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# Comparative toxicity study of leaf, bark and stem extracts of eucalyptus against *Sitophilus oryzae* L. (Curculionidae: Coleoptera)

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## ABSTRACT

Comparative toxicity of the extract of eucalyptus leaf, bark and stem as a botanical insecticides against rice weevil, *Sitophilus oryzae* L., the most common and destructive insect in all types of rice storage in Bangladesh, was evaluated based on toxicity, repellency and residual activity in the laboratory conditions (28±3°C and 70±5% RH). The extract was prepared with methanoic solvents and applied in four doses (0.5, 1.0, 1.5 and 2.0%) with control. Extracts from all three parts had toxic effects against rice weevil but the extracts of the eucalyptus stems showed the highest (mortality, 34.53%) toxic effect while extract of leaves showed the lowest (mortality, 11.20%) effect. The lower $LD_{50}$ values of the stem extract also showed the highest toxic effects against rice weevils. In the residual toxicity test, the stem extract also showed the lowest number of adult emergence (36.53) and the highest percent of infestation inhibition (67.42%), but the highest number of adult emergence was recorded in the control (78.33) treatment. Among the tested plant parts of eucalyptus, the stem extract showed the highest repellent effect (73.33%) and the leaves extract was the lowest (56.33%). All levels of extract had direct toxicity, residual and repellent effects, but the highest response was obtained using 2.0% and the toxicity increased in proportion to the level of concentration. Considering the toxicity, residual and repellency effects, toxicity were found in the order of stem>bark>leaf. We therefore, suggest that the stem extract of eucalyptus are promising for environment friendly management practices against stored grain pests.

**Keywords:** Eucalyptus, residual toxicity, repellency effect, *Sitophilus oryzae* L.

## INTRODUCTION

More than 600 species of Coleopteran pests attack stored products which cause quantitative and qualitative losses (Rajendran and Sriranjini 2008). Among the stored product pests, the rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) are the common and most destructive pest of the stored raw cereal grains in the world. Such types of insects are very active in warm and humid areas especially in Bangladesh. It is well known that both the adults and grubs are serious pests of stored grains and stored products such as rice, wheat, maize, sorghum, barley, lentil, biscuits, dried potatoes, cornflower, beans, pumpkin seeds, millets, etc (Hanies 1991). Infestation with the rice weevil also indirectly quality loss the grains by the encourage of mould growth and growth of other insect populations (Rees 2004). The estimated damage caused by these pests measured about 5 to 10 % in the temperate zone and 20 to 30 % in the tropical zone (Haque et al. 2000).

To reduce the loss of grains in stores due to this notorious pest, rice weevil and other important stored product pests, several of devices, principles, and management practices are taken by the people of many

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developing countries. Many researchers recommended to apply chemical insecticide for the weevil control in storage but it creates serious drawbacks such as ecological imbalance, health hazards, residue in food and environment, an outbreak of secondary pests, pest resurgence etc. (Adedire et al. 2011, Ileke and Olotuah 2012). Fumigation with poisonous gases for stored grains is also effective for controlling rice weevil, but not applicable at the farm level because the storage farm may not be always airtight in farmer's levels. Alternatives to chemical pesticides for controlling insect pest of storage are utmost necessary.

It is reported that the farmers save their crop and/or products with herbal substances such as oils, leaves, roots, seeds etc. of the different plant instead of synthetic chemical insecticides for a long time (Talukder and Howse1993). Many plant volatile essential oils and their constituents have been studied to possess potential as an alternative compound with gaining importance for the management of stored products and these are ecologically safe and biodegradable (Batish et al. 2008, Cosimi et al. 2009). Botanicals like eucalyptus which are grown by farmers with minimum expense and extracted by indigenous methods. This is helpful in minimizing the undesirable side effects of synthetic pesticides.

However, there are many research work done on the evaluation of different indigenous plant extracts against stored product pests (Ali et al. 2012, Arya and Tiwari 2013, Bachchu et al. 2013, Ghani et al. 2014). Surprisingly, a few information has supported the eucalyptus leaf as a stored grain protectant against pests. Therefore, it is now utmost importance to know the mechanism about the toxic effect of eucalyptus plant parts against stored grain pest as Botanical pesticides. The objective is to study the comparative toxicity, residual and repellent effect of leaf, bark and stem extracts of the eucalyptus plants, against rice weevil.

## MATERIALS AND METHODS

**Collection and preparation of plant extracts:** The leaf, bark and stem of eucalyptus, *Eucalyptus camaldulensis* L.were used for this experiment. The fresh leaves were collected from the HSTU campus and local area of Dinajpur, bark and stem dust were collected from different sawmills of Dinajpur area. After collection, the leaves were kept in the laboratory for 7 days for air drying followed by one-day sun drying before making powder. Powder was made with the help of an electric grinder. The powdered materials of leaf, bark and stem were extracted in methanol solvent. Hundred gram of each category of powder were taken in a 600 mL beaker and separately mixed with 300 mL of methanol. Then the mixture was stirred for 30 minutes in a magnetic stirrer and then allowed to stand for 72 hours followed by shaking several intervals. The mixture was then filtered through a filter paper (Whatman filter paper no. 1) and allowed to evaporate the solvent by rotary evaporator. Finally, the condensed extracts were found. The prepared extracts were preserved in tightly corked vials and stored in a refrigerator until further study.

