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Biological Activities of *Zingiber officinale* (Zingiberaceae) and *Piper cubeba* (Piperaceae) Essential Oils Against Pulse Beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae)

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Abstract: *Zingiber officinale* (Zingiberaceae) and *Piper cubeba* (Piperaceae) was essential oils were investigated for repellent, insecticidal, antiovipositional, egg hatching, persistence of its insecticidal activities against pulse beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). Essential oil vapours repelled bruchid adults significantly as oviposition was found reduced in choice oviposition assay. *Z. officinale* and *P. cubeba* essential oils caused both fumigant and contact toxicity in *C. chinensis* adults. In fumigation toxicity assay, median lethal concentrations (LC_{50}) were 0.34 and 0.27 μL cm^{-3} for *Z. officinale* and *P. cubeba* essential oils, respectively, while in contact toxicity assay, LC_{50} were 0.90 and 0.66 μL cm^{-2} for *Z. officinale* and *P. cubeba* essential oils, respectively. These two essential oils reduced oviposition in *C. chinensis* adults when treated with sublethal concentrations by fumigation and contact method. Oviposition inhibition was more pronounced when adults come in contact than in vapours. Both essential oils significantly reduced egg hatching rate when fumigated. Persistence in insecticidal efficiency of both essential oils decreased with time. *P. cubeba* showed less persistence than *Z. officinale* essential oil because no mortality was observed in *C. chinensis* adults after 36 h of treatment with *P. cubeba* and after 48 h of treatment of *Z. officinale* essential oil. Fumigation with these essential oils has no effect on the germination of the cowpea seeds. Findings of the study suggest that *Z. officinale* and *P. cubeba* essential oils can be useful as promising agent in insect pest management programme.

Key words: *Zingiber officinale, Piper cubeba*, oviposition deterrence index, hatching inhibition rate, callosobruchus chinensis

INTRODUCTION

With the beginning of agriculture, storage of food grains as a safeguard against poor harvests and famine started and since then insect pests are damaging stored grains both quantitatively and qualitatively. This damage amounts to 10-40% in countries depending on traditional storage technologies and this approaches 10% of total production in India at farm level (Lal, 1988; Shaaya *et al*., 1997). This creates a major problem in storing grains leading to wastage of food grains. Among important stored-product insect pests, *Callosobruchus chinensis* (Family: Bruchidae) is a serious pest infesting gram, cowpea, beans, lentil and other pulses. Under storage condition, *C. chinensis* causes 32-64% loss and the maximum damage takes place from April to October (Weaver *et al*., 1995). Only grubs are infective stages for the stored grains. These make holes in grains and consume inner part leaving empty kernel, and thus, damaged grains become unsuitable for human consumption, production of sprout and also lose its market value. Different synthetic chemicals in form of fumigants, sprays and dusts have been in use for the management of this pest, and some of them have been proved unsuccessfull because of high migration rate during transportation from field to godowns (Pandey *et al*., 1983). Besides this, excessive and continuous use of chemicals against insect pests developed resistance in them resulting in loss of several billion dollars worldwide each year (Elzen and Hardee, 2003; Benhalima *et al*., 2004; Islam and Talukder, 2005). Use of these insecticides increases the chances of ozone depletion, neurotoxicity, carcinogenicity, teratogenicity and mutagenic effects in non-target animals and cross-and multi-resistance in targets (WMO, 1991; Lu, 1995; UNEP, 2000; Beckel *et al*., 2002). These problems have diverted public interest regarding human safety and adverse effects on environment started using plant products in stored-grain insect pest management and since last two to three decades, efforts have been made to replace synthetic insecticides by plant products. For this purpose, different workers have used different plant based insecticidal agents especially essential oils against many insect pests (Caballero-Gallardo *et al*., 2011;

