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The persistence of residual toxicity of zinc, copper and silica green nanoparticles against important storage pests

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Abstract

Postharvest losses in India are estimated to be around 10 per cent in developed countries and 20 per cent or more in developing countries due to lack of proper storage facilities, improper handling of grains, and spillage of grains, besides contaminating food products through the presence of live insects, chemical excretions, dead insects and insect body fragments. The excessive use of pesticides and chemical compounds affects the grain quality and unsuitable for human consumption and also has an adverse effect on environment. This experiment was conducted at Centre for Nanotechnology Laboratory, UAS, Raichur. The green nanoparticles of zinc, copper and silica were biologically synthesized from Spinach leaves, tulasi leaves and paddy husk respectively, and these nanoparticles were characterized by Zetasizer, UV-Vis spectroscopy, X-ray diffraction (XRD), and Scanning electron microscope (SEM). The pesticidal effects of these green nanoparticles were used as stored product insect protectants compared to malathion as standard reference. Among the different nanoparticles the concentration of 1500 ppm of Silica nanoparticle proved to be superior by recording highest mortality, lowest number of eggs, lowest adults emerged, least seed damage, and seed weight loss in both in sorghum and chick pea seeds up to five months of storage then compare to zinc and copper nanoparticles.

Keywords: *Callosobruchus analis*, chick pea, sorghum, *Sitophilus oryzae*, nanoparticles

Introduction

Out of the several basic needs of mankind, food is the first and most important one. The world as a whole is in the grip of acute food crisis. In this context the preservation of food grain whatever is produced, is of paramount importance of food grains produced are often stored for future needs, but due to improper handling and unscientific storage methods it has led to economic loss. There are 854 million food insecure people globally, of which a third live in India (Borlaug, 2007) [2]. In addition to rising demand for food resulting from increasing population and economic growth, increased risks of food insecurity are foreseen from an estimated rising demand of global energy of 50 per cent 2030, a decline in per capita land from 0.40 ha in 1961 to 0.25 ha in 1999 and projected climate change impacts (Sastry *et al.*, 2011) [11].

Losses due to stored grain pests in India is 25 per cent in rice and sorghum, 5 per cent in wheat, and 15 per cent in pulses. Food grain production in India was reported to be 250 million tons in the year 2010-2011, in which nearly 20–25 per cent food grains are damaged by stored grain insect pests (Dhaliwal *et al.*, 2010) [3]. According to the Food and Agricultural Organization (FAO) estimate, 10 to 25 per cent of the world's harvested food is destroyed annually by insects and rodent pests.

Chick pea [*Cicer arietinum* (Linnaeus)] is an important legume crop, constitutes an integral part of Indian agriculture because it enriches human diet and soil fertility. Sorghum (*Sorghum bicolor* (L.) Moench) is a premier crop of the semi arid tropics which ranks fourth after rice, wheat and maize and is a major staple food in several parts of the world. It is a dry land crop grown in *kharif* and *rabi* seasons for the utility as food, feed, forage and industrial raw material

Rice weevil is economically important storage pest on sorghum and other cereals in tropical and sub-tropical regions of the world. Rice weevil infestation alone resulted in sorghum grain loss of 61.3 per cent over a period of five months (Jayakumar *et al.*, 2017) [6].

Callosobruchus analis is a very important pest of grain legumes both in storage and field. It is distributed throughout India. It attacks peas, chickpea, pigeon pea, black gram, horse gram, cowpea etc. Larval feeding on the cotyledons causes significant losses in seed weight and viability.

Green nanotechnology has goals to produce nanomaterials and products without harming the environment or human health and producing nano products that provide solutions to environmental problems. It uses existing principles of green chemistry and green engineering to make nanomaterials and nano-products without toxic ingredients at low temperatures using less energy and renewable inputs (Gnanasangeetha and Thambavani, 2014) [4]. 'Green synthesis' or 'Biogenic synthesis' of nanoparticles shows better advancement over chemical and physical methods as it is lesser toxic, cost effective, environmental friendly (Vidya *et al.*, 2013) [12]. Keeping in view of the above facts, the present investigation on The persistence of residual toxicity of zinc, copper and silica green nanoparticles against important storage pests was undertaken.