**Rearing of rice weevil:** Adult rice weevil, *Sitophilus oryzae* L. were collected from the naturally infested wheat grains of the local market of Dinajpur and was mass reared in the laboratory at ambient temperature (28±3°C) in glass jars. About 200 beetles were released in each glass jar containing 500 g of wheat seeds and the mouth of the jar was covered with a piece of cloth to prevent insect escape. They were allowing for free oviposition for 7 days, the beetles were removed from each jar and then the jars were left undisturbed for completing the life cycle. After adults emerged, one day old adults were sorted by sieving and transferred regularly into separate jars with wheat grains for continuous culture. Only three to five day old insects were used for the study.

**Insect bioassays:** Insect bioassays were conducted at 28±3°C temperatures and 70±5% relative humidity to determine the direct toxicity, residual toxicity and repellent effects of leaf, bark and stem extracts of eucalyptus. Four concentrations (0.5%, 1.0%, 1.5% and 2.0%) along with control of each category of leaf, bark, and stem extracts were prepared in the methanol solvent. Prior to insect bioassay, pilot experiments were done to obtain the appropriate dose.

**Direct toxicity tests:** Toxicity tests were conducted according to the Film residue method describe by Busvine (1971). One mL solution of each leaf, bark, and stem extracts of eucalyptus was applied to the petridish (60 mm dia.). The petridishes were then kept in the air for drying for a few minutes. The solvent was fully evaporated and 3 days old 10 adults were released in each petridish. Three replications were made for each treatment and each concentration including control. An equal number of insects with only methanol solvent were treated as control. Insect mortality was recorded every 24 hours after treatment (HAT) upto 120 HATs. The percentage of mortality was corrected using Abbott's (1987) formula before analysis.

$$P = \frac{\acute{P} - C}{100 - C} \times 100$$

Where,
$P$ = Percentage of corrected mortality
$P'$ = Observed mortality (%)

C = Mortality (%) at control

**Residual toxicity test:** Leaf, bark, and stem extracts of four different concentrations were mixed with wheat grain separately (1 mL/25 g wheat) followed by air-dried about 10 minutes. Five pairs of 3 days old beetles were released into the pot containing treated with leaf, bark and stem extracts and the pot was covered with a perforated lid. In control treatments, only methanol solvent was used. Three replications were maintained for each of the concentrations of the individual leaf, bark and stem extracts along with control. All pots were kept at ambient temperature (27±3°C) in the laboratory for oviposition. After 7 days, dead and alive beetles were removed from each container. When adults were emerged, the number of adult emergence was counted after every 7 days interval upto 42 days. Percent inhibition rate (%IR) was calculated by the following formula (Talukder and Howse 1993):

% IR= Cn-Tn/Cn x 100
Where,
% IR=Percentage of inhibition rate
Cn= Number of insect on control treatment
Tn= Number of insect on treated treatment

**Repellency test:** The repellent activities were evaluated using the filter paper impregnation method (Talukder and Howse 1994). Firstly, the Whatman no. 1 filter paper was cut into two halves and 1 mL solution of each concentration was applied to one half uniformly. Only the solvent was used in the control half of the filter paper. The treated half and control half was then air-dried. Three days old 10 insects were released at the centre of each petridish (90 mm diameter) and a cover was placed on the petridish. Three replications were used for each extract and each concentration. The number of insects on each portion was counted at hourly intervals upto the $5^{th}$ hour with control treatment. The data was expressed as % of repulsion (%PR) using the following formula:

$$\%PR = (Nc-50)\times 2$$

Where,

% PR= Percentage repulsion
Nc= Percent of insects present in the control half

Positive values expressed repellency and negative values attractancy. The average values were categorized as describe by McDonald et al. (1970). The average values were categorized as class 0: repellency 0>0.01 to 0.1%, class I: repellency 0.1 to 20.0%, class II: repellency 20.1 to 40.0%, class III: repellency 40.1 to 60.0%, class IV: repellency 60.1 to 80.0% and class V: repellency 80.1 to 100.0%, respectively.

**Statistical analysis:** The collected data were statistically analyzed by a completely randomized design (CRD) with the help of MSTAT-C statistical program. The mean values were adjusted by Duncan's New Multiple Range Test (DMRT). The insect mortality data were also analyzed by probit analysis program. All graphical works were done with Microsoft Excel program.

## RESULTS AND DISCUSSION

**Mortality effect of eucalyptus plant extracts against rice weevil:** The results of the direct toxic effects of leaf, bark and stem extracts of the eucalyptus plant, doses and their interactions at different time intervals against the adult of rice weevil are presented in Table 1 and 2. The toxicity effect of leaf, bark and stem showed different level of toxicity against the adult of rice weevil and toxicity was differed significantly (P>0.05) among the treatments. The average mortality percentage of rice weevil at 24, 48, 72, 96 and 120 hours after treatment (HAT) indicated that stem extract possessed the highest (mortality34.53%) toxic effect, whereas leaf extract possessed the lowest (mortality, 11.20%) toxic effect (Table 1). Mortality percentages were increased proportionally with the increased time intervals. The order of toxicity of different parts of eucalyptus plant extracts against rice weevil were stem>bark>leaf. Mortality of rice weevil also differed significantly among all the concentrations level at different time interval (Table 1). The highest average mortality (27.17%) was observed at the highest concentration (2.0%) of plant extract and the lowest average mortality (12.33%) was found at the lowest concentration (0.5%). No mortality was recorded in untreated control up to 120 HAT. The average mortality percentage of concentration directly proportional to the level of concentration of eucalyptus plant extracts. The interaction effects of different parts, doses and time is presented in Table 2. The highest average mortality (60.67%) of rice weevil was found from the extracts of stem at 2.0% and lowest (8.00%) from the extracts of the leaf at 0.5% concentration level. No adult mortality was found at control treatments in the interaction of doses and time at 24, 48, 72, 96 and 120 HATs.

