## Responses of *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae) against essential oils and pure compounds

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## Summary

The essential oils of *Zingiber officinale* rhizomes (*Zingiberaceae*) and *Piper cubeba* berries (*Piperaceae*) as well as pure compounds,  $\alpha$ -pinene and  $\beta$ -caryophyllene, were evaluated for their contact toxicity, persistence of insecticidal and antifeeding activities against *T. castaneum* and *S. oryzae*.  $\beta$ -Caryophyllene showed highest toxicity followed by *P. cubeba*, *Z. officinale* and  $\alpha$ -pinene against both insects. *S. oryzae* was more sensitive than *T. castaneum* to both essential oils and pure compounds.  $\alpha$ -pinene had least persistence followed in increasing order by  $\beta$ -caryophyllene while *Z. officinale* and *P. cubeba* essential oils showed same trends regarding persistence but more than pure compounds. In antifeedant assay, both essential oils and pure compounds exhibited antifeedant activities against *T. castaneum* and *S. oryzae* adults. Feeding deterrency was maximum in both insects by *P. cubeba* essential oil followed by *Z. officinale* essential oil,  $\beta$ -caryophyllene and  $\alpha$ -pinene.

Key words: Zingiber officinale, Piper cubeba,  $\alpha$ -pinene,  $\beta$ -caryophyllene, essential oils

## **INTRODUCTION**

Storage of food grains started with the beginning of agriculture as a safeguard against poor harvests and famine. Simultaneously, several insect species started

damaging stored grains both quantitatively and qualitatively and constitute major problem in storing food grains. This damage amounts to 10%-40% in countries lacking modern storage technologies [1]. In India, this damage at a farm level is approximate 10% of total production [2]. Among important stored product insect pests, red flour beetle, Tribolium castaneum Herbst (Coleoptera: Tenebrionidae) is a cosmopolitan, polyphagous and major secondary pest of processed or damaged grains [3]. This pest has been reported to attack the germ part of the grain. Their presence in stored foods directly affects both quantity and quality of commodity [4]. Rice weevil, Sitophilus orvzae L. (Coleloptera: Curculionidae) is also major cosmopolitan pest affecting stored rice. Both larval and adult stages devour kernel, causing weight losses and deterioration of quality and facilitating development of micro-organisms in stored cereals [5, 6]. Attacked seed has a lower germination and also is unlikely to meet stringent industry standards on milling quality. A number of insecticides has been developed for successful control of these pest but use of chemicals against insect pests of stored grains has become ineffective due to the development of resistance in them [7-9]. Ecological variations in the resistance status of different insect pests to diverse insecticides have been observed by various researchers [10-12]. Insecticide resistance and consequent losses of food arising from failure of chemicals in pest control have caused economic losses of several billion dollars worldwide each year [13]. It also increased risk of ozone depletion, neurotoxic, carcinogenic, teratogenic and mutagenic effects in non-targets and cross- and multi-resistance in insects [14-17]. This increased public awareness regarding human safety and environmental damage due to insecticides also diverted attention towards the use of plant products in stored-grain insect pest management. Amongst plant derived chemicals, essential oils have come into play since last two to three decades. Essential oils are natural complex secondary metabolites characterized by strong odour, volatility and have generally lower density than water [18]. Due to their volatility, essential oils are environmentally nonpersistent [19, 20]. Essential oils are 'generally recognised as safe' by United States Food and Drug Administration (FDA). Recent researches have reported insecticidal nature of several essential oils [21-27]. Essential oil producing plants are distributed in families such as Myrtaceae, Lauraceae, Rutaceae, Lamiaceae, Asteraceae, Apiaceae, Cupressaceae, Poaceae, Zinziberaceae and Piperaceae. Biological activities of essential oils depends upon its chemical composition which, in turn, varies with plant parts used for extraction, extraction method, plant phenological stage, harvesting season, plant age, soil nature and environmental conditions [28, 29]. Essential oils are very complex mixtures containing about twenty to sixty compounds at different concentrations, characterized by two or three major components at fairly high concentrations (20–70%) compared to others components present in trace amounts and generally major components determine biological activities of these essential oils. These components include two groups of distinct biosynthetical origin. The main group is composed of terpenes and terpenoids and other of aromatic and aliphatic constituents, all characterized by low molecular weight [18]. In the present study, Zingiber officinale (Zingiberaceae), Piper *cubeba* (*Piperaceae*),  $\alpha$ -pinene and  $\beta$ -caryophyllene were evaluated for their biological activities against red flour beetle, *T. castaneum* and rice weevil, *S. oryzae*.

