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Research Article

Synergistic Effect of Natural Plants Products as Essential Oils Against Stored Grain Insect Pests *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Abstract

Background and Objective: Due to the uncontrolled use of synthetic pesticides for the management of stored grain insect pests, in the present investigation, three essential oils have been evaluated for their insecticidal activities in binary combination against red flour beetle *Tribolium castaneum*. **Material and Methods:** For repellency and toxicity assay, the different combinations of essential oils were prepared in acetone and applied on filter paper and tested their repellent and toxicity activity against *T. castaneum*. **Results:** Highest repellent activity was observed by a binary combination of *A. marmelos* and *M. arvensis* are 8.6 at 0.40% concentration (χ^2 value = 3.263) ($p<0.05$). In binary combination, the essential oils of *A. marmelos* and *M. arvensis* are more effective against red flour beetles having minimum LC_{50} value against adults was 13.431 µL cm^{-3} at 48 hrs ($F = 31.835$) ($p<0.001$), respectively compared to other essential oils combination. The percent grains infection was reduced 85.69, 67.86 and 76.88 at 60% of the sub-lethal concentration of 48 hrs LC_{50}. **Conclusion:** From the present study it can be concluded that essential oils have a more efficient insecticidal tool against *T. castaneum* as a fumigant in binary combination.

Key words: *Aegle marmelos*, *Mentha arvensis*, *Citrus reticulata*, *Tribolium castaneum*, Insecticidal activity, binary combination, essential oils

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Stored-grain insects are one of the major causes of grain losses during storage[1-3]. Crops loss due to insect pests varies between 10 and 30% of total worldwide annually[4]. The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is one of the common pests found in indoor food storage facilities[5,6]. They can breed throughout the year in warm areas and live for two years during which time female produces nearly 1000 eggs[7,8]. Control of *T. castaneum* populations is primarily dependent on repeated applications of conventional insecticides or fumigants[9,10]. Synthetic chemical pesticides have been in use for the field and post-harvest protection of crops. However, these synthetic insecticides are toxic and show adverse effects to the environment by contaminating soil, water and air and the extensive use of these compounds has led to the development of resistance in several species[11,12]. Given the disadvantage of synthetic insecticides, small scale farmers are more inclined to use traditional approaches to protect their grains. There is a growing interest in research concerning the possible use of plant metabolites i-e essential oils as alternatives to synthetic insecticides that would be readily available, affordable, relatively less toxic and detrimental to humans and the environment[13-15].

The plant is secondary metabolites in the form of essential oils are known to have several biological activities against different insect species[16,17]. The insecticidal activity of essential oils against different stored-product pests has been evaluated and regarded as environmentally compatible[18-22].

The effect of some Rutaceae and Lamiaceae families show insecticidal activity against stored-grain insect pests[23-26]. In a previous study, the repellency and toxicity of *A. marmelos*, *M. arvensis* and *C. reticulata* essential oils have already been determined against *Cryptolestes ferrugineus*, stored-grain pests *T. castaneum* and *Sitophilus oryzae*[27-30].

In continuation of previous work and due to resistant behaviour, recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide in their synergistic effects these three plants have been selected to study their insecticidal activity against *T. castaneum*, one of the most abundant and detrimental insect pest.

MATERIALS AND METHODS

Study area: This study was carried out at Entomology Research Laboratory, Department of Zoology, D.D.U. Gorakhpur University, from 2014-2015.

Plant collection and isolation of essential oils: For the extraction of essential oils, leaves of *A. marmelos*, *M. arvensis* and peel of *C. reticulata* were collected from the local area of the city, India. The leaves and peels were dried in absence of sunlight at room temperature (30±5°C) and grounded using a mixer. The essential oils were extracted by hydro-distillation using a modified Clevenger apparatus with distilled water. Distillation was done continuously for five hours to yield essential oils. Anhydrous sodium sulphate was used to remove water after extraction. The superior phase was collected from the condenser in glass containers and stored in an Eppendorf tube at 5°C until their use for further experiments.

