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Fumigant Toxicity of Essential Oils Against Rice Weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae)

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Abstract: Essential oils being natural products, biodegradable and ecologically safe are emerging candidates as replacement of synthetic pesticides in pest management programme. In the present study, essential oils were isolated by hydrodistillating dried fruits of *Cuminum cyminum* (Apiaceae) and *Piper nigrum* (Piperaceae) and its repellent, fumigant toxicity and effect on acetylcholinesterase enzyme (AChE) activity was determined against rice weevil, *Sitophilus oryzae*. *C. cyminum* and *P. nigrum* essential oils showed significant ($p < 0.05$) repellent activity and caused fumigant toxicity in *S. oryzae* adults with median lethal concentrations (LC_{50}) 0.67 and 0.58 $\mu\text{L cm}^{-3}$ air, respectively. Fumigation of *S. oryzae* adults with sublethal concentrations of *C. cyminum* and *P. nigrum* essential oils significantly ($p < 0.05$) inhibited AChE activity. Reduction in AChE activity was 77.38 and 50.0 and 75.0 and 53.57% of the control after 24 h of fumigation with 40 and 80% of 24-h LC_{50} of *C. cyminum* and *P. nigrum* essential oils, respectively. In conclusion, these essential oils probably induce toxicity in insects by inhibiting AChE activity.

Key words: *Cuminum cyminum*, *Piper nigrum*, essential oil, acetylcholinesterase, *Sitophilus oryzae*

INTRODUCTION

With the beginning of agriculture, storage of food grains started as a safeguard to avoid situations like poor harvest and famine. Since ancient time insect pests have been damaging and causing heavy losses of stored grain quantitatively and qualitatively both throughout the world especially in tropical and semitropical countries (Madrid *et al.*, 1990; Tripathi *et al.*, 2009). In countries without modern storage technologies, insect pest cause 10-40% damage in stored grains (Shaaya *et al.*, 1991). In India, this damage accounts for 10% of the total production at the farm level (Lal, 1988). In such a critical situation, synthetic pesticides came into play, however, with increased risk of neurotoxic, carcinogenic, teratogenic and mutagenic effects in non-target animals and resistance in insects (Dyte and Halliday, 1985; Sharaby, 1988; Irshad and Gillam, 1990; Zettler and Cuperus, 1990; Zettler, 1991). These outcomes of synthetic insecticides application have increased public awareness regarding the human safety and possible environmental damage and focused researches towards other alternatives for the management of stored-grain insect pest.

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; Mahdneshtin *et al.*, 2009), plant extracts (Allotey and Azlekor, 2000; Sabbour, 2003; Upadhyay *et al.*, 2006; Haghtalab *et al.*, 2009) and insect growth regulators (Arthur and Hoeremann, 2004) have been proved effective tools in controlling stored grain insect pests. Beside, plant based chemicals especially plant derived volatiles have attracted focus of the scientific communities as an effective tool in pest management programme (Shaaya *et al.*, 1991, 1997; Huang and Ho, 1998; Lee *et al.*, 2001; Ikbali *et al.*, 2007; Koul *et al.*, 2007; Chaubey, 2007a,b; Abdelgaleil *et al.*, 2009; Chaubey, 2011). Aromatic plant are distributed in a limited number of families such as Asteraceae, Apiaceae, Cupressaceae, Lauraceae, Lamiaceae, Myrtaceae, Poaceae, Piperaceae, Rutaceae and Zinziberaceae and approximately 3,000 essential oils are known. Essential oils are complex secondary metabolic products with strong odour. These are lipophilic in nature having density lower than water and with metabolic, biochemical, physiological and behavioral role in insects (Bakkali and Averbeck, 2008). Biological activities of essential oils depends upon its chemical composition which in turn, varies with plant parts used, method of extraction, age and phenological stage of plant, harvesting season, nature of soil and environmental conditions (Angioni *et al.*, 2006; Isman *et al.*, 2007). These essential oils have larvicidal activity, antifeedant activity, capacity to delay development, adult emergence, fertility and deterrent

effects on oviposition in insects (Naumann and Isman, 1995; Marimutu *et al.*, 1997; Adebayo *et al.*, 1999; Larocque *et al.*, 1999; Oyedele *et al.*, 2000; Gbolade, 2001; Chaubey, 2007a,b; Isman *et al.*, 2007; Koul *et al.*, 2007; Abdelgaleil *et al.*, 2009; Caballero-Gallardo *et al.*, 2011; Liu *et al.*, 2011). In the present study, bioactivity of *Cuminum cyminum* (Apiaceae) and *Piper nigrum* (Piperaceae) essential oils were studied against rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae).

MATERIALS AND METHODS

Isolation of oils: The dried fruits of *C. cyminum* and *P. nigrum* were purchased from the local market of Gorakhpur, U.P., India. These were grounded and powdered material was hydrodistilled in Clevenger apparatus continuously for four hours to yield essential oils. The oils were collected and kept in eppendroff tube at 4°C until their use. The collection of essential oils was done in the months of February and March and its bioactivities were determined in the months from April to August.

