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Effect of PONNEEM[#] on *Spodoptera litura* (Fab.) and Its Compatibility with *Trichogramma chilonis* Ishii

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ABSTRACT

Antifeedant and growth regulating activities of PONNEEM, an oil formulation containing neem and pungam (karanj) oils were evaluated along with individual neem and karanj oils and Nimbicidine, a commercial neem-based pesticide against fourth instar larvae of Spodoptera litura *(Fab.). Among all the treatments, PONNEEM recorded the maximum antifeedant activity (88.6%) at 0.6%. Neem and karanj individual treatments as well as PONNEEM extended larval duration compared to control. Pupal weight and fecundity were significantly reduced and pupal period was greatly increased by PONNEEM treatment compared to other treatments. The PONNEEM was found to be compatible with* Trichogramma chilonis *Ishii, an egg parasitoid of many lepidopteran pests, at 0.15, 0.3 and 0.5% concentrations. PONNEEM did not affect the parasitoid emergence significantly at 0.3% concentration compared to control. PONNEEM also showed growth disruption activity against fourth instar larvae.*

Key words: Oil formulation, *Spodoptera litura,* antifeedant activity, growth regulating activity, *Trichogramma chilonis*

INTRODUCTION

Spodoptera litura (Lepidoptera: Noctuidae) is a major polyphagous pest of various economically important crops such as cotton, groundnut, chilly, tobacco, castor, pulses, etc. (Ayyangar and Rao 1989) in India, China and Japan (Kamaraj et al. 2008). Crop loss due to insect pests is estimated between 10 and 30% for major crops (Ferry et al. 2004). Due to the development of insect resistance to many chemical pesticides (Armes et al. 1992; Brewer and Trumble 1994; Kannaiyan 2000), alternative strategies to control the insects are gaining ground. Plants are important natural sources of bioactive compounds and many such plant compounds have been included in commercial botanical pesticides (Ballesta et al. 2008). Many plant products are safer to non-target organisms and effective against the diseases, nematodes and phytophagous insects (Kareem 1999). Plant products bring about wide range of behavioral and physiological effects on the insects (Koshiya and Ghelani 1993; Gökçe et al. 2010). Nearly 2000 plant species are known to possess antifeedant activity (Russell and Lane 1993). Neem tree, *Azadirachta indica* A. Juss, belonging to family Meliaceae, is an important source of 99 biologically active compounds, including azadirachtin, nimbin, nimbidin and nimbolides and most of these products have antifeedant, ovicidal, larvicidal, oviposition deterrent, growth regulating and repellent activities against the insects (Schmutterer 1990; Sharma and Dhiman 1993; Su and Mulla 1998; Schmutterer 2002; Isman 2006; Locantoni et al. 2006; Dua et al. 2009). Antifeedant compounds can be of great value in

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protecting the crops from insect attack. Neem formulations have significant reproductive inhibition on rice hoppers (Mahesh Kumar et al. 2001).

The compounds present in the neem oil are reported as strong antifeedants and growth inhibitors against lepidopteran larvae (Koul et al. 2004). Deota and Upadhyay (2005) reported that azadirachtin, the active ingredient of *A. indica* against *S. litura* showed toxicity and antifeedancy. Sighamony et al. (1984) reported that karanj oil (pungam oil), the oil obtained from the seeds of *Pongamia pinnata*, exhibited repellent property at 2.5 mg/cm^3 against insect pests and retained the efficacy up to eight weeks. Antifeedant activity of karanj oil against *S. litura* was reported by Rajasekaran and Kumaraswami (1985), Srimannarayana and Rao (1985) and Koshiya and Ghelani (1993). So far there is no report on the additive or synergistic effect of neem and karanj oils on feeding, survival, growth and development of *S. litura*. Hence, the present work was undertaken to evaluate the effects of PONNEEM, a newly developed oil formulation consisting of pungam oil and neem oil in 1:1 ratio on feeding, biological and growth regulating activities against *S. litura* and biosafety against the natural enemy *Trichogramma chilonis*.

MATERIALS AND METHODS

Insect Rearing

The egg masses of *S. litura* were collected from groundnut plants at Vellavedu village near Poonamallee, Chennai, India. The eggs were surface sterilized with 0.02% sodium hypochlorite solution and allowed to hatch in the insectary. After hatching the neonate larvae were reared on castor (*Ricinus communis*) leaves at 27 ± 2^0 C and 60 ± 5% relative humidity under light in the insectary and allowed to multiply. The newly emerged fourth instar larvae were used for the present investigation.