## MATERIALS AND METHODS

## Insects

Flour beetles, *T. castaneum* (Coleoptera: Tenebrionidae) and rice weevil, *S. oryzae* (Coleoptera: Curculionidae) were used to determine biological activities of essential oils and pure compounds. *T. castaneum* adults and larvae were reared on wheat flour while *S. oryzae* adults were reared on whole wheat grain in the laboratory at  $28\pm2^{\circ}$ C,  $75\pm5^{\circ}$  RH and a photoperiod of 12:12 (L:D) h.

## Isolation of oils

*Z. officinale* rhizomes (*Zingiberaceae*) and *P. cubeba* berries (*Piperaceae*) were purchased from local market of Gorakhpur, U.P., India. Grounded rhizomes and berries were hydrodistilled in Clevenger apparatus continuously for five hours to yield essential oils. The oils were collected and kept in eppendroff tube at 4°C until their use.

## Pure compounds

Two pure compounds,  $\alpha$ -pinene and  $\beta$ -caryophyllene (fig. 1) were purchased from Sigma Chemicals, USA.





Figure 1. α-pinene β-caryophyllene

## Contact toxicity of essential oils/pure compounds

Contact toxicity of *Z. officinale* and *P. cubeba* essential oils and  $\alpha$ -pinene and  $\beta$ -caryophyllene was determined against *T. castaneum* adults/larvae and *S. oryzae* 

adults. Formulations of essential oils/compounds were made in acetone, applied on bottom surface of glass Petri dish (7 cm diameter×1 cm height) and left for two minutes for evaporation of solvent. Ten freshly emerged adults/4<sup>th</sup> instar larvae were released at the centre of Petri dish, covered and kept in condition applied for rearing of insect. Mortality in adults/larvae was observed after 24 and 48 h of treatment. Four different doses were applied for each essential oil/pure compound and six replications were set for each concentration. In control only acetone was applied.

## Persistence of insecticidal activity of essential oils/pure compounds

To determine the persistence of insecticidal activity of essential oils/pure compounds, a dose causing 100% mortality (24h-LD<sub>100</sub>) was determined for each essential oil/pure compound against adults of *T. castaneum* and *S. oryzae*. Now, formulations of LD<sub>100</sub> of essential oils/pure compounds were prepared in acetone, applied on bottom surface of glass Petri dish as was done in toxicity assay. At the beginning and after every 6 h, 10 insects were introduced in each Petri dish up to 48 h and each time, the mortality of insects was recorded. Each experiment was replicated six times.

## Antifeedant activity (AFA) of essential oils/pure compounds

Antifeedant activity of essential oils/pure compounds was determined according to Suthisut et al. method [30]. Flour disks were prepared by mixing 10 g wheat flour with 50 ml water until completely suspended. Wheat flour suspension was pipetted ( $200 \mu$ l) onto a plastic sheet, held for 24 h at room temperature and then dried in an oven at 60°C for 1 h. Flour disks were weighed between 70-76 mg each. Flour disk was treated with 5, 10, 15 and 20  $\mu$ l of essential oils/pure compounds, weighed, placed in glass *Petri dish* (7 cm diameter×1 cm height), and to each twenty-five adults were added. Insects were allowed to feed and after 4 days, flour disks were reweighed. Antifeedant activity (AFA) was calculated using: AFA = [C-T/C]×100, where C – consumption of flour disk in control group and T – consumption of flour disc in treated group.