It was observed from the above findings that the eucalyptus plant extracts were effective for controlling the rice weevil but stem extract was the most effective. The present study agreed with the previous finding of Sarac and Tunc (1995), Aggarwal et al. (2001) and Lee et al. (2004a and b). Sarac and Tunc (1995) observed that oil of *Pimpinella anisum* caused 95% mortality of *Tribolium confusum*, and *S. oryzae* within shorter exposure. Oils of *Tuymbra spicata var. spicata, Eucalyptus camaldulensis* and *Satureja thymbra* showed higher toxicity only to *S. oryzae*.Aggarwalet al. (2001) observed that the lethal dose and lethal concentration required to kill 50% of the beetles ($LD_{50}$ and $LC_{50}$,

respectively) varied with the toxicity assay method. $LD_{50}$ values of 0.03, 0.04, and 0.04 ml/insect against *C. maculatus*, *R. dominica* and *S. oryzae*, respectively, were found in the topical application assay, while the $LC_{50}$ values in the fumigant assay were 0.28, 0.33, and 0.46 ml/l against *C. maculatus*, *R. dominica*, and *S. oryzae*, respectively. In the study of Lee et al. (2004b) considered that 6 out of 42 essential oils extracted from species of the family Myrtaceae found in Australia were shown to have potent fumigant toxicity against three major stored grain insects: *S. oryzae*, *T. castaneum* and *R. Dominica*. They indicated fumigant effects of the essential oils rich in 1,8-cineole were considered to warrant further research into their potential for commercial use.

**Table 1.** Direct toxic effect of eucalyptus plant extracts and different levels of plant extracts against rice weevil at different HATs

| | Extracts used | Insect mortality (%) at different HAT | | | | | Average mortality(%) |
|---|---|---|---|---|---|---|---|
| | | 24HAT | 48HAT | 72HAT | 96HAT | 120HAT | |
| **Plant effect** | Leaf | 4.00 b | 8.67 b | 9.33 c | 15.33 b | 18.67 c | 11.20 c |
| | Bark | 6.00 b | 10.00 b | 14.67 b | 20.00 b | 28.00 b | 15.73 b |
| | Stem | 12.67 a | 21.33 a | 33.33 a | 44.67 a | 60.67 a | 34.53 a |
| | Control | 0.00 c | 0.00 c | 0.00 d | 0.00 c | 0.00 d | 0.00 d |
| | LSD | 2.858 | 3.690 | 3.565 | 4.667 | 5.636 | 3.278 |
| | CV % | 68.35 | 50.00 | 33.70 | 31.62 | 28.46 | 28.91 |
| **Levels effect** | level of extracts used | | | | | | |
| | 0.5% | 4.17 c | 8.33 c | 10.83 d | 15.00 d | 23.33 c | 12.33 d |
| | 1.0% | 5.83 bc | 10.00 bc | 15.00 c | 20.83 c | 29.17 c | 16.17 c |
| | 1.5% | 8.33 ab | 13.33 b | 20.00 b | 28.33 b | 35.83 b | 21.17 b |
| | 2.0% | 10.00 a | 18.33 a | 25.83 a | 35.83 a | 45.83 a | 27.17 a |
| | Control | 0.00 d | 0.00 d | 0.00 e | 0.00 e | 0.00 d | 0.00 e |
| | LSD | 3.196 | 4.126 | 3.986 | 5.218 | 6.302 | 3.665 |
| | CV % | 68.35 | 50.00 | 33.70 | 31.62 | 28.46 | 28.91 |

Within column values followed by different letter(s) are significantly different by DMRT at 5 % level of probability.

**Table 2.** Direct toxic effect of eucalyptus plant extracts of different doses against rice weevil at different HAT (Interaction of plant, dose and time)

