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## **Combinatorial Action of Essential Oils Towards Pulse Beetle *Callosobruchus chinensis* Fabricius (Coleoptera: Bruchidae)**

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### **ABSTRACT**

In the present study, combinatorial action of essential oils towards a serious insect pest of pulses, *Callosobruchus chinensis* has been investigated. Essential oils were isolated from dried fruits of *Trachyspermum ammi*, *Anethum graveolens* and *Nigella sativa* by hydrodistillation and its insecticidal and oviposition inhibitory activities were determined by fumigating adults of the *C. chinensis* with different balanced combinations of essential oils. Mortality in adults was observed in concentration dependent manner when fumigated with different combinations of different essential oil combinations. The 24-h  $LC_{50}$  (Median lethal concentration) values for combinations of *T. ammi* and *A. graveolens*; *A. graveolens* and *N. sativa* and *N. sativa* and *T. ammi* and essential oils were 11.6, 8.7 and 11.2  $\mu$ L, respectively. Fumigation with sublethal concentrations of *T. ammi* *A. graveolens*; *A. graveolens* and *N. sativa* and *N. sativa* and *T. ammi* essential oil combinations significantly reduced the oviposition potential of the *C. chinensis* in comparison to the control group. From the results of the present study, it can be concluded that combinations of essential oils shows synergism and give desired toxicity even at lower concentration than used alone.

**Key words:** *Trachyspermum ammi*, *Anethum graveolens*, *Nigella sativa*, Synergism, *Callosobruchus chinensis*

### **INTRODUCTION**

The practice of storing food grains began about 4,500 years ago as a safeguard against poor harvests and famines due to adverse weather conditions. Since time immemorial several species of storage insect pest attacked granaries and other food structures and even today storage losses remain notoriously high. According to an estimate, the overall damage caused by stored-grain insect pests accounts for 10-40% worldwide annually. In India, food grain losses during storage at the farm level approximate 10% of the total production. In spite of improved storage structures and modern chemical and physical control techniques employed for the 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 quite essential. To achieve this goal, the use of several synthetic pesticides came into existence. But residues of such pesticides accumulate at different trophic levels in food chain and exceed the tolerance limit. Experimental studies of chronic exposure to pesticide have revealed increased risk of neurotoxic, carcinogenic, teratogenic and mutagenic effects among others. Also, many insects have acquired resistance against various synthetic insecticides. In addition, efficacy of these synthetic insecticides against stored-grain insect pests varies greatly after treatment. Uncontrolled

use of these synthetic insecticides causes great environmental hazards due to their persistent nature and also various biochemical and behavioral changes in non-target animals (Gupta *et al.*, 2001).

*Callosobruchus chinensis* is a serious pest of stored grain which infests cowpea, beans, lentils and other pulses. In India, it is responsible for 32-64% loss under storage conditions and maximum damage occurs from April to October (Mukherjee and Joseph, 2000). Only grubs are infective stages for the storage grains. These make holes in the grains and consume the inner part leaving empty kernel. The damaged grains become unsuitable for human consumption, production of sprout and also lose its market value. For the control of these specific and other stored grain insect pests, efforts have been made to develop new alternatives of synthetic pesticides. In such strategies, inert dusts (Mahdi and Khalequzzaman, 2006), ionizing radiations (Valizadegan *et al.*, 2009), ozonization (Hollingsworth and Armstrong, 2005), microbial products (Buda and Peculyte, 2008; Khashaveh *et al.*, 2008; Mahdeshin *et al.*, 2009), plant extracts (Allotey and Azalekor, 2000; Sabbour, 2003; Upadhyay *et al.*, 2006; Haghtalab *et al.*, 2009), insect growth regulators (Arthur and Hoeremann, 2004) and biological agents have been proved effective tools in controlling stored grain insect pests. From the last two decades, plant products especially essential oils have been gaining much attention in insect pest management programme. Till now, many essential oils and its constituents have been reported for insecticidal activities against stored grain insect pests (Lee *et al.*, 2001; Tripathi *et al.*, 2003; Koul *et al.*, 2007; Chaubey, 2007, 2008; Aboua *et al.*, 2010; Manzoomi *et al.*, 2010). Present investigation aimed to study insecticidal activities of different combinations of *T. ammi*, *A. graveolens* and *N. sativa* essential oils against *C. chinensis*.

