Effect of Methanol Leaf Extract of Nishinda, *Vitex negundo* on the Development of Ovary of Rice Weevil, *Sitophilus Oryzae*

Nithiyagowry R* and Thulashi K
Department of Zoology,Faculty of Science,University of Jaffna, SriLanka

*Email: rngowry@univ.jfn.ac.lk

Abstract: The rice weevil, *Sitophilus oryzae* Linnaeus is one of the most important pests in stored rice. Since rice is a crucial staple food with seasonal availability, ensuring a continuous supply for consumers is essential. Therefore, it is imperative to protect against pest infestations during storage. In order to find efficacy of methanol leaf extracts of Nishinda, *Vitex negundo* on development of ovary of rice weevil *Sitophilus oryzae* (L.), methanol leaf extracts of *V.negundo* prepared at laboratory and tested at three concentrations of 0.025g/ml, 0.075g/ml, and 0.125g/ml along with solvent control and untreated control by measuring the length of germerium, vitellarium and ovariole. Methanol leaf extract of *V.negundo* at 0.125g/ml significantly ($P<0.05$) reduced the lengths of gemarium, vitellarium, and ovariole than that of controls as a result fecundity of *S. oryzae* is reduced. This study revealed that high concentration of (0.125g/ml) *V.negundo* found as most effective in reduction in the development of the ovary by reduction of length of gemerium, vitellarium and ovariole. Thereby it leads to a reduction in fecundity of *S.oryzae* and *V. negundo* plant can be considered as a potential pesticidal plant for control the pest.

Keywords: *Sitophilus* sp., *Vitex negundo*, ovariole, ovarian development

1. INTRODUCTION

The rice weevil, *Sitophilus oryzae* Linnaeusis one of the most important pests of the majority of common post-harvest cereals in storages. Since rice is an important stable food and they are seasonal and want to be a continuous supply for the consumers, it is essential to protect against pest infestation in storage. Changes in the ovarian system can give a measure of the physiological age of the insect. Age – grading techniques based on the physiological changes in the reproductive system including physical changes in ovaries lead to a decline in fecundity (Tyndale- Biscoe, 1984; Hayes and Wall, 1999). Rate of oviposition has been found to be influenced by several factors such as humidity and temperature and also depends on the quality of food (Khare and Agrawal, 1963). Botanicals influence insects by several manners such as behaviour, development and physiology of insects (Sharma *et al.*, 1984) and control pest population. In 1980, Golob and Webly made an excellent review on the use of botanicals and minerals to control storage pests by reducing oviposition, adult emergence, and act as antifeedant, repellent, growth regulator etc. Reduction in fecundity depend on the physiology of development of ovary. Insecticidal property of *V. negundo* leaves against stored grain pests was reported by Ahmed and Koppel (1987) but lack of studies on the development of the ovarian system in rice weevil. In this view, this study was undertaken to study the effect of methanol leaf extract of *V. negundo* on the development of ovary in rice weevil as a measure of control of rice weevil.

2. MATERIALS AND METHODS

2.1. Rearing of rice weevil, *Sitophilus oryzae*

2.1.1 Mass culture

In order to maintain the culture of rice weevil, *S. oryzae* rearing was carried out in the laboratory of the Department of Zoology, University of Jaffna under the prevailing environmental conditions of 27-31°C and 70-75% RH. Undamaged rice was obtained from the local market and was placed in direct sunlight for 3 days to disinfection. Fifty rice weevils were introduced into a glass container (Jam bottle – 300ml) containing 80g of infestation free rice and covered with muslin cloth secured with rubber bands to prevent contamination and escape of insects and was kept in a dark place under laboratory condition

to obtain new emerging adults. After 20 days all the adults were removed and kept for adult emergence.

2.1.2 Subculture

In order to obtain a pure culture, 0–24 hours old adults from the mass culture were collected by using an aspirator and introduced into another glass container (300ml) containing 50g infestation free rice. The same condition was maintained and left to breed. Then same-aged (1 day old) adults from this culture were used for the experimental purpose.