Liu *et al*., 2011; Stefanazzi *et al*., 2011; Chaubey, 2011, 2012; Jiang *et al*., 2012). Essential oils are secondary metabolites produced in plant parts of different plant species. These have strong odour, volatility and lower density (Bakkali *et al*., 2008). Due to their volatility, essential oils are nonpersistent environmentally and 'generally recognized as safe' by United States Food and Drug Administration (Tripathi *et al*., 2009; Nerio *et al*., 2010). Essential oils are produced in a number of plant species belonging to families like Myrtaceae, Lauraceae, Rutaceae, Lamiaceae, Asteraceae, Apiaceae, Cupressaceae, Poaceae, Zinziberaceae and Piperaceae. The chemical constituents and their biological activities vary with plant parts, extraction method, plant phenology, harvesting season, plant age, soil and environmental conditions (Angioni *et al*., 2006; Isman *et al*., 2007). The biological activities of essential oils depend on the major constituents present. Recent researches have reported insecticidal nature of several essential oils against bruchids (Chaubey, 2008, Chaubey, 2011; Jiang *et al*., 2012; Islam *et al*., 2009; Aboua *et al*., 2010; Ahmed, 2010; Caballero-Gallardo *et al*., 2011). In the present study, *Z. officinale* and *P. cubeba* essential oils were evaluated for their repellent, insecticidal, antiovipositional and egg hatching inhibitory activities against pulse beetle, *C. chinensis*.

MATERIALS AND METHODS

Essential oils: Essential oils were isolated from *Z. officinale* rhizome and *P. cubeba* berries. Rhizomes and berries were purchased from local market of Gorakhpur, (U.P.), India, grounded by domestic mixer and hydrodistilled in Clevenger apparatus continuously for 5 h to yield essential oils. Oils were collected and kept in Eppendorf tubes at 4°C until their use.

Insects Pulse beetles, *C. chinensis* were used to investigate the biological activities of essential oils. The insects were reared on cowpea seeds in laboratory at 30±2°C, 75±5% RH and a photoperiod of 12:12 (L:D) h.

Repellency assay: Twenty 0-24 h old bruchid adults were taken in a rounded plastic box (20 cm diameter×10 cm height) with two glass petri dishes (9 cm diameter) containing 20 cowpea seeds each. A filter paper disc (9 cm diameter) impregnated with 1,000 μL aliquot of solutions (prepared in ethanol) containing 0.31, 0.63, 0.94 and 1.26 μL cm^{-2} essential oil was placed on a petri dish (test). Simultaneously, a filter paper disc treated with ethanol only was placed on the opposite petri dish (control). After 48 h, the number of eggs laid on cowpea seeds was counted. Repellency was measured by comparing the number of eggs laid on cowpeas in test petri dish against the number of eggs laid on cowpeas in control. For each concentration of essential oil and control three replicates were set.

Fumigation toxicity assay: Fumigant toxicity of essential oils was determined against 2-4 days old bruchid adults using glass vials (10 cm long and 3 cm diameter) with screw cap. Test solutions of different concentrations were prepared by diluting essential oils with ethanol. For fumigation, filter paper strip (2 cm diameter) impregnated with 100 μL aliquot of test solution (containing 10, 20, 30 and 40 μL of essential oil) was pasted on the inner side of cap and solvent was allowed to evaporate for 5 min. Neck of vial was blocked with a metal mesh to avoid the contact effect of essential oils. Twenty cowpea seeds were taken in each vial and into it ten adults were released. Open end of vial was closed by screw cap so that oil treated filter paper remains inside vial and vials were kept in conditions maintained for insect culture. Mortality in adults was recorded after 24 h of treatment. In control, filter paper impregnated with solvent only was used. For each essential oil, four different concentrations and for each concentration of essential oil and control six replicates were set.

Contact toxicity assay: Contact toxicity of essential oils was determined against 2-4 days old bruchid adults using glass vials (10 cm long and 3 cm diameter) with screw cap. Test solutions of different concentrations were prepared by diluting essential oils with ethanol. A 1 mL aliquot of test solution (containing 5, 10, 15 and 20 μL of essential oil) was applied on inner surface of vials and under surface of screw cap by rolling it. The treated glass vial was kept open for 5 min to evaporate solvent. Twenty cowpea seeds were taken in each vial and into it ten adults were released, Vials were closed and kept in conditions maintained for insect culture. Mortality in adults was recorded after 24 h of treatment. In control group, glass vial treated with solvent only was used. For each essential oil, four different concentrations and for each concentration and control six replicates were set.

Oviposition inhibition assay

By fumigation method: In this assay, ten 0-24 h old bruchid adults were fumigated with two sublethal concentrations (40 and 80% of 24 h-LC_{50} determined in fumigation toxicity assay) of essential oil test solutions as was done in fumigation toxicity assay. After 24 h of fumigation, adults were transferred into clean vial with twenty fresh cowpea seeds. In control group only solvent was used. After 96 h, number of eggs laid over the

cowpea seeds was counted. For each concentration of essential oil as well as control group, six replicates were set.

