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Biocidal effects of acetone based plant extracts of *Murraya exotica*, *Murraya koenigii* and *Nicotiana tabacum* on stored Grain insect pest, *Tribolium castaneum*

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Abstract

Contact action and repellent effects of the acetone based leaf extracts of *Murraya exotica*, *Murraya koenigii* and *Nicotiana tabacum* were tested against the adult stage of stored grain insect pest, *Tribolium castaneum*. Different concentrations (5, 10 and 15%) were employed and the knockdown effect was checked after a period of 24, 48, 72, 96, 120, 144 and 168 hours while in the case of repellency, the area preference method was used and effect was evaluated after periods of 24, 48 and 72 hours. Experiments to check for the toxic effects revealed that *N. tabacum* (12.95%) proved to be more effective as compared to *M. exotica* (9.53%) and *M. koenigii* (4.31%), respectively. Repellent effect of plant extracts exhibited different trend and *M. exotica* (70.61%) proved to be most effective among the three extracts followed by *N. tabacum* (60.98%) and *M. koenigii* (51.97%) respectively. Toxicological and repellency bioassays proved a definite impact of plant extracts against the adult stage of rust red flour beetle, *T. castaneum*. Plant extracts also proved to be potent to induce knockdown and repellence against stored insect pests and also reflected their potential to be used as alternate of the synthetic insecticides.

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Introduction

Stored grains and processed commodities are always at risk of the insect invasion (Ukeh *et al.*, 2012) and due to insect attack a progressive deterioration in quality and quantity results (Nadeem *et al.*, 2012) which in turn reduces the weight and germination capacity (Phillips and Throne, 2010). Humidity of attacked grains increases (Keskin and Ozkaya, 2013) along with the temperature (Semeao *et al.,* 2012) resulting in fungus development and premature germination (Padin *et al.*, 2013). Unsafe or conventional storage increases the risk of insect attack (Upadhyay and Ahmad, 2011).

Dependence on the synthetic insecticides presents the only solution to cope up with the pest attack and the non-judicial use of these chemicals is aggravating the process of biotype development due to which a progressive increase in resistance has been observed (Perez *et al.*, 2010). Pesticides results negative changes in environment (Meena *et al.*, 2006; Hashim and Davi, 2003) and therefore new methods are under observation which will decrease the dependence on these synthetic chemicals and among possible natural control methods, use of plant extracts seems to be a better one (Isman, 2000).

Plant extracts are rich in secondary metabolites that defend the plant against foreign invasions (Bakkali *et al.*, 2008). Stored grain insects can be effectively controlled with these environmental friendly biocides (Sagheer *et al.*, 2011). Keeping in view of past studies, the present experiments were designed to evaluate the performance of some new plant extracts against the stored grain insect pest, *T. castaneum*.

Materials and methods

Experiments were performed in the Stored Grain Research, Training and Storage Management Cell of Department of Agricultural Entomology, University of Agriculture, Faisalabad, during the year 2012-13.

Insect collection

Mass Collection of Red Flour Beetle, *Tribolium castaneum*, was obtained from godowns and grain market of Faisalabad, Pakistan.

Insect rearing

Collected insects was being kept in the sterilized plastic jars under optimum conditions in an incubator (SANYO incubator MIR-254) having uniform temperature and relative humidity of 28±3°C and 70±5%, respectively. Wheat flour was utilized as culture media for *T. castaneum*. Adults were sieved out and hundred adults were released in each of the plastic jars having 300gm of sterilized flour and covered with muslin cloth. Adults were allowed to mate and lay eggs. After oviposition phase of 5 days, beetles were sieved out of the flour. The flour, having the eggs, was kept under the uniform conditions inside the incubator. Homogenous population was achieved after a time period of 28-35 days as illustrated by Islam and Talukder (2005).

Plant materials

Leaves of *Murraya exotica*, *Murraya koenigii* and *Nicotiana tabacum* were collected from different areas of Faisalabad and Layyah, Pakistan.

Preparation of plant extracts

Shade dried leaves were washed and dried under the shade to get the dried form of plant material. Electric grinder was used to crush the plant material to fine powder and then sieved with a fine mesh sieve. The extraction was made by mixing 50 g of ground sieved sample and 100 ml of acetone and shaking was ensured for 24 hours with the help of Rotary Shaker (IRMICO OS-10), adjusted at 250 rpm. After 24 hours, filtration was made with the help of filter paper. Preliminary extract was subjected to the Rotary evaporator to get 100% stock solution as described by Hasan *et al.* (2005) and Sagheer *et al.* (2013).

