African Journal of Agricultural Research Vol. 2 (11), pp. 596-600, November 2007
Available online at http://www.academicjournals.org/AJAR
ISSN 1991- 637X © 2007 Academic Journals

Full Length Research Paper

Insecticidal activity of *Trachyspermum ammi* (Umbelliferae), *Anethum graveolens* (Umbelliferae) and *Nigella sativa* (Ranunculaceae) essential oils against stored-product beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae)

Mukesh Kumar Chaubey*

Department of Zoology, Deen Dayal Upadhyay Gorakhpur University, Gorakhpur-273009, (U.P.), India. E-mail: chaubey.mukesh@rediffmail.com.

Accepted 8 October 2007

The essential oils from the dried fruits of three common spices, *Trachyspermum ammi*, *Anethum graveolens* and *Nigella sativa* were isolated by hydrodistillation and its repellent, toxic and developmental inhibitory activities were determined against wheat flour insect pest *Tribolium castaneum*. The three essential oils repelled the adults of *T. castaneum* at low concentrations in the filter paper repellency assay. The death of larvae and adults of *T. castaneum* was caused by fumigation with these essential oils. Median lethal concentrations (LC_{50}) of *T. ammi*, *A. graveolens* and *N. sativa* essential oils against larval stages of the insect were 11.62, 14.78 and 9.46 µl and against adults were 13.48, 16.66 and 10.87 µl, respectively. Median effective concentrations (EC_{50}) of *T. ammi*, *A. graveolens* and *N. sativa* essential oils that reduce to a half the transformation of larval population into pupa were 6.70, 7.86 and 5.62 µl, respectively. These essential oils reduced the oviposition potential and increased the developmental period of the *T. castaneum* in comparison to the control group. Fumigation of these essential oils inhibited development of larvae to pupae and the pupae to adults and also resulted in the deformities in the different developmental stages of the insect. All the responses were found concentration-dependent.

Key words: *Trachyspermum ammi, Anethum graveolens, Nigella sativa*, *Tribolium castaneum*, insecticidal activity, essential oils.

INTRODUCTION

Stored grain insect pests have been damaging our economy by infesting agricultural stored products. These are responsible for worldwide loss of 10 - 40% in the stored grains annually (Matthews, 1993). The continuous increasing pressure of expanding human population has also created a critical problem of food scarcity. In such a situation, to manage stored grains and other agricultural products from insect infestation, various synthetic insecticides have been used. But insects have acquired resistance against most of these synthetic pesticides (Zettler and Cuperus, 1990; Jembere et al., 1995). Besides, the efficacy of insecticides against storage pests varies greatly after treatment (Pinto et al., 1997). Also the uncontrolled use of these synthetic insecticides causes great hazard for environment and consumers due to residual property (White, 1995). Thus, it is an urgent need to develop new alternatives that must be ecologically sound with no residual activity and adverse effect on other non-target animals. In this regard, many plant products have been evaluated for their toxic properties against different stored grain pests (Su, 1990; Mukherjee and Joseph, 2000) especially in form of essential oils (Shaaya et al., 1991; Ngamo et al., 2007). The essential oils are the complex mixture of volatile organic compounds produced as the secondary metabolites whose functions are other than the nutrition. The essential oils of many botanical origins are known to have repellent and insecticidal activities against insect

pests (Tripathi et al., 2000a; Verma et al., 2000). Besides crude oils, toxic effects of many essential oil constituents have also been determined against many insect pests (Weaver et al., 1991; 1995). *Tribolium castaneum* is a major pest of wheat grain flour (Howe, 1965). For the control of this insect pest many synthetic chemicals as fumigants have been used which cause adverse effects on non-target animals in addition to toxicity to the users (Okonkwo and Okoye, 1996). Some botanical extracts and essential oils have been reported for their toxic effects against this insect pest (Emara and Ryan, 1997; Tripathi et al., 2000b). In the present study, I report laboratory studies on the repellent, toxic and developmental inhibitory effects of three common spices *Trachyspermum ammi*, *Anethum graveolens* and *Nigella sativa* essential oils against wheat flour pest *T. castaneum*.

