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Effect of zinc oxide nanoparticles on pulse beetle *(Callosobruchus maculatus)* (Col.: Chrysomelidae) in greengram

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Abstract
The effect of Zinc oxide nanoparticles at different concentrations [0 (T_0), 25 (T_1), 50 (T_2), 75 (T_3), 100 (T_4), 125 (T_5), 150 (T_6), 175 (T_7) and 200 (T_8) ppm] on pulse beetle *(Callosobruchus maculatus)* (Col.: Chrysomelidae) in greengram was studied in terms of different entomological parameters viz., adult mortality, number of eggs, seed weight loss and seed damage. The percent mortality was recorded on 1st, 2nd, 4th, 7th and 14th days of treatment. The highest mortality was found in treatment T_8 with 100% on 14th day which was statistically on par with treatment T_7 (95.00%). There was no mortality observed in control on 1st and 2nd day, whereas on 14th day, it was 36.67%. The number of eggs per 100 seeds were less in treatment T_8 (13.33) and the highest was recorded in control with 27.33 eggs/100 seeds. The weight loss in seed due to infestation recorded was lowest (1.83%) in treatment T_8 which was statistically on par with T_7 (2.38%) and T_6 (2.43%) and the highest in control (2.88%). Similar trend was also noticed in seed damage (14.00%), the lowest in treatment T_8 and the highest in control (34.00%).

Keywords: ZnO nanoparticles, pulse beetle, greengram, seed damage

Introduction
Nanoparticles exhibit new or improved properties with larger particles of the bulk materials and these novel properties are derived due to the variation in specific characteristics such as size, distribution and morphology of the particles (Ravindra *et al.*, 2011) [9]. The conventional methods of synthesizing nanoparticles using chemical method were found to be more expensive and also found to involve the use of toxic, hazardous chemicals which were responsible for various biological risks (Geoprincy *et al*., 2012) [4]. In this regard using "green" methods for the synthesis of Zinc oxide nanoparticles has increasingly become a topic of interest over conventional chemical methods (Mason *et al.*, 2012) [6].
Nanoparticles help to produce new pesticides, insecticides and insect repellants (Owolade *et al.*, 2008) [7]. Targeted nanoparticles often exhibit novel characteristics such as extraordinary strength, more chemical reactivity and high electrical conductivity. Bruchid beetle, *Callosobruchus maculatus* is a major stored pest responsible for considerable damage in stored pulses and make the pulses unfit for human consumption (Singh and Jackai, 1985) [14] The control of stored grain pests stands mostly on broad action of insecticides and fumigants. But, this leads to contamination of food with toxic pesticide residues (Debnath *et al.*, 2011) [3]. Moreover, the main problem in controlling pests in stored grain is the resistance to pesticides. Regarding the resistance of grain pests and pesticide residues, it seems that chemical control is not an appropriate approach for controlling these pests (Rouhani *et al.*, 2012b) [11]. Traditional strategies like integrated pest management used in agriculture are insufficient and application of chemical pesticides have adverse effects on animals and human beings apart from the decline in soil fertility (Ragaei and Sabry, 2014) [8]. Thus, nanotechnology has become one of the most promising new approaches for pest control in recent years. Also, researchers believe that nanotechnology will revolutionize agriculture including pest management in the near future. Over the next two decades, the green revolution would be accelerated by means of nanotechnology (Bhattacharyya *et al*., 2010) [2].
In this context, the objective of this research was to study the effect of biosynthesized Zinc oxide nanoparticles on pulse beetle (*Callosobruchus maculatus*) (Col.: Chrysomelidae) in

greengram.

Materials and methods

Raw material

Greengram seeds (variety, BGS-9) were collected from seed unit, UAS campus, Raichur.

Biosynthesis of ZnO nanoparticles

The ZnO nanoparticles were synthesized from spinach (*Spinacia oleracea*) leaves with Zinc nitrate hexahydrate solution as a precursor. A change in the colour from dark green to pale yellow indicated the formation of ZnO nanoparticles. The presence of ZnO nanoparticles were confirmed and characterized by Particle size analyzer, UV-Visible spectrophotometer and Scanning electron microscope.