Bioassay for percent mortality

Different concentrations (5, 10 and 15%) of acetone based extracts were applied on the filter paper and were allowed to dry for a reasonable time period. Control was maintained by treating the filter paper with acetone only. For examining the percent mortality, 30 adults (15 days old) were taken in the Petri dishes embedded with Whatman's filter paper, covered with lid and tightened with scotch tape on both sides. Small amount of wheat flour was provided to decrease chances of mortality due to starvation. Mortality of adult beetles was being recorded after period of 24, 48, 72, 96, 120, 144 and 168 hours. Experiment was replicated three times and Completely Randomized Design (Factorial) was followed.

Bioassay for percent repellency

Repellency of the plant extracts was checked against the T. castaneum by using the area preference method (Mohana and Fields, 2002) in which filter paper was cut into two equal halves. Different concentrations were made on the one half. After drying, the treated paper was stapled together and was adjusted in the Petri dishes. Twenty adult beetles of *T. castaneum* were released in the center of both halves. Repellency data was taken after a period of 24, 48 and 72 hours. Diet will be provided on both sides (treated and untreated end of filter paper) to decrease mortality due to starvation.

Results

Experimental observations proved a definite impact of acetone based extracts on the mortality and repellency of the target insect.

Table 1. Toxicological effects of plant extracts at three concentrations (5, 10 and 15%) against *Tribolium castaneum.*

	% Mortality ± SE		
Concentrations	*N. tabacum*	*M. exotica*	*M. koenigii*
5	6.29±1.17cd	7.23±1.03c	3.23±0.57d
10	13.54±1.91b	8.52±1.29c	3.39±0.67d
15	19.02±2.52a	12.85±1.55b	6.29±0.98cd

Table 2. Contact toxicity of plant extracts at various exposure periods (24, 48, 72, 96, 120, 144 and 168h) against *Tribolium castaneum.*

	% Mortality ± SE		
Time	*N. tabacum*	*M. exotica*	*M. koenigii*
24	2.59 ± 0.92gh	2.96 ± 1.17gh	0.37 ± 0.37h
48	4.81 ± 1.12fgh	5.18 ± 1.12fgh	1.48 ± 0.58h
72	7.49 ± 1.13efg	6.36 ± 1.46efgh	3.37 ± 0.56fgh
96	14.98 ± 2.49bcd	8.98 ± 1.86def	4.86 ± 0.74fgh
120	16.48 ± 2.68bc	12.36 ± 1.48cde	4.92 ± 0.68fgh
144	19.32 ± 2.78ab	14.60 ± 1.68bcd	6.06 ± 1.28fgh
168	25.00 ± 3.89a	16.28 ± 1.71bc	9.09 ± 1.39def

Table 3. Percent Repellency of plant extracts at various concentrations (5, 10 and 15%) against *Tribolium castaneum.*

	% Repellency ± SE		
Concentrations	*N. tabacum*	*M. exotica*	*M. koenigii*
5	58.88±1.24bc	70.00±4.04ab	40.37±3.78d
10	64.44±3.28abc	67.40±3.03abc	58.51±2.55bc
15	59.62±4.13bc	74.44±3.51a	57.03±4.56c

Contact toxicity of plant extracts

Table 1 shows the effects of different concentrations on the mortality of *T. castaneum*. The knockdown was highest on highest concentration of *N. tabacum* (19.02%) and the lowest was observed in the case of M. koenigii (3.23%). Overall the mortality tended to progress with the increase in the concentration.

Table 2 shows the effects of time intervals on the mortality of *T. castaneum*. Highest mortality was noted at interval of 168h and in case of the *N. tabacum* (25.00%) while the lowest was noted after 24h for the *M. koenigii* (0.37%). Time interval posed a definite impact on the biology of test insect and overall it was proved that it tended to increase the mortality factor.

Table 4. Percent Repellency of plant extracts at various exposure periods (24, 48 and 72h) against *Tribolium castaneum*.

	% Mortality ± SE		
Time	*N. tabacum*	*M. exotica*	*M. koenigii*
24	66.66±3.96b	80.00±2.54a	58.51±3.29bc
48	59.25±1.98bc	69.62±2.51ab	53.70±5.01cd
72	57.03±2.38bc	62.22±2.88bc	43.70±4.09d

Repellent activities of Plant Extracts

Repellence of plant extracts was also proved and it varied according to the. As indicated by Table 3, increased repellence (57.03%) was noted in case of *M. exotica* and lowest was observed in case of *M. koenigii* (40.37%). All the plant extracts showed effects on the repellence against the stored grain insect, *T. castaneum* and increased repellence were noted at higher concentrations.

