

Original Research Article

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Effect of Biosynthesized Zinc Oxide Green Nanoparticles on Pulse Beetle, *Callosobruchus analis* (Coleoptera: Chrysomelidae)

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ABSTRACT

Among the stored product insects, *Callosobruchus analis* (Fabricius) is a cosmopolitan and serious pest of pulses in field as well as in storage condition. The excessive use of pesticides and chemical compounds led to development of resistance, resurgence and has an adverse effect on environment. This experiment was conducted at Centre for Nanotechnology Laboratory, UAS, Raichur. In the recent years, nanotechnology has emerged as a promising tool for pest control. In the present study, we reported the biological synthesis of zinc oxide green nanoparticles from spinach leaves. The green synthesized zinc oxide nanoparticles were characterized by UV-Vis spectroscopy, X-ray diffraction (XRD), Zetasizer and Scanning electron microscope (SEM). The bio-physical characterization revealed that the zinc oxide nanoparticle has a hexagonal structure with a mean particle size of 20.67 nm. The pesticidal effects of Zinc oxide green nanoparticles (ZnO) were used as stored product insect protectants compared to chemical malathion as standard reference. Data obtained from different concentration (250, 500, 750, 1000, 1250 and 1500 ppm) of zinc oxide green nanoparticles indicated that the increasing of concentration and exposure period caused increasing in adult mortality (%). Among the different concentration 1500 ppm of zinc oxide nanoparticle proved to be superior by recording highest mortality, lowest number of eggs, least seed damage, and seed weight loss in chick pea seeds up to one month of storage.

Keywords

Callosobruchus analis,
Zinc oxide
nanoparticles, Chick
pea, Mortality

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Introduction

Stored grains are ravaged by a number of insect pests. The stored grain pests infest grains to fulfill their food and shelter requirements resulting in qualitative as well as quantitative losses. The tropical climate of India is highly favourable for continuous

occurrence of storage insect pests throughout the year. A number of insect pests gain access to the grain storage at various stages of processing of food grains/ seeds viz., during the process of development and maturation of seeds/ grains, processing in threshing yards, during transit or while in storage. Some insect pests initiate damage at the ripening stage of

crops and continue during storage. Major sources of infestations are old bags, storage structure, old containers, and cross over infestation (Chitra and Subramanian, 2016).

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. Larva which grows inside eats endosperm, and then the seed is totally damaged. Adults are non-feeding. Cross infestation is very common, as adults are capable of flying. It causes 33% infestation to legume seeds. Larval feeding on the cotyledons causes significant losses in seed weight and viability. Gujar and Yadav (1978) reported 55- 60% losses in seed weight and 45.5 to 66.3% losses in protein content due to bruchid infestation in storage and the infested seeds become unfit for human consumption.

Nanotechnology has revolutionized the world with tremendous advancements in many fields of science like engineering, biotechnology, analytical chemistry and agriculture. Their use in crop protection is just in its infancy (Resham *et al.*, 2015). Nanomaterials measure between approximately 1 and 100 nm. Over many decades, nanotechnology and nanomaterials have been employed successfully and safely in various fields like medicine, environmental science and food processing. However, the use of nanomaterials in agriculture, especially for plant protection and production, it is an under-explored research area (Khot *et al.*, 2012). It has been used as conductors and semi-conductors, medical devices, sensors, coatings, catalytic agents and also as pesticides (Jordan, 2010).

Nanoparticles have large surface to volume ratio, chemically alterable physical properties, and possess strong affinity to targets such as proteins (Kumar *et al.*, 2010). Nano biosensors and other field sensing devices can

be used in detection and measurement of crop nutrient status, detection of hidden infestation of storage insects, pathogens, weeds, moisture level, soil fertility, temperature, etc. which helps in real time monitoring of the crop growth and provide essential data for precision farming practices leading to minimize agricultural inputs and maximizing resource output and yield (Scott and Chen, 2003).

Materials and Methods

The spinach leaves (*Spinacia oleracea*) (Heena variety) were collected from University campus and chickpea seeds (JG-11) were collected from seed unit, UAS, Raichur and Zinc nitrate hexahydrate [$Zn(NO_3)_2 \cdot 6H_2O$] was procured from M/s. High Media, Bangalore.

