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Control of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) by Pulverized Leaves of *Punica granatum* (Lythraceae) and *Murraya koenigii* (Rutaceae)

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ABSTRACT

The pulverized leaves of *Punica granatum* (Pomegranate) and *Murraya koenigii* (Curry tree) were tested for their insecticidal activities against the stored grain pest *Rhyzopertha dominica* (Fabr.) under laboratory conditions. Five different concentrations of leaf powders ranging from 0.05 to 1 g per 10 g wheat grains and 10 g beaten rice (Poha) separately, were tested for their efficacy. Pulverized leaves of both plants produced high incidence of mortality and reduced the rate of development in both medium resulting in significant reduction in population. The percentage mortality over controls ranged from 18-71% with *P. granatum* ($p < 0.001$) and 18-65% with *M. koenigii* ($p < 0.001$) in wheat medium. However, it was 26-79% ($p < 0.001$) and 16-74% ($p < 0.001$) correspondingly in beaten rice. The percentage seed protection over controls in wheat extended from 27-75% with *P. granatum* (< 0.001) and 08-73% ($p < 0.001$) with *M. koinigii*. In beaten rice the protection was about 44-93% ($p<0.001$) when treated with *P. granatum,* while 19-75% ($p < 0.001$) with *M. koinigii*. This shows that in beaten rice, as the pests were more exposed to plant components, they exhibited high mortality, while in wheat they could bore and hide inside the grains from the effect of plant components. However, both plant leaves have exhibited insecticidal and seed protective effect to a promising level and can be opted as good alternatives to chemical pesticides. © 2011 Friends Science Publishers

Key Words: Biological control; Stored pest; Mortality, Insecticidal activity

INTRODUCTION

Farmers suffer heavy losses of stored grains due to insect pests. There are about thirty nine species of pests, which attack the stored grains and grain products. According to FAO estimates, about 10-25% of the world harvested food is destroyed by insects and rodent pests (Anonymous, 1980). Uncontrolled use of insecticides and pesticides results in the evolution of resistant strains in addition to environmental contamination and health hazards (Prakash & Rao, 2006; Rahman *et al*., 2009). In such situations a safe method is to use the bioactive pesticides for protection of stored grains (Tapondjou *et al*., 2001; Boeke *et al*., 2004; Talukdar *et al*., 2004; Tooba *et al*., 2005; Epidi *et al*., 2008). Unlike insecticides, which mostly kill insects, plant ingredients are known to suppress the feeding and breeding behaviour of insects in many ways in addition to direct mortality (Jilani, 1984).

Use of plant parts with insecticidal properties have been reported from all over the world as they are convenient, less expensive, highly effective and safer for the humans and environment. 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 against various stored grain pests like *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (Fabr.), *Trogoderma granarium* (Everts.) and *Callosobruchus chinensis* (L.) (Gautam *et al*., 2003; Deka & Singh, 2005; Singh & Singh, 2005; Hazan *et al*., 2006; Prakash & Rao, 2006; Kestenholz *et al*., 2007; Neoliya *et al*., 2007; Sankari & Swamy, 2007). They are widely used as traditional stored grain protectants in powder form, crude mixtures or extracts due to their easy accessibility and biodegradable nature (Dwivedi & Garg, 2003). Many plants like *A. squamosa, L. camara, C. inermis, C.fistula, A. indica* and *C. procera* have been proved to be lethal for various stored grain pests and delayed the developmental stages by interfering with their apolytic and molting processes (Tewari & Singh, 1978; Dwivedi & Garg, 2003; Dwivedi & Karsawara, 2003; Deka & Singh, 2005; Morya *et al*., 2010). Leaves f *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).

