Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(5): 38-42
Received: 15-07-2019
Accepted: 18-08-2019

S Selva Rani
Anbil Dharmalingam Agricultural College and Research Institute, TNAU, Tiruchirappalli, Tamil Nadu, India

C Gailce Leo Justin
Anbil Dharmalingam Agricultural College and Research Institute, TNAU, Tiruchirappalli, Tamil Nadu, India

K Gunasekaran
Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

S Sheeba Joyce Roseleen
Anbil Dharmalingam Agricultural College and Research Institute, TNAU, Tiruchirappalli, Tamil Nadu, India

Correspondence
K Gunasekaran
Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Efficacy of green synthesized silver nanoparticle, plant powders and oil against rice weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae) on sorghum seeds

S Selva Rani, C Gailce Leo Justin, K Gunasekaran and S Sheeba Joyce Roseleen

Abstract

Synthesis of nanomaterials involves various routes including physical, chemical and biological approaches. The biological green route was chosen to prepare silver nanoparticles from silver nitrate using *Moringa oleifera* F. *(*Moringaceae) leaf extract and Botanicals also severely in the stored product pest management. The present study was focused on the insecticidal activity of synthesized silver nanoparticles and botanical powder against *Sitophilus oryzae*. L. The maximum mortality of *S. oryzae* was observed with sweet flag rhizome AgNPs (100%), sweet flag rhizome powder (100%), Pungam oil (98.66%) and check Malathion (98.0%) 15 days after treatment. The effect of plant powder and AgNPs on germination of seed was also observed by germination test.

Keywords: Plant powder, AgNPs, *Sitophilus oryzae*, Sorghum seeds

Introduction

Sorghum, *Sorghum bicolor* L. is called as 'Camel of crops' because of its hardiness and ability to withstand prolonged droughts. The crop plays a major role in the food security of millions of people in marginal agricultural areas. It is the fifth most important crop in the world after rice, wheat, corn and barley. It is produced in hot region with a minimum temperature of 25°C to ensure maximum grain production. It accounts for an area of 5.65 million ha with production of 4.41 million tonnes in India (Pattanayak, 2016) [18].

The rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) is a major pest of stored sorghum in India, and has been spread worldwide by commerce. Both, the adults and larvae feed on whole grains. They attack wheat, corn, oats, rye, barley, rice and dried beans. The weevil infestation is encountered in on-farm storage where it causes heavy loss in grain weight in addition to deterioration in quality (Giga *et al*., 1991). Suleiman *et al*., (2017) reported an annual 13.12 per cent weight loss of threshed sorghum and 8.34 per cent of unthreshed sorghum.

Seed is the most vital and important input for crop production. It is necessary to protect the seeds through locally available plant powder, oil and AgNPs, which are ecofriendly too. The plant kingdom is a rich store house of chemicals, which can prohibit the pest activity particularly in tropics and sub-tropics due to their insecticidal properties and some are with medicinal properties (Ileke and Oni, 2011) [11]. In bulk storage, the storage pests can be controlled using plant leaves either as plant powder or essential oil or applied as fumigants (Hashim *et al*., 2017) [9].

Materials and Methods

Maintenance of stock culture

The stock culture of the rice weevil was maintained by collecting adult weevils from the infested sorghum seeds from the Godown. The rice weevils were reared in plastic containers of 1kg capacity containing the sorghum seeds under laboratory conditions at 27^{0}C and70% RH in continuous darkness. The mouth of container was covered by kada cloth fastened with rubber band. Fresh sorghum seeds were provided periodically for development of test weevils and test insects were drawn from the stock culture for various investigations.

Synthesis of silver nanoparticles

The AgNPs were synthesized through biological method. The leaves of *M. oleifera* were washed thrice with tap water followed by distilled water and kept at room temperature for air drying. After air drying, 25g of chopped leaves was taken in a 250ml conical flask containing 100ml of distilled water and the contents was boiled at 100°C for 30 minutes and filtered through Whatman No. 1 filter paper. For the preparation of 1mM AgNO3 solution, 0.0421g of AgNO3 was added to 100ml of double distilled water. The solution was mixed thoroughly and stored in amber bottle in order to prevent auto-oxidation of Ag. For the synthesis of plant mediated AgNP, the leaf extract and 1mM AgNO3 solution were taken at 1:4 ratio and kept on a water bath at 60°C for 30 minutes until the colour changes from yellow to brown (Saini *et al*., 2013) [20]. The resultant brown coloured synthesized nanoparticles containing solution was centrifuged at 1200 rpm for 15 minutes, washed and decanted with deionized water for several times and dried at 60°C for 24 h to obtain the nanoparticles.