| Extracts used | Doses (%) | Insect mortality (% ) at different HAT | | | | | Average mortality (%) |
|---|---|---|---|---|---|---|---|
| | | 24 HAT | 48 HAT | 72 HAT | 96 HAT | 120 HAT | |
| Leaf | 0.5 | 3.33 de | 6.67 fg | 6.67 gh | 10.00 hi | 13.33 h | 8.00 h |
| | 1.0 | 3.33 de | 6.67 fg | 10.00 fg | 16.67 fgh | 20.00 gh | 11.33 gh |
| | 1.5 | 6.67 cde | 13.33 def | 13.33 efg | 23.33 efg | 26.67 fgh | 16.67 fg |
| | 2.0 | 6.67 cde | 16.67 cde | 16.67 ef | 26.67 def | 33.33 fg | 20.00 f |
| | Control | 0.00 e | 0.00 g | 0.00 h | 0.00 i | 0.00 i | 0.00 i |
| Bark | 0.5 | 3.33 de | 6.67 fg | 10.00 fg | 13.33 gh | 20.00 gh | 10.67 gh |
| | 1.0 | 6.667 cde | 10.00 ef | 16.67 ef | 23.33 efg | 30.00 fg | 17.33 fg |
| | 1.5 | 10.00 bcd | 13.33 def | 20.00 de | 30.00 de | 40.00 ef | 22.67 ef |
| | 2.0 | 10.00 bcd | 20.00 bcd | 26.67 cd | 33.33 cde | 50.00 de | 28.00 de |
| | Control | 0.00 e | 0.00 g | 0.00 h | 0.00 i | 0.00 i | 0.00 i |
| Stem | 0.5 | 10.00 bcd | 20.00 bcd | 26.67 cd | 36.67 cd | 60.00 cd | 30.67 cd |
| | 1.0 | 13.33 bc | 23.33 bc | 33.33 c | 43.33 c | 66.67 bc | 36.00 c |
| | 1.5 | 16.67 b | 26.67 b | 46.67 b | 60.00 b | 76.67 b | 45.33 b |
| | 2.0 | 23.33 a | 36.67 a | 60.00 a | 83.33 a | 100.0 a | 60.67 a |
| | Control | 0.00 e | 0.00 g | 0.00 h | 0.00 i | 0.00 i | 0.00 i |
| | LSD | 6.391 | 8.251 | 7.971 | 10.44 | 12.60 | 7.330 |
| | CV % | 68.35 | 50.00 | 33.70 | 31.62 | 28.46 | 28.91 |

Within column values followed by different letter(s) are significantly different by DMRT at 5 % level of probability.

**Probit analysis for mortality effects of eucalyptus plant extracts against rice weevil:** The results of the probit analysis for the estimation of $LD_{50}$ values, chi-square values and their 95% fiducial limits at 24, 48, 72, 96

and 120 HAT for the mortality of rice weevil are presented in Table 3. The $LD_{50}$ values of the leaf (138.643 %), bark (32.626 %) and stem (15.033 %) at 24 HAT indicated that stem extract was the most toxic followed by bark and leaf extract. Stem extract was also maintained its highest toxicity, when the $LD_{50}$ values were compared at 48 HAT (7.618%), 72 HAT (1.609 %), 96 HAT (0.914 %) and 120 HAT (0.435 %) and almost a similar trend of results was observed where lower $LD_{50}$ values at 48, 72, 96 and 120 HAT in stem extracts. The results indicated that the eucalyptus plant extracts were toxic to rice weevil and the order of toxicity levels were found as stem> bark> leaf. The chi-square values of eucalyptus plant extracts at different HAT were insignificant at a 5% level of probability and did not show any heterogeneity of the mortality data.

The lower $LD_{50}$ values of stem extract indicated that the highest toxic effects against rice weevil. The present study agreed with the previous findings of Aggarwal *et al*. (2001). They observed that the lethal dose and lethal concentration required to kill 50% of the beetles ($LD_{50}$ and $LC_{50}$, respectively) varied with the toxicity assay method. $LD_{50}$ values of 0.03, 0.04, and 0.04 mL/insect against *C. maculatus*, *R. dominica* and *S. oryzae*, respectively, were found in the topical application assay, while the $LC_{50}$ values in the fumigant assay were 0.28, 0.33, and 0.46 ml/l against *C. maculatus*, *R. dominica*, and *S. oryzae*, respectively.

**Table 3.** Probit analysis of eucalyptus plant extracts (leaf, bark and stem) against rice weevil after 24, 48, 72, 96 and 120 HATs.

| Extract used | No. of insect used | $LD_{50}$ values (%) | 95% fiducial limits | | $\chi^2$values with 2df |
|---|---|---|---|---|---|
| | | | Lower | Upper | |
| | | 24 HAT | | | |
| Leaf | 30 | 138.643 | 1.673E-03 | 1.148E+07 | 0.268 |
| Bark | 30 | 32.626 | 0.110 | 9665.377 | 0.129 |
| Stem | 30 | 15.033 | 0.344 | 655.973 | 0.205 |
| | | 48 HAT | | | |
| Leaf | 30 | 23.299 | 0.226 | 2393.837 | 0.420 |
| Bark | 30 | 16.162 | 0.391 | 667.988 | 0.218 |
| Stem | 30 | 7.618 | 0.427 | 135.634 | 0.385 |
| | | 72 HAT | | | |
| Leaf | 30 | 31.580 | 0.107 | 9313.836 | 0.041 |
| Bark | 30 | 8.141 | 0.813 | 81.488 | 0.100 |
| Stem | 30 | 1.609 | 1.016 | 2.547 | 0.860 |
| | | 96HAT | | | |
| Leaf | 30 | 6.952 | 0.927 | 52.100 | 0.003 |
| Bark | 30 | 4.661 | 1.037 | 20.953 | 0.060 |
| Stem | 30 | 0.914 | 0.671 | 1.245 | 3.449 |
| | | 120HAT | | | |
| Leaf | 30 | 5.192 | 1.010 | 26.676 | 0.013 |
| Bark | 30 | 2.224 | 1.159 | 4.267 | 0.184 |
| Stem | 30 | 0.435 | 0.239 | 0.793 | 3.501 |

Values were based on four concentrations, three replications of 10 insects each, $\chi^2$ = Goodness of fit, The tabulated value of $\chi^2$ is 5.99 (d. f = 2 at 5% level)

**Probit regression lines for mortality effect of eucalyptus plant extracts rice weevil:** The probit regression lines for the effect of leaf, bark and stem extracts of eucalyptus plant against rice weevil, *Sitophilusoryzae* L. at 24, 48, 72, 96 and 120 HATs are shown in Figure 1. The rate of mortality of rice weevil showed positive correlations with the doses in all cases.

