World Journal of Zoology 6 (1): 22-25, 2011
ISSN 1817-3098

Toxicity of Essential Oil Combinations Against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae)

M.K. Chaubey

Department of Zoology, Pest Management Laboratory,
Mahatma Gandhi Post Graduate College, Gorakhpur-273001, (U.P.), India

Abstract: Essential oils from dried fruits of *Trachyspermum ammi* (Umbelliferae), *Anethum graveolens* (Umbelliferae) and *Nigella sativa* (Ranunculaceae) were isolated by hydrodistillation and toxicity of essential oil combinations was determined against *Tribolium castaneum*. Fumigation with essential oil combinations caused mortality of *T. castaneum* larvae and adults. Median lethal concentrations (LC_{50}) for combinations of *T. ammi* and *A. graveolens*; *A. graveolens* and *N. sativa*; and *N. sativa* and *T. ammi* essential oils against larvae were 7.82, 10.43 and 8.58 μL and against adults were 9.34, 11.83 and 9.96 μL, respectively. Combinations of *T. ammi* and *A. graveolens*; *A. graveolens* and *N. sativa*; and *N. sativa* and *T. ammi* essential oils significantly reduced oviposition in adults when fumigated with sublethal concentrations in comparison to control group in concentration dependent manner. The same essential oil combinations also reduced pupation and adult emergence in larvae when fumigated. These essential oils caused toxicity in insects more potently in synergism as compared to used alone. In conclusion, combinations of essential oils are more useful in pest management programme which make this approach cost-effective for poor communities.

Key words: Trachyspermum ammi · Anethum graveolens · Nigella sativa · Essential oil

INTRODUCTION

Insect pests have been causing damage to granaries and other food structures since ancient times [1]. Even today, storage losses remain notoriously high. The overall damage caused by stored-grain insect pests accounts for 10-40% of losses worldwide annually [2]. In India, food grain losses during storage at the farm level reach approximate 10% of the total production [3]. Despite of improved storage structures and modern chemical and physical control techniques applied for safe storage of stored grains, 70-90% of food grain is still stored for six months to a year at farmer's level in traditional storage structures. In such a critical situation, protection of stored grains from insect infestation is essential.

To achieve this goal, use of synthetic pesticides came into existence. But these chemicals have persistent nature at different trophic levels in food chains. Experimental studies of chronic exposure to pesticides have revealed increased risk of neurotoxic, carcinogenic, teratogenic and mutagenic effects [4]. Also, many insects have acquired resistance against various such chemicals [5, 6]. In addition, efficacy of these chemicals against stored-grain insect pests varies greatly after treatment [7]. Uncontrolled use of these chemicals causes great environmental hazards due to their persistent nature and also various biochemical and behavioral changes in non-target animals [8, 9]. However, many chemicals of plant origin have been used in insect pest management programme instead of synthetic pesticides since ancient time. Amongst such plant derived chemicals, new classes of insecticidal agent i.e. essential oils come into existence as fumigants in stored grain insect pest management [10].

Many essential oils have been reported for repellent and insecticidal activities against stored grain insect pests [10-14]. Besides crude oils, toxic effects of many essential oil constituents have also been determined against insect pests [15, 16]. The insecticidal and developmental inhibitory activities of *T. ammi*, *A. graveolens* and *N. sativa* essential oils have already been determined against *Tribolium castaneum* and *Callosobruchus chinensis* [12, 13]. Herein, synergistic action of combinations of *T. ammi*, *A. graveolens* and *N. sativa* essential oils against red flour beetle *T. castaneum* was evaluated.

Corresponding Author: M.K. Chaubey, Pest Management Laboratory, Department of Zoology,
Mahatma Gandhi Post Graduate College, Gorakhpur-273001, (U.P.), India,
E-mail: chaubey.mukesh@rediffmail.com.

MATERIALS AND METHODS

Isolation of Oils: The dried fruits of *T. ammi* (Umbelliferae), *A. graveolens* (Umbelliferae) and *N. sativa* (Ranunculaceae) were purchased from the local market of Gorakhpur, U.P. India. These were grounded and the powdered material was hydrodistilled in a Clevenger apparatus continuously for five hours to yield essential oils. The oils were collected in glass containers and kept in Eppendroff tube at 4°C until their use.