Preparation of Plant Powders

The leaf powders of tulasi (*Ocimum sanctum*), lakke (*Vitex negundo*), neem (*Azadirachta indica*), periwinkle (*Cathranthus roseus*), custard apple (*Annona squamosa*) prepared from leaf samples collected from nearby locality and the sweet flag rhizome (*Acorus calamus*), dry chillies (*Capsicum annum*) samples from Trichy local market was collected, washed with distilled water and shade dried at room temperature for seven days and crushed into fine powder using a pulveriser (0.05 mm mesh sieve). All the powders were kept in plastic containers at room temperature and properly sealed to prevent quality loss.

Bioassay on mortality of rice weevil treated silver nanoparticles, plant powders, oil and Malathion

The bioassay on *S. oryzae* was performed in small plastic container (7 x 5.5 cm) containing 100 g of sorghum seeds. Based on the preliminary laboratory experiments conducted, different doses were fixed for different plant materials. The sorghum seeds in each container were treated with the treatments (Table 1). Then, the containers were shaken manually for 1min to achieve uniform distribution of all materials on sorghum seeds. A separate control was maintained. The treatments were replicated thrice. The containers were kept undisturbed for 24 h before the release of 25 pairs of adult *S. oryzae*. All the bioassay studies were performed at 27 ± 3 °C, 70% RH. The insect mortality was recorded after 3, 5, 7, 9, 15 days of treatment. The corrected mortality was worked out using Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality percentage (\%)} = \frac{X - Y}{100 - Y} \times 100$$

X = Percentage mortality in the treatments
Y = Percentage mortality in the control

Effect of plant powders and AgNPs on the germination of sorghum seeds

Experiments on the germination of seed were carried out employing roll towel method, described by International Seed Testing Rules Association (ISTA) Manisha *et al*, (2015). Germination test was conducted at monthly interval for two months.

The paper was cut into a convenient size to hold one replicate of the seeds. The paper was labeled at one end with the accession number, replicate number and the testing date. A pencil or permanent marker was used for labeling. The paper was moistened with water. The seeds were arranged in rows at regular intervals about 4 cm from the top edge, leaving 3-4 cm gap on the sides. The seeds were covered with another sheet of moist paper towel. The paper was rolled loosely from opposite end to the label end. A paper clip or rubber band was used to hold the rolled papers to prevent unrolling. The rolls were kept upright in a deep-bottom plastic tray. Sufficient quantity of water was added to the tray. The trays were incubated at room temperature and the test was carried out for the recommended period (14 days). The towels were kept moist by spraying with water if necessary when temperature was high. The germinated seeds were counted by unrolling the paper carefully to avoid tearing or damaging the roots of young seedlings. The test was continued until all the seeds had germinated or until no further germination had occurred after two consecutive counts. The seeds that did not germinate were noted but they were firm and sound at the end of the first count and those that failed to germinate was presumed dead at the end of the germination test. Observations on the germination, seedling root length, shoot length and biomass were recorded.

Estimation of weight loss

The seed weight loss was estimated using count and weight method taking 100 sorghum seeds randomly from each replication from all treatments.

$$\text{Weight loss} = \frac{(Wu \times Nd) - (Wd \times Nu)}{Wu \times (Nd + Nu)} \times 100$$

Where

Wu = Weight of undamaged seeds
Nu = Number of undamaged seeds
Wd = Weight of damaged seeds
Nd = Number of damaged seeds

Statistical analysis

The data obtained from the experiments were statistically analyzed by one factor CRD with the help of computer based program AGRES software after arcsine transformation.