Biosynthesis of Zinc oxide green nanoparticles from spinach leaves

The leaf extract of spinach (50 ml) was boiled at 60-80 °C using magnetic stirrer. Zinc nitrate hexa hydrate [$Zn(NO_3)_2 \cdot 6H_2O$] was used as a precursor. 1 mM Zinc nitrate solution was prepared using distilled water. The solution was added to the leaf extract when temperature reached to 60° C and boiled for 30 min or until colour changed. A change in the colour from dark green to pale yellow indicates the formation of Zinc oxide nanoparticles (Amrita *et al.*, 2015).

Zinc oxide green nanoparticles were confirmed by UV-Visible spectrophotometer (Perkin Elmer, Lambda 35, Germany) in 350 - 410 nm wavelength range.

The size and morphology of the synthesized Zinc oxide nanoparticles was characterized by Zetasizer (Malvern, ZETA Sizer, nano383 issue 5.0, England) X-ray diffraction and scanning electron microscope (Carl Zeiss Microscopy, EVO 10, Germany).

Maintenance of pure culture of pulse beetle on chick pea seeds

Pulse beetle was collected from the infested chick pea seeds and the culture was further maintained in plastic jars of two kg capacity containing chickpea seeds (JG-11). The plastic jars were 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 temperature of 27° C. The insects emerged from this culture were used throughout the period of experimentation.

Preparation of green nanoparticle solutions

The zinc oxide green nanoparticle which synthesized from spinach leaves was obtained from Centre for Nanotechnology laboratory, University of Agricultural Sciences, Raichur for the present study.

Bioassay studies for pulse beetle on chickpea seeds

Effects of biosynthesized zinc oxide green nanoparticles on adults of *C. analis* were determined by contact toxicity assay at seven doses of 125, 250, 500, 750, 1000, 1250 and 1500 ppm of nanoparticle kg-1 chickpea seeds. The experiments were carried out in Completely Randomized Design with three replications each consisted of 20 adults of *C. analis* in small plastic screw capped jars containing 100g of chickpea seeds in each jar were treated individually with nanoparticles and the plastic box were closed with muslin cloth and fastened tightly with the help of rubber band. Then, the jars were shaken manually for approximately 60 seconds to achieve equal distribution of nanoparticles on chickpea seeds. In one additional set no nanoparticle was mixed with chickpea seeds and this set served as control. After that 20 unmated adults of *C. analis* were introduced

into each jar. All bioassays were performed at 30±1°C and 65±5% RH. Insect mortality was checked after 1, 3, 5, 7 and 10 days after treatment. The observation were also recorded 1 month after treatment on number of eggs laid per 100 seeds, number of adults emerged per 100g of seeds, per cent seed damage, per cent weight loss, germination per cent, Dehydrogenase enzyme activity and percent reduction over control.

Results and Discussion

The size reduction of Zinc ions into zinc oxide nanoparticles were observed as the colour changed from dark green to pale yellow. The sharp bands of zinc oxide nanoparticles were observed around 375.4 nm as shown in figure 1 and similar results were obtained by (Awwad *et al.*, 2016) with 374 nm and (Singh *et al.*, 2015) with 368 nm.

The average particle diameter (Zetasizer) zinc oxide nanoparticle was found to be 20.67 nm as shown in figure 2. (Sindhura *et al.*, 2015) obtained 53 nm and (Supraja *et al.*, 2015) obtained 20.3 nm for ZnO NPs. The morphological features of zinc nanoparticles were characterized by scanning electron microscope and observed to be spindle shape. Similar results were also reported by (Noorjahan *et al.*, 2015) for ZnO NPs.

Mortality of pulse beetle

In the present investigations, the effect of Zinc oxide nanoparticles was tested on mortality of pulse beetles at different concentrations. It was observed that with increase in concentrations and number of days after treatment, the mortality also increased. Green zinc oxide nanoparticle @1500ppm proved to be superior by recording highest mortality of 40.00, 76.66, 83.33, 93.33 per cent, on 1,3,5,7 and 10 days after treatment and it was on par with its lower dosage @ 1250ppm where in

mortality ranged from 36.67 to 96.66 per cent from one day to ten days after treatment, whereas significantly highest mortality occurred in chemical check malathion 5D @ 1% (100.00%) which was superior over all treatments and there was no mortality in control as seen in table 1 and figure 3.

Effectiveness of green zinc oxide nanoparticles may be attributed to the damage 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.*, 2015).

