

Formulation based management of plants product as essential oils against stored grain insect pests *Sitophilus oryzae* L. (Coleoptera: Curculionidae)

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ABSTRACT

One of the primary insects of stored goods that causes significant losses globally is *Sitophilus oryzae* L. (Coleoptera: Curculionidae), a pest of stored grains. Utilization of synthetic pesticides, which are effective but also dangerous for the environment and human health, and has caused stored product pests to acquire resistance and disrupt the balance of ecosystem. As a result, creation of novel natural insect-control methods utilizing plants products in form of plant essential oils in a binary combination to control *S. oryzae* as a substitute to chemical. The Preference index (PI) was observed by binary combination of *Aegle marmelos* and *Mentha arvensis* are -0.95 at 0.40% concentration (χ^2 value=3.582) ($P<0.05$) with 95.35 per cent repellency. In *A. marmelos* and *M. arvensis* binary combination of essential oils it also showed, minimum LC₅₀ value of 10.296 $\mu\text{l}/\text{cm}^3$ with maximum toxicity against adults at 48 hrs ($F=28.358$) ($P<0.001$), respectively comparison to other essential oils combinations. With the sub-lethal concentration 60% of 48 hrs. LC₅₀ value, the binary combination of essential oils showed an antifeedant effect, reaching 85.69% for *S. oryzae*. In accordance with the present investigation, insecticides derived from plants can be employed as a substitute for chemical pesticides in formulations, that measure their insecticidal effectiveness against *S. oryzae* as fumigants in the binary combinations.

Keywords: *Aegle marmelos*, *Mentha arvensis*, *Citrus reticulata*, *Sitophilus oryzae*, Insecticidal activity, Formulation, Essential oils.

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INTRODUCTION

In India, it is estimated that the food grain production was 308.65 million tonnes, for the year 2020–2021 where wheat 159.08 and rice 149.56 million tonnes respectively (Ministry of Agriculture & Farmers Welfare of Government of India, 2021). Food and Agriculture Organization of the United Nations (FAO) reported that total, 15 to 20% of the world food production is destroyed during storage by Stored-grain insects (Bouchelos, 2018; Parera, 2021; Kumar *et al.*, 2021). The Insect infestation of stored agricultural products due to insect pests causes significant quantitative and qualitative damage (Stathas *et al.*, 2023). They degraded in quality, due to the presence of insects or the presence of their body parts in the products

(Stathas *et al.*, 2023). The rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) is a major pest of stored sorghum in India usually found in grain storage facilities or processing plants, infesting wheat, oats, rye, barley, rice, and corn (Jamwal, 2022; Chaubey, 2024). They cause heavy losses of stored grains, especially cereals, at conditions favorable to their development (25–35°C and low RH). Both, the adults and larvae are voracious feeder on a different variety of grains and seeds (Campbell, 2005). They proliferate due to favorable storage conditions, temperature and humidity and availability of food in abundance. It is also a serious issue in processed products and packed commodities.

For the control of insect pest synthetic pesticides are used for the field and post-harvest protection of crops. The infestation with stored grain insect affects commercial value of stored cereals (Sinha and Sinha, 1992; Banga *et al.*, 2020). However, these synthetic pesticides are expensive, ineffective, and harmful to the environment. The major problem is if used continuously, can cause environmental problems, danger to human health, pollution, and has led to resistance development in stored product pests and disrupt the balance of the ecosystem. Given the disadvantage of synthetic insecticides, small scale farmers are more inclined to use traditional approaches to protect their grains for development of new natural insect-control alternatives with natural plants inform of plant essential oils as alternatives to synthetic insecticides that would be readily available, affordable, relatively less toxic and detrimental to humans and the environment (Souto *et al.*, 2020; Raveau *et al.*, 2020; Khursheed *et al.*, 2022).

Plants may provide potential alternatives to currently used insect-control agents because they constitute a rich source of bioactive chemicals. These plants are secondary metabolites in the form of essential oils have several biological activities. The insecticidal activity of essential oils against different stored-product pests has been evaluated and regarded as environmentally compatible (Chaubey, 2007; Mishra *et al.*, 2016; Fouad and Camara, 2017; Demeter *et al.*, 2021; Khursheed *et al.*, 2022).

