). Reduction of protein content results to diminished demand for aromatic and branched-chain amino acids synthesis, which may reduce the efficacy of many herbicides, including ALS and EPSPS inhibitors (Patterson et al., 1999; Varanasi et al., 2016). Changed environmental conditions, particularly rising of CO_2 concentration and temperatures, stimulate weed growth through modification of photosynthesis, pigment production, as well as overall metabolic activity. Because of that, herbicides photosystem I and II and pigment inhibitors may become more effective. However, the effects of rising CO_2 on herbicide efficacy is species determined. Namely, at double atmospheric CO_2 concentrations, efficacy of metsulfuron on *Amaranthus retroflexus* L. decreased by 4.6 %, efficacy of imazethapyr in control of *Stellaria media* (L.) Vill. was unchanged, whereas efficacy of imazamethabenz-methyl over *Avena fatua* L. improved by 15.7 % (Archambault et al., 2001). According same authors, in the same conditions, efficacy of linuron in control of *Polygonum convolvulus* L. was reduced by 15 %, whereas status quo in the efficacy was reported for metribuzin on *Chenopodium album* L. and bromoxynil on *Kochia scoparia* (L.) Schrad., respectively. The effects of rising CO_2 on ACCase inhibitors varied and depend on weed species. At double–environment CO_2 concentrations clodinafop efficacy in control of *Avena fatua* increased by 8.6 %, while *Avena fatua* L. control was not affected by sethoxydim. No change in the efficacy was reported for control of *Avena fatua* and *Setaria viridis* (L.) P.Beauv.by fluazifop (Archambault et al., 2001). Further, decreasing of clopyralid efficacy for 8.9 % was noted in control of *Senecio vulgaris* L., whereas increasing of efficacy of 2,4-D for 26.9 % was obtained in control of *Polygonum convolvulus* L. (Archambault et al., 2001). Increased frequency of herbicide applications might exceed CO_2 caused declines in efficacy, but might bring additional risks for human and animal health because it might increase the occurrence and concentration of these chemicals in the environment (Ziska et al., 2004).

3 INTERACTION TEMPERATURE − HERBICIDE EFFICACY

Temperature has multiple impacts on weed growth and development as well as herbicide efficacy. Alterations in the apparent photosynthesis rate, respiration, phloem translocation, and protoplasmic flux, as well as rate of water up take and transpiration, leaves formation, cuticle compactness and hydration, number and aperture of stomata will affect uptake, diffusion, and metabolism of herbicides (Bailey, 2004; Zanatta et al., 2008; Rodenburg, et al., 2011). Higher temperature encouraged stomata conductivity, reduced the viscosity of cuticle waxes, thus increasing the uptake and diffusion of herbicides as a result of modifications in the structure and the permeability of the cuticle (Price, 1983; Chandrasena, 2009). *Abutilon theophrasti* Medik. plants treated with acifluorfen at lower (20/15 °C) day/night temperature regime showed 70 % higher production of epicuticular wax on the leaf surface than plants in condition of higher (32/22 °C) day/night temperature. Decreasing of the wax production was connected with better efficacy of herbicides when temperature increased, corroborating the assumption of higher herbicide efficiency as cuticle structure altered. This study also confirmed that when temperature increased from 20/15 °C to 32/22 °C, there was a 25 % increase in the acifluorfen absorption applied alone and 99 % increase in acifluorfen absorption applied with oil–based surfactant (Hatterman-Valenti et al., 2011). Ganie et al. (2017) noted that the efficacy of 2,4-D and glyphosate in control of *Ambrosia artemisiifolia* L. and *Ambrosia* *trifida* L. might be enhanced if applied at higher (29/17 °C) day/night temperature regime, because of improved absorption and translocation compared with applications during lower (20/11 °C) day/night temperatures. Study in greenhouse conditions using different night/day temperatures (5/10 °C, 15/20 °C, and 20/25 °C) demonstrated that *Raphanus raphanistrum* L. grown in conditions of lower (5/10 °C) temperatures was poorly controlled with 1,200 g ai ha^{-1} of glufosinate. Contrary, 100 % mortality was achieved under higher temperatures15/20 °C and 20/25 °C, respectively for the same dose (Kumaratilake & Preston, 2005), indicating increased efficacy of glufosinate under increased air temperature. Flumiclorac exhibited higher activity on *Amaranthus retroflexus* (threefold) and *Chenopodium album* (sevenfold) with increasing of temperatures from 10 °C to 40 °C (Fausey & Renner, 2001). Johnson & Young (2002) reported for threefold increase in mesotrione efficacy in control of *Abutilon theophrasti* and *Xanthium strumarium* L. with increasing of temperatures from 18 °C to 32 °C. Similarly, at higher temperature, fluthiacet was twice and three times more effective in control of *Amaranthus retroflexus* and *Chenopodium album*, respectively, compared to the efficacy observed at 10 °C (Fausey & Renner, 2001). Atrazine applied at 15:00 h, when the air temperature was the highest, provided the greatest control of *Ambrosia artemisiifolia* L. and *Abutilon theophrasti* (Stewart et al., 2009). Stopps et al. (2013) confirmed that glyphosate efficacy in control of *Ambrosia artemisiifolia* L. and *Abutilon theophrasti*, *Amaranthus* spp., increased when herbicide was applied between noon and 6 pm, which coincides to the higher air temperatures during the day. Contrary, the efficacy of bromoxynil on *Abutilon theophrasti* declined by up to 45 % when applied at 24:00 h, when the air temperature was the lowest (Stewart et al., 2009). Irrespective of temperature increase, dicamba/diflufenzopyr provided > 95 % control of *Amaranthus retroflexus,* and *Ambrosia artemisiifolia*, *Chenopodium album*. On the other hand, lower temperatures reduced control of *Abutilon theophrasti* by 7 % to 15 % (Stewart et al., 2009). Similar, in research of Ziska et al. (1999) glyphosate efficacy was reduced in control *Ambrosia trifida* and *Ambrosia artemisiifolia* at low temperatures.

Although the tendency of higher atmospheric temperatures is to enhance absorption and translocation of most POST-em applied herbicides, in some circumstances higher temperatures might cause hastened metabolism, which consequently decreases herbicide activity on target weeds (Johnson & Young, 2002). Enhanced metabolism rate was the reason for reduction of pinoxaden efficacy on *Brachypodium hybridum* (L.) P. Beauv. control and other grasses in conditions of higher temperature (Matzrafi et al., 2016). Ou et al. (2018) tested effects of temperature on *Kochia scoparia* (L.) Schrad. growth treated with glyphosate and dicamba under three day/night temperatures: 17.5/7.5 °C; 25/15 °C; and 32.5/22.5 °C. Visual above-ground dry biomass, injury and mortality data indicated greater sensitivity to both glyphosate and dicamba when *Kochia scoparia* was grown in conditions the two cooler day/night temperature regimes. Similar trend was noted in investigation of Kleinman et al. (2016) when *Conyza bonariensis* (L.) Cronq., *Conyza canadensis* (L.) Cronq., and *Kochia scoparia* were treated with glyphosate. A significant variation in control of *Amaranthus palmeri* S. Watson with mesotrione was obtained when the weed was grown in conditions of low and high day/night temperature regimes (25/15 °C and 40/30 °C, respectively) compared to optimum day/night temperature (32.5/22.5 °C). Related to weed height, injury, and mortality, *Amaranthus palmeri* S. Watson was more susceptible to mesotrione at 25/15 °C and less susceptible at 40/30 °C compared to 32.5/22.5 °C (Godar et al., 2015). Pyrithiobac provided higher efficacy in control of *Amaranthus palmeri* at 18 °C (25 % dry mass accumulation) than at 40 °C (70 % dry mass accumulation), although the highest efficacy was recorded at 27 °C (only 2.5 % dry weight accumulation) (Mahan et al., 2004). Mesotrione efficacy in control of *Digitaria sanguinalis* (L.) Scop. and *Amaranthus rudis* J.D.Sauer decreased by six and seven times when temperature increased from 18 °C to 32 °C (Johnson & Young, 2002). Increased temperatures as well as increased metabolic activity of the weeds nullify increased herbicide translocation, because herbicide metabolisation increases at higher temperature, as well (Martini et al., 2015; Matzrafi et al., 2016). Higher temperatures also might generate diminishing of herbicide absorption due to quick drying of spray droplets to solid deposits (Devine et al., 1993) and volatility of some herbicides, such as growth regulators herbicides causing in vapor drift and possible injury on non target broadleaf crops (van Rensburg & Breeze, 1990; Strachan et al., 2010).