These findings are in agreement with those of Abo Arab *et al.*, (2014) who reported that malathion increased mortality and reduced the number of progeny of *S. oryzae* or *S. zeamais* compared to nanoparticles (Al₂O₃-ZnO) and control. Kirthi *et al.*, (2011) reported that the comparison between acaricidal, licicidal and larvicidal activities of zinc oxides and the synthesized ZnO nanoparticles, the mortality

effect of ZnO nanoparticles was significant. Malaikozhundun and Vinodhini (2017) reported that the treatment with Pp-ZnO NPs at 25 µg mL⁻¹ increased the mortality of *C. maculatus* to 100%, similarly Rouhani *et al.*, (2012a) reported efficiency of silver and Zinc nanoparticles at 20 mg/l in *Aphis nerii*, and Salem *et al.*, (2015) for aluminium and Zinc oxide nanoparticles at 2 g/kg in *T. castaneum*.

Oviposition of *Callasobruchus analis*

Efficacy of zinc oxide nanoparticles on egg laying by *C. analis* on chick pea seeds (Variety JG-11) revealed (Table 2) that the number of eggs laid per 100 seeds were nil in the malathion treatment after 30days as compared to the treatment with zinc oxide nanoparticles @1500ppm concentration with 2.66 eggs per 100 seeds, and @ 1250 ppm concentration with 6.33 eggs per 100 seeds. However, the maximum number of eggs was recorded in untreated check (T₉) (65.00 eggs / 100 seeds).

Table.1 Effect of zinc oxide green nanoparticles on pulse beetle in chick pea seeds

| Treatment details | Mortality of pulse beetle (%) | | | | |
|---|--------------------------------|--------------------------------|-------------------------------|--------------------------------|--------------------------------|
| | 1 DAT | 3 DAT | 5 DAT | 7 DAT | 10 DAT |
| T1: Green zinc oxide nanoparticle @125 ppm | 6.67 (12.39) ^f | 13.33 (21.48) ^f | 16.66 (23.86) ^e | 43.33 (41.15) ^d | 66.66 (54.78) ^e |
| T2: Green zinc oxide nanoparticle @250 ppm | 13.33 (21.14) ^e | 20.00 (26.57) ^e | 26.66 (31.00) ^e | 53.33 (46.92) ^d | 76.66 (61.55) ^{cd} |
| T3: Green zinc oxide nanoparticle @500 ppm | 16.67 (23.86) ^{de} | 33.33 (34.88) ^d | 43.33 (41.15) ^d | 66.66 (54.78) ^c | 83.33 (66.81) ^c |
| T4: Green zinc oxide nanoparticle @750 ppm | 20.00 (26.57) ^{de} | 36.33 (37.22) ^{cd} | 56.66 (48.85) ^c | 73.33 (59.34) ^c | 86.66 (68.86) ^c |
| T5: Green zinc oxide nanoparticle @1000 ppm | 26.67 (31.00) ^{cd} | 43.33 (42.49) ^c | 66.66 (54.78) ^c | 76.66 (61.89) ^c | 93.33 (77.71) ^b |
| T6: Green zinc oxide nanoparticle @1250 ppm | 36.67 (37.22) ^{bc} | 70.00 (57.33) ^b | 80.00 (63.93) ^b | 90.00 (71.57) ^b | 96.66 (83.52) ^{ab} |
| T7: Green zinc oxide nanoparticle @1500 ppm | 40.00 (39.15) ^b | 76.66 (61.22) ^b | 83.33 (66.14) ^b | 93.33 (77.71) ^b | 100.00 (90.00) ^a |
| T8: Malathion 5D @1 % | 66.67 (54.12) ^a | 90.00 (71.57) ^a | 96.66 (83.52) ^a | 100.00 (90.00) ^a | 100.00 (90.00) ^a |
| T9: Untreated control | 0.00 (0.00) ^g | 0.00 (0.00) ^g | 0.00 (0.00) ^f | 0.00 (0.00) ^e | 0.00 (0.00) ^f |
| S.Em± | 0.31 | 0.28 | 0.34 | 0.36 | 0.32 |
| CD@1% | 1.26 | 1.14 | 1.38 | 1.47 | 1.29 |

