Insecticidal property of terpenes against maize weevil, *Sitophilus zeamais* (Motschulsky)

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ABSTRACT

Synthetic pesticides are used indiscriminately in insect pest management, which damages the ozone layer and causes resistance in target organisms as well as neurotoxicity, carcinogenicity, teratogenicity and mutagenesis in non-target organisms. Due to these negative consequences, research is now focused on using plant-based techniques to control insect pests. The maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae), was tested in the laboratory to determine the insecticidal effects of two pure essential oil constituents, namely α-pinene and β-caryophyllene. These two terpenes were tested against *S. zeamais* for their toxic, ovipositional, developmental, and feeding inhibitory effects. When *S. zeamais* adults were fumigated for 24 and 48hrs, the median lethal concentrations (LC_{50}) of α-pinene and β-caryophyllene were 0.412 and 0.305 μlcm^{-3} and 0.486 and 0.315 μlcm^{-3} air respectively. When *S. zeamais* adults were exposed for 24 and 48hrs in a contact toxicity assay, the LC_{50} values for α-pinene and β-caryophyllene were 0.388 and 0.256 μlcm^{-2} and 0.308 and 0.216 μlcm^{-2} area respectively. Adults exposed to sub-lethal concentrations of both terpenes experienced decreased acetylcholine esterase (AChE) enzyme activity. In *S. zeamais*, α-pinene and β-caryophyllene decreased oviposition, progeny output and eating. According to this study, α-pinene and β-caryophyllene can be used to make environmentally acceptable formulations and as a substitute for synthetic insecticides.

Keywords: α-Pinene, β-Caryophyllene, *Sitophilus zeamais*, Oviposition inhibition

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INTRODUCTION

Grain insect pests cause significant qualitative harm to stored grains as well as yearly economic losses. Insecticides of synthetic type have been created and utilised in various ways to reduce these losses. But the continued and unchecked use of these synthetic chemicals has negatively impacted the health of people and the environment. In addition, these include ozone layer destruction, neurotoxicity, carcinogenicity, teratogenicity and mutagenicity in species unrelated to the target, as well as cross- and multi-resistance in insects that are both targets and non-targets (WMO, 1991; Lu, 1995; UNEP, 2000; Beckel, 2002).

These synthetic insecticides are killing over 355,000 people per year by entering continuously in our ecosystems and food chain (Alavanja and Bonner, 2012; EEA, 2013). The main culprit, organochlorines are persistent in nature and bio-accumulate in organisms at population level and kill bees, birds, amphibians, fish and small mammals (Köhler and Triebskorn, 2013). All these outcomes have shifted the focus towards the use of plant based insecticides for insect pest management.

Essential oils are produced as secondary metabolites in plants of families like Alliaceae, Apiaceae, Asteraceae, Cupressaceae, Lamiaceae, Lauraceae, Myrtaceae, Piperaceae, Poaceae, Rutaceae and Zingiberaceae. These are complex mixtures of compounds of various chemical natures whose concentration depends on parts of plant used for extraction, extraction method, plant phenological stage, harvesting season, plant age, genotype of plant, soil nature and environmental conditions (Atti-Santos *et al.,* 2004; Angioni *et al.,* 2006; Verma *et al.,* 2011). These essential oil and its constituents have been well known for their anti-insect activities (Negahban *et al.,* 2006; Rozman *et al.* 2006; Khalfi *et al.,* 2008).

Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae) is a significant pest frequently found in humid tropical regions of the world where maize is widely cultivated. It also harms wheat, rice, sorghum, oats, barley, rye, buckwheat, peas and cottonseed in addition to maize (Demissie *et al.,* 2008). Whole grains are attacked by the adult stage, while the developing larva feeds on the grains (Ileleji *et al.,* 2007).

