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Effect of biosynthesized copper green nanoparticles on pulse beetle, *Callosobruchus analis* (Coleoptera: Chrysomelidae)

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Abstract
Stored product insect *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 copper green nanoparticles from tulasi leaves. The green synthesized copper nanoparticles were characterized by UV-Vis spectroscopy, X-ray diffraction (XRD), Zetasizer and Scanning electron microscope (SEM). The bio-physical characterization revealed that the copper nanoparticle with a mean particle size of 82.41 nm. Data obtained from different concentration (250, 500, 750, 1000, 1250 and 1500 ppm) of copper green nanoparticles indicated that the increasing of concentration and exposure period caused increasing in adult mortality (%). Among the different concentration 1500 ppm of copper 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,* copper nanoparticles, chick pea, mortality

Introduction
Storage of grains is part of the post-harvest system through which food materials pass from field to consumer. One of the most important and essential issues with the storing process is the loss in quality and quantity of the grains caused by insects leading to damages and reduction of their dry weight and nutritional value. Thus, insect's pests that infect pulses are considered one of the main problem that cause damage of 10-40% of stored food pulses around the world (Zahir *et al*, 2012) [14].
Pulses are precious commodities used around the globe in variety of cuisines. They are rich source of plant proteins and contribute in improving nutrition security. They are now gaining attention in value added food market as functional ingredients and in nutraceutical industries. Pulses are annual and seasonal crops that can be stored for several months. However, these are at high risk of damage due to post harvest losses which can be up to 25-50%. These losses are linked to insufficient and poor storage facilities, lack of knowledge of advanced technology in post harvest pulse management and harsh climatic conditions (Singh and Larson, 2016).
Fumigants and residual insecticides are commonly used to combat stored grain pests. In recent years, consumer awareness of the health hazard from residual toxicity and the growing problem of insect resistance to these conventional insecticides have led the researchers to look for alternative strategies for stored grains protection. Nanoparticles represent a new generation of environmental remediation technologies that could provide cost-effective solution to some of the most challenging environmental clean-up problems (Chinnamuthu and Murugesa, 2009) [2].
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) [5]. '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].

Material and Methods

The tulasi leaves were collected from University campus and chickpea seeds (JG-11) were collected from seed unit, UAS, Raichur and Copper sulphate pentahydrate was procured from M/s. High Media, Bangalore.

Biosynthesis of Zinc oxide green nanoparticles from spinach leaves

The plant extract of tulasi leaves (25 ml) was mixed with 100 ml of 1mM aqueous copper sulphate pentahydrate ($CuSO_4.5H_2O$) solution under continuous string. After complete mixing of leaf extract with precursor the mixture was kept for incubation at 31˚ C for 24 h. A change in the colour from light green to dark green was observed and this indicated the formation of copper nanoparticles. The solution was then centrifuged at 6000 rpm for 30 min. (MPW Med. Instruments, MPW–350R, Poland) followed by re-dispersion of the pellet in deionised water to remove any unwanted biological materials (Mekal *et al.*, 2016) [8].

Copper green nanoparticles were confirmed by UV-Visible spectrophotometer (Perkin Elmer, Lamda 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 ºC. The insects emerged from this culture were used throughout the period of experimentation.

Preparation of green nanoparticle solutions

The copper 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 copper 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 ºC 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

In the process of biosynthesis of copper green nanoparticles the color changes from dark green to light green. The colour change was due to active molecules present in tulasi leaf extract which acted as a reducing and capping agent. The tulasi leaf extract reduced the copper metal ions into copper nanoparticles. The average particle diameter (Zetasizer) of copper nanoparticle was found to be 82.41 nm as shown in figure 1 and similar results were obtained by (Kulkarni and Kulkarni, 2013) [7] with 77 nm. Scanning electron microscopy analysis showed that the copper nanoparticles were spherical in shape. Similar results were observed by Saranyaadevi *et al*. (2014) [10] and Hariprasad *et al.* (2016) [6] *i.e.* spherical shape of copper nanoparticles. The X-ray diffraction pattern of the copper nanoparticles showed the broad halo at about $2θ = 20$-40° region which confirms the amorphous structure of Cu NPs as shown in fig 2 and present results were similar with Saranyaadevi *et al*. (2014) [10] $2θ = 39.1°$- 68.3° region and Fatma *et al*. (2017) [3] $2θ = 42.47°$ region confirms the copper nanoparticles.

