

Insecticidal efficacy of leaf extract of some medicinal plants on adult mortality and adult emergence of rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae)

Nithiyagowry R¹, Chaturica Thilini Ratnayake²

^{1,2} Department of Zoology, University of Jaffna, Sri Lanka

Abstract

The laboratory experiment was conducted to assess the efficacy of methanol leaf extract of five medicinal plants namely *Piper longum*, *Pongamia pinnata*, *Murraya koenigii*, *Acalypha indica* and *Justica gendarussa* at five concentrations (0.025g/ml, 0.75g/ml, 0.125g/ml, 0.175g/ml and 0.225g/ml) on adult mortality and adult emergence against *Sitophilus oryzae*. The results revealed that a significant ($P < 0.05$) increase in adult mortality and suppression of adult emergence in all concentration after 72 hours of treatment as compared to controls. The order of toxicity on adult weevil as follows *A.indica* > *P.pinnata* = *M.koenigii* = *J. gendarussa* > *P.longum*. LD₅₀ values after 72 hours of treatment was found as 0.182 g/ml (*A. indica*), 0.191g/ml (*P.pinnata*), 0.214g/ml (*J. gendarussa*) and reduction in adult emergence by all leaf extract at 0.225 g/ml ranged 60 -79%. These results suggest that these plants acted as potential insecticide against rice weevil and could be considered in the control of rice weevil.

Keywords: mortality, adult emergence, *Sitophilus oryzae*, medicinal plants, methanol leaf extract

1. Introduction

Rice is the most important starch food of the countries in the world particularly in Asia. Every year 30-50% of the total paddy production is stored by farmers for their consumption, future sales, wages and seed purposes [6]. Rice weevil, *Sitophilus oryzae* (L.) is a major pest of rice both in field before harvest and in storages. The white apodous grub and the reddish brown adults are internal feeders and cause deterioration in quality of the products [4], and presence of live and secretions adversely affects both appearance, taste and pose health risks and also influences seed vigor and reduce percentage of seed germination therefore managing grain in storages to prevent insect damage is essential as much as managing the crop while it's growing the field for future consumers. Indiscriminate usage of chemical pesticide has created serious problems not only to human and also to the environment hence there is an urgent need for effective biodegradable cheap, ecofriendly, safe alternatives to synthetic pesticides with greater selectivity thus in recent years considerable effort have been focused on plant materials, to protect the stored products against storage pests. Toxic effects of plant products on some pests have been studied by many researchers and identification of natural extracts exhibited detrimental effects on insect physiology and behaviour [2, 15]. Different types of plant preparations such as powders, solvent extracts, essential oils and whole plants are being investigated for their insecticidal activity. Although a large number of plant products in various formulations have been screened against pests of stored grains thousands are untouched. In this view the present study was under taken to evaluate the toxicity and progeny development of leaf extracts of five medicinal plants namely *Piper longum* (Thippili), *Pongamia pinnata* (Ponga/Karanj), *Murraya koenigii* (curry leaves), *Acalypha indica* (Kupaimeni) and *Justicia gendarussa* (Karu nochi) to develop environmentally safe, cheaper and effective control

that could replace synthetic insecticide for the control of rice weevil.

2. Materials and methods

2.1 Laboratory rearing of rice weevil *Sitophilus oryzae* (L.)

2.1.1 Mass culture

To maintain mass culture of rice weevil, rearing was carried out in the laboratory. Undamaged rice (Red nadu variety) was purchased from the local market and placed in direct sunlight for 2 days to disinfection and stored in air tight plastic bottles. Fifty unsexed adults of *S. oryzae* introduced into a bottle (500ml) containing 500 g disinfested whole rice grains. The top of each rearing bottle was covered with muslin cloth fastened tightly with rubber band, to prevent the contamination and escaping of insects and was kept in dark place under laboratory condition. After 2 weeks all the adults were removed and kept for adult emergence.

2.1.2 Sub culture

In order to obtain a pure culture 1-2 days old adults from the mass culture were collected using aspirator and introduced into another bottle (500ml) containing 100gram of infestation free rice. The rice weevil cultures were maintained at a temperature of 29±2°C and 84± 2% relative humidity and 12:12 D:L Photoperiod. Adults from the culture were used for the experiment.

2.2 Test plant material

The leaves of five medicinal plants *P. pinnata*, *A. indica*, *J. gendarussa*, *P. longum*, *M. koenigii* were collected from the University of Jaffna, Sri Lanka premises.

