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 *Zingiber 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: M1N1, M1N2, M1N3, M1N4, M2N1, M2N2, M2N3, M2N4, M3N1, M3N2, M3N3, M3N4, M4N1, M4N2, M4N3, M4N4. Fractions of M1N2, M2N3, M3N2 and M4N1 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 triquinane-1,4-bis (methoxy) (26.47%), Z-ocimene (21.97 %) and terpinen-4-ol (18.45%). Wanauppathamkul (2003) also reported the presence of Sabine (25-45%), γ-tepinene (5-10%), α-Tepine (5-2%), 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; Thiripathi 2008; Kamazeri et al., 2012; Yanbin Lu et al., 2005; Iswantini et al., 2011; Chauril, 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°C ± 0.5°C 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°C. 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.). The solvents were evaporated in rotary vacuum evaporator at 40°C under reduced pressure and the petroleum ether, acetone and methanol extracts were collected in small reagent bottle and preserved at 4°C in a refrigerator. The concentrated peroleum ether extract was mixed with a small amount of column grade Silica gel (70-230 mesh, E-MERCK) maintaining 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 ethyle acetate and methanol. The column was elute first with 100% toluene and increasing amount of chloroform and then ethyle 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    | Four spots (Rₐ 0.30, 0.56, 0.69, 0.9)                |
| MN-2     |        | Toluene : chloroform    | Four spots (Rₐ 0.28, 0.47, 0.59, 0.80)               |
| MN-3     |        | Toluene : ethyl acetate | One spot (Rₐ 0.48) with tailing from the baseline.   |
| MN-4     |        | Toluene : ethyl acetate | Two spots with tailing.                              |
| MN-5     |        | Toluene : ethyl acetate | One spot (Rₐ 0.58).                                  |
| MN-6     |        | Chloroform : Ethyl acetate | Three different spots with long tailing.             |
| MN-7     |        | Chloroform : Ethyl acetate | Tailing present, no clear spot.          |
| MN-8     |        | Ethyl acetate : Methanol | Long tailing present.                               |
Insecticidal activity of petroleum ether extract

Table IV. $\chi^2$ 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 | $\chi^2$ for heterogeneity | Regression equation | LD$_{50}$ $\mu m \text{cm}^{-2}$ | 95% confinement limit Lower | upper |
|--------------------------|-------------|----------------------------|---------------------|-----------------------------------|----------------------------|-------|
| 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$_f$ values of different fraction |
|--------------------------------|------------------------------------|
| Toluene: Chloroform (7:1)       | 0.42                               |
| Toluene: chloroform (8:1)       | 0.70                               |
| n-hexane: Ethyleacetate (7:1)   | 0.80                               |
| Toluene: Methanol (8:1)         | 0.87                               |
| 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 column and designated as M₁N₁, M₁N₂, M₁N₃, M₂N₁, M₂N₂, M₂N₃, M₃N₁, M₃N₂, M₃N₃, M₄N₁, M₄N₂, 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**

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Rhizome powder
Extraction with petroleum ether (40-60)°C

Petroleum ether extract

Residue
Extraction with acetone

Acetone extract

Residue
Extraction with Methanol

Methanol extract

Residue
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**SCHEMATIC PATHWAY OF DIFFERENT FRACTION OF PETROLEUM ETHER EXTRACT OF Z. cASSUMUNAR**

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Petroleum ether extract of Z. cassumunar
TLC-Developed

Fractionation by column chromatography total 78-seseraly fractions were combined to 8 fractions

MN-1 (4-12)
MN-2 (19-24)
MN-3 (28-34)
MN-4 (37-40)
MN-5 (44-48)
MN-6 (51-55)
MN-7 (59-62)
MN-8 (72-78)

Triturate
Solution in chloroform
PTL-Developed and choose of solvent system

M-1
M-2
M-3
M-4

M₁N₁ neg.  M₁N₂ con.  M₁N₃ neg.  M₁N₄ neg.  M₂N₁ con.  M₂N₂ neg.  M₂N₃ neg.  M₂N₄ neg.  M₃N₁ con.  M₃N₂ con.  M₃N₃ con.  M₃N₄ neg.  M₄N₁ con.  M₄N₂ neg.