Mortality of pulse beetle

The results of the present study showed the variation in the adult mortality of *C. analis* due to the different concentrations of copper nanoparticle and time of exposure. Treatment with higher concentration *i.e.*, 1500 ppm and 1250 ppm recorded highest adult mortality which ranged from 43.33 to 100 % and 36.66 to 100 % from 1st to 10th day after treatment respectively and 100 per cent mortality noticed in malathion 5D @ 1% on 5th day after treatment and there was no mortality recorded in untreated control as seen in table 1 and fig 3.

Since there are no reviews available on copper nanoparticles against adult mortality of *C. analis*, hence reviews of effect of copper green nanoparticles on other insects are presented here. Ghidan *et al*. (2017) [4] evaluated the activity of biologically synthesized copper oxide nanoparticles against the mortality of green peach Aphid, where 86 per cent of nymphal mortality was noticed at 800 mg/mL. Yang and Watts (2005) [13] reported that nanoparticles could be applied to facilitate pest control management (IPM) of stored grain pests as *C. maculates.*

Oviposition of *Callosobruchus analis*

The egg count revealed (Table 2) that number of eggs laid per 100 seeds were nil in malathion 5D @ 1% and copper green nanoparticle @ 1500 ppm concentration after 30 days followed by the copper green nanoparticle @ 1250ppm, 1000ppm and 750ppm concentration with 4.66, 11.33 and 17.66 eggs per 100g of seeds by *C. analis.* Whereas, significantly highest number of eggs were noticed in untreated check (68.00 eggs /100 seeds) 30 days of treatment.

Population build up of *Callosobruchus analis*

The population of *C. analis* at 30 days after storage ranged from 0.00 to 55.66 adults per 100 g of seeds (Table 2). There was no emergence of pulse beetle in the Malathion 5D @ 1% and copper green nanoparticle @1500 ppm (0.00 adults /100 g of seeds). This treatment followed by copper green nanoparticle at 1250 ppm (1.66 adults /100 g of seeds), at 1000ppm (6.66 adults / 100 g of seeds). However, the highest adult population build up was noticed in untreated check (55.66 adults / 100 g seeds) after 30 days of treatment.

Seed damage by *Callosobruchus analis*

From table 2 it was evident that there was no seed damage noticed in Malathion 5D @ 1% and copper green nanoparticle @ 1500 ppm and lowest seed damage in copper green nanoparticle @ 1250ppm concentration (0.33 %) followed by copper green nanoparticle @ 1000ppm concentration (3.33%). Whereas, the highest seed damage was recorded in untreated check (15.66 %). The seeds treated with copper nanoparticle @ 1500ppm showed highest seed germination (95.33%), dehydrogenase enzyme activity (2.147) and highest per cent reduction over control (100.00).

Seed weight loss due to *Callosobruchus analis*

The seed weight loss due to infestation recorded on 30th day after release (Table 2) of pulse beetle indicated that there was no seed weight loss in the Malathion 5D @ 1%, and copper green nanoparticle @1500 ppm concentration and they proved to be significantly superior to rest of the treatments. The untreated check recorded 5.33 per cent loss in seed weight on 30th day of exposure. Furthermore the per cent reduction over control was highest in copper green nanoparticle @1500 ppm as seen in fig 4.

Since there are no reviews available on copper 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) [1] 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) [9] 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.

