DOI: 10.14720/aas.2016.107.2.04
Agrovoc descriptors: Trialeurodes vaporariorum, new technology, zinc, insecticidal properties, Beauveria bassiana, entomogenous fungi, plant pests, biological control agents, Insecticides, bioassay

Agris category code: H01, H10

Insecticidal effects of zinc oxide nanoparticles and *Beauveria bassiana* TS11 on *Trialeurodes vaporariorum* (Westwood, 1856) (Hemiptera: Aleyrodidae)

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Received Janury 15, 2016; accepted April 29, 2016.
Delo je prispelo 15. januarja 2016, sprejeto 29. aprila 2016.

ABSTRACT

Greenhouse whitefly, *Trialeurodes vaporariorum* is a major pest of horticultural and ornamental plants and is usually controlled with insecticides or biological control agents. In the current study, we examined the effects of synthesized zinc oxide nanoparticles (ZnO NPs) and *Beauveria bassiana* TS11 on *T. vaporariorum* adults. ZnO NPs were synthesized by precipitation method. Field emission scanning electron microscope images indicated that ZnO NPs were non-compacted uniformly. X-ray diffraction results confirmed the hexagonal wurtzite structure of ZnO NPs. Fourier transform infrared analysis showed an intense absorption peak at a range of 434-555 cm^{-1} related to Zn-O bond. In bioassays, adults were exposed to different concentrations of ZnO NPs (3, 5, 10, 15, 20 mg l^{-1}) and fungi (10^4, 10^5, 10^6, 10^7, 10^8 spores ml^{-1}). LC_{50} values for ZnO NPs and fungi were 7.35 mg $l^{-1}$and 3.28×10^5 spores ml^{-1}, respectively. Mortality rates obtained with ZnO NPs and fungi at the highest concentration were 91.6 % and 88.8 %, respectively. The results indicate a positive effect of ZnO NPs and *B. bassiana* TS11on adults. The current study was conducted under laboratory conditions, therefore, more studies are needed in field.

Key words: entomopathogenic fungus; nanoparticle; metal oxide; insecticide; bioassay

IZVLEČEK

INSEKTICIDNI UČINKI NANO DELCEV CINKOVEGA OKSIDA IN TROSOV GLIVE *Beauveria bassiana* TS11 NA RASTLINJAKOVEGA ŠČITKARJA (*Trialeurodes vaporariorum* (Westwood, 1856) (Hemiptera: Aleyrodidae))

Rastlinjakov ščitkar (*Trialeurodes vaporariorum*) je eden izmed glavnih škodljivcev hortikulturnih rastlin in se ga navadno zatira z insekticidi ali biološkimi agensi. V tej raziskavi smo preučevali učinke nano delcev cinkovega oksida (ZnO NP) in glive *Beauveria bassiana* TS11 na njegove odrasle osebke. ZnO NP je bil pripravljen z metodo usedanja. Analiza nano delcev ZnO z vrstičnim elektronskim mikroskopom je pokazala njihovo neizenačenost. Njihova nadaljna analiza z rentgenskimi žarki je potrdila njihovo heksagonalno strukturo. Analiza s Fourierjevo prosevno infrardečo spektrometrijo je pokazala močan absorpcijski vrh v območju 434-555 cm^{-1}, ki se je nanašal na Zn-O vez. V preiskusu smrtnosti so bili odrasli osebki ščitkarja izpostavljeni različnim koncentracijam nano delcev ZnO (3, 5, 10, 15, 20 mg l^{-1}) in trosov glive (10^4, 10^5, 10^6, 10^7, 10^8 spor ml^{-1}). Vrednosti LC_{50} so bile za nano delce ZnO 7.35 mg l^{-1} in 3.28×10^5 ml^{-1} za trose glive. Smrtnost, ki je bila dosežena pri največjih koncentracijah nano delcev ZnO in trosov glive je znašala 91.6 % in 88.8 %. Ti izsledki kažejo pozitivni učinek obeh pripravkov na smrtnost odraslih osebkov rastlinjakovega ščitkarja. Glede na to, da je bila raziskava opravljena v laboratoriju je potrebno v bodoče opraviti še več raziskav v realnih razmerah.

Ključne besede: entomopatogene glive; nano delci; kovinski oksid; insekticid; biotest

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1 INTRODUCTION

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood, 1856) (Hemiptera, Aleyrodidae), is a globally distributed pest because of its quick multiplication, virus transmission, sap puncture, secretion of honeydew and promotion of sooty molds on the host leaves (Guzman et al., 1997). Excessive use of chemical pesticides and increased resistance to insecticides (Whalon et al., 2008), environmental pollutions, impacts on human health and other organisms and finally pesticides residues in nature and agricultural products have provided great impetus to the development of alternative techniques of pest control (Van Lenteren et al., 1996; Laznik et al., 2011; Sandhu et al., 2012).

Recent progress in nano-technology has provided new opportunities in the fields of science such as agriculture (Chaudhry et al., 2008). Application of nanomaterials can revolutionize agriculture by developing potential and effective methods for pest management (Rai and Ingle, 2012). Some studies have reported the toxic effects of metal nanoparticles (such as silver, zinc, aluminum, and titanium oxide) on plants, crustaceans, bacteria, fungi, pathogens and pests (Elchiguerra et al., 2005; Goswami et al., 2010; Kairyte et al., 2013; Kirthi et al., 2011; Manzo et al., 2011; Morones et al., 2005; Reddy et al., 2007; Rouhani et al., 2012; Samuel and Guggenbichler 2004). Among them, zinc oxide (ZnO) nanoparticle is best-known compound (Mitra et al., 2012) commonly used as agricultural fungicide (Waxman 1998). Furthermore, cytotoxicity in eukaryotic cells (Gupta and Gupta, 2005; Magrez et al., 2006), preventing growth in prokaryotic cells (Brayner et al., 2006), bactericidal and fungicidal activity and low effects of ZnO NPs on human cells have also been reported (Kairyte et al., 2013). Low cost synthesis, protective effect against ultraviolet (UV) rays, biocompatibility and non-toxicity has attracted the attention of many researchers (Brayner et al., 2006; Zhang et al., 2007; Ahmad Umar et al., 2009). In spite of the studies performed on NPs to control limiting factors in ecosystems, little research has been carried out to investigate the toxicity effect of ZnO nanoparticles on agricultural pests.

