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Insecticidal activity of different fractions of petroleum ether extract of *Zingiber cassumunar* rhizome against *Tribolium castaneum*

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Abstract

An experiment was carried out to investigate the efficacy of contact toxicity of different fractions of petroleum ether extract of *Zinziber cassumunar* Roxb. rhizome against *Tribolium castaneum.* Seventy-eight different fractions of petroleum ether extract were obtained from column chromatography. Elutes having the similar TLC behavior were combined in eight fractions and were named as: MN-1, MN-2, MN-3, MN-4, MN-5, MN-6, MN-7 and MN-8. Four separate fractions were collected from the MN-1 fraction by preparative thin layer chromatography. These four parts were washed with petroleum ether, chloroform, ethyl acetate and methanol. Thereafter, these were separated by small column and designated as: M_1N_1, M_1N_2, M_1N_3, M_1N_4, M_2N_1, M_2N_2, M_2N_3, M_2N_4, M_3N_1, M_3N_2, M_3N_3, M_3N_4, M_4N_1 and M_4N_2. Fractions of M1N2, M_2N_2, M_3N_2 and M_4N_2 were found to be the most effective against the beetle *T. castaneum* after 24, 48 and 72 hours. However, some fractions exhibited the moderate effect and other fractions did not work against the beetle.

Keywords: Petroleum ether extracts; *Z. cassumunar* rhizome; Chromatography; Insecticidal activity; *Tribolium castaneum* adults

Introduction:

The presence of insect infestation in stored products always posed unique problems. There are more or less 200 stored grain and stored products attacking insects and mites species are found (Khanam *et al.,* 2005). Among these, the red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae) and the confused flour beetle, *Tribolium confusum* Coleoptera: Tenebrionidae) are serious pest of a great variety of stored products, which are cosmopolitan in distribution. Both the adults and larvae cause serious damage to stored wheat, maize and wheat flour. Several insecticides are used indiscriminately to control this pest. But indiscriminate use of chemical pesticides produced many serious problems, *viz*. genetic resistance of pest species, toxic residues, threat to wild life, etc. (Talukder *et al.,* 2011). In fact this led a worldwide interest in the development of botanical pest control agent**.** The main advantages of botanicals are that, these can be produced easily by farmers and are potentially less expensive.

Zingiber cassumunar Roxb. commonly known as Bonada (Family: Zingiberaceae) is used in folklore remedies as a single plant or as component of herbal recipes for the treatment of inflammation, sprains, rheumatism, muscular pain wounds and also as mosquito repellent, a carminative, a mild laxative and an anti dysenteric agent in Bangladesh and many Asian countries (Bhuiyan *et al.,* (2008). *Z. cassumunar* grows abundantly in Bangladesh. It is a herb with elongated leafy stem. Stem is 1.2 to 1.8cm high. Leaves are sub sessile 23 to 35cm oblong. Bhuiyan and Co-workers (Bhuiyan *et al*., 2008) identified 32 volatile constituents in the rhizome oil of *Z. cassumunar*. The main components in rhizome oil were triquinance-1,4-bis (methoxy) (26.47%), Z-ocimene (21.97 % and terpinen-4-ol (18.45%). Wanauppathamkul (2003) also reported the presence of Sabinene (25-45%), γ-tepinene (5-10%), α-Tepinene (2-5%), Terpinen-4-ol (25-45%) and (E)-1-(3,4-dimethoxyphenyl) butadiene(DMPBD) (1-10%) as active chemicals in the *Z. cassumunar* essential oil.

Several workers reported the chemical composition, anti-inflammatory, antimicrobial and insecticidal activity of *Z. cassumunar* (Sukatta *et al.*, 2009; Giwanon *et al*., 2000; Pithayanukul *et al*., 2008; Thripathi 2008; Kamazeri *et al.*, 2012; Yanbin Lu *et al.*, 2005; Iswantini *et al.*,2011; Chairul, 2009 Khanam *et al.*, 2008; Nugroho *et al.*,1996; Bandara *et al.*, 2005; Talukder and Khanam, 2009; Somboom and Pimsamarn, 2011; Suthisut *et al.*, 2011). These encouraged the authors to find out the effective fraction of this plant having insecticidal activities against stored product pests. Therefore, the following investigation was undertaken to evaluate the insecticidal activity of different fraction of petroleum ether extract of *Z. cassumunar* rhizome against *T. castaneum.*

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Materials and methods

Stock culture of *T. castaneum* was maintained in plastic containers (1200mL) and sub-cultures in beakers (1000mL) with the food medium at $30^0C \pm 0.5^0C$ in an incubator. A standard mixture of whole-wheat flour with powdered dry yeast in a ratio of 19: 1 (Park, 1961) was used as food medium.