neg.→ Negligible
con.→ Considerable
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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 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/cm² 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°C ± 0.5°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/cm². 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/cm². The LD_{50} values of acetone extract were 2945.20, 700.81 and 454.75µg/cm². In case of methanol extract, these were 889.47, 767.94 and 297.96µg/cm². 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_{1}N_{1}, M_{2}N_{2}, M_{3}N_{3}, M_{4}N_{4}) only M_{4}N_{4} fraction were found to be effective against *T. castaneum*, whereas other fractions (M_{1}N_{1}, M_{2}N_{2}, M_{3}N_{3}, M_{4}N_{4}) did not show any effect against *T. castaneum* adult. The calculated LD_{50} values of M_{1}N_{1} fraction were 1220.96, 436.55 and 334.57µg/cm² after 24, 48 and 72 hours exposure time respectively against *T. castaneum*.

Toxic effects due to the application of four separated bands visualized by UV light and washed with chlororm fraction (M_{1}N_{1}, M_{2}N_{2}, M_{3}N_{3}, M_{4}N_{4}) against the adults *T. castaneum* were shown in Table-7. Results demonstrate that all these different fractions (M_{1}N_{1}, M_{2}N_{2}, M_{3}N_{3}, M_{4}N_{4}) were effective against the *T. castaneum* adults at all the duration. M_{3}N_{3} fraction exhibited lowest LD_{50} values at 24, 48 and 72 hours treatment. However, M_{4}N_{4} fraction did not show any effect at 24 hours treatment. The LD_{50} values of M_{1}N_{1} fraction were 222.73, 117.18 and 100.32µg/cm² and M_{4}N_{4} fractions were 317.20, 292.40 and 233.29µg/cm². M_{3}N_{3} fraction were 358.34, 277.51 and 168.30µg/cm² after 24, 48 and 72 hours exposure time respectively. The order of efficacy at 24hours was M_{1}N_{1}> M_{2}N_{2}> M_{3}N_{3}> M_{4}N_{4}. At 48 hours their efficacy followed the order as M_{1}N_{1}> M_{2}N_{2}> M_{3}N_{3}> M_{4}N_{4} fraction and 72 hours the efficacy followed the order as M_{1}N_{1}> M_{2}N_{2}> M_{3}N_{3}> M_{4}N_{4} fraction.

Toxicity data of the four separate bands visualized by UV light and washed with ethylacetate fraction (M_{1}N_{1}, M_{2}N_{2}, M_{3}N_{3}, M_{4}N_{4}) against adult *T. castaneum* were shown in Table-8. The results indicated that highest mortality was observed with M_{1}N_{1} fraction at 24, 48 and 72 hours treatment. However, other fractions (M_{1}N_{1}, M_{2}N_{2} and M_{4}N_{4}) did not show any effect against *T. castaneum* at 24 hours exposure time. The M_{1}N_{1} fraction also did not show any effect at all the duration. The LD_{50} values of M_{1}N_{1} fraction were 415.47, 185.22 and 154.54µg/cm² after 24, 48 and 72 hours exposure time respectively. The LD_{50} values of M_{1}N_{1} fraction were 277.51 and 168.30µg/cm². M_{3}N_{3} fraction were 4563.90 and 357.08µg/cm² after 48 and 72 hours treatment respectively. After 48 and 72 hours treatment their efficacy followed the order M_{3}N_{3}> M_{1}N_{1}> M_{4}N_{4}. Toxicity data of the four separate bands visualized by UV light and washed with methanol fraction (M_{1}N_{1}, M_{2}N_{2}, M_{3}N_{3}, M_{4}N_{4}) against *T. castaneum* adults are shown Table-9. Our results showed that only fractions, M_{1}N_{1} were exhibited the effective toxicity against *T. castaneum* adults whereas other fractions (M_{1}N_{1}, M_{2}N_{2} and M_{4}N_{4}) did not show any effect at all the duration. The LD_{50} values of the M_{1}N_{1} fraction were 326.13, 223.25 and 151.21µg/cm² 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*. Khamam et al.,
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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 | $\chi^2$ values for heterogeneity | Regression equation | LD$_{50}$ (µg/cm$^2$) | Fiducial limits |
|----------------------|-----------------|---------------------|-----------|----------------------------------|---------------------|----------------------|----------------|
|                      | No. 1 Petroleum ether fraction | M$_1$N$_1$ | 1.80 | $Y = -1.25 + 2.03X$ | 1220.96 | 341.15 - 4369.75 |
| 24 hours             | No. 2 Petroleum ether fraction | M$_1$N$_2$ | – | – | – | – |
|                      | No. 3 Petroleum ether fraction | M$_1$N$_3$ | – | – | – | – |
|                      | No. 4 Petroleum ether fraction | M$_1$N$_4$ | – | – | – | – |
|                      | No. 1 Petroleum ether fraction | M$_1$N$_1$ | 2.15 | $Y = -2.04 + 2.67X$ | 436.55 | 313.52 - 607.88 |
| 48 hours             | No. 2 Petroleum ether fraction | M$_1$N$_2$ | – | – | – | – |
|                      | No. 3 Petroleum ether fraction | M$_1$N$_3$ | – | – | – | – |
|                      | No. 4 Petroleum ether fraction | M$_1$N$_4$ | – | – | – | – |
|                      | No. 1 Petroleum ether fraction | M$_1$N$_1$ | 0.329 | $Y = 0.675 + 1.71X$ | 334.57 | 229.50 - 487.75 |
| 72 hours             | No. 2 Petroleum ether fraction | M$_1$N$_2$ | – | – | – | – |
|                      | No. 3 Petroleum ether fraction | M$_1$N$_3$ | – | – | – | – |
|                      | No. 4 Petroleum ether fraction | M$_1$N$_4$ | – | – | – | – |