## Data analysis

Median lethal doses ( $LD_{50}$ ) were calculated by POLO programme [31]. The correlation and linear regression analysis were conducted to define all dose-response relationships [32]. The analysis of variance (ANOVA) was performed to test the equality of regression coefficient [32].

## RESULTS

## Contact toxicity of essential oils/pure compounds

Essential oils and pure compounds caused contact toxicity in *T. castaneum* adults/larvae and *S. oryzae* adults. Median lethal doses  $(LD_{50})$  of *Z. officinale* and *P. cubeba* essential oils as well as  $\alpha$ -pinene and  $\beta$ -caryophyllene were 0.335, 0.24, 2.46 and 0.173  $\mu$ l/cm<sup>2</sup> after 24 h against *T. castaneum* adults, respectively (tab. 1). The values of LD<sub>50</sub> of *Z. officinale* and *P. cubeba* essential oils as well as  $\alpha$ -pinene and  $\beta$ -caryophyllene were 0.34, 0.277, 2.98 and 0.17  $\mu$ l/cm<sup>2</sup> after 24 h against *T. castaneum* larvae, respectively (tab. 1). Similarly, the values of LD<sub>50</sub> of *Z. officinale*, *P. cubeba* essential oils as well as  $\alpha$ -pinene and  $\beta$ -caryophyllene were 0.287, 0.209, 2.02 and 0.159  $\mu$ l/cm<sup>2</sup> after 24 h against *S. oryzae* adults, respectively (tab. 3).  $\beta$ -caryophyllene was the most effective against both insects, while  $\alpha$ -pinene was least effctive. *P. cubeba* was more toxic than *Z. officinale* essential oils. Regression analysis showed dose-dependent significant correlation between essential oils/pure compounds and larval and adult mortality (tab. 2, 4).

Table 1.

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Essential oil/ pure compound	Parameters	Exposure period	LD <sub>50</sub> [µl/cm <sup>2</sup> ]	LCL [µl/cm <sup>2</sup> ]	UCL [µl/cm²]	g-value	t-ratio	Heteroge- neity
7 officingle	adult mortality	24 h 48 h	0.335 0.306	0.302 0.287	0.368 0.325	0.15 0.18	4.82 4.34	0.31 0.33
Z. Officinale	larval mortality	24 h 48 h	0.340 0.312	0.319 0.296	0.361 0.328	0.11 0.14	4.77 5.02	0.29 0.32
P. cubeba	adult mortality	24 h 48 h	0.240 0.217	0.211 0.203	0.269 0.231	0.13 0.11	4.76 5.01	0.34 0.28
	larval mortality	24 h 48 h	0.277 0.234	0.263 0.212	0.289 0.256	0.16 0.14	4.89 4.26	0.31 0.29
α-Pinene	adult mortality	24 h 48 h	2.46 1.68	2.26 1.34	2.67 2.02	0.16 0.17	5.03 4.78	0.29 0.31
	larval mortality	24 h 48 h	2.98 2.36	2.26 2.17	3.30 2.55	0.13 0.17	5.21 4.87	0.33 0.31
β-Caryophyllene	adult mortality	24 h 48 h	0.173 0.153	0. 164 0. 142	0. 182 0. 164	0.17 0.14	5.22 4.69	0.33 0.29
	larval mortality	24 h 48 h	0.170 0.149	0. 162 0. 153	0. 178 0. 165	0.15 0.12	4.29 4.85	0.30 0.34

Contact toxicity of *Zingiber officinale*, *Piper cubeba* essential oils,  $\alpha$ -pinene and  $\beta$ -caryophyllene against *Tribolium castaneum* adults and larvae

 $LD_{50}$  represents lethal dose that cause 50% mortality

LCL and UCL represent lower confidence limit and upper confidence limit, respectively

g-value, t-ratio and heterogeneity are significant at all probability levels (p< 0.1, 0.05and 0.01)

