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FUMIGANT TOXICITY OF NATURAL PLANT PRODUCTS IN FORM OF ESSENTIAL OILS AGAINST PULSE BEETLE *CALLOSOBRUCHUS CHINENSIS* (L.) (COLEOPTERA:BRUCHIDAE)

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ABSTRACT: The cowpea weevil, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae), is an important pest of stored cowpea, *Vigna ungiculata* (L.) Walpers (Fabales: Fabaceae), with ample distribution in tropical and subtropical regions. Many plant essential oils have a broad-spectrum activity against pest insects, and these oils traditionally have been used in the protection of stored products. The present investigation was aimed to assess the impact of two essential oils for their insecticidal activity against *C. chinensis* and they were tested in laboratory. In this study, the lethal and sub-lethal effects of essential oils from *Aegle marmelos* (L) Correa (Rutaceae) and *Citrus reticulata* (Rutaceae) were evaluated against the larvae and adults of *C. chinensis* at 30 ± 2° C, 75 ± 5% RH, and a photoperiod of 10:14 L:D. The LC_{50} values of *A. marmelos* and *C. reticulata* were 14.351 µL and 15.231 µL after 72 hr against adults and 13.296 µL and 14.997 µL after 72 hr of exposure against larvae, respectively. Therefore, these essential oils can be suggested for controlling *C. chinensis* in storage systems.

INTRODUCTION

In developing countries, loss of cereal foods are considered the major problem due to pest infections through storage[1,2,3].With the beginning of agricultural practices, storage of food grains started as a safeguard against poor harvests and famine. Simultaneously, insect pests also started damaging stored grains, both quantitatively and qualitatively. At present, this damage accounts for 10–40% loss in countries depending on traditional storage technologies and has created a major problem in grain storage. Among the insect pests attacking stored pulses, the pulse beetle *Callosobruchus chinensis* L. is a serious one[4]. This insect has been reported from the Phllippines, Japan, Indonesia, Srilanka, Burma, India and Bangladesh. It is a notorious pest of chickpea, mung, cowpea, garden pea, black gram, lentil and arhar. The extent of damage to pulse seeds is very high both qualitatively and quantitatively[5]. The insect multiplies very rapidly in storage where it causes very high losses reach 50% after six month storage[6,7,8]. Therefore, protection of stored-grain agricultural products from insect infestation is quite essential.

To mitigate the economic damage chemical pesticides have been used from long time but residues of such pesticides accumulate at different tropic levels in food chain and exceed the tolerance limit. Many insects have acquired resistance against various synthetic pesticides. The uncontrolled use of these synthetic pesticides caused great hazards due to their persistent nature and also various biochemical and behavioral changes in non-target animals like humans[9,10,11,12]. The continuous use of chemical pesticides for control of stored grain pests has resulted serious problems such as hazards to the environment including human health and non-target organisms[13], residue in food grains[14], environmental pollution[15] and development of resistant strains[16,17,18,19]. Hence, there is urgent need for safe but effective, biodegradable pesticides with no toxic effect on non-target organisms.

From last two decades, botanical pesticides especially essential oils have been used for insecticidal activities against stored-grain insect pests.

Aegle marmelos L. (Rutaceae) and *Citrus reticulata* (Rutaceae) are important botanical products that available easily in low cost will be used in the form of natural pesticides have insecticidal, antimicrobial, antifungal and antibacterial activities[20,21,22]. Plant materials which arc being traditionally used by some farmers are quite safe and appear to be the most promising as grain protestants. The use of plant products are cheaper, they are easily available and safe. Keeping these views in mind, the present experiment was designed to investigate the insecticidal potency of some medicinal plants products against the pulse beetle on black gram seeds.

MATERIALS AND METHODS

Plant collection and isolation of essential oils

For the extraction of essential oils, leaves of *A. marmelos* and peel of *C. reticulata* was collected from the local area of the city. The leaves and peels were dried in absence of sun light 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 appendorff tube at 5°C until their use for further experiments.