Oils of karanj and neem used at different ratios

Five different oil formulations were prepared by using neem and pungam oils at different ratios, emulsifier, stabilizer and isopropyl alcohol. Neem and pungam oils were purchased from the oil dealers from Madurai. The oils were checked by High Performance Liquid Chromatography (HPLC) to determine the azadirachtin and karanjin contents, respectively. Neem oil had azadirachtin around 1000 ppm and pongam oil showed karanjin content around 500 ppm. Oils were taken at specified ratios as mentioned below in a stainless steel vessel with an electric stirrer and were stirred at 120 rpm for 10 minutes. Then 8% emulsifier + 1% stabilizer were added to the oils and again they were stirred at 120 rpm for 10 minutes. At last 2% isopropyl alcohol was added and again they were mixed thoroughly by stirring at 120 rpm for 10 minutes. A commercial neem-based pesticide, Nimbicidine, emulsifier control and water control were included in the screening experiments. The details of treatments are as follows:

Treatment 1: 85% neem oil + 15% emulsifier.
Treatment 2: 85% Karanj oil + 15% emulsifier.
Treatment 3: 21.25% karanj oil + 63.75% neem oil + 15% emulsifier.
Treatment 4: 63.75% karanj oil + 21.25% neem oil + 15% emulsifier.
Treatment 5: 42.5% karanj oil + 42.5% Neem oil + 15 % emulsifier.
Treatment 6: Nimbicidine (commercial neem-based pesticide) (Reference control).
Treatment 7: Emulsifier control (15%).
Treatment 8: Water control.

Antifeedant activity

The antifeedant activities of all the treatments were evaluated by the choice and no-choice methods at four different concentrations, *viz*., 0.15, 0.3, 0.45 and 0.6%. A stock concentration of 500 ppm of each treatment was prepared by mixing with dechlorinated water. From the stock, required concentrations were prepared and tested against the fourth instar larvae of *S. litura* using castor leaf discs. Fresh castor leaf discs of 3 cm in diameter were punched using cork borer and the leaf discs were dipped in different concentrations of different treatments separately for 5 minutes. Treated leaf discs were shade dried for 5 min and individually placed inside separate Petri dishes for no-choice method. Nimbicidine, water and emulsifier (15% with water) controls were also included in the experiment. A single pre-starved (for 4 h) fourth instar *S. litura* larva was released on the leaf disc. In choice method control and treated leaf discs were provided in each Petri dish and a single larva was introduced inside the Petri dish. For each control and treatment, 20 replications were maintained at a time. Progressive consumption of leaf area by the larva after 24 h was recorded using leaf area meter (Delta-T

Devices, Serial No. 15736 F96, UK). After the treatment period, the larva was reared continuously on fresh non-treated castor leaves to assess the impact of oil formulations during the development. The percentage of antifeedant index was calculated using the formula of Ben Jannet et al. (2001).

Antifeedant index = Area protected in control leaf - Area protected in treated leaf / Area protected in control leaf + Area protected in treated leaf × 100

Effect on Development

The effect on development associated with five of the treatments (T3, T4, T5, T6 and T7) was determined at 0.3% concentration against fourth instar larvae of *S. litura.* Ten larvae were introduced in a Petri dish having treated castor leaves. Emulsifier with water treated leaves served as solvent control and the leaves treated with Nimbicidine® were used for comparison. After 24 h feeding, the larvae were transferred to non-treated castor leaves for studying their development. There were five replications per treatment. Deformed larvae, pupae and adults, and larval, pupal and adult mortality were recorded. In addition, time taken for pupation, pupal duration, pupal weight, adult longevity, fecundity and egg hatchability were also recorded.

Effects on fecundity and egg hatchability

The adult moths emerged from the treated and control categories were released inside the oviposition cages (30x30x30 cm) and castor leaves were kept inside the cages. The egg masses laid by the moths were separated daily until all the moths inside the cage died. The scales that covered the egg masses were removed carefully and the total numbers of eggs found in all the egg masses were counted. Finally, the total number of eggs laid by one female moth was calculated.

All the egg masses were incubated at laboratory conditions (25 ± 1^0 C; 65 ± 5% RH; 11±0.5 h photoperiod) and number of larvae emerged from the eggs were counted. Percent hatchability of eggs was calculated.

