Toxicity Of Piper Betle L. (Piperaceae) Against Spodoptera Litura Fab. (Lepidoptera: Noctuidae)

S. Lingathurai[1*], P. Arockia Mary Fernandez[1], R. Niranjani[2]

[1]Department of Zoology and Research Centre, Aditanar College of Arts and Science (Affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu) Tiruchendur– 628 216, Tamil Nadu
[2]PG and Research Department of Zoology, St. Mary's College (Autonomous & Affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu) Thoothukudi, Tamil Nadu

*__Corresponding Author:__ S. Lingathurai
*E-mail: lings02@gmail.com

ABSTRACT
The biological activity of *Piper betle* extracts (Hexane, chloroform, ethyl acetate and methanol) was used for treatment with *Spodoptera litura*. The experiments were carried out with concentrations of 0.625, 1.25, 2.5 and 5 percent in a leaf disc no choice method and compared with control *S. litura*. Ethyl acetate extract was high feeding deterrent and larvicidal activity for third instar larvae of *S. litura* (2.5 and 5 percent concentrations, 63.74 and 72.8 percent respectively). The ethyl acetate extract on third instar larvae of *S. litura* showed LC_{50} value was 2.41%. Food consumption, digestion, relative consumption rate, efficiency of conversion of ingested food, efficiency of conversion of digested food, and relative growth rate values declined significantly but approximate digestibility of treated larvae was significantly higher as a result of treatment. Larval survival, pupal survival, larval period duration, pupal period duration and pupal weight also inhibited. Qualitative analysis of *P. betle* ethyl acetate extract revealed that contains phytochemical such as, steroids and quinines.The high biological activity of these quinines from *P. betle* ethyl acetate extract could be used as an active principle during the groundwork of botanical insecticides for lepidopteran pests. Based on their growth inhibitory and feeding deterrent properties, some of this plant extract have higher for use as alternative crop protectants against a number of pest species.

KEY WORDS: *Spodoptera litura, Piper betle*, quinine, feeding deterrence, toxicity, nutritional variation, development physiology

INTRODUCTION

Insect pests destroy about twenty five percent of the world's annual crop production (Oerke, 1994). Most of the lepidopteran insects cause their damages caused vegetable crops and cereals and pulses. Therefore, in recent years, various researchers have been concentrating their efforts on the search for natural products derived from plants and plant sources as an alternative to conventional chemical insecticides for insect control. Plant based secondary molecules have been the subject of thorough exploration for the past 30 years in an effort to find out new sources of botanical insecticides and antifeedants.

Among the plant families studied, the Meliaceae, Rutaceae, Asteraceae, Labiateae, Piperaceae and Annonaceae are possibly the most promising (Schoonhoven, 1982; Jacobson, 1989; Isman, 1995). Azadirachtin, a triterpenoid compound, limonoid group from neem tree seeds (*Azadirachta indica*, Meliaceae), possesses most potent antifeedant and growth inhibitory effects against various insect pests (Isman, 1997). Screening for biological activity using simple and fast bioassays has now been added to give a better indication of the usefulness of the plants.

Various species of Piper are used in traditional medicine (Pio-Corrêa, 1984), and as food flavouring and pest control agents (Estrela *et al*., 2006). Phytochemical Investigations of different Piper species and plant parts have led to the isolation of numerous active components including alkaloids, amides, pyrones, dihydrochalcones, flavonoids, phenylpropanoids and lignans (Parmar *et al*., 1997).

Piper betle Linn. (Piperaceae) is a perennial dioecious creeper, probably native of Malaysia but cultivated in India for its leaves, used for chewing (CSIR, 1969). The leaf is carminative, aphrodisiac, tonic, laxative and improves appetite (Kirtikar and Basu, 1998). This plant is found widely growing in the tropical humid climate of South East Asia, and its leaves, with a strong pungent and aromatic flavour, are widely consumed as a mouth freshener. Leaves contained caryophyllene, cadinene, γ-lactone, allyl catechol, *p*-cymene and eugenol methyl ether in varying amounts (Sarkar et al., 2000).

The Indian traditional system of medicine has identified the *P. betel* leaves with digestive and pancreatic lipase stimulant activities (Chatterjee and Pakrashi, 1995; Prabhu et al., 1995).The alcoholic extract of the leaf-stalk showed significant antifertility effects in both male and female rats (Adhikary et al., 1989; Adhikary et al., 1998). Autran et al., 2009, leaves of *Piper marginatum* Jacq essential oil showed potential larval toxicity against *Aedes aegypti*.Some

