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Efficacy of pulverised leaves of *Annona squamosa* (L.), *Moringa oleifera* (Lam.) and *Eucalyptus globulus* (Labill.) against the stored grain pest, *Tribolium castaneum* (Herbst.)

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Abstract

The pulverized leaves *of Annona squamosa* (L.)*, Moringa oleifera* (Lam.) and *Eucalyptus globulus* (Labill.) were tested for their insecticidal and seed protective effect against the stored grain pest of wheat, *Tribolium castaneum* (Herbst)*.* Different concentrations ranging from 0.05 to 2.0 g (0.05, 0.1, 0.15, 1.0 and 2.0 g) per 10.0 g wheat grains were tested against larvae and adults. When larvae were introduced to pulverized leaves of *A. squamosa, M. oleifera* and *E. globulus* separately, mortality rate increased with increase in concentrations and resulted in 100% mortality within a short period of eight, nine and ten days respectively with the highest concentration. The larvae which were introduced to these cultures did not grow well or molt to the next developmental stage. They have also shown very effective seed protection as the extent of damage produced in treated cultures were significantly reduced in comparison to control cultures. The seed protective effect ranged from 39% to 82% in *A. squamosa*, 34% to78% in *M. oleifera* and 42% to 88% in *E. globulus*. Considering the insecticidal and seed protective effect, these three plant powders could be employed as alternatives for chemical and synthetic pesticides at small scale level by farmers.

Keywords: *Annona squamosa; Moringa oleifera; Eucalyptus globulus; Tribolium castaneum;* Mortality.

INTRODUCTION

Insect pests are a major concern in fields and warehouses. It is estimated that about 35% of crops all over the world are destroyed by them [35]. They cause severe damage to stored grains and processed products by reducing their quantity and nutritional quality making them unfit for human consumption and agricultural purposes [13]. Estimated loss of the world's supply of stored grains from insect damage ranges from 5-10% of world production. The tropical countries alone suffer a loss of 20% due to favorable climatic and storage conditions [32]. Due to great economic losses caused by stored grain pests, control of infestation in warehouses, factories, ships and mills is of main interest to the food manufacturers and distributors [13].

Around the world, residual chemical insecticides are mainly the method of choice for the control of stored grain insects[38]. But extensive use has made the strains resistant to many of them [37&39]. In addition they result in environmental contamination and health problems [17,27&33]. In overcoming these problems, biopesticides have gained immense importance in recent grain protection technology because of their medicinal, antifungal, antibacterial and insecticidal properties. They are widely employed by small scale farmers to protect their crops and grains from infestations [16,17,22,23&25]. Many plants have now been reported for their pesticidal properties of which the most promising one is the neem, *Azadirachta indica* (A. juss.) [31]. Others include *Cassia fistula* (L.)*, Lantana camara* (L.)*, Chrysanthemum coronarium* (L.)*, Calotropis procera* (Ait.) Punica granatum (L.) and Murraya koinigii (L.) with antifeedant, repellant and growth regulating effects [14, 15, 28,29 &31]. They are traditionally and widely used against stored grain pests due to their easy accessibility and biodegradable nature [9].

In the present study pulverized leaves of *Annona squamosa*(L.), *Moringa oleifera* (Lam.)), and *Eucalyptus globulus* (Labill.) were assessed for their toxicity and seed protective effect against a serious pest in milled produce, *Tribolium castaneum* (Herbst.). *T. castaneum* along with *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae) and *Rhyzopertha dominica* (Fabr.) (Coleoptera: Bostrichidae) cause severe economic loss to farmers and consumers in India [13]. *A. squamosa, M. oleifera* and *E. globulus* have shown many medicinal properties. Insecticidal properties of *A. squamosa* against many insect pests including stored grains is reported [3, 6 & 24]. The seed extracts have both toxic and antifeedant properties [26]. Essential oil and ground bark of *M. oleifera* are shown to be effective against *T. castaneum* and *Scirpophaga incertulus* [2 & 8]. Essential oils obtained from *E. globulus* have medicinal effects in addition to insecticidal activity [19].