Insect rearing

Larvae and adults of *C. chinensis* were reared in departmental laboratory and stock cultures maintained in an incubator at 30±2^{0}C, 75±5% RH and at photoperiod of 10:14 (L:D) without exposure to any insecticides. *C. chinensis* adults were reared on Cow pea *(Vigna unguiculata)* at 12–13% moisture content. Ten days old unsexed adults and newly larvae of *C. chinensis* were used to determine the insecticidal property of *A marmelos* and *C. reticulata* essential oils.

Adult mortality

Glass vials (10 cm long and 3 cm diameter) with polystyrene cap were used for the toxicity assay against adults of the pest. For fumigation, one filter paper strip (2 cm^2) treated with solution of different concentrations of essential oils prepared in acetone (15, 20, 25, and 30 ml in 100 ml), was pasted on the inner side of cap. Twenty grams of cow pea grains were taken in the each vial and twenty adults were transferred to each vial and the open end of the vial was closed by the cap so that the oil treated filter paper remained inside the vial. For each type of essential oil four different concentrations and for each concentration six replicated were used. The vials were kept at 30±2^{0}C, 75±5% RH and at photoperiod of 10:14 (L:D) hours. Mortality of the adults were recorded after 48 h of treatment up to 72 h. In control, untreated filter paper was used.

Larval mortality

The toxic effect of *A. marmelos* and *C. reticulata* essential oils were tested against larvae of *C. chinensis* by fumigation action. The methodology used was the same as that used in determining the toxic effect of adult mortality of *C. chinensis*.

Chronic Toxicity

In this assay, adults were treated with two sub-lethal concentration viz. 40 and 60% of 48 h LC_{50} of essential oils as was done in the toxicity assay. Insects surviving after treatment were taken and used immediately for this assay. 100 gm of cow pea grains were taken in 500 ml flask and transferred 20 fumigated insects to it and reared them for 10 days. After these treatments, infected grains were counted. For each concentration of essential oils and control, six replicate were set.

Data analysis

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.,[23]. Correlation and linear regression analysis were conducted to define all dose-response relationships[24]. Analysis of variance was performed to test the equality of regression coefficient[24].

RESULTS AND DISCUSSION

Toxicity

Fumigant toxicity of the essential oils from leaves of *A. marmelos* and peels of *C. reticulata* against larvae and adults of *C. chinensis* gradually increased with increasing exposure time and concentration (P<0.01). The Medium lethal concentration (LC_{50}) of *A. marmelos* and *C. reticulata* was 14.351 µl and 153231 µl against adults, whereas 13.296 µl and 14.997 µl against larvae of *C. chinensis*, at 72 h, respectively (Table-1).

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, 99%.

The regression analysis showed a concentration dependent significant

Table-1. Summary of *Aegle marmelos* and *Citrus reticulata* essential oils toxicity assays against adult of *Callosobruchus chinensis*

Essential oils	Parameters	Exposure period	LC_{50}[a] (μL)	LCL-UCL[b]	g-value[c]	t-ratio[c]	Heterogenity[c]	Chi square
Aegle marmelos	**Adult mortality**	48 h	16.312	11.732-18.213	0.132	2.921	0.105	2.123
		72 h	14.351	10.238-16.329	0.118	3.205	0.195	3.945
	Larval mortality	48 h	14.213	10.321-15.392	0.212	3.232	0.114	2.395
		72 h	13.296	9.021-14.027	0.205	4.989	0.197	4.347
Citrus reticulata	**Adult mortality**	48 h	17.132	13.925-18.989	0.243	3.132	.0176	2.439
		72 h	15.231	12.205-17.927	0.212	4.102	0.113	4.395
	Larval mortality	48 h	15.488	12.829-19.392	0.221	2.831	0.193	3.051
		72 h	14.997	10.333-18.132	0.372	4.025	0.161	2.857

