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Efficacy of Pulverized *Punica granatum* (Lythraceae) and *Murraya koenigii* (Rutaceae) Leaves against Stored Grain Pest *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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ABSTRACT

The pulverized leaves of *Punica granatum* (Pomegranate) and *Murraya koenigii* (Curry trees) were tested for their efficacy against the stored grain pest *Tribolium castaneum* (Herbst) under controlled laboratory conditions. Five different concentrations of leaf powders ranging from 0.05 to 1.0 g per 10.0 g wheat grains (0.05, 0.1, 0.15, 0.5 & 1.0 g) were tested against the above mentioned common pest of stored grains for their insecticidal and seed protective properties. Both plant leaf powders have resulted in high mortality, delay in development and subsequent significant population reduction. They have also shown substantial seed protection over control cultures. The present study suggests that leaves from these trees have insecticidal properties against one of the most serious pest of stored wheat and could be employed as alternatives for chemical pesticides. © 2010 Friends Science Publishers

Key Words: *Punica granatum; Murraya koenigii; Tribolium castaneum* (Herbst)*;* Insecticidal; Mortality

INTRODUCTION

Safe storage of grains and food products against insect damage is a serious concern (Haq *et al*., 2005). It has been estimated that about 9% of the world's grain production is lost to post harvest insect and mite's infestations (Tooba *et al*., 2005) due to favourable climatic and storage conditions (Rahman *et al*., 2009). Though spectacular progress in pest control has been achieved by chemical measures, its adverse effect on ecological system and human life has stressed the need to develop alternative methods for controlling the various arthropod pests (Risk *et al.*, 2001). Plants such as *Azadirachta indica* (A. Juss), *Cassia fistula* (L.), *Calotropis procera* (Ait), *Lantana camara* (L.) and *Chrysanthenum coronarium* (L.) have shown insecticidal, antifeedant, repellant and growth regulating properties (Deka & Singh, 2005; Singh & Singh, 2005; Prakash & Rao, 2006; Kestenholz *et al*., 2007; Neoliya *et al*., 2007; Sankari & Narayanswamy, 2007). They are traditionally and widely used as stored grain protectants due to their easy accessibility and biodegradable nature (Dwivedi & Garg, 2003).

T. castaneum along with *Trogoderma granarium* (Everts) and Rhyzopertha dominica (Fabr.) causes severe economic loss to farmers and consumers in India (Frenmore & Prakash, 1992). Although numerous pesticides have been used to control insect pests, their impact on the environment is alarming (Farage, 1989; Markowitz, 1992; Rajaskaran & Baker, 1994; Gupta *et al*., 2001). In the present study, pulverized leaves of *Punica granatum* (Lythraceae) and *Murraya koenigii* (Spreng.) were tested for their lethal and growth regulating effect against *Tribolium castaneum* (Herbst). Both these plants have high medicinal values and have also shown antibacterial and anti-fungal properties (Alanis *et al*., 2005; Melendez & Capriles, 2006). They are generally used in pulverized form as these powders contain complex chemicals, which may show overall bioactivity when compared to isolated plant constituents by extraction methods (Berenbaum *et al*., 1985; Baki *et al*., 2005). Insect resistance is less likely to develop with crude forms like powders and mixtures (Feng & Isman, 1995). If plant materials are readily available, it will be easy for farmers to prepare the pulverized form as this is the simplest and cheapest method for preparing botanicals (Paul *et al*., 2009).

Therefore an eco-friendly approach (Upadhyay & Jaiswal, 2007) is always encouraged. Hence these easily available and traditionally used medicinal plants in India have been selected for the present study.

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 particles and dried properly before use.

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Preparation of leaf powder: Fresh leaves of *P. granatum* and *M. koenigii* leaves were collected from the author's garden, where there was no history of pesticide application. They were washed in water, shade-dried and ground to fine powder in a mixer and kept in airtight containers. The selected concentrations added to 10 g wheat grains were in the range of 0.05 to 1.0 g (0.05, 0.1, 0.15, 0.5 & 1.0 g) and mixed thoroughly with the grains. These concentrations were determined after conducting preliminary experiments to standardize the doses and then used for assessing their insecticidal and seed protective effects.

Selected insect pest: A stock culture of *T. castaneum* maintained on wheat at 28°C±1°C, 60%±5% RH and 14/10 h photoperiod for many years in the laboratory (Howe, 1991) was used. First instars, pupae and adults were separated from the stock culture and used for each experiment.

Experimental design: Three sets of cultures were set up simultaneously with five concentrations of each plant leaf powder and a control in small plastic vials (250 mL volume) containing 10.0 g fresh and undamaged wheat grains (about 200 seeds) and 2 g wheat powder with few dried yeast granules in each and mixed thoroughly.

