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Efficacy of rice husk silica nanoparticles against *Sitophiliusoryzae* (L) and *Xanthomonasoryzae*

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Abstract
The nanoparticles have received much attention for controlling of insects pests instored grains. The efficacy of amorphous rice husk silica nanoparticles (93.14 nm) against *Sitophiliusoryzae* (L) and *Xanthomonasoryzae* were carried out. It was observed that, adult mortality of *S. oryzae* is dependent on dose and time after application. Adult mortality on 4thdays showed significantly highest adult mortality (100%) of *S. oryzae* with 175 and 200 ppm and negligible weight loss. The results of antibacterial study of SNPs against *Xanthomonasoryzae* revealed that the SNPs did not have antibacterial activity in *vitro* condition. Thus, the use of rice husk silica nanoparticles as pesticide in an alternative strategy to combat *S. oryzae* insect pest which have become resistance to conventional pesticides with ecofriendly way.

Keywords: Rice husk, Silica nanoparticles, *Sitophiliusoryzae*, *Xanthomonasoryzae*

Introduction
Silica nanoparticles (SNPs) have gained a greater attention because of its highly reactive surface area to volume ratio, physical and chemical stability, low toxicity and straightforward surface chemistry which allows them to combined or functionalized with a variety of functional species or molecules (Ghorbani *et al.*, 2015) [19]. SNPs are frequently used in industrial manufacturing, packaging, ceramic, catalysis, diagnostics, drug delivery, cancer therapy, disease labelling, imaging, biosensor, food and agriculture (Bottini *et al.*, 2006; Wang *et al.*, 2011; Sahoo *et al.*, 2007; Kasaai, 2015) [6, 41, 36, 25] also, it can be used as alternative to conventional insecticides against variety of stored grain and field insects and pests (Debnath *et al.*, 2009; Goswami *et al.*, 2010; Rouhani *et al.*, 2012; Arumugam *et al.*, 2015) [12, 21, 34, 3]. Sodium silicate applied as a silicon source in industrial production of SNPs and it requires a large quantity of energy and purification which causes of widespread environmental pollution. In contrast, low temperature synthesis of amorphous silicanano particles from plant biomass yields high quality, eco-friendly and cost effective product as opposed to the high energy processing of the in organics (Liou and Yang, 2011) [27]. Therefore, the use of rice husk as source of SNPs has both positive environmental and economic impact through the use of an abundant low-value agricultural by-product that canal leviate waste disposal problems.
Storage and upkeep of food grains is very important post-harvest activities. *S. oryzae*is classified as a primary insect pest damaging stored grains worldwide (Aitken, 1975) [2]. Alarva of *S. oryzae* consumes 14 mg grain/day and adult consumes 0.4 mg grain/day (Devi *et al.*, 2014) [14]. They also attack wheat, corn, oats, rye, sorghum, barley and dried beans. It causes extensive losses in the quality and quantity of commercial products as well as deterioration of seed viability (Owolad *et al.*, 2008) [31]. Traditionally use of synthetic insecticides as chemical control is the most commonly used strategy against stored insects and pests and long term application of these chemicals develops resistance to pesticides. *S.oryzae*is becoming resistance to conventional insecticides such as pyrethroid (Heather, 1986) [23], phosphine (Daglish *et al.*, 2002; Benhalima *et al.*, 2004) [10, 5]. Debnath *et al.*, (2011) [13] reported that nanocides were expected to reduce the volume of application and kinetics of development of resistance in pests. In recent years, consumer awareness of health hazards from residual toxicity and the development of insect resistance to these conventional insecticides have led

the researchers to look for alternaive strategies for stored grains protection (Sabbour, 2015). The nanoparticles (NPs) have received much attention for controlling of insects pests instored grains. The use of inert dusts, particularly those based on silica has been finding increasing use as stored grain protectants (Golob, 1997) [20]. United States Department of Agriculture (USDA) has already declared amorphous silica as bio safe (Stathers *et al.,* 2004) [39]. The use of rice husk silica nanoparticles as pesticide in an alternative strategy to combat insect pest which have become resistance to conventional pesticides which can be nonhazardous. Rice occupies a prominent place in food security management. Unfortunately, rice is hyper sensitivities to phytopathogenic genus *Xanthomonasoryzae*pv. *oryzae*. (*Xoo*) responsible for serious diseases such as and it can lead to 10-50 % reduction in yield. Various chemical bactericides have been used to control the infection and spread of *Xoo*, such as bismerthiazol and streptomycin (Li *et al.,* 2014) [26]. Long term and excessive exposure to the chemical bactericides have induced resistance in the bacteria, widespread instances of poor treatment efficacy and large economic losses (Ocsoy *et al.,* 2013) [29]. To solve these problems, researchers have been searching different antibacterial agents as alternatives to synthetic chemical bactericides. Silica nanospheres loaded with silver have been investigated for management of bacterial leaf blight in rice (Cui *et al.,* 2016) [9], but as per review no such studies have been reported to test the efficacy of rice husk silica nanoparticles against *Xoo* in *vitro* condition.

