J. bio-sci. 17: 149-153, 2009 ISSN 1023-8654
http://www.banglajol.info/index.php/JBS/index

# TOXICITY OF FOUR PLANT BASED PRODUCTS AGAINST THREE STORED PRODUCT PESTS

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## Abstract

Context: Plant essential oil play an important role in stored product pests protection. Emulsified oil can easily mix with water and also revealed the same insecticidal activity as essential oil of plants that could be used in store as well as in the field as an alternative synthetic insecticide.

Objectives: The objective of this study is to investigate the efficacy of emulsified petroleum ether extract of *Acorus calamus* L. rhizome alone and three other combination of plant materials against three stored product pests, viz. *Callosobruchus chinensis* L., *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) in adult phase in laboratories condition.

Materials and method– Petroleum ether extract of different plant parts were emulsified. The emulsified products were *A. calamus* rhizome alone, *A. calamus* + *Corchorus capsularis* L. seed, *A. calamus* + *Thevetia neriifolia* Juss. seed and *A. calamus* + *Zingiber cassumunar* Roxb. rhizome. The residual film technique method was conducted to determine the $LC_{50}$ value of above mentioned plant extract against three stored product pests.

Results: Noticeable mortality was noted for all species after 24 and 48 hours of the treatment with the emulsified products, although highest mortality of *C. chinensis* was observed with *A. calamus* alone after 24 hours but it varied at 48 hours where the *A. calamus* + *T. neriifolia* showed the most effective result. The $LC_{50}$ values of *A. calamus* alone products were 13.30 and 6.59 $\mu gcm^{-1}$ and for *A. calamus* + *T. neriifolia* were 18.37 and 4.45 $\mu gcm^{-1}$ against *C. chinensis* adults after 24 and 48 hours treatment respectively. *A. calamus* + *T. neriifolia* products showed the lowest $LC_{50}$ values (43.27 $\mu gcm^{-1}$) against *Sitophilus oryzae* at 24 hours treatment but it varied at 48 hours where *A. calamus* (L.) alone (13.72 $\mu gcm^{-1}$) was found to be the most effective toxicant. *A. calamus* alone showed the lowest $LC_{-50}$ values (166.78 and 123.55 $\mu gcm^{-1}$) against *T. castaneum* adults after 24 and 48 hours treatment.

Conclusion: Use of plants in controlling insect infestation would offer desirable solutions, especially in developing tropical countries, where plants are found in abundance everywhere, throughout the year.

Key words: Plant extract, Contact toxicity, *Callosobruchus chinensis*, *Sitophilus oryzae*, *Tribolium castaneum*.

## Introduction

The natural products of plants come as an alternative ecologically more compatible in substitution to the synthetic insecticides (Rupp *et al.* 2006). The use of botanical pesticides to protect plants from pests is very promising because of several distinct advantages. Pesticidal plants are generally much safer than conventionally used synthetic pesticides. In addition, plant-based pesticides are renewable in nature and cheaper (Parugrug and Roxas 2008).

In Bangladesh *Acorous calamus* L., *Zingiber cassumunar* Roxb., *Thevatia neriifolia* Juss. and *Corchorus capsularis* Linn. grow abundantly. All these plant have more or less toxic materials. The toxicity of *A. calamus* rhizome oil due to the presence of (Z)- and (E)- asarones in rhizome oil (Park *et al.* 2002, Yao *et al.* 2008).

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Bhuiyan *et al*. (2008) identified 32 volatile constituent in the rhizome oil of *Z. cassumunar*. The main components in rhizome oil were triquinance1, 4-bis (methoxy) (26.47%), Z- ocimene (21.97 % and terpinen-4-ol (18.45%). Nugroho *et al.* (1996) reported that two phenylbutanoid from rhizome of *Z. cassumunar* are active on chronic feeding bioassays and also active in the residue contact bioassay ($LC_{50}$ values of 0.5 and 3.6 µg $cm^{-1}$ respectively) against the neonate larvae of *Spodoptera litoralis*. *T. neriifolia* (Yelow oleander) is large evergreen shrubs grow in gardens and roadside as ornamental plant. The thevetin (non-toxic) and thevotoxin (very toxic) were found to be present in seed kernel oil (Malek *et al.* 1996). He also reported that the toxicity of *T. neriifolia* is due to the presence of thevotoxin in seed oil. Its seed kernel provided 68.7% non drying oil.