By contact method: In this assay, ten 0-24 h old bruchid adults were treated with two sublethal concentrations (40 and 80% of 24 h-LC_{50} determined in contact toxicity assay) of essential oil test solutions as was done in contact toxicity assay. After 24 h of treatment, adults were transferred into clean vial with twenty fresh cowpea seeds. In control group only solvent was used. After 96 hours, number of eggs laid over the cowpea seeds was counted. For each concentration of essential oil as well as control, six replicates were set.

%ODI (Oviposition Deterrence Index) was calculated as:

$$\text{ODI (\%)} = \frac{100(A-B)}{(A+B)}$$

where, A and B represent number of eggs laid in control and in test respectively.

Ovicidal assay: In ovicidal assay, 25 eggs were fumigated with test solutions prepared by diluting essential oils with ethanol. A 100 μL aliquot of test solution (containing 12, 24, and 36 μL of essential oil) was applied on filter paper strip (2 cm diameter) was and solvent was allowed to evaporate for 5 min. The filter paper was attached to the undersurface of screw cap of vial (10 cm long and 3 cm diameter). Cap of vial was screwed and incubated for 24 h in conditions maintained for insect culture. After fumigation, eggs were allowed to hatch and number of adults emerged was counted till one month of treatment. Four different concentrations of each essential oil were used and for each concentration and control six replicates were set.

% HIR (Hatching Inhibition Rate) was calculated as:

$$\text{Hatching Inhibition Rate (HIR) (\%)} = \frac{(Cn-Tn)}{Cn} \times 100$$

where, Cn and Tn represent number of larvae hatched from control and test.

Persistence of insecticidal activity of essential oils: To determine persistence of insecticidal activity of essential oils, concentration causing 100% mortality (24 h-LD_{100}) was determined against 2-4 days old bruchid adults by contact method. Now, test solutions containing LD_{100} of essential oils were prepared in ethanol and applied as was done in contact toxicity assay. From beginning and after every 6 h, ten adults were introduced in each vial up to 48 h and mortality in adults was recorded. Each experiment was replicated six times.

Effect of essential oils on seed germination: Forty cowpea seeds were fumigated for one month with *Z. officinale* and *P. cubeba* essential oil test solutions in glass vials (10 cm long and 3 cm diameter) with screw cap as was done in fumigant toxicity assay. Essential oil test solutions were prepared by dissolving 40 and 80 μL in 100 μL ethanol. The treated cowpea seeds were placed on moistened filter paper and allowed to germinate. Number of cowpea seeds germinated was recorded for 10 days. For each concentration of essential oils as well as control group, six replicates were set.

Data analysis: Median lethal concentration (LC_{50}) was calculated by POLO programme and one way analysis of variance (ANOVA) was performed to test significancy of data (Sokal and Rohlf, 1973; Russell *et al*., 1977).

RESULTS

Repellency assay: *Z. officinale* and *P. cubeba* essential oils inhibit oviposition in repellency assay in concentration dependent manner. Oviposition was reduced to 74.94, 57.59, 37.27, 26.31% and 66.52, 48.94, 31.57 and 15.86% at 0.31, 0.63, 0.94 and 1.26 μL cm^{-2} concentration of *Z. officinale* and *P. cubeba* essential oils respectively (Fig. 1).

Toxicity assay: *Z. officinale* and *P. cubeba* essential oils caused both fumigant and contact toxicity in *C. chinensis* adults. In fumigation toxicity assay, median lethal

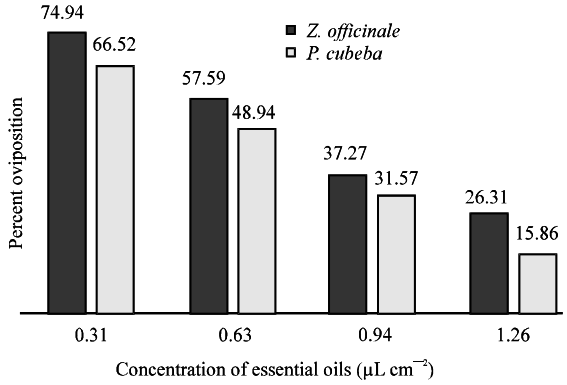

Fig. 1: Percent oviposition in repellency assay when *C. chinensis* adult were fumigated *Z. officinale* and *P. cubeba* essential oils by choice oviposition method

Table 1: LC_{50} values of *Z. officinale* and *P. cubeba* essential oils against *C. chinensis* adult determined by fumigation toxicity method

