Partial purification and characterization of phytoecdysone from *Chrystella parasitica* (L.) and screening its pesticidal properties on lepidopteran pests

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ABSTACT

In the present study the fern *Chrystella parasitica* (L.) Khun (Deunstaedtiaceae : Pteridophyceae) endemic to Western Ghats of Tamil Nadu was screened for its phytochemical constituents and evaluated its pesticidal properties against two lepidopteran pests *viz*., *Spodoptera litura* (Fab.) and *Helicoverpa armigera* (Hub.). The phytochemical constituents were extracted with two organic solvents such as chloroform and ethanol, then used for the isolation of phytoecdysteroids following the method of CSIRO, Australia. The extracts/fractions those recorded phytoecdysteroids were used for bioefficacy studies. The results of the preliminary phytochemical analysis revealed that all the fractions except AF contains steroids. Among the other groups of phytochemicals, saponins and santhoprotiens were recorded in CE, EE and AF fractions and tannins and flavonoids in EE and AF. HPLC results of the phytoecdysone characterization revealed that *C. parasitica* contains two major phytoecdysteroids α and β– ecdysteroids. It shows -ecdysone in CE and -ecdysone in EE and CEF fractions. Among the three extracts/ fractions screened for their pesticidal properties, EE recorded highest toxicity against the experimental insects followed by CEF and CE. Comparative studies between the two lepidopterans, *H. armigera* proved to be more susceptible than *S. litura*. The different extracts/fractions exerted different levels of impact on these experimental insects, as evident from the percent mortality, larval, pupal and adult developmental period, percent adult emergence, and developmental abonormalities. The deformities include larval-pupal intermediates, short and stumpy wings, retention of larval/pupal exuvia, head capsule etc.

Keywords: *Chyristella parasitica*, Phytoecdysone, *Helicoverpa armigera*, *Spodoptera litura*, *insecticidal, juvenometry*

INTRODUCTION

Health and environmental hazards imposed by the indiscriminate use of synthetic insecticides, their impact on non-target organisms and also the development of insecticide resistance in target pests, forced the scientists to explore natural resources for viable alternatives. In this juncture bioactive compounds plant origin are considered as ecologically safe alternative and the pant extracts with complex mixtures of bioactive compounds have been widely investigated for their insecticidal, repellent, ovicidal antifeedant and antiovoposition properties (Zue *et al.*, 2001; Isman *et al.*, 2001; Mehmet and Hakki 2003; Elisabeth and Katrin., 2004). *Helicoverpa armigera* (Hub.) and *Spodoptera litura* (Fab.) are the two major polyphagous insect pests in India, reported to attack more 200 different cultivated crop plants, ultimately causing severe crop loss (Manjunath *et al.*, 1989; Setiawati *et al.*, 2000). It has become difficult to control this pest because of widespread development of resistance to conventional insecticides As a part of the screening program for the insecticidal property of indigenous phytochemicals, we have chosen *Chrystella parasitica* an endemic fern species found in Western Ghats for this study, since pteridophytes are known to possess moulting hormone analogues (phytoecydosones) with insecticidal properties. In this study, impact of chloroform, ethanol, aqueous fraction, crude phytoecdysteroid fractions of *C.parasitica* on *S.litura* and *H.armigera* third instar mortality and development were studied.

MATERIALS AND METHODS

C. parasitica was collected from Kothaiyar Hills, Tirunelveli, Tamil Nadu, washed thrice in tap water and were shade dried for two weeks. The dried plants were powdered in a domestic grinder and stored in refrigerator for further use. From this stock, 50 gms of powder was extracted separately with 750 ml of solvent using soxhlet apparatus at 35° ± 5°C and 50° ± 5°C using chloroform and ethanol for about 24 hrs respectively. The extracts were concentrated using rotary evaporator and stored for further use.