Materials and Methods

Synthesis of silica nanoparticles from rice husk

Silica nanoparticles were synthesized from rice husk (RH) at the optimized conditions described by Rafiee *et al.* (2012) [32]. Pre-treated RH with 1NHCl subjected to heat treatment in muffle furnace ((Macro Scientific Works Pvt. Ltd., MSW-251, India) at 700 °C for 2 h to obtain the ash. Ash and 2.5 M NaOH were stirred in 1:8 proportions. The solution was heated in a covered beaker and filtered through filter paper. The obtained viscous, transparent and colourless filtrate solution was allowed to cool at room temperature and 10 M H2SO4 was then added under constant stirring at controlled conditions until it reached to pH 2, thenNH4OH was used to adjust pH level up to 8.5 and was allowed to stand at room temperature for 3 h. White silica participate was washed repeatedly with the distilled water until the filtrate was completely free from alkali. SNPs were prepared by using refluxing of obtained silica with 6 MHCl for 4 h and washed repeatedly using distilled water to make it acid free. Then it was dissolved in 2.5 M NaOH by continuous stirring and H2SO4 was added until it reached to pH 8. The precipitated silica was washed repeatedly with warm distilled water to make it alkali free and then dried in the hot air oven at 50 °C for 48 h.

Characterization

Silica nanoparticles have been characterized by using Zeta sizer (Malvern, ZETA Sizer, nano 383 issue 5.0, England), Fourier transform infrared (Shimadzu, FTIR-8400S, Japan) spectra was obtained on a Vertex 70 spectrometer equipped with a digital detector *via* the conventional potassium bromide (KBr) pellet method, X-ray diffraction (Theta-theta type X-ray diffract to meter, Rigaku corporation, Japan) and scanning electron microscope (Carl Zeiss Microscopy, EVO 10, Germany).

Stock culture of *S. oryzae* and bioassay

S. oryzae adults were collected from the infested whole rice seed obtained from a central store of Main Agricultural Research Station, Raichur, Karnataka (India). Insects were rearedunder laboratory conditions of 30±1 °C, 75±5% relative humidity in continuous darkness (Zahir *et al.,* 2012). The newly emerged adults from this culture were used for the presentexperiment.550 g of rice grains were kept in hot air oven at 50 ºC for 2 h to sterilize and make the sample free from previous infestation. 20 g of infestation free rice grains were placed in eachjar with different concentrations (25, 50, 75, 100, 125, 150, 175 and 200 ppm). Then, the jars were shaken manually for approximately 1 min to achieve equal distribution of SNPs on rice grains. The jar was kept for 24 h before introduce of adults of rice weevil were into each jar.

All bioassays were performed at room condition with release of 5 pair of newly emerged adults of rice weevil. The adult mortality were checked on 1st, 2nd, 3th and 4th days. The without silica nanoparticles served as control and each treatment were replicated by thrice. Adult mortality of rice weevil was calculated using the following formula described by Devi *et al.* (2014).

$$\text{Adult mortality (\%)} = \frac{\text{No. of dead insects}}{\text{Total No. of insects}} \times 100$$

Initial and final weight of the test sample was recorded and seed weight loss was computed by the following formula as suggested by Harris and Limblad (1978).

$$\text{Weight loss (\%)} = \frac{W_0 - W_1}{W_1} \times 100$$

Where, W0: Initial weight of sample, g, W1: Final weight of sample, g

Culture of *Xanthomonasoryzae* and zone of inhibition

The pure culture of *Xanthomonasoryzae (Xoo)* was isolated from *Xoo* diseased rice leaves. Diseased leaves were cleaned with potable water and air dried. These leaves were cut into small pieces about 5 to 7 cm and sterilized with 1% sodium hypochloride solution, then washed in sterilized distilled water. These pieces were cut into smaller pieces about 5 x 5 mm in size and put into the test tube containing 1 ml of sterilized distilled water for about 5 to 10 min and allowed to the bacteria *Xoo* out from the leaf tissue. Using the sterilized loop needle with bacterial suspension streaked onto petri dishes containing nutrient agar (NA) medium (1g beef extract, 1 g peptone, 0.5 g NaCl dissolved in 100 ml of double distilled water). The plates were incubated in room temperature (28-30 °C) for 3 to 4 days (Dinh *et al.,* 2008). The emerging colonies were sub cultured onto NA plates to get pure culture. Cultures were preserved at 4 °C on NA slants for further application.