The calculated probit regression equation lines for the effects of leaf, bark and stem extracts on rice weevil at 24 HAT were Y=0.859X + 3.077, Y= 1.055X + 2.418 and Y= 0.761X + 2.525 for stem, bark and leaf extracts respectively (Figure 1). Comparing among three lines, the regression line for stem extract showed the highest probit mortality and leaf extract showed the lowest probit mortality. The probit regression equations at 48 HAT were Y=0.768X + 3.568, Y=0.987X + 2.786 and Y=0.892X + 2.808 for stem, bark and leaf extracts, respectively. Comparing among these three lines, the regression line for stem extract showed the highest mortality effect whereas leaf extract showed the lowest mortality effect.

The calculated regression equations for the effects of eucalyptus plant extracts on rice weevil at 72, 96 and 120 HAT were Y=1.386X + 3.332, Y=1.055X + 2.978

and Y=0.849X + 2.903; Y=1.951X + 3.135, Y=1.177X + 3.063 and 1.119X + 2.935; Y=2.324X + 3.401, Y=1.367X + 3.169 and Y=1.140X + 3.054 for stem, bark and leaf extracts, respectively. The regression line for stem extract showed the highest mortality effect and the leaf extract showed the lowest mortality effect.

The probit regression lines for the effects of different parts of eucalyptus plant extracts against rice weevil showed a clear relationship between probit mortality and their doses and the probit regression lines become steeper as doses increased because the adult insects were treated with more toxins for the same period at higher doses. Comparing among these three lines, the highest probit mortality was found with stem extract at 24, 48, 72, 96 and 120 HATs.

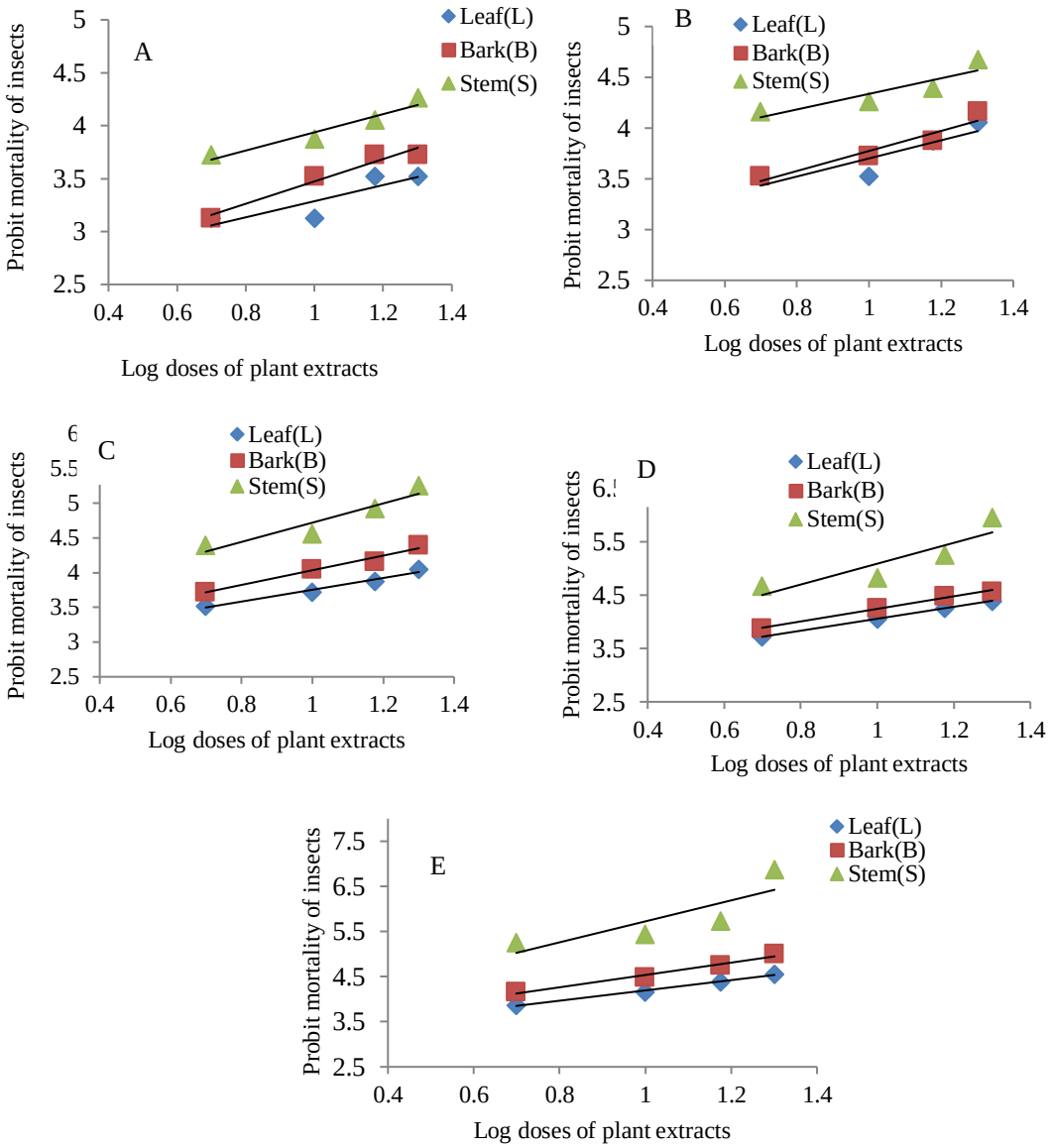


**Figure 1.** Relationship between probit mortality and log doses of eucalyptus plant extracts on rice weevilat24 (A), 48 (B), 72 (C), 96 (D) and 120 (E) HATs