α-Pinene, a monoterpene containing a reactive four-membered ring, is found in essential oils of *Nepta racemosa* (Dabiri and Sefidakon, 2003), Ferulago spp. (Khalighi-Sigaroodi *et al.,* 2005), *Syzygium aromaticum* (Alma *et al.,* 2007), *Biden pilosa* (Deba *et al.,* 2008), *Zingiber officinale* (Koroch *et al.,* 2007; Sasidharan and Menon, 2010), Eucalyptus spp. (Cheng *et al.,* 2009; Maciel *et al.,* 2010), *Citrus* spp. (Kamal *et al.,* 2011), *Vicia dadianorum* (Kahriman *et al.,* 2012) and *Picea abies* (Kamaityte *et al.,* 2021). β-Caryophyllene, a bicyclic sesquiterpene having cyclobutane ring, is found in essential oils of *Piper cubeba* (Lawless, 1995), *Scutellaria pinnati* (Ghannadi and Mehregan, 2003), Ferulago spp. (Khalighi-Sigaroodi *et al.,* 2005), *Syzygium aromaticum* (Alma *et al.,* 2007), *Biden pilosa* (Deba *et al.,* 2008), *Eucalyptus* spp. (Cheng *et al.,* 2009; Maciel *et al.,* 2010), *Citrus* spp. (Kamal *et al.,* 2011), *Pistacia lentiscus* (Burham *et al.,* 2011) and *Psidium guajava* (Arain *et al.,* 2019).

In this work, the effects of two volatile terpenes, α-pinene and β-caryophyllene, on the maize weevil, *S. zeamais,* were assessed. These effects included repulsion, insecticidal, AChE inhibitory, oviposition inhibitory, developmental inhibitory and antifeedant properties.

MATERIALS AND METHOD

Terpenes

Two pure terpenes viz. monocyclic monoterpene, α-pinene (2,6,6 Trimethylbicyclo [3.1.1] hept-2-ene) and bicyclic sesquiterpene, β-caryophyllene (4,11,11-trimethyl-8-methylene-bicyclo [7.2.0] undec-4- ene) were purchased from Sigma Chemicals, USA.

Insects

Maize weevil, *S. zeamais* was used to evaluate insecticidal properties of α-pinene and β-caryophyllene. The insects were reared on whole maize grain at 28±4^{0}C, 50±5% RH and photoperiod of 10:14 (L:D)hrs.

Repellent activity

Repellency assay was performed in glass petri dishes (diameter 8.5 cm, height 1.2 cm) (Chaubey, 2007). Experimental solutions of α-pinene and β-caryophyllene were prepared in acetone. Whatman filter papers were cut into two halves and each test solution was applied to half of the filter paper as uniform as possible using micropipette (Fine Care Corporation, Dantali, Gujarat, India). The other half of filter paper was treated with acetone only. Treated and untreated halves were dried to evaporate acetone completely. Both treated and untreated halves were then attached with cellophane tape and placed in each petri dish. Forty *S. zeamais* adults were released at the center of filter paper disc and petri dish was covered and kept in dark. Six replicates were set for each concentration of pure compounds. After 4hrs of exposure, adults in treated and untreated halves were counted. Percent repellency (PR) was calculated using formula: PR = [(C-T)/(C+T)] ×100, C = number of insects in the untreated halves and T = number of insects in treated halves. Preference index (PI) was calculated using formula: PI = (percentage of insects in treated

halves - percentage of insects in untreated halves)/ (percentage of insects in treated halves+percentage of insects in untreated halves). PI between - 1.0 and - 0.1 indicate repellant nature, - 0.1 to + 0.1 neutral nature and + 0.1 to + 1.0 attractant nature.

Fumigant toxicity

Experimental solutions of α-pinene and β-caryophyllene were made in acetone. Ten adults taken from laboratory culture were placed with 2 gm of maize grains in glass petri dish. Filter paper strip (2 cm diameter) was treated with terpene formulation and left for few minutes to evaporate acetone. Now, experimental test solution coated filter paper was pasted on undercover of petri dish, air tightened with parafilm and kept in conditions applied for rearing of insect in dark. Six replicates were set for each concentration of pure compounds and control. After 24 and 48hrs of exposure, mortality in adults was recorded.

