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Original article

Comparative insecticidal activity of different plant materials from six common plant species against Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae)



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ABSTRACT

Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) is one of the major insect pests of stored grains. Due to export legislation and zero-tolerance for live insect in trade commodities, extensive use of synthetic insecticides is in practice in order to eliminate pest infestations from the lots. Currently, the one and only acceptable chemical to be used in stored grain is phosphine but due to its excessive usage the stored grain pests are becoming resistant against it. Hence discovery of alternative compounds is much needed. In this study we have compared insecticidal efficacy of different plant materials from six commonly grown plants of Pakistan, viz. Allium sativum (Alliaceae), Zingiber officinale (Zingiberaceae), Cymbopogon citratus (Poaceae), Eucalyptus globulus (Myrtaceae), Nicotiana tabacum (Solanaceae), and Azadirachta indica (Meliaceae) against T. castaneum infesting stored wheat, rice, corn and gram pulse. Various plant parts were dried, powdered, and used as admixtures to the stored commodities in the experiments. The results have suggested that A. sativum (garlic) and Z. officinale (ginger) were more effective resulting into 15 times higher adult mortality and 4 to 5 times reduction in grain weight losses when mixed with rice grains. Similarly, A. indica when admixture with wheat checked the population growth in the resources resulting into 3.5 times less adult production compared to controls. A subsequent experiment was conducted to study the dose response of neem seed powder against the beetle pest infesting milled products. Surprisingly, better control was observed either at lowest (1% w/w) or the highest doses (5% w/w). This finding is of great interest to understand the underlying phenomenon which we assume is the ability of T. castaneum to feed selectively in flour mediums, however, further research on this aspect is required to be investigated. The results of this study support the use of botanicals for stored product pest management.

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1. Introduction

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Stored grains, cereals, and their products are important sources of the world food, therefore, effective conservation of this prime resource is important for subsistence of mankind (Stejskal et al., 2015). Maize, rice, and wheat are a few of the most consumed grains, while, chickpea supplement world food demands and is also

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a major source of animal feed (Wondatir et al., 2015). All of these crops are traditionally included in the cropping patterns of Pakistan and after harvesting, these commodities are stored for up to a year for getting good market rates for the produce. This prolonged storage leaves the precious resources vulnerable to various losses, especially caused by insect pest infestations.

Globally, insect pests of stored grains cause the highest qualitative and quantitative losses to stored commodities (Fields, 2006) that may range from 10 to 40% (Lorini and Filho, 2004). Most of this damage is caused by rust-red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) causing up to 40% reduction in grain weight (Ajayi and Rahman, 2006; Rees, 2007). It consumes endosperm of the seeds leaving them with coagulating consistency and moldy smell (Keskin and Ozkaya, 2013). In order to save these grains from spoilage, there is a need of an efficient control measure.

Stored grain industry currently rely upon synthetic grain protectants. Not only these chemicals have severe effects on environment but also cause serious health issues to the consumers (Salem et al., 2007). Also their extensive and indiscriminant use against stored grains pests has resulted in development of strong resistance in these insects against such chemicals. Such detrimental impacts of chemical control of insects warrants evaluation of natural but equally effective compounds that can be used these pests without posing much threat to human health and deteriorating grain quality (Mahdi and Rahman, 2009; Salem et al., 2007; Fields, 2006).

Plant based compounds, being natural and organic, may provide the starting point for such discoveries. In these regards, recent studies have identified several promising natural extracts of plant exhibiting insecticidal activities in stored grain systems (Tatun et al., 2014; Tripathi et al., 2009). Such botanical extracts may have various modes of action and can help in pest management by repelling the pest away, may act as feeding and oviposition deterrent, and at the same time may act as insecticides (Mohan and Fields, 2002). A well-documented example of such botanical compound is Azadirachtin, derived from Azadirachta indica seeds. Studies have proved its efficacy against aphids, lepidopteran larvae. several stored grain pests and mealy bugs (Morgan, 2009). It works as feeding deterrent, insect-growth regulator, repellent and sterilant, and may inhibit oviposition (Isman, 2006). Similarly, nicotine (derived from Nicotiana tabacum) is effective against insects causing the uncontrolled nerve firing and masking acetylcholine (Ujváry, 1999) with an LD₅₀ between 50 and 60 mg kg⁻¹ (Isman, 2006; Yamamoto, 1999). Similarly, Eucalyptus globulus leaves have metabolites that are toxic to Rhyzopertha dominica F. (Coleoptera: Bostrichidae) and T. castaneum and can be used as fumigant (Batish et al., 2008). Therefore, it is worth comparing their efficacy with other commonly available plants species (Nadra, 2006; Talukder, 2005; Udo, 2005; Aslam et al., 2002).