Extraction and phytochemical analyses

A common method developed by CSIRO, Australia for ecdysteroid extraction from the dried plant materials was

followed in this study. Chloroform and ethanol extracts and hexane, chloroform and aqueous fractions obtained during the process of phytoecdysteroid separation were used for qualitative analysis of phytochemicals like steroids, alkaloids, reducing sugar, phenolic compounds, saponins, xanthoprotien, tannins and flovonoids. One mg of *Chyristella parasitica* extract (chloroform, ethanol and crude ecdysteroid extracts) were dissolved separately in 1 ml HPLC grade methanol and the required quantity of each sample was injected into the HPLC unit (column type : Lichrospher 100 RP 18e; system-LaChrom) and the chromatograms were recorded.

Rearing of experimental animals

Larvae of *Helicoverpa armigera* and *Spodoptera litura* were collected from the groundnut fields of Tirunelveli and Kanyakumari Districts, Tamil Nadu and they were maintained in the laboratory conditions (29 ± 1°C temperature; 65 - 70% Rh and 11 L and 13 D photoperiod) on groundnut leaves. *S. litura* larvae were reared in plastic trough (21.0, 28.0, 9.0 cm) where as *H. armigera* larvae were reared individually in small plastic vials (50 ml volume) to avoid cannibalism. Laboratory emerged third instar *S. litura* (225 - 250 mg) and *H. armigera* larvae (200 - 225 mg) were used for the experiments.

Preparation and treatment of fern extracts

Chloroform and ethanol extracts and also the ecdysone fractions were used for the preliminary range finding tests to detect the concentra tions of extracts causing 100% mortality. Based on this, different concentrations *viz.* 0.05, 0.10, 0.20, 0.40, 0.60, 0.80, 1.00, 1.20, 1.40, 1.60, 1.80 and 2.00% were prepared from the stock solution by adding required quantities of respective solvents. Ten grams of groundnut leaves (variety TMV-7) were soaked in different concentrations of the fern extracts separately for five minutes. For the control, leaves were soaked in respective solvents. After five minutes, the leaves were air dried for another five minutes and were supplied to the pest larvae. Since later larval stages of these pests cause severe damage to the groundnut, third instar larvae were used for this study. Ten third instar *S. litura* larvae were released on the treated and non-treated (control) leaves taken in the plastic containers (600 ml) and the containers were covered with muslin cloth. Six replications were maintained for each concentration and control respectively. The larvae were allowed to feed the treated leaves for a period of 4 days (96 hrs) and the mortality was recorded for every 24 hrs. From the results LC_{50} was calculated (Finney, 1971). For *H. armigera*, 60 larvae were tested and the experiments were conducted individually in 50 ml vials. Live larvae were maintained on fresh groundnut leaves till pupation. After pupation weight of the pupa was measured using mono pan balance. These pupae were maintained under the above mentioned laboratory conditions till adult emergence. Larval developmental period, pupal period, pupal weight and adult emergence were recorded. Observed data was subjected two way ANOVA and their significances were expressed at 5% level.

RESULT AND DISCUSSION

Even though the use of plant materials to protect the crop and agricultural commodities are being practiced from the time immemorial, intensive search of eco-friendly pesticides as an alternative to the newly emerged chemical pesticides were initiated only after the commencement of environmental issues and health hazards.

Phytochemical analyses of *C. parasitica* extracts

The results of the preliminary phytochemical analyses revealed that all the fractions except AF contains steroids, whereas alkaloid test reported negative. Among the other groups of phytochemicals, saponins and santhoprotiens were recorded in CE, EE and AF fractions and tannins and flavonoids in EE and AF. The reducing sugar was present

Table 1. *C. parasitica* extracts on the LC_{50} parameters of *H. armigera* and *S. litura*

Extracts	Regression equation	LD_{50}	X^2	Variance	LFL	UFL
CE	3.8395x – 0.6456	0.2953	1.8804	0.0087	0.1940	0.4496
EE	3.8541x + 0.5774	0.1404	1.5110	0.0063	0.0980	0.2011
CEF	2.6976x + 1.7797	0.1562	2.1761	0.0094	0.1009	0.2417
CE	3.8395x – 0.6456	0.2953	1.8804	0.0087	0.1940	0.4496
EE	4.1018x – 0.3214	0.1982	1.0299	0.0171	0.1099	0.3577
CEF	2.5417x – 1.3786	0.2659	4.2563	0.0080	0.1774	0.3984

CE - Chloroform Extract; EE - Ethanol Extract and CEF - Crude phytoecdysone fraction; LFL – Lower fiducial limit; UFL – Upper fiducial limit.