scientists also reported gastrocytoprotective, antimicrobial prperties and healing properties of the leaf extract on experimentally induced gastric lesions (Majumdar et al. 2003; Nalina and Rahim, 2007; Bhattacharya et al.,2007).
The cluster caterpillar, *S. litura* (Fabricious) (Lepidoptera; Noctuidae) important polyphagous pest of cultivated crops primarily in tropical, subtropical regions (Brown and Dewhurt, 1975) and Western and Southeast Asia (Murata and Tojo, 2002). It has a wide range of host, feeding on 112 species worldwide, of which 40 species are known from India (Singh *et al*., 1998 and Paulraj, 2001). Many vegetables and other crops are damaged by *S. litura* crops like to be seriously damaged various taros, cabbage and its relatives and tomatoes (Schreiner, 2000). It is a polyphagous and has about 150 host species (Rao *et al*., 1993). *S. litura* South Indian strains exhibited high resistant levels 61- to – 148 fold to organic pesticides (Karanth *et al*., 2002).
Awareness on the deleterious effects of chemical insecticides has led to emphasis on biological agents for insect pest management that are eco-friendly, safe, economically viable and socially acceptable. There is growing interest in the use of biochemical / botanical pesticides (Rao et al., 1990; Koul et al.., 2004; Teway et al., 2005). Botanicals are active and such pest control tools that have been eliciting interest in recent times. They possess a complex of bioactive compounds that cause different behavioral and physiological responses in insects.
Plants in the Piperaceae are members of traditional pharmacopeia in many Asian and African tradedtional healers and have been used for pest control. *Piper guineese* (Ivbijaro and Bolaji, 1990) for insecticidal and molluskicides, *Piper longum* L, *Piper betle* L and *Piper cubeba* (Miyakado et al., 1989) for insecticidal activity against mosquitoes and flies. There is no report for lepidopteran pests. The search for plant-derived chemicals that have potential use as crop protectants (insecticides, antifeedants, and growth inhibitors) often begins with the screening of plant extracts. Initially, the test insects are fed the extracts and effects on insect behavior and development are monitored. We undertook this study to determine the *S. litura* to establish the phytocompound for pest control management. We discover to evaluate their antifeedant, development, growth regulation and nutritional indices against cluster caterpillar *S. litura.*

MATERIALS AND METHODS

Insects

Spodoptera litura egg mass and larvae were collected from Valajabad agriculture field, Kancheepuram district, Tamil Nadu, India. Collected egg mass and larvae were maintained on castor leaves (*Ricinus communis*) in the laboratory at 26 ± 1^0C: 11 ± 1hr photoperiod and 65 – 70% R.H. Adults were released into oviposition chambers for egg laying. Eggs were collected, kept separately and newly hatched larvae were maintained on castor leaves. Freshly emerged 3rd instar larvae were used for the experiment.

Preparation of hexane, chloroform ethyl acetate and methanol extracts

Fresh leaves of *Piper betle* was collected from Samayanallur, Madurai District, Tamil Nadu, India and were washed twice with tap water and once with distilled water and then shade-dried for two weeks. The dried leaves material ground into powder by an electronic blender and 300g of plant powder was soaked sequentially in 1000ml with increasing polarity of solvents (Hexane, Chloroform, Ethyl acetate and Methanol) for 72h with constant shaking. The soaked powder material was filtered through filter paper. The solvent in the filtrate was evaporated under reduced pressure by vacuum rotary evaporator (Evator, Buchi type, The Science House Instruments, Chennai, India) to yield crude hexane, chloroform and ethyl acetate extract. These concentrated three solvent crude extracts were analyzed for antifeedant bioassay and active crude extract was further tested for growth inhibition bioassay.

Antifeedant activity for *Piper betle* leaf extracts.

Antifeedant activity of crude extracts was studied using leaf disc no choice method (Isman, *et al*, 1990). The stock concentration of crude extracts (5%) was prepared by dissolving in acetone and mixing with dechlorinated water. From the stock, required concentrations were prepared and tested against *S. litura*. Fresh castor leaf discs of 4-cm diameter were punched using cork borer and dipped in 0.625, 1.25, 2.5 and 5. % concentrations of hexane, chloroform and ethyl acetate and methanol extracts individually and air dried for 5 minutes. After air-drying, treated leaf discs were kept in petridishes (1.5 cm X 9 cm) separately and single 2hr pre-starved 3rd instar larva of *S. litura* was introduced on each treated leaf discs. Leaf discs treated with acetone were considered as positive control. Ten replications were maintained for each treatment and control. Progressive consumption of leaf area by the larva in 24 hr period was recorded in control and treatments. Leaf area consumed in plant extract treatment was corrected from the control. The percentage of antifeedant index was calculated using the formula of Bentley et al. (1984):
Antifeedant activity = [(C-T)/C] x 100

Bioassay

The leaf disc method of bioassay was discussed with Binod et al., 2007. In contrast, fresh castor leaf discs were dipped in the different concentrations of plant extracts (0.625, 1.25, 2.5 and 5 %) of three solvent extracts. separately for 1min. Control leaves were treated with water and air-dried. The leaves were allowed to dry at room temperature for 1min and were then placed in 90cm diameter petri dishes. The experiments were carried out with newly moulted 4hr starved third instars (12 larvae per concentration 3 replication). The larvae were allowed to feed treated leaves as well as solvent control leaves. After 24 h, the larvae were transferred to fresh untreated castor leaves and maintained until they developed or died. The larvae were observed for mortality. The percent mortality data after correction (Abott, 1925) were estimated for a period of 4 days continuously. Moribund larvae were also considered as dead larvae.

Preliminary Phytochemical analysis

Ethyl acetate of *P. belte* extracts are examined preliminary phytochemical analysis. This method followed by Mukergy (2002) and Harborne (1983).

Nutritive food utilization of ethyl acetate treated *S. litura* larvae

Growth inhibitory activity and food consumption of effective extracts were studied for four days with the treatment and after treatment. The various food utilization efficiency measures were adopted from the progression of Waldbauer (1968). *S. litura* larval weight gain, food consumption, and faeces were measured every 24h. All weights were measured using a Monopan balance (Mettler Toledo - Switzerland) accurate to 0.2mg. The fresh castor leaves (*Ricinus communis*) sprayed with 0.625, 1.25, 2.5 and 5.0 percent concentrations of ethyl acetate extract of *P. betle*. Control leaves were treated with acetone and air-dried. The newly moulted third instar *S. litura* were used and starved for 4 h. After measuring the initial weight of the larvae, they were individually introduced into detach containers. The larvae (12 larvae per concentration) were allowed to feed period of 24h on castor leaves of weighed quantities of extracts treated and untreated.