Materials and Methods

Present Study was carried out during 2017-18 at the Department of Agricultural Entomology, College of Agriculture, and Centre for Nanotechnology, College of Agriculture engineering, UAS, Raichur, Karnataka.

Biosynthesis and characterization of different green nanoparticles from different plant sources

The biosynthesis of Zinc oxide nanoparticles was carried out using spinach leaf extract and Zinc nitrate hexahydrate solution as a precursor. The colour change was due to excitation of surface Plasmon vibration (Amrita *et al.*, 2015) [1].

The biosynthesis of copper nanoparticles was carried out using tulasi leaf extract and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. The colour change was due to active molecules present in the extract which reduced the $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ metal ions into copper nanoparticles (Mekal *et al.*, 2016) [7].

According to Rafiee *et al.* (2012) [8] biosynthesis of silica nanoparticles was undertaken from rice husk.

Characterization of biosynthesized green nanoparticles

These biosynthesized green nanoparticles were characterized by dynamic light scattering (Zetasizer) analysis, UV-visible spectrophotometer analysis, X-ray spectroscopy (XRD) and scanning electron microscopy (SEM).

Maintenance of pure culture

Stored grain pests were collected from the infested chick pea and sorghum seeds and the culture was further maintained in plastic jars of two kg capacity containing chickpea seeds and sorghum seeds. Fresh seeds were provided regularly and exposed separately for the multiplication of insects at room temperature of 27 °C. The insects emerged from this culture were used throughout the period of experimentation.

Bioassay studies for pulse beetle on chickpea seeds and rice weevil on sorghum seeds

Infestation free sound (one kg) sorghum and chickpea seeds were sun dried and later kept in hot air oven for one hour at 50 °C to sterilize to ensure that sample is free from previous infestation and such seeds were treated with the most effective concentration of green nanoparticles and insecticide

and kept in plastic container. In the container insects were released at monthly interval upto 6 months of storage. The observations were made on per cent seed damage, weight loss of the seeds, adult emergence and percent reduction over control at monthly interval up to 6 months after treatment. Observations were also recorded on germination percentage and dehydrogenase enzyme activity. It was replicated thrice. Damaged seeds were counted for each treatment by drawing a sample of 100 seeds at random. Adults that emerged from 100 g were obtained by deep freezing for about five minutes and sieved. Seed weight loss was computed by the following formula as suggested by Harris and Limblad (1978) [5].

$$\text{Per cent weight loss} = \frac{\text{O.W.} - \text{C.W.}}{\text{O.W.}} \times 100$$

Results and Discussion

Persistence of residual toxicity of zinc, copper and silica green nanoparticles on pulse beetle on chickpea seeds

Oviposition of *Callosobruchus analis* on chickpea seeds

Results of study on efficacy of different green nanoparticles at different days after treatment against the pulse beetle revealed that the number of eggs laid per 100 seeds were nil in silica and copper green nanoparticle @ 1500 ppm 30 days of treatment. Whereas significantly lowest egg laying was noticed in silica nanoparticle at 1500 ppm (3.55 eggs /100 seeds) followed by copper nano particle at 1500 ppm (26.25 eggs / 100 seeds) at 150 days after treatment. Whereas the highest egg laying was noticed in untreated check (260.7 eggs /100 seeds) and no egg laying was noticed in Malathion 5D @ 1% (table 1).

The overall mean eggs laid revealed that silica green nanoparticle at the dose of 1500 ppm concentration recorded least average number of eggs per 100 seeds (0.71 eggs/ 100 seeds) and the highest was in untreated check (173.22 eggs/ 100 seeds) and there was no egg laying in malathion treatment (table 1).

Population builds up of *Callosobruchus analis* on chickpea seeds

The adult population at 150 days after storage ranged from 0.00 to 246.50 adults per 100 g of seeds. Among the different nanoparticles the minimum adult emergence was noticed in silica nanoparticle at 1500 ppm (1.25 adults /100g seeds) followed by copper nano particle at 1500 ppm (16.00 adults / 100g seeds). Whereas the highest population build up was noticed in untreated check (246.50 eggs /100 seeds) and there was no egg laying in Malathion 5D @ 1% (Table 1).