## MATERIALS AND METHODS

**Isolation of oils:** The dried fruits of Ajowan (*Trachyspermum ammi*, Umbelliferae), dill (*Anethum graveolens*, Umbelliferae) and black cumin (*Nigella sativa*, Ranunculaceae) were purchased from the local market of Gorakhpur, (U.P.), India. These were grounded by domestic mixer and the powdered material was hydrodistilled in Clevenger apparatus continuously for five hours to yield essential oils. The oils were collected in glass containers and kept at 4°C until their use.

**Insects:** Pulse beetles *C. chinensis* was used to determine the insecticidal nature of different combinations of essential oils. The insects were reared on cowpea in laboratory at 30±2°C, 75±5% RH and a photoperiod of 10:14 (L: D) h.

**Toxicity assay:** Insecticidal properties of *T. ammi* and *A. graveolens*; *A. graveolens* and *N. sativa*; and *N. sativa* and *T. ammi* essential oil combinations were tested against *C. chinensis* adults by fumigation method. Glass vials (10 cm long and 8 cm diameter) with polystyrene cap were used for this assay. For fumigation, filter paper strips (1 cm<sup>2</sup>) treated with solutions (prepared in acetone) of different concentrations of different essential oil combinations was used. Ten grams of cow pea grains and ten adults were taken to each vial and the open end of the vial was closed by the cap so that the oil treated filter paper strip remained inside the vial. For each combination of essential oils, four different concentrations and for each concentration of essential oil combination, six replicates were used. In all combinations, different essential oils were mixed in equal proportion. The vials were kept at 30±2°C, 75±5% RH and a photoperiod of 10:14 (L: D) hours and mortality in insects were recorded every after 24 h of treatment up to 96 h. In control, untreated filter paper strip treated with acetone only was used.

**Oviposition inhibition assay:** Effects of *T. ammi* and *A. graveolens*; *A. graveolens* and *N. sativa*; and *N. sativa* and *T. ammi* essential oil combinations on oviposition potential was studied by fumigating newly emerged adults of *C. chinensis* with two sublethal concentrations viz. 40 and 80% of 24 h  $LC_{50}$  of the essential oils as was done in toxicity assay. For fumigation, filter paper strips (1 cm<sup>2</sup>) treated with solutions (prepared in acetone) of different concentrations of different essential oil combinations were used. Ten grams of cow pea grains and ten adults were taken 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 combination of essential oils, four different concentrations and for each concentration six replicates were used. In all combinations, different essential oils were mixed in equal proportion. The vials were kept at 30±2°C, 75±5% RH and a photoperiod of 10:14 (L: D) h and number of eggs were recorded after 96 h of treatment. In control, untreated filter paper was used. The %ODI was calculated as 100(A-B)/(A+B), where A and B represent the number of eggs laid in control and in the test, respectively.

**Data analysis:** Median lethal concentration  $LC_{50}$  was calculated by Probit Or Logit (POLO) programme (Russell *et al.*, 1977). Analysis of variance was performed to determine significance of the data and dose response relationship (Sokal and Rohlf, 1973).

## RESULTS

Fumigation with different combinations of essential oils caused death of *C. chinensis* adults. The  $LC_{50}$  values of different combinations of *T. ammi* and *A. graveolens*; *A. graveolens* and *N. sativa* and *N. sativa* and *T. ammi* essential oils adults were 11.6, 10.2, 9.6 and 8.5; 8.7, 7.4, 6.8 and 5.9 and 11.2, 10.5, 9.7 and 8.9 µL, respectively after 24, 48, 72 and 96 h of treatment (Table 1).

