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Efficacy of nanoparticles against rice weevil [*Sitophilus oryzae* (Linnaeus)] on maize seeds

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Abstract
An experiment was conducted at National Bureau of Plant Genetic Resources (NBPGR), Regional Research Station, Rajendranagar, Hyderabad and Seed Research and Technology Centre, PJTSAU Rajendranagar, during 2015-16, to study the effect of nano particles *viz*, nano silica, nano alumina and nano clay at thee different dosages @ 500, 250, 125 ppm kg^{-1} maize seed and Diatomaceous earth @ 1000 ppm kg^{-1} seed along with control on *Sitophilus oryzae* (Linnaeus). All the treatments were replicated thrice. Among the three nanocides, nano silica applied at 500 ppm kg-1 dosage showed the superior performance over other treatments resulting in cent percent mortality at one day after treatment followed by nano silica @ 250 and 125 ppm kg-1 which caused cent percent mortality at three days after treatment. Nano silica at 500, 250 and 125 ppm kg^{-1} inhibited oviposition and no adult emergence of *S. oryzae* was observed and has a great promise in pest management.

Keywords: Maize, nanoparticles, diatomaceous earth, *Sitophilus oryzae* (Linnaeus)

1. Introduction
Maize (*Zea mays*. L) is one of most versatile emerging crop having wider adoptability under varied agro climatic conditions. According to the Ministry of Agriculture & Farmers welfare, Government of India, Statistics, 2015-16 [1], the area, production and productivity of maize is 8.80 m ha, 22.56 mt and 2563 kg ha^1, respectively. The maize is cultivated throughout the year in all states of the country for various purposes.

Maize crop is mostly grown in all the districts of Telangana state. The predominant maize growing districts of Telangana are Mahaboobnagar, Medak, Warangal, Karimnagar and Nizamabad. The area, production and productivity of maize crop for 2015-16 in Telangana state are 5.73 lakh hectares, 17.51 Lakh tonnes and 3056 kg per hectare, respectively (www.indiastat.com) [2]. In India, Telangana stands sixth position in area and fifth position in production and productivity of maize crop. Maize hybrids and varieties are reported to be highly susceptible to insect pests both in the field and storage (Gimma *et al*., 2008) [10].

Most of the maize grain harvested is stored on the farm, where post harvest pest management practices are inadequate (Dubale**,** 2011) [7] leading to huge amounts of maize seed losses due to pests of stored grain. Among the several insects attacking maize grain during storage are rice weevil, *Sitophilus spp* (Linnaeus); lesser grain borer, *Rhizopertha domonica* (Fabricius); red flour beetle, *Tribolium castaneum* (Herbest); rice moth, *Corcyra cepholonica* (Stainton) and angoumois grain moth, *Sitotroga cereallela* (oliver) are of economic importance. *Sitophilus spp* (Linn) is the most destructive insect pest of the stored raw cereal grains in the world (Champ and Dyte, 1976) [4]. *S. oryzae* causes enormous losses upto 100 percent in stored maize in India and other countries (Irabagon, 1959 [12] and Singh *et al*., 1974) [19]. This evidently indicates the importance of *S. oryzae* in the storage of maize seed.

In the new state like Telangana, maize seeds are often traditionally stored in jute bags. This leads to significant increase of moisture during rainy seasons, thereby creating conducive conditions for weevil infestation (Hossain, *et al*., 2007 [11] and Zunjare *et al.*, 2014) [23]. Infested seed fetches lower market price due to reduced weight. Seed viability of the damaged grain is drastically reduced and affects subsequent planting (Tefera, 2012) [22].

Nano-technology has become one of the most promising new technologies in the recent decade. Use of inorganic inert dusts is considered as one of the environment friendly alternative to chemical pesticides in stored pest management (Stadler *et al.*, 2012) [20]. They are chemically stable, highly persistent and have low mammalian toxicity.