2.2.1 Preparation of methanol leaf extract

Fresh leaves from the following medicinal plants *P. pinnata*, *A. indica*, *J. gendarussa*, *P. longum*, *M. koenigii* were

collected from the University of Jaffna, Sri Lanka premises and were thoroughly washed separately in tap water and air dried in the shade for one week. Fully dried leaves were ground in an electric grinder separately and the resulting powder was pass through 500µm mesh size sieve to obtain powder.

50 grams leaf powder of *P. pinnata* were mixed with 450ml of methanol and the mixture was stirred about 3 hours using magnetic stirrer and left to stand for 24 hours. After 24 hours mixture was filtrate through muslin cloth. The methanol extract was allowed to evaporate on a hot water path (maximum temperature 40°C) until the constant weight of extract was gained. From this 2.5g divide into 0.1g, 0.3g, 0.5g, 0.7g, and 0.9g, which are dissolved with 4ml of methanol separately to get 0.025g/ml, 0.75g/ml, 0.125g/ml, 0.175g/ml and 0.225g/ml concentration of extracts. Likewise leaf extract of *A. indica*, *J. gendarussa*, *P. longum*, *M. koenigii* leaves were prepared separately.

2.3 Experimental Condition

All experiments were carried out in the laboratory of Department of Zoology, University of Jaffna, Sri Lanka under prevailing environmental conditions of 29 ± 2° C and 75±5% RH during the daytime and photoperiod D: L 12:12. Control experiments were conducted concurrently along with experiment trials.

2.4 Experiment 1.Effect of leaf extracts on mortality of adult rice weevil.

To study the effect of methanol leaf extracts of selected five medicinal plants on mortality of adult rice weevil, 1kg of rice brought from local shop were kept under direct sunlight for 3 days in order to kill the life stages of rice weevil which already present in it. From this 10 gram of pest free rice was taken into seven vials (3 cm diameter x8 cm height) separately. Methanol leaf extracts of *P.pinnata* 0.025g/ml, 0.075g/ml, 0.125g/ml,0.175g/ml and 0.225g/ml were added into 5 vials separately and was mixed well by shaking the vials manually one kept for control as untreated and one kept for solvent control. Then treated and untreated rice in the vials transferred into petridish (9cm height x 1.5cm diameter) separately and allowed to dry. Five pairs of 1-3 days old adults from the laboratory culture were introduced into each petridish. They were covered by lids which had a hole of 4cm in diameter covered over with a fine nylon mesh to provide ventilation. All petridishes were tied by rubber bands. Likewise 0.025g/ml,0.75g/ml, 0.125g/ml, 0.175g/ml and 0.225g/ml methanol leaf extract of *A. indica*, *J. gendarussa*, *P. longum*, *M. koenigii* were treated separately.

Each treatment was repeated three times along with the control test. All were kept in the wooden cupboard under laboratory conditions at 29 ± 2° C and 84±2 % relative humidity and 12:12 L:D photo period. After 7 hours, 24 hours, 48 hours, 72hours and one week of introduction of insects dead adults were removed from each treatment and counted separately and mortality data was corrected according to Abbott's formula [1] as follows.

$$\text{Corrected mortality} = \frac{\% \text{ mortality treated insects} - \% \text{ mortality in control}}{100 - \% \text{ mortality in control}} \times 100$$

After correcting the mortality value the medial lethal dose (LD₅₀) values were calculated by probit analysis method.

Experiment 2. Effect of methanol leaf extracts on suppression of adult emergence of rice weevil

Ten gram of pest free rice was taken into seven vials (3cm diameter x 8cm height) separately. 0.025g/ml, 0.075g/ml,0.125g/ml, 0.175g/ml, 0.225g/ml methanol leaf extract of *P. pinnata*, plant was added into 5 vials separately and was mixed well by shaking the vials manually. Four ml methanol was added in to sixth vial, shake well and seventh one kept for control as untreated. Then treated and untreated rice in the vials transferred into petridishes (9cm height x 1.5cm diameter) separately and allowed to dry.

Five pairs of (0-24 hrs old) adults from the laboratory culture were introduced into each petridish. They were covered by lid which had a hole of 4cm in diameter covered over with a fine nylon mesh to provide ventilation. All petridishes were tied by rubber bands. Likewise 0.025g/ml, 0.075g/ml, 0.125g/ml, 0.175g/ml, 0.225g/ml methanol leaf extract of *A. indica*, *J. gendarussa*, *P. longum*, *M. koenigii* were treated separately. Three replicates of each treatment were conducted concurrently along with solvent control and untreated control test. All were kept in the wooden cupboard under laboratory conditions at 29 ± 2° C and 84±2 % relative humidity. And 12:12 L: D photo period. After third week of treatment all adults both dead and live were removed and kept still adult emergence. The number of newly emerged adults of F₁ progeny from each treatment were removed and recorded daily until the number of emergence becomes zero.