Results

Evaluation of AgNPs and plant powders on the mortality of rice weevil

The results clearly indicated that all the treatments revealed a wide variation in mortality compared to the control (Table 1). Among the eleven treatments tested the standard check malathion recorded the maximum mortality of 93.33 per cent on third Day After Treatment (DAT) followed by sweet flag rhizome powder (20.66%), periwinkle leaf powder (19.33%), pungam oil (17.33%) and AgNPs (15.33%) significantly superior than the control. This was followed by lakke leaf powder (5.33%), tulasi leaf powder (3.33%), custard apple leaf powder (3.33%), chillies dry fruit powder (0.00%), neem leaf powder (0.00 %) on 3 DAT. The maximum mortality was recorded in sweet flag rhizome powder (100%) and Malathion (96.66%) treatments followed by pungam oil (63.33%), AgNPs (60.66%) and periwinkle leaf powder (53.33%) which were equally effective. Similar variations were noticed in chillies dry fruit powder (2.0%), tulasi leaf powder (8.0%), lakke leaf powder (8.66%), Neem leaf powder (13.33%) and custard apple leaf powder (30.00%) during 5 DAT. Subsequently, on 7 DAT, significant maximum mortality was seen in sweet flag rhizome powder (100%), Malathion (98.00%), pungam oil (97.33%) and AgNPs (86.00%). The other treatments *viz.*, periwinkle leaf powder showed 65.33 per cent mortality followed by custard apple leaf powder

(45.33%), chillies dry fruit powder (5.33%), lakke leaf powder (10.00%), tulasi leaf powder (13.33%) and neem leaf powder (26.66%). Further, observations on 9 and 15 DAT showed AgNPs (100%), sweet flag rhizome powder(100%), pungam oil (98.66%) and malathion (98.0 %) equally effective followed by periwinkle leaf powder (73.33%) and custard apple leaf powder (58.66%).

Effect of AgNPs and Effect of AgNPs and botanical powders on the germination, seedling vigour, biomass and weight loss of sorghum seeds

Data analysis indicated a highly significant difference in terms of germination, seedling length, vigour index, biomass and seed weight loss in all the treatments when compared to control (Table2). Among the treatments evaluated, AgNPs (100 mg) showed a significant increase in the germination (88.00%) against standard treated check Malathion 5D (73.30%) as well as sweet flag rhizome powder (84.0%) and periwinkle leaf powder (77.33%). However, the others quality parameters *viz*., seedling length, vigour index, biomass and seed weight loss as influenced by AgNPs and sweet flag rhizome powder were on par with Malathion.

Table 1: Effect of AgNPs, plant powders and oil on the mortality of rice weevil

Treatments	Dosage /100g seed	Mortality (%)*				
		3 DAT	5 DAT	7 DAT	9 DAT	15 DAT
T1- AgNPs	100 mg	15.33 (23.05)^{b}	60.66 (51.15)^{b}	86.00 (68.02)^{b}	100 (90.00)^{a}	100 (90.00)^{a}
T2- Tulasi leaf powder	5.0 g	3.33 (10.51)^{c}	8.0 (16.42)^{d}	13.33 (21.41)^{ef}	16.00 (23.57)^{d}	28.66 (32.37)^{d}
T3-Lakke leaf powder	1.0 g	5.33 (13.35)^{c}	8.66 (17.12)^{d}	10.00 (18.43)^{ef}	14.00 (21.97)^{d}	21.33 (27.50)^{d}
T4 – Sweet flag rhizome powder	5.0 g	20.66 (27.03)^{b}	100 (90.0)^{a}	100 (90.00)^{a}	100 (90.00)^{a}	100 (90.00)^{a}
T5 - Chillies dry fruit powder	5.0 g	0.00 (0.62)^{d}	2.00 (8.13)^{e}	5.33 (13.35)^{fg}	17.33 (24.60)^{d}	29.33 (32.79$^{)d}$
T6 - Pungam oil	1.0 ml	17.33 (24.60)^{b}	63.33 (52.73)^{b}	97.33 (80.60)^{a}	98.66 (83.36)^{a}	98.66 (83.36)^{a}
T7 - Neem leaf powder	5.0 g	0.00 (0.62)^{d}	13.33 (21.91)^{d}	26.6 (31.09)^{de}	42.00 (47.29)^{c}	54.00 (47.29)^{c}
T8 – Periwinkle leaf powder	5.0 g	19.33 (26.08)^{b}	53.33 (46.91)^{b}	65.33 (53.92)^{c}	73.33 (62.96)^{b}	79.33 (62.96)^{b}
T9 - Custard apple leaf powder	5.0 g	3.33 (10.51)^{cd}	30.00 (33.21)^{c}	45.33 (42.32)^{cd}	58.66 (55.14)^{b}	67.33 (55.14)^{c}
T10 - Malathion 5% D	1.0 g	93.33 (75.03)^{a}	96.66 (79.48)^{a}	98.00 (81.86)^{ab}	98.00 (81.86)^{a}	98.00 (81.16)^{a}
T11 – Control	-	0.00 (0.62)^{d}	0.00 (0.62)^{f}	0.00 (0.62)^{g}	0.00 (0.62)^{e}	1.33 (6.63)^{e}
SED		3.59	3.38	6.14	4.78	5.76
CD(p=0.05)		7.46	7.01	12.74	9.91	11.96