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 | $\chi^2$ values for heterogeneity | Regression equation | LD$_{50}$ (µg/cm$^2$) | Fiducial limits |
|----------------------|-----------------|---------------------|-----------|----------------------------------|---------------------|----------------------|----------------|
|                      | No. 1 Chloroform fraction | M$_2$N$_1$ | 0.175 | $Y = -3.19 + 3.20X$ | 358.34 | 286.75 - 447.79 |
| 24 hours             | No. 2 Chloroform fraction | M$_2$N$_2$ | – | – | – | – |
|                      | No. 3 Chloroform fraction | M$_2$N$_3$ | 3.12 | $Y = -6.18 + 4.76X$ | 222.73 | 198.96 - 249.33 |
|                      | No. 4 Chloroform fraction | M$_2$N$_4$ | 1.71 | $Y = -5.76 + 4.30X$ | 317.20 | 273.69 - 367.63 |
|                      | No. 1 Chloroform fraction | M$_2$N$_1$ | 2.23 | $Y = -2.79 + 3.19X$ | 277.51 | 233.19 - 330.25 |
| 48 hours             | No. 2 Chloroform fraction | M$_2$N$_2$ | 0.57 | $Y = -2.64 + 2.89X$ | 444.55 | 323.48 - 610.94 |
|                      | No. 3 Chloroform fraction | M$_2$N$_3$ | 2.35 | $Y = -2.30 + 3.53X$ | 117.18 | 100.55 - 136.56 |
|                      | No. 4 Chloroform fraction | M$_2$N$_4$ | 1.28 | $Y = -4.88 + 4.01X$ | 292.40 | 252.38 - 338.78 |
|                      | No. 1 Chloroform fraction | M$_2$N$_1$ | 3.18 | $Y = -4.37 + 4.21X$ | 168.30 | 149.05 - 190.03 |
| 72 hours             | No. 2 Chloroform fraction | M$_2$N$_2$ | 0.68 | $Y = -3.59 + 3.50X$ | 283.51 | 240.99 - 333.54 |
|                      | No. 3 Chloroform fraction | M$_2$N$_3$ | 0.411 | $Y = -2.28 + 3.64X$ | 100.32 | 84.80 - 118.69 |
|                      | No. 4 Chloroform fraction | M$_2$N$_4$ | 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 | χ² values for heterogeneity | Regression equation | LD₅₀ (µg/cm²) | Fiducial limits |
|----------------------|--------------------------------------|-----------|-----------------------------|---------------------|--------------|---------------|
|                      | No. 1 Ethyl acetate fraction         | M₁N₁      |                            |                     |              |               |
|                      | No. 2 Ethyl acetate fraction         | M₂N₂      |                            |                     |              |               |
|                      | No. 3 Ethyl acetate fraction         | M₃N₃      | 2.20                        | Y = - 2.27 + 2.78X  | 415.47       | 307.04        | 562.19        |
|                      | No. 4 Ethyl acetate fraction         | M₄N₄      |                            |                     |              |               |
| 24 hours             | No. 1 Ethyl acetate fraction         | M₁N₁      |                            |                     |              |               |
|                      | No. 2 Ethyl acetate fraction         | M₂N₂      |                            |                     |              |               |
|                      | No. 3 Ethyl acetate fraction         | M₃N₃      | 0.007                       | Y = 2.81 + 0.596X  | 4563.90      | 38.99         | 534116.30     |
|                      | No. 4 Ethyl acetate fraction         | M₄N₄      |                            |                     |              |               |
| 48 hours             | No. 1 Ethyl acetate fraction         | M₁N₁      | 3.18                        | Y = - 4.37 + 4.21X | 168.30       | 149.05        | 190.03        |
|                      | No. 2 Ethyl acetate fraction         | M₂N₂      | 3.96                        | Y = 1.61 + 1.33X  | 357.08       | 127.71        | 998.38        |
|                      | No. 3 Ethyl acetate fraction         | M₃N₃      | 6.12                        | Y = - 2.54 + 3.44X | 154.54       | 108.93        | 219.25        |
|                      | No. 4 Ethyl acetate fraction         | M₄N₄      |                            |                     |              |               |
| 72 hours             | No. 1 Ethyl acetate fraction         | M₁N₁      |                            |                     |              |               |