The uneaten leaves were weighed and removed after 24h and replaced with fresh untreated leaves. Larvae were again weighed and the difference in weight of the larvae was used as fresh weight gained during the period of study. Sample larvae were weighed fresh to found a percentage of the experimental larvae. The leaves remaining at the end of each day were weighed to establish a percentage conversion value to allow for the assessment of diet weight. The quantity of food ingested was estimated by subtracting the diet (dry weight) residual at the end of each experiment from the total dry weight of the diet provided. Faeces were collected and weighed, and then oven dried, and re-weighed to estimate the dry weight of excreta. The experiment was continued for 4 days and observations were recorded every 24 h. Consumption and post-ingestive food utilization efficiencies (dry weight) were calculated. Relative growth rate (RGR), consumption index (CI), approximate digestibility (AD), efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) were estimated until pupation of treated and control insects. Consumption index (CI) = E/ TA, Relative Growth rate (RGR) = P/ TA, Efficiency of conversion of ingested food (ECI)= P/E X 100, Efficiency of conversion of digested food (ECD) = P/ (E-F) X 100, Where, A is mean weight of animal during period, E is the weight of food eaten, F is the weight of feces produced, p is the weight gain of insect, T is the duration of experimental period.

Effect of ethyl acetate extracts of *P. betle* on *S. litura* larval development

Third instar larvae were used for *S. litura* larval development bioassay. Leaf discs (4cm diameter) were dipped in ethyl acetate extract of *P. betle* at different doses. Four concentrations, (0.625, 1.25, 2.5 and 5%) were dissolved in acetone and applied individual leaf discs were used (3 groups of 10 insects each). Controls were treated with acetone alone. The duration of larval, pupal and adult stages were noted after treatments with different concentrations of the ethyl acetate extracts were evaluated. Every 24h the castor leaf were replaced and for each individual the weight and the stage were recorded until it died.

Statistics

The lethal concentrations (both LC_{50} and LC_{90}) were calculated using probit regression analysis and values were expressed as means ± standard deviation (SD). Data from nutritional indices, antifeedant activity and larval development were subjected to analysis of variance (ANOVA). Probit analysis was done to calculate median lethal concentration (LC_{50}) and LC_{90} using SPSS 11.5 version software package.

RESULTS

Impact of four different solvent extracts (Hexane, chloroform, ethyl acetate and methanol) of leaves of *P. betle* extracts screened feeding deterrence tested against third instar larvae of *S.litura.* Among the different extracts, ethyl acetate extract exhibited promising result antifeedant activity followed by chloroform, hexane and methanol. Five percent concentration of ethyl acetate extracts shows 72.8%, hexane 51.63% chloroform 38.36%, and methanol35.3%. Feeding deterrence of *S. litura* third instar larvae on castor leaves measured percent leaf damage was significantly greater on *P. betle* ethyl acetate extract-treated leaves than on solvent treated control leaves in both experiments 22.85, 33.16, 65.74 and 72.8% feeding deterrent between control and treatment with 0.625 – 5% were noted 24h feeding. The observed higher feeding activity of larvae on control leaves compared to extract-treated leaves increased. The ethyl acetate extract were tested phytochemical analysis followed by Harborne (1983) and Mukergy (2002) contain steroids and quinines (Table 1).

So, we test toxicity, nutritional parameter and biology of *S. litura* assessed ethyl acetate extracts. The LC50 and LC95 values, confidence limit (95%) and regression slope at 96h exposure to *P. betle* ethyl acetate extract shown in Table 1. The LC50 and LC95 for third instar larvae is 2.41 and 18.0 % concentration (table 2). Consumption index (CI), relative growth rate (RGR) and nutritional efficiency measured (ECI and ECD) of treated individuals were reduced in comparison to those control. Relative growth rate did not show significant changes in treated larvae compared to control. But approximate digestibility (AD) increased with increased concentration of treated insects. Both ECI and ECD were significantly reduced at all treated concentrations. Table 3 shows no significant difference in relative growth rate between larvae in control (11.45mg), and larvae treated with 0.625 percent concentration of extract (10.42mg). the

difference was significant between control and treatment with 1.25 % concentration (9.14 mg), 2.5% (7.00mg) and highly significant with 5% extract (6.25mg). Consumption Index also same as at 5percent concentration level showed 1.18 and control insects 5.82. The approximate digestibility (AD) only increased by the increasing concentration of treatment. At 5% level (69.35 percent) and lower concentration 0.625% (52.49 percent) with comparisons of control 51.2 percent. The efficiency of ingested food was also affected significantly at the different concentration (0.625 – 5%) compared with control (28.3 percent). Differences were also found in the efficiency of digested food between control and different *P. betle* ethyl acetate extract concentrations.

All treatments reduced RGR, CI, ECI and ECD of from third instar to pupation. The treatment of *P. betle* into the castor leaves significantly reduced larval growth of armyworm compared to controls (Table 3). There was a concentration-dependent reduction in growth from 0.625 to 5%. Efficiency of conversion of ingested and digested food (ECI and ECD) into biomass of *S. litura* larvae was reduced except the control. The reduction in these parameters was irrespective of any significant change in relative consumption rates and the only significant reduction in consumption relative to controls was observed at the highest treatment dose of 5% (Table 3). Approximate digestibility (AD) of 5% extract treated larvae was significantly higher than the control in the 0.625 and 1.25% treatments during the experimental periods. Ethyl acetate extracts on the total larval duration, pupal duration, pupal weight, adult emergence and malformed adults are given Table 4.