Although the botanicals are used in many ways against pests [30 &34], in the present study, they were used in pulverized form as these powders contain complex chemicals which may show overall bioactivity when compared to isolated plant constituents by extraction or other methods [4 & 5]. Moreover, insect resistance is less likely to develop with crude forms like powders and mixtures [30]. If plant materials are readily available, it would be easy for farmers to

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prepare the pulverized form as this is the simplest and cheapest method for preparing botanicals [5].

MATERIALS AND METHODS

Samples of wheat grains

Local variety of wheat grains, *Triticum aestivum* (L.) cultivated in this region, were collected from market, washed thoroughly with water to remove any dust or other residual particles and dried properly before use.

Preparation of leaf powder

Fresh leaves of *A. squamosa, M. oleifera* and *E. globulus* were collected from author's garden where there was no history of pesticide application. They were washed, shade- dried and ground to fine powder in a mixer and kept in air tight containers. Different concentrations of these powders ranging from 0.05 to 0.15 g (0.05,0.1, 0.15,1.0 and 2.0 g) per 10 g wheat grains were selected for assessing their insecticidal and seed protective effects after conducting preliminary experiments.

Selected insect pest

A stock culture of *T. castaneum* maintained on wheat at 28°C ± 1°C, 60% ± 5% r.h and 14L:10 D photoperiod for many years in the laboratory [21] were used. First instars and adults were separated from the stock cultures and used for each experiment.

EXPERIMENTAL DESIGN

Mortality rate

Three sets of cultures were set up simultaneously with five concentrations of each plant leaf powder (0.05, 0.1, 0.15, 1.0 and 2.0 g) and a control in small plastic vials (250ml vol.) containing 10 g fresh and undamaged wheat grains (about 200 seeds) + 2 g wheat powder along with few yeast granules in each. Freshly emerged first instars were used in this experiment. Twenty larvae per vial were separated from stock cultures and transferred to pretreated vial (just prior to experiment). Control cultures without any leaf powder treatment were maintained under same experimental conditions. All the vials were covered with wire meshed lids. They were checked at regular intervals of 24 h to see the toxic effect of these plant powders. Dead larvae were identified by their brownish black colour and were removed from the cultures after noting their number. This was continued till all the twenty larvae were dead and noted the time needed for 100% mortality in each set. Experiments were repeated in three replicates and the data was subjected to statistical analysis.

Seed protective effect

Experimental set up was similar to that of previous one only with an exception of adults being used instead of larvae. Twenty adults were separated from stock cultures and transferred to each experimental and control vial and maintained for a period of fifty days so as to assess their seed protective effect. The cultures were checked at regular intervals for egg laying, larval emergence and their growth. The details were noted down and after a period of fifty days the damaged seeds were counted and compared with those in controls to evaluate their seed protective effect. Percentage seed damage and percentage seed protection over controls was calculated. Experiments were repeated in triplicates and the data was subjected to statistical analysis.

Statistical analysis

The percentage mortality and the seed protection over controls and the mean ± SE for each triplicate was calculated [1&18]. The data was then subjected to one way ANOVA test using Bonferroni's multiple comparison tests for the post hoc separation using the software PRISM 3.0 and the graphs were produced accordingly.

RESULTS

The effect of pulverized leaves of *A. squamosa, M. oleifera* and *E. globulus* are depicted in Figure.1 When larvae were introduced to different concentrations of *A. squamosa,* 100 % mortality was obtained at day eight after exposure to the highest concentration (2.0 g), while it was eighteen days with the lowest concentration (0.05 g). The time taken for 100% mortality for the remaining concentrations ranged from 10-15 d. In case of *M . oleifera,* the minimum days required for 100% mortality with 2.0 g concentration was 11, while with 0.05 g it was 18 d. For the remaining concentrations it ranged from 11-13 d. Similarly with *E. globulus* the highest concentration produced 100% mortality at day nine and the lowest concentration at day twenty. For the rest of the concentrations it ranged from 13- 16 d. It was evident that with all the three leaf powders the rate of mortality increased with increase in concentrations and the time required for 100% mortality decreased correspondingly (Figure 1) ($p < 0.0001$). Out of the three leaf powders tested, *A. squamosa* was found to be the most effective and produced 100% mortality within a short time with more than 50% mortality exhibited within first two days (data not shown).