In addition to nanotechnology, biological control is another alternative method to chemical pesticides due to its non-toxicity for human and other non-target organisms, decreasing toxic residue in nature and food (Lacey et al., 2001). Aleyrodidae entomopathogens are limited to fungi since they are capable to penetrate the insect cuticle of sucking insects such as *Aphis gossypii* (Glover, 1877) (Gurulingappa et al., 2011), *Rhynchophorus ferrugineus* (Olivier, 1790) (Sewify et al., 2009), *Laniifera cyclades* (Druce, 1895) (Lozano-Gutierrz and Espana-Luna, 2008) and *Galleria melonella* (Linnaeus, 1758) (El-Sinary and Rizk, 2007). Among entomopathogenic fungi, *Beauveria bassiana* (Bals.-Criv.) Vuill. with broad host range is known as an effective organism to control medical and agricultural pests (Inglis et al., 2001). The aim of this research was to investigate the insecticidal activity of synthesized ZnO nanoparticles and *B. bassiana* TS11 on *Trialeurodes vaporariorum* under greenhouse conditions.

2 MATERIALS AND METHODS

2.1 Preparation of ZnO nanoparticles by precipitation

To prepare ZnO nanoparticles, first, 1 g of zinc oxide powder was dissolved in 100 ml of 1 % acetic acid oxidizing zinc-to-zinc cations. Then, the mixture was sonicated for 30 minutes. After 5 minutes, sodium hydroxide solution (1 M) was added drop wise to the above solution until the pH of solution reached to 10. The solution was heated in a water bath at 40-80 °C for 3 hours. Then, it was filtered through a filter paper and the precipitate was washed twice with distilled water. Finally, it was placed in an oven at 50 °C for 1 hour to form ZnO nanoparticles (Abdelhady, 2012).

2.2 Characterization of the synthesized ZnO nanoparticles

An analysis of nanoparticles was performed using Fourier transform infrared (FTIR) spectrometer (Bruker Optics Ft Tensor 27, Germany) by KBr (potassium bromide). The XRD analyses were performed using Bruker D8 X-ray diffractometer. FESEM (Hitachi S4160) analysis was carried out to observe the morphology of ZnO nanoparticles.

2.3 Entomopathogenic fungus culture and suspension preparation

Isolate TS11 of fungus *B. bassiana isolate* TS11 was obtained from Department of Plant Protection, University of Tehran, Iran. The fungus was inoculated on Sabouraud dextrose agar (SDA) medium in 8 cm Petri dishes and grown at 27 °C. After 12 days, to prepare inoculum, 10 ml of sterile distilled water containing 0.2 % of aqueous Tween 20 solution (Sigma Alrdich, Spain) was spread over the petri dishes using a suitable tool. The suspension was filtered through cheesecloth to separate conidia from remnants of the mycelium. The suspension was vortexed to separate spores from each other and to prevent mass formation during counting. Haemocytometer was used to count spores and prepare various concentrations of spores per unit volume.

2.4 Insect rearing

Adults of *T. vaporariorum* were collected from the surface of cucumber leaves (*Cucumis sativus* L) attacked with this pest from Sistan region (31.0256 °N, 61.5011 °E, and average of 480 meters above the sea level) located in the east of Iran. The adults were reared on young green bean (*Phaseolus vulgaris* L.) plants in a laboratory greenhouse (University of Zabol, Zabol, Iran) under controlled conditions (27±2 °C, 60±10 % RH and a photoperiod of 16:8 (L: D) h). In order to perform bioassays, Muniz and Nombela`s method (2001) was followed.

2.5 Bioassay and determination of lethal concentration of ZnO nanoparticles

To find concentrations with 10-90 % mortality, primary tests were done with concentration ranges between 1-30 mg l^{-1} of the synthesized ZnO NPs. 20 adults of *T. vaporariorum* with same age were considered for each concentration. Following method was carried out in both primary and final tests. To diminish the activity of greenhouse whiteflies, the numbers of insects per concentration were released into the plastic tubes using an aspirator. The tubes were then put in the incubator at 5 °C for 2 minutes (personal observations). Then, the specific numbers of insects were transferred to glass petri dishes already with filter paper-covered floor, but before getting started, the desired concentrations of 5 ml were sprayed on them using a 5 ml handy sprayer. To avoid precipitation of nanoparticles in solutions, before spraying, all the prepared concentrations were sonicated for 10 minutes (Velayutham et al., 2013). After finishing, the insects were transferred to leaf cages installed over green bean leaves. The numbers of dead insects were counted after 24 hours and mortality was computed after three replications. In this experiment, distilled water was used for control.

2.6 Bioassays of *B. bassiana* TS11

Preliminary bioassays were conducted on adult insects with 10^2-10^{10} spores ml^{-1} concentrations of *B. bassiana* TS11. Concentrations of 10^4 and 10^8 spores/ml caused 30 and 80 % mortality were selected as minimum and maximum concentrations, respectively and between them three logarithmic concentrations of 10^5, 10^6 and 10^7 spores/ml were selected for final bioassays. In each treatment, 0.2 % Tween 20 (Sigma Aldrich, Spain) as an emulsifying agent was added to suspension of fungal conidia. Distilled water containing 0.2 % Tween 20 was used as control treatment. The experiments were conducted in a completely randomized design with three replicates of treatments.