**Residual effect of eucalyptus plant extracts against rice weevil:** The efficacy of leaf, bark and stem extracts of eucalyptus plant as protectants for wheat grains were evaluated and are shown in Table 4 and 5. The average numbers of adult emergence were statistically different among the plant extracts (Table 4). The highest number

of adult emergence was recorded in control (78.33). The lowest average numbers of adult emergence in stem (36.53) while the highest from leaf (39.56) extracts. The highest infestation inhibition was found in stem (67.42%). The lowest infestation inhibition was found in leaf (62.47%) followed by bark (64.42%) which was statistically different (Table 4). The number of adult emergence was found the highest (43.39) and the infestation inhibition was found the lowest (59.71%) at the dose level of 0.5% whereas the lowest number of adult emergence was found (38.39) at the dose level of 2.0% and the infestation inhibition rate was found the highest (66.33%) at the dose level of 2.0%. The interaction of plant extracts, doses at different HAT shown in Table 5. The lowest adult emergence was found on 2.0% dose of stem extract (22.00) where the highest infestation inhibition was found 72.87% while the highest adult emergence was found at 0.5%dose of leaf extract (33.56) and the lowest inhibition was found 57.30% followed by bark extract at the dose level of 0.5% adult emergence (31.55) and inhibition was found at 59.94% which was statistically different. The average adult emergence and the percentage of infestation inhibition were statistically significant different from the eucalyptus plant extracts at all levels of concentration. The results indicated that the residual effects of different plant materials decreased gradually with the increase of time interval.

From the above results, it was clear that the stem extract was the most effective for controlling the rice weevil. The present finding also agreed with that of Mani *et al.* (1993). They observed that the adult females of *Corcyra cephalonica* exposed to the volatile leaves of tulsi (*Ocimum basilicum)* and eucalyptus (*E.rostrata)* laid reduced the number of eggs. El-Atta and Ahmed (2002) established that leaf powder, seed kernel powder and oil extracted from the seeds of *A. indica* and leaf powder and oil extracted from the leaves of *E. canialdulensis* and benzene hexachloride (BHC) were tested at 1, 3 and 5% (w/w or v/w) against *S. oryzae*. Neem oil (NO) and eucalyptus leaf oil (ELO) at 3 and 5% were as efficient as BHC and significantly (P<0.001) reduced egg laying by *S. oryzae*, whereas eucalyptus leaf powder (ELP) had no significant effect.

**Table 4.** Residual toxicity effect of eucalyptus plant extracts and different doses of plant extractstreated against rice weevil

| | Extracts used | Number of adult emergence (DAT) and inhibition rate (%) | | | | | | Average emergence | Inhibition rate (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | 28 DAT | IR (%) | 35 DAT | IR (%) | 42 DAT | IR (%) | | |
| **Plant effect** | Leaf | 26.87 b | 65.47 b | 39.47 b | 62.14 b | 52.33 b | 59.81 b | 39.56 b | 62.47 b |
| | Bark | 25.87 bc | 67.68 ab | 38.33 b | 63.85 b | 50.80 b | 61.72 b | 38.33 bc | 64.42 b |
| | Stem | 24.20 c | 71.38 a | 36.47 c | 66.83 a | 48.93 c | 64.05 a | 36.53 c | 67.42 a |
| | Control | 56.33 a | - | 78.33 a | - | 100.3 a | - | 78.33 a | - |
| | LSD | 1.948 | 4.069 | 1.838 | 2.921 | 1.739 | 2.042 | 1.810 | 2.964 |
| | CV % | 7.92 | 7.08 | 5.17 | 5.39 | 3.73 | 3.92 | 5.09 | 5.43 |
| **Dose effect** | Doses used | | | | | | | | |
| | 0.5 % | 30.67 b | 60.80 c | 43.25 b | 59.73 c | 56.25 b | 58.59 c | 43.39 b | 59.71 c |
| | 1.0 % | 27.83 c | 67.54 b | 40.83 c | 63.99 b | 54.50 bc | 60.92 bc | 41.06 c | 64.15 b |
| | 1.5 % | 26.75 cd | 70.12 ab | 39.67 cd | 65.85 ab | 52.92 cd | 63.03 ab | 39.78 cd | 66.33 ab |
| | 2.0% | 25.00 d | 74.24 a | 38.67 d | 67.54 a | 51.50 d | 64.91 a | 38.39 d | 68.90 a |
| | Control | 56.33 a | - | 78.33 a | - | 100.3 a | - | 78.33 a | - |
| | LSD | 2.178 | 4.699 | 2.054 | 3.373 | 1.944 | 2.358 | 2.023 | 3.422 |
| | CV % | 7.92 | 7.08 | 5.17 | 5.39 | 3.73 | 3.92 | 5.09 | 5.43 |

Within column values followed by different letter (s) are significantly different by DMRT at 5% level of probability.