MATERIALS AND METHODS

Isolation of oils

The dried fruits of *T. ammi*, *A. graveolens* and *N. sativa* were purchased from the local market of Gorakhpur, (U.P.), India. These were grounded by domestic mixer 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 at 4°C until their use.

Insects

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

Repellency

Repellency assays of essential oils were carried out in 80 mm glass Petri plates. Test solutions were prepared by dissolving different volumes of essential oils in acetone (5, 10, 20, and 30 µl dissolved in 1 ml acetone). Whatman filter paper was cut into two halves of 80 mm discs and each oil solution was applied to a filter paper half as uniform as possible using a micropipette. The other half of the filter paper was treated with acetone only. The essential oil treated and acetone treated halves were dried to evaporate the solvent completely. After that both treated and untreated halves were attached with cellophane tape and placed at the bottom in the petriplate. Twenty adults of *T. castaneum* were released at the center of the filter paper disc and then Petri plates were covered and kept in dark. Four replicates were set for each concentration of essential oil. Number of the insects on both the treated and untreated halves was recorded after four hours in mild light.

Larval mortality

Larvicidal property of *T. ammi*, *A. graveolens* and *N. sativa* essential oils was tested against newly molted 4th instars *T. castaneum* larvae by fumigation. Ten larvae taken from the laboratory culture were placed with 1 gram of wheat flour in 80 mm petriplate. Flour was spread uniformly along the whole surface of the petriplate. A filter paper strip (1 cm^2), treated with solutions of different concentrations of essential oils prepared in acetone (4, 8, 12, 16, and 20 µl in 100 µl), was pasted on the inner surface of the cover of each petriplate. All the closed petriplates were kept in dark and six replicates were set for each concentration. After 24 h of fumigation, larval mortality was recorded.

Adult mortality

The toxic effect of *T. ammi*, *A. graveolens* and *N. sativa* essential oils was tested against adults of *T. castaneum* by fumigation. Ten adults taken from the laboratory culture were placed with 1 g of wheat flour in 80 mm petriplate. Flour was spread uniformly along the whole surface of the petriplate. A filter paper strip (1 cm^2), treated with solutions of different concentrations of essential oils prepared in acetone (4, 8, 12, 16, and 20 µl in 100 µl), was pasted on the inner surface of the cover of each petriplate. All the closed petriplates were kept in dark and six replicates were set for each concentration. After 24 h of fumigation, adult mortality was recorded.

Oviposition inhibition

Oviposition inhibitory activity of *T. ammi*, *A. graveolens* and *N. sativa* essential oils was tested against *T. castaneum* by fumigation. Twenty 1 - 2 week old adults of mixed sexes were placed in 1 gram of wheat flour in 80 mm petriplate. Flour was spread uniformly along the whole surface of the petriplates. A paper strip (1 cm^2) treated with 100 µl of different sublethal concentrations (6, 12, 24, 36, 48 µl of *T. ammi*; 6, 12, 24, 36, 48 µl of *A. graveolens*; and 3, 6, 12, 24, 36 µl of *N. sativa* dissolved in 600 µl acetone) of essential oils was pasted on the inner surface of the cover of each petriplate. All the closed petriplates were kept in dark and six replicates were set for each concentration. After 24 h of fumigation, the treated adults were transferred to fresh petriplates having fresh wheat flour. After 7 days of treatment, 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 four days continuously.

Developmental inhibition

Developmental inhibitory activity of *T. ammi*, *A. graveolens* and *N. sativa* essential oils was tested against 4th instars larvae of *T. castaneum*. Ten larvae were fumigated with 100 µl of different concentrations (6, 12, 24, 36, 48 µl of *T. ammi*; 6, 12, 24, 36, 48 µl of *A. graveolens*; and 3, 6, 12, 24, 36 µl of *N. sativa* dissolved in 600 µl acetone) of essential oils in 80 mm petriplates for 24 h as was done in larvicidal assay and then the treated larvae were transferred to fresh wheat flour in other petriplates. Number of survived larvae, transformed pupae from treated larvae and emerged adults from transformed pupae were recorded. The number of days taken from treatment to emergence of adults was also counted. Six replicates were set for each concentration.

Data analysis

Chi-square test was applied to establish the repellent activity of the essential oils tested (Sokal and Rohlf, 1973). The LC_{50} and EC_{50} were calculated by POLO programme (Russel et al., 1977). 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).