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.2 Effect of zinc oxide green nanoparticle on pulse beetle in chick pea seeds

| Treatment details | 30 Days after treatment | | | | Germination (%) | Dehydrogenase enzyme activity (OD value) | Per cent reduction over control |
|--|-------------------------------|--------------------------------------|---------------------------------|-------------------------------|------------------|--|---------------------------------|
| | Number of eggs/100 seed | Number of adults emerged /100g seeds | Seed damage (%) | Seed weight loss (%) | | | |
| T ₁ : Green zinc oxide nanoparticle @125 ppm | 31.33* (5.64) ^b | 15.00* (3.94) ^b | 11.33** (19.67) ^b | 2.66** (9.36) ^b | 90.17 (72.38) | 1.997 | 26.10 |
| T ₂ : Green zinc oxide nanoparticle @250 ppm | 27.00 (5.24) ^c | 12.66 (3.63) ^c | 9.33 (17.78) ^c | 2.33 (8.74) ^b | 91.00 (73.05) | 2.013 | 35.26 |
| T ₃ : Green zinc oxide nanoparticle @500 ppm | 21.33 (4.67) ^d | 11.00 (3.39) ^d | 7.66 (16.07) ^d | 2.00 (8.13) ^{bc} | 92.33 (73.93) | 2.019 | 44.25 |
| T ₄ : Green zinc oxide nanoparticle @750 ppm | 18.66 (4.38) ^e | 9.33 (3.13) ^e | 4.66 (12.46) ^e | 1.66 (7.37) ^{cd} | 93.17 (75.04) | 2.106 | 53.87 |
| T ₅ : Green zinc oxide nanoparticle @1000 ppm | 12.66 (3.63) ^f | 5.66 (2.48) ^f | 3.66 (11.02) ^f | 1.33 (6.54) ^{de} | 94.33 (76.23) | 2.111 | 63.03 |
| T ₆ : Green zinc oxide nanoparticle @1250 ppm | 6.33 (2.60) ^g | 3.33 (1.95) ^g | 1.66 (7.33) ^g | 1.00 (5.74) ^e | 94.83 (76.86) | 2.128 | 72.02 |
| T ₇ : Green zinc oxide nanoparticle @1500 ppm | 2.66 (1.77) ^h | 0.66 (1.05) ^h | 0.33 (1.91) ^h | 0.00 (0.00) ^f | 95.33 (77.53) | 2.143 | 100 |
| T ₈ : Malathion 5D @1 % | 0.00 (0.71) ⁱ | 0.00 (0.71) ⁱ | 0.00 (0.00) ^h | 0.00 (0.00) ^f | 91.67 (73.22) | 2.102 | - |
| T ₉ : Untreated control | 65.00 (8.09) ^a | 52.66 (7.29) ^a | 14.66 (22.52) ^a | 3.6 (11.02) ^a | 89.67 (71.25) | 2.035 | - |
| S.Em± | 0.07 | 0.08 | 0.32 | 0.18 | 0.18 | 0.001 | - |
| CD@1% | 0.29 | 0.33 | 1.31 | 0.72 | 0.73 | 0.005 | |