The rhizomes of Bonada, *Z. cassumunar* were procured from different areas of Rajshahi, Bangladesh. The rhizomes were chopped off into small pieces and dried in a shade. Finally, it was dried in an oven at 40^0C. After drying these parts were crushed (200mesh) by using a cyclotech grinding machine. After crushing, the plant materials were extracted in a soxhlet apparatus separately with petroleum ether, acetone and methanol. The extraction process was carried out by refluxing the solvent for twenty hours (A_1). The solvents were evaporated in rotary vacuum evaporator at 40^0C under reduced pressure and the petroleum ether, acetone and methanol extracts were collected in small reagent bottle and preserved at 4^0C in a refrigerator. The concentrated peroleum ether extract was mixed with a small amount of column grade Silica gel (70-230 mesh, E-MERCK) maintaing the ratio as: concentrated mass : Silica gel = 2:1 and dried in air. After drying, the mixture was powdered in a mortar. This powder was then ready for fractionation by column.

The petroleum ether extract was prepared for column chromatography using mobile phase toluene, chloroform, ethyle acetate and methanol. The column was elute first with 100% toluene and increasing amount of chloroform and then increasing amount of ethyl acetate, finally methanol (Table I). Elute were collected in an amount of about 50mL in a series of conical flask. Elute of similar behaviour were

Table I. Solvent used for eluting the column chromatography

Toluene (mL)	Chloroform (mL)	Ethyl acetate (mL)	Methanol (mL)	Total (mL)	Fraction no.
600(100%)	0 (0%)	0 (0%)	0 (0%)	600	1-9
190 (95%)	10 (5%)	0 (0%)	0 (0%)	200	10-14
270 (90%)	30 (10%)	0 (0%)	0 (0%)	300	15-19
240 (80%)	60 (20%)	0 (0%)	0 (0%)	300	20-25
280 (70%)	120 (30%)	0 (0%)	0 (0%)	400	26-32
180(60%)	120 (40%)	0 (0%)	0 (0%)	300	33-37
50 (50%)	50 (50%)	0 (0%)	0 (0%)	100	38-40
0 (0%)	100(100%)	0 (0%)	0 (0%)	100	41-42
0 (0%)	196 (98%)	4 (2%)	0 (0%)	200	43-45
0 (0%)	475 (95%)	25 (5%)	0 (0%)	500	46-54
0 (0%)	90 (90%)	10 (10%)	0 (0%)	100	55-57
0 (0%)	160 (80%)	40 (20%)	0 (0%)	200	58-60
0 (0%)	140 (70%)	60 (30%)	0 (0%)	200	61-66
0 (0%)	50 (50%)	50 (50%)	0 (0%)	100	67-69
0 (0%)	392 (98%)	0 (0%)	8 (2%)	400	70-76
0 (0%)	190 (95%)	0 (0%)	10 (5%)	200	78-80
0 (0%)	180 (90%)	0 (0%)	20 (10%)	200	81-84

combined together based on Thin layer chromatography (TLC) analysis. There were seventy-eight serially fraction obtained from column chromatography (CC) which were combined in eight fraction were designated as: MN-1, MN-2, MN-3, MN-4, MN-5, MN-6, MN-7 and MN-8 (Table II.). Thin layer chromatography of the above eight fractions were observed (Table III.). Then fraction, MN-1 was subjected to preparative thin layer chromatography (PTLC) using toluene: Chloroform (7:1) solvent system. The separated bands were visualized by the use of UV light (350nm). Four sharp bands were marked with a pin and were collected in different 100mL beakers, which were numbered

Table II. Designation of fractions of petroleum ether extracts having similar TLC behaviour, obtained after column