## Table 2.

Essential oil/pure compound	Parameters	Exposure period	Intercept	Slope	Regression equation	Correlation coefficient	F-value $(df=4,25)^{\circ}$
	adult mortality	24 h	-3.67	10.33	Y = -3.67 + 10.33X	0.99	133.74
7 officinala		48 h	-3.33	11.82	Y = -3.33 + 11.82X	0.99	166.08
2. Officinate	larval	24 h	-2.25	7.96	Y = -2.25 + 7.96X	0.99	133.04
	mortality	48 h	4.33	11.50	Y = 4.33 + 11.5X	0.99	69.17
	adult mortality	24 h	-5.0	10.83	Y = -5.0 + 10.83X	0.99	139.94
Dauhaha		48 h	-3.34	12.25	Y = -3.34 + 12.25X	0.99	121.05
P. CUDEDa	larval	24 h	-3.42	7.22	Y = -3.42 + 7.22X	0.98	172.92
	mortality	48 h	3.33	10.67	Y = 3.33 + 10.67X	0.99	44.84
	adult mortality	24 h	-6.67	0.67	Y = -6.67 + 0.67X	0.97	138.55
D		48 h	-3.8	0.68	Y = -3.8 + 0.68X	0.98	67.75
α–Pinene	larval	24 h	-9.72	0.53	Y = -9.72 + 0.53X	0.94	226.61
	mortality	48 h	-8.74	0.61	Y = -8.74 + 0.61X	0.95	79.59
β–Caryophyllene	adult mortality	24 h	-3.67	18.83	Y = -3.67 + 18.83X	0.99	134.45
		48 h	0.66	23.17	Y = 0.66 + 23.17X	0.99	80.43
	larval	24 h	-4.67	20.17	Y = -4.67 + 20.17X	0.99	157.33
	mortality	48 h	-3.0	21.67	Y = -3.0 + 21.67X	0.99	67.56

Regression parameters of adult mortality and larval mortality *Tribolium castaneum* when treated with *Zingiber officinale*, *Piper cubeba* essential oils,  $\alpha$ -pinene and  $\beta$ -caryophyllene

Regression analysis was performed between different doses of essential oils/pure compounds and response of adults/larvae

\*significant (p<0.01)

#### Table 3.

Contact toxicity of *Zingiber officinale*, *Piper cubeba* essential oils,  $\alpha$ -pinene and  $\beta$ -caryophyllene against *Sitophilus oryzae* adults

Essential oil/pure compound	Exposure period	LD <sub>50</sub> [µl/cm <sup>2</sup> ]	LCL [µl/cm <sup>2</sup> ]	UCL [µl/cm <sup>2</sup> ]	g-value	t-ratio	Heteroge- neity
Z. officinale	24 h	0.287	0.262	0.312	0.18	4.61	0.32
	48 h	0.242	0.219	0.264	0.16	4.73	0.30
P. cubeba	24 h	0.209	0.187	0.231	0.17	4.36	0.33
	48 h	0.166	0.154	0.278	0.14	4.54	0.29
α-Pinene	24 h	2.02	1.87	2.17	0.15	4.08	0.30
	48 h	1.32	1.16	1.48	0.16	4.95	0.33
β-Caryophyllene	24 h	0.159	0.144	0.174	0.18	5.09	0.30
	48 h	0.132	0.120	0.144	0.17	4.77	0.33

 $LD_{50}$  represents lethal dose that cause 50% mortality

LCL and UCL represent lower confidence limit and upper confidence limit, respectively

g-value, t-ratio and heterogeneity are significant at all probability levels (p<0.01, 0.05 and 0.01)