DAT – Days After Treatment

Figures in parantheses are arcsine transformed values

Means in a column followed by the same letters are not significantly different (p = 0.05) by LSD

*Mean of the replication

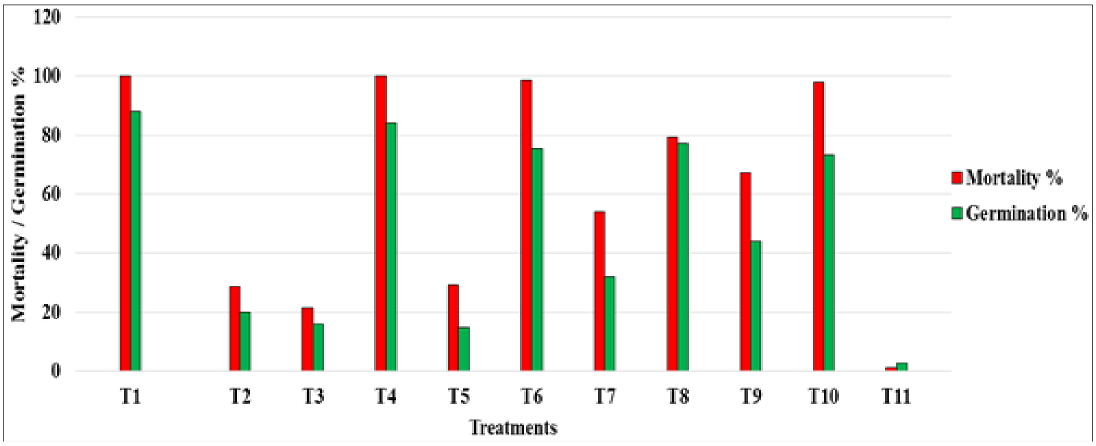
Table 2: Effect of AgNPs plant powders and oil on the biological parameters of sorghum seeds

Treatment	Dosage /100g seed	Weight loss* (%)	Germination* (%)	Seedling length* (cm)	Vigour* index	Biomass* (g)
T_1 - Silver nanoparticles	100mg	0.00 (0.62)^{a}	88.00 (69.73)^{a}	30.95^{a}	2723^{a}	2.42 (8.95)^{a}
T_2 - Tulasi powder	5g	6.97 (15.31)^{cd}	20.0 (26.56)^{ef}	22.85^{b}	457^{f}	0.91 (5.47)^{cd}
T_3 - Lakke powder	5g	9.62 (18.07)^{d}	16.0 (23.57)^{f}	21.33^{b}	341^{fg}	0.31 (3.21)^{ef}
T_4 - Sweet flag rhizome powder	1g	0.00 (0.62)^{a}	84.0 (66.42)^{ab}	32.02^{a}	2689^{a}	2.47 (9.04)^{a}
T_5 - Chilli dry fruit powder	5g	8.91 (17.37)^{d}	14.66 (22.51)^{f}	17.81^{c}	261^{g}	0.37 (3.48)^{de}
T_6 - Pungam oil	1ml	0.00 (0.62)^{ab}	75.53 (60.35)^{c}	28.32^{a}	2139^{c}	1.07 (5.94)^{c}
T_7 - Neem leaf powder	5g	4.34 (12.03)^{bc}	32.00 (34.44)^{de}	23.76^{b}	760^{e}	0.93 (5.54)^{c}
T_8 - Periwinkle leaf powder	5g	2.03 (8.19)^{b}	77.33 (61.56)^{abc}	30.21^{a}	2336^{b}	1.27 (6.47)^{bc}
T_9 - Custard apple leaf powder	5g	3.16 (10.24)^{b}	44.00 (41.55)^{d}	25.96^{ab}	1142^{d}	0.95 (5.59)^{c}
T_{10} - Malathion 5%D	5g	0.00 (0.62)^{a}	73.3 (58.88)^{bc}	30.04^{a}	2202^{c}	1.96 (8.05)^{ab}
T_{11} – Control	1g	21.73 (27.78)^{e}	2.66(9.38)^{g}	5.00^{c}	13^{h}	0.02 (0.93)^{f}
SED		2.22	5.24	2.37	60.51	0.90
CD(p = 0.05)		4.62	10.88	4.90	125.50	1.88