Insect rearing: Stock cultures of *T. castaneum* were maintained in an incubator at 30±2°C, 75±5% RH and a photoperiod of 10:14 (L:D) without exposure to any insecticides. *T. castaneum* adults were reared on grain and flours of wheat (*Triticum aestivum* L.) at 12-13% moisture content. Ten days old unsexed adults of *T. castaneum* were used to determine the insecticidal property of *A. marmelos*, *M. arvensis* and *C. reticulata* essential oils in binary combination i.e., *A. marmelos*, *M. arvensis* (1:1 ratio), *A. marmelos* and *C. reticulata* (1:1 ratio) and *M. arvensis* and *C. reticulata* (1:1 ratio).

Repellency: The repellency test used was adopted via following Mishra and Tripathi[27], Mishra *et al.*[29], study. Four solutions of 0.10, 0.20, 0.30 and 0.40% of binary combination of *A. marmelos*, *M. arvensis* and *C. reticulata* essential oils in (1:1) ratio, were prepared by dissolving essential oils in acetone. Whatman no. 1 filter papers were cut into two halves one half of each dish was treated with the binary combination of essential oils solution as uniform as possible by using a micropipette. The other half of the filter paper was treated with acetone only. The essential oils treated and acetone treated filter papers halves were dried to evaporate the solvent completely. Essential oils treated and acetone treated half-dishes were then attached lengthwise, edge-to-edge with adhesive tape and placed at the bottom in the glass petri dish (height 15 mm×radius 45 mm). Twenty adults of insects were released at the centre of the Petri dishes and then Petri dishes were covered and kept in dark. Six replicates were set for each concentration of essential oils. The number of insects on both treated and untreated halves was recorded after four hours in the mild light.

Toxicity: The toxic effect of the binary combination of *A. marmelos*, *M. arvensis* and *C. reticulata* essential oils were tested against adults of *T. castaneum* by fumigation action. A filter paper strip (2 cm^2) treated with a solution of a binary combination of both essential oils (prepared in acetone) was pasted on the inner surface of the cover of each Petri dish using a micropipette. Twenty adults taken from the laboratory culture were placed with 5 g of wheat flour in Petri dishes. Flour was spread uniformly along the whole surface of the Petri dishes. All the closed Petri dishes were kept in the dark and six replicates were set for each concentration. After 48 hrs, adult mortality was recorded.

Chronic toxicity: The chronic toxicity of the binary combination of *A. marmelos*, *M. arvensis* and *C. reticulata* essential oils was tested against adults of *T. castaneum* by fumigation action with sub-lethal concentration (30 and 60% of LC_{50} of 48 hrs). The methodology used was the same as that used in determining the toxic effect of adult mortality of *T. castaneum*.

Data analysis: A chi-square test was applied to establish the repellent activity of the essential oils tested. The Lethal concentration (LC_{50}), lower and upper confidence limits (LCL-UCL), Slope value, t-ratio, g-value heterogeneity factor and chi-square value were calculated using computer software of Robertson *et al.*[31]. Correlation and linear regression analysis were conducted to define all dose-response relationships. Analysis of variance was performed to test the equality of regression coefficient.

RESULTS

Repellency: In repellency assay, percentage of *T. castaneum* in treated filter paper disc half was 8.6 at 0.40% concentration of *A. marmelos* and *M. arvensis* (1:1) ratio of essential oils followed by 16.8 at 0.40% concentration of *M. arvensis* and *C. reticulata* (1:1) ratio and 12.7 at 0.40% concentration of *A. marmelos* and *C. reticulata* essential oils (1:1) ratio in Table 1.

Toxicity: Fumigation of *T. castaneum* adults with binary combination of essential oils caused toxicity by vapour action. In binary combination of *A. marmelos* and *M. arvensis* (1:1) ratio of essential oils median lethal concentration (LC_{50}) against adult were 13.431 µL cm^{-3}, followed by 18.483 µL cm^{-3} against *M. arvensis* and *C. reticulata* and 15.362 µL cm^{-3} against *A. marmelos* and *C. reticulata* at 48 hrs against *T. castaneum*, respectively in Table 2.