Many workers mentioned the presence of glucoside, corchorin, corchsularin in the jute seed oil (Saha and Choudhury 1922, Karrer and Benerjee 1949, Khalique and Ahmed 1954, Khan *et al.* 2006). Khuda *et al.* (1963) describes the presence of strophanthidin 0.5% raffinose 4.5% and several glucosides one of which indentical with β-sitosterol-d-glucoside in the *C. capsularis* seed powder. Hossen *et al.* (2008) suggested that the extracted jute seed oil may use as insecticides due to its bitter test.

Most insecticide products contain a synergist whose role is to amplify the chemical activity of the other compounds in the formulation (Wanyika *et al.* 2009). It has also been suggested that vegetable oils act as synergists in bio-pesticide formulations (Tembo and Murfitt 1995). Toxicity studies on mixtures of different essential oils or their constituents against stored-product insects to demonstrate additive, synergistic or antagonistic effects are rare. Therefore, the following investigation was undertaken to evaluate the toxicity of different emulsified mixtures of plant essential oils, *A. calamus, C. capsularis, T. neriifolia* and *Z. cassumunar* against *Callosobruchus chinensis* (L.), *Sitophilus oryzae* L. and *T. castaneum* adults.

## Materials and Methods

Stock culture of *C. chinensis* and *S. oryzae* were maintained in separate 1000ml beaker on sterilized cowpea and maize at 30 ± $0.5^0$C in an incubator respectively. A standard mixture of whole wheat flour with powdered dry yeast in a ratio of 19:1 was used as food medium in case of *T. castaneum* reared.

Seeds of *C. capsularis*, *T. neriifolia*, rhizomes of *Z. cassumunar* and *A. calamus* were procured from the different area of Rajshahi, Bangladesh. All the parts were dried in a shade and finally dried in an oven at $40^0$C. After drying these parts were crushed in a mortar and pestle and extracted by petroleum ether separately. Then extracted liquid was dried in a rotary evaporator and collected in a small reagent bottle, preserved at $4^0$C in a refrigerator. 5 ml *A. calamus* extract were mixed with 5ml of other plant extract separately. Then 5ml of 2.6% Sodium hydroxide solution in distil water were added and was stirred vigorously for 15 minutes. In case of *A. calamus*, 10 ml extract were mixed with 5 ml Sodium hydroxide solution (2.6%) in distilled water and stirred vigorously. Finally 15 ml of emulsified product of *A. calamus* + *C. capsularis* (Ac+Cc), *A. calamus* + *T. neriifolia* (Ac+Tn), *A. calamus*+ *Z. cassumunar* (Ac+Zc) and *A. calamus* (Ac) alone were prepared.

To test insect mortality seven doses from four plants based emulsified product were prepared including control (only water). Ten days old of *T. castaneum*, *S. oryzae,* and *C. chinensis* were taken from the stock culture. Depending upon the susceptibility of the beetle's different experimental doses was taken which were selected after a preliminary test. The doses were 39.29, 78.59, 157.19, 314.38 µg $cm^{-1}$ for *S. oryzae* and *C. chinensis*; 157.19, 314.38, 417.55, 628.72 µg $cm^{-1}$for *T. castaneum*. All the above doses were prepared by mixing the requisite amounts of the plant based products with 10 ml water. The experiment was carried out adopting the method of residual film technique. For each test dose 1ml liquid was dropped on a Petri dish (9

cm dia surface area 62.63 $cm^2$) and then dried in an oven at $40^0$C for 4 hours. Ten adults insect from each of above group were then released on to the Petri dish and these were kept in an incubator at $30\pm1^0$C, 70% relative humidity. The mortality was assessed after 24 and 48 hours of the treatment. The percentage of the mortality was corrected using Abbott's formula (Abbott 1925) and $LC_{50}$ values were determined by probit analysis (Busvine 1971).