Figures in parantheses are arcsine transformed values

Means in a column followed by the same letters are not significantly different (p = 0.05) by LSD

*Mean of the replication



T1 – Silver nanoparticles 100 mg / 100g seeds
T2 –Tulasi leaf powder 5g/ 100 g seeds
T3 - Lakke leaf powder 5 g / 100 g seeds
T4 - Sweet flag rhizome powder 1 g/100 g seeds
T5 – Chillies dry fruit powder 5 g/100 g seeds
T6 – Pungam oil 1ml/100 g seeds
T7 – Neem leaf powder 5g/100 g seeds
T8 – Periwinkle leaf powder 5 g/ 100 g seeds
T9 – Custard apple leaf powder 5 g/ 100 g seeds
T10 - Malathion 5D 1g/ 100g seeds
T11 - Control

Fig 1: Influence of AgNPs, plant powders, non - edible oil and Malathion on rice weevil mortality and quality parameters

Discussion

Effect of AgNPs and plant powders on mortality and seed biological parameters of rice weevil on sorghum seeds

The plant extracts can be used efficiently in the synthesis of AgNPs as a green route. The elucidation of the mechanism of plant mediated synthesis of nanoparticles has been very promising area of research (Vijay Kumar *et al.*, 2014) [22]. The nanoparticles have been utilized in production of newer insecticide (Owalade *et al.*, 2008).

In the present study, bioassay was carried out for assessing the insecticidal activity of AgNPs, plant powders, non-edible oils on *S. oryzae*. AgNPs, sweet flag rhizome powder and pungam oil were equally effective and on par with the treated check malathion against rice weevil on 15 DAT. AgNPs exhibited complete mortality of rice weevil at 2000 mg kg-1 of sorghum seeds on 15 DAT. This was in accordance with earlier findings of Kaveri, (2014) who reported complete mortality of *S. oryzae* at 1500 mg kg-1 on 15 DAT in stored maize. Rouhani *et al.*, (2012) also reported 75 per cent mortality of *C. maculatus* F. at 2500 mg kg-1 of cowpea seeds on 14 DAT. However, complete mortality of *S. oryzae* was reported at lower dose of 250 and 100 mg kg^{-1} of rice on 14 DAT, respectively (Zahir *et al.*,2012, Sankar and Abideen, 2015). The sweet flag rhizome powder documented complete mortality on 15 DAT. Similarly, 97 per cent mortality has been reported with sweet flag rhizome powder treated against *S. oryzae* in sorghum (Gadewar *et al.*, 2017) [8]. Pungam oil was found to give 98.66 per cent of mortality of rice weevil in the present observation. Similarly reports efficacy of pungam oil has been reported with reduced adult emergence after six months of storage (Deb, 2016) [4].

The damage of the mouthparts was not the main reason behind insect mortality because mortality was observed at all doses of SNPs treatments within 24 h, though it was noticed that the weevils could remain alive for more than one day without any food (Debnath *et al.*, 2012) [6]. The attachment of AgNPs all over the body of rice weevil caused scratches and splits on the cuticle. This subsequently led to the loss of water through dehydration as the water barrier in the cuticle was damaged and die out of desiccation and silica nanoparticles caused damage to insect cuticle by adsorbing cuticular lipids (Ebeling, 1971) [7]. Similarly damage occurs to the insects' protective waxy coat on the cuticle, both by sorption and abrasion (Debnath *et al*., 2011) [5].