2.2 Preparation of methanol leaf extracts of *Vitex negundo*

Leaves of *V.negundo* were thoroughly washed separately in tap water, air dried in the shade for 7 days. Fully dried leaves of *V.negundo* were ground in an electric grinder separately and the resulting powder was passed through 500µm mesh size sieve to obtain powder.

Fifty grams of leaves powder of *V.negundo* was mixed with 450ml of methanol and the mixture was stirred for about 3 hours in a magnetic stirrer and left to stand for 24 hours. Then the mixture was filtered through filter paper. The methanol extract was allowed to evaporate on a hot water bath (maximum temperature 40°C) until the constant weight of the extract was gained. 0.1g, 0.3g, and 0.5g precipitate dissolved with 4ml of methanol separately to get 0.025 g/ml, 0.075g/ml, and 0.125g/ml concentration of extracts.

2.3. Bioassay

Rice (500 g) brought from a local shop was kept under direct sunlight for 3 days in order to kill the life stages of weevil which already present in it. From this, 5g of pest free rice was taken into five vials (5cm diameter × 4cm height) separately. 4ml of 0.025g/ml, 0.075g/ml and 0.125g/ml methanol leaf extracts of *V.negundo* were added into first 3 vials separately and was mixed well by shaking the vial manually. 4ml methanol was added into fourth vial, shaked well and kept for solvent control. Fifth one kept for control as untreated.

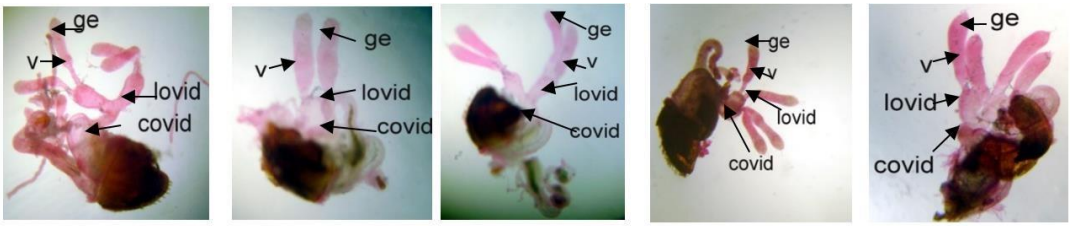
Treated and untreated rice in the vials transferred into petridishes (9cm diameter × 1.5cm height) separately and allowed to dry. 5 pairs of 0-24 hours old adults from the culture were introduced into each petridish and covered by a lid which had a hole of 4cm in diameter covered over with a fine nylon mesh to provide ventilation. All petridishes were tied by rubber bands. 3 replicates of each treatment were conducted concurrently along with solvent control and untreated control.

All were kept under laboratory condition at 29 ± 2°C temperature and 70-75% relative humidity and 12:12 L: D photoperiod.

After 20 days of treatment all the adults were removed and were kept still adult emergence. After 4 weeks of adult introduction, 0-24 hours old adults were removed from each treatment chambers and were kept in the separate vials containing infestation free rice. Every day 10 adults from each vial treated as well as untreated were taken out and dissected by using a stereomicroscope equipped with an ocular micrometer then reproductive system of female was removed with fine forceps and mounted on slide permanently for further study. The effect on development of ovary was assessed by measuring the length of gemarium, length of vitellarium and length of ovariole from each female dissected. This was carried out for 1 day old adults to 10 days old adults. Photos of all the dissected ovary was taken using digital camera under compound light microscope.

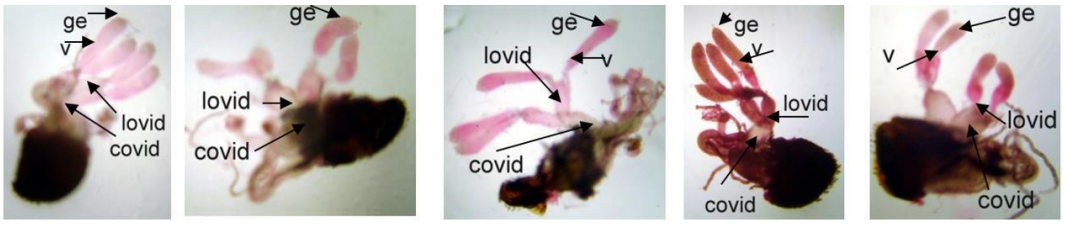
3. RESULTS AND DISCUSSION

The female reproductive system in *Sitophilus* is of the meristic/telotrophic type in which nurse cells or trophocytes are present in the germarium and are connected to oocytes in early stages of their development by tropic filaments (King and Buning,1985).