Compatibility of PONNEEM and Nimicidine with *Trichogramma chilonis*

The toxicity of PONNEEM and the commercial neem based insecticide Nimbicidine® was evaluated against the egg parasitoid *T. chilonis* using *Tricho* cards obtained from Sun Agro biotech Pvt. Ltd., Chennai. Tricho cards were obtained one day after parasitization. Different treatments used in this study were: PONNEEM (0.15%); PONNEEM (0.3%); PONNEEM (0.6%); Nimbicidine (0.15%); Nimbicidine (0.3%); Nimbicidine (0.6%); emulsifier (DMA-NE) (0.6%); water control and untreated check.

Different concentrations of all the above mentioned treatments were sprayed separately on one day old parasitized *Tricho* cards using a hand spray and the cards were placed in the polythene covers (25 x 15 cm). The number of adult *T. chilonis* emerged was recorded up to four days. Three replications were maintained for the control and each concentration of treatment.

Statistical Analysis

Mean and standard deviations were calculated from the replication data. Data were analyzed in a single factor analysis of variance (ANOVA) and effective means were separated by Least Significant Difference (LSD) using SPSS (11.5 for windows) software.

RESULTS

Antifeedant activity

Results of the choice and no-choice tests are presented in Table 1. The results clearly indicated that PONNEEM (neem + Karanj oil in 1:1 ratio) was the most effective treatment at all the concentrations tested. PONNEEM recorded maximum antifeedant activity in both the choice and no-choice experiments and the activity was significantly high ($p \leq 0.05$), compared to other treatments. The antifeedant activity of PONNEEM by the choice experiments was 83.7, 88.3, 88.7 and 96.1% at 0.15, 0.3, 0.45 and 0.6% concentrations, respectively. In the no-choice experiments, PONNEEM showed 67.8, 73.2, 75.9 and 88.6% antifeedant activity at 0.15, 0.3, 0.45 and 0.6% concentrations, respectively against the fourth instar *S. litura* larvae. In both the choice and no-choice experiments, antifeedant activity was directly related to the concentration in all the treatments. Furthermore, the antifeedant effect was less in the no-choice experiments than the choice experiments in all the treatments. Nimbicidine was the second effective treatment as evident from the antifeedant activity results (Table 1). At 0.6% Nimbicidine recorded 82.0 and 73.5% antifeedant

activity in the choice and no-choice tests, respectively. A notable observation in this experiment was that the individual neem and karanj oil treatments were significantly less effective compared to the PONNEEM. At the highest concentration (0.6 %) neem and karanj oils recorded 69.7 and 63.4% antifeedant activity, respectively in the choice experiments, which were significantly lower compared to the PONNEEM at the same concentration. Emulsifier control showed antifeedant activity of 0.5 and 2.7% in the choice and no-choice tests, respectively.

Table 1- Percent antifeedant activity of neem oil and karanj oil treatments at different ratios against 4th instar larvae of *Spodoptera litura* in no-choice and choice methods (Mean ± SD) (n=20).

Treatments	Concentrations (%)							
	0.15		0.3		0.45		0.6	
	Choice	No-choice	Choice	No-choice	Choice	No-choice	Choice	No-choice
Neem oil	53.0 ± 0.8^{b} (46.71)	37.2 ± 1.3^{a} (37.58)	57.3 ± 2.8^{b} (49.26)	44.2 ± 1.7^{a} (44.67)	63.1 ± 4.0^{a} (52.59)	50.0 ± 2.4^{a} (45.0)	69.7 ± 4.8^{b} (56.6)	68.8 ± 4.8^{b} (56.04)
Karanj oil	46.7 ± 3.8a (43.11)	35.8 ± 1.0^{a} (36.75)	52.6 ± 4.3^{a} (46.54)	44.3 ± 1.7^{a} (41.73)	61.4 ± 3.7^{a} (51.59)	53.4 ± 3.1^{a} (46.94)	63.4 ± 1.9^{a} (52.77)	60.5 ± 4.2^{a} (51.06)
Karanj + neem oil (1:3)	55.3 ± 3.4^{c} (48.04)	35.8 ± 1.0^{a} (36.75)	62.1 ± 2.7^{c} (52.0)	44.3 ± 1.7^{a} (41.73)	69.0 ± 1.6^{b} (56.17)	53.4 ± 3.1^{a} (46.94)	78.0 ± 1.0^{c} (62.03)	63.4 ± 1.9^{a} (52.77)
Karanj oil + neem oil (3:1)	49.6 ± 1.5^{a} (44.77)	35.0 ± 1.4^{a} (36.27)	55.0 ± 0.9^{ab} (47.87)	43.3 ± 1.1^{a} (41.15)	69.3 ± 4.1^{b} (56.35)	52.3 ± 3.3^{a} (46.37)	76.5 ± 1.8^{c} (61.00)	61.6 ± 2.4^{a} (51.71)
Karanj oil + neem oil (1:1) (PONNEEM)	83.7 ± 6.0^{d} (65.19)	67.8 ± 5.1^{c} (55.43)	88.3 ± 6.2^{e} (70.0)	73.2 ± 0.9^{c} (58.82)	88.7 ± 4.8^{d} (78.61)	75.9 ± 1.7^{c} (60.60)	96.1 ± 5.1^{d}	88.6 ± 5.3^{d} (70.77)
Nimbicidine (Commercial neem-based pesticide)	65.3 ± 3.8^{c} (53.97)	49.6 ± 2.1^{b} (44.77)	68.9 ± 3.5^{d} (56.10)	54.6 ± 1.7^{b} (47.64)	76.3 ± 0.8^{c} (60.87)	64.3 ± 1.1^{b} (53.37)	82.0 ± 8.1^{d} (64.90)	73.5 ± 1.9^{c} (59.02)