The elongation 5.54 days was observed in larval period (19.75 d) at 5% ethyl acetate extracts of *P. betle*. Lower concentrations of *P. betle* extracts showed concentration dependent increased larval period (14.25, 17.10 and 18.50 days for 0.625, 1.25 and 2.5% concentration respectively), of which 0.625% concentration was insignificant when compared to the control ($p \geq 5\%$). Experimental treated pupal stages also increased, the highest elongation of is pupal period was observed in *P. betle* (11.39 days) followed by (6.85 – 9.42 days) at 0.625 – 2.5% concentration of treatment. Since the treatment showed concentration dependent positive response of pual weight also decreased accordingly. Among the experimental insects minimum pupal weight was recorded 126.67mg at 5% concentration of treatment followed by 146.29, 167.20 and 180.48 in the concentration of 2.5, 1.25 and 0.625%.

Adult duration was observed treated insect life span significantly decreased. Lower concentration (0.625%) showed 6.35 days, higher concentration 5% treatment 2.62 days only. Deformed adult *S. litura* was also noted in their respective concentration 5% level 38.66 abnormal adults were showed. Larval duration of control insects showed 13days. The larval duration increased insect ($p \leq 5\%$ level) except for comparison of control. (Table 4). Pupal life duration did show significant difference in all treated groups as compared with control except at 0.625% concentration of ethyl acetate extract of *P. betle*. Pupal weight also decreased by increasing concentration of treatment. Adult life span sharply decreased at higher concentrations did show pronounced differences as compared with the control insect. Growth regulatory effect such as a deformed adults (deformed wings) occurred only at higher concentrations. The deformed insect exhibited major growth retardation of further development.

DISCUSSION

Plant secondary metabolites synthesized by plants an important role in protecting plants against insect pests. These compounds affect insects by being toxic causing a delay in larval growth and can act as antifeedant Isman (2006). *S. litura* larvae consumed less foods and gained lesser weight after the *P. betle* treatment when compared control. Reduced consumption of leaves in treated is likely to be the consequence of toxicity rather than cause of growth inhibition.

The present study indicates that ethyl acetate extract of *P. betle* is reduced feeding rate of *S. litura*. The rate of feeding varied significantly depending on the concentration of the plant extract. Ethyl acetate extract of this plant caused malformation of pupal and adult stages. Similar intermediates (larval – pupal and pupal - adult) were obtained when treated larvae of *S. litura, S. mauritia, Ephestia kuehniella*.and *M. sexta* (Gujar and Mehrotra, 1983; Jegannadh and Nair, 1992; Schluter et al., 1985; Barby and Klocke, 1990; Kumar et al., 2001).

This information well supported by the data from nutritional experiments where *P. betle* resulted in lower RGR and concomitant reductions in ECI and ECD. Interestingly, the RGR was significantly reduced by ethyl acetate extract of *P. betle* treatment which indicates that feeding depression was caused by behavioral effects (Jeyabalan and murugan, 1995). Reduction of ECI and ECD confirms the deleterious effects of post-ingestive toxicity.

This study clearly revealed that *P. betle* highly reduces the food consumption index, growth rate, efficiency of conversion of ingested food and efficiency of conversion of digested food. Hence *P. betle* leaf ethyl acetate extract can be explored in *S. litura* management. The extended larval and pupal duration and reduced longevity suggest that extract may disturb the endocrine function either to the blockage of haemolymph ecdysteroid peak, or extracts interfere with other biochemical / physiological processes through binding to critical macromolecules is highly probable(Koul and Isman 1991; Mordue et al., 1986).

The excellent antifeedant activity of the ethyl acetate extract of *P. betle* demonstrates their potential use as natural insecticides. Additionally, this extract exhibited larval mortality against of *S. litura*. This antifeedant and growth-inhibiting activity can therefore be incorporated into other insect control techniques in the strategy of integrated pest management (IPM). It would be interesting to investigate whether *Piper betle* contains substances similar to the antifeedant and growth inhibiting compounds present in the fruits of *Azadirachta indica* (Rembold, 1984, Schmutterrer, 1995) and *Melia volkensii* (Mwangi, R.W. and H. Rembold, 1998 and Kabaru,1996)

In conclusion, our results indicate that *P. betle* extract has toxic, as well as growth regulatory; feeding deterrence caused

pupal and adult malformation in *S. litura*. The use of this plant extract may play a more prominent role in integrated pest management programs in the future.