Essential oils	LC_{50} (μLcm^{-3})	LCL (μLcm^{-3})	UCL (μL cm^{-3})	t-ratio	Slope value	g-value	Heterogeneity
Z. officinale	0.34	0.29	0.40	3.98	1.83	0.32	0.31
P. cubeba	0.27	0.21	0.34	4.17	1.72	0.27	0.29

LC_{50} represents lethal concentrations that cause 50% mortality; UCL and LCL represent upper and lower confidence levels; t-ratio, heterogeneity, slope values and g-values are significant ($p<0.01$)

Table 2: LC_{50} values of *Z. officinale* and *P. cubeba* essential oils against *C. chinensis* adult determined by contact toxicity method

Essential oils	LC_{50} (μL cm^{-2})	LCL (μL cm^{-2})	UCL (μL cm^{-2})	t-ratio	Slope value	g-value	Heterogeneity
Z. officinale	0.90	0.76	1.02	3.21	1.92	0.33	0.30
P. cubeba	0.66	0.51	0.83	3.65	1.84	0.29	0.32

LC_{50} represents lethal concentrations that cause 50% mortality; UCL and LCL represent upper and lower confidence levels; t-ratio, heterogeneity, slope values and g-values are significant ($p<0.01$)

Table 3: Effect of *Z. officinale* and *P. cubeba* essential oils on oviposition of *C. chinensis* determined by fumigation method

Essential oils	Treatment	No. of eggs laid per insect (Mean±SE)	%ODI	F-value (df = 2,17)
Control	Ethanol	19.75±1.95(100)	-	-
Z. officinale	40% of 24 h-LC_{50}	15.01±1.71(76.0)	13.63	
	80% of 24 h-LC_{50}	9.15±1.30(46.33)	36.67	50.76
P. cubeba	40% of 24 h-LC_{50}	13.36±1.57(67.64)	19.30	
	80% of 24 h-LC_{50}	6.83±1.25(34.58)	48.61	78.59

%ODI was calculated as 100(A-B)/(A+B), where A and B represent the number of eggs laid in the control and in the test respectively, Values in parentheses indicate percent change with respect to control taken as 100%, F-values were significant ($p<0.01$)

Table 4: Effect of *Z. officinale* and *P. cubeba* essential oils on oviposition of *C. chinensis* determined by contact method

Essential oils	Treatment	No. of eggs laid per insect (Mean±SE)	%ODI	F-value (df = 2,17)
Control	Ethanol	18.070±1.84(100)	-	-
Z. officinale	40% of 24 h-LC_{50}	6.88±1.12(38.07)	44.85	178.89
	80% of 24 h-LC_{50}	3.53±0.78(19.53)	67.31	
P. cubeba	40% of 24 h-LC_{50}	5.11±0.96(28.27)	55.91	259.78
	80% of 24 h-LC_{50}	2.80±0.61(15.49)	73.17	

%ODI as calculated as 100(A-B)/(A+B), where A and B represent the number of eggs laid in the control and in the test respectively, Values in parentheses indicate percent change with respect to control taken as 100%, F-values were significant ($p<0.01$)

Table 5: Effect of fumigation of *Z. officinale* and *P. cubeba* essential oils on egg hatching rate of *C. chinensis*

Essential oils	Treatment (μL cm^{-3})	Egg hatching (Mean±SE)	% HIR[a]	F-value (df = 3,23)
Control	Ethanol	22.16±1.99(100)	-	-
Z. officinale	0.169	17.66±1.77(79.69)	20.3	144.74
	0.339	13.00±1.59(58.66)	41.33	
	0.509	6.83±1.15(31.09)	69.17	
P. cubeba	0.169	15.66±1.64(70.66)	29.33	289.54
	0.339	9.16±1.31(41.33)	58.66	
	0.509	4.83±0.94(21.79)	78.20	

[a]% Hatching Inhibition Rate (HIR) = [(Cn-Tn)/Cn]×100, where, Cn and Tn represent number of adults emerged from control and test, Values in parentheses indicate per cent change with respect to control taken as 100%, F-values were significant ($p<0.01$)

concentrations (LC_{50}) were determined 0.34 and 0.27 μL cm^{-3} for *Z. officinale* and *P. cubeba* essential oils respectively (Table 1). In contact toxicity assay, median lethal concentrations (LC_{50}) were determined 0.90 and 0.66 μL cm^{-2} for *Z. officinale* and *P. cubeba* essential oils, respectively (Table 2).