Mean values followed by same letters in a column are statistically not significant by LSD at p=0.05.
Figures in parentheses are arc-sine transformed values.

Effect on development

The larval developmental period, pupal duration and pupal weight of the test insect after the treatment with neem oil, karanj oil, PONNEEM and Nimbicidine at 0.3 % concentration are given in Table 2. The larval period of the PONNEEM treated larvae increased significantly ($p \leq 0.05$) compared to Nimbicidine and control. However, the effect of the PONNEEM was statistically the same as that of neem and karanj oils. Maximum larval duration in the neem and PONNEEM treatments was 15.6 days. In Emulsifier control and water control, the larval duration was 9.6 and 9.2 days, respectively. Pupal period was significantly increased ($p = 0.05$) in PONNEEM (16.0 days) treated insects. In contrast the pupal weight was highly reduced in the PONNEEM treatment, which was statistically significant. Adult moths which emerged from the treatments generally lived only for short time. In the PONNEEM treatment, the mean adult longevity was 3.6 days, whereas in the emulsifier and water controls, the adult longevity was 7.8 and 7.7 days, respectively. In all the treatments, deformed stages of *S. litura* were recorded and this effect was maximum in the PONNEEM treatment, in which several larvae did not grow normally and they showed reduced growth. Adult moths which emerged in the PONNEEM treatment showed poorly developed wings and reduction of body size. Small sized pupae and larval-pupal intermediates were also produced in the PONNEEM treatment.

Fecundity and egg hatchability

The present experiments clearly indicated that the neem and karanj oils had deleterious effect on the fecundity and egg development of *S. litura*. From Table 2 it was clear that the PONNEEM treatment significantly reduced the fecundity (266.8 eggs/female moth) and egg hatchability (39.8 %). The fecundity reduction in the PONNEEM treatment was highly significant compared to all other treatments and the egg hatchability in the PONNEEM was significantly different from the karanj oil (53.4 %), Nimbicidine (63.4 %), emulsifier control (95.0 %) and water control (97.8 %); neem oil showed the same hatchability with the PONNEEM.

Compatability of PONEEM and Nimbicidine with *Trichogramma chilonis*

PONNEEM did not affect the parasitoid emergence up to 0.3% concentration. At 0.6% concentration, the emergence was slightly reduced (86.4 %) but Nimbicidine greatly reduced the

emergence (77.7 %) at this concentration (Fig. 1). At 0.1% concentration, the effects of both PONNEEM and Nimbicidine were statistically similar. At 0.3%, nimbicidine showed 85.8% emergence and PONNEEM showed 87.3 % emergence of *T. chilonis*. Among all the treatments, untreated control showed the maximum per cent parasitoid emergence (89.3%). All concentrations of PONNEEM were safe to *T. chilonis* since the parasitoid emergence was not seriously affected.