Maintenance of stock culture

Adult of *C. maculatus* from the infested greengram seeds were collected from the National Food Security Mission lab, Raichur and the culture was maintained in plastic jars of one litre capacity. The mouth of the container was covered with muslin cloth and fastened tightly with the help of rubber band. Fresh seeds were provided regularly and exposed separately for the multiplication of insects at room condition. The insects emerged from this culture were reared throughout the period of experimentation.

Bioassay

The bioassay of *C. maculatus* was performed in greengram seeds with different concentration of Zinc oxide nanoparticles in small plastic screw capped jars. Each jar had a radius of 6 cm and height of 6.5 cm. Infestation free sound seeds (20 g) of greengram were weighed by electronic balance, sun dried and kept in hot air oven for 2 hours at 50^{o}C to sterilize and make the sample free from previous infestation. Twenty gram of greengram seeds were primed with ZnO NPs with different concentrations (0, 25, 50, 75, 100, 125, 150, 175 and 200 ppm) and was placed in jar and kept for 24 h before introducing adults (*C. maculatus*) into each jar. All bioassays were performed at room condition with release of 5 pairs of newly emerged adults of *C. maculatus* as shown in Plate 1. Insect mortality was checked after 1st, 2nd, 4th, 7th and 14th days after treatment (Rouhani *et al.*, 2012b) [11]. The observations were recorded in terms of number of eggs laid per 100 seeds, weight loss and seed damage of the seeds. Each treatment was replicated three times.

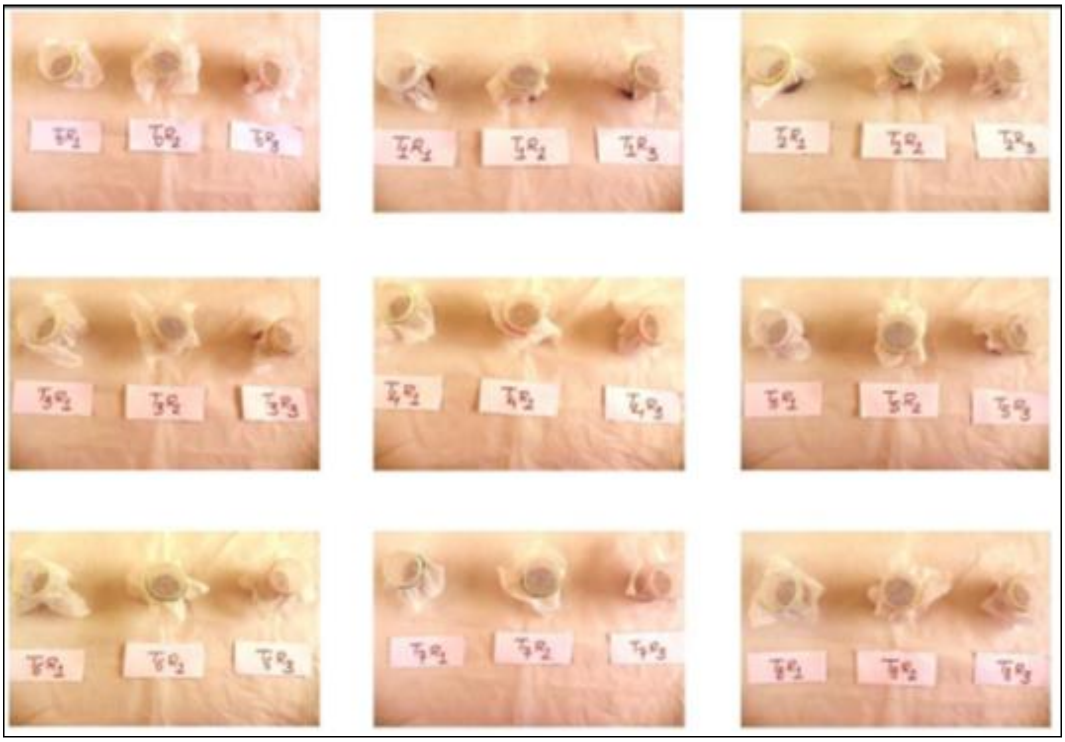

Plate 1: Experimental setup of bioassay

Adult mortality

Callosobruchus maculatus mortality was calculated using the following formula described by Rouhani *et al.* (2012b) [11].