Insects: Red flour beetles *T. castaneum* were used to determine the insecticide nature of essential oils. The insects were reared on wheat flour in the laboratory at 30±2°C, 75±5% RH and a photoperiod of 10:14 (L: D) h.

Lethality Assay: Insecticidal properties of combinations of *T. ammi* and *A. graveolens*; *A. graveolens* and *N. sativa*; and *N. sativa* and *T. ammi* essential oils were evaluated against 4th instars and adults of *T. castaneum* by fumigation. Larvae/adults were taken from the laboratory culture and were placed with 1 g of wheat flour in 80 mm diameter Petri dish, with their surface spread uniformly with wheat flour. Whatmann filter paper 1 cm^2 strips treated with solutions of different concentrations (2, 4, 6, 8, 10, 12 and 14 μL dissolved in 100 acetone μL for each replica) of essential oil combinations were pasted on the inner surface of cover of each Petri dish. In each binary combination proportion of essential oil was equal (1:1). All the closed Petri dishes were kept in dark with six replicates set for each concentration of essential oil combination as well as control groups. In control group, filter paper strips were treated with acetone only. Mortality was recorded after 24 hours of fumigation.

Oviposition Inhibitory Assay: Oviposition inhibitory activities of the essential oil combinations were tested against *T. castaneum* by fumigation. The 1 cm^2 Whatmann filter paper strips treated with 40 and 80% of 24-h LC_{50} essential oil combinations were used for fumigation of 20 adults of mixed sex in 80 mm Petri dishes as was done in toxicity assay. After 24 h fumigation, the adults were transferred to fresh Petri dishes having only wheat flour. After ten days daily observation, the adults were removed and discarded. The number of the larvae hatched was counted for the treated as well as for control groups. The counting was done for ten days continuously. Six replicates were set for each concentration of essential oil combinations. In control group, filter paper strips were treated with acetone only.

Developmental Inhibitory Assay: Developmental inhibitory activities of the essential oil combinations were determined against 4th instars of *T. castaneum*. Ten larvae were fumigated with 40 and 80% of 24-h LC_{50} of essential oil combinations in 80 mm Petri dishes as was done in larvicidal assay. After 24 hours of fumigation treated larvae were transferred to Petri dishes having wheat flour only. Number of transformed pupae from treated larvae and emerged adults from transformed pupae were recorded. Six replicates were set for each concentration of essential oil combination as well as control groups. In control group, filter paper strips were treated with acetone only.

Data Analysis: LC_{50} was calculated using the POLO programme [17]. Analysis of variance was performed to test the significancy of the data [18].

RESULTS

Median lethal concentration (LC_{50}) of combinations of *T. ammi* and *A. graveolens*; *A. graveolens* and *N. sativa*; and *N. sativa* and *T. ammi* essential oils against larvae were 7.82, 10.43 and 8.58 μL and against adults were 9.34, 11.83 and 9.96 μL, respectively (Table 1). Oviposition was reduced to 60.17 and 37.66; 62.2 and 36.74; and 58.13 and 38.05 when adults were fumigated with 40 and 80% of 24-h LC_{50} of *T. ammi* and *A. graveolens*; *A. graveolens* and *N. sativa*; and *N. sativa* and *T. ammi* essential oil combinations respectively (Table 2). The reduction in oviposition potential of *T. castaneum* was significant when fumigated with sub-lethal concentrations of the combinations of *T. ammi* and *A. graveolens* (F = 193.66); *A. graveolens* and *N. sativa* (F = 305.87); and *N. sativa* and *T. ammi* (F = 334.18) essential oils (Table 2). Pupation was reduced to 73.95 and 45.98; 78.03 and 49.94; and 72.03 and 45.99 when 4th instar larvae were fumigated with 40 and 80% of 24-h LC_{50} of *T. ammi* and *A. graveolens*; *A. graveolens* and *N. sativa*; and *N. sativa* and *T. ammi* essential oil combinations, respectively. Similarly, adult emergence was reduced to 61.27 and 28.55; 63.25 and 32.6; and 59.19 and 34.68 when 4th instar larvae were fumigated with 40 and 80% of 24-h LC_{50} of *T. ammi* and *A. graveolens*; *A. graveolens* and *N. sativa*; and *N. sativa* and *T. ammi* essential oil combinations respectively (Table 2). The decrease in pupation (F = 138.13, 174.75 and 249.34 Table 2) and adult emergence (F = 95.64, 171.09 and 195.32; Table 2) was increased significantly with increase in concentration of *T. ammi*: *A. graveolens*, *A. graveolens*: *N. sativa* and *N. sativa*: *T. ammi* essential oil combinations.