The t-ratio values were greater than 1.96, indicating a significant regression of each dose-response line. The heterogeneity factor was less than 1.0, demonstrating that the log-dose-probit lines are within the 95% confidence limits and thus the model fittest the data. Value of g less than 0.5 indicated that mean was within the limit at all probability levels of 90, 95 and 95%.

Concerning adult mortality, regression analysis showed a concentration-dependent significant positive correlation of *A. marmelos* and *M. arvensis* (F = 31.835) ($p<0.001$), *M. arvensis* and *C. reticulata* (F = 18.184) ($p<0.001$) and *A. marmelos* and *C. reticulata* (F = 39.655) ($p<0.001$) in Table 3.

Chronic toxicity: The percent grains infection was reduced by the combination of *A. marmelos* and *M. arvensis* (1:1) ratio of essential oils were 85.69%, followed by 67.86% against *M. arvensis* and *C. reticulata* and 76.88% against *A. marmelos* and *C. reticulata* at 60% of the sub-lethal concentration of 48 hr LC_{50}, respectively in Table 4.

Table 1: Repellency caused by the binary combination of essential oils against adults of *Tribolium castaneum* after four hours in filter paper test

Combination of essential oils	Con. (%) vol: vol	Mean (%) of insect untreated±SE	Mean (%) of insect treated±SE	χ^2 value p<0.05 (df = 5)
Aegle marmelos+Mentha arvensis	0.10	58.3±4.12	41.7±4.12	0.108^{NS}
	0.20	71.6±3.01	28.4±3.01	0.932^{S}
	0.30	79.3±3.60	20.7±3.60	1.582^{S}
	0.40	91.4±3.31	8.6±3.31	3.263^{S}
Mentha arvensis+Citrus reticulata	0.10	63.8±3.90	36.2±3.90	0.581^{NS}
	0.20	72.5±4.92	27.5±4.92	1.283^{S}
	0.30	79.3±5.71	20.7±5.71	1.439^{S}
	0.40	83.2±2.56	16.8±2.56	2.581^{S}
Aegle marmelos+Citrus reticulata	0.10	50.66±2.13	49.34±2.13	0.052^{NS}
	0.20	68.20±3.07	31.80±3.07	0.785^{S}
	0.30	74.05±2.10	25.95±2.10	1.235^{S}
	0.40	87.39±0.42	12.61±0.42	2.873^{S}

Adult of *Tribolium castaneum* was used in filter paper repellency assay. For each concentration of essential oils, six replicate were carried out and ten adults were used per replicate, mean of untreated and treated halves in filter paper repellency assay, NS: Not significant as the calculated values of χ^2 value was less than the table values at probability levels 99% and S: Significant at probability level 99%

Table 2: Summary of binary combination essential oils in toxicity assays against adults of *Tribolium castaneum* after 48 hrs

Combinations	Parameters (%)	Exposure period (hrs)	LC_{50} (µL)[a]	LCL-UCL[b]	g-value[c]	t-ratio[c]	Heterogeneity[c]	Chi square
Aegle marmelos+Mentha arvensis	Adult mortality	48	13.437	10.829-16.676	0.231	4.809	0.281	2.495
Mentha arvensis+Citrus reticulata	Adult mortality	48	18.483	11.253-21.035	0.293	2.585	0.175	3.212
Aegle marmelos+Citrus reticulata	Adult mortality	48	15.362	11.758-18.994	0.252	3.720	0.015	2.495

[a] LC_{50}: Median lethal concentration, [b] UCL-LCL: Upper confidence limit and lower confidence limit, [c] g-value, [c] t-ratio and [c] Heterogeneity were significant at all probability levels (90, 95 and 99%)

Table 3: Regression parameters of lethal activity on stored-grain insect pest *Tribolium castaneum* with essential oils in binary combination

Treatments	Parameters (%)	Intercept	Slope	Regression equation	Regression coefficient	f-value
Aegle marmelos+Mentha arvensis	Adult mortality	-6.756	4.762	Y = -6.756+4.762	0.995	31.835
Mentha arvensis+Citrus reticulata	Adult mortality	-10.549	5.930	Y = -10.549+5.930	0.999	18.184
Aegle marmelos+Citrus reticulata	Adult mortality	-5.457	4.916	Y = -6.756+4.916	0.998	39.655