REFERENCES

1. Adhikary, P., Banerji, J., Chowdhury, D., Das, A.K., Deb, C.C., Mukherjee, S.R., Chatterjee, A., 1989. Antifertility effect of *Piper betle* Linn. extract on ovary and testis of albino rats. Ind. J. Exp. Biol. *27*. 868-870.
2. Adhikary, P., Chowdhury, D., Banerji, J., Chatterjee, A., 1998. Antifertility effect of crude alcoholicextract of *Piper betle* stalk. Ind. J. Physiol. Allied Sci. 52, 22-27.
3. Alaux, J., Vassal, J.M., Vaissayre, M., Suivi de la., 1997. Sensibilite aux pyrethroid chez *Helicoverpa armigera* (Hubner) (Lepidoptera ; Noctuidae) en cote d'lvoire, J. Afr. Zool. 111, 1, 63 – 69.
4. Amin, P.W., 1983. Major field and storage insect pests of groundnut in India and their control. Occasional paper 1/83, Groundnut improvement programme, ICRISAT, Patancheru, Hyderabad.
5. Arms, J., Jadhav, D.R., Bond, G.S., King, A.B.S., 1992. Insecticide resistance in *Helicoverpa armigera* in South India. Pesti. Sci. 34, 355 – 364.
6. Autran, E.S., Neves, I.A., da Silva, C.S.B., Santos, G.K.N., da Câmara, C.A.G., Navarro, D.M.A.F., 2009. Chemical composition, oviposition deterrent and larvicidal activities against *Aedes aegypti* of essential oils from *Piper marginatum* Jacq. (Piperaceae) Biores. Technol. 100 2284–2288.
7. Barnby, M.A., Klocke, J.A.,1990. Effects of Azadirachtin on levels of ecdysteroids and prothorocicotropic hormone-like activity in *Heliothis virescens* (Fab.) larvae. J. Insec. Physiol. 36, 125- 131.
8. Baslas, R.K., Baslas, K.K., 1971. Chemistry of Indian essential oils - Part VIII. *Flavour Ind*. *1*, 473-474p, [*Chem. Abstr.*, *74*, 34538z].
9. Benjennet, F.H., Skhiri, Z., Mighri, Simmonds, M.S.J., Blaney, W.M., 2001. Antifeedant activity of plant extracts and of new natural diglyceride compounds isolated from *Ajuga psevdoiva* leaves against *Spodoptera littoralis* larvae. Indust. Crop. Prod. 14, 213 – 222.
10. Bhattacharya, S., Banerjee. D., Bauri, A.K., Chattopadhyay, S., Bandyopadhyay, S.K., 2007. Healing property of the *Piper betel* phenol, allylpyrocatechol against indomethacin-induced stomach ulceration and mechanism of action, *World J. Gastroenterol* 13,27, 3705-3713.
11. Binod, P., Sukumaran, R. K., Shirke, S.V., Rajput, J.C., Pandey A., 2007. Evaluation of fungal culture filtrate containing chitinase as a biocontrol agent against *Helicoverpa armigera.* J. Appl. Microbiol. 103. 1845–1852.
12. Blaney, W.M., Klocke, J.A., 1987. Effects of azadirachtin on the nutrient and development of the tobacco budworm *Helionthis virscens* (Fabr.) (Noctuidae). J. Insect Physiol. 33, 69 – 75.
13. Brindha, P., Sasikala, B., Purushothman, K.K., 1981. Pharmacognostic studies on *Murugan kizhangu.* Bull. Medic. Ethen. Botanic. Res. 3: 1, 84 – 96.
14. Brown, E. S., Dewhurust, C. F., 1975. The genus *Spodoptera litura* (Lepidoptera ; Noctuidae) in Africa and the Northeast. Bull. Entomol. Res. 65, 221 – 262.
15. Chari, M. S., Patel, N. G., 1972. Efficacy of some newer insecticides against the tobacco leaf-eating caterpillar, *Spodoptera litura* (Fabricius), Ind. J. Entomol. 34, 261 - 262.
16. Chatterjee, A., Pakrashi. S.C.,1995. Treatise of Indian Medicinal Plants. New Delhi: CSIR Publication, p 26.
17. CSIR., 1969. *The Wealth of India: Raw Materials*; Publications and Information Directorate. New Delhi, Vol. VIII. 84-94pp.
18. Estrela, J.L.V., Fazolin, M., Catani, V., Alécio, M.R., Lima, M.S., 2006. Toxicidade de óleos essenciais de *Piper aduncum* e *Piper hispidinervum* em *Sitophilus zeamais*. Pesq. Agropec. Bras. 41, 217–222.
19. Gujar, G.T., Mehrotra, K.N., 1983. Juvenilizing effect of azadirachtin on a noctuid moth, *Spodoptera litura* Fabr. Ind. J. Exp. Biol. 21, 292- 293.
20. Harbone, J.B., 1984. Phytochemical methods. Chapman and Hall, London, pp37.
21. Harborne, J. B., 1983. Phytochemical Methods; A guide to modern techniques of plant analysis. 3rd edition. Chapman and Hall. New York.
22. Isman, B., Koul, O., Lucyzynski, A., Kaminski, J., 1990. Insecticidal and antifeedant bioactivities of neem oils and their relationship to Azadirachtin content. J. Agric. food Chem. 38, 1407-1411.
23. Isman, M.B., 1993. Interspecific difference in the susceptibility of noctuid larvae to behavioural and physiological effects of azadirachtin. In Neem and Environment Vol I (eds. Singh, R.P., Chari, M.S., Raheja, A.K., Kraus. W.,) Science Publishers, Lebanon, New Hampshire. 11 – 18 pp.
24. Ivbijaro, M.F., Bolaji, O.O., 1990. Effects of Cypermethrin+dimethoate and extracts of *piper guineese* and *Azadirachta indica* on the pests of *Vigno ungiculata*. J. Agric. Sci.115, 227 – 231.
25. Jagannadh, V., Nair, V., 1992. Azadirachtin –induced effects on larval-pupal transformation of *Spodoptera mauritia.* Physiol. Entomol. 17, 56-61.
26. Jeyabalan, D., Murugan, K., 1997. Effect of Neem limnoids on feeding and reproduction of *Helicoverpa armigera* (Hubner) (Lep, Noctuidae). Entomon, 22, 1, 15 -20.
27. Kabaru, J.M. A toxicological study of *Melia volkensii* fruit extracts on *Locusta migratoria migratoriodes*. Ph.D. Thesis, University of Nairobi. 1996, 144 pp.
28. Kathuria, V., Kaushik, N., 2004. Comparison of different materials as *Helicoverpa armigera* (Hübner) oviposition substrates. Int. J. Tropic. Insec. Sci. 24, 336-339.