In this study, we compared the efficacy of lemongrass, garlic and ginger with neem, tobacco and eucalyptus, against rust-red flour beetle. The study was conducted in three logically interrelated experiments where the efficacy of various plant parts was tested in whole meal wheat flour, white flour, rice, and corn flour in one experiment while in the other, the same were tested in wheat, rice and gram-pulse seeds to evaluate their effect on the development and mortality of *T. castaneum*. Finally, the neem efficacy was checked by using different doses in whole meal flour and white flour.

2. Materials and methods

Freshly harvested wheat (*Triticum aestivum*), rice (*Oryza sativa*) corn (*Zea mays*), and gram pulse (*Cicer arietinum*) were collected. The seeds were frozen, to prevent insect infestation at -20 °C under the air tight conditions for at least a week or until they were used in the experiments. At the time of experiment, wheat was milled to yield whole meal and white flours. The plant parts to be used as botanicals in the experiments i.e. dried bulbs of garlic, rhizomes of ginger, and leaves of lemongrass and eucalyptus, and neem seed kernels (Table 1) were also collected, cleaned and powdered in coffee grinder.

Five hundred adults of *T. castaneum* were collected from infested wheat grains from Multan ($+30^{\circ}11'52''N$, $+71^{\circ}28'11''E$). These beetles were cultured in whole meal wheat flour with 5% brewer's yeast. The cultures were maintained at 30 ± 5 °C and $40 \pm 5\%$ RH. The newly pupated larvae were retrieved and sexed based on the abdominal characteristics (Halstead, 1963). The experiments started with introducing ten-day old adult beetles in test resources.

2.1. Efficacy of botanicals in seed forms of resources

This experiment was setup in glass bottles (500 mL) containing a mixture of 95 g of wheat, rice or gram pulse and 5 g of the powdered test plant material and a control with 100 g of the seeds only. Eight pairs of newly emerged adults (3°) of *T. castaneum* were introduced in each jar. The treatments were replicated three times and all the jars were incubated at 30 ± 5 °C and 40 ± 5% RH. for four months. After the incubation period average weight loss per one hundred seeds of each resource was recorded and the number of adult beetles was counted.

2.2. Efficacy of botanicals in flour forms of resources

The experiment was run in glass jars (50 mL) containing 5% w/ w mixture of resources and botanical (19 g flour of test resource (corn kernel, rice or wheat grains) and 1 g of plant material (neem, tobacco or eucalyptus). A control for each treatment with 20 g flour of a particular resource was included. This experiment was set in three blocks due to time and resource constraints and was replicated six times.

Table 1

List of the plant species and their parts used as botanical insecticides in different studies against Tribolium castaneum.

Plant species	Family	Parts used	Active ingredient	References
Allium sativum (Garlic)	Alliaceae	Bulbs	Methyl allyl disulphide, Diallyl trisulphide	Rahman and Talukder (2006) and Upadhyay et al. (2011)
Zingiber officinale (Ginger)	Zingiberaceae	Rhizome	Gingerol, β-Phellandrene, Camphene	Epidi and Odili (2009)
Cymbopogon citrates (Lemongrass)	Poaceae	Leaves	Neral, Gerenial, Linalool, Nerol, Geranyle Acetate	Manzoor et al. (2011)
Azadirachta indica (Neem)	Meliaceae	Seed kernels	Azadirachtin	Rahman and Talukder (2006) and Upadhyay et al. (2011)
Nicotiana tabacum (Tobacco)	Solanaceae	Leaves	Nicotine	Ujváry (1999) and Yamamoto (1999)
Eucalyptus globulus (Eucalyptus)	Myrtaceae	Leaves	Eucalyptin, Rutin, Gentisic acid, 1,8-cineole	Upadhyay et al. (2011) and Rahman and Talukder (2006)



Fig. 1. Percent weight loss in stored gram, wheat, and rice due *Tribolium castaneum* infestation after treatment with botanicals (garlic, ginger, and lemongrass). Bars represent means and the error bars are 95% CI. The lowercase superscript letters above each bar represent the results from post *hoc* pairwise comparisons between botanicals, and the uppercase superscript letters above each bar represent post *hoc* pairwise comparison between resource types. Bars with same letter were not statistically different from one another.