Fraction	Fraction No.	Designation
1	4-12	MN-1
2	19-24	MN-2
3	28-34	MN-3
4	37-40	MN-4
5	44-48	MN-5
6	51-55	MN-6
7	59-62	MN-7
8	72-78	MN-8

Table III. TLC behavior of the fractions obtained from column chromatography of petroleum ether extract

Fraction No.	Solvent system	Observation
MN-1	Toluene : chloroform (7:1)	Four spots (R_f 0.30, 0.56, 0.69, 0.9).
MN-2	Toluene : chloroform (9:1)	Four spots (R_f 0.28, 0.47, 0.59, 0.80).
MN-3	Toluene : ethyl acetate (8:2)	One spot (R_f 0.48) with tailing from the baseline.
MN-4	Toluene : ethyl acetate (8:2)	Two spots with tailing.
MN-5	Toluene : ethyl acetate (5:2)	One spot (R_f 0.58).
MN-6	Chloroform : Ethyl acetate (5:2)	Three different spots with long tailing.
MN-7	Chloroform : Ethyl acetate (5:2)	Tailing present, no clear spot.
MN-8	Ethyl acetate : Methanol (5:2)	Long tailing present.

Table IV. χ^2 value regression equation, LD_{50} and 95% confident limits of rhizome extract of *Zingiber cassumunar* against *Tribolium castaneum* adult after 24, 48 and 72 hours of treatment

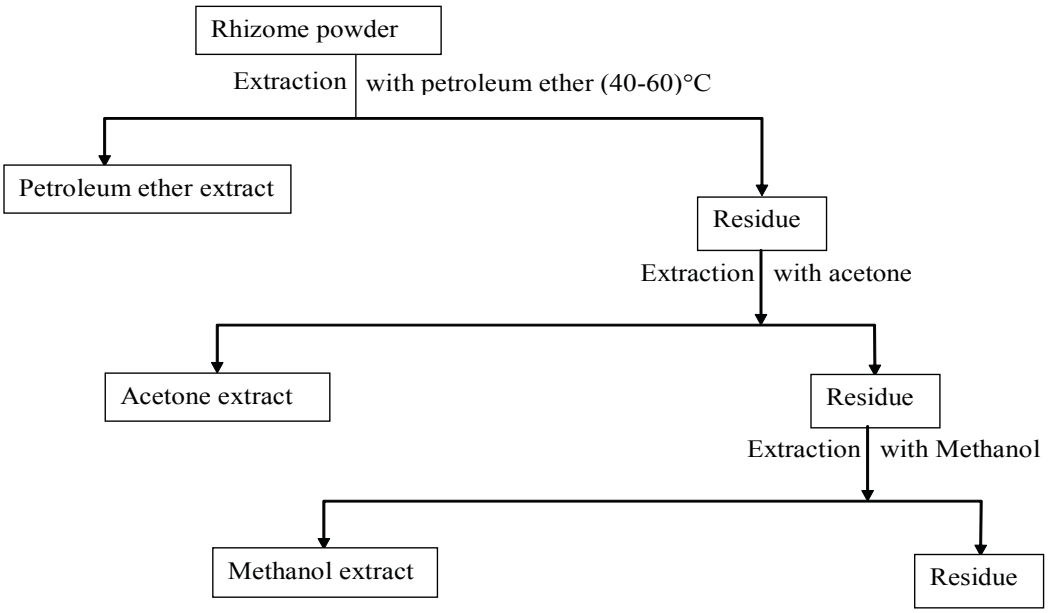
	Duration after treatment	Solvent used	χ^2 for heterogeneity	Regression equation	LD_{50} µm cm^{-2}	95% confinement limit	
						Lower	upper
T. castaneum	24 hours	Petroleum	9.66	Y=0.26+2.234X	225.91	183.07	278.77
	48 hours	Acetone	1.81	Y=0.682+1.244X	2945.20	1171.83	7422.44
	72 hours	Methanol	3.76	Y=-1.43+2.18X	889.47	686.67	1152.17
	24 hours	Petroleum	1.99	Y=-0.848+2.9X	102.34	89.79	116.64
	48 hours	Acetone	4.89	Y=-1.52+2.29X	700.81	580.72	845.73
	72 hours	Methanol	3.48	Y=-0.011+1.73X	767.94	594.10	992.65
	24 hours	Petroleum	2.88	Y=0.102+2.59X	78.08	65.54	934.30
	48 hours	Acetone	1.93	Y=0.227+1.91X	454.75	454.55	643.32
	72 hours	Methanol	3.35	Y=-4.15+3.79X	297.96	226.97	290.79