The effect of some Rutaceae and Lamiaceae family show insecticidal activity against stored-grain insect pests (Kabrambam *et al.*, 2021; Mishra *et al.*, 2012 and 2016; Ebadollahi *et al.*, 2020). In our previous study, the repellency and toxicity of *A. marmelos*, *M. arvensis* and *C. reticulata* essential oils have already been determined against stored-grain pests and *Sitophilus oryzae* (Mishra and Tripathi., 2011; Mishra *et al.*, 2011, 2012, 2016). In continuation of previous work and due to resistant behavior of insects, recently increased interest to developing plant origin insecticides as an alternative to chemical insecticide. The finding of the present

work, can be concluded the essential oils have more efficient and good alternative for management of stored-grain insect pest in binary combination in formulation based.

MATERIALS AND METHODS

Plant collection and isolation of essential oils

The *A. marmelos*, *M. arvensis* leaves as well as the *C. reticulata* peel had been collected from the local area of Madhepura district of Bihar situated in the Plains of River Koshi located in the Northeastern part of Bihar at latitude between 25°.34 to 26°.07' and longitude between 86°.19' to 87°.07' for formulation based investigation. After being allowed to dry at room temperature (30±5°C) without exposure to sunlight, leaves and peels had been crushed utilizing a mixer. The essential oils were extracted by hydro-distillation using a modified Clevenger apparatus with distilled water. To produce essential oils, 5hours of continuous distillation had been conducted. Anhydrous sodium sulphate was used to remove water after extraction. After being extracted from the condenser as well as placed in glass containers, the superior phase was kept at 5°C in an appendorff tube until it was needed for future investigation.

Insect rearing

S. oryzae stock culture were maintained in an incubator at 30±20C, 75±5% RH and at photoperiod of 10:14 (L:D) without exposure to any insecticides. Adult insects had been reared in *Oryza sativa* as well as *Triticum aestivum* grain and flours with a moisture level of 12–13 per cent. *M. arvensis*, *A. marmelos*, as well as *C. reticulata* essential oil's insecticidal properties have been examined in 10 day old unsexed adults of *S. oryzae* utilizing formulation-based methods, that include *A. marmelos* and *M. arvensis* (1:1 ratio), *A. marmelos* and *C. reticulata* (1:1 ratio), along with *M. arvensis* and *C. reticulata* (1:1 ratio).

Repellency

For repellent activity the method was adopted McDonald *et al.*, 1970 as modified by Talukder and Howse., 1993. Four solutions of 0.10%, 0.20%, 0.30% and 0.40% in formulation 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 equal halves one half of each dish was treated with binary combination of essential oils solution as uniform as possible by using micro pipette. 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 glass petri dish (height 15 mm $\times$ radius 45 mm). Twenty adults of insects were released at the center of the petri dishes and then petri dishes were covered and kept in dark. Six replicates were set for each concentration of essential oils. Number of the insects on both treated and untreated halves was recorded after four hours in mild light.

Toxicity

The toxic effect of binary combination of *A. marmelos*, *M. arvensis* and *C. reticulata* essential oils were tested against adults of *S. oryzae* by fumigation action. A filter paper strip (2 cm²) treated with solution of 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 10 g of rice 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 binary combination of *A. marmelos*, *M. arvensis* and *C. reticulata* essential oils was tested against adults of *S. oryzae* by fumigation action with sub lethal concentration (30% and 60% of LC₅₀ of 48 hrs). The methodology used was the same as that used in determining the toxic effect of adult mortality of *S. oryzae*.

Data analysis

A chi-square test was applied to establish the repellent activity of the essential oils tested Sokal and Rohlf., 1973. The Lethal concentration (LC₅₀), 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.*, 2007. Correlation and linear regression analysis were conducted to define all dose-response relationships Sokal and Rohlf., 1973. Analysis of variance was performed to test the equality of regression coefficient Sokal and Rohlf., 1973.

RESULTS

Repellency

In repellency assay, percent repellency of *S. oryzae* in treated filter paper disc half was 95.35% at 0.40% concentration of *A. marmelos* and *M. arvensis* (1:1) ratio of essential oils followed by 90.00% at 0.40% concentration of *M. arvensis* and *C. reticulata* (1:1) ratio and 84.30% at 0.40% concentration of *A. marmelos* and *C. reticulata* essential oils (1:1) ratio with Preference index (PI) -0.95, -0.90 and -0.84 from *A. marmelos* and *M. arvensis*, *M. arvensis* and *C. reticulata* and *A. marmelos* and *C. reticulata* respectively (Table 1).