Inert dusts mainly contain synthetic silica (silica dioxide) and natural silica such as diatomaceous earth (DE), kaolinite and silica gel which predominantly consists of amorphous and shapeless silica. They kill the arthropods by removing or adsorbing the epicuticular lipid layers causing excessive water loss through cuticle (Stadler *et al.*, 2012) [20]. Diatomaceous earth becomes more effective against insects if it possesses high amorphous silica content with uniform size distribution. Nano particles represent new technology that could provide cost effective solution to some of the most challenging environment clean up problem (Chinnamuthu and Murgesa Boopathi, 2009) [5]. Nanoparticles help to produce new pesticides and insect repellants (Owolade *et al*., 2008) [14]. Although there are numerous studies on the toxic effects of nanoparticles on bacteria, fungi and animal pathogen (Reddy *et al*., 2007) [16]. Little research has been carried out to investigate the toxic effects of nanoparticles on insects especially storage insect pests hence the present investigation was taken up.

2. Materials and Methods

Management of rice weevil by Nano particles of silica (10-20 nm), alumina (< 50 nm) and clay (< 100 nm) was tested on maize seed during 2015-17 at National Bureau of Plant Genetic Resources (NBPGR), Regional Research Station, Rajendranagar, Hyderabad and Seed Research and Technology Centre (SRTC), Professor Jayashankar Telangana State Agricultural University (PJTSAU), Rajendranagar. Nano silica, nano alumina, nano clay and diatomaceous earth were procured from Sigma Aldrich, USA. Each nano treatment was tested at three concentrations *viz*., 500, 250 and 125 ppm whereas the normal diatomaceous earth was tested at one level *viz*., 1000 ppm per kg maize seed.

2.1 Effect of nanocides on adult mortality of *Sitophilus oryzae* (L.)

Laboratory bioassay was conducted to test the toxicity of nano particles using dust application. The thoroughly dried and disinfested seed with moisture content ten percent were treated with the test nano particles and twenty freshly emerged adults were released on to the nano particles treated maize seeds. Adult mortality was assessed at 1, 2, 3, 5, 7, 9, 13 and 15 days after treatment. After studying the adult mortality, the seeds were placed back in the containers and kept undisturbed for the emergence of F_1 progeny and the number of adults emerged from each treatment was recorded and analysed statistically as suggested by panse and sukhatme (1978) [15] by using completely randomized design (CRD).

2.2 Effect of nanocides on *S. oryzae* adults by using Scanning Electron Microscopy (SEM)

The three nano particles along with diatomaceous earth used for the management of *S. oryzae*, were studied for their effect on the abrasion of cuticle and other parts of *S. oryzae* adults. For conducting the experiment, petri plates holding (nano silica, nano alumina, nano clay and diatomaceous earth) each treatment at 1 mg cm^{-1} were taken and twenty adults of *S. oryzae* were introduced into the nano treated plates. In another set, freshly emerged twenty adults were taken and this set served as control. Insects were taken out from the above treatments after 24 hours of exposure from both control and treated insects. Samples were fixed in 2.5 percent glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 hours at 4^{o}C and post fixed in 2 percent aqueous osmium tetroxide for four hours and dehydrated in series of graded alcohols and dried to critical point drying (CPD) with CPD unit. The processed samples were mounted over the stubs with double-sided carbon conductivity tape and thin layer of gold coat over the samples was done by using an automated sputter coater (Model - JEOL JFC-1600) for three minutes and scanned under scanning electron microscopy (SEM Model- JOEL-JSM 5600) at required magnifications as per the standard procedures (John and Lonnie, 1998) [13] at RUSKA Lab, College of Veterinary Science, PV Narsimha Rao Telangana State Veterinary University (PVNRTSVU). Rajendrangar, Hyderabad, India.