[a]LC_{50} represent the median lethal concentration. [b]UCL and LCL represent upper confidence limit and lower confidence limit. [c]g-value,t-ratio and heterogeneity were significant at all probability levels (90%, 95%, 99%).

correlation of the oils with adult mortality against *A. marmelos* ($F_{3,20}$=31.800, P<0.01) and against *C. reticulata* are ($F_{3,20}$=33.080, P<0.01), at 72 h exposure, and larval mortality against *A. marmelos* ($F_{3,20}$=26.877, P<0.01) and against *C. reticulata* are ($F_{3,20}$=28.935, P<0.01), at 72 h exposure respectively (Table 2).

Fumigation of *C. chinensis* adults with two sub lethal concentration (40% and 80% of 48 h LC_{50}) of *A. marmelos* and *C. reticulata* essential oil significantly reduced AChE activity. Inhibition in AChE activity against *A. marmelos* and *C. reticulata* was 9.11% and 9.32 % of control with 40% and 10.01% and 10.93% of control with 80% of 48 h LC_{50}, respectively (Table 3).

Plants products in the form of essential oils having considerable potential as insecticidal compounds are gaining tremendous importance in recent years. The insecticidal constituents of many plants extracts and essential oils are monoterpenoids. Due to their high volatility, the monoterpenoids have fumigant activity that might be importance for controlling

Table-2. Regression parameters of insecticidal effects of *Aegle marmelos* and *Citrus reticulata* essential oils against larvae and adults of *Callosobruchus chinensis*

Essential Oils	Parameters	Exposure period	Intercept	Slope value	Regression cofficient	*F*-value* (df=3,20) *P*<0.01
Aegle Marmelos	Adult mortality	48 h	-4.547	3.961	0.992	38.565
		72 h	-5.576	4.627	0.996	31.800
	Larval Mortality	48 h	-9.459	4.039	0.999	18.148
		72 h	-12..059	5.599	0.999	26.877
Citrus reticulata	Adult mortality	48 h	-5.792	4.012	0.998	40.654
		72 h	-6.852	3.923	0.996	33.080
	Larval Mortality	48 h	-9.325	4.839	0.999	20.841
		72 h	-12.831	4.035	0.999	28.935

Regression analysis was performed between different concentrations of essential oils and responses of the insect pest. *Significant at 99% probability level.

stored-grain pests[25]. The present results corroborate the findings of Mishra et al.,[26] who reported the repellent activity of mentha and citrus oils against on *Tribolium castaneum* and *Sitophilus oryzae*. More research that is current illustrated that the essential oils and their constituents may have potential as alternative compounds to currently used fumigants[27,28,29]. In the present study, the essential oils under investigation caused death of adults and larvae of *C. chinensis* when fumigated. In the toxicity assay, the mortality rate are found to increase with an increase in concentration and the LC_{50} values decreased at different grade exposure periods indicating that the response was concentration and time dependent. Low LC_{50} values of essential oils against larval stage indicated that larval stage of insect are more susceptible in comparison to adults stage. This result was similar to the work of Safavi and Mobki[30] who found that the essential oils of *C. reticuleta* peels show toxic effects against adults of *T. castaneum* and insecticidal activity of this oil against larvae of *T. castaneum* and adult of *T. castaneum* and *S. oryzae* Mishra et al.[31]. Response of *T. castaneum* and *S. oryzae* against *A. marmelos* essential oil was observed by Mishra et al[32] that found the oil of *A marmelos* show insecticidal activity against *T. castaneum* and *S. oryzae*.