A pair of *T. castaneum* was introduced to each of the test resource and was held at 30 ± 5 °C and $40 \pm 5\%$ RH for reproduction and oviposition. The adults were discarded after a week of oviposition and the media were incubated at 30 ± 5 °C and $40 \pm 5\%$ RH until the larvae hatched. The data for number of larvae that hatched and survived to pupate, and the number of adults, that eclosed, were counted to identify the role of botanicals on beetles' life cycle.

2.3. Efficacy of A. indica at different concentrations

Effect of five concentrations (*viz.* 1, 2, 3, 4, and 5% w/w) of neem seed kernel powder and wheat flour was tested on the fecundity of *T. castaneum.* Twenty grams of each mixture and a control medium with no botanical were taken in glass jars (50 mL) and were kept at 30 ± 5 °C and $40 \pm 5\%$ RH. A single pair of newly emerged male and female adults was introduced to each jar and was transferred to a fresh substrate every 24 h, to avoid egg cannibalism. This process was repeated for five days and the respected media were incubated until the larvae hatch and accounted for.

After two weeks of incubation, the larvae became visible to the naked eye and were counted. Similarly pupae were counted, sexed (Halstead, 1963) and observed till they adults emerged.

3. Results

3.1. Efficacy of botanicals in seed forms of resources

The results demonstrated no significant effect of interaction between commodity types and the botanicals for percent weight loss in each commodity due to feeding by *T. castaneum* (resource: $F_{6, 24} = 2.485$, p = 0.517). Similarly, percent weight loss to different resources due to beetle feeding was also no different to each other ($F_{2, 24} = 1.02$, p = 0.376). However, it was significantly different among different botanicals used ($F_{2, 24} = 33.36$, p < 0.001). In general, minimum weight loss was observed in the seeds treated with garlic and ginger, followed by those treated with lemongrass, whereas in the control treatments highest percent weight loss was recorded (Fig. 1).

Adult mortality after treatment and incubation also demonstrated the effectiveness of various botanicals used in different seeds where percent adult mortality in each treatment was significantly different to each other ($F_{3, 24} = 20.13$, p < 0.001) (Fig. 2).



Fig. 2. Efficacy of garlic, ginger and lemongrass against *Tribolium castaneum* infestation in gram, wheat, and rice seeds expressed in terms of larval mortality. Bars represent means and the error bars are 95% Cl. The lowercase superscript letters above each bar represent the results from post *hoc* pairwise comparisons between treatments. Bars with same letter were not statistically different from one another.



Fig. 3. Efficacy of neem, tobacco and eucalyptus against *Tribolium castaneum* infestation in corn, rice, and wheat flours expressed in terms of number of larvae (A), pupae (B), and adults (C) that developed in each treatment. Bars represent means and the error bars are 95% CI. The lowercase superscript letters above each bar represent the results from post *hoc* pairwise comparisons between botanicals, and the uppercase superscript letters above each bar represent post *hoc* pairwise comparison between resource types. Bars with same letter were not statistically different from one another.

However, the effect of botanicals was not independent of resource type (Resource × Botanical interaction: $F_{6, 24}$ = 3.57, p = 0.011). The highest mortality was observed in ginger and lemongrass treated rice (Fig. 2), and in gram pulse treated with garlic, ginger and lemongrass (Fig. 2). Control treatments showed the least adults mortality (Fig. 2).



Fig. 4. Efficacy of neem against *Tribolium castaneum* infestation in wholemeal (shaded bars) and white flours (unshaded bars) expressed in terms of number of larvae (A), pupae (B), and adults (C) that developed in each treatment. Bars represent means and the error bars are 95% CI. The lowercase superscript letters above each bar represent the results from post *hoc* pairwise comparisons between treatments. Bars with same letter were not statistically different from one another.