The antibacterial assays of SNPs against *Xoo* was performed by standard disc diffusion method. Nutrient agar was used to cultivate bacteria. After 30 min, the overnight culture of inoculums (100 µL) spread on to solidified nutrient agar plates. Sterile paper disc made of Whatman filter paper of 5 mm diameter (dipped in different concentration of silica nanoparticles as 25, 50, 75, 100, 125, 150, 175 and 200 ppm) were placed in each plate.

Control was used as distilled water without SNPs. The cultured agar plates were incubated at 37 ºC for 24 h. After 24 h of incubation the zone of inhibition was observed and recorded.

The diameter of the inhibition zone was recorded in mm. (Dar *et al.*, 2016).

Statistical analysis

The obtained data were analysed by using completely randomized design. The results were presented as means ± Standard Error (SE). Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) method were used to exam the means between treatments and then statistically significant differences between means by using SPSS software version 16.0 at $P \leq 0.01$.

Results

Zetasizer revealed an average particle diameter of biosynthesized silica nanoparticles was 93.14 nm (Fig. 1). FT-IR spectra of SNPs as shown Fig. 2 revealed that, the broad band between 2800 and 3750 cm^{-1} was due to silinol OH groups and adsorbed water and 2100 to 2300 m^{-1} was due to silane group Si-H. The predominant absorbance peak at 1320 cm^{-1} was due to siloxane bonds (Si-OSi), the peaks between 1200 and 700 cm^{-1} were attributed to vibration modes of the gel network. IR spectrum was clearly shown the pure silica. X-ray diffraction pattern showed broad halo band of absorbance at about 2θ = 15-25° region which confirms the amorphous nature (Fig. 3) and SEM image revealed that uniformly distributed biosynthesized silica nanoparticles were in the agglomerated with spherical morphology (Fig. 4).

Table 1 shows that, the adult mortality of *S. oryzae* significantly varied due to the different concentration of SNPs applications and time of exposure after treatment. The higher concentrations 200 and 175 ppm adult mortality were varied in range of 20 to 100 % and 10 to 100 % from 1st to 4th day of exposure respectively, as against 0 to 20 % in control. On 4th day highest mortality was found in treatment 125 and 175 ppm which was 80 % over the control. Application of SNPs showed minimum of weight loss (%) in rice grains ranged 0.63 to 0.00, respectively, as against in 0.07 % in control. In 200 ppm concentration no weight loss recorded and 175 ppm concentration was effective allowing only 0.03 % weight loss. The Pearson's correlation coefficients showed that negative linear relationship (r = -0.98) between per cent adult mortality and weight loss.

The results of antibacterial study of SNPs against bacteria leaf blight (*Xanthomonas oryzae*) revealed that, SNPs did not show any zone of inhibition in *vitro* condition.