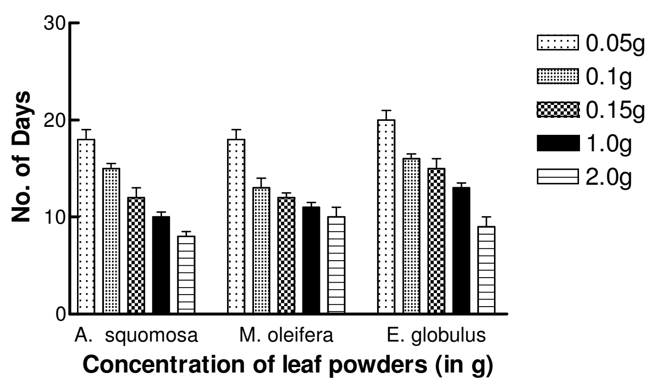

Fig 1.Time required for 100% Mortality for the larvae of *T. castaneum* with different concentrations (in g) of *Annona squamosa, Moringa oleifera* and *Eucalyptus globulus* leaf powders in 10.0 g of fresh wheat grains. ($p < 0.0001$)

Effect on Development and Seed protection

Seed protective effect of pulverized leaves was evaluated after a period of 50 d of exposure of the adult *T. castaneum* to plant materials. Since the control cultures have taken a period of about 35 ± 2 d to complete one life cycle, a little longer duration was given for evaluating the seed protective effect. As the insects multiply and grow in the medium, they feed on the grains which produce damage resulting in powdering and holes in the grains. The cultures were checked at weekly intervals. The cultures treated with pulverized leaves showed a reduction in egg laying and larval emergence which was relatively very less in the cultures treated with higher concentrations (1.0 and 2.0 g). Since toxicity of powdered leaves has been already established with larvae, this set of experiment was evaluated for the seed protective effect of the plant components. However, after third week of exposure there was no larval growth observed in the treated cultures except in controls. Further development in the treated cultures is prevented by the exogenous plant materials in the medium.

After a period of 50 d the damaged seeds were separated from treated and control cultures and their number recorded. Percentage seed damage and then the percentage seed protection over controls was calculated using Abbott's formula [1]. Mean values of percentage seed protection over controls from all the three replicates was subjected to statistical analysis and the data has been expressed in Figure 2. Promising amount of seed protection over controls was exhibited by treated cultures as there was significant decrease in the number of damaged seeds with different concentrations of leaf powders. This can be correlated with the mortality studies where the plant components resulted in mortality of the exposed larvae. As the mortality increased, the damage produced to wheat grains is considerably reduced. The seed protective effect varied with different concentrations. The higher concentrations showed high rate of seed protection over controls. In case of *A. squamosa,* the protective effect ranged from 39%- 82% while in *M. oleifera* it ranged from 34% -78%. *E. globulus* showed 42%- 88% seed protection over controls (Figure 2) ($p < 0.0001$). All the three plants tested were proved to be highly seed protective while *E. globulus,* due to its aroma, acted as more effective food deterrent and protected the seeds from insect infestations compared to the other two plants.

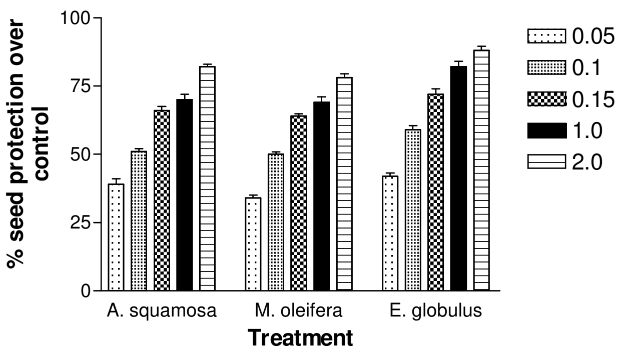

Fig 2. Percentage Seed protection over control after exposure to *T. castaneum* treated with different concentrations (in g) of *Annona squamosa* ,*Moringa oleifera* and *Eucalyptus globulus* leaf powders in 10.0g fresh wheat grains ($p<0.0001$)