Table 2- Impact of neem oil and karanj oil formulations on development, fecundity and egg hatchability in *Spodoptera litura* after 24 hours of treatment with 0.3% (Mean ± SD) (n=5)

Treatments	Growth Parameters					
	Time taken for Pupation (days)	Pupal duration (days)	Pupal Weight (mg)	Adult Longevity (days)	Fecundity No. of eggs/moth	Hatchability (%)
Neem oil	15.6 ± 1.7^{c}	12.2 ± 1.5^{b}	85.2 ± 3.9^{b}	5.0 ± 0.7^{a}	295.8 ± 9.5^{b}	42.2 ± 3.0^{a}
Karanj oil	15.0 ± 1.4^{c}	12.8 ± 0.8^{b}	107.6 ± 3.0^{c}	4.6 ± 1.1^{a}	315.4 ± 11.2^{c}	53.4 ± 3.1^{b}
PONNEEM	15.6 ± 1.7^{c}	16.0 ± 1.2^{c}	76.4 ± 3.9^{a}	3.6 ± 1.1^{a}	266.8 ± 5.0^{a}	39.8 ± 3.3^{a}
Nimbicidine	13.0 ± 1.0^{b}	12.2 ± 1.3^{b}	125.4 ± 2.8^{d}	6.6 ± 1.1^{b}	538.8 ± 3.0^{d}	63.4 ± 4.1^{c}
Emulsifier control	9.6 ± 1.1^{a}	10.0 ± 0.7^{a}	238.0 ± 5.8^{e}	7.8 ± 1.1^{b}	1451.8 ± 10.0^{e}	95.0 ± 3.1^{d}

Mean values followed by same letters in a column are statistically not significant by LSD at p=0.05.

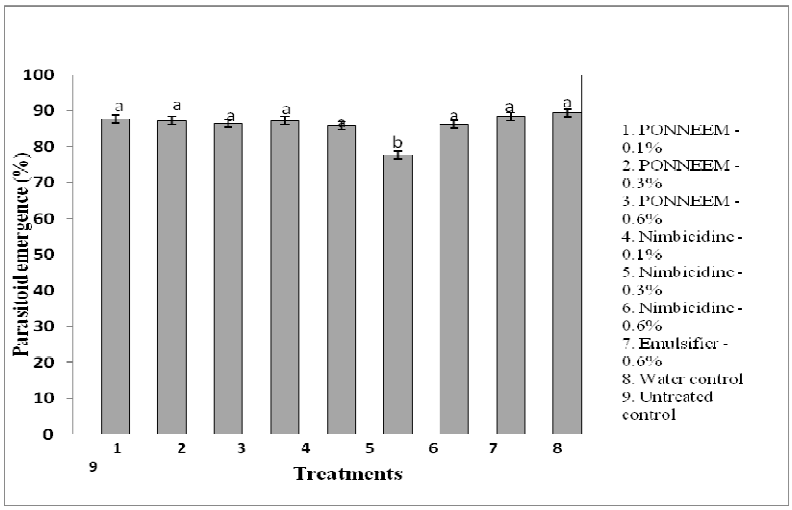

Figure 1 - Percent adult emergence of *Trichogramma chilonis* from Trichocards treated with PONNEEM and Nimbicidine at different concentrations.

DISCUSSION

Plant kingdom is a rich source of biologically active natural chemicals. More than 10,000 secondary metabolites have been chemically identified from the plant kingdom (Kareem 1999). Neem products such as neem seed kernal extract, neem leaf extract, neem oil and neem cake are widely used as insect repellents and insecticides against a vast number of pests (Larson 1989; Koul et al. 1990). Azadirachtin, a ring C-seco tetranortriterpenoid, is the major active principle and is the most potent natural insect antifeedant discovered to-date (Isman et al. 1990). According to Isman et al. (1990), the azadirachtin content in neem oil varied between 188 to 4026 ppm in HPLC analysis. Besides azadirachtin, neem oil also contains different fatty acids, namely oleic acid, linoleic acid, linolenic acid, palmitic acid and stearic acid (Valenzuela et al. 2007). In the present study, the neem oil used for PONNEEM preparation was found to contain around 1000 ppm of azadirachtin in addition to fatty acids.

The deleterious effects of neem products on lepidopteran pests have been well documented by many researchers (Kirsch 1987; Klocke 1987; Blaney et al. 1990). The antifeedant, growth regulating and insecticidal activities of neem oil

were mainly due to the action of azadirachtin present in it (Isman et al. 1990; Govindachari et al. 1996). Pungam oil possesses toxic components such as glabrin, karajin, karanjae and pongaglabrone, which are effective against insect pests of stored grains, field and plantation crops. Gibbs (1974) reported that pongam possessed saponins, several chalcones and related compounds. Karanchin is the major principle in pungam oil (Sahrawat 1982).