Table 1: The toxicity of essential oil combinations against *T. castaneum* larvae and adults.

Essential oil		LC_{50}^{*} (μL)	LCL-UCL** (μL)	g-value***	Slope***	t-ratio***	Heterogeneity***
T. ammi : *A. graveolens*	larvae	7.82	6.56 - 10.08	0.29	1.87	3.69	0.34
	adults	9.34	8.11 - 10.57	0.31	1.98	3.47	0.29
A. graveolens : *N. sativa*	larvae	10.43	8.96 - 12.0	0.28	2.01	3.84	0.33
	adults	11.83	10.42 - 13.26	0.29	2.14	4.01	0.38
T. ammi : *N. sativa*	larvae	8.58	7.29 - 10.87	0.30	1.95	3.56	0.31
	adults	9.96	8.56 - 11.27	0.27	1.83	3.21	0.34

*LC_{50} represents the median lethal concentration; **UCL and LCL represent upper confidence limit and lower confidence limit

*** g-value, slope, t-ratio and heterogeneity are significant at all probability levels

Table 2: Effect of *T. ammi*, *A. graveolens* and *N. sativa* essential oil combinations on oviposition, pupation and adult emergence of *T. castaneum*.

Essential oil combinations	Treatment	Oviposition (Number of larvae produced per twenty adults)	Pupation (Number of pupa transformed per ten fumigated larvae)	Adult emergence (Number of adults emerged per ten fumigated larvae)
T. ammi :	Control	254.0±18.48 (100)	8.33±0.83 (100)	8.16±0.98 (100)
A. graveolens	40% of 24h-LC_{50}	152.83$^{*\#}$±16.47 (60.17)	6.16$^{*\#}$±0.75 (73.95)	5.0$^{*\#}$±1.09 (61.27)
	80% of 24h-LC_{50}	95.66$^{*\#}$±13.80 (37.66)	3.83$^{*\#}$±0.75 (45.98)	2.33$^{*\#}$±0.52 (28.55)
	F-value (df = 2,15)	193.66$^{\#\#}$	138.13$^{\#\#}$	95.64$^{\#\#}$
A. graveolens:	40% of 24h-LC_{50}	158.0$^{*\#}$±20.44 (62.20)	6.50$^{*\#}$±0.75 (78.03)	5.16$^{*\#}$±1.47 (63.25)
N. sativa	80% of 24h-LC_{50}	97.33$^{*\#}$±9.87 (36.74)	4.16$^{*\#}$±0.75 (49.94)	2.66$^{*\#}$±0.82 (32.60)
	F-value(df = 2,15)	305.87$^{\#\#}$	174.75$^{\#\#}$	171.09$^{\#\#}$
T. ammi :	40% of 24h-LC_{50}	147.66$^{*\#}$±18.51 (58.13)	6.0$^{*\#}$±0.89 (72.03)	4.83$^{*\#}$±1.17 (59.19)
N. sativa	80% of 24h-LC_{50}	96.66$^{*\#}$±14.36 (38.05)	3.83$^{*\#}$±0.40 (45.99)	2.83$^{*\#}$±0.52 (34.68)
	F-value(df = 2,15)	334.18$^{\#\#}$	249.34$^{\#\#}$	195.32$^{\#\#}$

Significant ($P<0.05$, 0.025 and 0.01; ANOVA); *# Significant ($P<0.05$, 0.025 and 0.01; Student's t-test))