#### Table 4.

Essential oil/pure compound	Exposure period	Intercept	Slope	Regression equation	Correlation coefficient	F-value (df=4,25)°
Z. officinale	24 h	7.21	10.28	Y = 7.21 + 10.28X	0.99	64.41
	48 h	13.19	13.24	Y = 13.19 + 13.24X	0.98	80.79
P. cubeba	24 h	4.0	6.58	Y = 4.0 + 6.58X	0.98	49.53
	48 h	7.67	10.58	Y = 7.67 + 10.58X	0.98	78.39
α-Pinene	24 h	0.0	0.59	Y = 0.0 + 0.59X	0.99	81.89
	48 h	1.66	0.71	Y = 1.66 + 0.71X	0.97	79.45
β-Caryophyllene	24 h	5.33	19.67	Y = 5.33 + 19.67X	0.98	94.20
	48 h	6.0	23.33	Y = 6.0 + 23.33X	0.98	132.69

Regression parameters of adult mortality *Sitophilus oryzae* when treated with *Zingiber officinale*, *Piper cubeba* essential oils,  $\alpha$ -pinene and  $\beta$ -caryophyllene

Regression analysis was performed between different doses of essential oils/pure compounds and response of adults.

\*Significant (p<0.01)

## Persistence of insecticidal efficiency of essential oils/pure compounds

Persistence in insecticidal efficiency of *Z. officinale* and *P. cubeba* essential oils as well as  $\alpha$ -pinene and  $\beta$ -caryophyllene against *T. castaneum* and *S. oryzae* adults decreased with time. Percent mortality was reduced during 48 h treatment of *T. castaneum* and *S. oryzae* adults with *Z. officinale* and *P. cubeba* essential oils, respectively (tab. 5). After 36 h of treatment with  $\alpha$ -pinene, no mortality was observed in *T. castaneum* and *S. oryzae* adults (tab. 5). Similarly, after 42 h of treatment with  $\beta$ -caryophyllene no mortality was obseerved in *T. castaneum* and *S. oryzae* adults. Therefore,  $\alpha$ -pinene showed the least persistence, while *Z. officinale* and *P. cubeba* essential oils were more persistent than  $\beta$ -caryophyllene.

#### Table 5.

Persistence of insecticidal activity (per cent mortality) of *Zingiber officinale*, *Piper cubeba* essential oils,  $\alpha$ -pinene and  $\beta$ -caryophyllene at different exposure periods against *Tribolium castaneum* and *S. oryzae* adults

Essential oils/		T. castaneum					S. oryzae									
pure compounds	6 h	12 h	18 h	24 h	30 h	36 h	42 h	48 h	6 h	12 h	18 h	24 h	30 h	36 h	42 h	48 h
Z. officinale	100	88.33	76.66	61.66	50	33.33	25	13.33	100	86.66	71.66	55	23.33	13.33	10	6.66
P. cubeba	100	90	81.66	70	56.66	38.33	30	16.66	100	85	73.33	58.33	33.33	15	6.66	5
α-Pinene	100	66.66	50	30	18.33	0	0	0	100	60	43.33	23.33	11.66	0	0	0
β-Caryophyllene	100	75	61.66	36.66	21.66	13.33	0	0	100	66.66	56.66	35	16.66	10	0	0

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## Antifeedant activity of essential oils/pure compounds

*Z. officinale* and *P. cubeba* essential oils as well as  $\alpha$ -pinene and  $\beta$ -caryophyllene decreased consumption of flour disc by *T. castaneum* and *S. oryzae* adults. Consumption of flour disk by *T. castaneum* was reduced when treated with *Z. officinale* (F = 59.63), *P. cubeba* (F = 83.37) essential oils,  $\alpha$ -pinene (F = 40.95) and  $\beta$ -caryophyllene (F = 23.6) (tab. 6). Similarly, the consumption of flour disk by *S. oryzae* was reduced when treated with *Z. officinale* (F = 32.14), *P. cubeba* (F = 42.26) essential oils,  $\alpha$ -pinene (F = 21.20) and  $\beta$ -caryophyllene (F = 28.29), respectively (tab. 6). Antifeedant activity (AFA) was recorded highest for *P. cubeba* (61.96 and 64.66 for *T. castaneum* and *S. oryzae*) followed by *Z. officinale* (56.63 and 53.38 for *T. castaneum* and *S. oryzae*) and  $\alpha$ -pinene (42.46 and 36 for *T. castaneum* and *S. oryzae*) (tab. 6).