First instars, pupae and adults were separated (20 in number) and transferred to each vial. Respective controls from each stage were maintained without any leaf powder treatment. At regular intervals of 24 h, the cultures were assessed for mortality. The number of dead insects in each vial was noted and they were removed. The cultures were maintained until one generation was completed or up to a maximum period of 50 days. Percentage mortality and any delay in developmental stages with respect to controls were also determined. The extent of seed damage in treated groups and the seed protective effect of the plant leaf powders were evaluated by comparing the percentage of damaged seeds to undamaged ones from treated and control groups. The experiments were repeated thrice.

Statistical analysis: The number of days required for 100% mortality, percentage mortality in adults and percentage seed damage from each set of experiment had been tabulated and the mean±SE for each triplicate was calculated (Gurumani, 2005). These data were then subjected to one way ANOVA using Bonferroni's multiple comparison test for the post hoc separation using the software PRISM 3.0 and the graphs were produced accordingly.

RESULTS

Rate of mortality: The effect of powdered leaves of *P. granatum* and *M. koenigii* are shown in Figs. 1-3. When larvae were introduced to different concentrations of *P. granatum*, they failed to metamorphose into pupae and all died within a period of 8-27 days. Number of days required for 100% mortality decreased with increased concentrations (Fig. 1a). However the control insects completed their life-cycle within a period of 30 d, with a larval duration of 18 d and a pupal duration of 11 d. In case of *M. koenigii*, 100% mortality was observed within a period of 14-35 d. With 0.05 g concentration, moulting of larva to pupa took place at day 18 and pupal-adult moult at day 16 (data not shown). With further concentrations (0.15, 0.5 & 1.0 g), no metamorphosis from larva to pupa took place and all were dead within a period of 14-18 d (Fig. 1b).

When pupae were introduced to 0.05, 0.1 and 0.15 g of *P. granatum* and *M. koenigii*, respectively 100% mortality was observed within 3–5 d, while with 0.5 and 1.0 g concentration, the time taken for complete mortality was reduced to 24 h (Fig. 1a b). When the adults were introduced to different concentrations of leaf powders, the mortality rate increased from lower to higher concentrations. The lowest concentration of 0.05 g produced a mortality of 40%, while it was 85% with 1.0 g of *P. granatum.* In case of *M. koenigii* the percentage mortality ranged from 30-80% (Fig. 2a b). The effect was considerably less in comparison to larval or pupal mortality probably due to the sturdy nature of adults. With increase in concentrations the rate of mortality increased and was significant with respect to controls ($p<0.001$, Fig. 2a b).

Seed protective effect: To obtain the seed protective effect, undamaged grains, which were introduced at the beginning of each experiment (10.0 g wheat grains contain about 200 grains in number) were collected after a period of fifty days of exposure and checked for the extent of damage. The damaged seeds were separated, counted and compared with the control sets. There was significant reduction in the number of damaged seeds after treatment with *P. granatum* and *M. koenigii*. The percentage damage by control insects was 40%, while the insects treated with 1.0 g of *P. granatum* and *M. koenigii* leaf powder significantly reduced the damage to between 5 and 6% over a period of 50 days. Almost all concentrations were effective in reducing the damage significantly ($p<0.001$, Fig. 3a b) showing the seed protective effect of the plant, which extended from 85-95 % in *P. granatum* and 83-94% in *M. koenigii* over controls (Fig. 3a b).

DISCUSSION

Insect pests are a major concern. It is estimated that about 35% of crops all over the world (Shani, 2000) and 5-10% of the stored grains in India alone are lost due to insect damage (Frenmore & Prakash, 1992). Due to great economic losses caused by stored grain pests, control of infestation of warehouses, factories, ships and mills by them is of main interest to the food manufacturers and distributors (Frenmore & Prakash, 1992). Around the world, residual chemical insecticides are currently the method of choice for the control of stored-product insects (White & Leesch, 1995; Epidi & Odili, 2009), but extensive use has resulted in the conversion of innocuous species to pests and the

Fig. 1: Time required for 100% Mortality for the Larvae and Pupae of *T. castaneum* with different concentrations (in g) of *P. granatum* and *M. koenigii* leaf powders in 10.0 g of fresh wheat grains

(a) With *P. granatum* (b) with *M. koenigii*. Control insects have completed their life cycle within a period of 30 d