Toxicity

Fumigation of *S. oryzae* adults with formulations 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 concentrations (LC₅₀) against adult were 10.296 μ l/cm³, followed by 14.114 μ L/cm³ against *M. arvensis* and *C. reticulata* and 17.113 μ L/cm³ against *A. marmelos* and *C. reticulata* at 48 h. against *S. oryzae* respectively (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

Table 1. Repellency caused by *Aegle marmelos*, *Mentha arvensis* and *Citrus reticulata* in formulation against adults of *Sitophilus oryzae* after four hours in filter paper test

Combination of essential oils	Concentration (%) vol: vol	Mean % of insect Untreated $\pm$ SE	Mean % of insect treated $\pm$ SE	Preference Index**	χ^2 value $P < 0.05$ (df=5)
<i>Aegle marmelos</i> + <i>Mentha arvensis</i>	0.10%	66.60 $\pm$ 3.30	33.30 $\pm$ 3.30	-0.66	0.944 ^{NS}
	0.20%	74.30 $\pm$ 2.01	25.60 $\pm$ 2.01	-0.74	1.651 ^S
	0.30%	83.00 $\pm$ 2.20	17.00 $\pm$ 2.20	-0.83	3.555 ^S
	0.40%	95.35 $\pm$ 0.36	04.65 $\pm$ 0.36	-0.95	3.582 ^S
<i>Mentha arvensis</i> + <i>Citrus reticulata</i>	0.10%	55.00 $\pm$ 0.43	45.00 $\pm$ 0.23	-0.55	0.038 ^{NS}
	0.20%	56.60 $\pm$ 4.10	43.30 $\pm$ 4.10	-0.56	0.104 ^S
	0.30%	80.00 $\pm$ 5.10	20.00 $\pm$ 5.10	-0.80	2.679 ^S
	0.40%	90.0 $\pm$ 2.50	10.0 $\pm$ 2.50	-0.90	2.698 ^S
<i>Aegle marmelos</i> + <i>Citrus reticulata</i>	0.10%	50.00 $\pm$ 3.60	50.00 $\pm$ 3.60	-0.50	0.038 ^{NS}
	0.20%	60.20 $\pm$ 0.365	39.80 $\pm$ 0.365	-0.60	0.200 ^{NS}
	0.30%	75.00 $\pm$ 3.30	25.00 $\pm$ 3.30	-0.75	1.779 ^S
	0.40%	84.30 $\pm$ 4.10	15.70 $\pm$ 4.10	-0.84	2.956 ^S

Adult of *S. oryzae* were 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 were less than the table values at probability levels 99%. S: Significant at probability level 99%.

**Preference index (PI) = Percentage of insects in treated halves - percentage of insect in untreated halves / Percentage of insects in treated halves + percentage of insect in untreated halves).

Table 2. Summary of *Aegle marmelos*, *Mentha arvensis* and *Citrus reticulata* essential oils in formulation based toxicity assays against adults of *Sitophilus oryzae* after 48 hrs

Combination	^a LC ₅₀ (μ L)	LCL-UCL ^b	g-value ^c	t-ratio ^c	Heterogeneity ^c	Chi square
<i>Aegle marmelos</i> + <i>Mentha arvensis</i>	13.437	10.829-16.676	0.231	4.809	0.281	2.495
<i>Mentha arvensis</i> + <i>Citrus reticulata</i>	18.483	11.253-21.035	0.293	2.585	0.175	3.212
<i>Aegle marmelos</i> + <i>Citrus reticulata</i>	15.362	11.758-18.994	0.252	3.720	0.015	2.495

^aLC₅₀ represent the median lethal concentration. ^bUCL and LCL represent upper confidence limit and lower confidence limit. ^cg-value, t-ratio and heterogeneity were significant at all probability levels (90%, 95%, 99%).

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, 95%. With regard to adult mortality, regression analysis showed a concentration dependent significant positive correlation of *A. marmelos* and *M. arvensis* ($F = 28.358$) ($P < 0.001$), *M. arvensis* and *C. reticulata* ($F = 25.877$) ($P < 0.001$) and *A. marmelos* and *C. reticulata* ($F = 42.886$; $P < 0.001$) (Table 3).

Chronic Toxicity

The per cent grains infection was reduced by combination of *A. marmelos* and *M. arvensis* (1:1) ratio of essential oils were 89.25 %, followed by 78.86 % against *M. arvensis* and *C. reticulata* and 69.88 % against *A. marmelos* and *C. reticulata* at 60% of sub-lethal concentration of 48 hrs LC₅₀, respectively (Table 4).