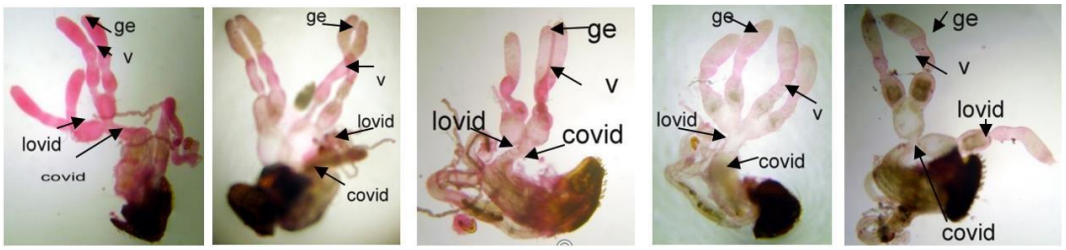
T1: 0.025 g/ml T2: 0.075 g/ml T3: 0.125 g/ml T4: Methanol control T5: untreated control

Plate 1: Ovariole of one day old adult *S.oryzae* from rice treated with methanol leaf extract of *Vitex negundo*and untreated rice (covid- common oviduct; ge- gemarium; loid – lateral oviduct; v-vitellarium)



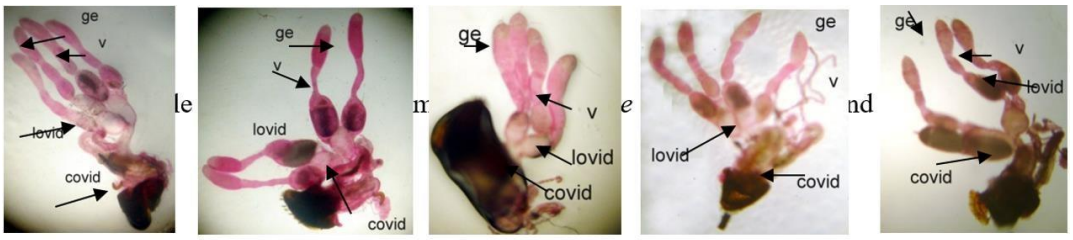
T1: 0.025 g/ml T2: 0.075 g/ml T3: 0.125 g/ml T4: Methanol control T5: untreated control

Plate 2: Ovariole of three day old female adult *S.oryzae* from rice treated with methanol leaf extract of *Vitex negundo* and untreated rice



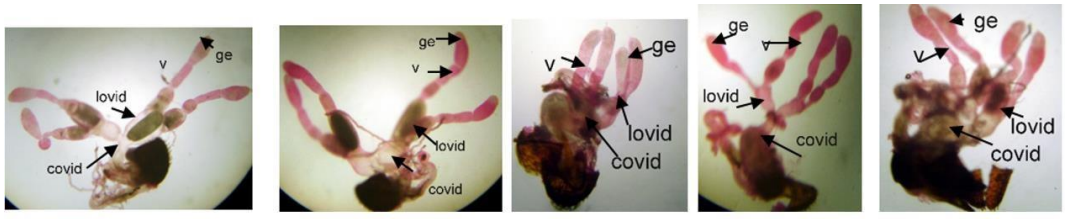
T1: 0.025 g/ml T2: 0.075 g/ml T3: 0.125 g/ml T4: Methanol control T5: untreated control

Plate 3: Ovariole of five day old female adult *S.oryzae* from rice treated with methanol leaf extract of *Vitex negundo* and untreated rice



T1: 0.025 g/ml T2: 0.075 g/ml T3: 0.125 g/ml T4: Methanol control T5: untreated control

Plate 4: Ovariole of seven day old female adult *S.oryzae* from rice treated with methanol leaf extract of *Vitex negundo* and untreated rice