Values in parentheses represent per cent change with respect to control taken as 100 %

DISCUSSION

Several essential oils have shown repellant, toxic and developmental inhibitory effects on *T. castaneum* like *Artemisia annua* [11], *Lippia alba* [19] and *Elletaria cardomum* [20]. Shukla *et al.* (2008) have reported developmental inhibitory activities of *Myristica fragrans* and *Illicium verum* essential oils against *T. castaneum* [21]. *T. ammi*, *A. graveolens* and *N. sativa* essential oils alone are repellant and toxic to growing larvae and adults of *T. castaneum* and reduce the egg laying capacity and development [12]. In this study, *T. ammi*, *A. graveolens* and *N. sativa* essential oils have been used in various combinations to evaluate their synergistic action against *T. castaneum*. Different essential oil combinations caused death of larvae and adults of *T. castaneum* when fumigated. In the toxicity assay, mortality rate was increased with increase in concentration of essential oils. The index of significance potency estimation, g-value indicates that the value of mean is within the limits at all probability levels ($P<0.05$, 0.025 and 0.01) as it is less than 0.5. Values of t-ratio greater than 1.96 indicate that the regression is significant. The steep slope values observed in toxicity assay demonstrated that a small increase in the concentration of essential oil cause a large mortality in insects. Values of heterogeneity factor were less than 1.0 denotes that model fits the data adequate. *T. ammi*, *A. graveolens* and *N. sativa* essential oil combinations reduced oviposition potency in insects when fumigated with sub-lethal concentration. These essential oil combinations reduced pupation and adult emergence from fumigated larvae in concentration dependent manner. F- Values (193.66, 305.87 and 334.18 for *T. ammi*: *A. graveolens*, *A. graveolens*: *N. sativa* and *N. sativa*: *T. ammi* essential oil combinations) were highly significant for all stimuli at probability levels $P<0.05$, 0.025 and 0.01 and indicate that oviposition behaviour and development in insects was significantly reduced by essential oil combinations. Combinations of essential oils were more effective as compared to the essential oils used alone as combinations have low median lethal concentration and produced desired effects in insects even at lower concentration. The rapid action of essential oils against insects is indicative of their neurotoxic mode of action interfering with neuromodulator octopamine [22] or with GABA-gated chloride channels [23].Combinations of essential oils probably targets both pathway of toxicity in insects. Therefore, combinations of essential oils are more useful in pest management programme which make this approach cost-effective for poor communities.

ACKNOWLEDGEMENT

Author is highly thankful to University Grants Commission, New Delhi for providing financial assistance under Minor Research Project Grant [8-2(136)/2011(MRP/NRCB].