29. Kirtikar, K.R., Basu, B.D., 1998**.** *Indian Medicinal Plants, 2nd ed*. Bishen Singh Mahendra PalSingh, Dehradun, India, Vol. III. 2131-2133pp.
30. Koul, O., Isman, M.B., 1991. Effects of azadirachtin on the dietry utilization and development of variegated (*Peridroma saucia*) J. Insect Physiol. 37, 591 – 598.
31. Koul, O., Shankar, J.S., Mehta, N.., Teneja, S.C., Tripathi, A.K., Dhar, K.L., 1997. Bioefficacy of crude extracts of *Aglaia* species (Meliaceae) and some active fractions against lepidopteron larvae: J. Appl. Entomol. 121, 245-248.
32. Koul. O., Multani, J.S., Goomber, S., Daniewski, W.M., Berlozecki. S., 2004. Activity of some non-azadirachtin limnoids from *Azadirachta indica* against lepidopteran larvae. Aust. J. Entomol. 43, 189 – 195.
33. Kranthi, D.R., Jadhav, D.R., Kranthi, S., Wanjari, R.R., Ali, R.R., Russel, D.A., 2002. Insecticide resistance in five major pests of cotton in India, Crop Protect. 216, 449 – 460.
34. Kranthi, K. R., Jadhav, D.R., Wanjari, L.R., Ali, S.S., Russell, D., 2001. Carboamate and organophosphate resistance in cotton pests in India 1995 -1999. Bull. Entomol. Res. 91, 37 – 46.
35. Kranthi, K., Arms, N.J., Rao, N.G.V., Raj, S., Sundaramurthy, V.T., 1997. Seasonal dynamics of metabolic mechanisms mediating pyrethroid resistance in *Helicoverpa armigera* in Central India. Pesti. Sci. 50, 91 – 98.
36. Kumar, M.K., Krishnaiah, N. V., Lingaiah, T., Pasalu, I.C., Krishnaiah,K., 2001. Effect of neem formulations on reproduction and oviposition of rice *hoppers Nilaparvata lugens, Sogatella furcifera* and Nephotettix virescens. Pestic. Res. J. 13, 48.
37. Majumdar, B., Ray Chaudhuri, S.G,, Ray, A., Bandyopadhyay, S.K., 2003. Effect of ethanol extract of *Piper betel* Linn leaf on healing of NSAID-induced experimental ulcer, a novel role of free radical scavenging action. Ind. J. Exp. Biol. 41, 311-315.
38. management. Ph. D. thesis, St. Joseph's College (Autonomous) Bharathidasan University. Trichy, Tamil Nadu, India.
39. Martin, T.F., Chandre, O.G., Ochu, M., Vaissayre., Fournier, D., 2002. Pyrethroid resistance mechanisms in the cotton bollworm *Helicoverpa armigera* (Hubner) (Lepidoptera ; Noctuidae) from west Africa. Pest. Biochem. Physiol. 74. 17 – 26.
40. McCaffery, A.R., King, A.B.S., Walker, A.J Nayir H.E.I., 1998. Resistance to synthetic pyrethroids in the bollworm, *Helionthis armigera* from Andhra Pradesh, India, Pesti. Sci. 27, 65 – 76.
41. Miyakado, M., Nakayama, M.I., Ohno, N., 1989. Insecticidal unsaturated isobutylamides: from natural products to agrochemical leads. In; Arnason, J.T., Philogene, B.J.R., Morand, P. (Eds.), Insecticides of Plant Origin. ACS symposium Series 387. American Chemical Society, New York. 183 -187 pp.
42. Morimoto, M., Tanimoto, K., Sakatani, A., Komai, K., 2002. Antifeedant activity of an anthraquinone aldehyde in *Galium aparine* L against *Spodoptera litura* F. Phytochem. 60, 163 – 166.
43. Mukergy, P., 2002. Quality control herbal drug; an approach to evaluation of Botanicals, 1st edition. Business Horizones, New Delhi.
44. Murata, M., Tojo, S., 2002. Utilization of lipid for flight and reproduction in *Spodoptera litura* (Lepidoptera ; Noctuidae) Eur. J. Entomol. 99, 221 – 224.
45. Mwangi, R.W. and H. Rembold. Growth inhibiting and larvicidal effects of *Melia volkensii* extracts on *Aedes aegypti* larvae. Ent. Exp. Appl. 1988, 46: 103-108.
46. Nair, M.G., Burke, B.A., 1990. Antimicrobial Piper metabolite and related compounds. J. Agric. Food Chem. 38, 1093–1096.
47. Nalina, T., Rahim, Z.H.A., 2007. The crude aqueous extract of *Piper betle* L. and its antibacterial effect towards *Streptococcus mtants.* Am. J. Biotech. Biochem. 3, 1, 10 -15.
48. Oerke, E.C., 1994. Estimated crop losses due to pathogens, animal pests and weeds, In; Oerke E.C Dehne, H. W., Schonbeck, Ewbberm /A. (Eds) Crop production and crop protection - Estimated losses in major food and cash crops, Elsevier, Amsterdam, 72- 78pp.
49. Park, B.S., Lee, S.E, Choi, S.W., Jeong, C.Y., Song, C., Cho, K.Y., 2002. Insecticidal and acaricidal activity of pipernonaline and piperoactadecaalidine derived from dried fruits of *Piper longum* L. Crop Protec. 21, 249 – 251.
50. Parmar, V.S., Jain, S.C., Bisht, K.S., Jain, R., Taneja, P., Jha, A., Tyagi, O.D., Prasad, A.K.,
51. Paulraj, M.G. (2001). Integration of intercrops and plant product on chosen groundnut pests
52. Pio-Corrêa, M., 1984. Dicionário das Plantas Úteis do Brasil e das Exóticas Cultivadas, vol. 3. Imprensa Nacional, Rio de Janeiro.
53. Prabhu, M.S., Platel, K., Saraswathi, G., Srinivasan, K., 1995.Effect of orally administered betel leaf (*Piper betle* Linn.) on digestive enzymes of pancreas and intestinal mucosa and on bile production in rats. Ind. J. Exp. Biol. 33, 752-756.
54. Rao, G., Wightman, V.R., Rangarao, J.R., 1993. World review of the natural enemies and diseases of *Spodoptera litura* (F.) (Lepidoptera ; Noctuidae), Insec. Sci. Appl.14, 274 – 284.
55. Rao, N.V., Reddy., A.S., Reddy, P.S,. 1990. Relative efficiency of some new insecticides on insect pests of cotton. Ind. J. Plant Prot. 18, 53 – 58.
56. Rastogi, R.P., Mehrotra, B.N., 1993. *Compendium of Indian Medicinal Plants, Vol. 3*. Publications and Information Directorate. New Delhi. 502-503pp.
57. Rembold, H. Secondary plant compounds in insect control with special reference to azadirachtins. Advances in Invertebrate reproduction. 1984, 3: 481- 491.