Table V. TLC behaviour of different fraction of petroleum ether extracts of *Zingiber cassumunar* rhizome

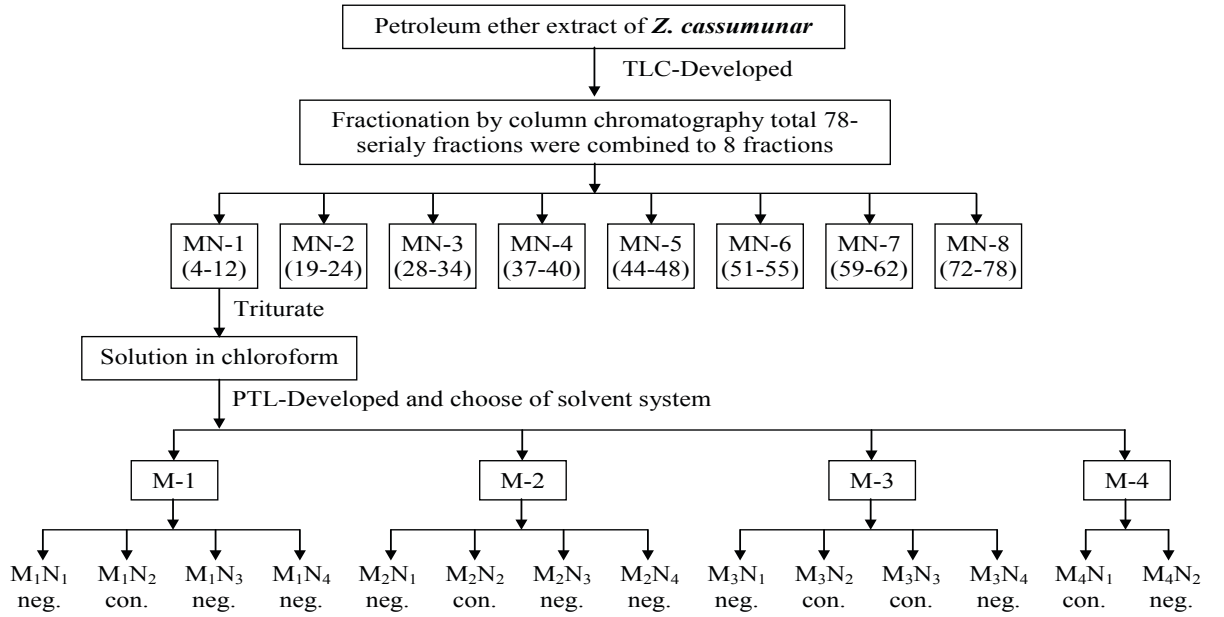
Solvent System	R_f vallues of different fraction													
	M_1N_1	M_1N_2	M_1N_3	M_1N_4	M_2N_1	M_2N_2	M_2N_3	M_2N_4	M_3N_1	M_3N_2	M_3N_3	M_3N_4	M_4N_1	M_4N_2
Toluene:Chloroform (7:1)	0.42								0.30, 0.33 and 0.39					
Toluene: chloroform(8 :1)		0.70				0.67								
n-hesane: Ethyleacetate(7:1)			0.80											0.73, 0.79 and 0.83
Toluene: Methanol (8:1)				0.87	0.67(a) 0.49(b)			0.54						
n-hexane : Ethyl acetate (8 : 1)							0.75							
Toluene : Chloroform (7 : 2).										0.67				
n-hexane : Ethyl acetate (9 : 1)											0.69			
Toluene : Methanol (8 : 2)												0.74		
Toluene : Chloroform (7 : 3)													0.68	

as M-1, M-2, M-3 and M-4. These four part were washed with petroleum ether, chloroform, ethyl acetate and methanol respectively and separated by small colum and designated as M_1N_1, M_1N_2, M_1N_3, M_1N_4, M_2N_1, M_2N_2, M_2N_3, M_2N_4 , M_3N_1, M_3N_2, M_3N_3, M_3N_4, M_4N_1, M_4N_2 etc. and the content were dried.TLC behaviour of these fourteen fractions were observed (Table V). The schematic pathway of fraction described as follows.