Higher antifeedant index normally indicates decreased rate of feeding. In the present study, the antifeedant activity varied significantly based on the concentration and combinations used for the formulations. Antifeedant is a chemical that inhibits the feeding without killing the insect directly, while the insect remains near the treated foliage and dies through starvation (Yasui et al. 1998). Most potent insect antifeedants are sesquiterpene lactones, diterpinoids, triterpinoids, quinoline and indole alkaloids (Schoonhoven 1982). In the present study, an additive effect in the antifeedant activity was recorded in the PONNEEM treatment (combination of neem and pungam oils), which was significantly high compared to the individual neem and pungam oil treatments. This result coincided with the findings of Kumar et al. (2007) who reported that a combined formulation of methanolic extracts of neem and karanj oils showed 70 and 11.36 - fold increase in the activity (LC50 = 0.11%) over the individual neem and karanj oils against *Tetranychus* species in the laboratory studies. They further stated that the neem and karanj combination showed synergistic activity against aphid *Macrosiphoniella sanborni*, providing 100% protection compared to neem (68.4%) and karanj (52.9%) alone after 48 h at 0.5 % concentration.

Neem and karanj oils also showed synergistic or additive effects with synthetic chemicals against the insects. Rao and Dhingra (2000) have reported that neem oil produced an additive effect with cypermethrin against two different populations of *S. litura* that were susceptible or resistant to synthetic pyrethroids. They also reported that karanj oil showed synergistic activity against susceptible populations of *S. litura* when combined with either cypermethrin or fenvalerate, but showed antagonistic effect against the resistant populations.

The active principles, including karanjin and azadirachtin present in the PONNEEM inhibited larval feeding. Previous studies hav shown evidences that botanicals exhibit feeding deterrent activity against *S. litura* (Caasi Lit and Rejesus 1990; Yasui et al. 1998; Morimoto et al. 2002). Pavela and Herda (2007) reported that pongam oil was an effective repellent against the common greenhouse whitefly (*Trialeurodes vaporariorum* Westwood). They also reported that pongam oil was an oviposition deterrent and recorded between 80 and 100% oviposition deterrence at varying concentrations from 0.5 to 2%

Insect growth regulation properties of plant extracts are very unique in nature, since insect growth regulator works on juvenile hormone. The enzyme ecdysone plays a major role in shedding of old skin and the phenomenon is called ecdysis or moulting. When the active plant compounds enter into the body of the larvae, the activity of ecdysone is suppressed and the larva fails to moult, remaining in the larval stage and ultimately dying (Koul and Isman 1991). In the present study, morphological deformities at larval-pupal intermediate, pupae and adult stages were recorded in *S. litura*. These deformities might be due to the interference of karanjin and azadirachtin that were present in the PONNEEM on the growth and developmental processes of the test insect. These results are consistent with the earlier reports on various lepidopteran species (Fagoonee 1984; Barnby and Klocke 1987; Koul and Isman 1991). The reduction in pupal weight due to plant product treatments has been reported by some investigators (Fagoonee and Lange 1981; Mukherjee and Sharma 1993). The number of eggs laid by the adults was reduced; normal adult emergence was suppressed; sometimes adults laid eggs before the copulation because of improper development of oozyte. Because of the infertile egg, the hatchability was reduced.

A favourable result from the present study was that the PONNEEM did not affect the parasitoid emergence in *T. chilonis*. The higher concentration (0.5%) alone reduced the parasitoid emergence but it was very low. However, the field concentration of the PONNEEM has been considered as 0.3%, which was found to be not deleterious to the parasitoid. Isman et al. (1992) reported that neem had no detrimental effects on predatory coccinellids, chrysopids and syrphids. The safety of neem oil 50 EC and NSKE at different concentrations to grubs of *C. carnea* was shown by Bhuvaneshwari et al. (1993). Kaethner (1990) reported that neem formulations did not influence the fecundity and egg hatchability of parasitoids.

The safety of the neem products to the parasitoid *Telenomus principium* has been recorded by Klemm and Schmutterer (1993).

CONCLUSION

In conclusion, the newly developed oil formulation PONNEEM exhibited good antifeedant and growth regulation activities against *S. litura* larvae. The new formulation was not harmful to the parasitoid *T. chilonis*. Hence, this formulation could be used as an efficient botanical pesticide against *S. litura*. Field studies are necessary to confirm the bioefficacy of PONNEEM in the management of *S. litura* population and to check the effect of PONNEEM on crop yield.

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