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Effect of Biologically Synthesized Silver Nanoparticles from *Murraya koenigii* (Curry leaf) on *Callosobruchus maculates* (cowpea weevil)

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The present investigation was aimed to study the Biologically Synthesized Silver Nanoparticles from *Murraya koenigii* (Curry leaf) on *Callosobruchus maculates* (cowpea weevil) to determine adult mortality, seed damageand seed weight loss. The effect of Ag NPs on *Callosobruchus maculates* (cowpea weevil) was studied by rearing the insects and preparing bioassay bytreating chickpea seeds with two concentrations of synthesized and standard Ag NPs (25-30 and 65-70 ppm) at 1.0:0.4 seed to solution ratio. 100% mortality was observed after one week of treatment S_2@ 65-70 ppm and after two weeks of B_2 @ 65-70 treatment. The least seed damage was traced in treatment S_2 (41.63%) followed by B_2 (45%) @ 65-70 ppm and the least seed weight loss was traced in treatment S_2 (1.24%) followed by B_2 (1.68%) @ 65-70 ppm after two weeks of treatment.

Keywords: Silver Nanoparticles; Murraya koenigii; Callosobruchus maculates; adult mortality; seed damage seed; weight loss.

INTRODUCTION

Storage of grains is a part of post-harvest system through which food materials pass from field to consumer. One of the most important and essential issues during storage is the loss in quality and quantity of the grains caused by insects leading to damage and reduction of their dry weight and nutritional value (Zahir et al., 2012).

Callosobruchusmaculatus is a species of beetles known commonly as the cowpea weevil or cowpea seed beetle. This common pest of stored legumes has a cosmopolitan distribution (Beck and Blumer, 2013). A female adult can produce over a hundred eggs, and most of them will hatch. It emerges after a larval period of 3 to 7 weeks, depending on conditions (Mano and Toquenaga, 2008). It is used as a model organism for both research and education due to its quick generation time, sexual dimorphism, and ease of maintenance. The beetle is considered "medically harmless" to humans. It is a damaging agricultural pest (Murdock et al., 2012).

Insect infestation in grains and dry food products is currently controlled by chemicals that can effect on ozone as depletion compound [5]. The persistent use of chemicals can lead to serious problems associated with human and environmental health (sparks et al., 2012) So, there is an urgent need to find an alternative for the purpose of insect management that is eco-friendly, safer and economically viable. Nanotechnology is one of the most novel new approaches for pest management in recent years particularly nanoparticles. Ag NPs may be an alternative to control growth of insects and pests during storage, as they are proved to have potential antibacterial, antifungal and larvicidal properties (Ragaei and Sabry, 2014). Thus, the aim of the present study was to study the effect of biologically synthesized Ag NPs on storage insects in comparison with standard Ag NPs.

MATERIALS AND METHODS

Biological synthesis of Ag NPs using *Murraya koenigii*

Ten grams of fresh curry leaves were weighed in an electronic balance were thoroughly rinsed with distilled water and chopped into small pieces using stainless steel knife. The chopped leaves were boiled in distilled water for 15 min at 55°C using water bath. The leaf extract was cooled to room temperature (24-25°C) and filtered using Whattman filter paper yielding about 45-500 mL of leaf broth and it was stored in a refrigerator for further study. 5 mL of prepared leaf broth was added to 100 mL of 1.50 mMAgNO$_3$ solution. The mixture was incubated at 100°C using rectangular hot plate until color changed from transparent yellow to dark brown indicating the formation of Ag NPs. The pH of synthesized Ag NPs was adjusted using pH meter and stored in a refrigerator (8±1°C) for further study (Al-Othman et al., 2014).

Effect of Ag NPs on *Callosobruchus maculates* (cowpea weevil) in chickpeas

The effect of Ag NPs on cowpea weevil was studied byrearing the insects and preparing bioassay by treating chickpea seeds with two concentrations of synthesized and standard Ag NPs (25-30 and 60-70 ppm). The detailed procedure followed in carrying out this experiment is presented below

Maintenance of cowpea weevil culture

The infested chickpea with weevil culture was maintained in a plastic container separately. Initially 1 kg of disinfested seeds was used and adults of *Callosobruchus maculates*were released from the infested lot. The plastic containers for maintaining culture were covered with the muslin cloth and fastened with the rubber band. Likewise, the culture was established and continued till they complete two generations. The experiment was conducted at Department of zoology, maintained at 32±2°C and 70% relative humidity in continuous dark condition.

Bioassay

The bioassay of weevil was performed with chickpea seeds with two different concentrations of Ag NPs in plastic screw capped jars. Fifty grams of fresh seeds, which were free from infestation were weighed using electronic balance and kept in hot air oven 50^{0}C for 120 minutes to sterilize and make the sample free from field infestation. Sterilized seeds were primed with both synthesized and standard Ag NPs at 1.0:0.4 seed to solution ratio with two concentrations (25-30 and 65- 70 ppm). Treated seeds were placed in jars and kept for one day, and then 5 pairs of newly emerged adults were transferred to each box. The experiment was replicated thrice and the untreated seeds were taken as control (Sankar and Abideen, 2015).