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

** 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

Fig.1 UV - Visible spectrum analysis of biosynthesized zinc oxide nanoparticles

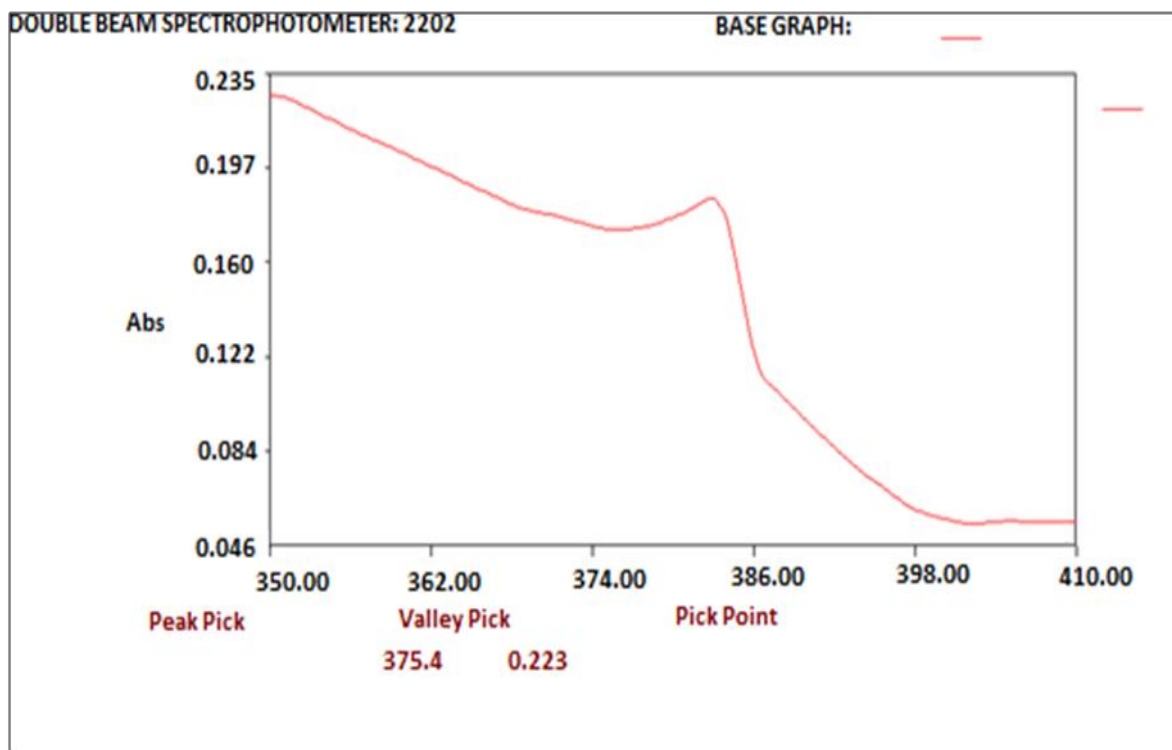


Fig.2 Average particle diameter of zinc oxide green nanoparticle

Results

| | Size (d.n... | % Intensity: | St Dev (d.n... |
|--------------------------------|----------------------|--------------|----------------|
| Z-Average (d.nm): 20.67 | Peak 1: 90.51 | 78.9 | 32.97 |
| Pdl: 1.000 | Peak 2: 1.618 | 21.1 | 0.3610 |
| Intercept: 0.338 | Peak 3: 0.000 | 0.0 | 0.000 |