Contact toxicity

Experimental solutions of α-pinene and β-caryophyllene were made in acetone, applied on bottom surface of glass petri dish (diameter 8.5 cm, height 1.2 cm) and left for few minutes to evaporate acetone. Ten adults taken from laboratory culture were released at the center of petri dish, covered and kept in conditions applied for rearing of insect in dark. After 24 and 48hrs of exposure, mortality in adults was recorded.

Acetylcholine esterase (AChE) activity

S. zeamais adults were fumigated with two sub-lethal concentrations *viz.* 40 and 80% of 24h-LC_{50} of α-pinene and β-caryophyllene. After 24hrs of fumigation, adults were used for determination of enzyme activity (Ellman *et al.,* 1961). Fumigated adult insects were homogenized and centrifuged in phosphate buffer saline (50mM, pH8). Supernatant was used as source of enzyme. To 0.1mL of enzyme source, added 0.1mL substrate acetylthiocholine iodide (0.5mM), 0.05 ml chromogenic reagent, 5,5-Dithio-bis 2-nitrobenzoic acid (0.33 mM) and 1.45 mL phosphate buffer (50mM, pH8). Enzyme activity was determined by measuring changes in the optical density at 412 nm by incubating the reaction mixture for 3 min at 25^0C. Enzyme activity was expressed as mmol of 'SH' hydrolyzed $min^{-1}mg^{-1}$ protein.

Oviposition inhibition

Ten *S. zeamais* adults of mixed sex were fumigated with two sub-lethal concentrations viz. 40% and 80% of 24hrs-LC_{50} and 48hrs-LC_{50} of α-pinene and β-caryophyllene for 24hrs and 48hrs respectively and reared on maize grain in 250 mL plastic box. After 10 days, adults were discarded and number of F1 progeny was counted after 45 days. Six replicates were set for each concentration of pure compounds and control. The percentage oviposition deterrence (POD) was calculated using the formula:

POD = [(EC-ET)/EC] ×100, where E_C = number of adults emerged in control and E_T = number of adults emerged in test.

Developmental inhibition

Ten *S. zeamais* adults of mixed sex were placed with 20 gm of maize grains in a 250 mL plastic container with plastic lid and allowed to mate and lay eggs in laboratory condition applied for rearing of insects. After 7 days, adults were removed from the container. Now, a filter paper strip (2 cm diameter) impregnated with α-pinene and β-caryophyllene was pasted on undercover of the plastic lid and kept the container in laboratory condition applied for rearing of insects. The eggs and juveniles were fumigated with three concentrations (0.2, 0.4 and 0.6 μlcm^{-3}) of α-pinene and β-caryophyllene and number of adults emerged in control as well as in test was counted during the observation. Six replicates were set for each concentration of α-pinene, β-caryophyllene and control. Inhibition rate (IR) was calculated using formula (Tapondju *et al.,* 2002):

IR = [(Cn-Tn)/Cn] ×100, where Cn = number of adults emerged in control and Tn = number of adults emerged in test.

Antifeedant activity

Antifeedant activity of α-pinene and β-caryophyllene was determined by flour disc method (Suthisut *et al.,* 2011). Flour discs were prepared by mixing 10 gm of maize flour with 50 mL water until completely suspended. Maize flour

suspension was pipetted out (200 µL) onto a plastic sheet, held for 24hrs at room temperature and then dried in an oven for one hour at 60^0C. Each flour disc was treated with two sub-lethal concentrations viz. 40% and 80% of 96-hrs LC_{50} of α-pinene and β-caryophyllene, weighed, placed in glass petri dish and twenty-five *S. zeamais* adults were introduced into it. Adult insects were allowed to feed. After four days, flour discs were reweighed and antifeedant activity (AFA) was calculated using formula: AFA = [C-T/C] × 100, where C = consumption of flour disc in control group and T = consumption of flour disc in treated group. Six replicates were maintained for each concentration of pure compounds and control.

Statistical analysis

Median lethal concentration (LC_{50}) was calculated using POLO programme (Russel *et al.,* 1977). One-way analysis of variance (ANOVA) and correlation and linear regression were conducted to define concentration-response relationship (Sokal and Rohlf, 1973).