The suffering of adult insects due to desiccation and spiracle blockage by the exposure of silica nanoparticles could be the cause for reduced fecundity (Arumugam *et al.,* 2016) [2].

The AgNPs gets attached all over the body of adult weevils which would have prevented subsequent mating. (Arumugam *et al.*, 2016) [2] substantiated the fact that insects are supposed to release a greasy substance on their body surface, which may be involved in physical interactions between the organisms especially during mating. In mating, males frequently attach to the female's dorsal body by means of their base, where lubricants play an important role for the attachment of base (Voigt, 2009) [23], Debnath *et al*. (2011) [5] reported the surface enlargement of the integument as a consequence of dehydration or blockage of spiracles and trachea. Thus it was clear that the mortality encountered in the present study may be attributable to the abrasion of the cuticles, depletion of the waxy layer and blockage of spiracles.

In the present study, the efficacy of sweet flag rhizome powder (1.0 g) and pungam oil (1.0 ml) in causing maximum mortality could have resulted due to destruction of physical barriers and spiracle blockage. Mulungu *et al.* (2007) [15] have reported the toxicity properties of these plant products due to tendency of blocking the spiracle of the insects and thus impairing respiration leading to death of insects. Chilli dry fruit powder that proved less effective. Similar results finding *C. annuum* fruit powder generally performed less than *A. sativum* oil against *C. maculatus* infestation (Mailafiya *et al.,* 2014) [14]. Oni. (2009) [16] have reported The generally lower performance of *C. annuum* fruit powder against *Sitophilus zeamais* and *callasobruchus maculatus.* Further, they reiterated that *S. oryzae* feeding on whole grain picks up the lethal dosage of the plant products thus resulting in stomach poisoning.

The AgNPs (100 mg), sweet flag rhizome powder (1.0 g) and pungam oil (1.0 ml) treated 100 g of sorghum seeds did not produce any weight loss and had good seed quality parameters on par with standard check Malathion 5D after 60 days of storage. Similarly, Gadewar *et al.* (2017) [8] reported that the sweet flag rhizome powder (2.5%) treated seeds produced good seed quality parameters. Further, Sunilkumar *et al.*(2005) could obtain sweet flag rhizome powder (1%) with maximum protection to the seeds up to 60 DAS. Hampanna *et al.* (2006) [10] reported that sweet flag rhizome powder (2%) gave complete protection without any damage to chickpea. The seed weight, vigour index, germination percentage decreased with increasing infestation of stored grain pest (Ashish *et al.*, 2011) The present observations clearly illustrated that the sweet flag rhizome powder (1.0 g) and pungam oil (1.0 ml) apart from affording maximum mortality provide some promising effect on the seed quality attributes of sorghum seeds.