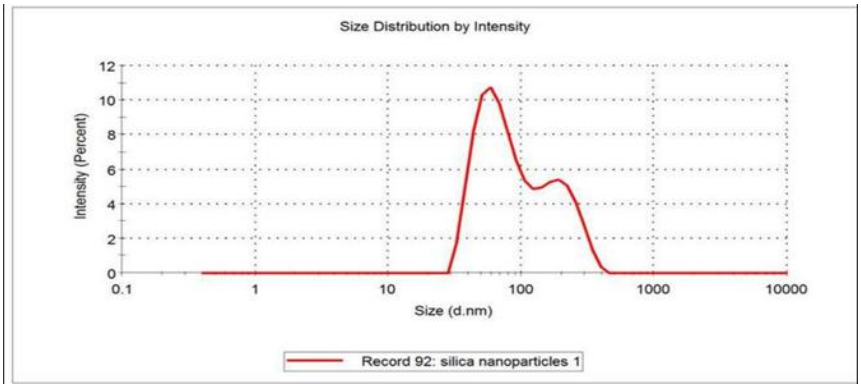

Fig. 1: Average particle diameter of SNPs

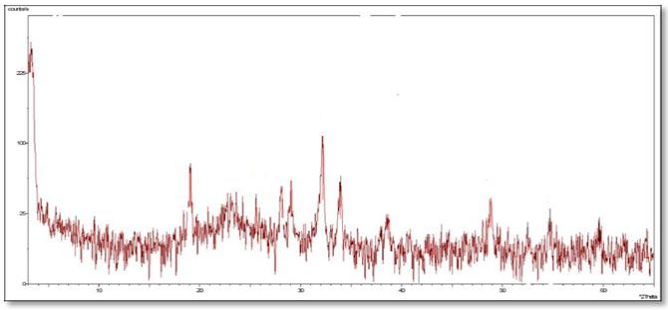

Fig. 2: XRD pattern of SNPs

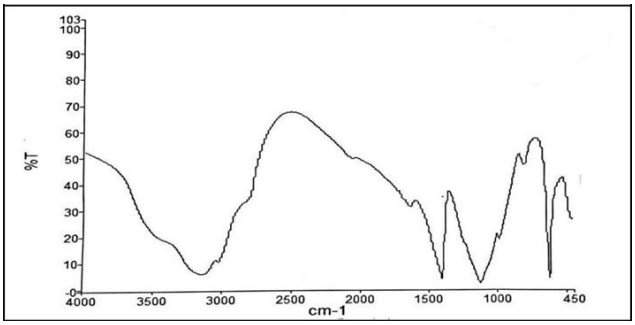

Fig. 5: FT-IR spectra of SNPs

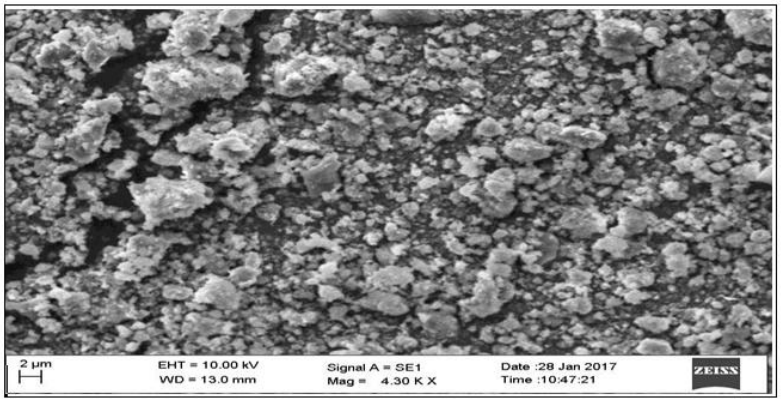

Fig. 4: SEM image of SNPs

Table 9: Efficacy of silica nanoparticles on rice weevil (*S. oryzae*)

Treatments (Concentrations of SNPs, ppm)	Adult Mortality (%)				Weight Loss (%)
	1 DAT	2 DAT	3 DAT	4 DAT	
T_0 (0)	0.00 (0.00)^{c}	0.00 (0.00)^{i}	10.00 (18.44)^{h}	20.00 (26.57)^{h}	0.70 (4.80)^{i}
T_1 (25)	0.00 (0.00)^{c}	6.67 (14.95)^{h}	20.00 (26.57)^{g}	26.33 (30.87)^{g}	0.63 (4.56)^{h}
T_2 (50)	0.00 (0.00)^{c}	10.00 (18.44)^{g}	21.67 (27.71)^{fg}	33.33 (35.26)^{f}	0.53 (4.18)^{g}
T_3 (75)	0.00 (0.00)^{c}	13.33 (21.41)^{f}	23.33 (28.86)^{f}	36.67 (37.27)^{e}	0.40 (3.63)^{f}
T_4 (100)	0.00 (0.00)^{c}	16.67 (24.09)^{e}	26.33 (30.87)^{e}	43.33 (41.17)^{d}	0.36 (3.44)^{e}
T_5 (125)	0.00 (0.00)^{c}	20.00 (26.57)^{d}	30.00 (33.21)^{d}	66.67 (54.74)^{c}	0.32 (3.24)^{d}
T_6 (150)	10.00 (18.44)b	23.33 (28.88)^{c}	43.33 (41.17)^{c}	75.00 (60.00)^{b}	0.23 (2.75)^{c}
T_7 (175)	10.00 (18.44)^{b}	30.00 (33.21)^{b}	63.67 (52.93)^{b}	100.00 (90.00)^{a}	0.03 (0.64)^{b}
T_8 (200)	20.00 (26.32)^{a}	40.00 (39.23)^{a}	66.67 (54.74)^{a}	100.00 (90.00)^{a}	0.00 (0.00)^{a}
SE (m) ±	0.24	0.21	0.56	0.29	0.05
C.D. @1%	1.00	0.86	2.31	1.21	0.88