FLOW CHART OF PLANT EXTRACTION



SCHEMATIC PATHWAY OF DIFFERENT FRACTION OF PRTROLEUM ETHER EXTRACT OF *Z. cassumunar*



neg.→ Negligible
con.→ Considerable

Experiment setting

Residual film technique (Busvine, 1971) was used to test the mortality rate of the larvae and adults of *T. castaneum.* The doses were prepared by the mixing the requisite quantities of different solvent extract and different fraction of petroleum ether extract of *Z. cassumunar* with 10mL acetone or methanol. Methanol was used in the case of methanol extract because these extracts do not dissolve properly in acetone. The experimental doses were 78.60, 157.19, 314.38, 471.57 and 628.76 mg/cm^2 for all the solvents. The doses were prepared by mixing the requisite quantities (5000, 10000, 20000, 30000 and 40000mg per petridish) of extracted materials with 1mL acetone or methanol. For testing the mortality, each dose with 1mL solvent was dropped on a petridish (9.5cm dia.). After drying, three petridishes were taken each with forty adult insects (one to two week old adult beetles of *T. castaneum*) considered as three replications. Other three petridishes contained only solvent and same number of insects considered as control. The experiment was performed at 30^{0}C ± 0.5^{0}C. The doses were calculated by measuring the weight of extracted materials (mg) in 01ml of the solvent divided by the surface area of the petridish and it is converted in to mg/cm^2. Mortality was assessed after 24, 48 and 72 hours of the treatment applied. The percentage of mortality was corrected using Abbott's formula (Abbott, 1925) and LD_{50} values were determined by probit analysis (Busvine, 1971).

Result and discussion

The result of the contact toxicity, LD_{50}, regression equation, and fiducial limits, due to the effect of different solvent extract of *Z. cassumunar* against *T. castaneum* are summarized in Table, 4, 6-9. From the result, it can be seen that all the solvent extracts were exhibited toxic effect to the beetle, *T. castaneum*, (Table IV). However, the highest mortality was observed in petroleum ether extract at all the intervals. The LD_{50} values in case of petroleum ether extract were 225.91, 102.34 and 78.80μg/cm^2. The LD_{50} values of acetone extract were 2945.20, 700.81 and 454.75μg/cm^2. In case of methanol extract, these were 889.47, 767.94 and 297.96μg/cm^2 Their efficiency followed the order Petroleum ether> Methanol > Acetone.

Toxicity data of the different fraction of the petroleum ether extract against the adult *T. castaneum* were shown in table, 6. Among the four separated bands visualized by UV light and washed with petroleum ether fractions (M_1N_1, M_1N_2, M_1N_3, M_1N_4) only M_1N_1 fraction were found to be effective against *T. castaneum*, whereas other fractions (M_1N_2, M_1N_3, M_1N_4) did not show any effect against *T.castaneum* adult. The calculated LD_{50} values of M_1N_1 fraction were 1220.96, 436.55 and 334.57μg/cm^2 after 24, 48 and 72 hours expousure time respectively against *T. castaneum*.

Toxic effects due to the appliction of four separated bands visualized by UV light and washed with chloromorm fraction (M_2N_1, M_2N_2, M_2N_3 and M_2N_4) against the adults *T. castaneum* were shown in table-7. Results demonstrate that all these different fractions (M_2N_1, M_2N_2, M_2N_3 and M_2N_4) were effective against the *T. castaneum* adults at all the duration. M_3N_2 fraction exhibited lowest LD_{50} values at 24, 48 and 72 hours treatment. However, M_2N_2 fraction did not show any effect at 24 hours treatment. The LD_{50} values of M_2N_3 fraction were 222.73, 117.18 and 100.32μg/cm^2 and M_2N_4 fractions were 317.20, 292.40 and 233.29μg/cm^2, M_2N_1 fraction were 358.34, 277.51 and 168.30μg/cm^2 after 24, 48 and 72 hours exposure time respectively. The order of efficacy at 24hours was M_2N_3> M_2N_4> M_2N_1> M_2N_2 fraction. At 48 hours their efficacy followed the order as M_2N_3> M_2N_1> M_2N_4> M_2N_2 fraction and 72 hours the efficacy followed the order as M_2N_3> M_2N_1> M_2N_4> M_2N_2 fraction.