Further, soil temperature has an effect on the absorption and translocation of PRE-em herbicides within the weed plant, as well as their persistence in the soil (Rodenburg et al., 2011). Warmer soil temperatures might reduce efficacy of PRE-em herbicides through rising volatility and degradation by soil microorganisms. For example, higher temperature had a great impact on the volatilization of the triallate from the soils. According Atienza et al. (2001) triallate losses increased from 7 % to 41 % in loamy soil and 14 % to 60 % in sandy soil, respectively with rising temperatures from 5 °C to 25 °C. Opposite, in the controlled trial conditions, low soil temperatures (around 10 °C) decreased the efficacy of alachlor and EPTC (Mulder & Nalewaja, 1978).

4 INTERACTION RELATIVE HUMIDITY – HERBICIDE EFFICACY

Relative humidity (RH) is mainly important for the activity of POST-em herbicides through its effects on herbicide absorption, including interactions between the herbicide droplets, leaf cuticle, and accessibility of water in or round droplets (Devine et al., 1993). In conditions of higher RH, cuticle hydrating and stomatal conductivity increases, consequently increases the penetrability and translocation particularly of hydrophilic herbicides into the leaf surface (Kudsk et al., 1990; Wichert et al., 1992; Shaw et al., 2000; Hatterman-Valenti et al., 2011). Penetration as well as efficacy of most POST-em herbicides is usually higher when weeds were exposed to higher RH after spraying than before, concluding that slowly droplets drying might be the reason for higher efficacy at higher RH levels rather than cuticle hydrating (Ramsey et al., 2002). The susceptability of *Digitaria sanguinalis* and *Amaranthus rudis* to mesotrione was two and four–times higher at 85 % RH compared with 30 %, respectively (Johnson & Young, 2002). Glufosinate ammonium efficacy in control of *Avena fatua* significantly increased (> 95 %) at higher RH compared with its efficacy at lower (40 %) RH. Additionally, penetration of glufosinate ammonium was higher when *Avena fatua* plants were exposed to higher RH for 30 min before and after application compared with those left at constantly lower RH (Ramsey et al., 2002). Efficacy of acifluorfen on *Ambrosia artemisiifolia* and *Xanthium strumarium* was 30 % higher when it was applied at 85 % RH compared with its efficacy at 50 % RH (Ritter & Coble, 1981). Likewise, acifluorfen, fomesafen, and lactofen provided higher efficacy in control of *Ipomoea lacunosa* L., *Ipomoea hederacea* Jacq. var. *integriuscula, Sida spinosa* L., and *Xanthium strumarium* at 85 % RH, compared to the condition of 50 % RH (Wichert et al., 1992). Similarly, when the efficacy of acifluorfen was estimated on trials carried–out for two consecutive years, it was concluded that there was higher control of *Xanthium strumarium* obtained in the year of higher RH condition (Shaw et al., 2000). Casley & Coupland (1985) stated that higher RH increased glyphosate performance due to slower evaporation from the plant surface, while Mathiessen & Kudsk (1996) claimed that higher RH had no significant influence on glyphosate efficacy.

5 INTERACTION SUNLIGHT INTENSITY – HERBICIDE EFFICACY

Alterations in sunlight intensities influence on the plants anatomy, morphology, and physiology, which consequently have an effect on herbicide performance in the plants. Stomatal conductivity and formation of leaf cuticle are positively correlated with sunlight intensity (Hull et al., 1975; Raschke et al., 1978). Under conditions of higher irradiation, stomata stay open, photosynthetic rate increases consequently increasing uptake, penetration and subsequent phloem translocation of POST-em applied herbicides in weed plant tissue (Fausey & Renner, 2001; Hwang et al., 2004; Camargo et al., 2012). Efficacy of clethodim, talkoxydim and bentazon proportionally increased with increasing of sunlight intensity (McMullan, 1996, Hatterman-Valenti et al., 2011). In study of Fausey & Renner (2001) flumiclorac provided nine times higher control of *Chenopodium album* at light intensity of 1,000 μmol m^{-2} s^{-2} than at 4 μmol m^{-2} s^{-2}. Control of *Amaranthus retroflexus* was 15 times more effective with the same herbicide under higher light intensity compared to the lower one. In same study, fluthiacet was also more effective in control of these two species at irradiance condition of 1000 μmol m^{-2} s^{-2}, as compared to the efficacy obtained at 4 μmol m^{-2} s^{-2}. Similar, oxadiazon and oxadiargyl reduced the growth of *Echinochloa crus–galli* (L.) P.Beauv. in the presence of light, but were completely ineffective in the dark (Hwang et al., 2004). UV light reduced the efficacy of tralkoxydim and clethodim, which indicates that application of these graminicides when sunlight intensity is higher during the day might increase their efficacy. Filtering UV light for 4 h after application improved efficacy of these herbicides between 13 and 55 % (McMullan, 1996). UV light is obviously significant to cyclohexanedione herbicide efficacy because these herbicides are unstable in UV light (Campbell & Penner, 1985; Falb at al., 1990; McInnes et al., 1992). Similar, ^{14}C-paraquat penetration and efficacy in control of *Abutilon theophrasti*, *Chloris virgata* Sw. and *Digitaria sanguinalis* was reduced during the UV-B treatment because of increasing leaf epicuticular wax deposition (Wang et al., 2006). On the other hand, in lower irradiance conditions, tendency of plants is to form thinner leaves with greater specific leaf surface and plant height to catch accessible sunlight required for photosynthesis. These adjustments in weed growth and leaf mofphology determinate the herbicide amount that is received and retained by the weed (Upasani & Barla, 2018). For example, surface coverage as well as absorption of POST-em herbicides is enhanced in weed with higher branching, whereas leaves with thicker structure retard herbicides penetration causing decreased herbicide efficacy (Riederer & Schonherr, 1985).

6 INTERACTION DROUGHT AND RAINFALL PATTERN − HERBICIDE EFFICACY

Herbicides might become less effective because of alteration of the external environment (drier and warmer conditions) or alterations in anatomy, physiology, and phenology of the weed flora (Clements et al., 2014; Chauhan et al., 2014; Ziska & McConnell, 2015). In this context, POST-em herbicide efficacy might be significantly influenced by drought. Drought might cause enlarged cuticle thickness and intensify grow of leaf pubescence, with consequent reductions in herbicide penetration into the leaves (Patterson, 1995). For example, the weed cuticle under arid conditions was 50-80 % thicker relative to optimal available water situations (Hatterman-Valentiet al., 2011). Increasing aridity and drought might reduce herbicide penetration, intensify herbicide volatilization, and consequently reduce its effectiveness. Drought influenced weeds are more challenge for control with POST-em herbicides than weeds that are actively growing in conditions without environmental stress. For example, for systemic POST-em applied herbicides is necessary active weed growth to be effective. In that context, in conditions of drought spells efficacy of glyphosate in control of *Abuthilon theophrasti* was reduced two and eight–fold when it was applied in two and six leaves weed growth stages, respectively (Zhou et al., 2007). Survival of glyphosate-resistant biotype of *Echinochloa colona* (L.) Link treated with double glyphosate rate (1440 g ha^{-1}) in condition of no water deficiency was only 19 %, but under water deficiency this value increased by 62 % (*Mollaee et al., 2020)*. Likewise, under dry soil conditions usually activity of PRE-em herbicides is reduced the due to strong herbicide soil adsorption (Arıkan et al., 2015). These herbicides are highly dependent on accessible water for relocation into the zone of weed seed germination (Olson et al., 2000). Herbicide photodecomposition is common process which takes place on the soil surface, and if optimal moisture does not become accessible in period of few days after application, weed control is often inadequate. Even for considerably persistent herbicides, inability to penetrate into the soil surface because of the moisture shortage give weeds opportunity to germinate without any herbicide injuries. Jursık et al. (2013) claimed a reduced pethoxamid efficacy under dry soil conditions. Contrary, increased soil moisture promotes the efficacy of many, PRE-em herbicides, including PROTOX inhibitors (Hatterman-Valenti et al., 2011).

