Int J Pharm Bio Sci 2013 July; 4(3): (B) 1108 - 1118

Research Article Biotechnology

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

SILICA NANOPARTICLES AS NANOCIDES AGAINST *CORCYRA CEPHALONICA* (S.), THE STORED GRAIN PEST

VANI C[*] AND BRINDHAA U

**Department of Biotechnology, School of Biotechnology and Health Sciences, Karunya University, Coimbatore – 641 114, Tamil Nadu, India*

.

ABSTRACT

Diatomaceous earth and synthetic silica have been used as insecticides in the form of inert dusts. Diatomaceous earth becomes more effective against insects if it possesses high amorphous silica content with uniform size distribution. Nanoparticles are more reactive than their bulk counterpart because of their increased surface to volume ratio. The objective of our study is to evaluate the effect of silica nanoparticles against the stored grain pest *Corcyra cephalonica*. Silica nanoparticles were synthesized by modified Stober's sol-gel method. They were characterized by Scanning Electron Microscope, X-ray Diffraction and Energy Dispersive X-ray Spectroscopy. Characterization studies showed silica nanoparticles of size range 70-80nm were synthesized which are spherical in shape and amorphous in nature. The entomotoxic effect of silica nanoparticles were evaluated against the stored grain pest *Corcyra cephalonica.* Amorphous silica nanoparticles were found to be highly effective against this insect pest causing 100% mortality, indicating the effectiveness of silica nanoparticles to control insect pests.

KEYWORDS: Silica nanoparticles, spherical, amorphous, entomotoxicity, *Corcyra cephalonica*

VANI C
Department of Biotechnology, School of Biotechnology and Health Sciences, Karunya University, Coimbatore – 641 114, Tamil Nadu, India

INTRODUCTION

In developing countries agriculture is the driving force for broad-based economic growth. One of the major problems with agriculture now-a-days is demand for the production of more and more agricultural products in order to provide food for the population, which is in permanent augmentation. In realizing this, one of the stumbling blocks seems to be the yield losses due to pests. During storage, food grains and products are severely destroyed by insects and other pests. So priority should be given to post harvest studies, particularly in humid tropical climates, where at least half of the food supply may be lost between harvest and consumption[1]. Storage and upkeep of agricultural products are very important post harvest activities. Considerable amount of food grains is being spoiled after harvest due to lack of sufficient storage and processing facilities [2]. Food and Agriculture Organization of United Nations estimate of worldwide annual losses in stored produce has been given as 10% of all stored grain. Routine management of stored produce pest through fumigation, chemical pesticides and plant based deterrents, is intended to contain this loss [3]. Losses of grain in storage due to insects are the final components of the struggle to limit insect losses in agricultural production. These losses can exceed those incurred while growing the crop. Losses caused by insects include not only the direct consumption of kernels, but also include accumulations of frass, exuviae, webbing, and insect cadavers. High levels of this insect detritus may result in grain that is unfit for human consumption. Insect-induced changes in the storage environment may cause warm, moist 'hotspots' that are suitable for the development of storage fungi that cause further losses. Worldwide losses in stored products, caused by insects, have been estimated to be between five and ten percent [4]. Insect infestation also causes a reduction in nutrients in the grain.The ricemoth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae), is an important stored-product pest in Asia and South America. It is probably one of the most catholic feeders among the storage pests which feed on a wide variety of dried vegetable materials, rice, corn, cocoa, dried fruits like almonds, date palm, nuts, chocolates, biscuits, oilcakes, coffee and other seeds [5]. In addition to consumption, as they become fully grown, larvae contaminate the grain by producing dense webbing containing their fecal material and cast skins [6.]