Bioassays in these experiments started with control treatment; next, they were continued from lowest level of concentration to the highest level. In order to infect the insects, similar as in bioassay procedure with ZnO nanoparticles, the insects were first deactivated at 5 °C (2 min). 30 adult whiteflies were considered for each concentration. The specific numbers of the insects were individually placed in glass petri dishes with filter paper-covered floor; the desired spore concentrations were then sprayed on insects (5 ml of each concentration using a 5 ml handy sprayer). After application of fungal suspension, whiteflies were placed inside glasses containing water-agar and

green bean leaf. In order to provide moisture for primary germination of spores, the glasses were put in the germinator (27 °C, 80 % RH) for 24 hours. After 24 hours, whiteflies were released into leaf cages installed over young green bean leaves outside germinator and mortality of insects was recorded under controlled condition (27±2 °C, 60±10 % RH, a photoperiod of 16: 8 ([L: D] h) for 10 days.

2.7 Statistical analysis

To determine LC_{50} and LC_{25}, SPSS 21 software (IBM, New York, US) with confidence limits of 95 % and Probit analysis were used.

3 RESULTS AND DISCUSSION

The current study presents the results of insecticidal activity of the synthesized ZnO nanoparticles after 24 hours and pathogenicity of entomopathogenic *B.bassiana* TS11 after 10 days. Mortality rate of insects was evaluated using Probit analysis. Table 1 illustrates values of LC_{25} and LC_{50}, confidence limits (CL), slope and Chi-square (χ^2) for ZnO nanoparticles and *B.bassiana* TS11.

Table 1: Mean values of *T. vaporariorum* mortality by lethal concentrations of ZnO nanoparticles and *B. bassiana* TS11

Test Samples	Intercept ± s_e	LC_{25} (95 % CL)	LC_{50} (95 % CL)	Slope ± s_e	χ^2 (df)
ZnO nanoparticles	-2.007 ± 0.264	3.76 mg l^{-1} (2.80 - 4.61)	7.35 mg l^{-1} (6.21 - 8.58)	2.32 ± 0.27	5.18 (3)
B. bassiana TS11	-2.499 ± 0.288	0.106×10^5 spores ml^{-1} (0.28×10^4 - 0.26×10^5)	3.28×10^5 spores ml^{-1} (1.62×10^5 - 6.2×10^5)	0.453 ± 0.048	1.37 (3)

LC_{25} and LC_{50}: lethal concentration that kills 25 and 50 % of *T. vaporariorum* after exposure to nanoparticle concentration (mg l^{-1}) and *B.bassiana* TS11 (spores ml^{-1}), respectively. The estimated lethal concentration values (mg/l for ZnO NPs and spores ml^{-1} for *B. bassiana* TS11 for treatment was given using Probit analysis. Values in parentheses indicate 95 % confidence limits (CL). Df and s_e refers to degrees of freedom and standard error, respectively.

3.1 ZnO nanoparticles

Insecticidal activity of the synthesized ZnO nanoparticles revealed that values of LC_{50} and LC_{25} (7.35 and 3.76 mg l^{-1}, respectively) had significant lethal effects on *T. vaporariorum* adults. Statistical results showed a significant difference between concentrations of ZnO nanoparticles at 5 % level. Moreover, mortality rate depended on concentration, in other words, as concentration increased, lethality also significantly increased. Mortality rates of concentrations of 3, 5, 10 15 and 20 mg l^{-1} were 21.6 %, 35 %, 53.3 %, 73.3 % and 91.6 %, respectively (Figure 1).

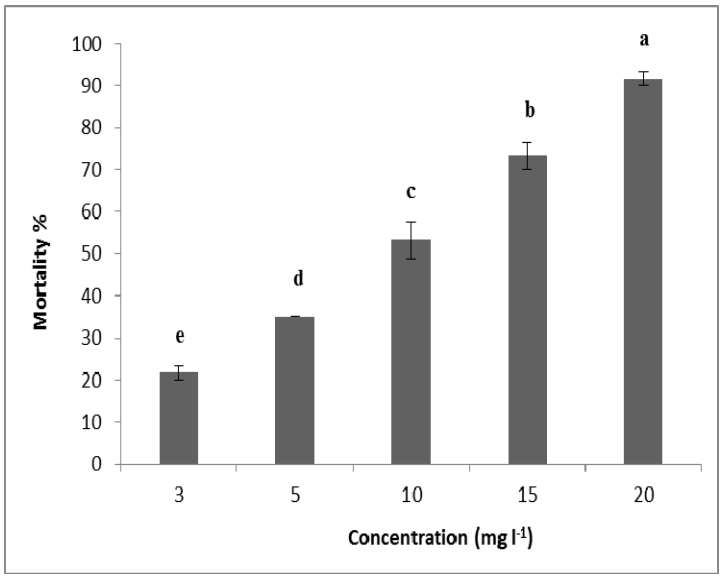

Figure 1: Mortality of *T. vaporariorum* adults, 24 hours after application of ZnO nanoparticles. Values followed by same letter indicate there is no overlap in 95 % confidence interval.

The FESEM images taken from ZnO nanoparticles sample indicated that ZnO nanoparticles are well-distributed with the lowest agglomeration of nanoparticles (Figure 2).

The peaks at 2θ values including 31.85, 34.5, 36.3, 47.65, 56.7, 62.95 with (100), (002), (101), (102), (103) and (110) diffraction, respectively are shown in the XRD patterns of ZnO nanoparticles (Figure 3). Furthermore, the results of XRD show the presence of ZnO crystals with hexagonal wurtzite structure. Average size of the synthesized ZnO nanoparticles was found to be 23.34 nm using Debye-Scherrer equation (1): $D=k\lambda/\beta \cos \theta$.

Where k is equal to 0.89; λ is X-ray wavelength (1.54 A), β is peak width at half maximum height in radian and θ is bragg diffraction angle of the maximum peak (Zhu et al., 2005; Suwanboon et al., 2013).