## Results and Discussions

Toxicity data of the products against adult of *C. chinensis* are shown in Table 1. All the product is toxic to the adult mortality and increased with the increasing exposure time. After 24 hours of treatment highest mortality was recorded with the Ac on *C. chinensis* adults. The $LC_{50}$ values of Ac were 13.30 and 6.59µgcm$^{-1}$ at the respective time intervals. Whereas the Ac+Tn revealed the lowest $LC_{50}$ values, 4.45 µgcm$^{-1}$, against *C. chinensis* adults at 48 hours after treatment. The $LC_{50}$ values of Ac+Tn were 18.37 and 4.45 µgcm$^{-1}$, Ac+Zc were 74.26 and 6.67 µgcm$^{-1}$and Ac+Cc were 93.82 and 5.63 µgcm$^{-1}$ after 24 and 48 hours exposure time respectively. During 24 hour treatment their efficacy followed the order Ac > Ac+Tn > Ac+Zc > Ac+Cc product. After 48 hours, their order of efficacy were Ac+Tn > Ac+Cc > Ac > Ac+Zc.

The data in the Table 1 provided evidence that all the plant based product used in the experiment were found to be effective in suppressing the *S. oryzae* adults. Ac+Tn exhibited the lowest $LC_{50}$ value on *S. oryzae* adults after 24 hours where Ac was found to be the most effective toxicant against the beetle at 48 hours. The calculated $LC_{50}$ values of Ac+Cc, Ac+Tn, Ac+Zc and Ac were 77.63, 43.27, 84.61 and 54.46 µg cm$^{-1}$respectively against the *S. oryzae* adults after 24 hours. At 48 hours interval Ac+Cc, Ac+Tn, Ac+Zc and Ac were 23.32, 16.43, 32.87 and 13.72 µg cm$^{-1}$ respectively against the same species. The order of the efficacy at 24 hours was Ac+Tn > Ac > Ac+Cc > Ac+Zc products and at 48 hours the efficacy followed the order Ac > Ac+Tn > Ac+Cc > Ac+Zc.

Toxic effects due to the application of four plant based products against *T. castaneum* adult are shown in Table 1. Results demonstrate that these entire plant based product were effective against the *T. castaneum* adult at all the duration. Ac exhibited lowest $LC_{50}$ values at 24 and 48 hours treatment. The $LC_{50}$ values of Ac was 113.44 and 7.82 µgcm$^{-1}$, Ac+Cc was 403.66 and 313.95 µgcm$^{-1}$, Ac+Zc was 547.08 and 452.51 µgcm$^{-1}$ and Ac+Tn 887.68 and 592.69 µgcm$^{-1}$ after 24 and 48 hours exposure time respectively. Their efficacy followed the order Ac > Ac+Cc > Ac+Zc > Ac+Tn during 24 and 48 hour treatment.

Tembo and Murfitt (1995) reported that treatment with vegetable oils of groundnut, rape seed and sunflower combined with pirimiphos-methyl were effective than the pirimiphos methyl alone. Krishnarajah *et al.* (1985) observed that a 1:1 mixture of the essential oils of *Cymbopogon nardus* (L.) and *Vitex negundo* L. was more toxic than the individual oils against *Sitotroga cerealella* (Olivier). Kim *et al.* (2003) revealed the significant insecticidal activity of *Cinnamonum cassia* bark, oil, horse radish (*Cocholeria aroracia*) oil and mustard oil (*Brassica juncea*) with in 1 day after treatment against adults of *S. oryzae* and *C. chinensis* using direct contact application methods. Result of the present study is in agreement with the results of Park *et al.* (2002) who reported that essential oil of *A. calamus* oil was toxic to *S. oryzae* and *C. chinensis* adults when applied tropically. In the filter paper diffusion test ethanol extract of *A. calamus* caused 95.56% and 17.78% mortality to *S. zamias* at 314.54 µgcm$^{-1}$and 78.63 µgcm$^{-1}$ respectively after 4 days treatment (Yao *et al.* 2008).Our results are in conformity with the result of Mahfuz and Khanam (2007), who reported that methanol extracts of *C. capsularis* showed tremendous toxicity against *T. confusum* adults. The present results supported the finding of Khanam *et al.* (2006), who reported that petroleum ether extract of *Z. cassumunar* rhizome, *Thevetia neriifolia* leaf and *Thevetia neriifolia* root caused highest mortality than those of other solvent extract against *S. oryzae*.