Insects: Rice weevil *S. oryzae* was used to determine the insecticidal nature of essential oils. The insects were reared on whole wheat grains in the laboratory at 28±2°C, 75±5% RH and a photoperiod of 12:12 (L: D) h.

Repellant activity of essential oils on *S. oryzae* adults:

Repellency assay was carried out in glass petri dishes (diameter 8.5 and height 1.2 cm). Test solutions of serial dilution of *C. cyminum* and *P. nigrum* essential oils were prepared in acetone. Whatmann filter paper was cut into two equal halves and each essential oil solution was applied to filter paper half as uniform as possible using micropipette. The other half of filter paper was treated with acetone only. The essential oil treated and acetone treated halves were dried to evaporate the solvent completely. Both treated and untreated halves were attached with cellophane tape and placed at the bottom in petri dishes. Twenty adults of *S. oryzae* were released at the centre of filter paper disc and then petri dishes were covered and kept in dark. Four replicates were set for each concentration of essential oil solution. Number of insects on both treated and untreated halves was recorded after 4 h in mild light.

Fumigant activity of essential oils on *S. oryzae* adults:

Fumigant toxicity of *C. cyminum* and *P. nigrum* essential oils was tested against adults of *S. oryzae*. Ten adults taken from the laboratory culture were placed with 2 g of

wheat grains in glass petri dishes (diameter 8.5 and height 1.2 cm). A filter paper strip (2 cm diameter) treated with essential oil solutions prepared in acetone (30, 40, 50, 60 µL essential oil in 100 µL solvent per replicate), was pasted on the under cover of petridish. All the petri dishes were closed and kept in dark. Six replicates were set for each concentration of essential oil. After 24 h of fumigation, mortality in adults was recorded.

Acetylcholinesterase enzyme (AChE) activity

determination: Adults of *S. oryzae* were fumigated with two sublethal concentrations viz. 40 and 80% of 24 h LC₅₀ of *C. cyminum* and *P. nigrum* essential oils as was done in fumigant toxicity assay. After 24 h of fumigation, adults were utilized for determination of acetylcholinesterase enzyme activity using (Ellman *et al.*, 1961) method. Enzyme activity was expressed as µ mol of SH hydrolysed min⁻¹ mg⁻¹ protein. Each assay was replicated three times.

Data analysis: Chi-square test was applied to establish the repellent activity of essential oils (Sokal and Rohlf, 1973). Median Lethal Concentration (LC₅₀) was calculated using the POLO programme (Russell *et al.*, 1997). Student t-test was performed to test the significant changes in enzyme activity with respect to control (Armitage *et al.*, 2002).

RESULTS

Repellant activity of essential oils on *S. oryzae* adults:

In repellency assay, percentage of insects in treated filter paper disc half was 37.5, 30, 17.5 and 7.5 at 0.003215%, 0.00625, 0.0125 and 0.025% concentration of *C. cyminum* essential oils (Table 1). Similarly, percentage of insects in treated filter paper disc half was 30, 20, 12.5 and 5 at 0.003215, 0.00625, 0.0125 and 0.025% concentration of *P. nigrum* essential oils (Table 1). Chi-square analysis indicated that *C. cyminum* and *P. nigrum* essential oils were repellent to *S. oryzae* adults. These essential oils showed significant repellent activity even at 0.00625 and 0.003215% concentrations (vol:vol) of *C. cyminum* and *P. nigrum* essential oils, respectively as the hypothesis of the ratio 1:1 was rejected (Table 1).

Fumigant activity of essential oils on *S. oryzae* adults:

C. cyminum and *P. nigrum* essential oils induced high mortality in *S. oryzae* adults when fumigated. Median lethal concentration (LC₅₀) of *C. cyminum* and *P. nigrum* essential oils against *S. oryzae* adults were 0.67 and 0.58 µL cm⁻³ air, respectively (Table 2).

Table 1: Repellant activity of *Cuminum cyminum* and *Piper nigrum* essential oils against *Sitophilus oryzae* adults

Concentration (%) (vol:vol)	<i>C. cyminum</i>			<i>P. nigrum</i>		
	Treated Mean±SE	Untreated Mean±SE	χ^2 -values	Treated Mean±SE	Untreated Mean±SE	χ^2 -values
0.025	7.5±0.23	92.5±0.23	31.4 ^a	5±0.25	95.0±0.25	35.8 ^a
0.0125	17.5±0.95	82.5±0.95	20.0 ^b	12.5±0.5	97.5±0.5	28.6 ^b
0.00625	30±0.95	70.0±0.95	10.4 ^c	20±0.5	80.0±0.5	16.8 ^b
0.003215	37.5±0.95	62.5±0.95	4.6 ^d	30±0.5	70.0±0.5	8.0 ^b

^aSignificant at $p < 0.001$, ^bSignificant at $p < 0.01$, ^cSignificant at $p < 0.05$, ^dInsignificant