Table 6.

_	Concentra-	Consumption of flour disk [mg±SD]							
Insect	tion [µl/ disk]	Z. officinale	P. cubeba	α-Pinene	β-Caryophyllene				
T. castaneum	0	11.83±3.31	11.83±3.31	11.83±3.31	11.83±3.31				
	5	9.83±3.01(16.90)	9.16±1.95(22.56)	$11.0 \pm 1.38(7.02)$	10.50±2.78(11.24)				
	10	8.16±2.22(31.02)	7.33±1.64(38.04)	9.83±0.75(16.91)	9.16±1.94(22.57)				
	15	$6.66 \pm 1.16(43.70)$	5.66±1.76(52.15)	8.0±0.63(32.37)	7.33±1.17(38.04)				
	20	5.16±1.50(56.63)	4.50±1.21(61.96)	6.83±1.03(42.46)	6.16±1.50(47.92)				
		$F = 59.63^{\circ}$	$F = 83.37^{\circ}$	$F = 40.95^{\circ}$	$F = 23.60^{\circ}$				
S. oryzae	0	22.16±1.17	22.16±1.17	22.16±1.17	22.16±1.17				
	5	19.50±0.75(12.0)	19.16±0.75(13.54)	21.0±0.55(5.23)	20.50±0.89(7.49)				
	10	17.16±0.51(22.56)	15.50±0.51(30.05)	18.83±1.03(15.03)	18.16±0.98(18.05)				
	15	14.16±0.81(36.10)	12.50±0.81(43.58)	16.66±1.03(24.81)	16.16±1.09(27.07)				
	20	10.33±0.75(53.38)	7.83±0.54(64.66)	14.16±0.98(36.1)	11.33±0.75(48.87)				
		$F = 32.14^{\circ}$	$F = 42.26^{\circ}$	$F = 21.20^{\circ}$	F = 28.29°				

Feeding inhibitory activities of *Zingiber officinale*, *Piper cubeba* essential oils,  $\alpha$ -pinene and  $\beta$ -caryophyllene at different concentration against *Tribolium castaneum* and *Sitophilus oryzae* adults

Values in parentheses indicate Antifeedant Activity (AFA)

\*F-values significant (p<0.01, df=4,25)

## DISCUSSION

Insecticides based on essential oils and its constituents have been proved effective against many stored-grain insect pests. These have been formulated and applied variously as repellant [21, 33, 34], antifeedants [30], growth inhibitors [35, 36], oviposition inhibitors [34, 37] and ovicides [38]. In present study, *Z. officinale* and *P.* 

cubeba essential oils as well as  $\alpha$ -pinene and  $\beta$ -caryophyllene show contact toxicity against both T. castaneum and S. oryzae. In earlier attempts, individual compounds that make up essential oils are also proved toxic to stored-product insects [24, 39-41]. In this study,  $\beta$ -caryophyllene is the most active against *T. castaneum* and *S*. oryzae adults (24 h -LD<sub>50</sub>, 0.173 and 0.159  $\mu$ l/cm<sup>2</sup>) while  $\alpha$ -pinene is least active (24 h -LD<sub>10</sub>, 2.46 and 2.02  $\mu$ l/cm<sup>2</sup>). S. oryzae adults are more sensitive than T. castaneum adults to both essential oils and pure compounds as evidenced by its low LD<sub>50</sub> values. Similar trends are seen with the essential oil of nutmeg, Myristica fragrans [42]. However, T. castaneum adults are more sensitive to C. longa oils than S. oryzae [43], and S. zeamais and T. castaneum have the same susceptibility to essential oils of *Elletaria cardamomum* [44]. 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 and 0.01) as it is less than 0.5. The values of t-ratio higher than 1.6 indicates that the regression is significant. Values of heterogeneity factor lower than 1.0 denotes that model fits the data adequate. Insecticidal activity of essential oils and pure compounds decreased with time because of high volatile property.  $\alpha$ -pinene showed least persistence causing toxicity only up to 36 h while Z. officinale and P. cubeba essential oils were more persistent causing toxicity up to 48 h.  $\beta$ -carvophyllene is more persistent than  $\alpha$ -pinene but less than both essential oils evaluated in the present study. The persistence of the insecticidal activity depends on the chemical properties and nature and position of the functional groups of essential oil constituents [45, 46]. Essential oils having high content of hydrogenated compound loss their activity more quickly than those containing high content of oxygenated compounds [47, 48]. Z. officinale and P. cubeba essential oils as well as  $\alpha$ -pinene and  $\beta$ -caryophyllene decreased consumption of flour disk by T. castaneum and S. oryzae adults. Antifeedant activity (AFA) was recorded highest for *P. cubeba* followed by *Z. officinale* essential oil,  $\beta$ -caryophyllene and  $\alpha$ -pinene (tab. 6). Feeding was reduced in *S. oryzae and T. castaneum* both by essential oils and pure compounds. Similar results have been observed in case of Schinus molle, Alpinia conchigera, Zingiber zerumbet and Curcuma zedoaria essential oils both in T. castaneum and S. oryzae adults [30, 49].