Table 1. Filter paper repellency assay using *T. ammi*, *A. graveolens* and *N. sativa* essential oils against *T. castaneum* adults.

Conc. (%) vol:vol	*T. ammi*			*A. graveolens*			*N. sativa*		
	Untreated Mean*± SE	Treated Mean*±SE	X^2- Value	Untreated Mean*±SE	Treated Mean*±SE	X^2- Value	Untreated Mean*±SE	Treated ean*±SE	X^2- Value
0.05	63.75±0.5	36.25±0.5	15.5 [a]	62.50±0.6	37.50±0.6	13.0 [a]	63.75±1.0	36.25±1.0	16.5[a]
0.10	85.50±0.9	14.50±0.9	99.0 [b]	72.50±0.5	27.50±0.5	41.0 [b]	82.50±1.4	17.50±1.4	87.0[b]
0.20	97.50±0.6	2.50±0.6	181.0[c]	88.75±1.0	11.25±1.0	121.5 [c]	93.75±1.0	6.25±1.0	154.5[c]
0.30	100.00±0.0	0.0±0.0	200.0[c]	97.50±1.0	2.50±1.0	181.4 [c]	98.75±0.5	1.25±0.5	190.5 [c]

Adults of *T. castaneum* were used in filter paper repellency assay. For each concentration of essential oil, four replicates were carried out and 20 adults were used per replicate. *Mean of adult percentage on the untreated and treated halves in filter paper repellency assay. [a] Not significant, [b] Significant at 95% probability level, [c] significant at 99% probability level.

Table 2. Summary of *T. ammi, A. graveolens* and *N. sativa* essential oils toxicity assays against *T. castaneum* larvae and adults.

Essential oils	Parameters	LC_{50}/EC_{50} [a]	LCL-UCL [b]	g-value [c]	t- ratio [c]	Heterogeneity [c]
T. ammi	Larval mortality	11.62 µl	10.52 – 12.72 µl	0.09	6.70	0.33
	Adult mortality	13.48 µl	12.12 – 14.84 µl	0.10	5.90	0.34
	Larval survival	6.70 µl	5.62 – 7.78 µl	0.14	4.81	0.25
A. graveolens	Larval mortality	14.78 µl	13.44 – 16.12 µl	0.07	6.22	0.30
	Adult mortality	16.66 µl	15.36 – 17.96 µl	0.12	5.80	0.29
	Larval survival	7.86 µl	6.64 – 9.08 µl	0.15	5.64	0.38
N. sativa	Larval mortality	9.46 µl	8.62 – 10.48 µl	0.11	6.51	0.33
	Adult mortality	10.84 µl	9.49 – 12.19 µl	0.13	6.23	0.29
	Larval survival	5.62 µl	4.72 – 6.52 µl	0.16	4.78	0.25

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

RESULTS

Repellency

Chi-square analysis indicated that the essential oils of *T. ammi*, *A. graveolens* and *N. sativa* tested were repellent to *T. castaneum* adults. These three essential oils showed significant repellent activity even at low concentrations as the hypothesis of the ratio 1:1 was rejected (Table 1).

Larval and adult mortality

The essential oils of the *T. ammi*, *A. graveolens* and *N. sativa* killed the larvae and adults of the *T. castaneum* by vapour action. The LC_{50} of *T. ammi* oil was found 11.62 and 13.48 µl for larvae and adult respectively (Table 2). Regression analysis showed a concentration dependent significant correlation of the oil fumes with larval mortality (F = 115.04) and adult mortality (F = 126.46) (Table 3). The LC_{50} of *A. graveolens* oil was found 14.78 and 16.66 µl for larvae and adult respectively (Table 2). Regression analysis showed a concentration dependent significant correlation of the oil fumes with larval mortality (F = 110.28) and adult mortality (F = 147.82) (Table 3). The LC_{50} of *N. sativa* oil was found 9.46 and 10.84 µl for larvae and adult respectively (Table 2). Regression analysis showed a concentration dependent significant correlation of the oil fumes with larval mortality (F = 136.59) and adult mortality (F = 131.36) (Table 3).