Fumigants and residual insecticides are commonly used to combat stored grain pests. Fungicides are the primary means of controlling post harvest diseases. The current methods for managing stored grain pests depend heavily on synthetic pesticides. However, repeated use of certain chemical fungicides in packing houses has led to the appearance of fungicide-resistant populations of storage pathogens. Unfortunately this will lead to contamination of food with toxic residues [7]. 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[8] .One such alternative is the use of Inert dusts such as clay, sand, rock phosphate, ashes, diatomaceous earth, and synthetic silica that have been used as insecticides for thousands of years by aboriginal peoples in North America and Africa and are also used in modern grain storage facilities. Modern day research on inert dusts as a stored-grain protectant began in the 1920's [9]. Diatomaceous earth (DE) is the remains of micro-scopic one-celled plants (diatoms) that lived in the oceans that once covered the western part of the United States and other parts of the world. Huge deposits were left behind when the water receded. The insecticidal quality of DE is due to the razor sharp edges of the diatom remains. As the insects crawl through treated grain and dusted bins, the DE comes contact with the insects and the sharp edges puncture the insect's exoskeleton. The powdery DE then absorbs the body fluids causing death from dehydration[10]. Inert dusts are chemically unreactive dusts that have insecticidal capability, killing by physical rather than chemical means. Dryacide, " a diatomaceous earth inert dust, has been used since 1986 for admixture to grain stored in farm.The National Association of Sustainable Agriculture Australia (NASSA) has approved Dryacide", for use on organic grain [11] .The main advantage of inert dusts is their low-mammalian toxicity. In Canada and the USA, diatomaceous earth is registered as an animal feed additive and silicon dioxide is registered as a human food additive. Also inert dusts are effective for long durations and they do not affect end use quality. Their main disadvantages are that they are dusty to apply, do not work at high relative humidity and impede the flow of grain [12]. Silicon is present in environment in different forms. Although it is not found in nascent form but it is always present in combination with oxygen (as in silica) or hydroxides (As in silicic acid). 78% of earth's crust consists of silicon and oxygen compounds, both amorphous and crystalline compounds for example quartz, flint, opal, silicates etc. Silicon is also present in dissolved form in the oceans as silicic acid. Also silica is found in living organisms like sponges, grasses, algae (for example, diatoms)[13]. DE becomes more effective against insects if it possess high amorphous silica content with uniform size distribution. Nanoparticles are ultrafine particles which have at least one dimension less than 100nm. A number of physical properties of materials change as their size approach nanoscale. NPs are more reactive than their bulk counterpart because of their increased surface to volume ratio as in any physical or chemical interaction only the surface exposed to the reaction condition participates in the process without any toxicity[14]. The synthesis and study nanoparticles, has become a major interdisciplinary area of research over the past 10 years. The size, morphology as well as the properties of nanoparticles basically depends on the methods of preparation. All the processes can be broadly divided into two processes, physical methods and chemical methods. The Stober process used for the preparation of mono dispersed silica colloids 'white carbon black" by means of hydrolysis of alkyl silicates and subsequent condensation of silicic acid in alcoholic solutions using ammonia as catalyst was first published in 1968 [15]. Ever since, there have been many research groups who applied those mono dispersed silica colloids as model material in various applications.[16] Sacks and Tseng utilized those colloids to pack ordered structure membrane and investigated its sintering behavior [16]. On the other hand [17] Unger *et al*., 2000 applied these submicron silica colloids as packing material for capillary chromatography. Diatomaceous earth and synthetic silica have been used as insecticides in the form of inert dusts. For all these different applications, it would always be desirable to use silica particles with a specified particle size and extremely narrow distribution. Therefore, it is expected that nanocides would be needed in lesser quantity for protection. Nanocarriers are being designed to reduce the volume of application and slow down the release kinectics of agrochemicals. However, nanotechnological applications in agriculture are still at a nascent stage [8].Our study aims at the synthesization and characterization of silica nanoparticles and to evaluate the effect of the synthesized nanoparticles to the stored product pest *Corcyra cephalonica.*

MATERIALS AND METHODS

(i) Insects Rearing

The stock culture of *Corcyra cephalonica* and the recipe for the standard medium were obtained from the Department of Entomology, Tamilnadu Agricultural University, Coimbatore, India. The stock cultures were maintained at 28 ± 1°C, 68 ± 3% RH and a photoperiod regime of 14L : 10D. [18]. Fourth instar stage of the larvae were used for the experiments.

(ii) Synthesis of silica nanoparticles

Silica nanoparticles were prepared by hydrolysis and condensation of Tetraethyl Ortho Silicate (TEOS) in ethanol, and in presence of ammonia as catalyst were used for the synthesis of silica nanoparticles as per the protocol [19]. Briefly, appropriate quantities of absolute ethanol, ammonia and deionised water were taken and mix thoroughly for 5 mins. Then proper amount of TEOS was added drop wise at 37 C for 24 h at room temperature. First, solution containing appropriate quantities of absolute ethanol, ammonia and deionized water were stirred for 5 minutes to ensure complete mixing. Then a proper amount of TEOS in absolute ethanol was added to the above solution and the reaction proceeded at ambient temperature for 24 hours according to reactants concentrations. Thereafter the colloidal solution was separated by high-speed centrifuge, and the silica particles were washed by absolute ethanol for three times to remove undesirable particles, Followed by drying in oven at 100 °C for 2 hrs to prevent continuous reaction.