The percentage reduction in the number of adults emerged was calculated by the following equation as described by Khoshnoud (2008) [8].

$$\% \text{ reduction of adult emergence} = (\text{Ec}-\text{Et}/ \text{Ec}) \times 100$$

Where: Ec- mean number of adult emerged in untreated control

Et – mean number of adults emerged in treated

Statistical analysis

Data obtained during choice and no-choice ovipositional tests were analyzed statistically using One-way ANOVA and the means were compared using Turkey's Pair wise Comparison test. Data obtained

during damage assessment were subjected to analysis of variance and where significant differences existed, treatment means were compared at 0.05 level of significance. Treatment means were compared by the least significant difference (LSD) test.

3. Results and Discussion

3.1 Effect of methanol leaf extracts on mortality of adult rice weevil

The result of the experiment (Fig. 1) showed that the mortality rate of adult rice weevil was significantly (P<0.05) increased with the increase of dose of methanol leaf extract of all tested plants after 72 hours and one week of treatment compared to controls. Adult mortality was ranged 46.6% - 63.3% and 53 %-73.3 % when rice treated with 0.225g/ml of methanol leaf extract after 72 hours and one week of exposure respectively. Leaf extract of *A. indica* showed highest mortality rate (63.3%) than the other four plants. There was no significant difference on adult mortality among treatments when rice treated with methanol leaf

extract of 0.025 g /ml ($P>0.05$), 0.075 g /ml ($P>0.05$), and 0.125 g/ml ($P>0.05$) after 72 hours of exposure. However significant difference on mortality was observed when rice treated with 0.175 g/ml ($P<0.05$) and 0.225 g/ml ($P<0.05$). From the LSD test highest toxic effect on adult rice weevil was observed when rice treated with methanol leaf extract of *A.indica* while *P.longum* was showed least toxic effect on adult rice weevil and *P.pinnata*, *M.koenigii* and *J.gendarussa* plants were showed equal plants toxic effect on adult after 72 hours of exposure. From the probit analysis, LD_{50} values after 72 hours of treatment was found as 0.182 g/ml (*A. indica*), 0.191g/ml (*P. pinnata*), 0.214g/ml

(*J. gendarussa*).

According to [2] *A. indica* against rice weevil 69.73% of adult mortality was observed between 7 and 14 days against rice weevil. From this study 63% of adult mortality was observed after 72 hours of exposure. This variation may be due to the solvent effect and different concentration used for the test. Vishal kumar *et al.* (2006) [16] has reported that methanol fraction of seed oil and leaf extract exhibit toxicity and repellency against *Tribolium castaneum*. This study also confirmed the toxic activity of *A. indica* and *P. pinnata* against rice weevil. But activity is depending on the solvent and concentration of extract.