Regression analysis was performed between different concentrations of essential oil and responses of the insect pest, *significant at 99% probability level, *f-values were significant at all probability levels (90, 95 and 99%), *df = 3, 20 and **df = 2, 20

Table 4: Effect of fumigation of essential oils in binary combination on damage caused by *Tribolium castaneum*

Essential oil (binary combination)	48 hrs LC_{50} (µL)	Treatments	Grain infected (Mean±SE) (%)
Aegle marmelos	13.437 µL	30% of 24 hrs LC_{50}	53.28±0.69
Mentha arvensis		60% of 24 hrs LC_{50}	85.69±0.33
Mentha arvensis	18.483 µL	30% of 24 hrs LC_{50}	47.22±0.63
Citrus reticulata		60% of 24 hrs LC_{50}	67.86±0.67
Aegle marmelos	15.362 µL	30% of 24 hrs LC_{50}	51.92±0.42
Citrus reticulata		60% of 24 hrs LC_{50}	76.88±0.56

DISCUSSION

Different essential oils and their chemical component have been evaluated for their role in the IPM programme. *M. arvensis* oil containing linalool and linalyl acetate exhibits repellency and fumigant toxicity against stored-grain insect pests[29]. Although the repellent activity of essential oils is generally attributed to some particular compound, a synergistic phenomenon among these metabolites may result in a higher bioactivity compound to the isolated compound[32]. This indicates that minor constituents also contribute to repellent activity and reflects the importance of compositional complexity in conferring bioactivity to the natural mixture.

Linalool has been demonstrated to act on the nervous system affecting ion transport and the release of acetylcholine esterase in insects[33]. Jianhua *et al.*[28] has studied the effect of citrus peel oils against stored-grain insect pests. Several compounds including the major component of all citrus peel oils, limonene are insecticidal[34]. In the present study binary combination of *A. marmelos*, *M. arvensis* and *C. reticulata* essential oils have been evaluated for their repellency, toxicity, in binary combination against flour beetle *T. castaneum*. The result of the present study, indicate that a binary combination of these three essential oils has insecticidal properties against stored product pest in synergistic form as evidenced by their low LC_{50} value in the present study as compared to the activity of essential oils used alone in the previous study[28-30]. The present study is based on the evidence that essential oils are a complex mixture of the volatile organic compound of different chemical groups and insects cannot develop resistance against this due to their synergism of all compounds. These essential oils alone also caused fumigant toxicity in adults and larvae both[35-37]. Lee *et al.*[38] have reported toxicity of menthol, methionine, limonene, α-pinene, β-pinene and linalool against *Sitophilus oryzae* and proved that these essential oil components exert their toxicity by inhibiting acetylcholine esterase enzyme. In reducing the dose of essential oils, targeting multiple sites of action and resistance in insects, synergism can play an important role.

These earlier reported finding[39] support the result of the present study. Rapid action of essential oils and its component against insect pests is indicative of neurotoxic actions[16,40]. The mode of action of these essential oils is yet to be confirmed but it appears that the death of the adults may be due to the suffocation and inhibition of different biosynthetic processes of the insect metabolism[30]. The result indicated that *A. marmelos*, *M. arvensis* and *C. reticulata* used singly deterred its insecticidal activity. This combined action of repellency, toxicity will therefore after greater protection of stored grain against flour beetle.

Thus, it can be suggested that fumigants from volatile oils of plant origin in synergistic form could have greater potential in future based on their efficacy, economic value and use in large-scale storage.

CONCLUSION

In conclusion, it can be suggested that natural plant products in form of volatile essential oils in synergistic form could have greater potential compared to other insecticides in future based on their efficacy, economic value and use in large-scale storage.

SIGNIFICANCE STATEMENT

This study discovers the possible synergistic effect of natural plant products in the form of essential oils that can be beneficial for the management of stored grain insect pests. This study will help the researcher to the used a binary combination of natural plant products due to the development of resistance against insecticides.

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