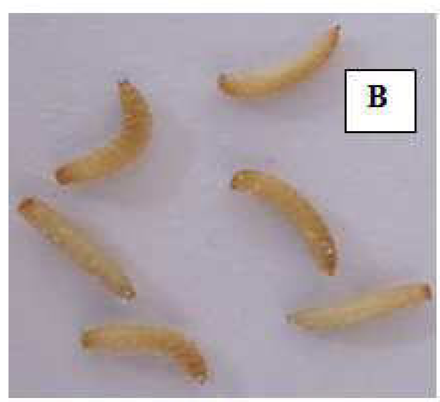
(iii) Characterization

Silica nanoparticles synthesized in our laboratory were analyzed by Scanning Electron Microscope (SEM, JEOL JSM-6390), X-ray Diffraction (XRD), Energy Dispersive X-ray Spectroscopy (EDAX), Dynamic Light Scattering (DLS, Molvern Zetasize Nano ZS90) in the Central Research Facilities, Department of Nanotechnology, Karunya University, Coimbatore, Tamilnadu The characterization was carried out as per the protocol of [20] Rahman *et al*., 2007.

(iv) Bioassay of Corcyra cephalonica.

The bioassay on *Corcyra cephalonica* was performed in small plastic screw capped jars. Each jar had a radius of 6 cm and height of 6.5 cm. The caps were perforated to allow aeration. 20 g of broken pearl millet grains was placed in each jar. Each jar was treated individually with laboratory made Stober Silica nanoparticles (SNPs) at four dose rates SNP I (10mg), SNP II(20mg), SNP III(40mg), SNP IV(80mg) nanoparticles per 20g of pearl millet grains. Then, the jars were shaken manually for approximately 1 min to achieve equal distribution of nanoparticles on pearl millet grains [21]. For each dose, there were five replicates. In one additional set no nanoparticle was mixed with the grains and this set served as control. The jars were kept for 24 h before 10 larvae of *C. cephalonica* were introduced into each jar (Plate 1). All bio-assays were performed at 30^0 C ± 1^0C, 75 ± 5% r.h. Insect mortality was checked after 1, 2, 4 and 8 days [8].

Plate 1
(A)- Pearl millet grains, (B)- Larvae of Corcyra cephalonica, (C)-Bioassay of Corcyra cephalonica exposed to silica nanoparticles treated grains



(v) Data analysis
All data were arcsine-transformed to standardize means and normalize variances. Means were compared with the control by using the Dunnett test.