Table 1: Effect of green copper nanoparticle on pulse beetle in chick pea seeds

Treatment details	Mortality of pulse beetle (%)				
	1 DAT	3 DAT	5 DAT	7 DAT	10 DAT
T_1: Green copper nanoparticle @125 ppm	10.00 (18.43)^{e}	16.66 (23.86)^{e}	26.66 (31.00)^{g}	46.66 (43.08)^{e}	73.33 (59.34)^{c}
T_2: Green copper nanoparticle @250 ppm	16.66 (23.19)^{d}	23.33 (28.78)$_{e}$	36.66 (37.22)^{f}	63.33 (52.78)^{d}	80.00 (63.43)^{c}
T_3: Green copper nanoparticle @500 ppm	20.00 (26.57)^{cd}	36.66 (37.22)^{d}	46.66 (43.08)^{e}	76.66 (61.22)^{c}	86.66 (68.86)^{a}
T_4: Green copper nanoparticle @750 ppm	23.33 (28.78)^{cd}	40.00 (39.15)^{cd}	56.66 (48.85)^{d}	83.33 (66.81)^{c}	90.00 (71.57)^{a}
T_5: Green copper nanoparticle @1000 ppm	26.66 (31.00)^{c}	53.33 (46.92)^{c}	66.66 (54.78)^{c}	86.66 (68.86)^{c}	93.33 (77.71)^{a}
T_6:Green copper nanoparticle @1250 ppm	36.66 (36.89)^{b}	73.33 (59.34)^{b}	83.33 (66.81)^{b}	93.33 (77.71)^{b}	100.00 (90.00)^{a}
T_7:Green copper nanoparticle @1500 ppm	43.33 (41.15)^{b}	83.33 (66.81)^{b}	86.66 (68.86)^{b}	96.66 (83.86)^{ab}	100.00 (90.00)^{a}
T_8: Malathion 5D @1 %	66.66 (54.99)^{a}	90.00 (75.67)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}
T_9:Untreated control	0.00 (0.00)^{f}	0.00 (0.00)^{f}	0.00 (0.00)^{h}	0.00 (0.00)^{f}	0.00 (0.00)^{d}
S.Em±	0.28	0.32	0.33	0.36	0.42
CD@1%	1.16	1.28	1.34	1.45	1.72

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 copper green nanoparticle on quality parameters of 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 copper nanoparticle @125ppm	28.66* (5.40)^{b}	13.33* (3.72)^{b}	9.33** (17.78)^{b}	2.33** (8.74)^{b}	90.17 (71.73)	2.070	56.28
T_2 : Green copper nanoparticle @250ppm	26.66 (5.21)^{c}	11.66 (3.49)^{c}	6.66 (14.95)^{c}	2.00 (8.13)^{bc}	91.00 (72.55)	2.095	62.47
T_3: Green copper nanoparticle @500ppm	24.33 (4.98)^{d}	9.33 (3.13)^{d}	5.33 (13.34)^{cd}	1.66 (7.33)^{cd}	92.33 (73.93)	2.105	68.84
T_4: Green copper nanoparticle @750ppm	17.66 (4.26)^{e}	7.66 (2.86)^{e}	4.66 (12.46)^{d}	1.33 (6.54)^{d}	93.17 (74.85)	2.109	75.04
T_5: Green nanoparticle @1000 ppm	11.33 (3.44)f	6.66 (2.68)f	3.33 (10.50)e	1.00 (5.74)d	94.33 (76.23)	2.118	81.23
T_6: Green copper nanoparticle @1250 ppm	4.66 (2.27)^{g}	1.66 (1.46)^{g}	0.33 (1.92)^{f}	0.00 (0.00)^{e}	94.83 (76.86)	2.121	100
T_7: Green copper nanoparticle @1500 ppm	0.00 (0.71)^{h}	0.00 (0.71)^{h}	0.00 (0.00)^{g}	0.00 (0.00)^{e}	95.33 (77.53)	2.147	100
T_8: Malathion 5D @1%	0.00 (0.71)^{h}	0.00 (0.71)^{h}	0.00 (0.00)^{g}	0.00 (0.00)^{e}	91.67 (73.22)	2.101	-
T_9:Untreated control	68.00 (8.28)^{a}	55.66 (7.49)^{a}	15.66 (23.31)^{a}	5.33 (13.31)^{a}	89.67 (71.25)	2.035	–
S.Em±	0.06	0.06	0.28	0.14	0.20	0.001	_
CD@ 1%	0.24	0.23	1.15	0.58	0.83	0.004	_