RESULTS

Repellent activity

Percent Repellency (PR) and Preference Index (PI) were increased with increase in the concentration of terpenes, α-pinene and β-caryophyllene and were recorded maximum at 0.8% concentrations (Table1). Both α-pinene and β-caryophyllene showed significant repellency against *S. zeamais* adults (F = 215.17 for α-pinene; F = 179.42 for β-caryophyllene; P<0.01; Table 1).

Fumigant toxicity

Median lethal concentrations (LC_{50}) were recorded 0.412 and 0.305 µlcm^{-3} air for α-pinene after 24 and 48hrs exposure period respectively (Table 2). On the other hand, LC_{50} values were 0.486 and 0.315 µlcm^{-3} air for β-caryophyllene oil after 24 and 48hrs exposure period respectively (Table 2). The index of significancy of potency estimation, g-value indicates that the mean value is within the limits of all probability levels (P<0.1, 0.5 and 0.01) as it is less than 0.5. Values of t-ratio greater than 1.6 indicated that regression was significant. Values of heterogeneity factor less than 1.0 denotes that model fits the data adequate. Regression analysis showed concentration-dependent mortality in *S. zeamais* adults as lethality was found to increase with increase in concentration of terpenes (Table 2). Fumigation of α-pinene and β-caryophyllene caused significant lethality in *S. zeamais* adults (For α-pinene, F = 2226.32 for 24hrs and 200.16 for 48hrs; For β-caryophyllene, F = 256.22 for 24hrs and 189.30 for 48hrs; P<0.01; Table 2).

Contact toxicity

Median lethal concentrations (LC_{50}) were 0.388 and 0.256 µlcm^{-2}; and 0.308 and 0.216 µlcm^{-2} area for α-pinene and β-caryophyllene after 24 and 48hrs exposure period respectively (Table 2). Regression analysis showed concentration-dependent mortality in *S. zeamais* adults by α-pinene and β-caryophyllene (Table 2). Treatment of *S. zeamais* adults with both α-pinene and β-caryophyllene in contact toxicity assay caused significant lethality in *S. zeamais* adults (For α-pinene, F = 236.17 for 24hrs and 203.18 for 48hrs; for β-caryophyllene, F = 256.38 for 24hrs and 192.63 for 48hrs; P<0.01; Table 2).

Acetylcholine esterase (AChE) activity

Fumigation of *S. zeamais* adults with 40 and 80% of 24h-LC_{50} of α-pinene reduced AChE activity to 73.94 and 54.56% of control respectively (Table3). Similar treatment of *S. zeamais* adults with β-caryophyllene significantly reduced AChE activity to 63.89 and 42.76% of control (Table 3). Both α-pinene and β-caryophyllene significantly inhibited activity of AChE enzyme in *S. zeamais* adults (For α-pinene, F=161.32; for β-caryophyllene, F = 143.71; P<0.01; Table 3).

Oviposition inhibition

When *S. zeamais* adults were fumigated with 40 and 80% of 24hrs-LC_{50} of α-pinene and β-caryophyllene oviposition was reduced to 79.11 and 59.53%; and 78.56 and 61.20% of control respectively (For α-pinene, F = 232.24; for β-caryophyllene, F = 214.32; P<0.01; Table 4). Similarly, oviposition was reduced to 57.90 and 38.24%; and 62.18 and 38.41% of control respectively when *S. zeamais* adults were fumigated with 40 and 80% of 48hrs-LC_{50} of α-

pinene and β-caryophyllene (For α-pinene, F = 254.18; for β-caryophyllene, F = 238.27; P<0.01; Table 4). Fumigation of *S. zeamais* adults with α-pinene and β-caryophyllene significantly reduced oviposition capacity of insects (P<0.01).