3. Results

3.1 Effect of Nano particles on adult mortality of *Sitophilus oryzae* (L.)

The results indicated that among the three nano treatments nano silica @ 500 ppm was highly effective and caused cent percent mortality after one day of treatment while, nano silica applied @ 250 ppm and 125 ppm resulted in 76.67 percent and 73.33 percent mortality, respectively after one day of treatment and complete mortality was observed after three days of treatment (Table 1). Nano alumina was less effective than nano silica and caused cent percent mortality after five days of treatment when applied @ 500 ppm, while 250 ppm caused complete mortality after seven days of treatment. Lower concentration of 125 ppm caused cent percent mortality after nine days of treatment. No mortality was observed in nano clay @ 500, 250 and 125 ppm after one day of treatment. The adult mortality observed in nano clay @ 500 ppm after second day of treatment was 18.33 percent and reached to 95.00 percent at fifteen days after treatment. Diatomaceous earth @ 1000 ppm recorded 21.67 percent mortality after first day of treatment and caused cent percent mortality after eleven days of treatment. Among all the treatments nano clay was least effective in controlling pests.

3.2 Effect of nano particle on adult emergence of *Sitophilus oryzae*

The adult emergence studies also indicated the superior performance of nano silica over other treatments (Table 2). Nano silica particles @ 500 ppm resulted in complete mortality of adults after one day of treatment.The lower concentration of nano silica @ 250 and 125 ppm which caused cent percent mortality of adults after three days of treatment resulted in no adult emergence. This might be due to very few eggs were laid by insect and did not develop into adults. In nano alumina and nano clay adult emergence ranged from 2.33 to 6.67 and 10.67 to 26.00, respectively in different concentrations. While adult emergence observed in diatomaceous earth @1000 ppm was 8.33. Nano clay was least effective in protecting the seed against *S. oryzae* damage.

3.3 Effect of nano dusts on *Sitophilus oryzae* by Scanning Electron Microscope (SEM)

The scanning electron microscope (SEM) images of adult insect exposed to nano silica particles (1 mg cm^{-2}) clearly showed the abrasion of the cuticle of the abdomen over the normal cuticle of abdomen in untreated check (Plate 1). The silica nano particles also caused the abrasion of the elytra surface. Similarly the nano particles (nano silica, nano alumina and nano clay) resulted in degeneration of the mouth parts (Plate 2).