RESULTS

1. Synthesis and Characterization of Silica nanoparticles.

Plate 2:Silica Nanoparticles

Figure 1
SEM image of silica nanoparticles

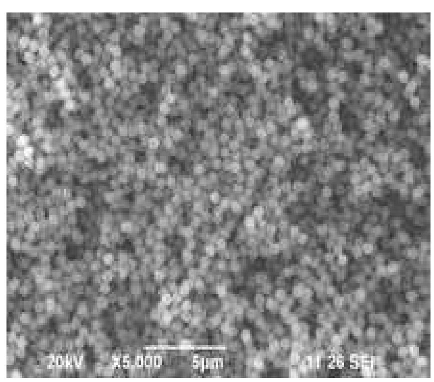
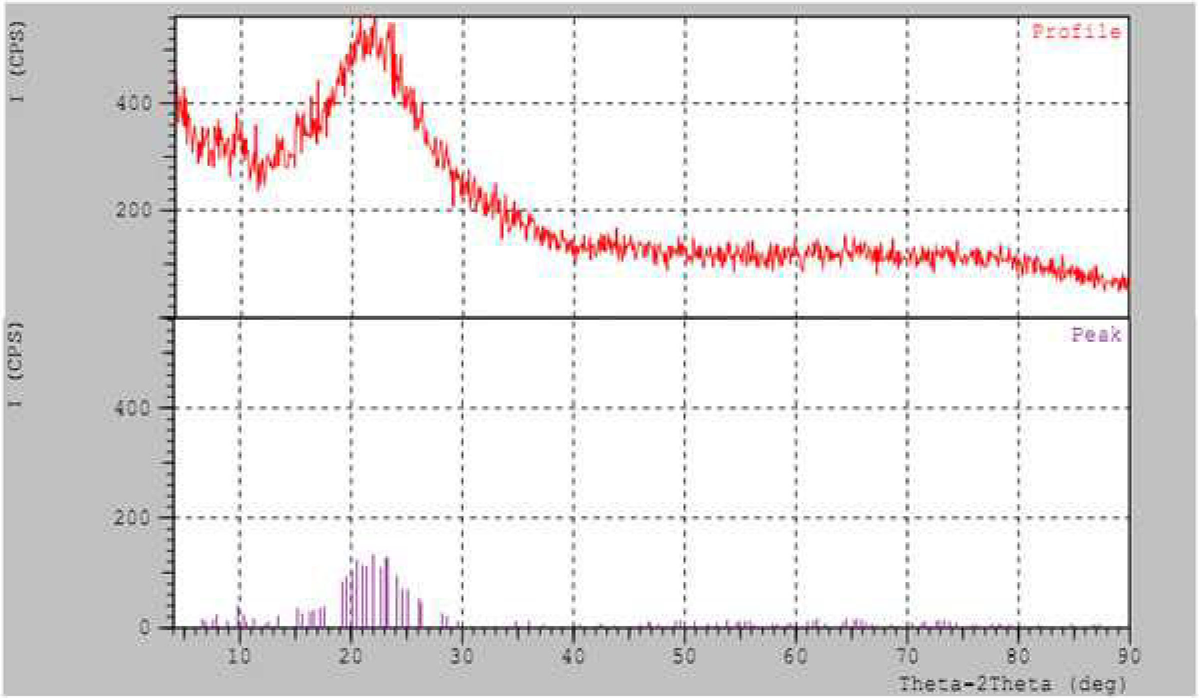


Figure 2
XRD pattern of silica nanoparticles



Silica nanoparticles were prepared by Stober's sol-gel method which involves the hydrolysis and condensation of TEOS in ethanol, and the presence of ammonia, acts as catalyst at room temperature and the resulting product was white in colour (Plate 2). Scanning Electron Microscope micrograph (Fig.1) revealed that SiO2 nanoparticle are well separated with spherical morphology and the average particle size range is 70-80nm. XRD pattern of the as-prepared SiO_2 nanoparticle is shown in Fig.2. There is a weak broaden peak corresponding to the characteristic of an amorphous SiO_2 located in the range of 20–22.5°. It implies that the core region of the composite particles is an amorphous SiO_2, which is consistent with the observation of SEM images. EDAX carried out on nanoparticle indicated qualitatively the presence of SiO_2 (Fig. 3) The particles size of silica nanoparticles prepared by sol- gel method has been characterized by light scattering experiments. Fig.4 represents the size distribution curve of silica nanoparticles in which the particle distribution is more uniform with a narrow distribution range. The particle size distribution range for particles is in the range of 60-110 nm.