Table 2. Efficacy of *C. parasitica* extracts (CE-Chloroform and EE-Ethanol extracts and CEF-Crude phytoecdysone fraction) on the life cycle parameters of of *S. litura*

Extracts/ fraction	Para-meters	Water	Solvents	0.05	0.10	0.20	0.40	0.60	0.80
CE	LP	17.4 ± 0.258	17.45 ± 0.652[a]	17.42 ± 0.63[a]	17.00 ± 0.34[a]	16.60 ± 0.43[a]	16.20 ± 0.33[a]	15.80 ± 0.50[a]	14.80 ± 0.63[a]
	PP	9.2 ± 0.33	9.43 ± 0.74[a]	8.40 ± 0.42[a]	7.57 ± 0.39[a]	6.60 ± 0.51[a]	5.80 ± 0.63[a]	-	-
	AL	9.0 ± 0.51	8.17 ± 0.27	5.8 ± 0.26	5.6 ± 0.27	2.8 ± 0.31	2.4 ± 0.25	-	-
	P	95.0 ± 5.0	91.67 ± 6.87	86.67 ± 4.7	80.0 ± 8.16	56.67 ± 4.71	35.0 ± 5.0	23.3 ± 4.71	18.3 ±3.73
	P M	-	12.22 ± 3.52[a]	38.4 ± 4.58[a]	47.34 ± 6.92[a]	72.25 ± 10.33[a]	84.72 ± 15.53[a]	100.0 ± 0.0[a]	100.0 ± 0.0[a]
	AE	100	87.78 ± 3.52[a]	61.6 ± 4.58[a]	52.66 ± 6.92[a]	27.75 ± 10.33[a]	15.28 ± 15.53[a]	-	-
	PW	312.5 ± 2.9	280.8 ± 5.2[a]	270 ± 4.38[a]	261.3 ± 19.3[a]	235 ± 11.1[a]	228.0 ± 7.16[a]	210.7 ± 19.7[a]	204.0 ± 12.2[a]
EE	LP	17.4 ± 0.55	17.0 ± 0.55[a]	16.6 ± 0.45[a]	16.2 ± 0.45[a]	15.4 ± 0.55[a]	14.5 ± 0.45[a]	-	-
	PP	9.2 ± 0.45	9.0 ± 1.0[a]	7.0 ± 0.83[a]	6.0 ± 0.83[a]	6.4 ± 1.0[a]	6.0 ± 0.5[a]	-	-
	AL	9.0 ± 1.0	8.4 ± 1.80	3.4 ± 0.55	2.2 ± 0.45	-	-	-	-
	P	95.0 ± 5.0	93.3 ± 4.71	56.67 ± 4.71	50.0 ± 8.16	40.0 ± 8.16	16.67 ± 4.71	-	-
	P M	-	8.52 ± 3.83[a]	39.47 ± 5.56[a]	50.0 ± 5.77[a]	61.91 ± 9.74[a]	100.0 ± 0.0[a]	-	-
	AE	100.0	91.48 ± 3.82[a]	60.53 ± 5.56[a]	50.0 ± 5.77[a]	38.09 ± 9.74[a]	-	-	-
	PW	312.4 ± 5.12	287.3 ± 6.2[a]	258.5 ± 4.5[a]	230.0 ± 7.5[a]	206.0 ± 6.0[a]	194.5 ± 12.0[a]	-	-
CEF	LP	17.4 ± 0.55	17.0 ± 0.55[a]	16.8 ± 0.45[a]	16.5 ± 0.70[a]	16.0 ± 0.55[a]	14.8 ± 0.45[a]	-	-
	PP	9.2 ± 0.45	9.0 ± 1.0[a]	8.0 ± 1.0[ac]	6.6 ± 0.55[a]	6.2 ± 0.83[a]	5.5 ± 0.5[a]	-	-
	AL	9.0 ± 1.0	8.4 ± 1.80	5.4 ± 0.55	3.8 ± 0.55	1.5 ± 0.5	-	-	-
	P	95.0 ± 5.0	93.3 ± 4.71	68.3 ± 3.73	56.67 ± 4.71	50.0 ± 5.77	38.3 ± 3.73	16.67 ± 4.71	-
	P M	-	8.52 ± 3.83[a]	36.89 ± 8.6 7[a]	50.0 ± 5.77[a]	65.85 ± 5.07[a]	100.0 ± 0.0[a]	-	-
	AE	100.0	91.48 ± 3.82[a]	63.11 ± 8.67[a]	50.0 ± 5.77[a]	34.15 ± 5.07[a]	-	-	-
	PW	312.4 ± 5.12	287.3 ± 6.2[a]	263.33 ± 20.0[a]	245.3 ± 8.33[a]	225.67 ± 28.9[a]	205.67 ± 12.66[a]	-	-