Result quality Refer to quality report

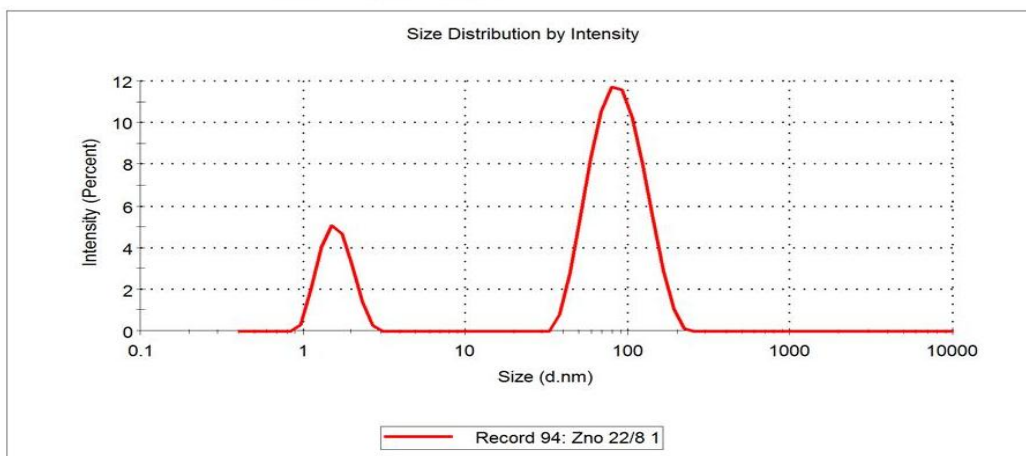


Fig.3 Effect of zinc oxide green nanoparticles on pulse beetle

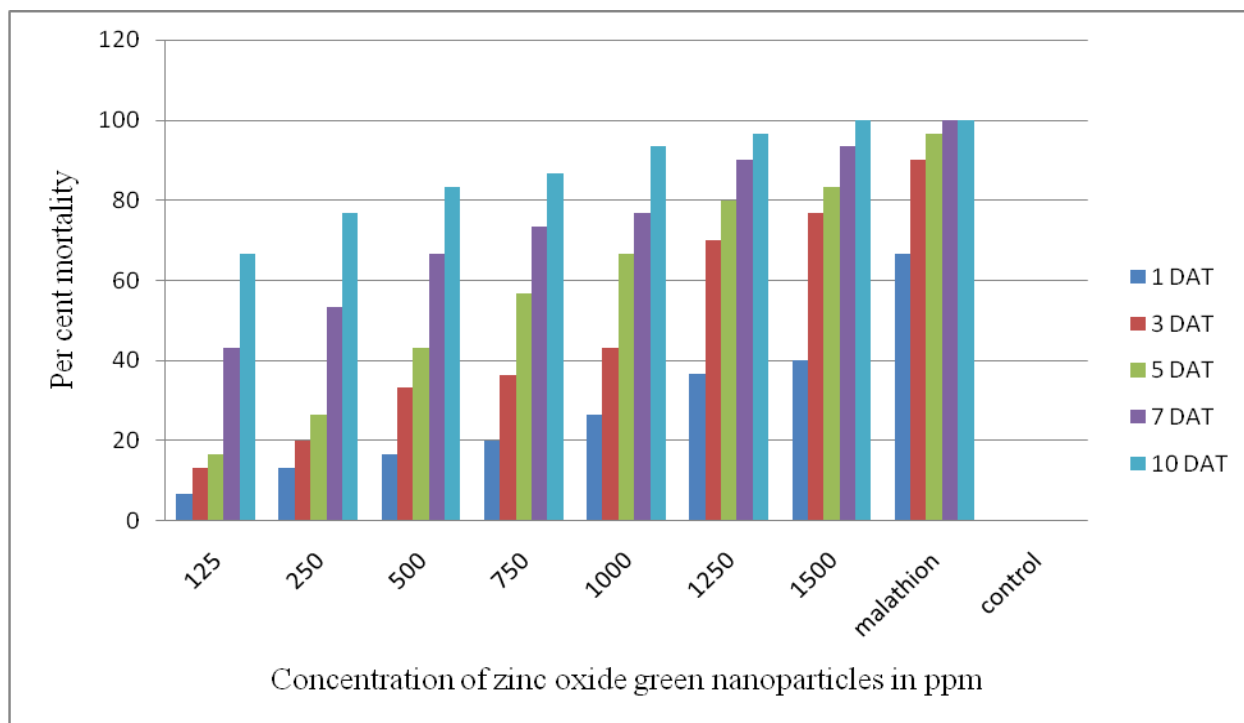
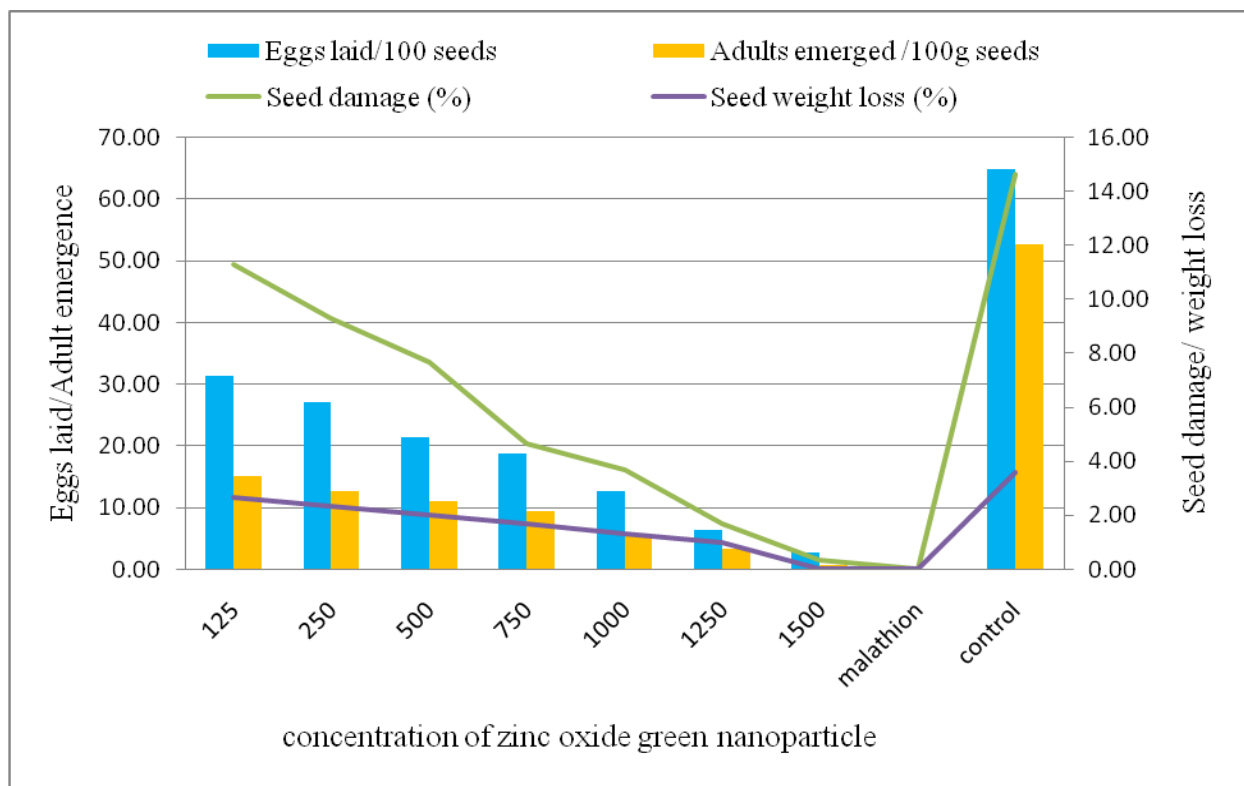