References

1. Abbott WS. A method of computing the effectivness of an insecticide. Journal of Economic Entomology. 1925; 18:265-267.
2. Arumugam G, Velayutham V, Shanmugavel S, Sundaram J. Efficacy of nanostructured silica as a stored pulse protector against the infestation of bruchid beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae). Applied Nanoscience. 2016; 6(3):445-450.
3. Ashish Lambat, Rajesh Gradewar SC, & PL. Evaluation of organic grain protectants in seed storage against rice weevil in wheat. Proc. International Conference on Sustainable, 2011.
4. Deb S. Efficacy of vegetable oils as grain protectants against *Sitophilus oryzae* L. on maize under storage condition, Journal of Insect Science. 2016; 6:43-54.
5. Debnath N, Das S, Seth D, Chandra R, Bhattacharya SC, Goswami A. Entomotoxic effect of silica nanoparticles against *Sitophilus oryzae* (L.). Journal of Pest Science. 2011; 84(1):99–105.
6. Debnath N, Mitra S, Das S, Goswami A. Synthesis of surface functionalized silica nanoparticles & their use as entomotoxic nanocides. Powder Technology. 2012; 221:252–256.
7. Ebeling W. Sorptive dusts for pest control. Annual Review of Entomology. 1971; 16:123–158.
8. Gadewar R, Babhulkar V, Lambat P. The influence of some botanicals against rice weevil during storage in rabi sorghum, International Journal of Research Biological Agriculture and Technlogy. 2017; 5(1):28-30.
9. Hashim NA, Samsuddin NN, Saad K, Thanh V, Anh T. Effects of several plant leaves on rice weevil, *Sitophilus Oryzae* (Coleoptera: Curculionidae) productivity and stored rice qualities. Asian Journal of Agriculture and Food Sciences. 2017a; 5(3):2321–1571.
10. Hampanna YL, Naganagoud A, Patil BV. Evaluation of animal origin inert materials against rice weevil and pulse beetle in stored sorghum and chickpea. Karnataka Journal of Agricultural Science. 2006; 19(1):54-57.
11. Ileke KD, Oni MO.Toxicity of some plant powders to maize weevil, *Sitophilus zeamais* (Motschulsky) [Coleoptera: Curculiondae] on stored wheat grains (T*riticum aestivum*). African Journal of Agricultural Research. 2011; 6(13):3043-3048.
12. Kaveri. Effect of nanomaterials in the management of *Sitophilus oryzae L. i*n stored maize. *M.Sc Thesis Tamil nadu Agricultural University*, Coiambature, 2014, 98-100.
13. Manisha Meena. Biology,varietal screening and management of *Rhyzopertha dominica* (Fabricius) on stored sorghum. *M.Sc Thesis*, *Navsari Agricultural University*, Navsari, 2013, 100-121.
14. Mailafiya MD, Degri MM, Maina YT, Sharah HA. Bioefficacy of *Allium sativum* (L.) Oil and *Capsicum annum* Miller (Chili Pepper) fruit powder against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) infestation in stored cowpea grains. World Journal of Agricultural Science. 2014; 10(1):18-24.
15. Mulungu LS, Lupbenza G, SOWMR Uben, RNM. Evaluation of botanical products as stored grain protectant against maize weevil, *Sitophilus oryzae* (L.) on maize. Journal of Entomology. 2007; 4((3)):258–263.
16. Oni MO. Insecticidal activity of extracts from fruits of three local cultivars of pepper (*Capsicum* species) on cowpea seed beetle, (*Callosobruchus maculatus* [Fabricius]) and maize weevil (*Sitophilus zeamais* (Motschulsky)). Ph.D. *Thesis*, The Federal University of Technology, Akure, 2009, p105.
17. Owolade OF, Ogunleti DO, Adenekan MO. Titanium dioxide affects disease development and yield of edible cowpea. EJEAF Chemistry. 2008; **7**(50):2942–2947.
18. Pattanayak. Area, Production and Yield of Principal Crops. Agricultural statistic at a glance, 2016, 73-216.
19. Rouhani M, Samih M, Kalantari S. Insecticidal effect of silica and silver nanoparticles on the cowpea seed beetle, *Callosobruchus maculatus* F.(Col.: Bruchidae). Journal of Entomological Research. 2012; 4(4):297–305.
20. Saini J, Kashyap D, Batra B, Kumar S, Malik DK. Green synthesis of silver nanoparticles by using neem (*Azadirachta Indica*) and amla (*Phyllanthus Emblica*) leaf extract. Indian Journal of Applied Science. 2013; 3(5):209-210.
21. Sankar MV, Abideen S. Pesticidal effect of Green synthesized silver & lead nanoparticles using *Avicennia marina* against grain storage pest *Sitophilus oryzae*, International Journal of Nanomaterial & Biostructure. 2015; 5(3):32–39.
22. Vijay Kumar PPN, Pammi SVN, Kollu P, Satyanarayana KVV, Shameem U. Greensynthesis and characterization of silver nanoparticles using *Boerhaavia diffusa* plant extract and their anti bacterial activity. Industrial Crops and Products. 2014; 52:562-566.
23. Voigt D, Peisker HGS. Visualization of epicuticular grease on the covering wings in the Colorado potato beetle: a scanning probe approach. In: Bhushan B, Fuchs H, (eds). In Applied scanning probe methods XIII. Nano Science and Technology, Heidelberg, 2009.
24. Zahir AA, Bagavan A, Kamaraj C, Elango G, Rahuman AA. Efficacy of plant-mediated synthesized silver nanoparticles against *Sitophilus oryzae*, Journal of Biopesticide. 2012; 5:95-102.