58. Sarkar, M., Gangopadhyay, P., Basak, B., Chakrabarty., K., Banerji, J., Adhikary, P., Chatterjee, A., 2000. The reversible antifertility effect of *Piper betle* Linn. on Swiss albino male mice. Contracep. 62, 271-274.
59. Schluter, U., Bidmon, H.J.,Grewe., 1985. Azadirachtin affects growth and endocrine events in larvae of the tobacco hornworm *Manduca sexta.* J.Insec. Physiol. 31;,773-777.
60. Schmutterer, H., 1995. The Neem Tree. Source of unique Natrual Products for Integrated Pest Management, Medicine, Industry and other purpuses. (ed). VCH verlagsgessellschaft, weinheim.
61. Schmutterrer, H. The neem tree *Azadirachta indica* A. Juss. and other Meliaceous plants. VCH Publishers, Weinheim, Germany. 1995, 696 pp.
62. Schreiner, I., 2000. Cluster caterpillar (*Spodoptera litura* [Fabricious]). Agricultural development in the American Pacific, ADAP 3.
63. Singh, A.K., Parasnath and Ojha, J.K., (1998). Antifeeding response of some plant extract against *Spodoptera litura* (Fab.) of groundnut. Indian Journal of Applied Entomology 12: 9-13.
64. Singh, A.K., Rembold, H., 1992. Maintenance of the cotton bollworm, *Heliothis armigera* Hübner (Lepidoptera: Noctuidae) in laboratory culture-I. Rearing on semi-synthetic diet. Insec. Sci. Appl, 13, 333-338.
65. Srinivas, R., Vdikeri, S. S., Jayalakshmimi, S. K., Sreeramulu, K., 2004. Identification of factors responsible for insecticide resistance in *Helicoverpa armigera* (Hubner) Comp. Biochem. Physiol. 137, 261 – 269.
66. Su, H.C.F., Horvat, R., 1981. Isolation, identification and insecticidal properties of *Piper nigram* amides. J. Agric. Food Chem. 29, 115 – 118.
67. Tauzubil, P.B., 1993. Potential for Neem (*Azadirachta indica* A. Juss) in army worm control in Africa In ; Neem and Environment Vol I (eds. Singh, R.P., Chari, M.S, Raheja, A.K., Kraus. W.,). Science Publishers, Lebanon, New Hampshire. 91 - 101 pp.
68. Tewany, D. K., Bharadwaj, A., Shanker, A., 2005. Pesticidal activities in five Medicinal plants collected from mid hills of Western Himalayas . Indust. Crop Procd. 22, 241 – 247.
69. Vassal, J.M., Vaissayre, M., Martin, T., 1997. Decrease in the suceptiblity of *Helicoverpa armigera* (Hubner) (Lepidoptera ; Noctuidae) to pyrethroid insecticides. Resis. pest. Manag. 9, 2, 14 -15.
70. Waldbauer, G. P., 1964. The consumption, digestion and food utilization of solanaceous and non- solanaceous plants by Biology of *Lacanobia oleracea* Larvae of the tobacco hornworm, *Protoparce sexta* (Johan) (Lepidoptera : Sphingdae). Entomol. Exp. Appl. 7, 253- 269.
71. Wengel, J., Olsen, C.E., Boll, P.M., 1997. Phytochemistry of the genus Piper. Photochem. 46, 597–672.
72. Wheeler, D.A., Isman, M.B., 2000. Effect of *Trichilia americana* extract on feeding behavior of Asian armyworm, *Spodoptera litura*. J. Ecol. 26, 12, 2791 – 2800.