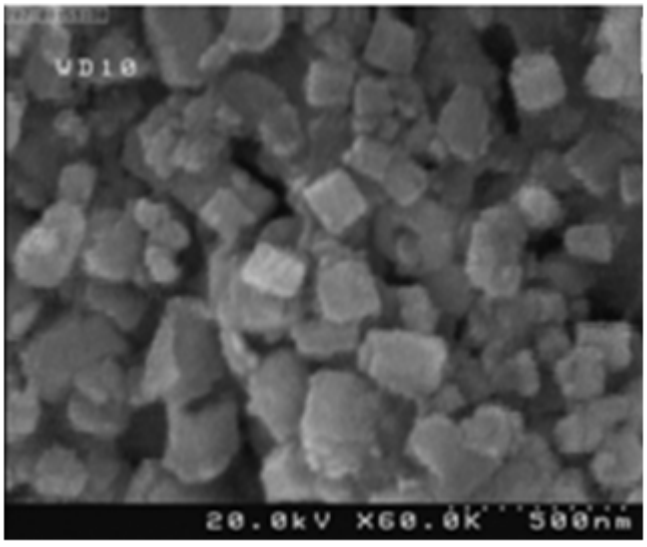

Figure 2: FESEM images of synthesized ZnO nanoparticles. Scale bar is 500 nm.

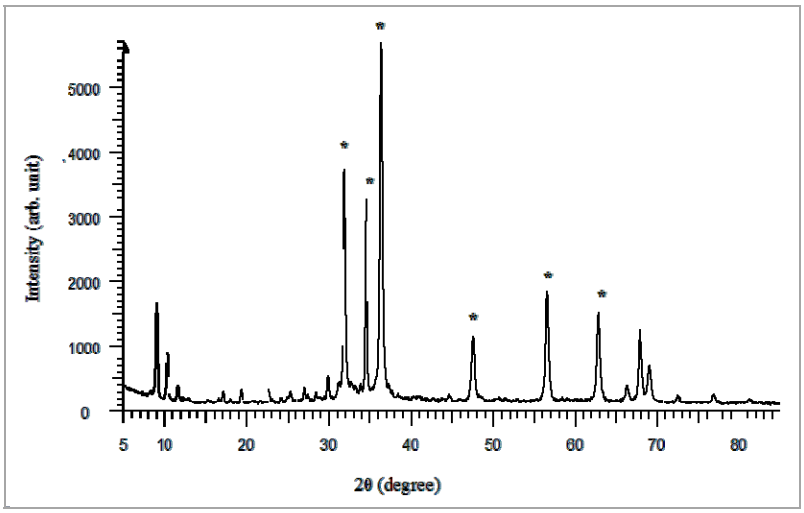

Figure 3: XRD spectrum of synthesized ZnO nanoparticles (Star symbols indicated the presence of ZnO NPs).

In FTIR spectrum of ZnO nanoparticles sample, two strong absorptions are seen at 503 cm^{-1} and 432 cm^{-1} in which 432 cm^{-1} peak represents tensile bond of ZnO and 503 cm^{-1} peak represents oxygen vacancies in ZnO (Figure 4).

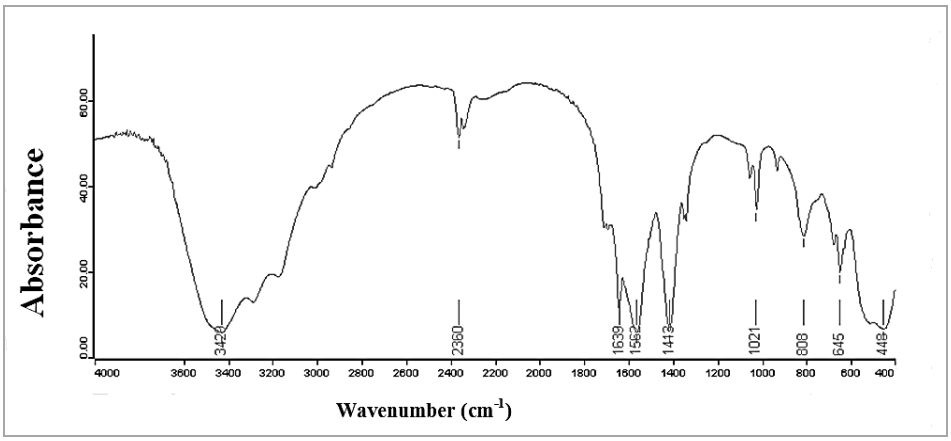

Figure 4: FTIR of synthesized ZnO nanoparticles

The current study revealed the positive effect of insecticidal activity of ZnO NPs on *T. vaporariorum* adults. Acaricidal, licicidal and larvicidal activities of the synthesized ZnO nanoparticles on blood - feeding parasites - *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini, 1888), *Pediculus humanus capitis* (De Geer,1767), *Anopheles subpictus* (Grassi, 1899) and *Culex quinquefasciatus* (Say, 1823) revealed an increase in mortality when concentration increased; moreover, considering optimal time for lethal effects of ZnO nanoparticles after 24 hours, it was 100 % proved. In the comparison made between acaricidal, licicidal and larvicidal activities of zinc oxides and the synthesized ZnO nanoparticles, the mortality effect of ZnO nanoparticles was significant (Kirthi et al., 2011).

Rouhani et al. (2012) studied the insecticidal effects of imidacloprid, Ag and Ag-Zn nanoparticles on *Aphis nerii* (Boyer de Fonscolombe, 1841) and LC_{50} values after 24 hours were seen to be 0.13 µl ml^{-1}, 424.67 and 539.46 mg ml^{-1}, respectively. In a similar study, Samih et al. (2011) investigated the insecticidal effect of Amitraz, ZnO nanoparticles and $ZnAl_2O_3$ against Pistachio psylly (*Agonoscena pistaciae* (Burckhardt and Lauterer, 1989)) and found out a

greater insecticidal effect of Amitraz than the above nanoparticles. It should be taken into consideration that nanoparticles, especially the synthesized zinc nanopartilces, besides their insecticidal activity and also their slower effect than imidacloprid and Amitraz insecticides, have a lower risk of resistance to these nanoparticles in comparison to commercial insecticides (Rouhani et al., 2011).