Table 1: Effect of nanocides on adult mortality of *Sitophilus oryzae*

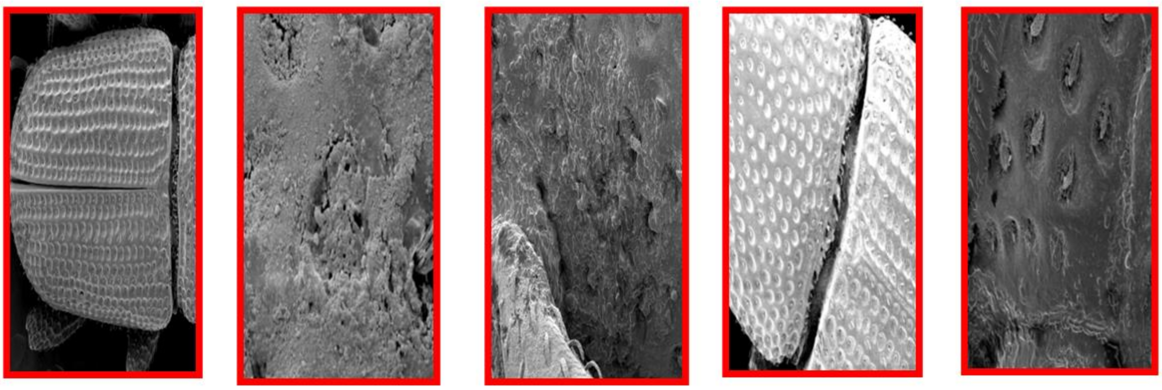
Treatments	Percent mortality								
	Days after treatment (DAT)								
	1	2	3	5	7	9	11	13	15
T_1-Nano silica @ @500 ppm	100.00 (85.9)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)
T_2-Nano silica @ 250 p	76.67 (61.14)	96.67 (80.03)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)
T_3-Nano silica @ 125 ppm	73.33 (58.93)	88.33 (70.11)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)
T_4-Nano alumina @ 500 ppm	71.67 (57.86)	81.67 (64.69)	85.00 (67.21)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)
T_5-Nano alumina @ 250 ppm	56.67 (48.84)	61.67 (51.76)	65.00 (53.73)	81.67 (64.69)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)
T_6-Nano alumina @ 125 ppm	43.33 (41.16)	55.00 (47.87)	60.00 (50.79)	70.00 (56.84)	86.67 (68.66)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)
T_7-Nano clay @ 500 ppm	0.00 (4.05)	18.33 (25.31)	26.67 (31.07)	43.33 (41.16)	76.67 (61.14)	85.00 (67.21)	90.00 (71.57)	91.67 (73.40)	95.00 (77.08)
T_8-Nano clay @ 250 ppm	0.00 (4.05)	0.00 (4.05)	11.67 (19.89)	23.33 (28.86)	35.00 (36.27)	43.33 (41.16)	53.33 (46.90)	61.67 (51.76)	73.33 (58.93)
T_9-Nano clay @ 125 ppm	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	11.67 (19.89)	21.67 (27.71)	31.67 (34.23)	41.67 (40.20)	53.33 (46.91)	61.67 (51.76)
T_{10}-Diatamaceous earth @1000 ppm	21.67 (27.71)	31.67 (34.23)	43.33 (41.16)	63.33 (52.74)	80.00 (63.43)	90.00 (71.95)	100.00 (85.95)	100.00 (85.95)	100.00 (85.95)
T_{11}-Untreated check	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)
SEm±	0.79	1.21	0.8	0.96	0.66	0.96	0.41	0.69	0.44
CD (P=0.05)	2.3	3.54	2.35	2.82	1.92	2.81	1.2	2.07	1.29
CV (%)	3.76	4.87	2.88	3	1.81	2.49	1.02	1.7	1.05

Figures in the parentheses are angular transformed values

Table 2: Effect of nanocides on adult emergence of *Sitophilus oryzae*

Treatments	Adult emergence (number)
T_1-Nano silica @ 500 ppm	0.00 (0.71)
T_2-Nano silica @ 250 ppm	0.00 (0.71)
T_3- Nano silica @125 ppm	0.00 (0.71)
T_4-Nanoalumina @ 500 ppm	2.33 (1.68)
T_5-Nano alumina @ 250 ppm	4.00 (2.11)
T_6-Nano alumina @ 125 ppm	6.67 (2.68)
T_7-Nano clay @ 500 ppm	10.67 (3.34)
T_8-Nano clay @ 250 ppm	15.67 (4.02)
T_9-Nano clay @ 125 ppm	26.00 (5.15)
T_{10}-Diatamaceous earth @1000 ppm	8.33 (2.97)
T_{11}-Untreated check	38.33 (6.23)
SEm±	0.07
CD (P=0.05)	2.07
CV (%)	4.35

Figures in the parentheses are square root transformed values



A) Control B) Nano silica C) Nano alumina D) Nano clay E) Diatomaceous

Plate 1: SEM image of cuticle of *S. oryzae* showing abrasion and impregnation of nano silica, nano alumina, nano clay and diatomaceous earth