LP – Larval period; PP – Pupal period; AL – Adult longevity; P – Pupation; PM – Pupal mortality; AE – Adult emergence; PW – Pupal weight., values carrying same alphabets are statistically in significant at 5% level. (a – insignificant between extracts - two way anova; b – insignificant between concentration - two way anova).

in CE extract. Carbohydrates were present in the CE, EE and AF extracts. Except CE and CEF, all other extracts contain phenolic compounds. HF and CEF of *C. parasitica* reported positive results in saponin test. Tannins and flavonoids were recorded in EE and AF.

The two solvents used for the extraction showed differences in solubility of various compounds. Since the polarity of ethanol is higher than chloroform, most of the compounds dissolved in ethanol. The hexane fraction obtained during the process of phytoecdysone extraction recorded only steroids and phenolic compounds. However the aqueous extract recorded all the compounds present in ethanol extract except steroids. Steroids alone were recorded in ecdysone fraction. These results suggested that during the process of phytoecdysone separation from the ethanolic extract, waxy impurities were removed by hexane partitioning. Therefore, hexane fraction recorded steroids and phenolic compounds. During the water partitioning most of the water soluble compounds were separated and found in aqueous phase.

Bioactive constituents such as tannins, steroids, flavonoids and phenolic compounds were widely distributed throughout the plant kingdom at least in 400 species belonging to more than 80 families (Swain, 1978). They were known to inhibited various metabolic enzymes in living organisms. For instance, soyabean saponins inhibit mammalian protease such as trypsin, chymotrypsin and papaine as well as the midgut digestive proteases of *Tribolium castaneum, Tenebrio molitor* and *Dermestes* sp. (Ishaaya *et al.*, 1969). Swain (1978) reported that the saponin derivatives inhibit the larval growth and development and the tannin derivatives combines with protein and thus it inhibited the enzyme activities and reduce the availability of protein in haemoloymph.

Identification of phtoecdysteroids using HPLC

In the present study, HPLC analysis of the *C. parasitica* for the experiment were recorded to have similar compounds which were comparable to the and ecdysones recorded by Koreeda and Teicher (1977). In addition to the ecdysone (RT = 2.43) EE of *C. parasitica* contain ecdysone (RT = 1.77). As observed in the EE extracts, CEF also contains both and ecdysones.