Clausen et al., (2011) investigated the efficiency of $ZnSO_4$ and ZnO nanoparticles on mortality of *Reticulitermes flavipes* (Kollar, 1837) (Isoptera, Rhinotermitidae). Their results confirmed that *R. flavipes* feeding on wood impregnated with ZnO nanoparticles, decreased to less than 4 % in comparison to control treatment. Despite low usage of wood impregnated with ZnO nanoparticles, 94-99 % of mortality rate was seen in termites. In contrast, using wood impregnated with $ZnSO_4$, 10-12 % was consumed and low mortality rate (10-29 %) was seen in these termites. Thus, ZnO nanoparticles have the necessary potential for application in wood preservatives for protecting woods against termites.

LC_{50} value of the synthesized ZnO nanoparticles on *T. vaporariorum* were consistent with the results of the above researches. Therefore, it can be said that ZnO nanoparticles have the necessary potential for insecticidal activity on *T. vaporariorum* and causes maximum lethality (91.6 %) at the highest concentration.

Nowadays, the only successful control strategy for the greenhouse whitefly is the combined use of pesticides and natural enemies (Hayashi, 1996); therefore, applications of some concentrations of pesticides with minimal impacts on natural enemies seem quite necessary (Laznik and Trdan, 2014). In the current study, through calculation of LC_{25}, we can obtain a concentration of the synthesized ZnO nanoparticles to control *T. vaporariorum,* which is likely to distort its physiology. Since no research has been done on the impact of ZnO nanoparticles on natural enemies of the greenhouse whitefly, therefore, we conclude that using LC_{25}, survival rate of *T. vaporariorum* decreases; however, this concentration would have minimal impact on natural enemies. With application of LC_{25} (3.76 mg l^{-1}), which is quite less compared to LC_{50} (7.35 mg l^{-1}), we can prevent the occurrence of adverse effects on natural enemies of whiteflies.

Based on new and significant properties of nanoparticles, these materials are widely used in industrial and agricultural sectors; therefore, assessment of their potential toxic effects on human health and environment seems quite necessary (Auffan et al., 2009). As a discussion, the best approach to avoid adverse effects on the environment and ecotoxicological effects of nanoparticles on beneficial insects such as parasitoids wasps is using low concentrations of nanoparticles to control pest insects such as *T. vaporariorum*.

3.2 Bioassay of *B. bassiana* TS11

The results obtained from statistical analysis of bioassays on the greenhouse whitefly revealed the susceptibility of *T. vaporariorum* adults to *B. bassiana* TS11 isolate; however, the amount of mortality was different based on determined spore concentrations. More than 50 % of mortality was observed in concentrations of 10^6, $10^7$and 10^8 spores ml^{-1} during the experimental period (10 days). Generally, the least amount of mortality after 10 days was obtained with 10^4 spore ml^{-1} concentration with an average of 33.3 %. Mortality rate of whiteflies depended on concentration and mortality rate increased along with the increase in concentration. Maximum mortality (88.8 %) rate was obtained with 10^8 spore ml^{-1}. In concentrations of 10^5, 10^6 and 10^7 spore ml^{-1}, the mortality rates were seen to be 42.2 %, 58.8 % and 78.8 %, respectively (Figure 5). Statistically, a significant difference was seen between concentrations of 10^5 and 10^6 spore ml^{-1}. However, in terms of mortality rate, there were no significant differences between concentrations of 10^7 and 10^8 and concentrations of 10^4 and 10^5 spore ml^{-1}.

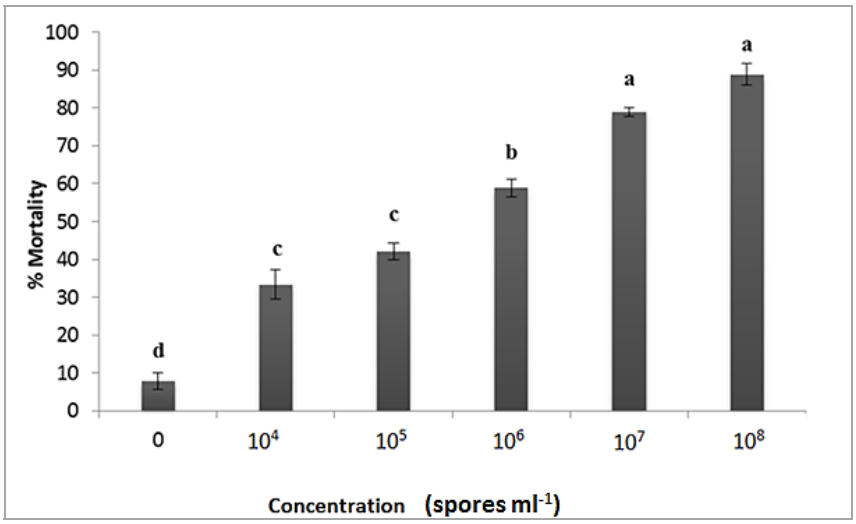

Figure 5: Mean of mortality of *T.vaporariorum* adults 10 days after treatment by *B.bassiana*