**Repellency effect of eucalyptus plant extracts against rice weevil:** The repellency effects of eucalyptus plant extracts against rice weevil showed that leaf, bark and stem extracts has strong repellent action (Table 6). Among the different parts of eucalyptus plant extracts tested, stem extracts showed the highest mean repellent effect (73.33%) followed by bark (63.00%) whereas leaf extracts showed the lowest mean repellent (56.33%) effect. On the basis of repellency rate, it was found that stem and bark extracts were in the same repellency class i.e. Class IV and leaf extracts werein Class III.The mean repellent (%) effect of eucalyptus plant extracts in different dose levels on rice weevil is also presented in Table 6. The repellency rate did not increase uniformly with the doses. The highest mean repellency effect was found with 2.0% dose extract (75.56%) and the lowest was found with 0.5% dose extract (52.89%). The results also indicated that repellency action increased proportionally to the concentration. The interaction repellent effect of

eucalyptus plant extract and their doses against rice weevil at different HATs is presented in Table 7. The repellent effect influenced by different levels of doses at different HAT and had significant differences. The highest mean repellency effect was found on a 2.0% dose of stem extract (82.67%) whereas the lowest mean repellency effect was found on 0.5% dose of leaf extract (42.67%).

**Table 5.** Combined residual toxicity effect of plant and doses at different time treated against rice weevil

| Extracts used | Doses (%) | Number of adult emergence (DAT) and inhibition rate (IR) (%) | | | | | | Average emergence | Inhibition rate (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | 28 DAT | IR (%) | 35 DAT | IR (%) | 42 DAT | IR (%) | | |
| Leaf | 0.5 | 23.67 b | 58.04 e | 33.67 b | 57.03 d | 43.33 b | 56.82 e | 33.56 b | 57.30 e |
| | 1.0 | 19.67 b-e | 65.18 b-e | 29.33 b-e | 63.00 bcd | 40.33 bcd | 59.81 cde | 29.78 bcd | 62.66 b-e |
| | 1.5 | 18.33 cde | 67.55 bcd | 28.33 cde | 63.85 bc | 39.33 bcd | 60.81 cde | 27.44 cde | 64.07 bcd |
| | 2.0 | 16.33 def | 71.10 abc | 27.67 cde | 64.70 abc | 38.33 cde | 61.81 bcd | 28.67 cde | 65.87 bcd |
| | Control | 56.33 a | - | 78.33 a | - | 100.3 a | - | 78.33 a | - |
| Bark | 0.5 | 22.00 bc | 60.99 de | 31.33 bc | 60.02 cd | 41.33 bc | 58.82 de | 31.55 bc | 59.94 de |
| | 1.0 | 18.33 cde | 67.53 bcd | 28.67 cde | 63.42 bcd | 38.67 cde | 61.48 bcd | 28.56 cde | 64.14 bcd |
| | 1.5 | 17.33 cde | 69.34 bcd | 27.33 cde | 65.14 abc | 37.67 cde | 62.47 bcd | 27.45 cde | 65.65 bcd |
| | 2.0 | 15.33 ef | 72.88 ab | 26.00 def | 66.84 ab | 36.00 de | 64.13 bc | 25.78 def | 67.95 abc |
| | Control | 56.33 a | - | 78.33 a | - | 100.3 a | - | 78.33 a | - |
| Stem | 0.5 | 20.67 bcd | 63.37 cde | 29.67 bcd | 62.14 bcd | 40.00 bcd | 60.14 cde | 30.11 bcd | 61.88 cde |
| | 1.0 | 17.00 de | 69.90 a-d | 27.00 c-f | 65.55 abc | 38.67 cde | 61.47 bcd | 27.56 cde | 65.64 bcd |
| | 1.5 | 15.00 ef | 73.48 ab | 24.67 ef | 68.55 ab | 34.33 ef | 65.80 ab | 24.67 ef | 69.28 ab |
| | 2.0 | 12.00 f | 78.75 a | 22.67 f | 71.08 a | 31.33 f | 68.79 a | 22.00 f | 72.87 a |
| | Control | 56.33 a | - | 78.33 a | - | 100.3 a | - | 78.33 a | - |
| | LSD | 4.356 | 8.138 | 4.109 | 5.841 | 3.888 | 4.085 | 4.047 | 5.928 |
| | CV % | 7.92 | 7.08 | 5.17 | 5.39 | 3.73 | 3.92 | 5.09 | 5.43 |

Within column values followed by different letter (s) are significantly different by DMRT at 5% level of probability.

**Table 6.** Repellent effect of eucalyptus plant extracts and doses against *Sitophilus oryzae* L. using treated and untreated filter paper at different HAT (Interaction of plant extracts and doses)