DISCUSSION

In botanical extracts used as insecticides, the essential oils and their active component have

Table 3. Regression parameters of lethal activity on stored-grain insect pest *Sitophilus oryzae* with *Aegle marmelos*, *Mentha arvensis* and *Citrus reticulata* essential oils in formulation based by fumigation method

Treatment	Intercept	Slope	Regression Equation	Regression coefficient	F-value
<i>Aegle marmelos</i> + <i>Mentha arvensis</i>	-6.756	4.762	$Y = -6.756 + 4.762$	0.995	31.835
<i>Mentha arvensis</i> + <i>Citrus reticulata</i>	-10.549	5.930	$Y = 10.549 + 5.930$	0.999	18.184
<i>Aegle marmelos</i> + <i>Citrus reticulata</i>	-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;

**df=2, 20.

Table 4. Effect of fumigation of *Aegle marmelos*, *Mentha arvensis* and *Citrus reticulata* essential oils in formulation based on damage caused by stored-grain insect pest *S. oryzae* infestation, Mean $\pm$ SE

Essential oil	48 hr LC ₅₀ (μ l)	Treatments	% grain infected
<i>Aegle marmelos</i> + <i>Mentha arvensis</i>	13.437	30% of 48h LC ₅₀	57.53 $\pm$ 1.63
		60% of 48h LC ₅₀	89.25 $\pm$ 1.32
<i>Mentha arvensis</i> + <i>Citrus reticulata</i>	18.483	30% of 48h LC ₅₀	51.28 $\pm$ 1.53
		60% of 48h LC ₅₀	78.86 $\pm$ 0.67
<i>Aegle marmelos</i> + <i>Citrus reticulata</i>	15.362	30% of 48h LC ₅₀	48.97 $\pm$ 1.42
		60% of 48h LC ₅₀	69.88 $\pm$ 1.53

promising alternative to chemical pesticides in Insect Pest Management Programme. They play a key role in plant signaling process. Now a days many types of plants are used as insecticides for control of stored-grain insect pests (Isman, 2007; Asmanizar and Djamin., 2012; Demeter *et al.*, 2021). Although 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. This indicates that minor constituents also contribute to repellent activity and reflects the importance of compositional complexity in conferring bioactivity to natural mixture.

The mode of action of essential oils acts on the nervous system affecting ion transport and the release of acetylcholine esterase in insects (Barocci *et al.*, 2000) which hydrolyzes

acetylcholine responsible for signal (Lopez and Pascual villalibos., 2010). Mishra *et al.* (2011) has studied effect of citrus peel oils against stored-grain insect pests. Several compounds including the major component of all citrus peel oils, limonene has been found to be insecticidal (Mursiti, 2019; Ciriminna *et al.*, 2021; Showler *et al.*, 2019). In the present study, formulation of *A. marmelos*, *M. arvensis* and *C. reticulata* essential oils in different ratio have been evaluated for their repellency, toxicity against rice weevil *S. oryzae*. It is found that the essential oils inhibit gamma-aminobutyric acid (GABA) receptors that are the primary neurotransmitter of CNS of insects (Grünewald and Siefert., 2019). The result of present study, indicate that formulation of these three essential oils in different ratio have insecticidal properties against stored product pest in synergistic form as evidence by their low LC₅₀

value with maximum toxicity against *S. oryzae* as compared to alone used in previous research (Mishra and Tripathi., 2011; Mishra *et al.*, 2011; Mishra *et al.*, 2012; Mishra *et al.*, 2012; Fouad, and da Camara, 2017; Yang *et al.*, 2020). Present study is based on the evidence that essential oils are a complex mixture of volatile organic compound with different chemical groups and insect cannot develop resistance against this due to their synergism form. These essential oils alone also caused fumigant toxicity in adults and larvae both (Mishra and Tripathi, 2011; Mishra *et al.*, 2011, 2012). Lee (2001) have reported toxicity of menthol, methonene, limonene, α -pipene, β -pipene and linalool against *S. oryzae* and proved that these essential oil components exert its toxicity by inhibiting acetylcholine esterase enzyme. After reducing the dose of essential oils, target multiple site of action and resistance in insects, formulation can play an important role.

The previous finding clearly supports the result of the present study. Rapid action of essential oils and its component against insect pests is an indicative of neurotoxic actions (Youssef, 1997; Yang Jeong, 2008). The mode of action of these essential oils is yet to be confirmed but it appears that death of the adults may be due to the suffocation and inhibition of different biosynthetic processes of the insect metabolism (Don-Perdo, 1997). The result indicated that *A. marmelos*, *M. arvensis* and *C. reticulata* used singly deterred its insecticidal activity. The combined action of repellency, toxicity will therefore after greater protection of stored-grain against rice weevil. Thus, it can be suggested that due to resistant nature of insects fumigants from volatile oils of plant origin in formulation form could have greater potential in future on the basis of their efficacy, economic value and use in large-scale storage.

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