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 | χ² values for heterogeneity | Regression equation | LD₅₀ (µg/cm²) | Fiducial limits |
|----------------------|--------------------------------------|-----------|-----------------------------|---------------------|--------------|---------------|
|                      | No. 1 Methanol fraction              | M₁N₁      |                            |                     |              |               |
|                      | No. 2 Methanol fraction              | M₂N₂      |                            |                     |              |               |
|                      | No. 3 Methanol fraction              | M₃N₃      | 0.13                        | Y = - 2.51 + 2.99X | 326.13       | 263.08        | 404.28        |
|                      | No. 4 Methanol fraction              | M₄N₄      |                            |                     |              |               |
| 24 hours             | No. 1 Methanol fraction              | M₁N₁      |                            |                     |              |               |
|                      | No. 2 Methanol fraction              | M₂N₂      |                            |                     |              |               |
|                      | No. 3 Methanol fraction              | M₃N₃      | 1.18                        | Y = - 4.02 + 3.84X | 223.25       | 195.82        | 254.51        |
|                      | No. 4 Methanol fraction              | M₄N₄      |                            |                     |              |               |
| 48 hours             | No. 1 Methanol fraction              | M₁N₁      |                            |                     |              |               |
|                      | No. 2 Methanol fraction              | M₂N₂      |                            |                     |              |               |
|                      | No. 3 Methanol fraction              | M₃N₃      | 0.01                        | Y = - 4.63 + 4.42X | 151.21       | 134.39        | 170.14        |
|                      | No. 4 Methanol fraction              | M₄N₄      |                            |                     |              |               |
| 72 hours             | No. 1 Methanol fraction              | M₁N₁      |                            |                     |              |               |
|                      | No. 2 Methanol fraction              | M₂N₂      |                            |                     |              |               |
|                      | No. 3 Methanol fraction              | M₃N₃      |                            |                     |              |               |
|                      | No. 4 Methanol fraction              | M₄N₄      |                            |                     |              |               |
conchigera, Zingiber aerumbet essential oils from rhizomes of zingiberaceae (Alpinia were toxic to S. zeamis deion A. conchigera oils larvae. They also reported that, (PTLC). Four fractions were found (M-1, M-2, M-3, M-4) subjected to Preparative Thin Layer Chromatography (PTLC) extracted. Result of the present study is in agreement than those of other extracts (Methanol and leaf and rhizome showed highest repellency to cassumunar (Talukder et al., 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 LC50 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 LD50 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 LD50 values 121 and 127ppm respectively against neonate larvae of S. littoralis. Talukder and Co-workers (Talukder et al., 2009) reported that the emulsified products of petroleum ether extract of Acorus calamus combined with Zingiber cassumunar exhibited moderate effect against Callosobruchus chinensis, Sitophilus oryzae and Tribolium castaneum adults. They also reported that the LD50 values of emulsified products were 547.08 and 452.51μg/cm2 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, 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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