Figure 3
EDAX spectrum of the nanoparticles

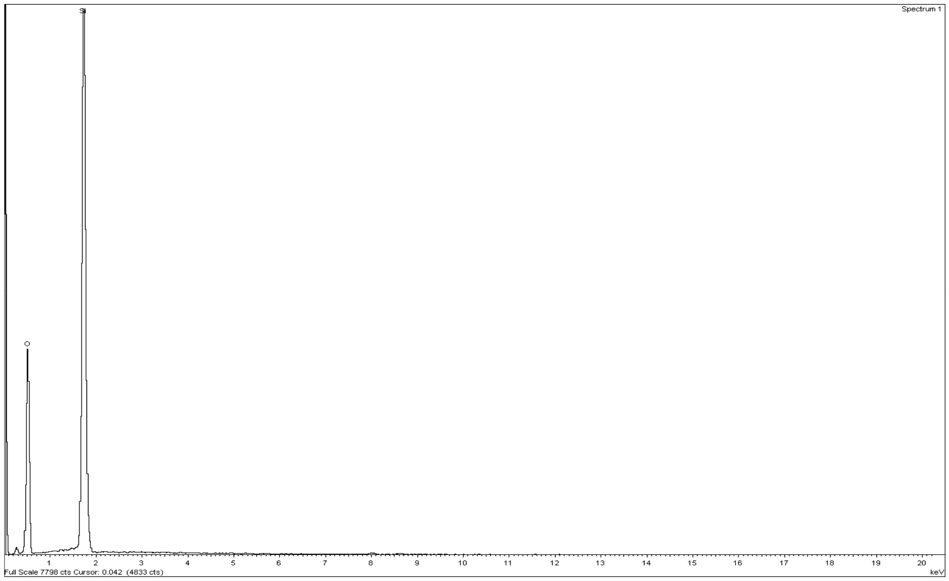
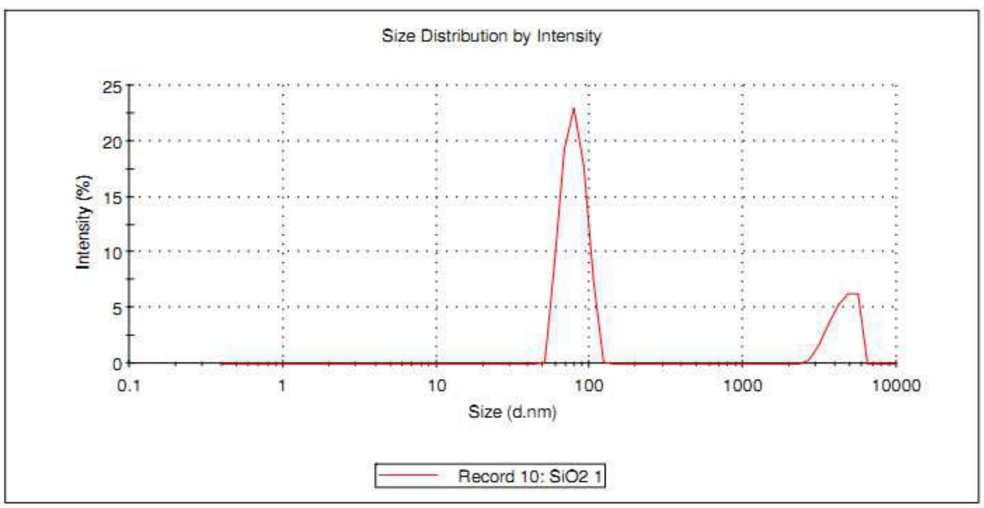


Figure 4
Particle size distribution curve of silica nanoparticles



2. *Bioassay of Corcyra cephalinica.*

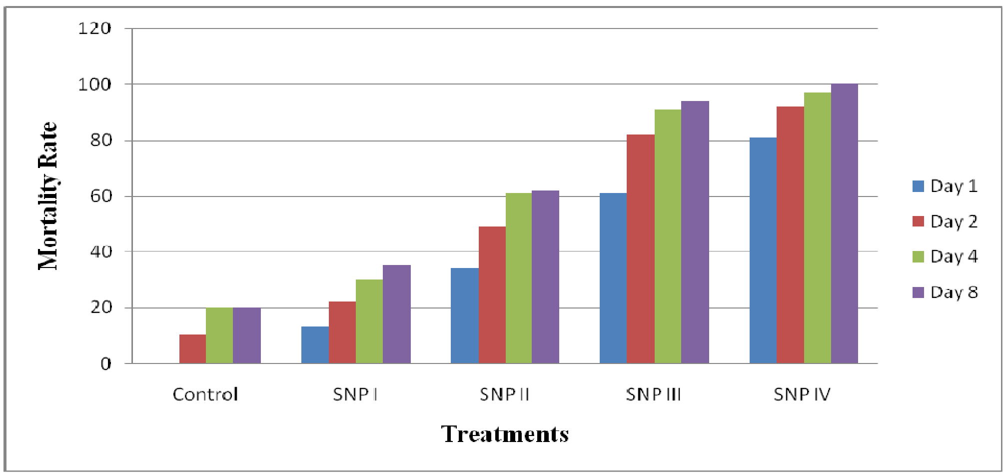
Table 1
Mean mortality (±S.E) of Corcyra cephalonica exposed on pearl millet grains treated with nano silica at four dose rates with control for different days