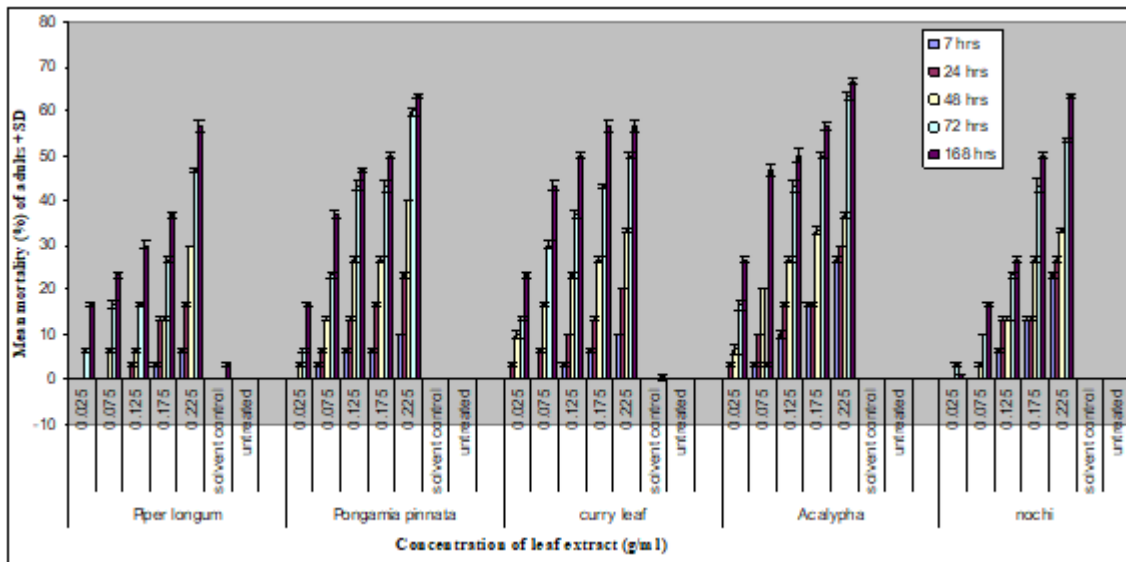


Fig 1: The percentage mortality of adults *S. oryzae* exposed for different periods on rice treated with different concentration of methanol leaf extract of medicinal plants Bar indicates \pm SD, N= 30

Haidri *et al.* (2014) [7] described mortality effect *M. koenigii* against *Callosobruchus maculatus* as 20 % acetone leaf extract caused 67 % adult mortality. This also confirmed by this study. *M.koenigii* leaves are a rich source of volatile oils. Duke (1990) [5] and Baser *et al.* (1998) [3] reported that the plant compounds terpenes, steroids and alkaloids shows toxic effects against a wide range of insect pests by disrupt major metabolic pathways and cause death or act as antifeedant leads to starvation and death of insects. The mortality rate was also found to be Dependant on dose and exposure time (Fig.1).

3.2 Effects of leaf extracts on adult emergence of rice weevil, *S. oryzae*

One of the effective grain protectant against insect pest is the ability to reduce progeny development. This is clearly revealed by the mean number of *S. oryzae* adults that emerged and percentage reduction in adult emergence after 35 days of treatment is presented in Table 1 & 2, Fig.2. The result revealed that significantly lesser ($P<0.05$) mean number of adults emerged in treated grain than the control. All the extracts showed > 42 % and 60 % reduction in adult emergence at 0.175 g/ml and 0.225 g/ml respectively.

Highest suppression of adult emergence was observed at 0.225 g/ml by *P. longum* (78.6 %) and followed by as *P.pinnata* (75%), *A. indica* (69.2 %), *M. koenigii* (66.7 %), and *J. gendarussa* (60 %) compared to control (Table 2). There was no significant difference on adult emergence among the treatment when rice treated with methanol leaf extract of 0.025 g /ml ($P>0.05$), 0.075 g/ml($P>0.05$), 0.125 g /ml($P>0.05$) while there was a significant difference among treatments when rice treated with 0.175 g/ml($P<0.05$) and 0.225 g/ml($P<0.05$). From the LSD test *P.longum* was showed least adult emergence than other plant leaf extracts. So *P.longum* is most effective on adult emergence at 0.225 g/ml while other plant leaf extracts showed equal effect on adult emergence. Adult emergence of adult rice weevil was decrease with increase the dose of methanol leaf extract of all plants.(Table 1 and Fig.2). Plant extracts reduce the oviposition act as repellent, ovicides or larvicides and oviposition deterrent as a result it suppress the progeny production [9, 11, 14]. High suppression of adult emergence was shown by *P. longum* and *P.pinnata* this is may be due to the presence of active ingredients piperine, piperlongumine in the leaf extract of *P. longum* act on insect as oviposition inhibitor [9, 10] as a result the number of adults emerged reduced.