LC_{50} on *Helicoverpa armigera* and *Spodoptera litura*

Insecticidal property of chloroform extracts (CE), ethanol extracts (EE) and crude phytoecdysone fraction (CEF) were evaluated on *H. armigera* and *S.litura* presented in the table 1. The results clearly indicated that among the three extracts tested EE of *C. parasitica* was found to be the most effective, followed by CEF. The LC_{50} concentration for *H. armigera* was 0.295, 0.156 and 0.140 percentage for

Table 3. Efficacy of *C. parasitica* extracts (CE-Chloroform and EE-Ethanol extracts and CEF-Crude phytoecdysone fraction) on the life cycle parameters of of *H. armigera*

Extracts/ fraction	Para meters	Water	Solvents	0.05	0.10	0.20	0.40	0.60	0.80
CE	LP	18.2 ± 0.43	18.0 ± 0.65[a]	17.8 ± 0.55[a]					
	PP	8.17 ± 0.27	8.2 ± 0.40[a]	8.0 ± 0.311[a]	7.2 ± 0.265[a]	6.8 ± 0.48[a]	6.2 ± 0.51[a]	6.0 ± 0.39[a]	-
	AL	7.0 ± 0.39	6.8 ± 0.43	4.2 ± 0.43	3.4 ± 0.43	3.2 ± 0.31	2.6 ± 0.45	-	-
	P	100.0 ± 0.0	93.3 ± 4.71[a]	88.3 ± 3.73[a]	78.3 ± 3.7[a]	56.67 ± 4.7[a]	45.0 ± 5.0[a]	18.3 ± 3.73[a]	-
	PM	-	9.1 ± 4.07[a]	36.08 ± 6.2[a]	47.24 ± 6.88[a]	73.37 ± 9.25[a]	85.6 ± 6.56[a]	100.0 ± 0.0[a]	-
	AE	100.0 ± 0.0	90.9 ± 4.07	63.92 ± 6.2	52.76 ± 6.88	26.63 ± 9.25	14.4 ± 6.56	-	-
	PW	229.67 ± 21.14	223.0 ± 9.27[a]	217.0 ± 18.06[a]	202.5 ± 22.5[a]	185.0 ± 16.3[a]	163.3 ± 17.08[a]	151.5 ± 8.5[a]	-
EE	LP	18.2 ± 0.43	18.2 ± 0.60a	17.6 ± 0.43a	16.8 ± 0.53a	16.2 ± 0.50a	16.0 ± 0.71a	-	-
	PP	8.17 ± 0.27	8.4 ± 0.43a	7.6 ± 0.416a	6.8 ± 0.62a	6.17 ± 0.52a	-	-	-
	AL	7.0 ± 0.39	6.8 ± 0.62a	4.2 ± 0.43a	1.4 ± 0.26a	-	-	-	-
	P	100.0 ± 0.0	96.67 ± 4.71a	83.3 ± 4.71a	68.3 ± 3.7a	36.67 ± 4.7a	16.67 ± 4.71a	-	-
	PM	-	7.4 ± 5.2a	46.28 ± 6.16a	65.88 ± 6.4 a	84.52 ± 13.68a	100.0 ± 0.0a	-	-
	AE	100.0	92.6 ± 5.2	53.72 ± 6.16	34.12 ± 6.4	15.48 ± 13.68	-	-	-
	PW	229.67 ± 21.14	226.67 ± 28.23[a]	214.0 ± 10.2[a]	169.0 ± 14.99[a]	154.0 ± 17.35[a]	146.0 ± 12.0[a]	-	-
CEF	LP	18.2 ± 0.43	18.2 ± 0.60a	17.2 ± 0.45a	17.0 ± 0.70a	16.4 ± 0.55a	16.0 ± 1.2a	-	-
	PP	8.17 ± 0.27	8.4 ± 0.43a	8.25 ± 1.6a	7.6 ± 0.57a	6.3 ± 0.57a	5.4 ± 0.83a	-	-
	AL	7.0 ± 0.39	6.8 ± 0.62a	5.0 ± 1.0a	3.2 ± 0.45a	-	-	-	-
	P	100.0 ± 0.0	96.67 ± 4.71a	76.67 ± 4.71a	66.67 ± 4.71a	50.0 ± 5.77a	15.01 ± 5.0a	-	-
	PM	-	7.4 ± 5.2a	49.07 ± 2.1a	60.33 ± 4.51a	78.3 ± 3.7a	100.0 ± 0.0a	-	-
	AE	100.0	92.6 ± 5.2	50.83 ± 2.1	39.63 ± 4.51	21.7 ± 3.7	-	-	-
	PW	229.67 ± 21.14	226.67 ± 28.23a	180.0 ± 15.0a	163.3 ± 13.08a	150.0 ± 5.0a	136.0 ± 12.0a	-	