$$\text{Adult mortality (\%)} = \frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100$$

Seed weight loss

Seed weight loss was computed by the following formula as suggested by Harris and Lindblad (1978) [5].

$$\text{Seed weight loss (\%)} = \frac{\text{Original weight} - \text{Final weight}}{\text{Original weight}} \times 100$$

Seed damage

Seed damage was calculated by following formula as suggested by Tamiru *et al.* (2016) [15].

$$\text{Seed damage (\%)} = \frac{\text{Number of damaged seeds}}{\text{Total number of seeds}} \times 100$$

Results and discussion

Effect on adult mortality

It was observed that with increase in concentrations and number of days after treatment, the mortality also increased. The highest mortality was observed in treatment T_8 with 100% mortality on 14th day as shown in Fig 1. There was no mortality observed in control (T_0) in first and second day, whereas on 14th day, it was 36.67%. The reason might be due to the release of a greasy layer by insects on their body surface, which might be involved in physical interactions between the organisms especially during mating. This could have caused incomplete mating in the case of lower concentrations and prevented mating at higher concentrations of Zinc oxide nanoparticles. The damage could have occurred to the protective wax coat on the cuticle of insects, both by sorption and abrasion so that the insects begin to lose water and die due to desiccation (Arumugam *et al.*, 2016). Similar results were also obtained by Debnath *et al.* (2011) [3] for silica nanoparticles in *Sitophilus oryzae*, Rouhani *et al.* (2012a) [10] for silver and Zinc nanoparticles at 20 mg/l in *Aphis nerii* and Salem *et al.* (2015) [13] for aluminium and Zinc oxide nanoparticles at 2 g/kg in *T. castaneum.*

Effect on number of eggs

The egg count revealed that the number of eggs per 100 seeds were less in treatment T_8 (13.33) after 14 days and the highest was recorded in control (T_0) with 27.33 eggs/100 seeds by *C. maculatus* as shown in Fig 2. These results are in agreement with Sabbour (2015) [12] nano entomopathogenic fungi in *S. oryzae,* Arumugam *et al.* (2016) [1] for silica nanoparticles at 1000 ppm in *Callosobruchus maculatus.*

Effect on seed weight loss

The seed weight loss due to infestation was recorded after 30 days of release of adults. The lower weight loss of greengram seeds was recorded in treatment T_8 (1.83%) as against control (2.88%) as shown in Fig 3. Similar results were also obtained by Salem *et al.* (2015) [13] for aluminium and Zinc oxide nanoparticles at 2 g/kg in *T. castaneum.*

Effect on Seed damage

From Fig 4, it was evident that the seed damage in greengram seeds reduced in all the treatments over control. The seed damage recorded in treatment T_8 was 14.00% and T_7 was 17.00% as against 34.00% in control (T_0). The reason for reduction in number of eggs, seed weight loss and seed damage in T_8 might be due to early mortality of pulse beetles. These results are in agreement with Arumugam *et al.* (2016) [1] for silica nanoparticles at 1000 ppm in *Callosobruchus maculatus.*

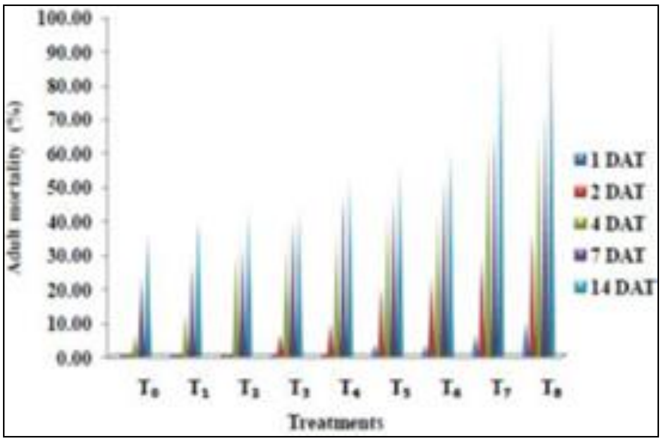

Fig 1: Effect of Zinc oxide nanoparticles on mortality of pulse beetle in greengram seeds