T1: 0.025 g/ml T2: 0.075 g/ml T3: 0.125 g/ml T4: Methanol control T5: untreated control

Plate 5: Ovariole of ten day old female adult *S.oryzae* from rice treated with methanol leaf extract of *Vitex negundo* and untreated rice

In all treatments there was a dramatic increase in mean length of gemarium within the first 5 days. After 5 days mean gemarium length seem to be changed slightly upto tenth day after emergence. This is due to the formation of oocytes and passes to the proximal region of vitellarium in the ovariole during the early period of emergence. That is reproductive rate is peak during this period (Plates 1 & 2).

However, there was a significant difference in gemarium length was observed among the treatment upto 3 day old adult. There was a significant difference in the length of gemarium in female adult rice weevil among the rice treated with different concentrations of *V.negundo* leaf extracts and untreated in day 1 to day 3 old adult. For day 1 ($F=33.291$, $df=49$, $P<0.05$); for day 2 ($F=3.8$, $df=49$, $P<0.05$) and for day 3 ($F=20.5$, $df=49$, $P<0.05$ (Fig. 1). Significantly lesser mean length of gemarium was observed in each concentration (0.025g/ml, 0.075g/ml, and 0.125g/ml) in day1, day 2 and day3 old adult weevils when compared with that of untreated control. (Fig. 1)

There was no significant difference ($P>0.05$) in length of gemarium between each concentration treated and untreated in 4^{th} and 5^{th} days old adult (Fig. 1). This is may be due to the follicular development.Perez- Mendoza, *et al*., (2004) reported that through the successive ovulations, the follicular relics become compressed by the action of follicles passing through the constricted opening into the lateral oviduct. As more ovulations occur, distinctly darker particles become apparent within the follicular relics.

Dark colour in the follicle of the adult ovary was observed in the 4^{th} day – 10^{th} day old adult of untreated. Among the treatment it was observed in 7^{th} day – 10^{th} day old adult of 0.025g/ml and 4th day, 6^{th}day – 10^{th} day old adult of 0.075g/ml. There was no darkening observed in adult of 0.125g/ml (Plate 4). This indicates that ovulation is slowed down or slightly reduced in adults emerged from the rice treated with leaf extracts when compared with that of untreated. However, at high concentration (0.125g/ml) ovulation got reduced than the other concentrations. There was a gradual increase in mean length of vitellarium from day 1 to day 5 old adult. Then suddenly increase (day 5- day 7) and decrease. This increase may be due to the increase in a number of follicles and the decrease may be due to ovulation (Fig. 2).

There was a significant difference in the length of vitellarium in female adult rice weevil among the rice treated with different concentrations of *V.negundo* leaf extracts and untreated in day 2 to day 10 old adults. For day 2 ($F=6.31$, $df=44$, $P<0.05$); For day 3 ($F=21.45$, $df=49$, $P<0.05$); For day 4**(**$F=4.56$, $df=44$, $P<0.05$); For day 5 ($F=3.55$, $df=49$, $P<0.05$); For day 6 ($F=48.99$, $df=49$, $P<0.05$); For day 7 ($F=43.23$, $df=44$, $P<0.05$); For day 8 ($F=2.78$, $df=44$, $P < 0.05$); For day 9 ($F=16.71$, $df=44$, $P<0.05$) and for day 10 ($F=5.85$, $df=39$, $P<0.05$) (Fig. 2).

Vitellarium contains a series of follicles in successive stages of development. Each follicle contains oocytes. So the rate of fecundity increased with the number of follicles in the vitellarium. There was a lesser vitellarium length was observed among the treatment with leaf extract in day 2 to day 9 old adults compared with that of untreated and solvent controls. So, the reduction in length leads to a reduction in fecundity as a result number of adult emergence reduced in rice treated with leaf extract of *V.negundo*.

There was a dramatic increase in the mean length of ovariole within the first 7 days. After 7 days' mean ovariole length seems to be changed slightly up to the 10^{th} day after emergence. There was a significant difference in the length of ovariole in female adult rice weevil among the rice treated with different concentrations of *V.negundo* leaf extracts and controls in day 6 and day 7 old adult. For day 6 ($F=42.06$, $df=49$, $P<0.05$); For day 7 ($F=42.06$, $df=49$, $P<0.05$) (Fig. 3).