Rainfall after POST-em herbicides application might reduce their efficiency through washing out. Increased frequency and intensity of precipitation will have a negative effect on penetration, translocation, and activity of PRE-em herbicides (Bailey, 2004; Rodenburg et al., 2011). An unusual increase in precipitation might cause leaching of PRE-em herbicides (Soukup et al., 2004; Pacanoski & Mehmeti, 2021), and consequent crop injury (Pacanoski et al., 2020) and under soil water contamination (Froud-Williams, 1996). From the other side, scarce rainfall amounts during the season might cause water–deficit conditions that impact herbicide efficacy (Zanatta et al., 2008; Keikotlhaile, 2011). For example, situations of water deficit reduced the absorption of acifluorfen (Hatterman-Valenti et al., 2011). Pereira et al. (2011) reported that *Eleusine indica* (L.) Gaertn. grown under water–stress conditions was not effectively controlled by sethoxydim. Similarly, control of *Eleusine indica* with fenoxaprop-p-ethyl, topramezone, foramsulfuron, 2,4-D + dicamba + MCPP + carfentrazone, and thiencarbazone-methyl + foramsulfuron + halosulfuron-methyl at soil moisture contents < 12 % was unsatisfactory (Shekoofa et al., 2020). *Urochloa plantaginea* (Link) R.D.Webster grown under water–deficit stress was less susceptible to ACCase–inhibiting herbicides when applied during the later growth stages (Pereira, 2010).

7 MULTIPLE INTERACTIONS

Atmospheric CO_2 and air temperature elevate simultaneously. The result can be completely different when both factors are taken into account together in comparison when only one factor is considering**.** In weeds, alteration in temperatures and CO_2 concentrations might modify net photosynthesis rates resulting with modification in carbohydrate accessibility and stability causing in altered weed physiological and biochemical capabilities. Increased CO_2 and atmospheric temperature might decrease herbicide efficacy by changing herbicide penetration, translocation and metabolism, subsequently increasing herbicide decomposition in weeds and decreasing herbicide availability for the target weed (Matzrafi, 2019). For example, reduced glyphosate susceptibility was observed in *Chenopodium album* and *Conyza canadensis* in response to elevated temperature, (32/26 °C) combined with raised CO_2 (720 ppm). According obtained results by Matzrafi et al. (2019), 61.1

%, 69.0 % and 64.0 %, respectively of the plants tested survived in conditions of mutual effects of higher temperature/elevated CO_2 concentration. Further, the efficacy of cyhalofop-butyl was reduced about 50 % in multiple–resistant *Echinochloa colona* plants grown under higher CO_2 concentration (700 ± 50 ppm) or high (35/23 °C) day/night temperature regime compared to multiple–resistant plants at ambient conditions. Higher CO_2 and temperatures increased the level of resistance to multiple–resistant *E. colona* to cyhalofop–butyl, as well (Refatti et al., 2019). Opposite, mutual effects of ambient CO_2 concentration (400-450 ppm) and day/night temperature (20/10 °C) and increased CO_2 concentrations (400-450 ppm, 800–900 ppm) and day/night temperature (25/15 °C), did not reduce efficacy of glyphosate in control of *Lactuca serriola* L., *Hordeum murinum* L., and *Bromus tectorum* L. (Jabran & Doğan, 2018). Interaction between CO_2 concentrations and water deficiency was studied by *Weller et al. (2019). According their results, e*fficacy of glyphosate in control of glyphosate resistant and susceptible *Chloris truncata* R.Br. biotypes in condition of moisture stress (50 % field capacity) and increased CO_2 level (750 ppm) was significantly reduced. Few studies have examined correlation between temperatures and RH. When higher temperatures are related with higher RH levels, there is increased cuticle hydrating, which consequently increases herbicides penetration and efficacy (Price, 1983). With simultaneous temperature and RH increasing, the efficacy of metribuzin also increased, while at lower temperatures (10 °C and 20 °C) caused no significant decreasing in its efficacy (Gealy & Buman, 1989). Opposite, glufosinate ammonium provided higher efficacy on *Setaria faberi* Herrm. at higher RH as well as higher temperature (Anderson et al., 1993).

8 CONCLUSION

The successfulness of weed management is predicted to change together with the changing of environmental conditions. In conditions of rising CO_2 and air temperature, and unpredictable drought spells and prolonged rainfall forecasts, the possibility of herbicides either to generate crop injure or being ineffective at weed control is expected, as well. Elevated CO_2 and temperatures might cause anatomical, morphological and physiological changes in weeds, their growth and development, all that could impact on absorption, translocation, and metabolism of herbicides and on the entire efficacy of herbicides. Conditions of soil water deficiency decrease the activity of PRE-em herbicides, affect their persistence in the soil and the "windows" for herbicide effectiveness due to strong herbicide adsorption, while severe or frequent rainfall after the application may cause herbicide leaching and dilution. One–sided and repeated herbicide use is estimated to result in appearance of resistant weed biotypes. Changes of environmental conditions might hasten this. In these circumstances, additional herbicide applications at higher rates might be needed to control such weeds, but additional activities increase the cost of control. Modification strategies are existing, but the expenditures of realizing such strategies (e.g. herbicide with new active ingredient, higher herbicide rates) are uncertain. Specific national legislation regulated herbicides use. In case of changing environmental conditions, which encourage weed species spreading out of their geographical boundaries, new herbicidal active ingredient might be essential to control them effectively. Commonly it takes a lot of time to obtain state agreement for a new herbicide active ingredient or an active ingredient that is not been previously used locally.

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Možnosti nekemičnega zatiranja virusonosnih ogorčic *Xiphinema index* Thorne & Allen, 1950

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Non-chemical control options against the virus vector nematodes *Xiphinema index* Thorne & Allen, 1950

Abstract: Nematodes are widespread organisms that exhibit remarkable ubiquity, biodiversity, and adaptability in diverse ecosystems. While most nematodes are beneficial, there are also some parasitic species that have harmful effects. Among these plant-parasitic nematodes is the species *Xiphinema index*. It primarily colonizes the root environment of grapevines (*Vitis vinifera* L.), as these vines serve as its primary host. Although the nematode's direct effects on roots are not particularly problematic, it poses a significant threat to grapevine because it can transmit and introduce *Grapevine Fanleaf Virus* (GFLV), a member of the genus *Nepovirus*. Infection with GFLV can result in yield losses of over 80 %. Therefore, it is imperative to take preventive measures to contain the uncontrolled spread of nematodes and the resulting infections in vineyards. In addition to transmission through planting material, agricultural machinery and implements in vineyards are also important vectors. Traditional chemical methods of controlling *X. index* have proven ineffective due to the nematodes' resilience and widespread distribution in the soil. Future efforts should therefore focus on pursuing alternative, more effective approaches. In addition to intercropping, the efficacy of bacterial and fungal preparations has also been tested and offers great potential for further research.

Key words: virus-transmitting nematodes, *Xiphinema index*, nepoviruses, *Vitis vinifera*, GFLV, nematicides, biological control

Možnosti nekemičnega zatiranja virusonosnih ogorčic *Xiphinema index* Thorne & Allen, 1950

Izvleček: Ogorčice so organizmi, ki jih zaradi njihove številčnosti, raznolikosti in prilagodljivosti najdemo praktično povsod. Medtem ko je večina ogorčic koristnih, pa poznamo tudi take, ki s svojim parazitiranjem povzročajo škodo. Med škodljive ogorčice uvrščamo tudi rastlinsko-parazitsko vrsto *Xiphinema index*. Najdemo jo lahko v bližini korenin žlahtne vinske trte (*Vitis vinifera* L.), saj je prav ta njena glavna gostiteljica. Ogorčica ni tako problematična z vidika neposrednega napada korenin, ampak vinski trti predstavlja grožnjo zaradi prenosa in vnosa virusa pahljačavosti listov vinske trte (GFLV) iz rodu *Nepovirus*. Virus namreč na vinski trti povzroči bolezen kužne izrojenosti vinske trte, kar vodi v ekonomsko nekonkurenčnost vinogradov. Okužba lahko privede tudi do več kot 80 % izpada pridelka. V izogib nenadzorovanemu širjenju ogorčic in posledično okužbam v vinogradih je pomembna preventiva, saj poleg prenosa s sadilnim materialom, pomembnega prenašalca predstavljata tudi kmetijska mehanizacija in fizični prenos z orodjem. Ker se je kemično zatiranje ogorčice *X. index* zaradi njene trdoživosti in razporeditve v tleh izkazalo za neučinkovito, je potrebno v prihodnje stremeti k alternativnim in predvsem učinkovitejšim pristopom. Poleg vmesnih posevkov so preverjali delovanje pripravkov na podlagi nekaterih bakterij in gliv, ki predstavljajo velik potencial za nadaljnja raziskovanja.