However, considering the realities experienced by small scale farmers, who can depend only on simple methods for preparing botanicals, in the present study,

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pulverized leaves of *Punica granatum* (L.) (Lythraceae) and *Murraya koinigii* (L.) (Rutaceae) were used for controlling the pest *Rhyzopertha dominica* (Fabr.) (Coleoptera: Bostrichidae). These two plants are widely distributed and available in all parts of India and are known for their insecticidal, antifungal, antibacterial and medicinal properties (Alanis *et al*., 2005; Melendez & Capriles, 2006). Our earlier studies also have shown that they are very effective against *T. castaneum* (Herbst) (Gandhi *et al.*, 2010). Moreover, the odor of the curry leaves usually repel many of the stored grain insects and hence traditionally used for short term storage of grains in India (Kiruba *et al*., 2008).

R. dominica is one of the most injurious pest of stored grains both in larval and adult stage. It also infest other stored food items like beaten rice (Poha), dry fruits etc., (Kuzumenov *et al*., 1984; Raju, 1984). The adults are sturdy fliers, which fly from warehouse to warehouse, causing severe infestation and convert the stored grains to mere frass (Frenmore & Prakash, 1992). Hence, in the present study *R. dominica* is selected for testing the efficacy of *P. granatum* and *M. koenigii* in two different culture medium such as wheat and beaten rice. Two food media were chosen with a view to assess their efficacy against different types of stored food items and also to evaluate the food preference, if any, exhibited by the pests.

MATERIALS AND METHODS

Samples of wheat grains: Local variety of wheat grains, *Triticum aestivum* (L.) (Poaceae) cultivated in this region, were collected from market, washed thoroughly with water and dried properly in sunlight before use.

Beaten rice: Good quality beaten rice was collected from shop, sieved to remove any dust particles and powder, dried in sunlight for 1 h to remove moisture, if any, and used for experiments.

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 for further use. Five different concentrations ranging from 0.05 to 1 g (0.05, 0.1, 0.15, 0.5 & 1 g) were standardized after conducting some preliminary experiments and then used for assessing their insecticidal and seed protective effects.

Culture of insect pest: For wheat culture, stock cultures of *R. dominica,* reared on wheat as per the method of Howe (1991) at $28^oC\pm1^oC$, 60%±5% RH and 14/10 h photoperiod for many years in the laboratory were used. Adults of *R. dominica* were separated from these stock cultures and used for various experiments. For beaten rice culture, few adults of *R. dominica* reared on wheat were separated and cultured in beaten rice for three generations at $28^oC\pm1^oC$, 60%±5% RH and 14/10 h photoperiod. Fresh adults from these cultures were used for conducting experiments in beaten rice.

Experimental design: Two sets of experiments were conducted using wheat as well as beaten rice. *Rhizopertha* causes severe damage to both. This also gives insight into the food preference exhibited by the insect pest and its influence on its survival. With wheat experiments, 20 adults of *R. dominica* were separated from stock cultures to each small plastic jar (250 mL volume) containing 10 g wheat grains and 2 g wheat flour mixed with few dried yeast granules and different concentrations (0.05-1 g) of powdered leaves of *P. granatum* and *M. koenigii* separately. Same number of insects was transferred to control vials, which were maintained at same conditions except with leaf powders.

With beaten rice, experiments were set up using beaten rice (10 g) as the medium of culture to evaluate the efficacy of plant powders in different medium. Twenty adults of *R. dominica* reared on beaten rice were separated and transferred to each vial containing 10 g beaten rice mixed with different concentrations of powdered leaves of *P. granatum* and *M. koenigii* separately and labeled properly. Same number of insects were transferred to cultures without any leaf powder treatment and maintained as controls.

All these cultures were assessed at 24 h intervals to evaluate the efficacy of these plant powders on *R. dominica*. Dead insects in each vial were noted and discarded and the cultures were continued until one life cycle was completed or up to a period of 50 days. In both the sets, experiments were repeated thrice.

Statistical analysis: Percentage mortality and percentage seed protection over controls were calculated as per Abbott's (1925) formula. The mean values from three replicates were subjected to one way ANOVA test and Bonferroni's multiple comparison test was used for the post hoc separation. Student 't' test was used for comparing the significance between the plants and media. The analysis was performed using the software PRISM 3.0 and the graphs were produced accordingly.