The average population build up revealed that silica green nano particle at 1500 ppm recorded least average population build up (0.25adults/ 100 g of seeds) and highest average population build up was noticed in untreated check (146.04 adults/ 100 g of seeds). The data on population build up clearly depicted that malathion followed by silica green nanoparticle at 1500 ppm showed superiority in preventing the emergence of pulse beetle (Table 1).

Seed damage by *Callosobruchus analis*

Among the different nanoparticles the minimum seed damage percentage was noticed in silica nanoparticle at 1500 ppm (0.75%) followed by copper nanoparticle at 1500 ppm (8.25%). Whereas the highest seed damage was noticed in untreated check (98.50%) (Table 2).

Silica green nanoparticle @ 1500 ppm concentration shows least average seed damage (0.15%) which was followed by copper nanoparticle @1500 ppm (1.15%). Whereas highest seed damage was recorded in untreated check (48.10%). The data clearly indicated that malathion followed by silica green nanoparticle at 1500 ppm showed superiority in reducing the seed damage by pulse beetle (Table 2).

Seed weight loss by *Callasobruchus analis*

The seed weight loss due to infestation recorded after 150 days of release of beetles indicated that the silica nanoparticle at 1500 ppm recorded the least (0.25%) which was followed by copper nano particle at 1500 ppm (4.00%). Whereas the highest seed weight loss was noticed in untreated check (88.00%) and there was no seed weight loss noticed in Malathion 5D @ 1% (Table 2).

The average weight loss was computed and it revealed that silica green nanoparticle at 1500 ppm had the least average seed weight loss (0.05%) and highest average seed weight loss was in case of untreated check (48.10%). The data clearly depicted that malathion followed by silica green nanoparticle at 1500 ppm showed superiority over other treatments in reducing the seed weight loss and cent per cent reduction over control (Table 2)..

Persistence of residual toxicity of zinc, copper and silica green nanoparticles on rice weevil on sorghum seeds

Population builds up of *Sitophilus oryzae*

Among the different nanoparticles the minimum adult emergence was noticed in copper nanoparticle @ 1500 ppm (27.50 adults / 100g seeds) followed by zinc nanoparticle @ 1500 ppm (69.75 adults per 100g of seeds). Whereas the highest population build up was noticed in untreated check (224.25 adults per 100g seeds) and there was no egg laying noticed in Malathion 5D @ 1% and silica nanoparticle @ 1500 ppm (Table 3). The average population build up revealed that silica green nanoparticle @ 1500 ppm and malathion 5D @1% recorded nil population build up and highest average population build up was noticed in untreated check (146.75 adults/ 100 g of seeds). The data on population build up clearly depicted that malathion followed by silica green nanoparticle at 1500 ppm showed superiority in preventing the emergence of rice weevil (Table 3).

Seed damage by *Sitophilus oryzae*

Seed damage by *Sitophilus oryzae* after 150 days after treatment revealed that the minimum seed damage per cent was noticed in copper nanoparticle @ 1500 ppm (15.50%), Whereas the highest seed damage was noticed in untreated check (91.25%) and there was no seed damage noticed in Malathion 5D @ 1% and silica green nanoparticle @ 1500 ppm (Table 4). Average seed damage among all the different nanoparticles the silica green nanoparticle @1500 ppm concentration and malathion 5D @ 1% showed nil seed damage. Whereas least seed damage (5.05%) was noticed in copper nanoparticle @1500 ppm and highest seed damage recorded in untreated check (58.76%). The data clearly indicated that malathion followed by silica green nanoparticle @ 1500 ppm showed superiority in reducing the seed damage by rice weevil (Table 4).

Seed weight loss by *Sitophilus oryzae*

The observation recorded after 150 days of release showed that the minimum seed weight loss was noticed in copper nanoparticle @ 1500 ppm (8.25%) followed by zinc nanoparticle @1500 ppm (14.50%), Whereas the highest seed weight loss was noticed in untreated check (59.50%) and there was no loss in seed weight in Malathion 5D @ 1% and silica green nanoparticle @ 1500 ppm (Table 4).

Average seed weight loss among all the different nanoparticles the silica green nanoparticle @1500 ppm concentration and malathion 5D @ 1% showed no seed weight loss. Whereas least weight loss of (2.30%) was noticed in copper nanoparticle @1500 ppm and highest seed damage was recorded in untreated check (35.31%). The data clearly indicated that malathion followed by silica green nanoparticle @ 1500 showed superiority by reducing rice weevil infestation without any damage and without any loss in seed weight (Table 4).