Essential oils inhibited the oviposition response of the insect when fumigated. In oviposition inhibitory assay, oviposition was reduced to 55.39 and 28.6%; 53.97 and 32.44% and 57.81 and 28.13% of the control after treatment with 40 and 80% of 24 h  $LC_{50}$  of the *T. ammi* and *A. graveolens*; *A. graveolens* and *N. sativa* and *N. sativa* and *T. ammi* combination of essential oils (Table 2). The maximum per cent oviposition deterrence index (%ODI±SE) was observed in case

Table 1: Summary of *Trachyspermum ammi*, *Anethum graveolens* and *Nigella sativa* essential oils toxicity assays against *Callosobruchus chinensis*

Combinations of essential oils	Exposure period (h)	$LC_{50}$ (µL)	LCL-UCL <sup>b</sup> for larvae (µL)	t-ratio <sup>c</sup>	Heterogeneity <sup>c</sup>	Slope value <sup>c</sup>	g value <sup>c</sup>
<i>T. ammi</i> : <i>A. graveolens</i>	24	11.6	10.7-12.5	3.61	0.28	2.11	0.29
	48	10.2	9.5-10.9	3.46	0.24	2.03	0.34
	72	9.6	9.1-11.1	3.24	0.29	2.16	0.31
	96	8.5	6.56-10.08	3.57	0.25	2.06	0.29
<i>A. graveolens</i> : <i>N. sativa</i>	24	8.7	8.1-9.3	3.46	0.23	2.09	0.26
	48	7.4	6.9-7.9	3.27	0.26	2.22	0.31
	72	6.8	6.4-7.2	3.63	0.25	2.28	0.37
	96	5.9	5.5-6.3	3.49	0.22	2.11	0.25
<i>T. ammi</i> : <i>N. sativa</i>	24	11.2	10.6-11.8	3.44	0.27	2.08	0.27
	48	10.5	9.8-11.2	3.29	0.23	2.19	0.33
	72	9.7	9.3-10.1	3.62	0.25	2.01	0.35
	96	8.9	8.4-9.4	3.17	0.28	2.17	0.28

<sup>a</sup> $LC_{50}$  represent the median lethal concentration. <sup>b</sup>UCL and LCL represent upper confidence limit and lower confidence limits. <sup>c</sup>t-ratio, heterogeneity, slope values and g-values are significant at all probability level

Table 2: Effect of different synergistic combinations of *Trachyspermum ammi*, *Anethum graveolens* and *Nigella sativa* essential oils on per cent oviposition of *Callosobruchus chinensis*

Combinations of essential oils	Treatment (%) (24-h LC <sub>50</sub> )	Number of eggs laid per insect	% eggs laid per insect	%ODI <sup>a</sup>	F-value (df = 2,15) (p<0.0001)
<i>T. ammi</i> : <i>A. graveolens</i>	40	11.66±0.84	55.39±3.83	30.40±4.04	99.85
	80	6.25±0.68	28.60±2.98	59.58±4.28	
<i>A. graveolens</i> : <i>N. sativa</i>	40	11.20±0.70	53.97±3.37	29.65±4.58	60.02
	80	6.73±0.43	32.44±2.07	51.64±4.41	
<i>T. ammi</i> : <i>N. sativa</i>	40	11.72±0.78	57.81±3.86	33.82±3.14	63.7
	80	5.60±0.48	28.13±2.63	56.46±3.48	

Values are represented as Mean±SD. <sup>a</sup>%ODI was calculated as 100(A-B)/(A+B), where A and B represent the number of eggs laid in control and in the test, respectively

of *T. ammi* and *A. graveolens* (59.58±4.28) followed by *N. sativa* and *T. ammi* (56.46±3.48) and *A. graveolens* and *N. sativa* (51.64±4.41). The decrease in oviposition potential of *C. chinensis* was significant when fumigated with the combinations of *T. ammi* and *A. graveolens* (F = 99.85; df = 2, 15; p<0.0001); *A. graveolens* and *N. sativa* (F = 60.02; df = 2,15; p<0.0001) and *N. sativa* and *T. ammi* (F = 63.7; df = 2,15; p<0.0001) essential oils (Table 2).