Future research is required to determine the active components of the essential oils responsible for insecticidal activity. Also the possibility of antagonism and synergism must be taken into consideration [50, 51]. Biological activities of essential oils depends on its chemical composition which, in turn, varies with plant parts used for extraction, extraction method, plant phenological stage, harvesting season, plant age, soil nature and environmental conditions [28, 29]. It must be kept in mind that essential oils/constituents should be toxic to target insects and not to non-target organisms such as beneficial insects and other animals like fish, birds and humans. There are several other factors that determine 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

In conclusion, use of essential oils and its constituents as an alternative to control stored grain insects is a sustainable alternative as they can be obtained from nature. Essential oils and its constituents cause contact toxicity, fumigant toxicity, repellent, antifeedant, oviposition inhibitory and developmental inhibitory activities and act at multiple levels in the insects, so the possibility of the generation of the resistance is low. For these reasons, essential oils could be considered as a natural alternative in the control of stored grains insects.

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# ODPOWIEDŹ *TRIBOLIUM CASTANEUM* (COLEOPTERA: TENEBRIONIDAE) I *SITOPHILUS ORYZAE* (COLEOPTERA: CURCULIONIDAE) NA DZIAŁANIE OLEJKÓW ETERYCZNYCH I CZYSTYCH ZWIĄZKÓW

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## Streszczenie

Badano olejki eteryczne kłącza Zingiber officinale (Zingiberaceae) i jagody Piper cubeba (Piperaceae), a także czyste związki: α-pinen i β-kariofilen pod względem ich toksyczności kontaktowej, trwałości działania owadobójczego i działania deterentnego w stosunku do Tribolium castaneum i Sitophylus oryzae. Najwyższą toksyczność w stosunku do obu owadów wykazał β- kariofilen, a następnie *P. cubeba*, *Z. officinale* i α-pinen. *S. oryzae* był bardziej wrażliwy niż *T. castaneum* zarówno na olejki eteryczne, jak i czyste związki. Działanie α-pinenu było najkrótsze, kolejny był β-kariofilen, natomiast czas działania olejków eterycznych wykazywał podobne trendy, lecz był dłuższy niż olejków eterycznych. W testach na działanie deterentne oba olejki eteryczne i czyste związki wykazywały właściwości odstraszające od jedzenia w stosunku do dorosłych osobników *T. castaneum* i *S. oryzae*. Działanie deterentne było najwyższe u obu owadów po zastosowaniu olejku eterycznego z *P. cubeba*, następnie olejku eterycznego z *Z. officinale*, β-kariofilenu i α-pinenu.

Słowa kluczowe: Zingiber officinale, Piper cubeba,  $\alpha$ -pinen,  $\beta$ - kariofilen, olejki eteryczne

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