Discussion

It was evident from this insecticidal assay; rice husk SNPs were much more effective because of enormously increased insect cuticle contact. According to Ebeling and Wagne (1959) [16] insecticidal efficacy dust can be becomes enhanced if the particles are finely divided. SNPs caused damage to external cuticle of *S. oryzae* body which forms barrier by sorption and abrasion. Also much smaller size, SNPs become impregnated into cuticle wall and digestive tract effectively which caused break its integrity and damage occurs to protective wax coat. (Smith, 1969; Rouhani *et al.,* 2012; Ziaee and Ganji, 2016) [38, 34, 43]. The greasy layer on body of insect pest plays important role in physical interactions between the organis (Voigt *et al.,* 2009) [40]. ms especially during mating, which helps to attach of males feet to the female' dorsal body This could have incomplete mating in case of lower concentrations and prevent matting at higher concentration of SNPs. It was cause the desiccation or blockage of spiracles also the surface enlargement of the integument as a consequence of dehydration (Rouhani *et al.,* 2012; Arumugam *et al.,* 2015) [34, 3].

Inert dusts have been showed to control number of common storage insect pests. They are most effective in low humidity conditions because of the induce mortality by causing desiccation; water is lost because the dusts remove the waxy layer of the cuticle of the exoskeleton by adsorption (Maceljski and Korunic, 1972; Le *et al.,* 1989) [28]. The present findings corroborate the results of Debnath (2011) [13] reported that 1 g Kg^{-1} of amorphous SNP was found 95% mortality after 7 days. Rouhani *et al. (*2012) [34] reported Insecticidal activity of SNPS was more than the silver NPs against adult of *C. maculates.* Rouhani *et al.* (2011) [33] showed themost mortality effect pertained to 28% ZnO-70% TiO2-2% Ag against *Frankliniellaoccidentalis* (Pergande). Sabbour (2012) found that alaminium oxide had the highest cumulative mortality (73.3%) followed by titanium dioxide reached (59.7%) after 7 days against *S. oryzae*. The variation in insecticidal activity might be due to different particle size, particle morphology and surface area of nanoparticles (Buteler *et al.,* 2015) [7].

According to Debnath *et al.* (2011) [13] reported the in *vitro* cellular toxicity in human fibroblast cell line and acute oral toxicity study in mice revealed that similar to the amorphous silica nanoparticles (25, 51, 128, 320, 800, 2000, and 5000 ppm) relatively non-toxic. So, SNPs con be used to control rice weevil in stored grains. Epstein (1994) [17] reported Silica is non-reactive, it will not contaminate ground water or pollute soil; either it will enhance structural strength and rigidity of plant. This might be one of possible reason silica has been used as protective agent for stored seed since from age old tradition by different ethnic races all over the world.

The SNPs did not show antibacterial activity in *vitro* condition are in good agreement to previous reported by Garcia-Saucedo *et al.* (2011), who reported dispersed HfO2, Al2O3 and SiO2 NPs were not toxic to O2 uptake of to the yeast, *Saccharomyces cerevisiae* even when NPs were supplied at concentrations as high as 1000 mg/L, concluded these NPs were no effective on this yeast. Karimiyan *et al.* (2015) [24] also concluded SNPs did not have antifungal effect against *Candida albicans* at in *vitro* condition. Silicon does not inhibit the growth of bacterial pathogens in *vitro*, Ferreria *et al.* (2015) [18] observed that silicon did not affect *A. citrulli* directly. Oliveira *et al.* (2012) [30] found that calcium silicate did not inhibit *Xanthomonascitri* subsp. *Malvacearum* growth in culture medium at any of the testedsilicon concentrations. However, the high pH 9.2, at the rate of 0.5 µL the silicon solution affected *Pseudomonas syringaepv* tomato growth in *vitro* (Andrade *et al.* 2013) [4]. The pH of rice husk SNPs was in the range of 6.9 to 7.1 and according to Cho (1975) [8] pH level of 7.0 to 7.5 is best supported to the growth of the *X. oryzae*pv. *Oryzae*, might be this reason SNPs it did not affect against *X. oryzae* in *vitro*, but in case of *vivo* condition the effect of silicon on plant resistance to bacterial pathogens is considered to be due to either a deposition of siliconon cell walls acting as a physical barrier making bacteria penetration difficult when soil amendment is applied, or biochemical changes related to plant defenses when a foliar sprayor soil amendment is applied (Sakr, 2016) [37]. Further study is needed to find out the detailed mechanisms of action of rice husk SNPs as nanocides. The removal of SNPs from the grains prior to further processing was also not attempted.

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Compliance with Ethical Standards

Conflict of interest: The authors declared that they have no conflict of interest.

Author Contribution

The corresponding author Nita Baba so Patil has carried out actual work and other reaming authors were guided the during this research work.

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