## DISCUSSION

Chemical control using synthetic insecticides has dominated control measures against cowpea bruchids. However, these insecticides have serious problems like genetic resistance in the pest, toxic residue property of insecticides and toxicity to consumers and other non-target animals. To avoid such conditions, in recent years use of plant derived insecticides as a replacement of synthetic insecticides is gaining importance. Earlier, several successful attempts have been made by different scientific groups to use plant based insecticides to control pulse beetle bruchids (Upadhyay *et al.*, 2006; Aboua *et al.*, 2010; Ofuya *et al.*, 2010). Present study is based on the fact that essential oils are complex mixture of a large number of compounds of different chemical groups and their biological effects are the results of synergism of all components. The activity of individual components of the essential oil is modulated by other components of the oil and possibly, the target site and mode of action of the essential oil components vary (Hoet *et al.*, 2006).

Results of the present study indicate that combinations of *T. ammi*, *A. graveolens* and *N. sativa* essential oils have insecticidal properties against adults of *C. chinensis* in synergistic manner as evidenced by their low median lethal concentration values in present study as compared to efficacy of essential oils used alone in the previous study (Chaubey, 2008). 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 (90, 95 and 99%) 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 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. Ngamo *et al.* (2007) have reported similar results against *Sitophilus oryzae* when applied essential oils alone or in balanced combinations. The rapid action of essential oils against insects is indicative of their neurotoxic mode of action interfering with neuromodulator octopamine (Kostyukovsky *et al.*, 2002) or with GABA-gated

chloride channels (Priestley *et al.*, 2003). Combinations of essential oils probably target both pathway of toxicity in insects and essential oils could be the source of active components showing synergism. Furthermore, exploration of mode of actions of essential oil constituents in combination will definitely help in understanding the mechanism of action in synergism. On the basis of observations, it can be advised for the use of *T. ammi*, *A. graveolens* and *N. sativa* essential oils in different combinations as insecticides as it is safe for environment, health of the consumers and economically cost effective.

## REFERENCES

- 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 Cote D'Ivoire. Eur. J. Sci. Res., 39: 243-250.
- Allotey, J. and W. Azalekor, 2000. Some aspects of the biology and control using botanicals of the rice moth, *Corcyra cephalonica* (Stainton), on some pulses. J. Stored Prod. Res., 36: 235-244.
- Arthur, F.H. and C.K. Hoeremann, 2004. Impact of physical and biological factors on susceptibility of *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae) to new formulations of hydroprene. J. Stored Prod. Res., 40: 251-268.
- Buda, V. and D. Peculyte, 2008. Pthogenicity of four fungal species to Indian meal moth *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae). Ekologija, 54: 265-270.
- 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. Agric. Res., 2: 596-600.
- 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.
- Gupta, A., R.K. Upadhyay and P.N. Saxena, 2001. Toxicity evaluation of Zectran on certain blood biochemical parameters in *Pessier domesticus*. J. Sci. Indus. Res., 60: 668-674.
- Haghtalab, N., N. Shayesteh and S. Aramideh, 2009. Insecticidal efficacy of castor and hazelnut oils in stored cowpea against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). J. Biol. Sci., 9: 175-179.
- Hoet, S., C. Stevigny, M.F. Herent and J. Quetin-Leclereq, 2006. Antiparasomal compounds from leaf essential oil of *Strychnos spinosa*. Planta Med., 72: 480-482.
- Hollingsworth, R.G. and J.W. Armstrong, 2005. Potential of temperature controlled atmospheres and ozone fumigation to control thrips and mealybugs on ornamental plants for export. J. Econ. Entomol., 98: 289-298.
- Khashaveh, A., M.H. Safaralizade and Y. Ghosta, 2008. Pathogenicity of three Iranian isolates of the fungus, *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes) against granary weevil, *Sitophilus granarius* L. (Coleoptera: Curculionidae). J. Boil. Sci., 8: 804-808.
- Kostyukovsky, M., A. Rafaeli, C. Gileadi, N. 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 Manage. Sci., 58: 1101-1106.
- Koul, O., G. Singh, R. Singh and J. Singh, 2007. Mortality and reproductive performance of *Tribolium castaneum* exposed to anethol vapours at high temperature. Biopestic. Int., 3: 126-137.