Among the different nanoparticles like zinc, copper and silica nanoparticle tested against *C. analis* silica nanoparticle @ 1500ppm was found to be most effective by inhibiting egg laying by pulse beetle 120 DAT and recorded minimum number of eggs at 150 DAT which was on par with malathion 5D @ 1% followed by copper green nanoparticle @ 1500ppm. Further more there was no adult emergence, no seed damage, and seed weight loss upto 120 DAT. Hence silica green nanoparticle @ 1500ppm can be effectively used as seed protectant which affords complete protection upto 120 DAT.

Among the different nanoparticles like zinc, copper and silica nanoparticle tested against *S. oryzae* silica nanoparticle @ 1500ppm was found to be most effective by inhibiting emergence of adults, no seed damage and no seed weight loss upto 150 DAT which was on par with malathion 5D @ 1% followed by copper green nanoparticle @ 1500ppm. Further more there was no adult emergence, no seed damage, and seed weight loss upto 150 DAT. Hence silica green nanoparticle @ 1500ppm can be effectively used as seed protectant which affords complete protection upto 150 DAT.

There were no such studies on persistence effect of different nanoparticles on pulse beetle and rice weevil, hence the studies with respect to different store gain pests are discussed below.

Because of their short edge and amorphous in nature, these silica green nanoarticles cause the abrasion of the cuticle and formation of slits on the elytra of adults pulse beetle which leads to mortality of the pulse beetles. The adult mortality of pulse beetle could be attributed to the impairment of the digestive tract or to surface enlargement of the integument as a consequence of dehydration or blockage of spiracles and tracheas. Also it refers to their enormously increased exposed surfaces which could interact with the insect cuticle. Damage occurs to the insects protective wax coat on the cuticle, both by sorption and abrasion (Rouhani *et al.*, 2012)^[9]. Sabbour *et al.* (2015)^[10] investigated the mean number of deposited eggs per female and per cent adult emergence (F1), per cent seed damage and per cent weight loss caused by *Tribolium castaneum* in wheat seeds were greatly affected by natural DE and nano-DE at 1g/kg of wheat seeds in comparison to untreated control with highly significant differences during 20, 90 and 120 days of stored conditions.

Table 1: Effect of zinc, copper and silica green nanoparticles on oviposition and population build up of pulse beetle in chickpea seeds

Treatment details	Number of eggs/100seeds						Number of adults emerged /100g seeds					
	30 DAT	60 DAT	90 DAT	120 DAT	150 DAT	MEAN	30 DAT	60 DAT	90 DAT	120 DAT	150 DAT	MEAN
T1: Zn nps@1500 ppm	2.75 (1.80) ^b	8.25 (2.95) ^b	18.00 (4.30) ^b	33.00 (5.79) ^b	65.75 (8.14) ^b	25.55	1.25 (1.12) ^b	2.75 (1.79) ^b	7.25 (2.78) ^b	14.25 (3.84) ^b	27.00 (5.24) ^b	10.50
T2: Cu Nps @1500 ppm	0.00 (0.71) ^c	0.00 (0.71) ^c	4.00 (2.11) ^c	9.00 (3.08) ^c	26.25 (5.17) ^c	7.85	0.00 (0.71) ^c	0.00 (0.71) ^c	1.50 (1.40) ^c	6.50 (2.64) ^c	16.00 (4.06) ^c	4.8
T3:Si Nps@1500 ppm	0.00 (0.71) ^c	0.00 (0.71) ^c	0.00 (0.71) ^d	0.00 (0.71) ^d	3.55 (1.98) ^d	0.71	0.00 (0.71) ^c	0.00 (0.71) ^c	0.00 (0.71) ^d	0.00 (0.71) ^d	1.25 (1.12) ^d	0.25
T4: Malathion 5D @1%	0.00 (0.71) ^c	0.00 (0.71) ^c	0.00 (0.71) ^d	0.00 (0.71) ^d	0.00 (0.71) ^e	0.00	0.00 (0.71) ^c	0.00 (0.71) ^c	0.00 (0.71) ^d	0.00 (0.71) ^d	0.00 (0.71) ^e	0.00
T5: Untreated control	68.00 (8.28) ^a	123.55 (11.14) ^a	183.33 (13.57) ^a	230.50 (15.21) ^a	260.75 (16.37) ^a	173.22	55.50 (7.48) ^a	85.75 (9.29) ^a	122.75 (11.10) ^a	220.00 (14.85) ^a	246.50 (15.71) ^a	146.04
S.Em ±	0.03	0.04	0.05	0.05	0.10	-	0.04	0.05	0.08	0.08	0.07	-
CD@1%	0.14	0.18	0.21	0.19	0.41	-	0.17	0.20	0.32	0.31	0.29	-