DISCUSSION

The present study shows that the pulverized leaves of the three plants tested were effective in producing mortality of *T. castaneum*. The time required for 100% mortality was decreased with increasing concentrations of leaf powder suggesting that the normal process of growth and development was inhibited by the plant components. The adults transferred to grains treated with higher concentrations produced very less number of eggs or larvae in the cultures during the 50 d period suggest their ovicidal and larvicidal properties. Even further developmental stages were not observed in any of the treated cultures, suggesting their interference with the molting process. Many plants like *A. squamosa, L. camara, C. inermis, C. fistula, A. indica* and *C. procera* are proved to be lethal for various stored grain pests and delay the developmental stages by interfering with their apolytic and molting process [7,9 &10]. The ovicidal, larvicidal, and molt inhibiting properties of *L. camara* extract on *Corcyra cephalonica* (Staint.) and *R. dominica* have already been reported [26, 28 & 36]. The molt inhibiting properties of the active ingredients, especially alkaloids, were due to their adverse effect on the apolytic process.

Larvicidal property of *A. squamosa, M. oleifera* and *E. globulus* was also evident from the mortality studies, where the mortality of insects increased with increase in concentrations while the time taken for 100% mortality was decreased. Still higher concentrations could further boost the rate of mortality within a short time span. This is in conformity with the results reported with crude seed extracts and powdered leaves of *A. squamosa* and powdered leaves of *L. camara, P. granatum, M. koenigii, C. fistula* and *C. procera* against diamond moth, *Plutella xylostella* (L.), *T. castaneum, R. dominica* and *Sitophilus oryzae* [14, 15, 24 & 28]. Extracts from *A. squamosa* kernel have shown pesticidal properties for a range of insect pests like *Chilo pertellus* (Swinhoe), *Nilaparvata lugens* (Stal.), *Spodoptera litura* (Fabr.) and *D. koenigii* (F.) [3,6&20]. It was also effective against stored grain pests like *Callosobruchus chinensis*, *R. dominica*, *S. oryzae, T. castaneun* and *C. cephalonica* (Staint.) [24].

High rate of seed protection was exhibited by the tested leaf powders over controls. The amount of seed protection was high with higher concentrations. Periodic observation of cultures showed a drastic decline in the production of eggs and larvae in the treated cultures. This automatically reduced the food consumption by the insects in culture and thus the damage to seeds. These findings corroborate with the results of other workers who have reported the effectiveness of various plant leaves against insect pests of stored grains including *T. castaneum.* [14, 15, 24 & 30]. The powdered leaves of *Ageratum conyzoides* (coatweed) brought about significant grain protection against *Sitotroga cerealella, Sitotroga oryzae* and *R. dominica* under controlled and natural conditions of infestation in stored rice [31]. The toxicity, antifeedant activity and seed protective effect of crude seed extracts of *A. squamosa* against lepidopteran pests was also reported [26]. The pulverized leaves of *Eucalyptus sp., Bougaivillea glabra, A. indica, Saraca indica* and *Ricinus communis* were effective against *T. castaneum* [28] .They were also found to be seed protective as the damage to seeds was reduced significantly. Similar results with pulverized plant parts of *Telferia occidentalis, Zingiber officianale, Vitex grandifolia* and *Dracaena arborea* (Wild) against *T. castaneum* were reported and such post harvest treatments are recommended for stored groundnuts (*Arachis hypogaea* L.) [11 &19].

From the present study, the effectiveness of *A. squamosa*, *M. oleifera* and *E. globulus* to control post harvest food grain losses during storage is highly recommendable. This is of practical importance to the farmers who could improve their traditional methods of seed protection with the use of pulverized leaves as they are easily available and potentially less expensive.

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