Ključne besede: virusonosne ogorčice, *Xiphinema index*, nepovirusi, vinska trta, GFLV, nematocidi, biotično varstvo

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1 UVOD

Ogorčice so najbolj raznolike in najštevilčnejše predstavnice večceličnih organizmov, ki so prisotne tako v kopenskih kot tudi vodnih ekosistemih, in lahko parazitirajo večino rastlinskih in živalskih vrst (Smythe in sod., 2019; Lazarova in sod., 2021). Prav zaradi velike raznolikosti in fenotipske prilagodljivosti ocenjujejo, da se je tekom evolucije razvilo do približno milijona različnih vrst (Hugot in sod., 2001; Smythe in sod., 2019). Najštevilčnejše so v tleh živeče ogorčice (Lazarova in sod., 2021). Večina vrst talnih ogorčic je v kmetijstvu koristnih, saj prispevajo k razgradnji organske snovi v tleh in so pomemben člen v prehranjevalni verigi (Smiley, 2005). Vsaj polovica vseh ogorčic je prostoživečih (Baldwin in sod., 2000; Hugot in sod., 2001; Smythe in sod., 2019), pomembne pa so predvsem ogorčice, ki parazitirajo živali in rastline (Archidona-Yuste in sod., 2020). Največji poudarek in večina raziskav je namenjenih prav parazitskim ogorčicam, predvsem v povezavi z zdravstvom in kmetijstvom (Smythe in sod., 2019). Rastlinsko-parazitske ogorčice predstavljajo približno 15 % vseh trenutno znanih ogorčic (Wyss, 1997; Decreamer in Hunt, 2006; Wick, 2012; Archidona-Yuste in sod., 2020).

V tleh živeče ogorčice povzročajo škodo na številnih vrtninah, okrasnih rastlinah, sadnem drevju in vinski trti, kar povzroča velike izgube na ravni svetovnega gospodarstva. Ocenjujejo, da rastlinsko-parazitske ogorčice zmanjšajo količino kmetijskih pridelkov za 8,8–15 %, kar se odraža z izgubami od 90 do 160 milijard evrov po celem svetu (Abad in sod., 2008; Nicol in sod., 2011; Jones in sod., 2013; Singh in sod., 2013; Singh in sod., 2015; Andret-Link in sod., 2017; Coyne in sod., 2018; Wernet in Fischer, 2023). Poleg fizičnih poškodb, ki jih ogorčice povzročajo na rastlinah, so lahko tudi prenašalke virusov ter zaradi povzročenih poškodb na rastlinskem tkivu vplivajo tudi na intenzivnejši vdor fitopatogenih bakterij in gliv (Garcia in sod., 2019; Hajji-Hedfi in sod., 2019; Wernet in Fischer, 2023).

Ena pomembnejših virusnih bolezni vinske trte je kompleks kužne izrojenosti vinske trte. To bolezen povzroča 15 virusov iz družine Secoviridae, ki jih prenašajo talne ektoparazitske vrste ogorčic iz rodov *Longidorus*, *Paralongidorus* in *Xiphinema*, ki so uvrščeni v družino Longidoridae (Andret-Link in sod., 2004; Martelli in Boudon-Padieu, 2006). Med temi ogorčicami ima največji vpliv na pridelavo vinske trte vrsta *Xiphinema index* Thorne & Allen, 1950, ki je prenašalka virusa pahljačavosti listov vinske trte (GFLV) (Andret-Link in sod., 2004). Med več kot 4.100 znanimi vrstami rastlinsko-parazitskih ogorčic je vrsta *X. index* uvrščena na 8. mesto na seznamu 10 najpomembnejših ogorčic glede na njihov znanstveni in gospodarski pomen (Jones in sod., 2013; Andret-Link in sod., 2017).

Tako ogorčica *X. index*, kot virus GFLV sta od leta 2019 na seznamu nadzorovanih nekarantenskih škodljivih organizmov v Evropski uniji (EPPO, 2023). V Sloveniji je bila prisotnost ogorčice *X. index* prvič potrjena leta 1978 (Hržič, 1978), kasneje je bila najdena in preučevana v obdobju med letoma 2002 in 2004, ko so njeno najdbo potrdili v Vipavski dolini in na Krasu (Urek in Širca, 2005). Prisotnost *X. index* je zaenkrat omejena le na Primorski vinorodni okoliš, številčnost populacije pa je v nekaterih vinogradih zelo visoka, tudi več kot 1000 osebkov na 1 kg vinogradniške zemlje (Širca in Theuerschuh, 2020).

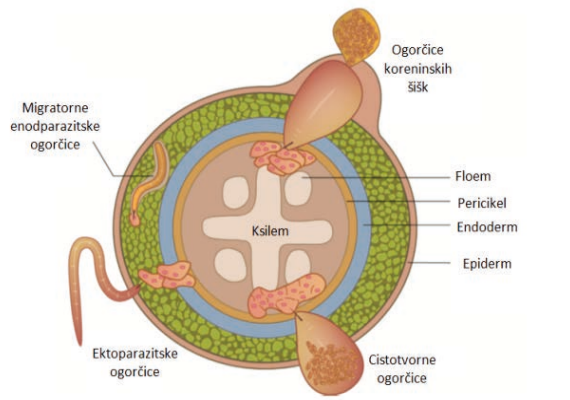
2 RASTLINSKO PARAZITSKE OGORČICE

Ogorčice uvrščamo v deblo Nematoda Cobb, 1932. Na podlagi morfoloških raznolikosti so med ogorčicami določili dve glavni skupini, kopenske ogorčice – Secernentea in vodne ogorčice – Adenophorea (Smythe in sod., 2019). Sledile so molekularne analize 18S rDNA, ki so filogenetsko drevo oblikovale v tri glavne skupine oz. podrazrede: Chromadoria, Dorylaimia in Enoplia (De Ley, 2006; Blaxter in sod., 2011; Smythe in sod., 2019). Več kot 4.100 vrst je prepoznanih in identificiranih kot rastlinsko-parazitskih ogorčic, kar predstavlja nekje 15 % vseh trenutno znanih ogorčic (Wyss, 1997; Decreamer in Hunt, 2006; Wick, 2012; Archidona-Yuste in sod., 2020). Kot navaja Smythe s sodelavci (2019), fitoparazitske oz. rastlinsko-parazitske ogorčice uvrščamo v dva reda – Rhabditida (podred Tylenchina) in Dorylaimida (podred Dorylaimina), medtem ko De Ley (2006) dodaja še red Triplonchida (podred Diphtherophorina).

Ogorčice, ki se prehranjujejo kot fitofagi oz. rastlinski paraziti, se lahko hranijo na različne načine (Slika 1) (Urek in Hržič, 1998; Vieira in Gleason, 2019):

- ektoparazitsko (črpanje rastlinskih sokov z vbodom v rastlinsko tkivo od zunaj),
- semiendoparazitsko (sesanje rastlinskih sokov z delnim prodorom v rastlinsko tkivo) ali
- endoparazitsko (izčrpavanje rastline celotno življenjsko obdobje s popolnim vdorom v rastlinsko tkivo).