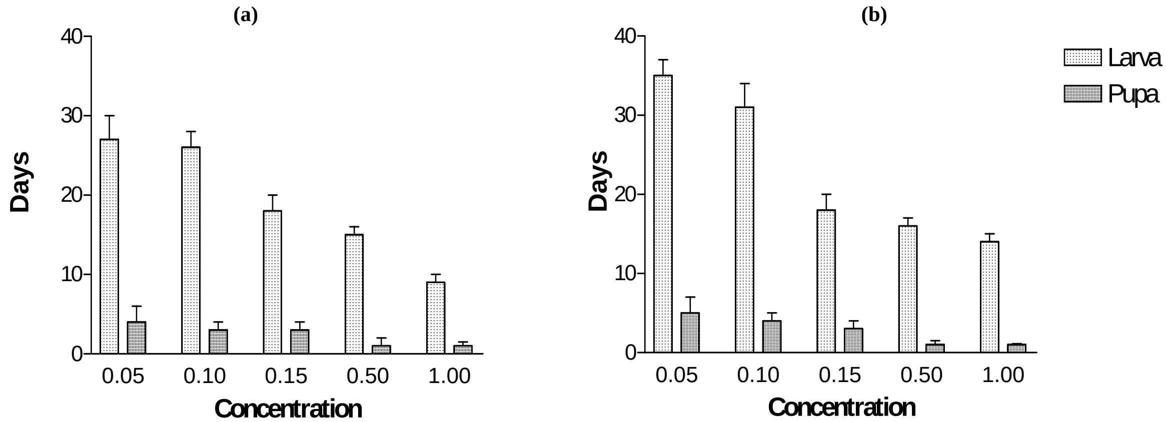


Fig. 2: Percentage mortality in adult *T. castaneum* treated with different concentrations (in g) of *P. granatum* and *M. koenigii* leaf powders in 10.0g of fresh wheat grains

(a) With *P. granatum* [Anova $F_{(5, 54)} = 357.4$, ($p<0.0001$), Bonferrroni's test ($p<0.001$)] (b) with *M. koenigii* [Anova $F_{(5, 54)} = 377.8$, ($p<0.0001$), Bonferrroni's test ($p<0.001$)]

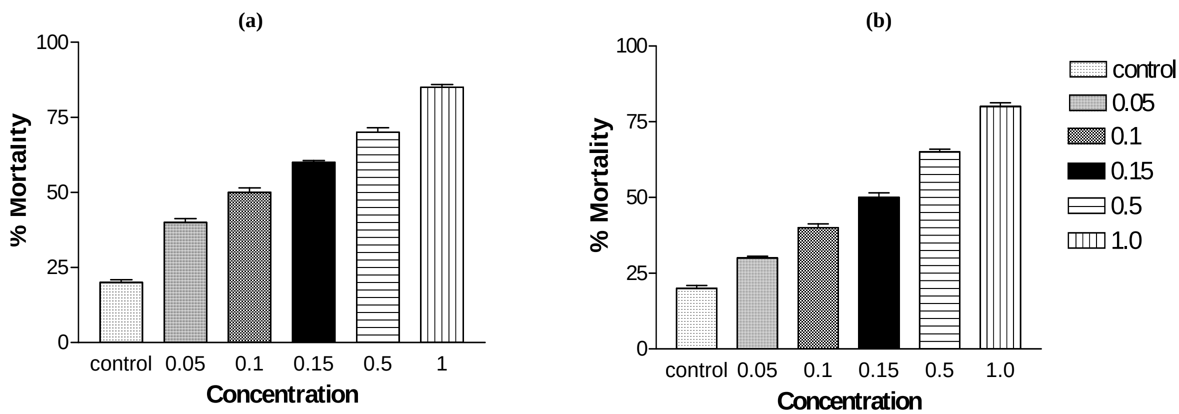
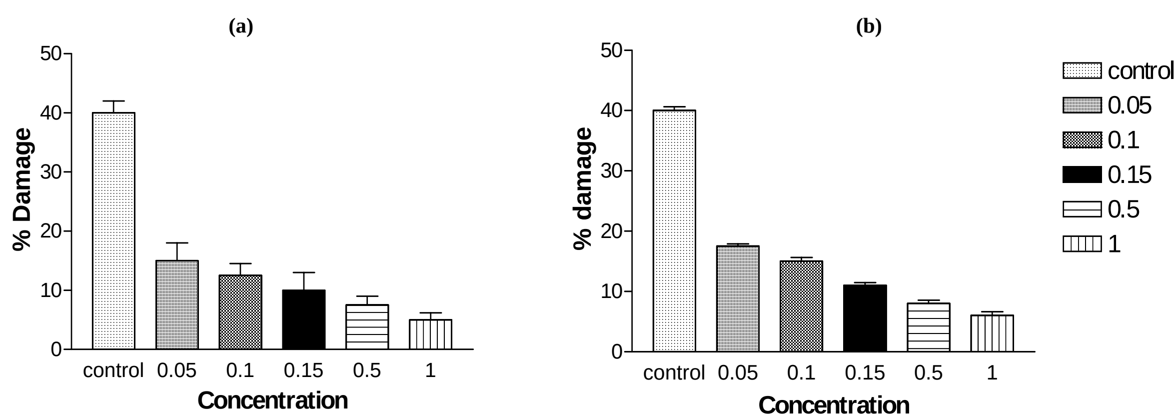