RESULTS

Rate of mortality: Percentage corrected mortality over control in wheat and beaten rice with *P. granatum* and *M. koenigii* are represented in (Figs. 1a & b). There was an increase in percentage mortality with increase in concentrations and *P. granatum* was found to be more effective than *M. koenigii* in both media except with 0.15 and 0.5 g concentration in wheat medium (Fig. 1a & b). The percentage mortality ranged from 18-71% with *P. granatum* ($F_{(4,10)}$ 195.6 $p<0.001$) and 18-65% with *M. koenigii* ($F_{(4,10)}$ 151.2 $p<0.001$) in wheat medium. While it was 26-79% ($F_{(4,10)}$ 306.3 $p<0.001$) and 16-74% ($F_{(4,10)}$ 288.8 $P<0.001$), respectively in beaten rice. However, the graph plotted for the effect of individual plants on both media showed an increased rate of mortality in beaten rice with both plant components (Fig. 2a & b; $p<0.05$ & 0.01). This indicates that in beaten rice the pests are directly exposed, while in wheat seeds they are getting more protection as they could hide inside the grains to escape from the deterrent activity of plant powders.

Effect on developmental cycle: Both leaf powders were effective in producing considerable delay in development. In case of controls, the life-cycle was completed within 25±1 d in wheat medium. But 0.05 to 0.15 g of *P. granatum* and *M. koenigii* treated adults completed their life-cycle with a delay of about 4 to 10 days. The higher concentrations of 0.5 g and 1.0 g of both plant powders further delayed the life-cycle and it was not completed even after a period of 50 d (Fig. 3a & b).

There was not much variation in the life cycle of insects treated with 0.05 and 0.1 g of *P. granatum* while 0.15 g shown a delay of almost 10 d in development. The higher concentrations of 0.5 and 1 g significantly delayed the life cycle and prevented the molting to next stage even after 50 d. Significant delay in development was observed with *M. koenigii* also and molting was inhibited from 0.15 g onwards (Fig. 3a & b).

Seed protective effect: The wheat seeds and beaten rice which were used in the beginning of experiment was checked after 50 d to evaluate the damage produced by the pest. In case of wheat, damaged seeds were separated and counted, while in case of beaten rice the weight was taken into consideration. A good amount of seed protection was observed in both media (Fig. 4a & b) as the extent of damage produced was considerably reduced after mixing with plant powders. The percentage protection in wheat extended from 27-75% with *P. granatum* (*$F_{(4,10)}$ 110.5 p<0.001*) and 08-73% ($F_{(4,10)}$ 192.1 p<0.001) with *M. koinigii*. In beaten rice the protection was about 44-93% ($F_{(4,10)}$ 368.3 p<0.001) when treated with *P. granatum,* while 19-75% ($F_{(4,10)}$ 164.7 P<0.001) with *M. koinigii*. The extent of damage was found to be more in wheat cultures than in that of beaten rice as these insects were protected within the grains of wheat, while in beaten rice their exposure to plant components was more. Significant variations in protection was even found between different plants and media (Fig. 4 & 5, P<0.05-0.001). The percentage protection was more pronounced in beaten rice.

DISCUSSION

In the present study, significant rate of mortality was observed in adults with both plants suggesting their effectiveness against the pest. This is in conformity with our earlier results already reported for *T. castaneum* where both *P. granatum* and *M. koenigii* were shown to be highly insecticidal and seed protective (Gandhi *et al*., 2010). Similarly, Belmain *et al*. (2001) have reported that dry leaf powder of *Cassia sophera* (L.) increased the adult mortality of *C. maculates* and *R. dominica* when mixed at 5% w/w to cowpea or wheat. The same treatment significantly reduced adult emergence of *C. maculates* and *R. dominica.*

Fig. 1: Percentage corrected mortality over control in adult *R. dominica* treated with different concentrations of *P. granatum* ($F_{(4,10)}$ 195.6 p<0.001) and *M. koenigii* leaf powders ($F_{(4,10)}$ 151.2 p<0.001) in 10.0 g of fresh wheat grains and beaten rice ($F_{(4,10)}$ 306.3 p<0.001). ($F_{(4,10)}$ 288.8 p<0.001; * & ** 't' test significance between compounds)