* 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

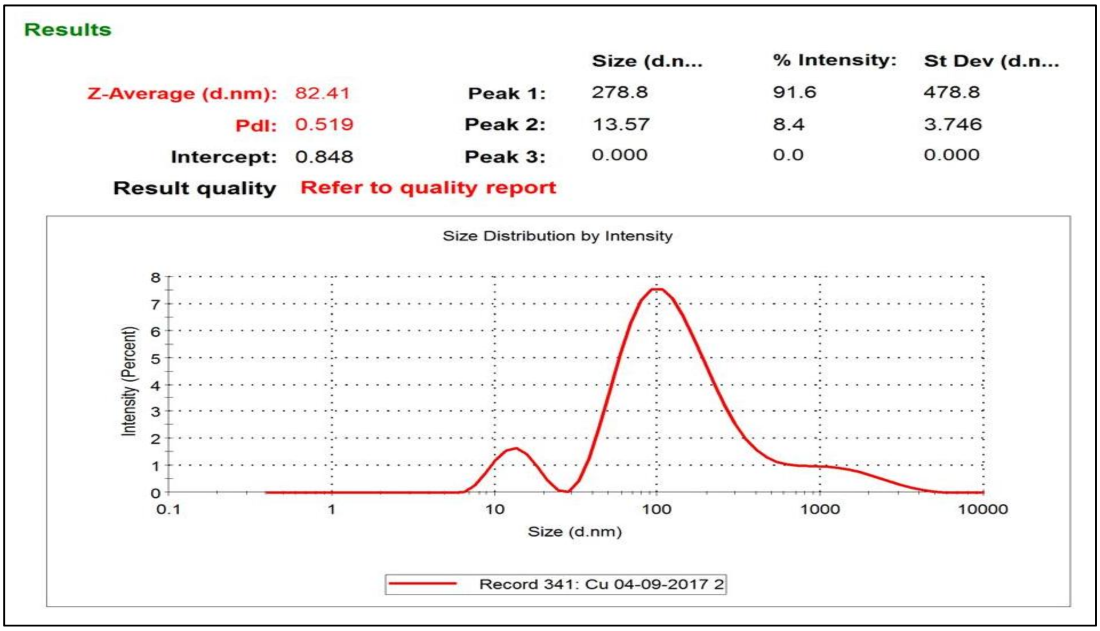

Fig 1: Average particle diameter of copper green nanaoparticle

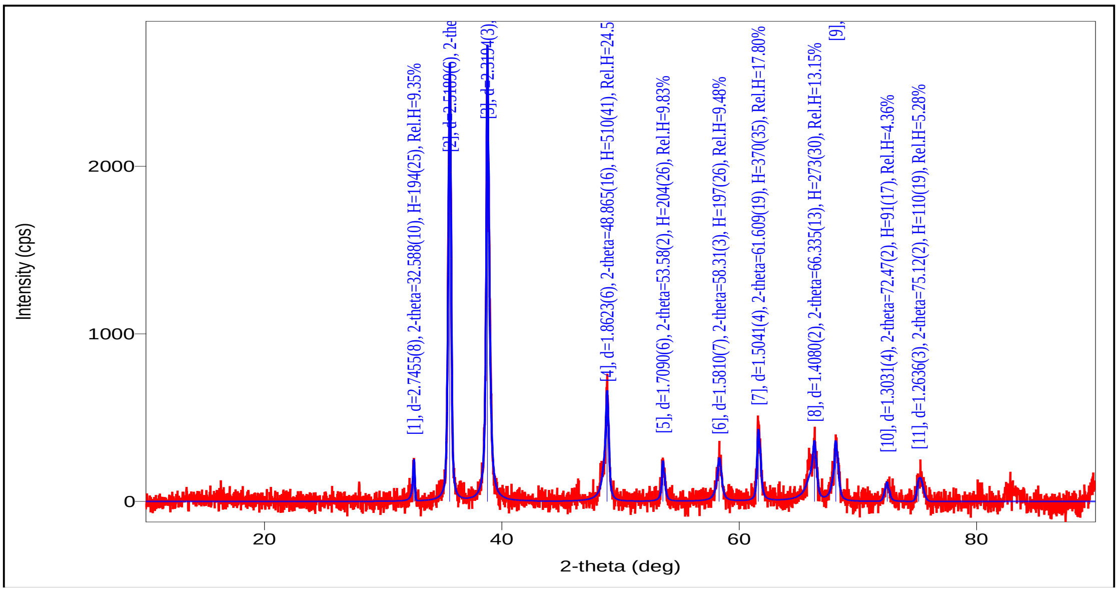

Fig 2: X- ray diffraction image of biosynthesized copper green nanoparticles

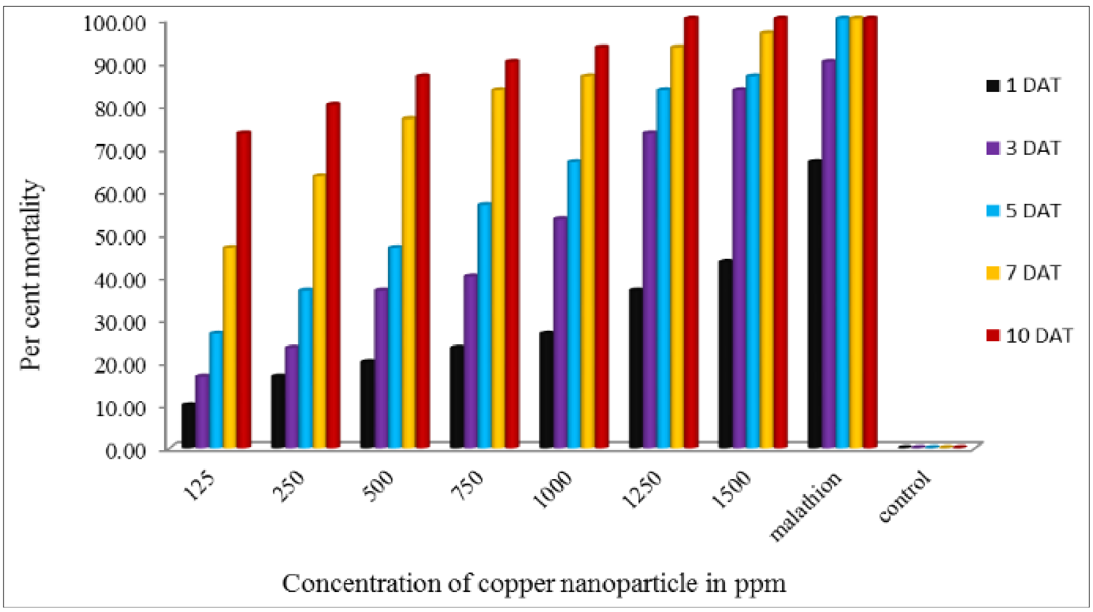