Figure 1. Percent feeding deterrence of hexane, chloroform, ethyl acetate and methanol solvent of *P. betle* leaf extracts treated against 3rd instar larvae of *S. litura*

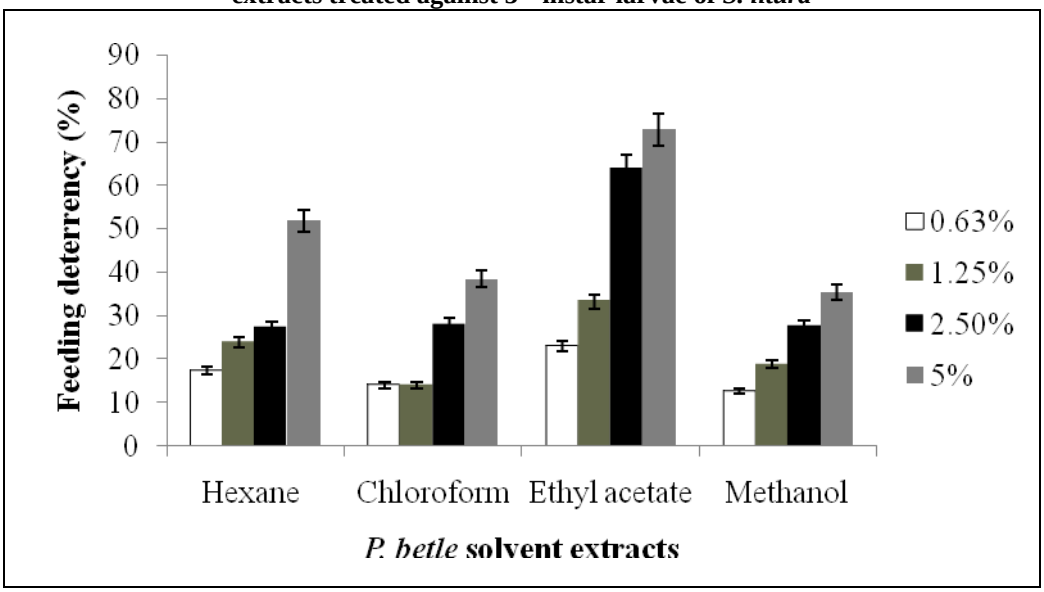

Table1. Preliminary phytochemical analysis *P. betle* extract

Extract	Quantity of extract (gm)	Phytochemicals
Hexane	6.83	Steroids
Chloroform	14.42	Steroids,
Ethyl acetate	8.71	Steroids and quinone
Methanol	12.58	Saponin

Table 2. Toxicity of *P. betle* ethyl acetate leaf extract against third instar larvae of *S. litura*

Insect	LC_{50}[a]	LC_{95}[a]	Slope ± SE	Chi square (X^2)
S. litura	2.41 (1.23 – 8.48)	18.0 (6.24 – 16225.9)	1.88 ± 0.72	0.170

Units LC_{50} and LC_{95} = % / w, applied for 96h. [a]95% lower and upper fiducial limits are shown in parenthesis.

Table 3. Nutritional indices of *P. betle* ethyl acetate extract treated with third instar larvae of *S. litura*

Treatment (%)	RGR (mg)	CI (mg)	AD (%)	ECI (%)	ECD (%)
Control	11.45 ± 2.97[a]	5.82 ± 0.48[a]	51.2 ± 2.30[a]	28.34 ± 1.83[a]	48.4 ± 4.48[a]
0.625	10.42 ± 2.75[a]	5.42 ± 0.99[a]	52.49 ± 3.82[a]	15.54 ± 2.46[b]	32.94 ± 5.63[b]
1.25	9.14 ± 0.89[b]	4.59 ± 0.58[b]	59.04 ± 4.65[ab]	17.08 ± 1.35[a]	27.69 ± 1.24[b]
2.50	7.00 ± 1.11[b]	2.60 ± 0.81[bc]	65.24 ± 3.49[b]	11.21 ± 0.58[b]	20.54 ± 2.04[bc]
5.00	6.25 ± 1.93[bc]	1.18 ± 0.02[c]	69.35 ± 5.56[c]	8.34 ± 1.68[c]	21.04 ± 3.54[c]

(Mean ± SD)Values carrying same alphabets in a column are statistically not significant by LSD at 5% level. RGR, relative growth rate: CI, consumption index: AD, approximate digestibility; ECI, efficiency of conversion of ingested food; ECD, efficiency of conversion of digested food.

Table 4. Biological characteristics of *S. litura* on ethyl acetate extract of *P. betle* treated castor leaves.

Treatments (%)	Larval duration (days)	Pupal Duration (days)	Pupal weight (mg)	Adult duration (days)*	Percent deformed adults
Control	13.21 ± 1.78a	6.50 ± 2.11a	219.24± 3.30a	7.75 ± 0.91a	0a
0.625	14.25 ± 1.45a	6.85 ± 1.62a	180.48 ± 2.67a	6.35 ± 1.47a	2.33 ± 0.24a
1.25	17.10 ± 1.53b	8.43 ± 1.88ab	167.20 ± 2.88bc	6.22 ± 1.04b	8.66 ± 0.52b
2.5	18.50 ± 1.86bc	9.42 ± 2.38b	146.29 ± 6.10c	5.83 ± 1.30bc	16.34 ± 1.86
5	19.75 ± 2.52	11.39 ± 1.43	126.67 ± 4.21	2.62 ± 0.64	38.66 ± 3.76

Values carrying same alphabets in a column are statistically not significant by LSD at 5% level