Table 5. Active ingredients found in the plants used in the bioassay experiments.

Plant	Focal Part	Active Ingredient	Reference
Murraya exotica	Leaf	Murraxocin	Li *et al.*, 2010
Murraya koenigii	Leaf	Koenigine	Ma *et al.*, 2013
Nicotiana tabacum	Leaf	Nicotine	Zhao *et al.*, 2013

Table 4 indicates the effect of time factor on repellence. Overall the 80.00% of repellence was noted in case of *M. exotica*. 43.70 % repellency was showed by *M. koenigii*. Repellency factor tended to decrease with time indicating a low persistence of plant extracts.

Discussion

Use of plant extracts can be helpful for the environmental integrity and conservation and can also help to (Tapondjou *et al.*, 2001; Hasan *et al.*, 2006). Results are in accordance with Gandhi *et al.*, 2010 who evaluated a significant effect of *M. koenigii* on the adult stage of *T. castaneum*. They utilized lower concentrations of the plant powders as compared to our experiment in which higher concentrations of plant extracts were used. Greater number of days was required in their experiment while in our case lower time period posed greater mortality of the test insect. Results are overall in similarity with another research of Gandhi and Pillai (2011) who checked for the extracts of *M. koenigii* on another stored grain insect, *Rhyzopertha dominica* but the mortality is much higher as compared to our experiments. Significant effect on mortality was observed in case of use of powders even at lower concentrations. Higher mortality may be due to the use of crude plant powders which provide greater area of contact. In our experiments *M. koenigii* showed an average mortality of 4.31% which is much lower. Li *et al.* (2010) found the toxic potential of *M. exotica* which was used in China as a medicinal herb. Insecticidal properties of *M. exotica* were tested on the red flour beetle, *T. castaneum* and maize weevil, *Sitophilus zeamais*. Essential oils obtained from the aerial portions of plant was obtained and analyzed by different chromatographic methods, i.e. GC-MS and GC. Active ingredients found in highest concentration were spathulenol (17.7%) followed by α-pinene, caryophyllene oxide and α-caryophyllene present in percentage of 13.3, 8.6 and 7.3%, respectively. Experiments revealed that the natural plant extract has fumigant properties and against both of the test organisms. Our findings are also in accordance with Danjumma *et al.* (2009) who tested for the efficacy of *N. tabacum* powder against the stored grain weevil pest, *Sitophilus zeamais*. Efficacy of the plant extracts can be increased by extracting the natural ingredient or the active metabolites found in them as given in Table 5.

By utilizing the active ingredients, lower concentrations will be required. Synthetic production of these bio-actives can further be helpful in this regard. From the result inferred, it can be concluded that the plant extracts can be used as suitable alternate of synthetic insecticides provided that they are produced at commercial levels and in suitable formulations.

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References

Danjumma BJ, Majeed SB, Yabaya MA, Dike MC, Bamaiyi L. 2009. Effect of Some Plants Powders in the Control of *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae) Infestation on Maize Grains. American-Eurasian Journal of Scientific Research **4,** 313-316.

Bakkali F, Averbeck S, Averbeck D, Idaomar M. 2008. Biological effects of essential oils: A review. Journal of Food Chemistry and Toxicology **46,** 446-475.
http://dx.doi.org/10.1016/j.fct.2007.09.106

Gandhi N, Pillai S. 2011. Control of *Rhyzopertha dominica* (coleoptera: bostrichidae) by pulverized leaves of *Punica granatum* (Lythraceae) and *Murraya koenigii* (Rutaceae). International Journal of Agriculture and Biology **13,** 535–540.

Gandhi N, Pillai S, Patel P. 2010. Efficacy of pulverized *Punica granatum* (Lythraceae) and *Murraya koenigii* (Rutaceae) leaves against stored grain pest *Tribolium castaneum* (Coleoptera: Tenebrionidae). International Journal of Agriculture and Biology **12,** 616–620.

Hasan M, Siddique MA, Sagheer M, Aleem M. 2005. Comparative efficacy of ethanol leaf extracts of *Amaranthus viridis, Salsola baryosma* and Cypermethrin against *Trogoderma granarium* (Everts). Pakistan Journal of Agricultural Sciences **42,** 61-63.

Hasan M, Sagheer M, Ullah A, Wakil W, Javed A. 2006. Response of *Trogoderma granarium* (Everts) to different doses of *Haloxylon recurvum* extract and Deltametrin. Pakistan Entomologist **28,** 25-29.