Fig 3: Effect of copper green nanoparticle on pulse beetle

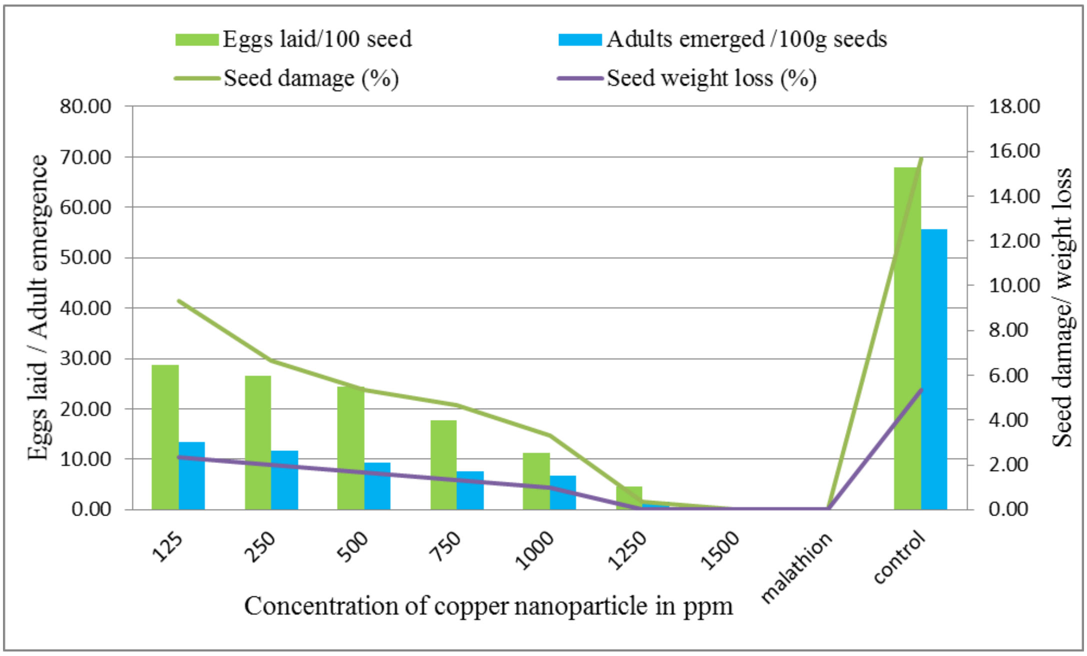

Fig 4: Effect of copper green nanoparticle on quality parameters of chickpea seeds

Conclusion

Copper 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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