Oviposition inhibition

The oviposition potential of the *T. castaneum* was decreased significantly when fumigated with the essential oils of *T. ammi* (F = 357.55), *A. graveolens* (F = 393.08) and *N. sativa* (F = 373.24) (Table 3).

Developmental inhibition

The percentage of larvae transformed into the pupae and the percentage of pupae transformed into the adult stage were decreased significantly with an increase in concentration of essential oils. The EC_{50} value that reduced to a half the number of larvae transferred to pupae was found 6.70, 7.86 and 5.62 µl for *T. ammi*, *A. graveolens* and *N. sativa* oil, respectively (Table 2). Regression analysis showed a concentration-dependent significant correlation of the *T. ammi* oil fumes with larval survival (F = 21.78), pupal survival (F = 44.89) and adult emergence (F = 148.94) (Table 3). For *A. graveolens* oils regression

Table 3. Regression parameters of insecticidal, oviposition and developmental inhibitory effects of *T. ammi*, *A. graveolens* and *N. sativa* essential oils against *T. castaneum*.

Essential oils	Parameters	Intercept	Slope	Regression coefficient	F- value*
T. ammi	% Larval mortality	- 5.85	3.03	0.97	115.04
	% Adult mortality	- 4.98	2.91	0.97	126.46
	% Larval survival	142.40	- 1.68	- 0.86	21.78
	% pupal survival	100.00	- 2.37	- 0.99	44.89
	% Adult emergence	103.06	- 7.26	- 0.99	148.94
	% Oviposition	104.30	- 8.13	- 0.97	357.55
	Developmental period	12.68	0.64	0.99	56.79
A. graveolens	% Larval mortality	- 4.89	3.94	0.97	110.28
	% Adult mortality	- 4.51	4.07	0.97	147.82
	% Larval survival	90.38	- 3.51	- 0.86	30.24
	% pupal survival	101.35	- 5.57	- 0.99	57.32
	% Adult emergence	122.72	- 18.32	- 0.99	152.15
	% Oviposition	107.27	- 6.67	- 0.98	393.08
	Developmental period	12.73	0.69	0.99	49.86
N. sativa	% Larval mortality	- 5.62	3.56	0.97	136.59
	% Adult mortality	- 6.99	3.98	0.96	131.36
	% Larval survival	119.43	- 2.53	- 0.89	27.23
	% pupal survival	132.23	- 6.99	- 0.98	49.72
	% Adult emergence	101.98	- 17.64	- 0.99	163.79
	% Oviposition	107.88	- 6.82	- 0.98	373.24
	Developmental period	12.69	0.67	0.99	47.90

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

analysis showed a concentration-dependent significant correlation of the oil fumes with larval survival (F = 30.24), pupal survival (F = 57.32) and adult emergence (F = 152.15) (Table 3). Similarly for *N. sativa* oils, regression analysis showed a concentration-dependent significant correlation of the oil fumes with larval survival (F= 27.23), pupal survival (F = 49.72) and adult emergence (F = 163.79) (Table 3). The development period also increased significantly when samples were fumigated with the essential oils of *T. ammi* (F = 56.79), *A. graveolens* (F = 49.86) and *N. sativa* (F = 47.90) (Table 3).

DISCUSSION

It is clear from the results that *T. ammi*, *A. graveolens* and *N. sativa* essential oils are repellant and toxic to growing larvae and adults of *T. castaneum*. These repel the adult beetles significantly even at very low concentration. The essential oil fumes inhibit the egg laying capacity and development of the insects. Previously for the management of economic loss caused by *T. castaneum*, several essential oils of botanical origin have been reported for their repellant, toxic and developmental inhibitory activities. Essential oils of *Anethum sowa* (Tripathi et al., 2000a), *Artemisia annua* (Tripathi et al., 2000b), *Lippia alba* (Verma et al., 2000) and *Elletaria cardomum* (Huang et al., 2000) have been reported for their repellant and toxic behavior against *T. castaneum*. These earlier reported findings clearly support the result of the present study. The mode of action of these essential oils is yet to be confirmed but it appears that death of the adults, larvae, oviposition inhibition and development inhibition may be due to the suffocation and inhibition of different biosynthetic processes of the insect metabolism (Don-Perdo, 1989).