Among all antimicrobial agents, entomopathogenic fungi due to frequency in pathogenic races, broad host range and methods of pathogenicity can control a broad range of pests (Fan et al., 2007). The current study revealed that *B. bassiana* TS11 isolate can lead to infection and mortality of *T. vaporariorum*. Several studies have reported *B. bassiana* to control agricultural pests such as *Tetranychus cinnabarinus* (Boisduval, 1867) (Shi and Feng, 2004), *Chilo partellus* (Swinhoe, 1885) (Tefera and Pringle, 2004), whiteflies (Wraight et al., 1998, 2000; Ramos et al., 2000; Mascarin et al., 2013), *Helicoverpa zea* (Boddie, 1850), *Spodoptera exigua* (Hubner,1808), *Spodoptera frugiperda* (J.E. Smith, 1797) (Wraight et al., 2010), and *Aelia rostrata* (Boheman, 1852) (Mustu et al., 2011). Khosravi et al., (2014) studied the pathogenicity of four isolates, IRAN 403C, SP 566, SPT 22 and IR-K-40 of *B. bassiana* on *Arge rosae* (Linnaeus 1970). The results showed the great effect of these isolates on this hymenopteran species; however, IRAN 403C isolate with LC_{50} value of 5.54×10^5 spore ml^{-1} and mortality rate of 82.5 % was highly more effective.

Mascarin et al., (2013) reported that *B.bassiana* CG1229 isolate in a concentration of 1×10^7 spore ml^{-1} led to more than 80 % of mortality rate in silver-leaf whitefly (*Bemisia argentifolii* (Gennadius, 1889)) populations. Therefore, *B.bassiana* has a high pathogenicity potential for this insect while it can significantly control this whitefly (Mascarin et al., 2013).

In the present study, *B. bassiana* TS11 had a significant pathogenic effect on *T. vaporariorum* adults, because it had a low LC_{50} value and led to high mortality rate (88.8 %) in concentration of 10^8 spore ml^{-1}. Mortality in control treatment was so low and no fungal growth was seen on dead adult whiteflies. In terms of pathogenicity of *B. bassiana* for *T. vaporariorum*, similar to the effects of synthesized ZnO nanoparticles, calculation of lethal concentrations such as LC_{25} (0.106×10^5 spore ml^{-1}) is in turn particularly important.

We should pay attention to the interactions of the entomopathogenic fungi and whiteflies natural enemies (i.e. *Encarsia* spp., *Eretmocerus* spp., etc.) to minimize adverse effects on them. Therefore, despite the best effect of *B. bassiana* on pest insects such as *T. vaporariorum*, we should also take into account the low lethal effects of this entomopathogenic fungus so that they have minimal impact on natural enemies.

4 CONCLUSION

The current study was performed to demonstrate the insecticidal effects of ZnO nanoparticles and *B. bassiana* on the greenhouse whitefly. The obtained results proved the efficiency of synthetic

nanoparticles and entomopathogenic fungi as effective control agents, which can lead also to the delay in pest resistance mechanisms to chemical insecticides. It is possible that by adding nanoparticles and entomopathogenic fungi to formulations of insecticides, toxicity of chemical insecticides for humans and other non-target organisms would be mitigated. Further study will need to focus on methods to increase stability and on physiological mechanisms of nanoparticles and entomopathogenic fungi interactions to increase their effects in integrated pest management programs at large greenhouse and field levels.

5 ACKNOWLEDGMENT

The authors are grateful to Department of Plant Protection and Department of Chemistry, University of Zabol, Iran, for financing and providing the laboratory facilities.

6 REFERENCES

AbdElhady M.M. 2012. Preparation and characterization of chitosan/zinc oxide nanoparticles for imparting antimicrobial and UV protection to cotton fabric. International Journal of Carbohydrate Chemistry: 1-6. Retrieved from DOI: 10.1155/2012/840591.

Auffan M., Rose J., Bottero J.Y., Lowry G.V., Jolivet J.P., Wiesner M.R. 2009. Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. Nat. Nanotechnol. 4: 634-641. DOI: 10.1038/nnano.2009.242.

Brayner R., Ferrari-Iliou R., Brivois N., Djedia S., Benedetti M.F., Fievet F. 2006. Toxicological impact studies based on *Escherichia coli* bacteria in ultra ZnO nanoparticles colloidal medium. Nano. Lett. 6: 866.

Chaudhry Q., Scotter M., Blackburn J., Ross B., Boxall A., Castle L., Aitken R., Watkins R. 2008. Applications and implications of nanotechnologies for the food sector. Food Addit. Contam. A 25 (3): 241-258. DOI: 10.1080/02652030701744538.

Clausen C.A, Kartal. SN., Arango R.A., Green F. 2011. The role of particle size of particulate nano-zinc oxide wood preservatives on termite mortality and leach resistance. Nanoscale Res. Lett. 6: 42. DOI: 10.1186/1556-276X-6-427.

El-Sinary N.H., Rizk S.A. 2007. Entomopathogenic fungus, *Beauveria bassiana* (Bals.) and gamma irradiation efficiency against the greater wax moth, *Galleria melonella* (L.). Am-Euras. J. Sci. Res. 2 (1): 13-18.

Fan Y., Fang W., Guo S., Pei X., Zhang Y., Xiao Y., Li D., Jin K., Bidochka M.J., Pei Y. 2007. Increased insect virulence in *Beauveria bassiana* strains overexpressing an engineered chitinase. Appl. Environ. Microbiol. 73(1): 295-302. DOI:10.1128/AEM.01974-06.

Goswami A., Roy I., Sengupta S., Debnath N. 2010. Novel applications of solid and liquid formulations of nanoparticles against insect pests and pathogens. Thin Solid Films. 519 (3): 1252-1257. DOI:10.1016/j.tsf.2010.08.079.

Gupta A.K., Gupta M. 2005. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. Biomaterials. 26 (18): 3995-4021. DOI:10.1016/j.biomaterials.2004.10.012.

Gurulingappa P., McGee P.A., Sword G. 2011. Endophytic *Lecanicillium lecanii* and *Beauveria bassiana* reduce the survival and fecundity of *Aphis gossypii* following contact with conidia and secondary metabolites. Crop Prot. 30: 349 - 353. DOI: 10.1016/j.cropro.2010.11.017.