The toxic effects of peppermint oil may be due to suffocation and inhibition of various biosynthesis processes of the insects at developmental stages. Mesbah et al.[33] reported that all the efficiency tested essential and or volatile oils acted principally as insect growth inhibitors

Table-3. Effect of 40% and 80% of 48h LC_{50} of *Aegle marmelos* and *Citrus reticulata* essential oils on acetylcholinesterase enzyme (AChE) activity in *Callosobruchus chinensis*

Essential oils	Control	AChE Activity	
		40% of 48 h of LC_{50}	80% of 48 h of LC_{50}
Aegle marmelos	0.091±0.005 0.(100%)t= 7.91	0.063±0.002t= 9.11#*	0.049±0.005t= 10.01#*
Citrus reticulata	0.096±0.005 0.(100%)t= 8.23	0.069±0.002t= 9.32#*	0.052±0.005t= 10.93#*

Note: Enzyme activities were expressed as ì mol of 'SH' hydrolyzed min-1 mg protein-1. Values are mean± SE of six replicates. A value in parentheses indicates the percentage of enzyme activity with untreated control taken as 100%. #Paired t-test was applied.*Significant ($P < 0.01$).

causing disruption of insect development and abnormal adults that were lead finally to death.

The current findings are similar to the results of Tripathi et al.[34] who has also reported oviposition reduction effects of orange peel oil against *T. castaneum*. The essential oils of *Mentha* species show insecticidal activity against stored-grain insect pests. The mortality of *Rhyzopertha dominica* was 100% when *Mentha spicata* essential oil are used[35]. The essential oils can vary quality, quantity and in chemical composition according to the process of distillation climate, soil composition, plant organ, age[36,37]. This result was supported to our findings that *C. reticuleta* and *A. marmelos* essential oils have fumigant toxicity, oviposition and developmental inhibitory activity against *C. chinensis.*

In conclusion, the development and wide use of natural or biological insecticides may help to decrease negative effects of synthetic chemicals such as residues in the stored products, insect resistance and environmental pollution. In this respect, natural insecticides appear as easily biodegradable solutions with relatively low environmental pollution. Therefore, in the light of the present findings, it can be suggested that these plant essential oils can be used as new and effective insecticidal reagents against adults of *C. chinensis*. However, further studies need to be conducted to evaluate the cost and safety of these reagents, along with the efficiencies under various environmental conditions.

REFERENCES

1. Fornal, J., T. Jeli'nski, J. Sadowska, S. Grundas, J. Nawrot, A. Niewiada, J.R. Warchalewski & W. B³aszczak, 2007. Detection of granary weevil Sitophilus granarius (L.) eggs and internal stages in wheat grain using soft X-ray and image analysis. *J. Stored Prod. Res.*, 43, 142–148.
2. Haff, R.P. & D.C. Slaughter, 2004. Real-Time X-Ray Inspection of Wheat for Infestation by the Granary Weevil, *Sitophilus granarius* (L.). Trans. ASAE, 47:531–537.
3. Demeter, S., O. Lebbe, F. Hecq, S. Nicolis, T.K. Kemene, H. Martin, M.L. Fauconnier & T. Hance, 2021. Insecticidal Activity of 25 Essential Oils on the Stored Product Pest, *Sitophilus granarius. Foods*, 10: 200.

4. Alam, M.Z., 1971. Pests of stored grain and other stored products and their control, Bangladesh; pp. 361.

5. Atwal, A.S., 1976. Insect Pests of stored grain and other stored products. In: Agricultural Pests of India and South East Asia. pp: 389-415. Kalyani Pub. New Delhi, India.

6. Caswell, G.H., 1980. A review of the work done in the entomology section. Institute. Agricultural Research. Pests of stored grains. *Samaru miscellan*, 99: 12.

7. Sanon, A., P.A. Ouedraogo, Y. Tricault, P.F. Credland & J. Huignard, 1998. Biological control of bruchids in cowpea stores by release of *Dinarmusbasalis*(Hymenoptera: Pteromalide) adults. *Environ. Entomol.*, 27: 717-725.