Days	Control	SNP I (10mg/20g)	SNP II (20mg/20g)	SNP III (40mg/20g)	SNP IV (80mg/20g)
Day 1	0.0±0.0	13.0±5.8	34.0±8.2	61.0±8.2	81.0±4.2***
Day 2	1.0±2.3	22.0±5.9	49.0±8.4	82.0±5.7	92.0±7.6***
Day 4	2.0±2.7	30.0±8.2	61.0±7.4	91.0±4.2	97.0±2.7***
Day 8	2.0±2.7	35.0±8.3	62.0±5.6	94.0±4.2	100.0±0.0***

***** indicates significance (p<0.001 level) when compared with the control group**

Mortality rate of different dosage rates of SNPs against *Corcyra cephalonica* with grains were presented in Table 1. All main effects and their associated interactions were significant at P>0.01 level. Treatment of silica nanoparticles caused mortality of 35% and 100% at the doses of SNP I (10mg/20g) and SNP II(80mg/20g) at eight days of exposure. All doses were significantly different from untreated control. Nearly 60% of the insects were killed when the grains (cumbu) were treated with SNP II (20mg/20g) on day 4 and 8. Greater than 90% mortality was obtained with SNP III (40mg/20g) on day 4 and 8. Mortality rate of SNP III and SNP IV of days 2, 4 and 8 was similar and significantly better than the other treatments. The rate of mortality of larvae was increased with increase in exposure time (Fig 5). Eight days of exposure to SNP IV caused higher mortality than others that is 100% mortality and was significantly superior over the untreated control (plate 2). Using Probit analysis the LC_{50} value was found that 36mg is lethal to cause 50 per cent mortality.

Figure 5
Mean mortality of Corcyra cephalonica exposed on pearl millet grains treated with nano silica at four dose rates with control of different days



DISCUSSION

1. *Synthesis and Characterization*

The particle size was determined by the synthesis parameters. Close examination of the silica particles by scanning electron microscopy (SEM) revealed that all the particles used in this study were spherical and the primary particle sizes were approximately uniform. The particle size of the silica particles prepared from TEOS by Stober synthesis in our present work is from 70 to 80 nm. According to [19], the resulting particles in the product suspension were highly monodispersed in size and spherical in shape which was prepared. Amorphous silica nano particles tend to adopt the spherical shape so as to reach a minimum of interfacial surface area[22]. The size distribution spectrum of each set of silica particles in a neutral solvent showed a single peak. Moreover, the average particle size corresponded almost precisely to the anticipated size for each sample. The results suggested that the silica particles synthesized remained as stable well-dispersed particles in solution described by [2]. Elemental confirmation was done by EDAX which confirmed the presence of Si and O in the nanoparticles synthesized which indicated qualitatively the presence of SiO_2 as showed by [24].The powder diffraction pattern in our work indicates a broad peak at $2\theta = 22^{o}$, which reveals the amorphous nature of the silica nanoparticles. Further, the XRD pattern confirms the absence of any ordered crystalline structure and the results were in accordance with the work done by Gobi [25]. According to the International Union of Pure and Applied Chemistry (IUPAC), the atoms must be arranged periodically with long-range order (at least 10 repeats in all directions) and produce sharp maxima in a diffraction experiment to observe x-ray diffraction (XRD) crystallinity [26]. The threshold for observing crystallinity depends on the unit cell size (size of the repeated unit in a crystal). For materials with large unit cells, such as porosils, the minimum particle size required is about 10 nanometers to observe a distinct, sharp XRD pattern. Amorphous silica may present some short-range order but lacks long-range order in 3 dimensions and does not exhibit a sharp XRD pattern. Thus, particles with an ordering at limited-length scales or with amorphous regions may be classified as amorphous [22].