REFERENCES

1. Levinson, Z.H. and Levinson, 1985. Storage and insect species of stored grains and tombs in ancient Egypt. Zeitschrift für Angewandte Entomologie, 100: 321-339.
2. Matthews, G.A., 1993. Insecticide application in the stores. In: Application technology for crop protection. CAB, London.
3. Lal, S., 1988. Saving grain after harvest. In: The Hindu Survey of Indian Agriculture. National Press. Madras, India, pp: 246-248.
4. Kamrin, M.A., 1997. Pesticide profiles: toxicity, environmental impact and fate. Lewis Publishers, Boca Raton, Florida, USA.
5. Zettler, J.L. and G.W. Cuperus, 1990. Pesticide resistance in *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in wheat. J. Econ. Entomol., 83: 1677-1681.
6. Jembere, B., D. Obeng-Ofori, A. Hassanali and G.N.N. Nyamasyo, 1995. Products derived from the leaves of *Ocimum kilimanndscharium* (Labiatae) as post-harvest grain protectants against the infestation of three major stored product insect pests. Bull. Entomol. Res., 85: 361-367.
7. Pinto, A.R.J.R., Furiatti, P.R.V.S. Pereira and F.A. Lazzari, 1997. Avaliac, ao de insecticidas no controle de *Sitophilus oryzae* (Coleoptera: Curculionidae) em Arroz Armazenado. Anais da Sociedade Entomologica do Brasil., 26: 285-290.
8. Forage-Elawer, M., 1989. Enzymatic and behavioural changes in young chicks as a result of carbaryl treatment. J. Toxicol. Environ. Health, 26: 119-131.
9. Gupta, A., R.K. Upadhyay and P.N. Saxena, 2001. Toxicity evaluation of Zectran on certain blood biochemical parameters in Pesser domesticus. J. Sci. Indust. Res., 60: 668-674.
10. Shaaya, E., Ravid, U.N. Paster, B. Juven, U. Zisman and V. Pistarev, 1991. Fumigant toxicity of essential oils against four major stored product insects. J. Chem. Eco., 17: 499-504.
11. Tripathi, A.K., V. Prajapati, K.K. Aggrawal, S.P.S. Khanuja and S. Kumar, 2000. Repellency and toxicity of oil from *Artemisia annua* to certain stored product beetles. J. Econ. Entomol., 93: 43-47.
12. Chaubey, M.K., 2007. Insecticidal activity of *Trachyspermum ammi* (Umbelliferae), *Anethum graveolens* (Umbelliferae) and *Nigella sativa* (Ranunculaceae) against stored-product beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). Afr. J. Agri. Res., 2: 596-600.
13. Chaubey, M.K., 2008. Fumigant Toxicity of essential oils from some common spices against pulse beetle *Callosobruchus chinensis* (Coleoptera: Bruchidae). J. Oleo. Sci., 57: 171-179.
14. Aboua, L.R.N., B.P. Seri-Kouassi and H.K. Koua, 2010. Insecticidal Activity of Essential Oils from Three Aromatic Plants on *Callosobruchus maculatus* F. in Côte D'ivoire. Euro. J. Sci. Res., 39: 243-250.
15. Weaver, D.K., F.V. Dunkel, L. Ntezurubanza, L.L. Jakson and D.T. Stock, 1991. Efficacy of lianlool, a major component of freshly milled *Ocunum canum* Sims. (Legiminaceae) for protection against post harvest damage by certain stored product Coleoptera. J. Stored Prod. Res., 27: 213-220.
16. Weaver, D.K., T.W. Phillips, F.V. Dunkel, T. Weaver, R.T. Grubb and E.L. Nance EL, 1995. Dried leaves from the rocky mountain plants decrease infestation by stored product beetles. J. Chem. Ecol., 21: 127-142.
17. Russel, R.M., J.L. Robertson and S.A. Savin, 1977. POLO: A new computer programme for probit analysis. Bull. Entomol. Res., 23: 209-213.
18. Sokal, R.R. and F.J. Rohlf, 1973. Introduction to biostatistics. W.H. Freeman and Co, San Francisco, CA, USA. pp: 185-207.
19. Verma, N., A.K. Tripathi, V. Prajapati, J.R. Bahl, S.P.S. Khanuja and S. Kumar, 2000. Toxicity of essential oil from *Lippia alba* towards stored grain insects. J. Med. Arom. Plant Sci., 22: 50.
20. Huang, Y., S.L. Lam and S.H. Ho, 2000. Bioactivities of essential oil from *Ellataria carodum* (L.) Maton to *Sitophilus zeamais* and *Tribolium castaneum* (Herbst). J. Stored Prod. Res., 36: 107-117.
21. Shukla, J., S.P. Tripathi and M.K. Chaubey, 2008. Toxicity of *Myristica fragrans* and *Illicium verum* essential oils against flour-beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). Elec. J. Environ. Agri. Food Chem., 7: 3059-3064.
22. Kostyukovsky, M., M. Rafaeli, C. Gileadi, C. Demehenko and E. Shaaya, 2002. Activation of octopaminergic receptors by essential oil constituents isolated from aromatic plants: possible mode of action against insect pests. Pest Manag. Sci., 58: 1101-1106.
23. Priestley, C.M., E.M. Williamson, K.A. Wafford and D.B. Sattelle, 2003. Thymol, a constituent of thyme essential oil, is a positive allosteric modulator of human GABA receptors and a homo-oligomeric GABA receptors from *Drosophila melanogaster*. Br. J. Pharmacol., 140: 1363-1327.