8. Ouedraogo, A.P., S. Sou, A. Sanon, J.P. Monge, J. Huignard, B. Tran & P.F. Credland, 1996. Influence of temperature and humidity on population of *Callosobruchus maculates* (Coleoptera: Bruchidae) and its parasitoid *Dinarmusbasalis*(Pteromalidae) in two climatic zones of Burkina Faso. *Bull. Entomol. Res.*, 86: 695-702.

9. Muniz, J.F., L. Mc Cauley, J. Scherer, M. Lasarev, M. Koshy & Y.W. Kow *et al.*, 2008. Biomarkers of oxidative stress and DNA damage in agricultural workers: a pilot study. *Toxicology and Applied Pharmacology*, 227(1):97-107.

10. Jembere, B., D. Obeng-Ofori, A. Hassanali & G.N.N. Nyamasyo, 1995. Products derived from the leaves of *Ocimum kilimandscharicum* (*Labiatae*) as post-harvest protectants against the infestation of three major stored product insect pests. *Bulletin of Entomological Research*, 85:361-367.

11. Boyer, S., H. Zhang & G. Lempérière, 2012. A review of control methods and resistance mechanisms in stored product insects. *Bulletin of Entomological Research*, 102:213-229.

12. Simoniello, M.F., E.C. Kleinsorge, J.A. Scagnetti, R.A. Grigolato, G.I. Poletta & M.A. Carballo, 2008. DNA damage in workers occupationally exposed to pesticide mixtures. *Journal of Applied Toxicology*, 28(8):957-65.

13. Sighamony, S., I. Anees, T. Chandrakala & Z. Osmani, 1986. Efficacy of certain indigenous plant products as grain protectants against *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (F.). *Journal of Stored Product Research,* 22:21–23.

14. Fishwick, F.B., 1988. Pesticide residues in grain arising from postharvest treatments. Aspect *Appl. Biol.*, 17: 37-46.

15. Muir, D.C.G, C. Teixeira & F. Wania, 2004. Empirical and modeling evidence of regional atmospheric transport of current-use pesticides. *Environ Toxico Chem.,* 23:2421–2432.

16. Champ, B.R. & C.E. Dyte, 1976. Report of the FAO global survey of pesticides susceptibility of stored grain pests. In: FAO Plant Production and Protection series; No. (5), pp. 257. Food and Agriculture Organization of the United Nations, Rome

17. Zettler, J.L. & G.W. Cuperus, 1990. Pesticide resistance in Tribolium castaneum (Coleoptera: Tenebrionidae) and Rhyzopertha dominica (Coleoptera: Bostrichidae) in wheat. *J. Econ. Entomol.*, 83:1677–1681.

18. Yusof, O. & S.H. Ho, 1992. A survey of insecticide resistance in Sitophilus zeamais Motsch in Malaysia and Singapore. *J. Plant Prot. Tropic.*, 9: 219-225.

19. Zettler, J.L., 1982. Insecticide resistance in selected stored product insects infesting Peanuts in the southern United States. *J. Econ. Entomol.*, 75: 359- 362.

20. Mishra, B.B., S.P. Tripathi & C.P.M. Tripathi, 2014. Chronic activity of plant volatiles essential oils in management of rice weevil *Sitophilus oryzae* (Coleoptera: Curculionidae). *Journal of Entomology*, 11:76-83.

21. Mishra, B.B., S.P. Tripathi & C.P.M. Tripathi, 2012. Repellent effect of leaves essential oils from *Eucalyptus globules* (Mirtaceae) *and*

Ocimum basilicum (Lamiaceae) against two major stored grain insect pests of Coleopterons. *Nature and Science*, 10(2):50-54.

