Efficacy of green synthesized silver nanoparticle, plant powders and oil against rice weevil

*Sitophilus oryzae* L. (*Coleoptera: Curculionidae*)
on sorghum seeds

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**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). 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). 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).

**Materials and Methods**

**Maintenance of stock culture**

The stock culture of the rice weevil was maintained by collecting adult weevils from the Godown. The rice weevils were reared in plastic containers of 1kg capacity containing the sorghum seeds under laboratory conditions at 27°C and 70% 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 (Catharanthus 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 pulversiser (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 SD (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 biological parameters of sorghum seeds

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dosage /100g seed</th>
<th>3 DAT</th>
<th>5 DAT</th>
<th>7 DAT</th>
<th>9 DAT</th>
<th>15 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1- AgNPs</td>
<td>100 mg</td>
<td>15.33</td>
<td>60.66</td>
<td>86.00</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>T2- Tulasi leaf powder</td>
<td>5 g</td>
<td>3.33</td>
<td>8.00</td>
<td>13.33</td>
<td>16.00</td>
<td>28.66</td>
</tr>
<tr>
<td>T3- Lakke leaf powder</td>
<td>1.0 g</td>
<td>5.33</td>
<td>8.66</td>
<td>10.00</td>
<td>14.00</td>
<td>21.33</td>
</tr>
<tr>
<td>T4- – Sweet flag rhizome powder</td>
<td>5 g</td>
<td>20.66</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>T5- Chilies dry fruit powder</td>
<td>5 g</td>
<td>0.00</td>
<td>2.00</td>
<td>5.33</td>
<td>17.33</td>
<td>29.33</td>
</tr>
<tr>
<td>T6 - Pungam oil</td>
<td>1.0 ml</td>
<td>17.33</td>
<td>63.33</td>
<td>99.66</td>
<td>98.66</td>
<td>98.66</td>
</tr>
<tr>
<td>T7 - Neem leaf powder</td>
<td>5 g</td>
<td>0.00</td>
<td>13.33</td>
<td>42.00</td>
<td>54.00</td>
<td></td>
</tr>
<tr>
<td>T8 – Periwinkle leaf powder</td>
<td>5 g</td>
<td>19.33</td>
<td>65.33</td>
<td>73.33</td>
<td>79.33</td>
<td></td>
</tr>
<tr>
<td>T9 - Custard apple leaf powder</td>
<td>5 g</td>
<td>3.33</td>
<td>50.00</td>
<td>58.66</td>
<td>67.33</td>
<td></td>
</tr>
<tr>
<td>T10 - Malathion 5% D</td>
<td>1.0 g</td>
<td>93.33</td>
<td>99.66</td>
<td>99.80</td>
<td>99.80</td>
<td></td>
</tr>
<tr>
<td>SED</td>
<td></td>
<td>3.59</td>
<td>3.38</td>
<td>6.14</td>
<td>4.78</td>
<td></td>
</tr>
<tr>
<td>CD (p=0.05)</td>
<td></td>
<td>7.46</td>
<td>7.01</td>
<td>12.74</td>
<td>9.91</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dosage /100g seed</th>
<th>Weight loss* (%)</th>
<th>Germination* (%)</th>
<th>Seeding length* (cm)</th>
<th>Vigour* index</th>
<th>Biomass* (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 - Silver nanoparticles</td>
<td>100mg</td>
<td>0.00 (0.62)</td>
<td>88.00 (69.73)</td>
<td>30.95</td>
<td>2723</td>
<td>2.42 (8.95)</td>
</tr>
<tr>
<td>T2 - Tulasi powder</td>
<td>5 g</td>
<td>6.97 (15.31)</td>
<td>20.00 (26.56)</td>
<td>22.85</td>
<td>457</td>
<td>0.91 (5.47)</td>
</tr>
<tr>
<td>T3- Lakke powder</td>
<td>5 g</td>
<td>9.62 (18.07)</td>
<td>16.00 (23.57)</td>
<td>21.33</td>
<td>341</td>
<td>0.31 (3.21)</td>
</tr>
<tr>
<td>T4 - Sweet flag rhizome powder</td>
<td>1 g</td>
<td>0.00 (0.62)</td>
<td>84.00 (66.42)</td>
<td>32.02</td>
<td>2689</td>
<td>2.47 (9.04)</td>
</tr>
<tr>
<td>T5 - Chili dry fruit powder</td>
<td>5 g</td>
<td>8.91 (17.37)</td>
<td>14.66 (22.51)</td>
<td>17.81</td>
<td>261</td>
<td>0.37 (3.48)</td>
</tr>
<tr>
<td>T6- Pungam oil</td>
<td>1ml</td>
<td>0.00 (0.62)</td>
<td>75.53 (60.35)</td>
<td>28.32</td>
<td>2139</td>
<td>1.07 (5.94)</td>
</tr>
<tr>
<td>T7 - Neem leaf powder</td>
<td>5 g</td>
<td>4.34 (12.03)</td>
<td>32.00 (34.44)</td>
<td>23.76</td>
<td>760</td>
<td>0.93 (5.54)</td>
</tr>
<tr>
<td>T8 - Periwinkle leaf powder</td>
<td>5 g</td>
<td>2.03 (8.19)</td>
<td>77.33 (61.56)</td>
<td>30.21</td>
<td>2336</td>
<td>1.27 (6.47)</td>
</tr>
<tr>
<td>T9 - Custard apple leaf powder</td>
<td>5 g</td>
<td>3.16 (10.24)</td>
<td>44.00 (41.55)</td>
<td>25.96</td>
<td>1142</td>
<td>0.95 (5.59)</td>
</tr>
<tr>
<td>T10 - Malathion 5% D</td>
<td>5 g</td>
<td>0.00 (0.62)</td>
<td>73.33 (58.88)</td>
<td>30.04</td>
<td>2202</td>
<td>1.96 (8.05)</td>
</tr>
<tr>
<td>T11 – Control</td>
<td>1g</td>
<td>21.73 (27.78)</td>
<td>2.69 (9.38)</td>
<td>5.00</td>
<td>13</td>
<td>0.02 (0.93)</td>
</tr>
<tr>
<td>SED</td>
<td></td>
<td>2.22</td>
<td>5.24</td>
<td>2.37</td>
<td>60.51</td>
<td>0.90</td>
</tr>
<tr>
<td>CD (p=0.05)</td>
<td></td>
<td>4.62</td>
<td>10.88</td>
<td>4.90</td>
<td>125.50</td>
<td>1.88</td>
</tr>
</tbody>
</table>

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
through dehydration as the water barrier in the cuticle was damaged and die out of dessication 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 (Arunugam et al., 2016) [2]. The AgNPs gets attached all over the body of adult weevils which would have prevented subsequent mating. (Arunugam 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 frequency 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) [22]. 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 of rice weevil could be the cause for reduced fecundity (Debnath et al., 2011) [5]. 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 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 of rice weevil could be the cause for reduced fecundity (Debnath et al., 2011) [5]. 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
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.

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