Adult mortality

In each treatment, the number of insects taken was 10. The number of insects dead from each sample was counted manually and the mean of three replications were taken to calculate the adult mortality (Rouhani et al., 2012). Insect mortality was checked after 1st, 3rd, 5th, 7th and 14thday

$$= \frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100$$

Seed damage

Here, the treated seeds from each treatment were taken and the number of seeds damaged by the insects after 1st, 3rd, 5th, 7th and 14th day was counted (tamiru et al., 2016). The mean of three replications were taken to calculate the seed damage (%) for each treatment.

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

Seed weight loss

The weight of each sample was measured using an electronic balance and replicated thrice. The mean of replications was taken to determine the seed weight loss from each treatment (Sajeshkumar et al., 2015).

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

RESULTS AND DISCUSSION

For biological Synthesis, curry leaf extract was added to $AgNO_3$ solution and a visible color change from transparent yellow to dark brown was observed, which indicated the formation of Ag NPs. This might be due to the reduction of silver ions in the solution by the presence of terpenoids (Ashtaputrey et al., 2017) .The color changed from colorless to yellowish and then to dark brown for curry leaves(Raju and Ebency, 2017; Wazid et al., 2018).

The effect of synthesized and standard Ag NPs at two different concentrations against *Callosobruchus maculates* by considering different entomological parameters such as adult mortality, seed damage and seed weight loss. From the results, it is observed that, with increase in concentrations and number of days after treatment, the adult mortality also increased. Biologically synthesized Ag NPs showed similar effect on adult mortality as standard Ag NPs @ 65-70 ppm. Similar results were observed that, the Ag NPs @ 50 ppm showed mortality of 100.00% on 14th day for adult of *Sitophilusoryzae*. The Ag NPs @ 50 ppm showed 100.00% mortality on 4th day after treatment imposition on *Sitophilusoryzae.*ZnO NPs @1250 ppm recorded mortality of 96.66% on pulse beetle 10 days after treatment (Arumugam et al., 2016). This might be due to ingestion of seed treated with nanoparticles which might have caused abrasion of mid gut leading to death of adults (Saleem et al., 2015).

Table.1 Effect of Ag NPs on *Callosobruchus maculates* (cowpea weevil) in chickpeas

Treatment	Adult mortality(%) at different days of observation					Seed Damage (%)	Weight Loss (%)
	1DAY	4 DAY	3 DAY	1st week	2nd week	1st fortnight	1st fortnight
Untreated control	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	14.44 (21.14)	62.56 (38.34)	2.21 (8.34)
B1 @ 25-30 ppm	0 (0.00)	25.56 (40.98)	34.44 (45.90)	65.56 (51.19)	94.44 (66.59)	35.56 (30.00)	1.14 (5.10)
S1 @ 25-30 ppm	0 (0.00)	30 (44.98)	60 (35.65)	84.44 (55.11)	95.56 (84.84)	49.44 (44.39)	1.05 (3.91)
B2 @ 65-70 ppm	4.14 (5.14)	30 (44.89)	64.44 (38.98)	85.56 (58.82)	100 (90)	45 (40.46)	0.95 (3.51)
S2 @ 65-70 ppm	5.56 (12.28)	54.44 (32.63)	84.44 (55.11)	100 (90)	100 (90)	41.63 (49.02)	1.68 (1.06)
Mean SEm (±)	2 2.10	48 3.36	33.99 2.38	59.44 2.38	80.55 2.38	31.85 2.94	1.24 0.09
C.D. @1%	5.42	16.84	8.24	8.24	8.24	9.46	0.40

Seed damage (%)

Application of synthesized and standard Ag NPs resulted in reduced seed damage potential in all the treatments over control. It is observed that biologically synthesized Ag NPs showed similar effect on reduction of seed damage as standard Ag NPs @ 65-70 ppm. The per cent seed damage was reduced (4%) for wheat grain using Ag NPs (200 ppm) treatment over control. ZnO NPs @ 1000 ppm reduced the seed damage by 75% over the control (Arumugam et al., 2016). The reduction in seed damage by the infestation of pulse beetle *Callosobruchuschinensis*(L.) treated withsilica nanoparticles. There was inverse relationship between adult mortality (%) and seed damage (%).

Seed weight loss (%)

Reduced seed weight loss was observed due to application of Ag NPs at two different concentrations compared to control for silica nanoparticles at 1000 ppm in pulse beetle, *Callosobruchuschinensis*(L.).The reasonfor reduction in seed weight loss (%) and seed damage (%) might be due to early mortality of pulse beetles (Saleem et al., 2015).

CONCLUSION

Biological synthesis of Ag NPs was carried out by using curry leaves, *Murraya koenigii* (L.) as reducing agent. Formation of Ag NPs was confirmed by the colour change from transparent yellow to dark brown. The effect of synthesized and standard Ag NPs on *Murraya koenigii* (Curry leaf) on *Callosobruchus maculates* (cowpea weevil) in chickpea seed by priming method by considering different entomological parameters. The adult mortality of 100 % was observed in treatment S_2 after one week of treatment and B_2 after two weeks of treatment. The minimum seed damage was recorded in treatment S_2 followed by B_2 @ 65-70 ppm and the minimum seed weight loss was recorded in treatment S_2 followed by B_2 @ 65-70 ppm after two weeks of treatment. Hence, it was found that biologically synthesized Ag NPs showed better entomological parameters and recorded almost similar results as standard Ag NPs.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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