Developmental inhibition

Progeny production was reduced to 84.87%, 64.44% and 43.70%; and 86.35%, 66.54% and 42.80% as compared to the control when

Table 1. Repellent activity of α-pinene and β-caryophyllene against *S. zeamais* adults

Compound	Concentration (%)	Percent Repellency (PR)* Mean±SD	Preference Index** (PI)	F-value***
α-Pinene	0.1	21.75±2.72	- 0.21	215.17
	0.2	42.50±1.37	- 0.42	
	0.4	80.00±0.31	- 0.80	
	0.8	100±0.0	- 1.0	
β-Caryophyllene	0.1	31.50±1.88	- 0.31	179.42
	0.2	52.50±1.01	- 0.52	
	0.4	85.50±0.28	- 0.85	
	0.8	100±0.0	- 1.0	

*Percent repellency (PR) = [(C-T)/(C+T)] ×100, C =number of insects in the untreated halves and T = number of insect in treated halves

**Preference index (PI) = (percentage of insects in treated halves - percentage of insects in untreated halves)/ (percentage of insects in treated halves + percentage of insects in untreated halves).

PI value between -1.0 to -0.1 indicates repellent compound, -0.1 to +0.1 neutral compound and +0.1 to +1.0 attractant compound.

*** Significant (P<0.01)

Table 2. Fumigant and contact toxicity of α-pinene and β-caryophyllene against *S. zeamais* adults

Compound	Toxicity	Exposure period (h)	LC_{50}*	g-value	Heterogeneity	t-ratio	Regression Equation	Correlation coefficient	F-value**
α-Pinene	Fumigant toxicity	24	0.412	0.21	0.33	3.85	Y = - 3.59+4.94X	0.99	222.32
		48	0.305	0.20	0.34	4.24	Y = 5.36+6.31X	0.98	200.16
	Contact toxicity	24	0.388	0.19	0.33	4.67	Y = - 7.98+2.64X	0.99	236.17
		48	0.256	0.17	0.35	3.79	Y = 6.39+6.31X	0.99	203.18
β-Caryophyllene	Fumigant toxicity	24	0.486	0.18	0.34	3.67	Y = - 3.75+6.05X	0.99	256.22
		48	0.315	0.17	0.32	4.13	Y = 5.99+3.85X	0.98	231.26
	Contact toxicity	24	0.308	0.19	0.31	4.56	Y = - 6.81+6.34X	0.99	192.63
		48	0.316	0.18	0.33	3.72	Y = 76.33+6.96X	0.96	169.26

*μlcm^{-3} for fumigant toxicity and μlcm^{-2} for contact toxicity

** Significant (P<0.01)

Table 3. Effect of α-pinene and β-caryophyllene on AChE activity in *S. zeamais*

Compound	Concentration	Enzyme activity* (Mean±SD)	F-value (df=2,15) **
α-Pinene	Control	0.0975±0.0023(100)	161.32
	40% of 24hrs-LC_{50}	0.0721±0.00020(73.94)	
	80% of 24hrs-LC_{50}	0.0532±0.0013(54.56)	
β-Caryophyllene	Control	0.0975±0.0026 (100)	143.71
	40% of 24hrs-LC_{50}	0.0623±0.0015(63.89)	
	80% of 24hrs-LC_{50}	0.0417±0.0011(42.76)	

*mmol of 'SH'hydrolysed $min^{-1}mg^{-1}$ protein

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

**Significant at P<0.01(df=2,15)

Table 4. Oviposition inhibitory activities of α-pinene and β-caryophyllene in *S. zeamais*

Compound	Concentration	No. of progeny emerged (Mean±SD)	POD	F-value	Concentration	No. of progeny emerged (Mean±SD)	POD	F-value
α-Pinene	Control	91.46±2.68 (100%)	-	232.24**	Control	91.46±2.68 (100%)	-	254.18**
	40% of 24hrs-LC_{50}	72.36±2.71 (79.11)	20.88		40% of 48hrs-LC_{50}	52.96±2.32 (57.90)	38.50	
	80% of 24hrs-LC_{50}	54.32±2.13 (59.39)	40.60		80% of 48hrs-LC_{50}	34.98±1.67 (38.24)	61.75	
β-Caryophyllene	Control	91.46±2.68 (100%)	-	214.32**	Control	91.46±2.68 (100%)	-	238.27**
	40% of 24hrs-LC_{50}	74.32±2.14 (78.56)	18.74		40% of 48hrs-LC_{50}	56.87±2.27 (62.18)	37.82	
	80% of 24hrs-LC_{50}	55.98±2.03 (61.20)	38.79		80% of 48hrs-LC_{50}	35.13±1.34 (38.41)	61.59	