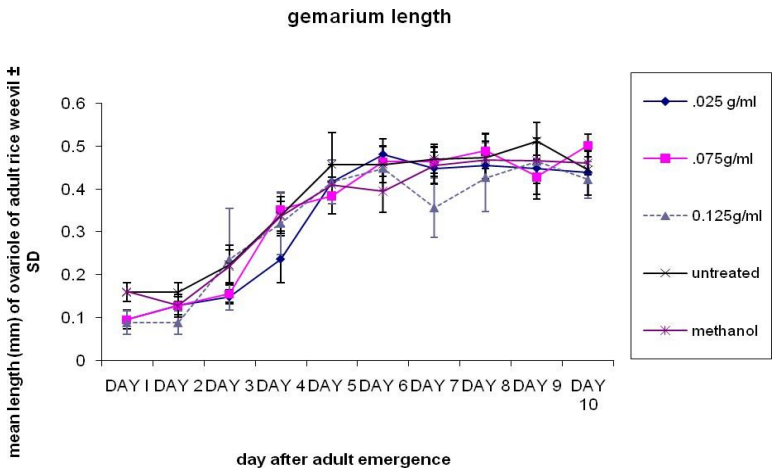


Figure 1: Effect of methnolic *Vitex negundo* leaf extract on development of gemarium in the ovary of *Sitophilus* sp. (Bar indicates standard deviation)

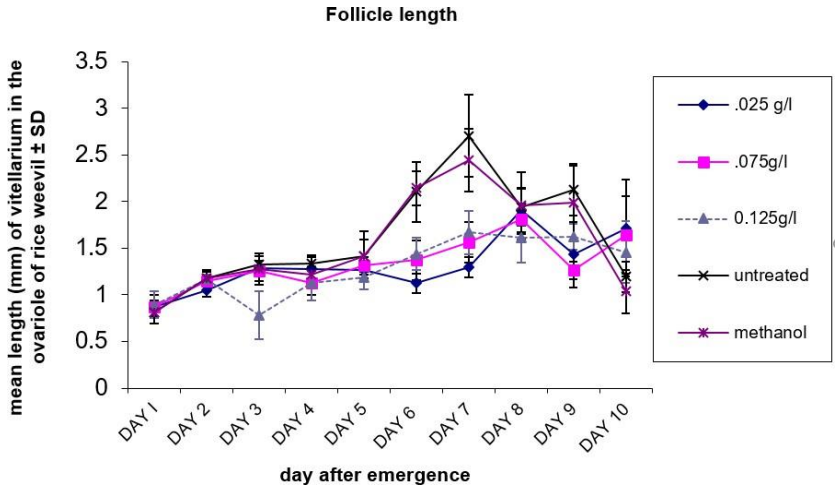


Figure 2: Effect of methnolic *Vitex negundo* leaf extract on the development of follicle in the ovary of *Sitophilus sp.* (Bar indicates standard deviation)

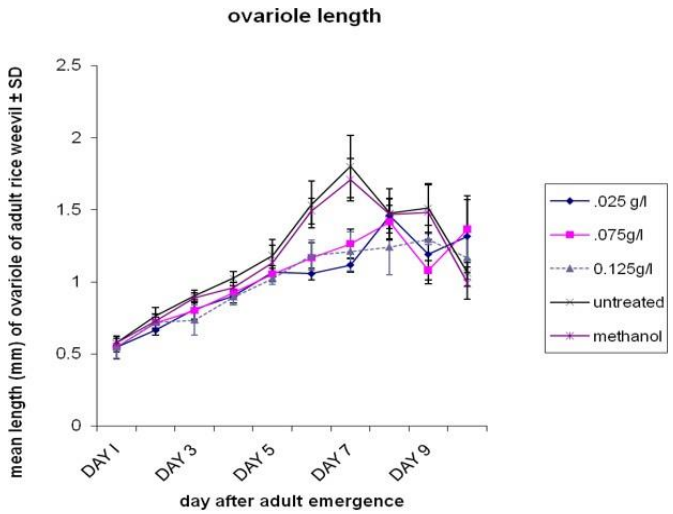


Figure 3: Effect of methnolic *Vitex negundo* leaf extract on development of ovariole in the ovary of *Sitophilus* sp.