22. Erler, F., 2005. Fumigant activity of six monoterpenoids from aromatic plants in Turkey against the two stored-product pests confused flour beetle, *Tribolium confusum* and Mediterannean flour moth, *Ephestia kuehniella*. *Journal of Plant Diseases and Protection*, 112: 602-611.
23. Robertson, J.L., R.M. Russell, H.K. Preisler & N.E. Savin, 2007. Bioassay with arthropods POLO Computer programme for analysis of bioassay data 2nd Edition, CRC Press, Talor and Francis, pp. 1-224.
24. Sokal, R.R. & F.J. Rohlf, 1973. Introduction to biostatistics.Freeman WH, San Francisco, Calif, USA.
25. Renault-Roger, C. & A. Hamraoui, 1995. Fumigant toxic activity and reproductive inhibition induced by monoterpenes on *Acanthoscelides obtectus* (Say) (Coleoptera), a bruchid of kidney bean (*Phaseolus vulgaris* L.). *J Stored Prod Res.,* 31: 291-299.
26. Mishra, B.B. & S.P. Tripathi, 2011. Repellent activity of plant derived essential oils against *Sitophilus oryzae* (Linnaeus) and *Tribolium castaneum* (Herbst). Singapour *Journal of Scientific Research*, 1(2):173-178.
27. Keszthelyi, S., R. Hoffmann, Z. Pónya & F. Pál-Fám, 2017. Acute and persistence effects of oil of *Hippophae rhamnoides* and *Calendula officinalis* on *Sitophilus granarius* (Coleoptera:Curculionidae) in stored maize," *Acta Phytopathologica et Entomologica Hungarica.*, 52(2): 255–264.
28. Pavela, R., 2015. Essential oils for the development of eco-friendly mosquito larvicides: a review," *Industrial Crops and Products*, 76:174–187.
29. Okonkwo, E.U., 2004. Plant materials used for controlling insect pests of stored products in Nigeria, families Annonaceae, Piperaceae, and Rutaceae," *Journal of Herbs, Spices and Medicinal Plants*, 11(1–2) 47–69.
30. Safavi, S.A. & M. Mobki, 2016. Susceptibility of Tribolium castaneum (Herbst, 1797) larvae to essential oils of Citrus reticulata Blanco fruit peels and the synergist, diethyl maleate. *Biharean Biologist.,* 10 (2): 82-85.
31. Mishra, B.B., S.P. Tripathi & C.P.M. Tripathi, 2011. Contact toxicity of essential oil of Citrus reticulate fruits peels against stored grain pests *Sitophilous oryzae* (Linnaeus) and *Tribolium castenium* (Herbst). World *J. Zool.,* 6(3):307–311.
32. Mishra, B.B., S.P. Tripathi & C.P.M. Tripathi, 2016. Impact of *Syzygium aromaticum* (L.) essential oil as fumigant against *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Journal of Entomology and Zoology Studies,* 4(6): 811-816.
33. Mesbah, H.A., A.K. Mourad & A.Z. Rokaia, 2006. Efficacy of some plant oils alone/ or combined with different insecicides on the cotton leaf-worm Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae) in Egypt. Commun. *Agric. Appl. Biol. Sci.*, 71: 305-328.
34. Tripathi, A.K., V. Prajaoati, S.P. Khanuja & S. Kumar, 2003. Effect of d-Lemonene on three stored-product beetles. *J. Economic Entomology*, 96: 990-995.
35. Khalfi O, Benyoussef E & Yahiaoui N. 2006. Extraction, analysis and insecticidal activity of spearmint essential oil from Algeria against *Rhyzopertha dominica* (F.). J. *Essen. oil-Bearing Plants*, 9(1): 17-21.
36. Angioni, A., A. Barra, V. Coroneo, S. Dessi & P. Cabras, 2006. Chemical composition, seasonal vari-ability, and antifungal activity of Lavandula stoechas L. ssp. stoechas essential oils from stem/leaves and flowers. *J. Agric. Food Chem.,* 54:4364–4370.
37. Masotti, V., F. Juteau, J.M. Bessie're & J. Viano, 2003. Seasonal and phenological variations of the essential oil from the narrow endemic species Artemisia molinieri and its biological activities. *J. Agric. Food Chem.,* 51:7115–7121.