Values in parentheses indicate per cent change with respect to control taken as 100%
* Percentage of oviposition deterrence (POD) = $[(E_C-E_T)/E_C] \times 100$
Where E_C = number of adults emerged in control and E_T = number of adults emerged in test
**Significant at $P<0.01$ (df = 2,15)

Table 5. Effect of α-pinene and β-caryophyllene on developmental period of *S. zeamais*

Compound	Conc.	No. of progeny emerged (Mean±SD)	PR*	F-value**
α-Pinene	Control	83.24±6.35 (100)	-	86.62
	0.2 μlcm^{-3}	70.65±5.94 (84.87)	15.12	
	0.4 μlcm^{-3}	53.64±4.65 (64.44)	35.55	
	0.6 μlcm^{-3}	36.38±2.43 (43.70)	56.29	
β-Caryophyllene	Control	83.24±6.35 (100)	-	74.24
	0.2 μlcm^{-3}	71.88±5.31 (86.35)	13.64	
	0.4 μlcm^{-3}	55.39±4.32 (66.54)	33.46	
	0.6 μlcm^{-3}	35.63±3.56 (42.80)	57.19	

Values in parentheses indicate per cent change with respect to control taken as 100%
* Inhibition rate (IR) = $[(Cn-Tn)/Cn] \times 100$
Where Cn = number of adults emerged in control and Tn = number of adults emerged in test
**Significant at $P<0.01$(df = 3,20)

Table 6. Antifeedant activity of α-pinene and β-caryophyllene against *S. zeamais*

Concentration	α-Pinene		β-Caryophyllene	
	Consumption of flour disc (mg) (Mean±SD)	AFA*	Consumption of flour disc (mg) (Mean±SD)	AFA*
Control	11.29±0.09 (100)	-	11.29±0.09 (100)	-
40% of 96hrs-LC_{50}	6.98±0.23 (61.82)	38.17	7.48±0.32 (66.25)	33.74
80% of 96hrs-LC_{50}	2.56±0.24 (34.18)	65.81	2.98±0.28 (35.25)	64.75
	F** = 412.68		F** = 367.98	

Values in parentheses indicate per cent change with respect to control taken as 100%
*Antifeedant activity was calculated using AFA = [C-T/C] × 100
Where C = consumption of flour disc in control group, and T = consumption of flour disc in treated group.
**Significant at $P<0.01$ (df = 2,15)

fumigated with 0.2, 0.4 and 0.6 µlcm^{-3} of α-pinene and β-caryophyllene respectively (For α-pinene, F = 86.62; for β-caryophyllene, F = 78.34; P<0.01; Table 5).

Antifeedant activity

Both α-pinene and β-caryophyllene significantly inhibited feeding in *S. zeamais* adults. Both terpenes decreased consumption of flour disc by *S. zeamais* adults. Antifeedant activity was reduced to 38.17% and 65.81; and 33.74% and 64.75% with respect to control at 40% and 80% of 96-hrs LC_{50} of α-pinene and β-caryophyllene respectively (For α-pinene, F = 412.68; for β-caryophyllene, F = 367.98; P<0.01; Table 6).