Rastlinsko-parazitske ogorčice hrano pridobivajo iz gostiteljskih rastlin. Kljub temu, da so organizmi zelo majhni (v dolžino običajno merijo 1 mm), lahko povzročijo velike izgube pridelka, kar predstavlja znatno ekonomsko škodo. Rastlinsko parazitske ogorčice se med sabo precej razlikujejo, lahko jih najdemo na koreninah, steblih kot tudi listih. V kmetijstvu je največji poudarek namenjen endoparazitskim ogorčicam, ki napadajo koreninski sistem in podzemna rastlinska tkiva (Vieira in



Slika 1: Ilustracija različnih načinov prehranjevanja rastlinsko-parazitskih ogorčic na prerezu korenine (Vir: Vieira in Gleason, 2019)
Figure 1: Illustration of a cross-section of a root with different feeding habits of plant-parasitic nematodes (Figure: Vieira and Gleason, 2019)

Gleason, 2019). Medtem ko se ektoparazitske ogorčice prosto gibljejo okrog rastlin in se hranijo z rastlinskimi celicami (primer vrste rodu *Longidorus* in *Xiphinema* – ogorčice prenašalke virusov), endoparazitske ogorčice prodrejo v tkivo rastline in eno ali več svojih življenjskih obdobij preživijo v rastlinskem tkivu. Ločimo migratorne in sedentorne endoparazite. Migratorne endoparazitske ogorčice prodrejo v rastlinsko tkivo in se nato znotraj tkiva hranijo in premikajo (primer vrste rodu *Pratylenchus* – ogorčice koreninske pegavosti). Sedentorne endoparazitske ogorčice pa prav tako vstopijo v rastlinsko tkivo, potujejo do mesta prehranjevanja in se tam ustalijo, se ne gibljejo več po rastlini (primer vrste rodu *Meloidogyne* – ogorčice koreninskih šišk in vrste rodu *Globodera* – cistotvorne ogorčice) (Urek in Hržič, 1998; Vieira in Gleason, 2019). Ob pojavu bolezenskih znamenj na rastlinah je za odkrivanje in identifikacijo ogorčic potrebna morfološka (pregled organizmov pod mikroskopom) in molekularna analiza, saj je zgolj na podlagi bolezenskih znamenj nemogoče natančno določiti, za kateri organizem gre (Jackson, 2020). Ogorčice se v grobem med sabo ločijo predvsem po ustnem aparatu in načinu prehranjevanja (Bilgrami in Brey, 2005).

2.1 OGORČICE, KI PARAZITIRAJO VINSKO TRTO

Številne vrste ogorčic lahko napadejo korenine žlahtne vinske trte (*Vitis vinifera* L.), vendar le nekatere izmed njih povzročijo znatno škodo (Urek in Hržič, 1998; Jackson, 2020). Ogorčice, ki napadajo vinsko trto, so omejene na prehranjevanje na koreninah. Prehranjujejo se s srkanjem citoplazemske tekočine iz koreninskih celic. To jim omogoča suličasto bodalo – stilet, ki predre v gostiteljske celice. Hranjenje je lahko omejeno na površje korenin, v kolikor pa ogorčice prodrejo v rastlino, se zadržujejo predvsem v koreninski skorji. Poleg neposredne škode, ki jo povzroči hranjenje, in posledične motnje delovanja koreninskega sistema, kar drugim patogenom lajša okužbo rastlin, lahko nekatere ogorčice prenašajo tudi viruse (Jackson, 2020).

Rastlinam so najbolj nevarne ogorčice koreninskih šišk iz rodu *Meloidogyne* in virusonosne ogorčice iz rodu *Xiphinema*. Medtem ko Meloidogyne spp. povzročajo znatne poškodbe na koreninah s tvorbo zadebelitev (šišk), ogorčice iz rodu *Xiphinema* prenašajo viruse vinske trte. Druge ogorčice, ki se občasno prehranjujejo na koreninah vinske trte, vključujejo vrste iz rodov *Pratylenchus* (ogorčice koreninske pegavosti), *Tylenchulus* (citrusove ogorčice) in *Criconemella* (obročaste ogorčice) (Urek in Hržič, 1998; Jackson, 2020). Gospodarsko škodo na vinski trti povzročajo rastlinsko-parazitske ogorčice več različnih vrst; najpogosteje zastopane ogorčice v vinogradih so: *Xiphinema index*, *Meloidogyne ethiopica* Whitehead, 1968, *Mesocriconema xenoplax* Raski, 1952 in *Tylenchulus semipenetrans* Cobb, 1913 (Aballay in sod., 2009; Baginsky in sod., 2013).

Kot je že omenjeno, lahko nekatere ogorčice, ki se hranijo z rastlinskim sokom preko ustnega aparata, prenašajo iz okuženih na zdrave rastline fitopatogene viruse, so njihovi prenašalci (Urek in Hržič, 1998). Ogorčice prenašalke virusov uvrščamo v dve družini (Taylor in Brown, 1997; Urek in Hržič, 1998):

- družina Longidoridae: rod *Longidorus*, *Paralongidorus* in *Xiphinema* (razred Dorylaimea)

in

- družina Trichodoridae: rod *Paratrichodorus*, *Trichodorus* (razred Enoplea).

Ogorčice prenašalke virusov so ektoparaziti, ki živijo prosto v tleh in se hranijo na koreninah rastlin (Urek in Hržič, 1998). Kot navajata Taylor in Brown (1997) so ogorčice iz rodov *Longidorus*, *Paralongidorus* in *Xiphinema* prenašalke nepovirusov (virusi iz rodu *Nepovirus*), medtem ko ogorčice iz rodov *Paratrichodorus* in *Trichodorus* prenašajo tobraviruse (virusi iz rodu *Tobravirus*). Najštevilčnejši rod ogorčic, prenašalk virusov je rod *Xiphinema*, sledi rod *Longidorus* s 183 vrstami, rod *Trichodorus* zajema 52 vrst, sledita pa rodova *Paralongidorus* s 34 vrstami in *Paratrichodorus* s 30 vrstami (Hodda, 2022). Rod *Xiphinema* je ena najbolj raznolikih skupinskih vrst virusonosnih ogorčic z več kot 280 vrstami (Coomans, 2000; Ye in sod., 2004; Decraemer in Hunt, 2006; Gutiérrez-Gutiérrez in sod., 2010; Hodda, 2022). Devet od približno 280 poznanih vrst *Xiphinema* so potrjeno prenašalke nepovirusov (Decraemer in Robbins, 2007; Gutiérrez-Gutiérrez in sod., 2013). Glede na veliko morfološko raznolikost te skupine razdelimo rod *Xiphinema* v dve različni skupini (Decraemer in Hunt, 2006; Gutiérrez-Gutiérrez in sod., 2013; Archidona-Yuste in sod., 2016):

- skupina *Xiphinema americanum* sensu lato (ameriška skupina), ki obsega kompleks približno 60 vrst (Ye in sod., 2004; Gutiérrez-Gutiérrez in sod., 2010; Gutiérrez-Gutiérrez in sod., 2013; EFSA in sod., 2018; Archidona-Yuste in sod., 2020) in

- skupina *Xiphinema* non-*americanum* (neameriška skupina), ki obsega kompleks več kot 220 vrst (Thorne, 1935; Coomans, 2000; Decraemer in Hunt, 2006; Archidona-Yuste in sod., 2020).

Xiphinema index je uvrščena v neameriško skupino ogorčic – *Xiphinema* non-*americanum* group (Taylor in Brown, 1997; EFSA in sod., 2018).

2.1.1 Ogorčice vrste *Xiphinema index*

Prvi dokaz o prenosu rastlinskega virusa z rastlinsko-parazitsko ogorčico sega v pozna petdeseta leta 20. stoletja. Dokazali so, da talna ektoparazitska vrsta *X. index*, prenaša virus pahljačavosti listov vinske trte (GFLV), ki je eden glavnih povzročiteljev bolezni kužne izrojenosti vinske trte (Hewitt in sod., 1958). Bolj kot direktne poškodbe ogorčic *X. index* so problematične zaradi prenosa virusa GFLV (Andret-Link in sod., 2004; Van Ghelder in sod., 2015). Bolezen, ki jo povzroča virus GFLV se na rastlinah odraža s skrajšanimi internodiji, slabša je kakovost plodov, izpad pridelka lahko presega 80 % (Andret-Link in sod., 2004; Van Zyl in sod., 2012; Rubio in sod., 2020).