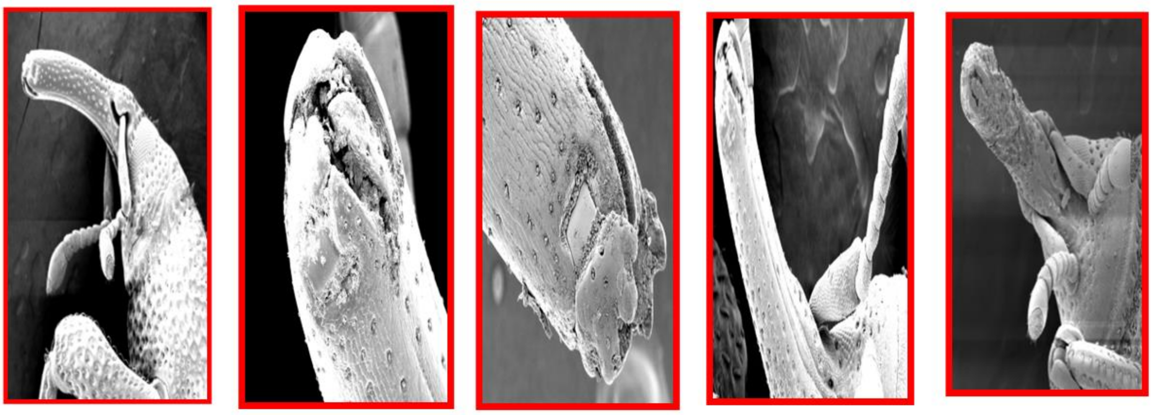


Plate 2: SEM image of mouth parts of *S. oryzae* showing abrasion and impregnation of nano silica, nano alumina, nano clay and diatomaceous earth

4. Discussion: Thus the results obtained from different nanocide treatments showed that nano silica was significantly superior over other treatments. Nano silica applied @ 500 ppm was the best treatment which resulted in complete mortality and prevented the subsequent development of the adults. The results are in accordance with the findings of Rouhani *et al*. (2013) [17] who reported that nano silica applied at 2.5 mg kg^{-1} cowpea seeds resulted in cent percent mortality of *C. chinensis*. Debnath *et al*. (2011) [6] also reported cent percent adult mortality of *S. oryzae* when, nano silica was applied @ 2 mg kg^{-1} of paddy.

The SEM images also clearly revealed that the amorphous uniform sized flat nano silica particles resulted in uniform distribution of the nano particles over the body resulting in abrasion of the insect cuticle there by resulting in loss of water from the body and also caused defacing of the mandibles which in turn resulted in death of the insect. The less efficacy of nano alumina and clay could be due to their physical properties with respect to the size, shape etc which would in turn effect the distribution of particles over the treated surface. The results are in accordance with the findings of Debnath *et al.* (2011) [6]. They reported that nano silica was far more effective than bulk silica because of their enormously increased exposed surfaces which could interact with the insect cuticle. Death of the insects could be due to the damage of protective wax coat on the cuticle, both by sorption and abrasion. Ebeling and Wagner (1959) [9] proposed that insecticidal efficacy of the dust becomes enhanced if the particles are finely divided. The insects begin to lose water through desiccation as the water barrier is damaged (Ebeling, 1971) [8] and die due to desiccation. This hypothesis for the physical mode of action makes the ease for the use of nanocides stronger. The nanocides can be removed by conventional milling process without leaving residues on the stored grain unlike sprayable formulations of conventional pesticides (Debnath *et al.,* 2011) [6]. Therefore, nano particles especially silica nano particles has an excellent potential as stored grain as well as seed protecting agent if applied with proper safety measures.

The use of nano particles as pesticide is an alternative strategy to combat pests which have become resistant to conventional pesticides. United States Department of Agriculture (USDA) has already declared non-crystalline silica as safest grain protectant (Stathers *et al.,* 2004) [21]. For the practical use of nano silica particles and test compounds as novel pesticide to proceed, further research is required on the safety issues of these materials on human health and bio safety to non target organ needs to the scrupulously assessed before they could be considered for commercialization as the nano structural material have more toxicological concerns (Borm *et al*., 2006 [3]; Scheufele *et al*., 2007) [18] due to the novel physico chemical properties arising from their size. Other areas requiring attention are its mode of action and development of formulations to improve potency and stability, as well as to reduce cost. This study could open up newer pathways of using nano material-based technology in pesticide industry.

5. Conclusions

Among the three nanocides, nano silica applied at dosage 500 ppm kg^{-1} caused immediate mortality and inhibited adult emergence of *Sitophilus oryzae***.** From the results, it is evident that nano silica can act as a promising alternative to commercially available inert dust.

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