2. Bioassay of Silica nanoparticles against Corcyra cephalonica

The use of inert dusts, particularly those based on silica, has been finding increasing use as stored grain protectants [9]. Among them, Diatomaceous earth—composed mainly of amorphous micron sized silica—has become most popular [27] as an alternative to the conventional pesticides which have low mammalian toxicity. The effect of nano alumina against two stored grain pests *Sitophilous oryzae* and *Rhyzopertha dominica* (F.). was studied by [28]. However, nano alumina in ground water inhibits the growth of carrot, cabbage, cucumber, corn, and soyb. Our study presents the entomotoxic potential of SNP which has no adverse effect on plant growth, rather silica enhances structural rigidity and strength of plant [30]. This may be one of the possible reasons for which there is an age old tradition of using silica dust as protective agent for stored seeds by different ethnic races all over the world[31].Mortality rate was increased with increase in exposure time and concentration of SNP when grains (chumbu) admixed with 10mg and 80 mg of SNP achieved better control in *C. cephalonica.* Eight days of exposure to SNP IV caused 100 percent mortality than the other treatments which was significantly superior over the untreated control. The LC_{50} value was 36mg/ 20 g of the grain. The above result was in accordance with the results of [8].SNP does not affect the looseness and bulk density of grain mass like DE even with the highest dose used in our bioassay. Proposed that insecticidal efficacy of the dust becomes enhanced if the particles are finely divided was [32]. This was also evident from our experiment, where nano-silica was effective because of 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. Modified Stober SNP (without any surface capping) and the other three surface-functionalized SNPs act with same efficacy on *S. oryzae.* This indicates that superior entomotoxicity is due to nano-sized silica itself, not due to the surface groups attached to them[8]. This result was in accordance with the present study that SNP synthesized showed efficacy in killing *C. cephalonica* and per cent mortality increases as the concentration increases. The lethal concentration for 50 % mortality was observed as 36mg/20 g of the grain.

The insects begin to lose water through desiccation as the water barrier is damaged [31] and die due to desiccation. We believe that this hypothesis for the physical mode of action makes the case for the use of nanocides stronger. The nanocides can be removed by a conventional milling process unlike sprayable formulations of conventional pesticides, leaving residues on the stored grain.

CONCLUSION

Therefore, Silica Nanoparticles will have an excellent potential as seed protecting agent if applied with proper safety measures. This study could lead to open up newer pathways of using nanomaterial-based technology for the control of stored grain pests.

ACKNOWLEDGEMENT

Authors would like to thank Department of Biotechnology, School of Biotechnology and Health Sciences, Karunya University and Central Research Facilities, Department of Nanotechnology, Karunya University. And also Department of Entomology, Tamilnadu Agricultural University, Coimbatore for their support and help throughout the course of work.

REFERENCES

1. Dubey N.K., Bhawana S., Ashok K., Current Status of Plant Products as Botanical Pesticides in storage pest management. Journal of Biopesticides, 1:182 – 186, (2008).
2. Singh RKP., Satapathy KK., Zero Energy Cool Chamber: a low cost storage structure. Journal of the North Eastern Council, 23:27-30, (2003).
3. Solomon K., Sathiadas S., Smaragdi P., Prospects of traditional seed storage strategies against insect infestation adopted by two ethnic communities of Tamil Nadu southern peninsular India, Bulletin of Insectology, 59:129-134, (2006).
4. David KW and Reeves PA. Introduction - Stored Grain Pest Management, third Edn, Macmillan, New York, (1982).
5. Jagadish PS., Nirmala P., Rashmi M.A., Neelu N., Biology of rice moth, *Corcyra cephalonica* Stainton on foxtail millet. Karnataka Journal of Agricultural Sciences, 22:674-675, (2009).
6. Fangneng H., Bhadriraju S., Responses of *Corcyra cephalonica* (Stainton) to pirimiphos-methyl, spinosad, and combinations of pirimiphos-methyl and synergized pyrethrins. Pest Managment Science, 60:191–198, (2003).
7. Dubey S.C., Suresh M., Singh B., Evaluation of *Trichoderma* species against *Fusarium oxysporum* f. sp. ciceris for integrated management of chickpea. Biological Control, 40:118-127, (2007).
8. Debnath N., Sumistha D., Dipankar S., Ramesh C., Bhattacharya S., Arunava G., Entomotoxic effect of silica nanoparticles against *Sitophilus oryzae* (L.). Journal of Pest Science, 84:99-105, (2010).
9. Golob P., Current status and future perspectives for inert dusts for control of stored product insects. Journal of Stored Products Research, 33:69-79, (1997).
10. Korunic, Z., Diatomaceous earths, a group of natural insecticides. Journal of Stored Products Research,34:87-97,(1997).
11. Donahaye EJ., Navarro S., Leesch JG., Integration of Inert dust into Control of Storage Pests in Bulk Grain in Storage in Australia. Proceedings of International Conference on Controlled Atmosphere and Fumigation in Stored Products,29:279-284,(2001).
12. Fields P., Zlatko K. Post-harvest insect control with inert dusts. Encyclopedia of Pest Management,48:650-653,(2002).
13. Patwardhan SV., Silicification and Biosilicification: The role of macromolecules in bioinspired silica synthesis. Ph.D. dissertation, University of Cincinnati, (2003).
14. Vani C., Sergy G., Annamalai A., A study on the Effect of Zinc Oxide Nanoparticles in *Staphyloccus aureus.* International Journal of Pharma Biosciences, 2:326-335, (2011).
15. Stober W., Fink A., and Bohn E., Controlled Growth of Monodisperse Silica Spheres in the Micron Size Range. Journal of Colloid and Interface Science,26: 62-69,(1968).
16. Sacks MD., Tseng TY., Preparation of SiO2 Glass from Model Powder Compacts: Formation and Characterization of Powders, Suspension and Green Compacts. Journal of the American Ceramic Society, 67:526-537, (1984).
17. Unger KK., Kumar D., Grun M., Renker S., Synthesis of Spherical Porous Silicas in