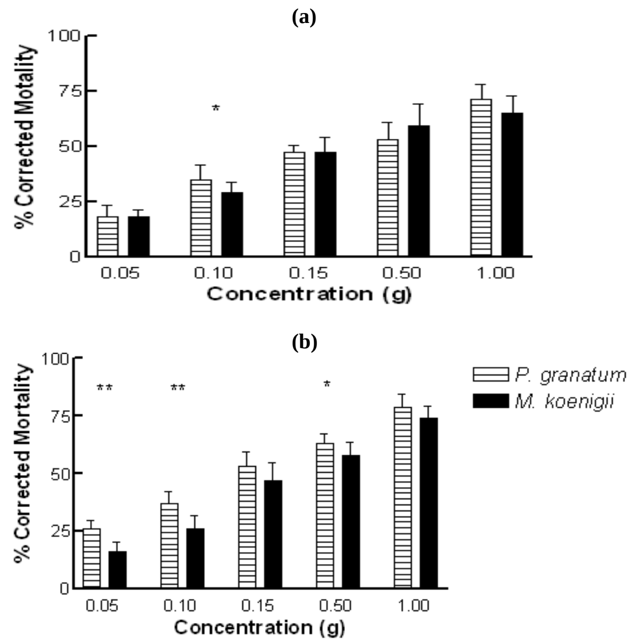


Fig. 2: Percentage corrected mortality over control with *P. granatum* and *M. koenigii* in two media separately (ANOVA and t test significance between media * p<0.05 **p< 0.01)

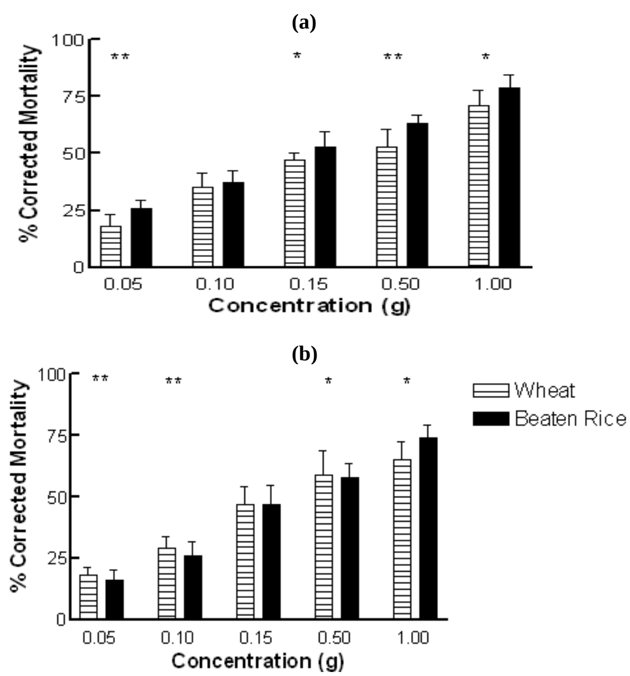


Fig. 3: Variation in the life-cycle of *R. dominica* with different concentrations (g) of *P. granatum* and *M. koenigii* leaf powders in 10.0 g fresh wheat grains and beaten rice

\# 0.5 and 1 gm treated adults have not completed one life-cycle even after 50 days and hence not shown

(a)