Table 1. The toxicity of four plant based product against *Callosobruchus chinensis, Sitophilus oryzae* and *T. castaneum* adults.

| Test insect | Hours after treatment | *Plant materials* | $LD_{50}$ (Fiducial limits) ($\mu g\ cm^{-1}$) | Regression equation ($\chi^2$ at 2 df) |
|---|---|---|---|---|
| *C. chinensis* | 24 | A | 93.82 (66.15 - 133.09) | Y= 2.31 + 1.37X (0.86) |
| | | B | 18.37 (4.09 - 82.47) | Y= 4.01 + 0.781X (1.04) |
| | | C | 74.26 (50.26 - 109.74) | Y= 2.53 + 1.32X (1.34) |
| | | D | 13.30 (3.99 - 44.33) | Y= 3.55 + 1.29X (0.47) |
| | 48 | A | 5.63 (0.59 - 53.66) | Y= 4.15 + 1.12X (0.09) |
| | | B | 4.45 (0.33 - 60.15) | Y= 4.29 + 1.08X (0.12) |
| | | C | 6.67 (0.49 - 90.07) | Y= 4.40 + 0.726X (0.61) |
| | | D | 6.59 (0.91 - 47.91) | Y= 3.95 + 1.28X (0.39) |
| *S. oryzae* | 24 | A | 77.63 (52.89-113.98) | Y= 1.40 + 1.90X (0.44) |
| | | B | 43.27 (23.67-79.09) | Y= 2.22 + 1.69X (0.27) |
| | | C | 84.61 (61.98-115.50) | Y= 0.485 + 2.34X (0.17) |
| | | D | 54.46 (32.60-90.97) | Y= 2.03 + 1.71X (0.04) |
| | 48 hours | A | 23.32 (7.62-71.31) | Y= 3.11 + 1.38X (0.22) |
| | | B | 16.43 (3.45-78.10) | Y= 3.48 + 1.25X (0.03) |
| | | C | 32.87 (16.03-67.39) | Y= 2.34 + 1.75X (0.10) |
| | | D | 13.72 (2.41-78.05) | Y= 3.60 + 1.23X (0.22) |
| *T. castaneum* | 24hours | A | 403.66 (331.55 - 491.46) | Y= -3.48 + 3.25X (1.29) |
| | | B | 887.68 (599.99 - 1313.29) | Y= -3.42 + 2.85X (3.19) |
| | | C | 547.08 (477.38 - 626.97) | Y= - 8.98 + 5.11X (1.09) |
| | | D | 166.78 (102.07 - 272.51) | Y= 0.065 + 2.22X (0.19) |
| | 48 hours | A | 313.95 (265.02 - 371.91) | Y= -6.74 + 4.70X (1.06) |
| | | B | 592.69 (489.39 - 717.78) | Y= -5.08 + 3.63X (3.58) |
| | | C | 452.51 (380.30 - 538.42) | Y= -6.14 + 4.19X (1.19) |
| | | D | 123.55 (64.99 - 234.83) | Y= 0.365 + 2.21X (0.97) |

A = *A. calamus* + *C. capsularis;* B = *A. calamus* + *T. neriifolia*; C= *A. calamus*+ *Z. cassumunar*; D = *A. calamus*

## Acknowledgement

The authors would like to express their sincere thanks to the Director, BCSIR Labs., Rajshahi for providing necessary laboratory facilities.

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