the Micron and Submicron Size Range: Challenges and Opportunities for Miniaturized High –Resolution Chromatographic and Electrokinetic Separations. Journal of Chromatography, 892:47-55,(2000).

18. Osman N., Development of the Rice Moth, *Corcyra cephalonica* (St.) on Different Grains. Pertanika, 9:155 -159,(1986).
19. Ismail A.M., Ibrahim AF., Mohamed A., Preparation of spherical silica nanoparticles: Stober silica. Journal of American Science, 6: 985-989, (2010).
20. Rahman IA., Vejayakumaran P., Sipaut CS., Ismail J., Bakar Abu M., Adnan R., Chee CK., An optimized sol-gel synthesis of stable primary equivalent silica particles. Colloids ans Surfaces A. Physicochemical Engineering Aspects, 294:102-110,(2007).
21. Subramanyam B and Roesli, R. Inert dusts: Alternatives to Pesticides in Stored-Product IPM, Kluwer Academic Publishers, Dordrecht, 25:24-29,(2000).
22. Dorota N.**,** Leen C.J.**,** Dominique L.**,** Johan A.M., Peter H.,The nanosilica hazard: another variable entity**.** Particle and Fibre Toxicology, **7**: 39-41, (2010).
23. Hiromi N., Tomoaki Y., Akihiro A., Tokuyuki Y., Effect of surface properties of silica nanoparticles on their cytotoxicity and cellular distribution in murine macrophages. Nanoscale Research Letters, 6(93), (2011).
24. Xiaolan S., Jiang N., Yukun L., Dayu X., Guanzhou Q., Synthesis of CeO2-coated SiO2 nanoparticle and dispersion stability of its suspension. Materials Chemistry and Physics,110: 128–135,(2008).
25. Gobi N., Thangavelu R., Venkatachalam R., Rajagoundar P., Effect of Silica Nanoparticles and BTCA on Physical Properties of Cotton Fabrics. Materials Research,14:552-559, (2011).
26. McCusker LB., Liebau F., Engelhardt G., Nomenclature of structural and Compositional characteristics of ordered microporous and mesoporous materials with inorganic hosts. Pure and Applied Chemistry, 73:381-394, (2001).
27. Mutambuki K., Ngatia C. M., Mbugua J. N., Likhago P., Evaluation of the efficacy of spinosad dust against major storage insect pest. Journal of Stored and Post Harvest Research, 3:19-23, (2011).
28. Stadler T., Buteler M., Weaver DK., Novel use of nanostructured alumina as an insecticide. Pest Management Science, 66: 577-9,(2010).
29. Yang L., Watts DJ., Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. Toxicology Letters, 158:122–132,(2005).
30. Epstein E., The anomaly of silicon in plant biology. Proceedings of the National Academy of Sciences, 91:11–17, (1994).
31. Ebeling W., Sorptive dusts for pest control. Annual Review of Entomology, 16:123–158, (1971).
32. Wagner RE., Ebeling W., Rapid desiccation of drywood termited with inert sorptive dusts and other substances. Journal of Economic Entomology, 52:190–207, (1959).