DAT: Days after treatment

Figures in the parentheses are $\sqrt{(x+1)}$ transformed values

Figures in the column followed by same letters are not-significant at p=0.01 by DMRT

Table 2: Effect of zinc, copper and silica green nanoparticles on seed quality of chickpea by pulse beetle

Treatment details	Seed damage (%)						Seed weight loss (%)					
	30 DAT	60 DAT	90 DAT	120 DAT	150 DAT	MEAN	30 DAT	60 DAT	90 DAT	120 DAT	150 DAT	MEAN
T1: Zn nps@1500 ppm	0.75 (2.78) ^b	1.50 (6.93) ^b	2.00 (7.99) ^b	8.00 (16.41) ^d	13.5 (21.59) ^b	1.95	0.25 (1.51) ^b	0.75 (4.30) ^b	1.00 (5.74) ^b	2.75 (9.44) ^b	5.00 (14.45) ^b	1.95
T2: Cu Nps @1500 ppm	0.00 (0.00) ^c	0.00 (0.00) ^c	0.50 (2.65) ^c	2.00 (7.99) ^d	8.25 (16.67) ^c	1.15	0.00 (0.00) ^b	0.00 (0.00) ^b	0.25 (1.43) ^{bc}	1.25 (5.62) ^c	4.00 (11.10) ^c	1.10
T3:Si Nps@1500 ppm	0.00 (0.00) ^c	0.00 (0.00) ^c	0.00 (0.00) ^c	0.00 (0.00) ^c	0.75 (2.78) ^d	0.15	0.00 (0.00) ^b	0.00 (0.00) ^b	0.00 (0.00) ^c	0.00 (0.00) ^d	0.25 (1.51) ^d	0.05
T4: Malathion 5D @1%	0.00 (0.00) ^c	0.00 (0.00) ^c	0.00 (0.00) ^c	0.00 (0.00) ^b	0.00 (0.00) ^d	0.00	0.00 (0.00) ^b	0.00 (0.00) ^b	0.00 (0.00) ^c	0.00 (0.00) ^d	0.00 (0.00) ^d	0.00
T5: Untreated control	14.75 (22.58) ^a	42.25 (40.54) ^a	66.75 (54.79) ^a	87.00 (68.87) ^a	98.50 (82.25) ^a	48.1	11.75 (20.04) ^a	32.00 (34.45) ^a	51.50 (45.86) ^a	57.25 (49.17) ^a	88.00 (67.67) ^a	48.10
S.Em ±	0.10	0.16	0.15	0.16	0.17	-	0.10	0.07	0.18	0.21	0.35	-
CD@1%	0.41	0.69	0.62	0.67	0.70	-	0.43	0.30	0.74	0.87	1.46	-

DAT: Days after treatment

Figures in the parentheses are angular transformed values

Figures in the column followed by same letters are not-significant at p=0.01 by DMRT

Table 3: Effect of zinc, copper and silica green nanoparticles on population buildup of rice weevil in sorghum seeds