Evropska populacija ogorčic iz rodu *Xiphinema* je tolerantnejša za nizke temperature in jih lahko v tleh najdemo praktično celotno koledarsko leto (Flegg, 1968a, b; Taylor in Brown, 1997). Vrsta *X. index* preživi med –11 in 35 °C, vendar je konstantna temperatura 45 °C ali –22 °C 10 dni zapored zanje smrtonosna (Van Zyl in sod., 2012).

Populacija *X. index* ima raje težka tla, ki so manj izpostavljena suši, njihovo prisotnost pa so potrdili tudi v vlažnih peščenih tleh (Esmenjaud in sod., 1992). Takšne rastne razmere so ugodne tudi za gojenje vinske trte, ki je njihova glavna naravna gostiteljica (Andret-Link in sod., 2017). Gibanje ogorčic je omejeno na nekaj centimetrov na leto (Pitcher, 1975; Taylor in sod., 1994). Ogorčice se v tleh premikajo vodoravno in navpično, večinoma v smeri rasti korenin, na katerih se hranijo (Thomas, 1981; Esmenjaud in sod., 1988). Na območju korenin je tudi gostota populacije največja (Feil in sod., 1997). Običajno jih je manj v plitvih slojih tal. V vinogradniških tleh se *X. index* najpogosteje nahajajo na globini 0,3–1,5 m, tam kjer je večina mladih korenin/koreninskih laskov (Esmenjaud in sod., 1992; Feil in sod., 1997; Villate in sod., 2008). Zadržujejo se lahko tudi na globini 3,6 m (Raski in sod., 1965).

Širijo se s kontaminirano opremo, sajenjem okuženih sadik in prenosom zemlje. Da bi preprečili širjenje ogorčic iz vinograda v vinograd je treba opremo, kot so traktorji, sadilniki, grebenarji in delovni škornji, po uporabi v vsakem vinogradu očistiti (Bileva in sod., 2009; Esmenjaud in Bouquet, 2009). Tudi voda povzroča aktivno migracijo ogorčic v tleh, saj se ogorčice lahko pasivno širijo s potoki, poplavnimi vodami in pronicajočo vodo v vinogradih (Roccuzzo in Cianco, 1991).

Razvojni krog ogorčic se razlikuje od vrste do vrste, nanj pa močno vplivajo okoljske razmere (Weischer, 1975; Taylor in Brown, 1997). Jajčeca odložijo v tla, blizu mesta hranjenja, ta pa se izležejo spomladi ali zgodaj poleti, ko odženejo nove korenine. *X. index* zaključi svoj življenjski krog na vinski trti v 7–9 mesecih. Ta čas pa je krajši, v kolikor so ogorčice prisotne v rastlinjaku (Andret-Link in sod., 2017). Med ogorčicami iz rodu *Xiphinema* so samci zelo redki, izjema je vrsta *X. diversicaudatum* Micoletzky, 1927. Razmnožujejo se nespolno – partenogenetsko. Posamezna ličinka vrste *X. index* lahko ustvari populacijo, kljub temu da zelo redko pride do spolnega razmnoževanja (Villate in sod., 2010).

Glavni morfološki parametri so (Andret-Link in sod., 2017):

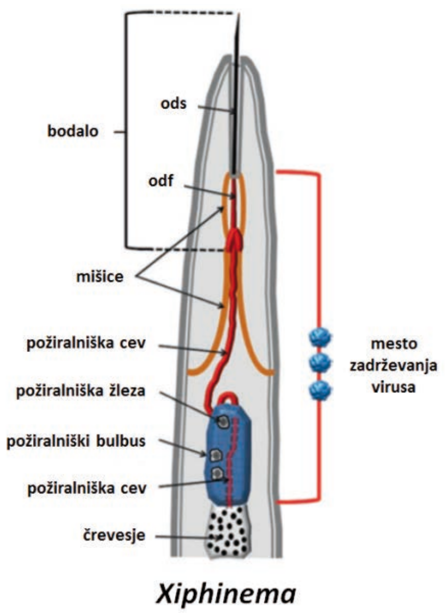
- dolžina telesa,
- oblika in velikost glave in ustnega aparata,
- struktura in dolžina bodala,
- struktura in položaj vodilnega obroča,
- položaj in tip genitalnih organov samice.

Dodatne morfološke značilnosti na ravni vrste so razvoj in struktura ženskega reproduktivnega organa, oblika repa v vseh razvojnih stadijih ter prisotnost oz. odsotnost samcev (Andret-Link, 2017).

Nepovirus GFLV se pri ogorčicah vrste *X. index* zadržuje v požiralniški cevi in v notranji plasti kutikule odontoforja (Slika 2) (Taylor in Robertson, 1970; Brown in sod., 1995; Wang in sod., 2002; Širca in Urek, 2016; Sanfaçon, 2020).

3 MOŽNOSTI ZATIRANJA OGORČIC VRSTE *X. index*

Pomembno je, da sadimo le neokužen sadilni material, ki je predhodno testiran na prisotnost virusov (Demangeat in sod., 2004; Pompe-Novak in sod., 2005). Predvsem je potrebna pazljivost pri možnostih prenosa ogorčic s prenašalci, saj na ta način lahko preprečimo pojavnost napadov in okužb v nasadih (Djennane in sod., 2021; Schurig in sod., 2021a; Schurig in sod., 2021b).



Slika 2: Morfologija sprednjega dela ogorčic rodu *Xiphinema* in zadrževalno mesto virusnih delcev (obarvano rdeče): v notranji plasti kutikule odontoforja (odf) in požiralniške cevi (prirejeno po Andret-Link in sod., 2017)
Figure 2: Morphology of the anterior region of nematodes genus *Xiphinema* and the viral particles retention site (red coloured): inner layer of the odontophore's cuticule (odf) and esophageal bulb (Figure: Andret-Link et al., 2017)

Zaradi vse večjih omejitev pri uporabi kemičnih nematocidov in njihove neučinkovitosti se je trenutno kot najučinkovitejša metoda za iztrebljanje *X. index* v tleh izkazala vsaj 7 letna praha oz. zemljišče v mirovanju, saj lahko ogorčice v vinogradniških tleh preživijo najmanj 4 leta brez prisotnosti gostiteljske rastline. Do prenosa virusa pa lahko pride tudi po predhodni 5 letni prahi (Demangeat in sod., 2005; Villate in sod., 2008; Meng in sod., 2017; Nguyen in sod., 2019; Wernet in Fischer, 2023), saj se lahko ogorčice v tleh ohranijo zaradi prisotnosti koreninskih ostankov po izkrčitvi trt (McKenry in Buzo, 1996; Jackson, 2020). Tako dolgotrajni postopki iztrebljanja ogorčic iz tal predstavljajo ekonomsko neprivlačno metodo (Demangeat in sod., 2004).

3.1 KEMIČNO ZATIRANJE – NEMATOCIDI

V Sloveniji so na seznamu registriranih fitofarmacevtskih sredstev – nematocidov navedeni štirje pripravki (Tabela 1) (FURS, 2023).

Učinkovitost nematocidov je slaba in ne vodi do želenih učinkov. Zatiranje ogorčic vrste *X. index* je problematično, saj njihova jajčeca v mirujočem stanju v tleh preživijo tudi več let. Vprašljivo je tudi prodiranje nematocidov v globino do 1,5 m in več, saj se tam nahaja večina koreninskega sistema. Predvsem fumiganti so veljali za potencial pri omejevanju širjenja in pojavnosti ogorčic, a se slabo aplicirajo do večjih globin, prav tako pa so slabo učinkoviti v ilovnatih tleh (Jackson, 2020). Na podlagi poskusov z uporabo kemičnih sredstev, karbamatov in organofosfatov, z nanosom na vinogradniška tla enkrat ali dvakrat letno so ugotovili, da ti niso dovolj učinkoviti. Neučinkovitost so pripisali vplivu namakanja, uporabi organskih dodatkov in načinu nanosa (Baginsky in sod., 2013).