Table 2: Summary of toxicity assays of *Cuminum cyminum* and *Piper nigrum* essential oils against *Sitophilus oryzae* adults

Essential oil	LC ₅₀ μL cm ⁻³	LCL-UCL μL cm ⁻³	g-value	t-ratio	Heterogeneity
<i>C. cyminum</i>	0.67	0.53 – 0.71	0.17	4.82	0.32
<i>P. nigrum</i>	0.58	0.49 – 0.67	0.19	5.08	0.33

LC₅₀ represents lethal concentration that causes mortality in half of population treated. LCL and UCL represent lower confidence limit and upper confidence limit, respectively. g-value, t-ratio and heterogeneity are significant at all probability levels (90, 95 and 99%)

Table 3: Effect of 40 and 80% of 24h-LC₅₀ of *Cuminum cyminum* and *Piper nigrum* essential oils on acetylcholinesterase enzyme (AChE) activity in *Sitophilus oryzae* adults

Essential oil	Control	40% of 24h-LC ₅₀	40% of 24h-LC ₅₀
<i>C. cyminum</i>	0.084± 0.007 (100)	0.064± 0.004 (77.38) t = 8.66**	0.042±0.004 (50.0) t = 10.39**
<i>P. nigrum</i>	0.084± 0.007 (100)	0.063± 0.003 (75) t = 5.04**	0.045±0.004 (53.57) t = 14.74**

Enzyme activity was expressed as μmol of 'SH' hydrolysed min⁻¹mg⁻¹ protein. Values indicated Mean±SD of three replicates. Values in parentheses indicate per cent change with respect to control taken as 100%. *Paired t-test was applied. **Significant ($p < 0.05$). **Significant ($p < 0.01$)

Acetylcholinesterase enzyme (AChE) activity determination: Fumigation of *S. oryzae* adults with two sublethal concentrations viz. 40 and 80% of 24-h LC₅₀ of *C. cyminum* and *P. nigrum* essential oils significantly reduced AChE activity. Reduction in AChE activity was 77.38 and 50.0 and 75.0 and 53.57% of the control after 24 h of fumigation with 40 and 80% of 24 h LC₅₀ of *C. cyminum* and *P. nigrum* essential oils, respectively (Table 3).

DISCUSSION

Chemical control using synthetic insecticides has dominated control measures against stored grain insect pests. However, these insecticides have serious problems like genetic resistance in the pest, toxic residue property 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. Among plant based insecticides, essential oil as fumigants have received much attention of scientific groups. Plants capable of

producing essential oil are distributed in a limited number of families such as Asteraceae, Apiaceae, Cupressaceae, Lauraceae, Lamiaceae, Myrtaceae, Poaceae, Piperaceae, Rutaceae and Zinziberaceae. Many essential oils have been reported for their larvicidal and antifeedant activity (Adebayo *et al.*, 1999; Larocque *et al.*, 1999; Chaubey, 2007a,b) capacity to delay development, adult emergence and fertility (Marimutu *et al.*, 1997; Chaubey, 2007a,b) and deterrent effects on oviposition (Chaubey, 2007a,b). In the present study, repellent and cidal activities of *C. cyminum* and *P. nigrum* essential oils against rice weevil, *S. oryzae* have been investigated. These essential oils have found to cause reduction in oviposition potential, inhibition of development of larvae to pupae and the pupae to adults, increase in developmental period and also result in the deformities in different developmental stages of the *Tribolium castaneum* (Chaubey, 2007a,b).

These two essential oils under considerations in the present study significantly repelled the adults of *S. oryzae* when exposed to oil fumes. In the fumigant toxicity assay, mortality rate was increased with increase in concentration of both *C. cyminum* and *P. nigrum* essential oils. The index of significant 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. Values of heterogeneity factor less than 1.0 denotes that model fits the data adequate. Regarding the mode of action of essential oils against insect pests, little information is available but treatment with various essential oils or their constituents cause symptoms that suggest a neurotoxic mode of action (Kostyukovsky *et al.*, 2002; Priestley *et al.*, 2003; Lu and Wu, 2010). In the present study, activity of AChE decreased significantly in fumigated *S. oryzae* adults in comparison to the non-fumigated insects. Lee *et al.* (2001) and Abdelgaleil *et al.* (2009) have reported the competitive inhibition of AChE activity by monoterpenes and monoterpenoids (Coats *et al.*, 1991; Lee *et al.*, 2001; Abdelgaleil *et al.*, 2009). Most of the essential oil components like cuminaldehyde, limonene, α -pinene and

β -phellendrene inhibiting AChE activity have been reported as constituents of *C. cyminum* and *P. nigrum* essential oils and probably might attribute AChE inhibitory activities to the oils under investigation.

CONCLUSION

On the basis of results of the present study, it can be concluded that the insecticidal activities of *C. cyminum* and *P. nigrum* essential oils against rice weevil, *S. oryzae* might be due to its AChE inhibitory activities.

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