DISCUSSION

Several plant derived volatile oils and pure compounds have been reported for their insecticidal properties against a variety of stored grain insect pests (Chaubey, 2012a,b,c;2013;2014;2016a,b; Patiño-Bayona *et al.,* 2021). Earlier studies with *Piper nigrum*, *Cuminum cyminum*, *Allium sativum* and *Aegle marmelos* oils have established their repellent, contact toxicity, fumigant toxicity, oviposition inhibitory and developmental inhibitory activities against *S. zeamais*. Fumigation of adult insects with these oils inhibited acetylcholine esterase activity in *S. zeamais* (Chaubey, 2017a,b). Besides oil's individual components have also been known for its repellent, contact toxicity, fumigant toxicity, oviposition inhibitory and developmental inhibitory activities against insects (Ogendo *et al.*, 2008; Chaubey, 2012a,c). Linalool, linalyl acetate, menthol, methonene, limonene, α-pipene, β-pipene, β-caryophyllene and linalool have been shown to cause toxicity in rice weevils (Enan, 2005, Ogendo *et al.,* 2008; Chaubey, 2012a). Linalool, carvacrol, terpinen-4-ol, limonene oxide, carvone, dihydrocarvone, fenchone, menthone, p-anisaldehyde, benzyl acetate and cinnamyl aldehyde have been reported to show toxic effects against the adults of *S. granaries* (Kordali *et al.,* 2017). Limonene has shown repellent, insecticidal and oviposition inhibitory activities in *S. zeamais*. It also inhibits acetylcholinesterase activity in *S. zeamais* adults when fumigated (Chaubey, 2021). 1,8-Cineole, sabinene, α-pinene, β-pinene, pulegone, limonene, α-phellandrene, γ-terpinene, fenchone, Δ-3-carene, terpinolene and carvone show fumigant action against *S. zeamais* (Patiño-Bayona *et al.,* 2021). In the present investigation, repellent, toxic, oviposition inhibitory, developmental inhibitory and feeding inhibitory activities of α-pinene and β-caryophyllene were studied against *S. zeamais*. Both α-pinene and β-caryophyllene repelled *S. zeamais* adults and caused mortality in them. The rapid action of these oil constituents shows their neurotoxic mode of action. α-pinene and β-caryophyllene reduced AChE activity in *S. zeamais* adults. Several essential oils and compounds have also been reported to inhibit AChE activity paralysis and death in insects (Chaubey, 2012a; 2017a,b). Researches (Enan, 2005; Tong and Coats, 2012) have been shown that these oils interference with neuromodulator octopamine or GABA-gated chloride channels. Some act on octopaminergic system of insects. Octopamine is a neurotransmitter, neurohormone and circulating neurohormone-neuromodulator. Disruption in its activity breaks down the nervous system in insects. Similarly, limonene inhibits acetylcholine esterase enzyme activity in *S. zeamais* adults when fumigated (Chaubey, 2021). α-Pinene and β-caryophyllene reduced oviposition potential of *S. zeamais* when fumigated, thereby, reduced progeny production. Reduction in oviposition could be the result of disruption of mating and sexual communication in *S. zeamais* adults. These two essential oil terpenes inhibited development of juvenile phases and increased developmental period of *S. zeamais*. Reduced adult emergence could be due to the egg and larval mortality while delay in development could be due to inhibition of metabolic processes or disturbances in hormonal effects and responsiveness. Both α-Pinene and β-caryophyllene reduced feeding in *S. zeamais* adults. This reduction was due to repellent activity of these terpenes. Similar results have been observed in *T. castaneum* and *S. oryzae* adults (Tripathi *et al.,* 2001; Sithisut *et al.,* 2011). Both

essential oil's terpenes under investigation have neurotoxicity indicating its rapid action and low persistence. The persistence of the insecticidal activity depends on the chemical nature of compounds (Kumbhar and Dewang, 2001). Compunds having high content of hydrogen loss their activity more quickly than those containing high content of oxygen (Huang and Ho, 1998). Further researches are essential to study the structure-activity of volatile oil constituents involved in insecticidal activity as well as possibility of their antagonism and synergism (Kordali *et al.,* 2006; Fields *et al.,* 2010). Finally, it must be kept in mind that essential constituents should be effective against target organism not against non-target organisms including humans. There are several other factors involved like risk associated to users, mode of exposure, degradation in the environment and chronic toxicity should also be considered for effective application of essential oils and its components for the management of stored-product insect populations.

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