Tabela 1: Seznam registriranih nematocidov v Sloveniji (Vir: FURS, 2023)
Table 1: List of registered nematicides in Slovenia (Reference: FURS, 2023)

Pripravek	Aktivna snov	Formulacija
Basamid granulat	dazomet	mikrozrnina
Profume insecticide	sulfuril fluorid	plin
Velum prime	fluopiram	koncentrirana suspenzija
Votivo FS 240	*Bacillus firmus* sev I-1582	koncentrirana suspenzija za tretiranje semena

Uporaba nematocidov je po svetu še vedno precej razširjena, a je njihovo delovanje vprašljivo tudi z vidika neciljnega delovanja, saj poleg ciljnih organizmov – ogorčic, v tleh negativno učinkujejo tudi na talne mikroorganizme in druge koristne organizme. Z njihovim uničenjem pa posledično siromašimo tla (Chitwood, 2003; Dong in Zhang, 2006; Atreya, 2008; Brun in sod., 2008; Pires in sod., 2022).

3.2 BIOTIČNO ZATIRANJE

Ker je uporaba kemičnih nematocidov precej omejena in neučinkovita, so potrebe po alternativnih, trajnostnih in okolju prijaznih pristopih vse večje (Wernet in Fischer, 2023). Kot možen pristop v boju proti vrsti *X. index* v vinogradih se je izkazala kombinacija kolobarjenja in strniščnih dosevkov (Aballay in sod., 2004). Prav tako lahko na populacijo ogorčic v vinogradniških tleh vplivajo antagonistične rastline s svojimi koreninskimi izločki (Bello in sod., 1998), hkrati pa lahko pozitivno vplivajo na vinsko trto in spodbujajo rast korenin (Birch in sod., 1993; Baginsky in sod., 2013). Biotično varstvo rastlin zajema tudi biotične agense različnega taksonomskega izvora: entomopatogene ogorčice, parazitoidne žuželke, patogene bakterije, glive, viruse ter plenilce (Mesa-Valle, 2020; Topalović, 2020; Pires in sod., 2022).

3.2.1 Vmesni posevki

V interakciji z ogorčico *X. index* je bilo preučevanih več posevkov rastlin iz družine nebinovk (Asteraceae) (Tsay in sod., 2004) in posevkov iz družine križnic (Brasicaceae) (Halbrendt, 1996). Večina jih je bila učinkovitih proti vrsti *X. index* (Insunza in sod., 2001; Aballay in sod., 2004). Učinek teh vmesnih posevkov je odvisen od vrste izbranih rastlin, tipa tal, ogorčice, ki je v tleh prisotna, in načina gojenja. V primeru neprimerne izbire vmesnega posevka lahko to vodi do škodljivih učinkov za glavni posevek (McLeod, 1994; Baginsky in sod., 2013). V raziskavi Baginskya in sodelavcev (2013) so v vinogradih preizkušali učinkovitost vmesnih posevkov oljne ogrščice (*Brassica napus* var. *napus* L.) in krmne repe (*B. rapa* var. *rapa* L.). Oba posevka sta tako kot nematocidi zmanjšala populacijo vrste *X. index* v vinogradniških tleh. Nekatere raziskave poljskih poskusov v vinogradu poročajo o obetavnih rezultati s posevki krmne grašice (*Vicia villosa* Roth) in žametnice (*Tagetes minuta* L.). Pod nadzorovanimi razmerami v rastlinjaku so se najbolj izkazali bela lupina (*Lupinus albus* L.), niger (*Guizotia abyssinica* (L. f.) Cass.) in žametnica (*Tagetes minuta* L.), saj so znatno zmanjšali populacijo vrste *X. index* v primerjavi z golimi tlemi, takimi brez posevka (Villate in sod., 2012). Aballay in Insunza (2002) poročata o rezultatih pridobljenih z gojenjem in vključevanjem oljne ogrščice (*Brassica napus*) v vinograde. Populacija vrste *X. index* se je v tleh ob prisotnosti oljne ogrščice znatno zmanjšala v primerjavi s kemičnim zatiranjem (fenamifos). Mulč pokrovnih posevkov, kot so kapusnice (*Brassica* spp.) lahko služi kot biofumigant, ki lahko zmanjša populacijo ogorčic v tleh (McLeod in Steel, 1999; Rahman in Somers, 2005). Kapusnice namreč tvorijo glukozinolate, ki so v živi rastlini neaktivni. Po mulčenju te spojine hidrolizira encim mirozinaza, pri čemer se sproščajo hlapni izotiocianati. Ti sodelujejo pri biofumigaciji vinogradniških tal (Kruger in sod., 2013). V poskusu Pensec in sod. (2013) pa se je sadrenka oz. šlajer (*Gypsophila paniculata* L.) izkazala z bionematocidnim delovanjem in ob tem ni vplivala na delovanje mikorize.

3.2.2 Glive

Alternativo kemičnemu zatiranju lahko predstavljajo tudi koristni talni mikroorganizmi. Eden od učinkovitih primerov je arbuskularna mikoriza (AM), ki omili škodo povzročeno s strani rastlinsko parazitskih ogorčic (Pozo in Azcon-Aguilar, 2007; Schouteden in sod., 2015, v Hao in sod., 2018). V poskusu Hao in sodelavcev (2012) so dokazali, da je izolat arbuskularne mikorizne (AM) glive *Rhizoglomus irregularis* Walker & Schüsler, 2010 imenovan BEG141 zmanjšal tvorbo šišk na koreninah, ki so jih povzročile brezvirusne ogorčice *X. index* in zmanjšal namnožitev ogorčic v tleh, ki so obdajale vinsko trto cepljeno na podlago SO4 (*Vitis berlandieri* × *V. riparia*). V kasnejši študiji je Hao s sodelavci (2018) preučeval učinke glive *R. irregularis* na razvoj virusonosne ogorčice *X. index* in okužbe z GFLV. Ugotovili so, da je prisotnost AM glive na koreninah vinske trte cepljene na podlago SO4 zmanjšala nastanek šišk na koreninah povzročenih s strani ogorčice *X. index*, zmanjšana pa je bila tudi namnožitev ogorčic v tleh. V primeru vnosa 10 osebkov vrste *X. index* v lonec, kjer je bila AM gliva, virusa GFLV pri vinski trti po 90 dneh niso potrdili, medtem ko so v primeru vnosa 100 osebkov potrdili GFLV tako pri rastlinah v loncih brez AM kot z AM glivo. Torej bi se z manjšanjem populacije vrste *X. index* in njihovega prodiranja v korenine zmanjšala tudi okuženost vinske trte z GFLV.

V poskusu Boosalis in Mankau (1965) so ugotovili, da je endoparazitska gliva *Catenaria anguillulae* Sorokin, 1876 parazitirala ogorčico vrste *X. index*. Kot parazit se je izkazala tudi pri ogorčicah *Panagrellus redivivus* Linnaeus, 1767 in samicah ogorčice *Heterodera schachtii* Schmidt, 1871, kar kaže na njihov velik potencial za biotično zatiranje ogorčic (Voss in Yyss, 1990). Galper s sodelavci (1991) so ugotovili, da je ekstrakt glive *Cunninghamella elegans* Lendner, 1907, gojene na kolagenu kot viru ogljika in dušika, zmanjšal gibljivost ogorčic *X. index* v *in vitro* razmerah. Prav tako se je potencial te glive pri biotičnem zatiranje ogorčic povečal, ko so kolagen dodajali v rastni substrat (0,1 % m/m).

Kot ekološko in stroškovno učinkovito alternativo varstvu pred ogorčicami so z različnimi raziskavami potrdili glivo *Arthrobotrys flagrans* Sidorova, Gorlenko & Nalepina, 1964 (prej *Duddingtonia flagrans*). Izkazala se je, da ima velik potencial za uporabo kot biotični nematocid (Araújo in sod., 2008; Vilela in sod., 2012; da Silva in sod., 2013; Braga in de Araújo, 2014; Mostafanezhad in sod., 2014).