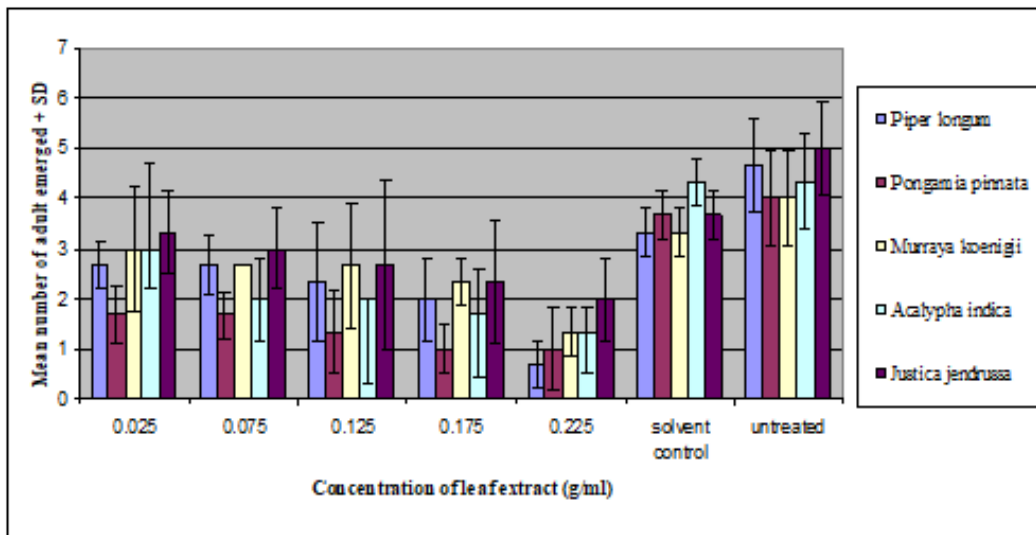


Fig 2: Effect of methanol leaf extract of medicinal plants on adult emergence on rice treated with different concentration Bar indicates ±SD

Table 1: Effect of methanol leaf extracts of medicinal plants on adult emergence of rice weevil, *S. oryzae* after admixing methanol leaf extract with rice grain at different concentrations.

Test plants	Concentration of leaf extract (g/ml)					Solvent control	Untreated
	0.025	0.075	0.125	0.175	0.225		
	Mean number of adults emerged ± SD						
<i>Piper longum</i>	2.67 ± 0.47	2.67 ± 0.57	2.33 ± 1.1	2 ± 0.81	0.67 ± 0.47	3.33 ± 0.47	4.66 ± 0.94
<i>Pongamia pinnata</i>	1.67 ± 0.57	1.67 ± 0.47	1.33 ± 0.81	1 ± 0.47	1 ± 0.81	3.67 ± 0.4	4 ± 0.47
<i>Murraya koenigii</i>	3 ± 1.2	2.67 ± 0	2.67 ± 1.2	2.33 ± 0.47	1.33 ± 0.47	3.33 ± 0.47	4 ± 0.47
<i>Acalypha indica</i>	3 ± 1.6	2 ± 0.81	2 ± 0	1.67 ± 0.94	1.33 ± 0.47	4.33 ± 0.81	4.33 ± 0.94
<i>Justicia jendarussa</i>	3.33 ± 0.81	3 ± 0.81	2.67 ± 1.6	2.33 ± 1.2	2 ± 0.81	3.67 ± 0.41	5 ± 0.94

SD- Standard Deviation

Table 2: Effect of methanol leaf extracts of medicinal plants on percentage reduction of adult emergence of weevil, *S. oryzae* after admixing methanol leaf extract with rice grain at different concentrations.

Test plants	Percentage reduction in adult emergence				
	Concentration of leaf extract (g/ml)				
	0.025	0.075	0.125	0.175	0.225
<i>Piper longum</i>	42.8	42.8	50.2	57.0	78.6
<i>Pongamia pinnata</i>	58.2	58.2	66.7	75.0	75.0
<i>Murraya koenigii</i>	25.0	33.3	33.3	41.8	66.7
<i>Acalypha indica</i>	30.7	53.8	53.8	61.4	69.2
<i>Justicia jendarussa</i>	33.4	40.0	46.0	53.4	60.0

4. Conclusion

Storage and preservation of medicinal herbs has been given more concern to use the herbal parts for preparation of medicine. But identifying the insecticidal properties of medicinal herbs would help to preserve the ecofriendly from insect pest damage. In this study the result obtained from the assay revaluated that leaf extracts of *P. pinnata*, *A. indica*, *J. gendarussa*, *P. longum*, *M. koenigii* were shown varying levels of bioactivity against *Sitophilus oryzae*. As a result methanol leaf extracts of *Acalypha indica* at highest dose shows residual effect on adult rice weevil while leaf extractions of *P. longum* have low toxicity effect on rice weevil at highest dose after 72 hours tested in this study. Methanol leaf extracts of all five plants were showed same effect on suppression of adult emergence of adult rice weevil after 72 hours of treatment. Methanol leaf extract of *P. longum* at highest concentration was showed effect on adult emergence of adult rice weevil. It was concluded that *A. indica*, *M. koenigii*, *J. gendarussa*, *P. pinnata* and *P. longum* possess insecticidal activity and potential of insecticidal activity and on mortality and progeny

production was propotional to the concentration and higher concentration had stronger effect. Methanolic leaf extract of *Acalypha indica*, *Murraya koenigii*, *Justicia gendarussa*, *Pongamia pinnata*. and *Piper longam* at 0.225 g/ml could be a considered for the management of *S.oryzae* infesting stored commodities. Further research efforts are suggested towards the screening and integration with other effective botanicals in order to formulate them for commercial purposes.