Fig.4 Effect of zinc oxide nanoparticles on quality parameters of chick pea seeds



Population buildup of *Callasobruchus analis*

The adult population builds up after 30 days of storage ranged from 0.00 to 52.66 adults per 100g of seeds (Table 2). There was no adult population recorded on chick pea seeds treated with Malathion 5D @ 1%. This treatment was followed by zinc oxide nanoparticle @ 1500 ppm (0.66 adults /100 g of seeds) which proved to be superior over other treatments, followed by zinc oxide nanoparticle at 1250 ppm concentration (3.33 adults / 100 g of seeds) and at 1000ppm concentration (9.33 adults / 100 g of seeds). However, the highest adult population build up was noticed in untreated check (T₉) (52.66 adults / 100 g seeds) after 30 days of treatment.

Seed damage by *Callasobruchus analis*

From results depicted in table 2 it was evident that there was no seed damage noticed in Malathion 5D @ 1 % treated seeds and lowest in zinc oxide nanoparticle (T₇) @ 1500ppm concentration (0.33 %) followed by zinc oxide nanoparticle (T₆) @ 1250 ppm concentration (1.66 %) and @ 1000ppm concentration (3.66 %). Whereas, significantly highest seed damage was recorded in untreated check (14.66 %). Highest seed germination (95.33%) and dehydrogenase enzyme activity (2.143) was noticed in zinc oxide nanoparticle @1500ppm proving that it did not exhibit any adverse effect on quality of seeds.

Seed weight loss due to *Callasobruchus analis*

The seed weight loss due to infestation by pulse beetle on chick pea seeds recorded on 30th day after release (Table 2) of pulse beetle indicated that the malathion 5D @ 1 % and zinc oxide green nanoparticle (T₇) @ 1500

ppm, proved to be significantly superior over all other treatments with no seed weight loss. The untreated check recorded 3.66 per cent loss in seed weight on 30 days of imposing treatments which was inferior to other treatments as seen in figure 4.

Since there are no reviews available on zinc oxide green nanoparticles against adult mortality and persistence studies of *C. analis*, hence reviews of other nanoparticles on other insects are presented here. Arumurugan *et al.*, (2015) noticed that fecundity, adult emergence, seed damage by pulse beetle was reduced when the seeds of gram treated with SNPs at 2g/ kg of seeds. Sabour (2012) studied the persistence effect of silica gel Cab-O-Sil-750 and silica gel Cab-O-Sil-500 tested against *S. oryzae* under laboratory condition. The number of eggs laid/ female were significantly decreased to 6±1.0 and 11±0.51 as compared to 99.1±1.43 and 97.2±1.82 in the control and adult emergence was reduced to 91 and 90% after 100 and 120 days of stored condition respectively.

Zinc oxide green nanoparticles can be effectively used for the management of pulse beetle in chickpea which inhibited egg laying, emergence of adults, seed damage, increased mortality of adults and this can be used an alternate strategy to chemical control.

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