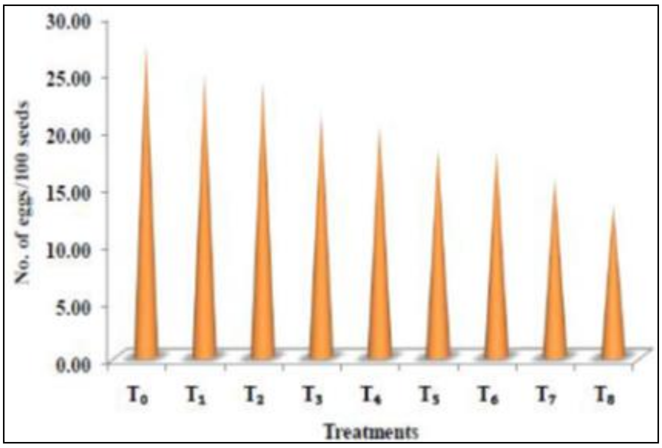

Fig 2: Effect of Zinc oxide nanoparticles on number of eggs in greengram seeds

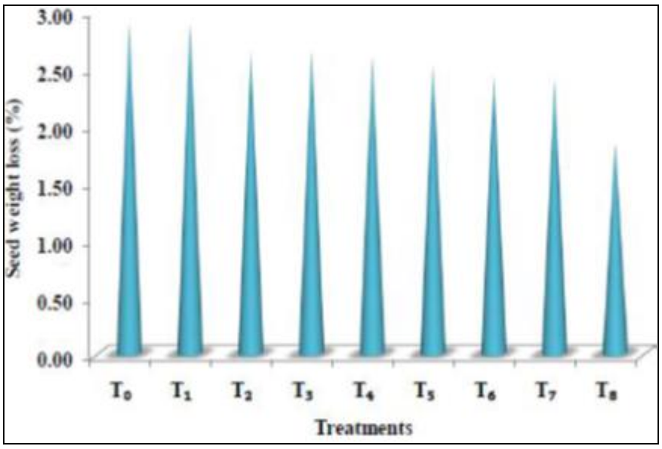

Fig 3: Effect of Zinc oxide nanoparticles on seed weight losss

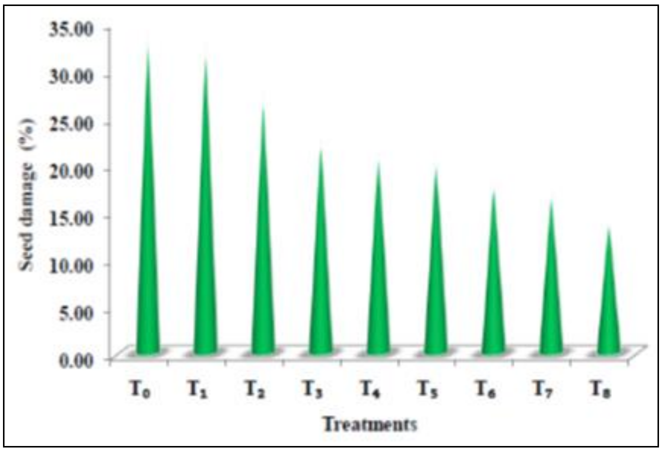

Fig 4: Effect of Zinc oxide nanoparticles on seed damage

Conclusion

This study investigated the effect of biosynthesized Zinc oxide nanoparticles from Spinach (*Spinacia oleracea*) leaves on pulse beetle (*Callosobruchus maculatus*) in green gram. Zinc oxide nanoparticles showed potential entomotoxicity against pulse beetle (*Callosobruchus maculatus*). Among different concentrations, 175 ppm was found to be effective to control *Callosobruchus maculatus*. This study could lead to open up newer pathways of using biosynthesized Zinc oxide nanoparticles for the control of stored grain insect pests, which could be alternative for insecticidal agents.

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