Toxicity data of the four separate bands visualized by UV light and washed with ethylacetate fraction (M_3N_1, M_3N_2, M_3N_3 and M_3N_4) against adult *T. castaneum* were shown in table-8. The results indicated that highest mortality was observed with M_3N_3 fraction at 24, 48 and 72 hours treatment. However, other fractions (M_3N_1, M_3N_2 and M_3N_4) did not show any effect against *T. castaneum* at 24 hours exposure time. The M_3N_4 fraction also did not show any effect at all the duration. The LD_{50} values of M_3N_3 fraction were 415.47, 185.22 and 154.54μg/cm^2 after 24, 48 and 72hours exposure time respectively. The LD_{50} values of M_3N_1 fraction were 277.51 and 168.30μg/cm^2, M_3N_2 fraction were 4563.90 and 357.08μg/cm^2 after 48 and 72 hours treatment respectively. After 48 and 72 hours treatment their efficacy followed the order M_3N_3>M_3N_1> M_3N_2.

Toxicity data of the four separate bands visualized by UV light and washed with methanol fraction (M_4N_1, M_4N_2, M_4N_3 and M_4N_4) against *T. castaneum* adults are shown Table-9. Our results showed that only fractions, M_3N_4 were exhibited the effective toxicity against *T. castaneum* adults whereas other fractions (M_4N_1, M_4N_2 and M_4N_4) did not show any effect at all the duration. The LD_{50} values of the M_3N_4 fraction were 326.13, 223.25 and 151.21μg/cm^2 at 24, 48 and 72 hours exposures time respectively.

Our findings are in accordance with the findings of Khamam and Co-workers (Khamam *et al.,* 2006), who reported that petroleum ether extract of *Z. cassumunar* rhizome, *Thevetia neriifolia* root caused highest mortality than those of other solvent extracts, methanol and acetone, against *S. oryzae*. Khanam *et al.,*

Table VI. Relative toxicity of different separated bands visualized by UV light and washed with petroleum ether fraction of *Zingiber cassumunar* rhizome against *Tribolium castaneum adults*

Hours after treatment	Plant materials *Zingiber cassumunar*	Fractions	χ^2 values for heterogeneity	Regression equation	LD_{50} ($\mu g/cm^2$)	Fiducial limits	
						Lower	Upper
24hours	No. 1 Petroleum ether fraction	M_1N_1	1.80	Y = - 1.25 + 2.03X	1220.96	341.15	4369.75
	No. 2 Petroleum ether fraction	M_1N_2	–	–	–	–	–
	No. 3 Petroleum ether fraction	M_1N_3	–	–	–	–	–
	No. 4 Petroleum ether fraction	M_1N_4	–	–	–	–	–
48 hours	No. 1 Petroleum ether fraction	M_1N_1	2.15	Y = - 2.04 + 2.67X	436.55	313.52	607.88
	No. 2 Petroleum ether fraction	M_1N_2	–	–	–	–	–
	No. 3 Petroleum ether fraction	M_1N_3	–	–	–	–	–
	No. 4 Petroleum ether fraction	M_1N_4	–	–	–	–	–
72hours	No. 1 Petroleum ether fraction	M_1N_1	0.329	Y = 0.675 + 1.71X	334.57	229.50	487.75
	No. 2 Petroleum ether fraction	M_1N_2	–	–	–	–	–
	No. 3 Petroleum ether fraction	M_1N_3	–	–	–	–	–
	No. 4 Petroleum ether fraction	M_1N_4	–	–	–	–	–

Table VII. Relative toxicity of different separated bands visualized by UV light and washed with chloroform fraction of *Zingiber cassumunar* rhizome against *Tribolium castaneum adults*