Hashim MS, Davi KS. 2003. Insecticidal action of the polyphenolic rich fractions from the stem barks of *Streblus asper* on *Dysdercus cingulalus*. Fitoterapia **8,** 670-676.

Islam MS, Talukder FA. 2005. Toxic and residual effects of *Azadirachta indica*, *Tagetes erecta* and *Cynodon dactylon* seed extracts and leaf powders towards *Tribolium castaneum*. Journal of Plant Disease and Protection **112,** 594-601.

Isman MB. 2000. Plant essential oils for pest and disease management. Crop Protection **19**, 603-608.

Li WQ, Jiang CH, Chu SS, Zuo MZ, Liu ZL. 2010. Chemical composition and toxicity against *Sitophilus zeamais* and *Tribolium castaneum* of the essential oil of *Murraya exotica* aerial parts. Molecules **15,** 5831-5839.

Ma Q, Tian J, Yang J, Wang A, Ji T, Wang Y, Su Y. 2013. Bioactive Carbazole Alkaloids from *Murraya koenigii* (L.) Spreng. Fitoterapia **87**, 1-6.

Meena M, Khalequzzaman M. 2006. Toxicity of essential oils against red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) International Journal of Biological Science **14,** 43-48.

Mohana S, Fields PG. 2002. A simple technique to assess compounds that are repellent or attractive to stored-product insects. Journal of Stored Product Research **33**, 23-31.
http://dx.doi.org/10.1016/S0022-474X(00)00039-4

Nadeem M, Iqbal J, Khattak MK, Shahzad MA. 2012. Management of *Tribolium castaneum* (Hbst.) (Coleoptera: Tenebrionidae) using neem (*Azadirachta indica* A. Juss) and tumha (*Citrullus colocynthis*) (L.). Pakistan Journal of Zoology **44,** 325-1331.

Padin SB, Fuse C, Urrutia MI, Bello GMD. 2013. Toxicity and repellency of nine medicinal plants against *Tribolium castaneum* in stored wheat. Bulletin of Insectology **66,** 45-49.

Perez SG, Lopez MAR, Sanchez MAZ, Ortega NCC. 2010. Activity of essential oils as a bio-rational alternative to control coleopteran insects in stored grains. Journal of Medicinal Plant Research **4,** 2827-2835.

Phillips TW, Throne JE. 2010. Bio-rational Approaches to Managing Stored Product. Annual Review of Entomology **55,** 375-397.

Sagheer M, Hasan M, Ali Z, Yasir M, Ali Q, Ali K, Majid A, Khan FZA. 2013. Evaluation of essential oils of different citrus species against *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae) collected from Vehari and Faisalabad districts of Punjab, Pakistan. Pakistan Entomologist **35**, 37-41.

Sagheer M, Hasan M, Latif MA, Iqbal J. 2011. Evaluation of some Indigenous medicinal plants as a source of toxicant, repellent and growth inhibitors against *Tribolium castaneum* (Coleoptera: Tenebrionidae). Pakistan Entomologist **33,** 87-91.

Semeao AA, Campbell JF, Whitworth RJ, Sloderbeck PE. 2012. Influence of Environmental and Physical Factors on Capture of *Tribolium castaneum* (Coleoptera: Tenebrionidae) in a Flour Mill. Journal of Economic Entomology **105,** 686-702.
http://dx.doi.org/10.1603/EC11322

Tapondjou LA, Adler C, Bouda H, Fontem DA. 2001. Efficacy of powder and essential oil from *Chenopodium ambrosioides* leaves as post-harvest grain protectants against six-stored product beetles. Journal of Stored Product Research **38,** 395-402
http://dx.doi.org/10.1016/S0022-474X(01)00044-3

Ukeh DA, Oku EE, Udo IA, Nta AI, Ukeh KA. 2012. Insecticidal Effect of Fruit Extracts from Xylopia aethiopica and Dennettia tripetala (Annonaceae) against *Sitophilus oryzae* (Coleoptera: Curculionidae). Chilean Journal of Agricultural Research **72,** 195-200.

Upadhyay RK and Ahmad S. 2011. Management strategies for Control of Stored Grain Insect Pests in Farmer Stores and Public Ware Houses. World Journal of Agricultural Sciences **7**, 527-549.

Zhao B, Agblevor FA, Ritesh KC, Jelesko JG. 2013. Enhanced production of the alkaloid nicotine in hairy root cultures of *Nicotiana tabacum* L. Plant Cell Tissue Organ Culture **12,** 1-9.