- Lee, B.H., W.S. Choi, S.E. Lee and B.S. Park, 2001. Fumigant toxicity of essential oils and their constituent compounds towards the rice weevil, *Sitophilus oryzae* (L.). Crop Prot., 20: 317-320.
- Mahdi, S.H.A. and M. Khalequzzaman, 2006. Toxicity studies of some inert dusts with the cowpea beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae). J. Boil. Sci., 6: 402-407.
- Mahdneshtin, Z., M.H. Safaralizadah and Y. Ghosta, 2009. Study on the efficacy of Iranian isolates of *Beauveria bassiana* (balsamo) vuillemin and *Metarhizium anisopliae* (metsch.) sorokin against *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae). J. Biol. Sci., 9: 170-174.
- Manzoomi, N., G.N. Ganbalani, H.R. Dastjerdi and S.A.S. Fathi, 2010. Fumigant toxicity of essential oils of *Lavandula officinalis*, *Artemisia dracuncululus* and *Heracleum persicum* on the adults of *Callosobruchus maculatus* (Coleoptera: Bruchidae). Munis Entomol. Zool., 5: 118-122.
- Mukherjee, S.N. and M. Joseph, 2000. Medicinal plant extracts influencing insect growth and reproduction. J. Med. Arom. Plant Sci., 22: 38-38.
- Ngamo, T.S.L., I. Ngatanko, M.B. Ngassou, P.M. Mapongmestem and T. Hance, 2007. Insecticidal efficacy of essential oils of 5 aromatic plants tested both alone and in combination towards *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Res. J. Biol. Sci., 2: 75-80.
- Ofuya, T.I., O.F. Olotah and O.J. Ogunsolo, 2010. Fumigant toxicity of crushed bulbs of two *Allium* species to *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae). Chilean J. Agric. Res., 70: 510-514.
- 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 homooligomeric GABA receptors from *Drosophila melanogaster*. Br. J. Pharmacol., 140: 1363-1372.
- Russell, R.M., J.L. Robertson and N.E. Savin, 1977. POLO: A new computer programme for probit analysis. Bull. Entomol. Soc. Am., 23: 209-213.
- Sabbour, M.M., 2003. Combined effects of some microbial agents mixed with botanical extracts on some stored product insects. Pak. J. Biol. Sci., 6: 51-56.
- Sokal, R.R. and F.J. Rohlf, 1973. Introduction to Biostatistics. W.H. Freeman and Co., San Francisco, pp: 365.
- Tripathi, A.K., V. Prajapati and S. Kumar, 2003. Bioactivity of l-carvone, d-carvone and dihydrocarvone towards three stored product beetles. J. Econ. Entomol., 96: 1594-1601.
- Upadhyay, R.K., L. Rohtagi, M.K. Chaubey and S.C. Jain, 2006. Ovipositional responses of the pulse beetle, *Bruchus chinensis* (Coleoptera: Bruchidae) to extracts and compounds of *Capparis deciduas*. J. Agric. Food Chem., 54: 9747-9751.
- Valizadegan, O., A.A. Pourmirza and M.H. Safaralizadeh, 2009. Combination of microwaves radiation and cold storage for control of *Oryzaephilus surinamensis* (L.) (Col. Silvanidae). J. Biol. Sci., 9: 231-236.