Guzman P., Arredondo C.R., Emmatty D., Gilbertson R.L. 1997. Partial characterization of two whitefly-transmitted geminiviruses infecting tomatoes in Venezuela. Plant Dis. 81 (3): 312-318. Retrieved from DOI: 10.1094/PDIS.1997.81.3.312A.

Hayashi H. 1996. Side effects of pesticides on *Encarsia Formosa* Gahban. Bulletin of the Hiroshima Prefectural Agriculture Research Center. 64: 33-43.

Inglis G.D., Goettel M.S., Butt T.M., Strasser H. 2001. Use of hyphomycetous fungi for managing insect pests. In Butt T.M., Jackson C., Magan, N. (Eds), Fungi as biocontrol agents: progress problems and potential (pp. 23-69). New York: CABI.

Kairyte K., Kadys A., Luksiene Z. 2013. Antibacterial and antifungal activity of photoactivated ZnO nanoparticles in suspension. J. Photochem.

Photobiol. B. 128: 78-84. DOI:10.1016/j.jphotobiol.2013.07.017.

Khosravi R., Sendi J.J., Zibaee A., Shokrgozar M.A., 2014. Virulence of four *Beauveria bassiana* (Balsamo) (Asc., Hypocreales) isolates on rose sawfly, *Arge rosae* under laboratory condition. J. King Saud Uni-Sci 27 (1): 49-53. Retrieved from DOI: 10.1016/j.jksus.2014.04.003.

Kirthi A.V., Rahuman A.A., Rajakumar G., Marimuthu S., Santhoshkumar T., Jayaseelan C., Velayutham K. 2011. Acaricidal, pediculocidal and larvicidal activity of synthesized ZnO nanoparticles using wet chemical route against blood feeding parasites. Parasitol. Res. 109: 461-472. DOI: 10.1007/s00436-011-2277-8.

Lacey L.A., Frutos R., Kaya H.K., Vail P. 2001. Insect pathogens as biological control agents: Do they have a future?. Biol. Control. 21 (3): 230-248. DOI: 10.1006/bcon.2001.0938.

Laznik Z., Trdan S. 2014. The influence of insecticides on the viability of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) under laboratory conditions. Pest Manag. Sci. 70(5): 784-789. DOI: 10.1002/ps.3614.

Laznik Z., Znidarcic D., Trdan S. 2011. Control of *Trialeurodes vaporariorum* (Westwood) adults on glasshouse-grown cucumbers in four different growth substrates: An efficacy comparison of foliar application of *Steinernema feltiae* (Filipjev) and spraying with thiamethoxam. Turk. J. Agric. For. 35: 631-640. DOI: 10.3906/tar-1007-1110.

Lozano-Gutierrez J., Espana-Luna M. 2008. Pathogenicity of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) against the white grub *Laniifera cyclades* (Lepidoptera: Pyralidae) under field and greenhouse conditions. Fla. Entomol. 91 (4): 664-668. Retrieved from DOI: 10.1653/0015-4040-91.4.664.

Magrez S., Kasas V., Salicio N., Pasquier J., Seo W., Celio M., Catsicas S., Schwaller B., Forro L. 2006. Cellular toxicity of carbon-based nanomaterials. Nano Lett. 6 (6): 1121-1125. DOI: 10.1021/nl060162e.

Manzo S., Rocco A., Carotenuto R., Picione F.D.L., Miglietta M.L., Rametta G.D., Francia G. 2011. Investigation of ZnO nanoparticles'ecotoxicological effects towards different soil organisms. Environ. Sci. Pollut. Res. 18: 756-763. DOI: 10.1007/s11356-010-0421-0.

Mascarin G.M., Kobori N.N., Quintela E.D., Delalibera J.I. 2013. The virulence of entomopathogenic fungi against *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae) and their conidial production using solid substrate fermentation. Biol. Control. 66: 209-218. DOI: 10.1016/j.biocontrol.2013.05.001.

Mitra S., Chandra S., Laha D., Patra P., Debnath N., Pramanik A., Pramanik P., Goswami A., 2012. Unique chemical grafting of carbon nanoparticle on fabricated ZnO nanorod: Antibacterial and bioimaging property. Mater Res. Bull. 47 (3): 586-594. DOI: 10.1016/j.materresbull.2011.12.036.

Morones J.R., Elechiguerra J.L., Camacho A., Holt K., Kouri J.B., Ramirez J.T., Yacaman M.J. 2005. The bactericidal effect of silver nanoparticles. Nanotechnology. 16 (10): 2346. DOI: 10.1088/0957-4484/16/10/059.

Muniz M., Nombela G. 2001. Differential variation in development of the B - and Q - Biotypes of *Bemisia tabaci* (Homoptera: Aleyrodidae) on sweet pepper at constant temperatures. Environ. Entomol. 30 (4): 720-727. Retrieved from DOI: 10.1603/0046-225X-30.4.720.

Mustu M., Demirci F., Kocak E. 2011. Mortality effects of *Isaria farinosae* (Holm.) and *Beauveria bassiana* (Balsamo) Vuillemin (Sordariomycetes: Hypocreales) on *Aelia rostrata* Boh. (Hemipterab: Pentatomidae). Turkish Journal of Entomology. 35(4): 559-568.

Rai M., Ingle A. 2012. Role of nanotechnology in agriculture with special reference to management of insect pests. Appl. Microbiol. Biotechnol. 94: 287-293.

Ramos E.Q., Alves S.B., Tanzini M.R., Lopes R.B. 2000. Susceptibilidade de *Bemisia tabaci* a *Beauveria bassiana* en condiciones de laboratorio. Manejo Integrado de Plagas. 56: 65-69.

Reddy K.M., Feris K., Bell J., Wingett D.G., Hanley C., Punnoose A. 2007. Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic system. Appl. Phys. Lett. 90: 213902. Retrieved from DOI: 10.1063/1.2742324.