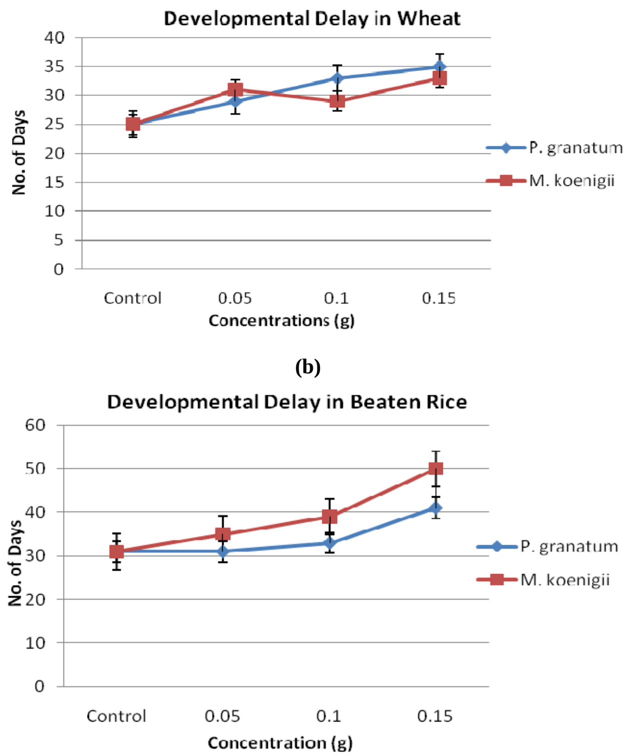
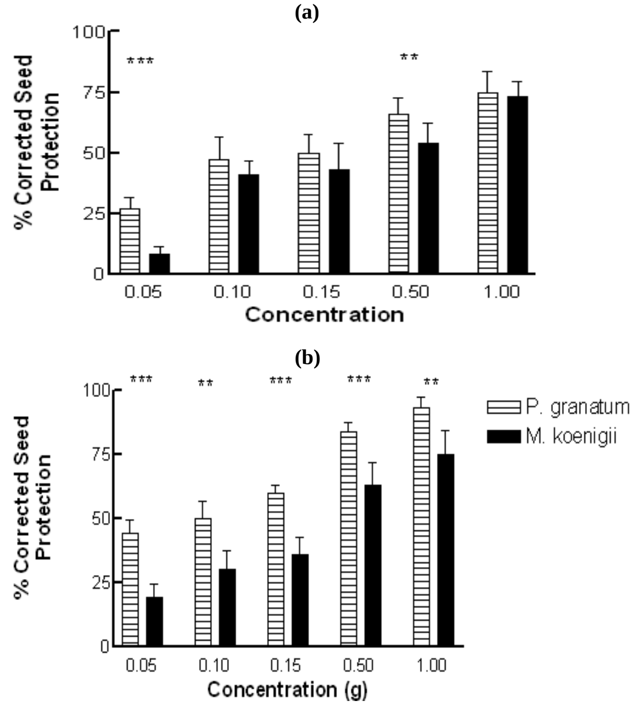


Fig. 4: Percentage Seed protection over controls in *R. dominica* treated with different concentrations (g) of *P. granatum* and *M. koenigii* leaf powders in 10.0 g fresh wheat grains (ANOVA *F* $_{(4,10)}$ *110.5 p<0.001)* ($F_{(4,10)}$ 192.1 p<0.001) and beaten rice (ANOVA F $_{(4,10)}$ 368.3 p<0.001) ($F_{(4,10)}$ 164.7 p<0.001; ** & * 't' test significance between compounds)**

(a)



Similar results were shown with *Licamera, C. inermis* and *C. procera* (Singh *et al*., 1996; Rao & Prakash, 2002; Dwivedi & Karsawara, 2003; Deka & Singh, 2005). Crushed leaves of *Cleome viscosa* (L.) have shown dose dependant insecticidal, ovicidal and oviposition inhibitory activities against *C. maculates* (Dabire *et al*., 2008).