Hours after treatment	Plant materials *Zingiber cassumunar*	Fractions	χ^2 values for heterogeneity	Regression equation	LD_{50} ($\mu g/cm^2$)	Fiducial limits	
						Lower	Upper
24hours	No. 1 Chloroform fraction	M_2N_1	0.175	Y= - 3.19 + 3.20X	358.34	286.75	447.79
	No. 2 Chloroform fraction	M_2N_2	–	–	–	–	–
	No. 3 Chloroform fraction	M_2N_3	3.12	Y= - 6.18 + 4.76X	222.73	198.96	249.33
	No. 4 Chloroform fraction	M_2N_4	1.71	Y= - 5.76 + 4.30X	317.20	273.69	367.63
48 hours	No. 1 Chloroform fraction	M_2N_1	2.23	Y= - 2.79 + 3.19X	277.51	233.19	330.25
	No. 2 Chloroform fraction	M_2N_2	0.57	Y = - 2.64 + 2.89X	444.55	323.48	610.94
	No. 3 Chloroform fraction	M_2N_3	2.35	Y= - 2.30 + 3.53X	117.18	100.55	136.56
	No. 4 Chloroform fraction	M_2N_4	1.28	Y= - 4.88 + 4.01X	292.40	252.38	338.78
72hours	No. 1 Chloroform fraction	M_2N_1	3.18	Y= - 4.37 + 4.21X	168.30	149.05	190.03
	No. 2 Chloroform fraction	M_2N_2	0.68	Y= - 3.59 + 3.50X	283.51	240.99	333.54
	No. 3 Chloroform fraction	M_2N_3	0.411	Y= - 2.28 + 3.64X	100.32	84.80	118.69
	No. 4 Chloroform fraction	M_2N_4	5.07	Y= - 6.13 + 4.70X	233.29	179.94	302.46

Table VIII. Relative toxicity of different separated bands visualized by UV light and washed with ethyl acetate fraction of *Zingiber cassumunar* rhizome against *Tribolium castaneum adults*

Hours after treatment	Plant materials *Zingiber cassumunar*	Fractions	χ^2 values for heterogeneity	Regression equation	LD_{50} ($\mu g/cm^2$)	Fiducial limits	
						Lower	Upper
24hours	No. 1 Ethyl acetate fraction	M_3N_1	–	–	–	–	–
	No. 2 Ethyl acetate fraction	M_3N_2	–	–	–	–	–
	No. 3 Ethyl acetate fraction	M_3N_3	2.20	Y = - 2.27 + 2.78X	415.47	307.04	562.19
	No. 4 Ethyl acetate fraction	M_3N_4	–	–	–	–	–
48 hours	No. 1 Ethyl acetate fraction	M_3N_1	2.23	Y = - 2.79 + 3.19X	277.51	233.19	330.25
	No. 2 Ethyl acetate fraction	M_3N_2	0.007	Y = 2.81 + 0.596X	4563.90	38.99	534116.30
	No. 3 Ethyl acetate fraction	M_3N_3	11.79	Y = - 2.91 + 3.49X	185.22	115.35	297.41
	No. 4 Ethyl acetate fraction	M_3N_4	–	–	–	–	–
72hours	No. 1 Ethyl acetate fraction	M_3N_1	3.18	Y = - 4.37 + 4.21X	168.30	149.05	190.03
	No. 2 Ethyl acetate fraction	M_3N_2	3.96	Y = 1.61 + 1.33X	357.08	127.71	998.38
	No. 3 Ethyl acetate fraction	M_3N_3	6.12	Y = - 2.54 + 3.44X	154.54	108.93	219.25
	No. 4 Ethyl acetate fraction	M_3N_4	–	–	–	–	–

Table IX. Relative toxicity of different separated bands visualized by UV light and washed with methanol fraction of *Zingiber cassumunar* rhizome against *Tribolium castaneum adults*