Rouhani M., Samih M.A., Aslani A., Beiki K. 2011. Side effect of nano-ZnO - Tio2 - Ag mix-oxide nanoparticles on *Frankliniella occidentalis* Pergande (Thys.: Thripidae). In Proceedings Symposium: Third International Symposium on Insect Physiology, Biochemistry and Molecular Biology. East China Normal University, Shanghai, China. 2-5.

Rouhani M., Samih M.A., Kalantari S. 2012. Insecticide effect of silver and zinc nanoparticles against *Aphis nerii* boyer de fonscolombe (Hemiptera: Aphididae). Chilean JAR. 72 (4): 590-594.

Samih M.A., Rouhani M., Aslani A., Beiki K., 2011. Insecticidal properties of amitraz, nano - amitraz, nano - ZnO and nano – ZnO - Al_2O_3 nanoparticles on *Agonoscena pistaciae* (Hem.: Aphelaridae). In Proceedings Symposium: Third International Symposium on Insect Physiology, Biochemistry and Molecular Biology. East China Normal University. Shanghai, China. 131.

Samuel U., Guggenbichler J.P. 2004. Prevention of catheter-related infections: The potential of a new nano - silver impregnated catheter. Int. J. Antimicrob. Ag. 23 (1): 75-78. DOI: 10.1016/j.ijantimicag.2003.12.004.

Sandhu S.S., Sharma A.K., Beniwal V., Goel G., Batra P., Kumar A., Jaglan S., Malhotra S., 2012. Myco - Biocontrol of insect pests: Factors involved, mechanism and regulation. J. Pathogens. 1-10. DOI: 10.1155/2012/126819.

Sewify G.H., Belal M.H., Al-Awash S.A. 2009. Use of the entomopathogenic fungus, *Beauveria bassiana* for the biological control of the red palm weevil, *Rhynchophorus ferrugineus* Olivier. Egypt J. Biol. Pest Control. 19 (2): 157-163.

Shi W.B., Feng M.G. 2004. Lethal effect of *Beauveria bassiana*, *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* on the eggs of *Tetranychus cinnabarinus* (Acari: Tetranychidae) with a description of a mite egg bioassay system. Biol. Control. 30 (2): 165-173. DOI: 10.1016/j.biocontrol.2004.01.017.

Suwanboon S., Amornpitoksuk P., Sukolrat A., Muensit N. 2013. Optical and photocatalyctic properties of La-doped ZnO nanoparticles prepared via precipitation and mechanical milling method. Ceram Int. 39: 2811-2819. DOI: 10.1016/j.ceramint.2012.09.050.

Tefera T., Pringle K.L. 2004. Evaluation of *Beauveria bassiana* and *Metarhizium anisopliae* for controlling *Chilo partellus* (Lepidoptera: Crambidae) in Maize. Biocontrol Sci. Techn. 14 (8): 849-853. DOI: 10.1080/0958315041000172707.

Umar A., Rahman A., Vaseem M., Hahn Y.B. 2009. Ultra-sensitive cholesterol biosensor based on low - temperature grown ZnO nanoparticles. Electrochem. Commun. 11 (1): 118-121. DOI: 10.1016/j.elecom.2008.10.046.

Van Lenteren J.C., Van Roermund H.J.W., Sutterlin S., 1996. Biological control of Greenhouse whitefly (*Trialeurodes vaporariorum*) with the Parasitoid *Encarsia formosa*: How does it work?. Biol Control. 6 (1): 1-10. DOI: 10.1006/bcon.1996.0001.

Velayutham K., Rahuman A.A., Rajakumar G., Roopan S.M., Elango G., Kamaraj C., Siva C. 2013. Larvicidal activity of green synthesized silver nanoparticles using bark aqueous extract of *Ficus racemosa* against *Culex quinquefasciatus* and *Culex gelidus*. Asian Pac. J. Trop. Med. 95-101. DOI: 0.1016/S1995-7645(13)60002-4.

Whalon M.E., Mota-Sanchez D., Hollingworth R.M. 2008. Analysis of global pesticide resistance in arthropods. In Whalon M.E., Mota-Sanchez D., Hollingworth R.M. (Eds.), Global pesticide resistance in Arthropods (pp. 5-31). CABI, Wallingford, UK.

Wraight S.P., Carruthers R.I., Bradley C.A., Jaronski S.T., Lacey L.A., Wood P., Galaini-Wraight S. 1998. Pathogenicity of the entomopathogenic fungi *Paecilomyces* spp. and *Beauveria bassiana* against the silverleaf whitefly, *Bemisia argentifolii*. J. Invertebr. Pathol. 71: 217-226. DOI: 10.1006/jipa.1997.4734.

Wraight S.P., Carruthers R.I., Jaronski S.T., Bradley C.A., Garza C.J, Galaini-Wraight S. 2000. Evaluation of the entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* for microbial control of the silverleaf whitefly, *Bemisia argentifolii*. Biol. Control. 17: 203-217. DOI: 10.1006/bcon.1999.0799.

Wraight S.P., Ramos M.E., Avery P.B., Jaronski S.T., Vandenberg J.D. 2010. Comparative virulence of *Beauveria bassiana* isolates against lepidopteran pests of vegetable crops. J. Invertebr. Pathol. 103: 186-199. DOI: 10.1016/j.jip.2010.01.001.

Zhang L., Jiang Y., Ding Y., Povey M., York D. 2007. Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnO nanofluids). J. Nanopart. Res. 9 (3): 479-489. DOI: 10.1007/s11051-006-9150-1.

Zhu Y., Yu F., Man Y., Tian Q., He Y., Wu N. 2005. Preparation and performances of nanosized Ta_2O_5 powder photocatalyst. J. Solid State Chem. 178: 224-229. DOI: 10.1016/j.jssc.2004.11.015.