Treatment details	Number of adults emerged /100g seeds					
	30 DAT	60 DAT	90 DAT	120 DAT	150 DAT	MEAN
T1: Green zinc nanoparticle @1500 ppm	0.75 (1.10) ^b	4.50 (2.23) ^b	15.50 (4.00) ^b	31.00 (5.61) ^b	69.75 (8.37) ^b	24.30
T2: Green copper nanoparticle @1500 ppm	0.00 (0.71) ^c	0.00 (0.71) ^c	4.25 (2.23) ^c	12.75 (3.64) ^c	27.5 (5.29) ^c	8.85
T3: Green silica nanoparticle @1500 ppm	0.00 (0.71) ^c	0.00 (0.71) ^c	0.00 (0.71) ^d	0.00 (0.71) ^d	0.00 (0.71) ^d	0.00
T4: Malathion 5D @1%	0.00 (0.71) ^c	0.00 (0.71) ^c	0.00 (0.71) ^d	0.00 (0.71) ^d	0.00 (0.71) ^d	0.00
T5: Untreated control	50.50 (7.14) ^a	121.25 (11.03) ^a	150.75 (12.30) ^a	185.5 (13.64) ^a	224.25 (14.99) ^a	146.75
S.Em ±	0.06	0.03	0.03	0.04	0.04	-
CD@1%	0.24	0.12	0.14	0.16	0.15	-

DAT: Days after treatment

Figures in the parentheses are $\sqrt{(x+1)}$ transformed values

Figures in the column followed by same letters are not-significant at p=0.01 by DMRT

Table 4: Effect of zinc, copper and silica green nanoparticles on seed damage and seed weight loss by rice weevil in sorghum seeds

Treatment details	Seed damage (%)						Seed weight loss (%)					
	30 DAT	60 DAT	90 DAT	120 DAT	150 DAT	MEAN	30 DAT	60 DAT	90 DAT	120 DAT	150 DAT	MEAN
T1: Zn nps@1500 ppm	0.5 (3.29) ^b	3.0 (9.90) ^b	9.75 (18.18) ^b	15.75 (23.36) ^b	35.5 (36.57) ^b	12.9	0.00 (0.00) ^b	1.25 (6.34) ^b	5.5 (13.55) ^b	8.75 (17.20) ^b	14.5 (22.38) ^b	6.00
T2: Cu Nps @1500 ppm	0.00 (0.00) ^c	0.00 (0.00) ^c	2.5 (9.05) ^c	7.25 (15.60) ^c	15.5 (23.18) ^c	5.05	0.00 (0.00) ^b	0.00 (0.00) ^c	0.75 (4.63) ^c	2.5 (9.05) ^c	8.25 (16.67) ^c	2.3
T3:Si Nps@1500 ppm	0.00 (0.00) ^c	0.00 (0.00) ^c	0.00 (0.00) ^c	0.00 (0.00) ^c	0.00 (0.00) ^b	0.00	0.00 (0.00) ^b	0.00 (0.00) ^c	0.00 (0.00) ^b	0.00 (0.00) ^c	0.00 (0.00) ^b	0.00
T4: Malathion 5D @1%	0.00 (0.00) ^c	0.00 (0.00) ^c	0.00 (0.00) ^c	0.00 (0.00) ^c	0.00 (0.00) ^b	0.00	0.00 (0.00) ^b	0.00 (0.00) ^c	0.00 (0.00) ^b	0.00 (0.00) ^c	0.00 (0.00) ^b	0.00
T5: Untreated control	20.5 (26.92) ^a	41.33 (40.11) ^a	60.5 (51.06) ^a	80.25 (63.62) ^a	91.25 (72.80) ^a	58.76	5.55 (14.05) ^a	26.50 (30.82) ^a	37.25 (37.61) ^a	47.75 (43.71) ^a	59.5 (50.48) ^a	35.31
S.Em ±	0.15	0.32	0.32	0.41	0.23	-	0.03	0.18	0.23	0.29	0.30	-
CD@1%	0.62	1.34	1.35	1.72	0.96	-	0.14	0.74	0.98	1.21	1.25	-

DAT: Days after treatment

Figures in the parentheses are angular transformed values

Figures in the column followed by same letters are not-significant at p=0.01 by DMRT

Conclusion

The biosynthesis of Zinc, copper and silica green nanoparticles from spinach leaves, tulasi and rice husk is an environmental friendly, simple and efficient route for synthesis of nanoparticles which could be an alternative to chemical and physical methods. Zinc, copper and silica green nanoparticles showed potential entomotoxicity against rice weevil and pulse beetle. Among different concentrations of green nanoparticles 1500 ppm proved to be effective by recording cent per cent mortality, inhibited the egg laying, no emergence of adults and without any seed damage and weight loss during the storage condition. This study could lead to open up newer pathways of using biosynthesized green nanoparticles for the control of stored grain insect pests, which could be alternative for insecticidal agents.

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