Oviposition inhibition assay: *Z. officinale* and *P. cubeba* essential oils significantly ($p<0.01$) reduced oviposition potential in *C. chinensis*. In fumigation oviposition assay, mean numbers of eggs laid per insect were 15.01 and 9.15; and 13.36 and 6.83 when bruchid adults were fumigated with 40 and 80% of 24 h-LC_{50} *Z. officinale* and *P. cubeba* essential oils respectively as compared to 19.75 eggs laid per insect in control (Table 3). The %ODI was calculated 13.63 and 36.67 and 19.3 and 48.61 when adults were fumigated with 40 and 80% of 24 h-LC_{50} *Z. officinale* and *P. cubeba* essential oils respectively (Table 3).

In contact oviposition assay, mean numbers of eggs laid per insect were 7.88 and 4.53; and 5.11 and 2.88 when bruchid adults were fumigated with 40 and 80% of 24 h-LC_{50} *Z. officinale* and *P. cubeba* essential oils respectively as compared to 18.07 eggs laid per insect in control (Table 4). The %ODI was calculated 39.27 and 52.18; and 55.91 and 73.17 when adults were fumigated with 40 and 80% of 24h-LC_{50} *Z. officinale* and *P. cubeba* essential oils, respectively (Table 4).

Ovicidal assay: *Z. officinale* and *P. cubeba* essential oils significantly ($p<0.01$) reduced hatching rate in *C. chinensis* eggs when fumigated. Mean number of eggs hatched per 25 eggs was reduced to 17.66, 13.0 and 6.83; and 15.66, 9.16 and 4.83 when fumigated with 0.169, 0.339 and 0.509 μcm^{-3} of *Z. officinale* and *P. cubeba* essential oils respectively as compared to 22.16 eggs hatched in control (Table 5). Increase in %HIR was 20.3, 41.33 and

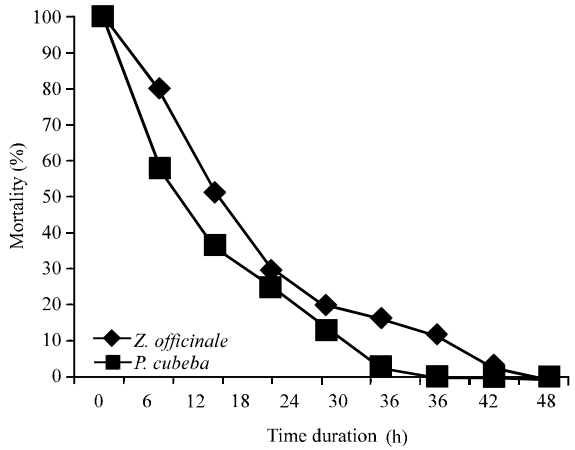

Fig. 2: Persistence of insecticidal activity of *Z. officinale* and *P. cubeba* essential oils against *C. chinensis* adults

Table 6: Effect of *Z. officinale* and *P. cubeba* essential oils on percent germination of cowpea seeds

Treatment	Conc. (μL cm^{-3})	Mean percent germination
Control	0.00	76.25
Z. officinale	0.56	75.00*
	1.13	77.07*
P. cubeba	0.56	76.65*
	1.13	79.15*

*Not significant

69.17; and 29.33, 58.66 and 78.2 when eggs were fumigated with 0.169, 0.339 and 0.509 μL cm^{-3} of *Z. officinale* and *P. cubeba* essential oils, respectively (Table 5).

Persistence of insecticidal activity of essential oils: Persistence in insecticidal efficiency of *Z. officinale* and *P. cubeba* essential oils against *C. chinensis* decreased with time. After 36 h of treatment with *P. cubeba* and after 48 h of treatment of *Z. officinale* essential oil, no mortality was observed in *C. chinensis* adults (Fig. 2). Therefore, *Z. officinale* showed more persistence than *P. cubeba* essential oil.

Effect of essential oils on seed germination: Fumigation with *Z. officinale* and *P. cubeba* essential oil had no significant effect on percent germination of cowpea seeds when compared to control (Table 6).