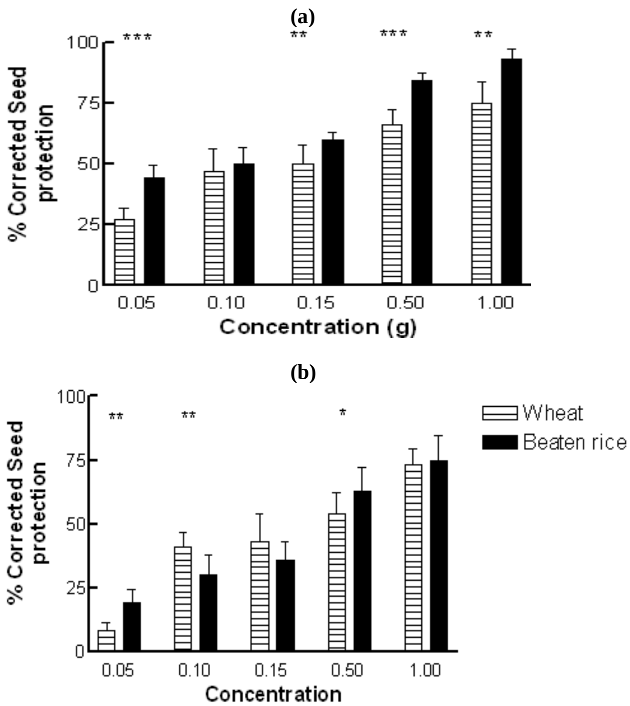
In *R. dominica*, the flower extracts of *L. camara* have reduced the fecundity, prolonged the developmental period and induced high larval mortality along with reduction in seed damage to a significant extent (Singh *et al*., 1996; Rao & Prakash, 2002). Asawalam *et al*. (2007) have also reported the efficacy of Acetone extract of *Piper guineese* seed extract on maize weevil *Sitophilus zeamays* (Motschulsky) in reducing the population and grain damage. It is known that normal metamorphosis in insects can be interrupted with the presence of exogenous materials in food, contact with chemicals or by radiation. Deformed characters are expressed or further molting is inhibited resulting in considerable reduction of insect population (Mohal *et al*., 2006). Significant delay in molting was exhibited by both plant leaf powders suggesting their interference with the normal growth process as reported for other plants like *L. camara* and *C. inerme* (Dwivedi & Garg, 2003; Morya *et al*., 2010).

Prakash and Rao (2006) have also reported that the leaf powder of *Ageratum conyzoides* (coat weed) brought about significant grain protection against *Sitotroga cerealella* (Oliv.), *Sitophilus oryzae* (L.) and *R. dominica* under controlled and natural conditions of infestation in stored rice.

In many Asian and African countries, it is an age old practice to mix plant parts with grains to manage stored grain pests (Kiruba *et al*., 2008; Paul *et al*., 2009). Usually plant factors show repellant, antifeedant and growth regulating effect. The larvae in such cases refuse to feed, become sluggish and do not bore into the seeds. If the plant components have toxic effect, the larvae may not do more than scarify the seeds before dying. If mortality does not occur the factor may have a debilitating effect on the larvae and their life cycle may be prolonged. In all these cases, the resulting effect is that there is a lesser amount of damage to the stored grains (Jilani, 1984).

Encouraging results were obtained on wheat grain as well as beaten rice stored for 50 d with different doses of pulverized leaves. The extent of damage was more in beaten rice than in wheat as the insects could bore and take shelter inside the wheat grains, while in beaten rice, the exposure was more fatal and the damage produced to beaten rice was naturally reduced. The pest exhibited a food preference for

Fig. 5: Percentage seed protection over controls in *R. dominica* treated with *P. granatum M. koenigii* in two media separately (ANOVA and t-test p<0.05-0.001;'t' test significance between media *p<0.05, **p<0.01, ***p<0.001)



wheat over rice as controls in wheat completed their life cycle within a short period than the controls in beaten rice. This can be attributed to their developmental behavior, which shows a preference for whole grains for larval and pupal life. However, a good rate of food protection could be brought about by both plants in both media suggesting their insecticidal effect. Higher doses can further increase the rate of mortality and boost up seed protection. In fact significant delay in development and inhibition of further molting is a good indication that they interfere with the growth and multiplication process of pest population.

CONCLUSION

Supporting our previous study, the pulverized leaves of *P. granatum* and *M. koenigii* have shown insecticidal and seed protective effect along with developmental delay against *R. dominica* in different media suggesting the effectiveness of these plant powders to control post harvest food grain losses during storage.

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