Hours after treatment	Plant materials *Zingiber cassumunar*	Fractions	χ^2 values for heterogeneity	Regression equation	LD_{50} ($\mu g/cm^2$)	Fiducial limits	
						Lower	Upper
24hours	No. 1 Methanol fraction	M_4N_1	–	–	–	–	–
	No. 2 Methanol fraction	M_4N_2	–	–	–	–	–
	No. 3 Methanol fraction	M_4N_3	0.13	Y = - 2.51 + 2.99X	326.13	263.08	404.28
	No. 4 Methanol fraction	M_4N_4	–	–	–	–	–
48 hours	No. 1 Methanol fraction	M_4N_1	–	–	–	–	–
	No. 2 Methanol fraction	M_4N_2	–	–	–	–	–
	No. 3 Methanol fraction	M_4N_3	1.18	Y = - 4.02 + 3.84X	223.25	195.82	254.51
	No. 4 Methanol fraction	M_4N_4	–	–	–	–	–
72hours	No. 1 Methanol fraction	M_4N_1	–	–	–	–	–
	No. 2 Methanol fraction	M_4N_2	–	–	–	–	–
	No. 3 Methanol fraction	M_4N_3	0.01	Y = - 4.63 + 4.42X	151.21	134.39	170.14
	No. 4 Methanol fraction	M_4N_4	–	–	–	–	–

(2008) also reported that petroleum ether extracts of *Z. cassumunar* leaf and rhizome showed highest repellency to *T. castaneum* than those of other extracts (Methanol and Acetone) tested. Result of the present study is in agreement with the results of Somboom and Co-workers (Somboom and Pimsamarn 2011), who reported that volatile oils from plants of zingiberaceae family, obtained by hydro-distillation method caused tremendous toxicity to rice weevil and flour weevil. The LC_{50} values at 48 hours were 10543 and 13693ppm respectively. Suthisut and Co-workers (Suthisut *et al.,* 2011) got effective fumigant toxicity of essential oils from rhizomes of zingiberaceae (*Alpinia conchigera*, *Zingiber aerumbet Curcuma zedoaria* and their major compounds) against *Sitophilus zeamais*, *Tribolium castaneum*, *Anisopteromalus calandrae* and *Trichogramma deion* larvae. They also reported that *A. conchigera* oils were toxic to *S. zeamis*, *T. castaneum* and *T. deion*. The LD_{50} values of *A. conchigera* oils 85μl/L and 73μl/L after 48 hours exposure time against *S. zeamais* and *T. castaneum* adults respectively. *T. castaneum* was more susceptible than *S. zeamais* to the eight pure compounds.

The present results supported the finding of Nugroho and Co-workers (Nugroho *et al.,* 1996) who reported that extracts from rhizomes of *Kaempferia rotunda* and *Zingiber cassumunar* displayed significant insecticidal activity in chronic feeding bioassays against neonate larvae of *Spodoptera littoralis.* They also reported the presence of two phenylbutanoids compounds in *Z. cassumunar* rhizome, which had LD_{50} values 121 and 127ppm respectively against neonate larvae of *S. littoralis*. Talukder and Co-workers (Talukder *et al.,* 2009) reported that the emulsifed products of petroleum ether extract of *Acorous calamus* combined with *Zingiber cassumunar* exhibited moderate effect against *Callosobruchus chinensis*, *Sitophilus oryzae* and *Tibolium castaneum* adults. They also reported that the LD_{50} values of emulsified products were 547.08 and 452.51μg/cm^2 after 24 and 48 hours exposure time respectively.

Conclusion

The phytochemical study of the plant extracts reveal that there used in successful control of noxious insects. It may be used as alternative to synthetic insecticides. Petroleum ether extract of *Z. cassumunar* rhizome were most effective against *T. castaneum* adult than acetone and methanol extract. The result also revealed that among the fractions of petroleum ether extract of *Z. cassumunar* obtained from colum chromatography (A_1) (MN-1, MN-2, MN-3, MN-4, MN-5, MN-6, MN-7 and MN-8) MN-1 fraction again was subjected to Preparative Thin Layer Chromatography (PTLC). Four fractions were found (M-1, M-2, M-3, M-4) by the use of UV-light (350nm). These fractions washed with chloroform and ethyl acetate showed the most effective result than other solvent against *T. castaneum* adults. The insecticidal property of the Z. *cassumunar* extract may be due to the presence of Phenolic compounds. There is a need to conduct farther study on above mentioned fraction against other stored product pests to establish its efficacy as plant based insecticide.

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