3.2. Efficacy of botanicals in flour forms of resources

The results from efficacy of different botanicals when used in flour forms of the resources are presented in terms of development of larvae, pupae, and adults (Fig. 3A–C). The data on larval development showed a significant difference in the number of larvae that developed in different resources ($F_{2, 60} = 3.55$, p = 0.045) and this was independent of any interaction with type of botanicals used (Resource × Botanical interaction: $F_{6, 60} = 1.363$, p = 0.244) (Fig. 3A). Similarly, a non-significantly different effect of botanicals was observed on the number of larvae that developed (Fig. 3A; see uppercase superscript letter on each histogram bar). The highest and significantly similar numbers of *T. castaneum* larvae developed in corn and rice flours (Fig. 3A; see lowercase superscript letter on each histogram bar) while the numbers of larvae that developed in wheat flour was significantly lower than other resources (Fig. 3A; see lowercase superscript letter above histogram bar).

In terms of the number of pupae that emerged in each treatment, no significant difference was observed due to resource type ($F_{2, 60} = 2.01$, p = 143), botanicals ($F_{3, 60} = 2.65$, p = 0.057) or their interaction (Resource × Botanical interaction: $F_{6, 60} = 1.10$, p = 0.374) (Fig. 3B).

The botanicals used in different resources had a significant effect on the number of adults that developed and eclosed in each treatment ($F_{3, 60} = 3.01$, p = 0.037) (Fig. 3C). The number of adults

that emerged was observed in control treatments (Fig. 3C; see uppercase superscripts above histogram bars). Highest numbers of adult emerged in untreated flours of all resources followed by those treated with tobacco and eucalyptus (Fig. 3C; see uppercase superscripts above histogram bars). While the highest control against *T. castaneum* adults was recorded in the flour treated with neem (Fig. 3C; see uppercase superscripts above histogram bars).

3.3. Efficacy of A. indica at different concentrations

The results have demonstrated a very significant interaction of both factors together resource × botanical interaction: $F_{5, 48} = 16.65$, p < 0.001), against the number of *T. castaneum* larvae that development (Fig. 4A).

The least number of larvae developed in white flours when treated with all doses of neem (Fig. 4A). Highest number of larvae developed in whole meal flour in control treatment followed by 3% mixture of neem powder and whole meal flour (Fig. 4A).

The rest of the treatments in whole meal flour had statistically similar control against *T. castaneum* development as compared to control treatment in white flour (Fig. 4A).

A very identical trend was observed in regards to number of pupae and adults that developed in each treatment (Fig. 4B and C). The resource type and neem dosage had demonstrated a significant interaction against pupal and adult development ($F_{5, 48}$ = 14.98, p < 0.001 and $F_{5, 48} = 13.90$, p < 0.001, respectively).

4. Discussion

The results have demonstrated that natural plant botanicals have a tendency to control *T. castaneum* population in stored commodities. Ginger and garlic have proved to be an effective botanical against the *T. castaneum* when mixed with seed form of resources (Figs. 2 and 3). These findings are in accordance with earlier studies where garlic and ginger have been proved to be insecticidal against *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae) and *T. castaneum* (Epidi and Odili, 2009), respectively.

Adult mortality was also dependent upon the type of resource used in the experiment. More adults failed to develop and survive in gram pulse and rice grains compared to wheat which demonstrates the better nutritive value of wheat. Similar results were observed in whole meal and white flour of wheat (Fig. 4) suggesting high nutritive value of wheat for *T. castaneum* development and effective against pests.

The inefficacy of neem against larval development and pupation represent that it does not have direct noticeable effect, however, may interact indirectly by deterring the larval feeding or affecting morphological development (Desmarchelier, 1994). The same is obvious from the data on adult survival (Fig. 4) where a significant effect was observed among different rates of neem. At 3%, the data showed much unexpected information. The number of surviving larvae and pupae increases at this rate but on increasing the concentration a higher mortality was observed (Fig. 4). This variability can be explained in terms of selective feeding by larvae due to deterring properties of neem. The probability of feeding on the botanical particle along with flour particle increases with increase in concentration. However, 3% neem mixture might initiate selective feeding by larvae and ultimately their better survival.

Our results are in accordance with many other studies (lqbal et al., 2015; Malik et al., 2012) which have reported *A. indica* to be an effective control against major pest species of stored grain system.

Being eco-friendly and bio-degradable product, botanicals may provide a complementary management method to limit chemical control in stored grain pest management.

5. Conclusion

It is concluded that the use of botanical insecticides can replace synthetic insecticides for many of the destructive stored grains pests. These powders can play an important role in protection of wheat, rice and grams pulse from insect invasion during storage. Their use is safe, environmental friendly, cheap and versatile. More research can be helpful in developing easy and effective application methodologies for pest management in domestic and smallscale field storage, as well as large-scale storage houses.

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