LP – Larval period; PP – Pupal period; AL – Adult longevity; P – Pupation; PM – Pupal mortality; AE – Adult emergence; PW – Pupal weight., values carrying same alphabets are statistically in significant at 5% level. (a – insignificant between extracts - two way anova; b – insignificant between concentration - two way anova).

CE, CEF and EE respectively. Lower and upper fiducial limit (LFL and UFL) of the LC_{50} values also followed the same trend. The order of toxicity was EE > CEF >CE. Efficacy of fern extracts on *S. litura* showed 50% mortality (LC_{50}) at 0.198% in EE followed by 0.266% in CEF and 0.295 in CE of *C. parasitica*. UFL and LFL of the LC_{50} concentrations also followed the same trend as observed in *H. armigera*. Of the two experimental animals tested, *H. armigera* showed more susceptibility to the fern extracts than *S. litura*.

Developmental period

In general, larval periods, pupal periods, adult longivities, pupation rate, adult emergence and pupal weight gradually decreased when the concentrations of the *C.parasitica* decreased in *S.litura* (Table 2) and *H.armigara* (Table 3). The different extracts of the same plant and same extracts of different plants showed differences in their toxic as well as growth disrupting responses. This could be due to the difference in concentration as well as the diversity of the secondary metabolites present in the extracts. Generally most of the reports suggested that synergistic effect was better than the effect of a single compound alone. Moreover, structural diversity among the phytoecdysones interfere with the detoxification process and the efficiency could be reduced (Kosovski *et al.*, 1989). They also reported that the amount of ecdysone in 500 Kg of silkworm is equal to the amount of phytoecdysone present in 25 gms of air-dried leaves or root of yew, an even richer source of phytoecdysone in the rhizome of common fern *Polipodium vulgare*. This report suggested that even though the animals have some detoxification process, the higher amounts and more structural diversity in phytoecdysone could compete with the detoxification process and it intern might have disrupted the normal development. Tanins, saponins, flavonoids etc accompanying the phytoecdysteroids synergistically acts on these pests Rajkumar *et al.*, 2000). Ingestion of phytoecdysteroids caused marked growth and developmental disruption (Arnault and Slama, 1986). Kubo and Klocke (1983) reported that *Pectinophora gossypiella* and *Bombyx mori* were highly susceptible to the ingested phytoecdysteroids.

Among the two experimental animals fed with fern phytochemical treated leaves *H. armigera* showed more sensitivity. Similarly Selvaraj *et al.* (2005) recorded the impact of *Pteridium aquilinum* (L) Kuhn on these to past. They also had maximum abnormalities in both larvae and adults. The highest rate of developmental disruptions in *H. armigera* might be due to the non/low level of ecdysteroid detoxifying enzyme activities in this insect (Rajkumar *et al.*, 2000). Rajkumar *et al.* (2000) reported

that ingestion of micrograms of ecdysteroidal fraction of *C. farinosa* into *H. armigera* and *S. litura* caused sterility *S.litura* deactivated the fern phytoecdysones when it was administered through food. The deactivation process takes place with the help of detoxifying enzymes like SGOT and SGPT. Hence it is more resistant than *H. armigera*. However the structural variation present in the phytoecdysone could protect these compounds from rapid detoxification. Therefore animals fed with higher concentration of fern phyto chemicals (above 0.8%) died at early periods of the treatment and those fed with lesser concentration (below 0.6%) started pupation and they failed to complete the process and died as larval-pupal intermediate. Those fed with least concentration (below 0.2%) transformed into normal pupae, of which some were failed to emerge into adult (expelled haemolymph and died) and the emerged ones displayed structural abnormalities (deformed wings, remnants of moult skin, pupal cover and head capsule). Further more, morphologically normal adults failed to mate, short lived and none of them laid eggs.