DISCUSSION

Use of plant products as pesticides is gaining importance in integrated pest management programmes because synthetic insecticides impose environmental and health hazard. Earlier attempts to explore the toxicity of essential oils against *C. chinensis* have been made by several scientific groups. Essential oils can affect insects by repellent activity (Islam *et al*., 2009), disrupting metabolic pathways leading to death, acting as contact insecticides (Jiang *et al*., 2012), fumigants (Chaubey, 2008) and inhibiting oviposition (Chaubey, 2008). Keita *et al*. (2001) have reported essential oils from *Tagetes minuta, Hyptis suaveolens, Ocimum canum* and *O. basilicum* cause mortality and complete inhibition of oviposition in *C. maculatus*, another pest of cow pea Keita *et al*., (2001). The dry ground leaves of *Chenopodium ambrosioides* inhibited F_1 progeny production and adult emergence (Tapondjou *et al*., 2002). Islam *et al*. (2009) have reported repellent, contact and fumigant toxicity, ovicidal and adult inhibitory activities of *Cinnamomum aromaticum* oil (Islam *et al*., 2009). Caballero-Gallardo *et al*. (2011) have reported toxic, antifeedant, antiovipositional and ovicidal activities of various essential oils (Caballero-Gallardo *et al*., 2011).

In the present study, *Z. officinale* and *P. cubeba* essential oils significantly repelled the bruchid adults at 0.31 μL cm^{-2} concentrations as the oviposition capacity decreased in choice oviposition assay. Both these essential oils caused fumigant and contact toxicity against bruchid adults. In the toxicity assay, the mortality rate was found to increase with an increase in concentration of essential oils indicating that the response was concentration dependent. In the toxicity assays, index of significancy of potency estimation, g-value indicates that the mean value is within the limits of all probabilities ($p<0.1$, 0.05, 0.001) as it is less than 0.5. Values of t-ratio greater than 1.6 indicate that the regression is significant. Values of heterogeneity factor less than 1.0 denotes that model fits the data adequate. The steep slope values indicated that even small increase in the concentration of the essential oil causes high mortality. *Z. officinale* and *P. cubeba* essential oils reduced egg laying capacity in *C. chinensis* adults as comparaed to the control in oviposition inhibition assay performed either by fumigation or contact method. Antiovipositional activities of both oils were more pronounced when bruchid adults were treated with essential oils by contact method. *Z. officinale* and *P. cubeba* essential oils reduced hatching rate in *C. chinensis* eggs when fumigated. Persistence in insecticidal efficiency of *Z. officinale* and *P. cubeba* essential oils against *C. chinensis* decreased with time. After 36 h of treatment with *P. cubeba* and after 48 h of treatment of *Z. officinale* essential oil, no mortality was observed in *C. chinensis* adults. Therefore, *Z. officinale* essential oil showed more persistence activity than *P. cubeba* essential oil. Persistence of the insecticidal activity depends on the chemical properties and nature and position of the functional groups of essential oil constituents (Obeng-Ofori *et al*., 1997;

Kumbhar and Dewang, 2001). Essential oils having high content of hydrogenated compound loss their activity quickly than those containing high content of oxygenated compounds (Huang and Ho, 1998; Regnault-Roger *et al*., 2002). The insecticidal constituents of essential oils are mostly monoterpenoids (Regnault-Roger *et al*., 2002; Ahn *et al*., 1998). Monoterpenoids are highly volatile and lipophilic compounds that can penetrate into insects rapidly and interfere with their physiological functions (Lee *et al*., 2003). Due to their high volatility, they are useful as fumigant for stored-product insects (Ahn *et al*., 1998). Therefore, insecticidal activity of *Z. officinale* and *P. cubeba* may be related to these components. It may also be possible that different minor components show synergism with other active components in any way (Yu *et al*., 2004). The toxicity exhibited by essential oils and their constituent monoterpenes makes them potential alternatives as fumigants (Huang *et al*., 2000). The mode of action of these essential oils has not yet been known but it may be due to suffocation and inhibition of various biosynthetic processes of the insect (Don-Pedro, 1989). It must be kept in mind that essential oils/constituents should be toxic to target insects and should not be toxic to non-target organisms such as other beneficial insects and other animals such as fish, birds and humans. There are several other factors that must be considered during the evaluation of insecticides like risk associated to users, mode of exposure, degradation in the environment and chronic toxicity to be used effective for control of stored-product insect populations.

CONCLUSION

From the present study, it can be concluded that essential oils can a potent replacement of the synthetic insecticides especially against stored grain insect pests since these are biodegradable, less toxic to mammals, more selective in action and may retard the development of insect resistance, these cause no ecological disturbances. Their main advantage is that they may be easily and cheaply produced by farmers on small scale industries.

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