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6. References

1. Abbott WS. A method of computing the effectiveness of an insecticide. J. econ. Ent. 1925; 18:265-267.
2. Ande AT. Biological activities of some plant materials against two stored product insect pest of wheat. Department of biological science, University of Florin, Ilorin, Nigeria, 2001.

3. Baser K, Demircakmak CB, Ermin N, Demirci F, Boyday I. The essential oil of *Bifora radians* Bied. *Journal of Essential Oil Research*. 1998; 10(4):451-452.
4. Dal Bello G, Padin S, Lopez Lastra C, Fabrizio M. Laboratory evaluation of chemical, biological control of the rice weevil (*Sitophilus oryzae* L.) in stored grains. *Journal of Stored Product Research*. 2001; 37(1):77-84.
5. Duke SO. Natural pesticides from plants in: "Advances in new crops. (Janick J, Simon JE.Eds)". Timber press. Portland, 1990, 511-517.
6. Gunarathna TVNM, Karunaratne MMSC. (Laboratory evaluation of some Sri Lankan plants as post-harvest grain protectants for the control of rice weevil *Sitophilus oryzae*), 2009.
7. Haidri SR, Sagheer M, Hasan M, Saeed S, Tahira Gul H, Ahmed Khan FZ. Bioefficacy of *Azadirachta indica* and *Murraya koenigii* against pulse beetle, *Callosobruchus chinensis* (Coleoptera:Bruchidae). *Applied Sciences and Business Economics*. 2014; 1(1):28-32.
8. Khoshnoud H, Ghiyasi M, Amimia R, Sadig Fard S, Tajbakhsh M, Salehza deh. And Alahyary. The potential of using insecticidal properties of medicinal plants against insect pests. *Pakistan Journal of Bio Science*. 2008; 11(10):1380-1384.
9. Nalini R, Rajavel DS, Geetha A. Effects of the Medicinal Plant Leaf Extract on the Rice Weevil, *Sitophilus oryzae* (L.). Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai 625 104 Tamil Nadu, 2009.
10. Narangoda SRCNK, Karunaratne MMSC. Oviposition deterrent and insecticidal activities of some indigenous plant extracts against the rice moth, *Corcyra cephalonica* (Stainton). Department of Zoology University of Sri Jayewardenepura Nugegoda, Sri Lanka, 2008.
11. Nithiyagowr R. Efficacy of different leaf formulations from five medicinal plants against rice weevil, *Sitophilus oryzae* (L.) (Coleoptera:Curculionidae). *Proceedings of Sri Lanka Association for the Advancement of Science*, 2015, 61.
12. Paranagama PA, Abeysekera KHT, Nugaliyadde, Abewickrama LK. Bioactivity of leaf volatiles of *Azadirachta indica* A. Juss and *Murraya koenigii* spreng against *Sitophilus oryzae* L.(Coleoptera:Curculionidae). *Tropical esearch and Extension*. 5(1&2):2002.
13. Rajapakse RHS. The Potential Of Plants And Plant Products In Stored Insect Pest Management. Rice weevil.htm-Public health vectors and pests, 2006.
14. Rajapakse R, Van Emden HR. Potential of four vegetable oils and ten botanical powders for reducing infestation of cowpea by *Callosobruchus maculatus*, *C. chinensis* and *C. rhodesianus*. *Journal of Stored Product Research*. 1997; 33(1):59-68.
15. Talukder PA. Plant products as potential stored product insect management agents- A mini review. *Journal of Agriculture Science*. 2006; 18(1):17-32.
16. Vishal Kumar, Chandrashekar K, Om Prakash Sidhu. Efficacy of karanjin and different extracts of *Pongamia pinnata* against selected insect pests. Biomass Biology Division, National Botanical Research Institute, Rana Pratap Marg - 226 001, Lucknow, Uttar Pradesh, India, 2006.
17. Yusuf SR, Ahmed BI, Chaudhary JP, Yusuf AU. Laboratory evaluation of some plant products for the control of maize weevil (*Sitophilus zeamais*) in stored maize. ESN occasional publications. 1998; 31:203-203.