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REFERENCE

Arnault, C., Slama, K., 1986. Dietary effects of phytoecdysone in the leek moth, Acrolepsis assectella Zell. (Lepidoptera:Acrolepiidae). *Journal of Chemical Ecology,* **12**:1979 – 1986.

Elisabeth, H,K. and Katrin, A. S., 2004. Effect of plants volatile son the feeding and oviposition of *Thrips tabaci*. In: *Poceedings of the seventh Internationla symposium on Thysanoptera*, 185-187 **PP.**

Ishaaya, I., Birk, Y., Bondi, A., Tencer, Y.1969 Soybean saponins IX. Studies of their effect on birds, mammals and coldblooded organisms. *Journal of Science and Food Agriculture*, **20:**433 – 436.

Isman, M.B., Wan, A.J. and Passreiter, C.M. 2001 Insecticida activity of essential oils to tobacco cutworm, *Spodoptera litura. Fitoterapia*, **72**: 65-68.

Koreeda M, Teicher M. 1977. Chemical analysis of insect moulting hormone. In: Turner RB, editor. Analytical biochemistry of insects. New York: Amsterdam-Oxford, Elsevier Scientific publishing Company; 207 – 240 **PP**.

Kosovski, MI., Syrov, VN., Mirakhmedov, MM., Katkova SP., Khushbaktova, ZA. 1989 Flavonoids from Leuzea carthamoides. *Problems in Endrokrinology* (Mosk) 35(5):77,81.

Kubo, I., Klocke, JA. 1983. Isolation of phytoecdysones as insect ecdysis inhibitors and feeding deterrents. In: Hedin P, editor. ACS symposium series 208. Plant Resistance to Insects. Washington, DC: *American Chemical Society.* 208 – 229 **PP**.

Manjunath, T.M.M. Bhatnagar, V.S., Pawar, C.S. and Sithanantham, S. 1989. Proceedings of the Biological control of Helipothis Increasing the effectiveness of Natrual Enemies (King, E.C.and Jackosn, R.D. eds), usda, New Delhi, 197-228 **PP.**

Mehmet, K. and Hakki, M.A. 2003. Insecaticaidal effect of essentialoils from various plant against larvae of pine processionary moth (*Thaumetopoea potyocampa* Schiff) (Lepidoptera: Thaumerropoeidae). *Pest Management Science*, **60:** 173-177.

Rajkumar, JA., Subramaniyam, B., Devakumar, C., 2000 Growth regulatory activity of silver fern extract on the cotton bollworm, *Helicoverpa armigera* (Hubner), *Insect. Science Application,* **20**(4): 295 – 302.

Selvaraj. P., John De Britto.A and Sahayaraj.K.2005. Phytoecdysone of *Pteridium aquilinum* (L.) Kuhn (Dennstaedtiaceae) and its pesticidal property on two major pests. *Archives of Phytopathology and Plant Protection.* **38**(2): 99 – 105.

Setiawati, W., Somantri, A. and Duriat, A.S. 2000. Effect of population density and infestation of *Helicoverpa armigera* Hubn. on tomato yield loss and its control. *Journal of Horticulture*, **10:** 112-120.

Swain, T. and Hillis, W. E. 1959. The phenolic constituents of *Prunus domestica* I.The qualitative analysis of phenolic constituents. *Journal of Science, Food and Agriculture*, **10**: 63 - 68.

Zhu, B.C., Henderson, G., Chen, F., Fei, H. and Laine, R. A. 2001. Evaluation of vetiver oil and seven insect active essential oils against the Formosan subterranean termite. *Journal of Chemical Ecology*, **27:** 1617-1625.

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