Miah *et al.,* (1993) reported that the leaf powder of Nishinda (*V.negundo* L.) proved to be most effective in reducing the number of eggs laid, adult emergence and seed weight loss by *C.chinensis*.and green gram treated with methanol leaf extract of *Vitex negundo* at 5% (w/w) level inhibiting oviposition, reduction in egg viability and caused reduction in adult emergence of *C.chinensis* this study also proves that reduction of length of ovariole, vetellaria, germarium leads to reduction in the number of eggs laid as a result it controls the adult population in storages.

4. CONCLUSION

To minimize the severe damage caused by insect pests, the traditional use of plant products, proved to be highly effective against stored- product insects. The findings of the present investigations indicate that botanical derivatives might be useful as insect control agents for commercial use.

Methanol leaf extracts of *V.negundo* at 0.125g/ml were effective to some extend in reducing the adult emergence by affecting the development of ovariole when compared with that of controls. Application of plant/vegetable oils to grain seeds for storage is an inexpensive and effective technique, and its easy adaptability will give additional advantages leading to the acceptance of this technology by farmers. The use of plant products will have purely to be advised for the safeguarding of the environment and the health of the user. A study to improve the effectiveness of botanical derivatives as insecticides will benefit agricultural sectors of developing countries, as these substances are not only of low cost but also have less environmental impact in term of insecticidal hazard.

Further research should be carried out on the residual effect of the radical on the commodities could then be critically evaluated and toxicity studies on the effects of the plant materials and their extracts on non-target organisms, and beneficial organisms will need to be undertaken.

5. REFERENCES

[1]. Ahmed, S., & Koppel, B. (1987). Use of neem and other botanical materials for pest control by farmers in India: summary of findings, Natural Pesticides from the Neem Tree, pp. 623-626.

[2]. Golob, B., & Webley, D.J. (1980). The Use of Plants and Minerals as Traditional Protectants of Stored Products. *Tropical Products Institute*, London. G-138, Now Post harvest pest and quality section. Natural Resources Institute, Chatham, United Kingdom.

[3]. Hayes, E. J., & Wall, R., (1999). Age-grading adult insects: a review of techniques. *Physiological Entomology* 24, 1–10.

[4]. Khare and Agrawal. (1963). Effect of temperature, relative humidity, food material and density of insect population on the oviposition of *S.oryzae* (Linn.) and *Rhyzopertha dominica* Fab. Bulletin of Grain Technology, vol.1: pp.48-60.

[5]. King, R.C. and Buning,J. (1985). The origin and functioning of insect oocytes and nurse cells. In: Kerkut, G.A., Gilbert, L I. (Eds). Comprehensive Insect Physiology, Biochemistry, and Pharmacology, Vol. 1, pp 37-82

[6]. Miah, M. R. U., Elias, M., Torotder, G. S., Islam, B. N., Sarder-MA, M. A., & Karim, A. M. (1993). Evaluation of local plant material against the pulse beetle (*Callosobruchus chinensis* Linn.) on chick pea. *Bangladesh Journal of Zoology* 21(2), pp.151-153.

[8]. Perez-Mendoza, J., Throne, J. E., & Baker, J. E. (2004). Ovarian physiology and age-grading in the *Sitophilusoryzae* (Coleoptera: Curculionidae). *Journal of Stored Products Research* 40, pp. 179-196

[9]. Sharma, R. N., Nagasampagi, B. A., Bhosle, A.S., Mulkarni, M. M., & Tungikar, V. B. (1984). “Neemrich”: the concept of enriched fractions from neem for behavioral and physiological control of insects. Proceedings of the second International Neem Conference, 115-128.

[10]. Tyndale-Biscoe, M. (1984). Age-grading methods in adult insects: a review. Bulletin of Entomological Research 74, 341–37