To sta potrdila tudi Wernet in Fischer (2023), saj se je gliva *Arthrobotrys flagrans* (*Duddingtonia flagrans*) v njuni raziskavi izkazala za učinkovit mikroorganizem za zatiranje ogorčice *X. index*. V lončnem poskusu s figo (*Ficus carica* L.) je prisotnost glive *A. flagrans* zmanjšala število ličink vrste *X. index*. Figa ni gostitelj virusa GFLV, je pa dober gostitelj ogorčice *X. index*, zato je mogoče zagotoviti, da brezvirusne ogorčice ostanejo neokužene z virusom tekom poskusa. Monteiro s sodelavci (2018) pa je potrdil pozitivno delovanje glive *A. flagrans* na sadike paradižnika, saj se je ob prisotnosti le-te v tleh povečala absorpcija elementov in mineralov, kar je pozitivno vplivalo na rast in razvoj sadik. Prav s poskusi na paradižnikih so dokazali nematocidno delovanje glive *A. flagrans*, saj se je po vnosu klamidiospor v rastni substrat zmanjšalo število ličink vrste *Meloidogyne javanica* Treub, 1885 za 73 % (Monteiro in sod., 2020; v Wernet in Fischer, 2023).

Wernet in Fischer (2023) sta s poskusi preverila interakcije gliv *A. conoides* Drechsler, 1937, *A. flagrans*, *A. oligospora* Fresen., 1850, *A. musiformis* Drechsler, 1937, *Drechslerella stenobrocha* Drechsler, 1950 in *Dactylelina haptoyla* Drechsler, 1950 z bakterijami iz rodov *Delftia*, *Bacillus*, *Pseudomonas*, *Enterobacter* in *Serratia*. Ugotovila sta, da so bakterije *Bacillus subtilis* Ehrenberg, 1835, *Pseudomonas stutzeri* Lehmann in Neumann, 1896, *Enterobacter cloacae* Jordan, 1890, *Serratia marcescens* Bizio, 1823 in *Delftia* po 48 urah na hifah gliv vrste *A. conoides* in *A. oligospora* tvorile »pasti« oz. zanke, ki ogorčice ujamejo in jih nato prebavijo. Ta interakcija gliv in bakterij, ter tvorba pasti na hifah gliv bi lahko v prihodnje predstavljala velik potencial biotičnega zatiranja patogenih ogorčic.

Darago in sodelavci (2013) pa so preverili nematocidno delovanje gliv iz rodu *Trichoderma*. Poskus so zasnovali v *in vitro* razmerah s šestnajstimi sevi šestih vrst: *T. atroviride* Bissett, 1984, *T. harzianum* Rifai, 1969, *T. rossicum* Bissett, Kubicek & Szakacs, 2003, *T. tomentosum* Bissett, 1991, *T. virens* Arx, 1987 in *T. asperellum* Samuels, Lieckf. & Nirenberg, 1999. Ocena testov smrtnosti ogorčic *X. index* je pokazala, da so glive *Trichoderma* spp. sposobne zmanjšati populacijo vrste *X. index* v *in vitro* razmerah. Najboljše rezultate so dosegli z glivo *T. harzianum.*

3.2.3 Bakterije

Eno od možnosti v boju proti ogorčicam *X. index* pa so preizkušali v raziskavi Aballaya in sod. (2011; 2012). Iz korenin vinskih trt so pridobili več kot 400 izolatov bakterij iz 25 rodov. Izolata bakterij *Brevibacillus brevis* Migula, 1900 in *Bacillus megaterium* de Bary, 1884 sta pokazala nematocidno delovanje tako v *in vitro* razmerah, kot pri lončnem poskusu. Rizobakterije, izolirane iz žlahtne vinske trte so zmanjšale škodo na mladih koreninah, ki jo povzroči vrsta *X. index* in so potencialni biotični agensi za nadaljnje raziskave o možnostih zatiranja oz. omejevanja pojavnosti ogorčice v vinogradih. Preprečitev tveganja za okužbo vinske trte z GFLV sicer ni dosežena, saj tudi pri majhni populaciji ogorčic lahko pride do okužbe, vendar prisotne bakterije pripomorejo k večji zaščiti korenin pred neposrednimi poškodbami ogorčic (Aballay in sod., 2012).

Potencial pri varstvu rastlin pred ogorčico *X. index* so ugotovili tudi pri bakteriji *Pasteuria penetrans* Thorne, 1940 (Sturhan, 1985). Prav tako se je pozitivno izkazala bakterija *Paenibacillus* sp. (sev B2), izolirana iz navadnega sirka (*Sorghum bicolor* L. Moench), saj izloča peptid penimiksin. Prisotnost bakterije je znatno zmanjšala populacijo vrste *X. index* v tleh in tvorbo šišk na koreninah vinske trte. Penimiksin je v *in vitro* razmerah zmanjšal aktivnost ogorčic *X. index* (Hao in sod., 2017).

Delovanje nekaterih rizobakterij na vrsto *X. index* so preverjali Aballay in sodelavci (2020). Korenine vinske trte v rastlinjaku so inokulirali z različnimi formulacijami rizobakterij (tekočina, prašek in izotonična raztopina). Ugotovili so, da so bili izolati rizobakterij *Brevibacterium frigoritolerans* Delaporte in Sasson, 1967, *Bacillus amyloliquefaciens* Fukomoto, 1943, *Bacillus megaterium*, *Bacillus thuringiensis* Berliner, 1915, *Bacillus weihenstephanensis* Lechner in sod., 1998 in *Pseudomonas fluorescens* Migula 1895 učinkoviti proti ogorčicam *X. index*, saj je bila njihova učinkovitost primerljiva z učinkovitostjo kemičnega nematocida Rugby®200 CS (kadusafos). Formulacija (prašek, tekočina, izotonična raztopina) na samo učinkovitost ni imela vpliva.

4 ZAKLJUČEK

Virusonosne ogorčice vrste *Xiphinema index* so v tleh sposobne preživeti več let. Kljubujejo različnim okoljskim in talnim razmeram, ob tem pa v svojem organizmu vseskozi ohranjajo virus GFLV, ter na ta način predstavljajo nevarnost za vinsko trto, ki je njihova glavna gostiteljica. Nepovirus GFLV, ki ga vrsta *X. index* prenaša, namreč povzroča virusno bolezen kužne izrojenosti vinske trte, ki postopno vodi do velikega izpada pridelka in kasneje v propad trt. Za zdaj učinkovitega trajnostnega pristopa za obvladovanje te bolezni ni. Najučinkovitejši pristop v boju z boleznijo predstavlja zmanjšanje populacije prenašalk virusa v tleh. Ker pa je značilna porazdelitev teh ogorčic v tleh tudi do globine 3 in več metrov, nakazuje na izjemno težavnost pri varstvu rastlin in predstavlja v »okuženih« vinogradih velik izziv. Priporočljiva je 10 letna praha pred ponovno zasaditvijo vinograda, kar pa je iz ekonomskega stališča nesprejemljivo. Prav zaradi obstojnosti, globinske porazdelitve in zmožnostjo preživetja v neugodnih razmerah je učinkovitost kemičnih pripravkov, nematocidov, v tleh, kjer so ogorčice prisotne, precej slaba. Vprašljivo pa je tudi njihovo neciljno delovanje in vpliv na ostale mikroorganizme v tleh. Vse več raziskav se nagiba k uporabi okolju prijaznejših in učinkovitejših strategij. Kot učinkoviti so se izkazali različni vmesni posevki, ki s svojimi koreninskimi izločki vplivajo na zmanjšanje populacije ogorčic v tleh. Prav tako se vse več raziskav nagiba k uporabi različnih mikroorganizmov, ki bi lahko v prihodnosti učinkoviteje nadomestili kemične pripravke. Preučevanje interakcij med vrsto *X. index,* virusom GFLV in vinsko trto predstavlja vsekakor velik izziv. Področje nekemičnega zatiranja virusonosnih ogorčic pa bo tudi v prihodnje deležno raziskovanja, saj bi z razvojem novih strategij zatiranja ogorčic ali kakršne koli drugačne prekinitve prenosa virusa na vinsko trto omogočili zmanjšanje izgube pridelka in dohodka na vinogradniških kmetijah.

5 ZAHVALA

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