REFERENCES

Don-Perdo KN (1989). Mechanism of the action of the some vegetable oils against *Sitophilus zeamais* (Motsch) (Coleoptera: Curculionidae) on wheat. J. Stored Prod. Res. 25: 217-223.

Emara TE, Ryan MF (1997). Effect of two crude botanical extracts on the red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). J. Egypt. Ger. Soc. Zool. 23:125-140.

Howe RW (1965). Losses caused by insects and mites in stored foods and foodstuffs. Nutr. Abstr. Rev. 35: 285-302.

Huang Y, Lam SL, Ho SH (2000). Bioactivities of essential oil from *Ellataria carodum* (L.) Maton to *Sitophilus zeamais* and *Tribolium castaneum* (Herbst). J. Stored Prod. Res. 36:107-117.

Jembere B, Obeng-Ofori D, Hassanali A, Nyamasyo GNN (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.
Matthews GA (1993). Insecticide application in the stores. In Matthews, G.A. and Hislop, E.C. (eds.). Application technology for crop protection. CAB, London, pp. 305-315.
Mukherjee SN, Joseph M (2000). Medicinal plant extracts influencing insect growth and reproduction. J. Med. Arom. Plant Sci. 22: p.38.
Ngamo TLS, Goudoum A, Ngassoum MB, Mapongmetsen, Lognay G, Malaisse F, Hance T (2007). Chronic toxicity of essential oils of 3 local aromatic plants towards *Sitophilus zeamais* Motsch (Coleoptera: Curculionidae). Afr. J. Agri. Res. 2: 164-167.
Okonkwo EU, Okoye WI (1996). The efficacy of four seed powders and the essential oils as protectants of cow-pea and maize grain against infestation by *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae) in Nigeria. Int. J. Pest Mang. 42: 143-146.
Pinto ARJr, Furiatti, Pereira PRVS, Lazzari, FA (1997). Avaliac, ao de insecticidas no controle de *Sitophilus oryzae* (Coleoptera: Curculionidae) em Arroz Armazenado. Anais da Sociedade Entomologica do Brasil. 26: 285-290.
Russel RM, Robertson JL, Savin SA (1977). POLO: A new computer programme for probit analysis. Bull. Entomol. Soc. Am. 23: 209-213.
Shaaya E, Ravid U, Paster N, Juven B, Zisman U, Pistarev V (1991). Fumigant toxicity of essential oils against four major stored product insects. J. Chem. Ecol. 17: 499-504.
Sokal RR, Rohlf FJ (1973). Introduction to biostatistics. Freeman WH, San Francisco, pp. 165, 231, 289.
Su HFC (1990). Biological activities of hexane extract of *Piper cubeba* against rice weevils and cowpea weevils (Coleoptera: Curculionidae). J. Entomol. Sci. 25:16-20.
Tripathi AK, Prajapati V, Aggrawal KK, Khanuja SPS, Kumar S (2000a). Toxicity towards *Tribolium castaneum* in the fraction of essential oil of *Anethum sowa* seeds. J. Med. Arom. Plant Sci. 22: p. 40.
Tripathi AK, Prajapati V, Aggrawal KK, Khanuja SPS, Kumar S. (2000b). Repellency and toxicity of oil from *Artemisia annua* to certain stored product beetles. J. Econ. Entomol. 93: 43-47.
Verma N, Tripathi AK., Prajapati V, Bahl JR, Khanuja SPS, Kumar S (2000). Toxicity of essential oil from *Lippia alba* towards stored grain insects. J. Med. Arom. Plant Sci. 22 : p.50.
Weaver DK, Dunkel FV, Ntezurubanza L, Jakson LL, Stock DT (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.
Weaver DK, Phillips TW, Dunkel FV, Weaver T, Grubb RT, Nance EL (1995). Dried leaves from the rocky mountain plants decrease infestation by stored product beetles. J. Chem. Ecol. 21:127-142.
White L (1995). Chemical control. Integrated management of insects in stored products. Dekker, Inc; New York. Basel. Hong Kong, pp. 287-330.
Zettler JL, Cuperus GW (1990). Pesticide resistance in *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in wheat. J. Econ. Entomol. 83: 1677-1681.