| | Extracts used | Repellency rate (%) at different HAT | | | | | % Mean repell. | Repell. classes |
|---|---|---|---|---|---|---|---|---|
| | | 1 HAT | 2 HAT | 3 HAT | 4 HAT | 5 HAT | | |
| **Plant effect** | Leaf | 41.67 b | 48.33 b | 58.33 b | 66.67 a | 66.67 b | 56.33 c | III |
| | Bark | 50.00 b | 56.67 b | 63.33 b | 70.00 a | 75.00 ab | 63.00 b | IV |
| | Stem | 65.00 a | 66.67 a | 78.33 a | 75.00 a | 81.67 a | 73.33 a | IV |
| | LSD | 10.51 | 9.729 | 10.51 | 9.729 | 9.729 | 5.148 | |
| | CV % | 23.88 | 20.18 | 18.71 | 16.37 | 15.51 | 9.51 | |
| **Dose effect** | Doses used | | | | | | | |
| | 0.5 % | 37.78 c | 44.44 c | 55.56 b | 62.22 b | 64.44 c | 52.89 d | III |
| | 1.0 % | 48.89 bc | 55.56 bc | 60.00 b | 68.89 ab | 71.11 bc | 60.89 c | IV |
| | 1.5 % | 55.56 ab | 57.78 b | 73.33 a | 73.33 ab | 77.78 ab | 67.56 b | IV |
| | 2.0 % | 66.67 a | 71.11 a | 77.78 a | 77.78 a | 84.44 a | 75.56 a | IV |
| | LSD | 12.13 | 11.23 | 12.13 | 11.23 | 11.23 | 5.945 | |
| | CV % | 23.88 | 20.18 | 18.71 | 16.37 | 15.51 | 9.51 | |

HAT = Hours after treatment, means in a column followed by different letter(s) are significantly different by DMRT at 5% level of probability.

From the above results, it was found that the eucalyptus plant extracts were effective for controlling the rice weevil but stem extract was the most effective. The present study agreed with the previous finding of Sharaby (1988) in where they reported that eucalyptus leaves showed more repellent action on *S. oryzae*. Khan and Shahjahan (1998) indicated that dried powder of *Eucalyptus teretocornis* leaves was extracted with hexane, acetone, ethanol and methanol and the extracts were tested to observe their effects against adults of *Sitophius oryzae* and *C. chinensis*. The results also stated that the percentages of repulsion for *S. oryzae* were 71.1, 74.7, 69.0 and 63.3% respectively. Lee et al. (2004a) showed that different solvent extracts of eucalyptus leaf

have repellent property against adults of *S. oryzae*. Mishra et al. (2012) indicated that the repellency of *E. globulus* and *O. basilicum* was 9.16±0.30 and 8.50±0.22 for *T. castaneum* and 8.66±0.33 and 8.16±0.30 for *S. oryzae*.

**Table 7.** Repellent effect of eucalyptus plant extracts at different dose level against *Sitophilus oryzae* L. using treated and untreated filter paper at different HAT (Interaction of plant extracts, dose and time)

| Extracts used | Doses (%) | Repellency rate % at different HAT | | | | | % Mean repell. | Repell. classes |
|---|---|---|---|---|---|---|---|---|
| | | 1 HAT | 2 HAT | 3 HAT | 4 HAT | 5 HAT | | |
| Leaf | 0.5 | 26.67 d | 33.33 c | 46.67 c | 53.33 b | 53.33 c | 42.67 g | III |
| | 1.0 | 33.33 cd | 46.67 bc | 46.67 c | 66.67 ab | 66.67 bc | 52.00 fg | III |
| | 1.5 | 46.60 bcd | 46.67 bc | 66.67 abc | 73.33 ab | 73.30 abc | 61.33 def | IV |
| | 2.0 | 60.00 ab | 66.67 ab | 73.33 ab | 73.33 ab | 73.33 abc | 69.33 bcd | IV |
| Bark | 0.5 | 33.33 cd | 46.67 bc | 53.33 bc | 66.67 ab | 66.67 bc | 53.33 efg | III |
| | 1.0 | 46.67bcd | 53.33 abc | 60.00 bc | 66.67 ab | 73.33abc | 60.00 def | III |
| | 1.5 | 53.33 abc | 53.33 abc | 66.67 abc | 73.33 ab | 73.33 abc | 64.00 cde | IV |
| | 2.0 | 66.67 ab | 73.33 a | 73.33 ab | 73.33 ab | 86.67 ab | 74.67abc | IV |
| Stem | 0.5 | 53.33 abc | 53.33 abc | 66.67 abc | 66.67 ab | 73.33 abc | 62.67 def | IV |
| | 1.0 | 66.67 ab | 66.67 ab | 73.33 ab | 73.33 ab | 73.33 abc | 70.67 bcd | IV |
| | 1.5 | 66.67 ab | 73.33 a | 86.67 a | 73.33 ab | 86.67 ab | 77.33 ab | IV |
| | 2.0 | 73.33 a | 73.33 a | 86.67 a | 86.67 a | 93.33 a | 82.67 a | V |
| | LSD | 21.02 | 19.46 | 21.02 | 19.46 | 19.46 | 10.30 | |
| | CV % | 23.88 | 20.18 | 18.71 | 16.37 | 15.51 | 9.51 | |

Means in a column followed by different letter(s) are significantly different by DMRT at 5% level of probability.

## CONCLUSION

The eucalyptus plant extracts of leaf, bark and stem were used as a botanical pesticide against stored grain pest, rice weevil, *Sitophilus oryzae* L. Farmer cannot always afford to buy synthetic pesticides to protect stored grain and products. The highest direct toxic effects were recorded in stem extract treated insect which indicated higher toxicity of stem extract for controlling rice weevil, *Sitophilus oryzae* L. From the present finding, it conclude that the eucalyptus plant of leaf, bark and stem extracts are effective in reducing the number of adult emergence and increasing infestation inhibition rates and farmers might apply for protecting their stored grain against rice weevil.

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