Fig. 3: Percentage Seed damage by *T. castaneum* treated with different concentrations (in g) of *P. granatum* and *M. koenigii* leaf powders in 10.0g fresh wheat grains

(a) With *P. granatum* [Anova $F_{(5, 54)} = 328.4$, ($p<0.0001$), Bonferrroni's test ($p<0.001$)] (b) with *M. koenigii* [Anova $F_{(5, 54)} = 480.4$, ($p<0.0001$), Bonferrroni's test ($p<0.001$)]



evolution of resistant forms (Schaafsma, 1990; Taylor, 1994; Zettler & Arthur, 1997; Rahman *et al*., 2009) in addition to environmental contamination and health hazards (White & Leesch, 1995). Under such conditions, the use of bioactive pesticides for protection of stored grains would be a safe alternative (Tapondjou *et al*., 2001; Boeke *et al*., 2004; Talukdar *et al*., 2004; Hasan *et al*., 2006; Epidi *et al*, 2008; Epidi & Odili, 2009).

Many plants like *Annona squamosa* (L.), *Lantana camara*, *Clerodendrun inerme*, *Cassia fistula*, *Azadirachta indica* and *Calotropis procera* are proved to be lethal to various stored grain pests and delay the developmental stages by interfering with their apolytic and molting processes (Tewari & Singh, 1978; Dwivedi & Garg, 2003; Deka & Singh, 2005). Leaves of *Ocimum sanctum* (L.), *Vitex negundo* (L.), *Aegle marmelos* (L.) and *Lippia geminata*(L.) have been used for the protection of stored rice forms in rural India (Prakash & Rao, 2006). Similarly leaf powders of *Annona squamosa* and *Balanites aegyptica* (L.) caused high mortality in *T. castaneum* and provided protection against seed damage (Sule & Ahmed, 2009).

With this view, the analysis of percentage mortality in the present study reveals the efficacy of the tested plant leaf powders on all developmental stages of *T. castaneum.* Larval mortality increased with increase in concentrations, while the time required for 100% mortality was reduced at higher concentrations. Given this trend in larval mortality, even higher doses of powdered leaves could be recommended for more favourable results. The pupae were found to be most sensitive of the tested stages, as they were killed within short durations. Adult mortality between 40-85% was observed with *P. granatum* and 30-80% in case of *M. koinigii* indicating that these plant materials were effective in reducing the normal growth and developmental processes of *T. castaneum.* They have also shown promising seed protection over controls even at the tested concentration of 0.15 g/10 g wheat grains. Percentage seed damage was reduced significantly after treatment with various doses of plant leaf powders, suggesting that both were effective in reducing wheat damage caused by *T. castaneum.* Increased concentrations can further boost the rate of mortality within a short time span and provide long lasting seed protection.

These results are in corroboration with findings by Haq *et al.* (2005), who showed that pulverized leaves of *Eucalyptus sp.*, *Bougaivillea glabra*, *Azadirachta indica*, *Saraca indica* and *Ricinus communis* were effective against *T. castaneum.* They were also found to be seed protective as the spoilage of seeds were reduced to a significant extent. Epidi and Odili (2009) have also shown similar results with pulverized plant parts of *Telferia occidentalis*, *Zingiber officianale*, *Vitex grandifolia* and *Dracaena arborea* (Willd) against *T. castaneum* and recommended such post harvest treatments on stored groundnuts (*Arachis hypogaea* L.).

From the present study, the effectiveness of these plant components to control post harvest food grain losses during storage is highly recommended. This is of practical importance to the farmers as they can improve their traditional method of seed protection with use of pulverized leaves of *P. granatum* and *M. koenigii*. Moreover removal of these powders from seeds before consumption is easy but not essential as both plants have medicinal properties and are non toxic to humans.

CONCLUSION

P. granatum and *M. koenigii* have shown promising signs of seed protection and insecticidal properties with the tested concentrations. Increased concentrations of these powders showed subsequent reduction of time to 100% mortality. It would therefore suggest the use of higher dosage as grain protectants to improve insecticidal property as well as help to control post harvest and food grain losses during storage at farm level. Use of these plant leaves as grain protectants besides being cost effective, may also abate the environmental pollution and health hazards.

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