

COMPATIBILITY OF PYRETHROID AND DIFFERENT CONCENTRATIONS OF NEEM SEED EXTRACT ON PARASITOID *TRICHOGRAMMA CHILONIS* (ISHII) (HYMENOPTERA: TRICHOGRAMMATIDAE) UNDER LABORATORY CONDITIONS

*M. H. KHAN, M. SARWAR, A. FARID¹ and F. SYED²

Nuclear Institute of Agriculture (NIA), Tando Jam-70060, Pakistan

¹Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan

²KPK Agricultural University, Peshawar³, Pakistan.

(Received April 30, 2010 and accepted in revised form December 08, 2010)

The parasitoid, *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae) is an important egg parasitoid, reported to reduce the lepidopteron pest populations considerably and often released agumentatively in integrated pest management (IPM) programs of crops and vegetables. Studies on the effect of pyrethroid (ripcard 0.5%) and different concentrations of neem (*Azadirachta indica*) seed extract on *T. chilonis* were conducted. Among different concentrations of neem seed extract (4, 2, 1, 0.5 and 0.25%), the highest mortality (68.29%) of *Trichogramma* was recorded with 4% neem seed extract and lowest (35.83%) with 0.25% neem seed extract. Overall, the highest mortality (97.52%) of *Trichogramma* was recorded with ripcard. Emergence time was non significantly different in all treatments of neem seed extract but was significantly different from ripcard where the emergence time was 9.75 days. Longevity and sex ratio was also non significantly different in all treatments of neem seed extract. It can be concluded that neem seed extract upto 4% concentration can be included in IPM to protect *T. chilonis* as biological control agent.

Keywords: *Azadirachta*, *Trichogramma*, Neem, Toxicity, Pyrethroid.

1. Introduction

Pesticides worth more than US\$ 30 billion are intentionally released into the global environment every year. Many of these are highly toxic and have immediate adverse effects on human health and wildlife or contaminate local food, water, soil and air. Growing without chemical pesticides is based on alternative preventive and curative pest control methods. If preventive measures are not sufficient, insecticides derived from natural plant extracts like neem and many more sources can be applied [1]. Pesticides have improved our life in many ways but they have also created problems for human and the environment. The main hazards are that synthetic pesticides are continuously affecting the biotic and abiotic components of the environment. It is a known fact, that often only 1% of the active ingredients reach the target pests, while 99% of these substances, some of which are highly toxic, burden

the environment [2]. Chemical control of insect pests has been successfully integrated with all other types of insect pest management tactics in many of the world's major food and fiber crops. However, in order to be of greatest utility, chemical control including pyrethroids must fit efficiently into IPM system [3].

Non-selective use of pesticides is responsible for water pollution, soil degradation, insect resistance and resurgence, destruction of native flora and fauna [4]. Although pesticides offer one way to manage pests, there are other alternatives that do not present the same environmental problems. These alternatives include integrated pest management, which is bringing together and utilizing all the control measures concurrently or successively in an integrated way against a particular pest and thereby minimizing the need for chemical control [5].

* Corresponding author : entomologist170@yahoo.com

Biological control is the method of pest management by the use of living organisms and there are numerous species of parasitoids and predators which are the natural enemies of pests. These have abilities to reduce the population of their prey or host and help to limit damage caused by the pest. Parasitoids of the genus *Trichogramma* are distributed worldwide and play an important role as natural enemies of lepidopterous pests on a wide range of agricultural crops such as fruits, vegetables, cereals and forests. *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae) are small wasps that are egg parasitoids of lepidopteron pests. They parasitize the eggs of lepidopterous pests by laying eggs inside their eggs. Larvae hatch from these eggs and feed on egg contents. Fully mature larvae pupate inside egg and emerge as adult. Almost 18 different *Trichogramma* species are being mass reared to use in sugarcane, rice, cotton, soyabean, sugarbeet, vegetables and pine in at least 16 countries for management of pests [1]. The *T. chilonis* is the typical gregarious egg parasitoid widely distributed in the world. It has been recorded to parasitize eggs of *Chilo infuscatellus*, *C. partellus*, *Helicoverpa armigera*, *Agrotis ipsilon*, *Autographa nigrisigna*, *Spodoptera litura*, *Acigona steniellus* and *Emmalocera depressella* [6]. During these days, *Trichogramma* species are the most widely used insect natural enemy in the world, partly because they are easy to mass rear and attack many important crop insect pests [7]. Certain insecticides have been noted dangerous showing different degrees of toxicity to the parasitoid [8]. The side-effects of pesticides were tested on adults and pupae of *Trichogramma* reared in the laboratory. Alpha-cypermethrin, carbosulfan, deltamethrin, endosulfan, profenofos and zeta-cypermethrin were highly noxious to the parasitoid, significantly reducing the percentage of emergence and parasitism of *Trichogramma* [3].

Botanical insecticides are promising alternatives for use in insect management. Botanicals are naturally occurring compounds derived from plant sources. Botanicals degrade rapidly in sunlight, air and moisture and rapidly broken down by detoxification enzymes. The rapid degradation of botanicals and their action as stomach poisons make them more selective in some instances for plant feeding pest insects and less harmful to beneficial insects. Neem products are derived from

the neem tree, *Azadirachta indica* A. Juss. (L.) that grows in arid tropical and subtropical regions on several continents. The principal active compound in neem is azadirachtin, a bitter, complex chemical that is both a feeding deterrent and a growth regulator. The meliantriol, salanin and many other minor components of neem are also active in various ways. For insects, neem is the most active as a feeding deterrent, but in various forms it also serves as a repellent, growth regulator, oviposition suppressant, sterilant, or toxin [9]. Very little work on the effects of neem seed extract on beneficial insects particularly side effects of pyrethroids and neem on *T. chilonis* has been conducted in this part of the world. To observe the effect of ripcard and neem on *Trichogramma*, present experiment was conducted under laboratory conditions to determine their effects on the life history parameters of *Trichogramma*.

2. Materials and Methods

2.1. Rearing of *Sitotroga Cerealella* (Oliv.)

Fresh eggs of *Sitotroga cerealella* were obtained from the culture maintained on wheat at the Entomology laboratory at Nuclear Institute for Food and Agriculture (NIFA), Peshawar.

2.2. Rearing of *Trichogramma Chilonis* (Ishii) on *S. Cerealella* (Oliv.)

The eggs of *S. cerealella* were glued on hard paper card (3×8 cm) and put in glass jars (55×12 cm) having *T. chilonis* (one day old 100 pairs) for parasitism. After parasitization, cards were removed and kept in an incubator at 25-30°C and 60-70% R.H for parasitoids development. After emergence the parasitoids were further used to parasitize host eggs.

2.3. Preparation of Neem Formulations

Crushed neem seed of 100 g with 5 g detergent were put in muslin cloth and placed in 1 lit., boiled water. The seed remained in the water for 24 h which gave 10% solution of neem seed extract. Different concentrations were made using the following formula: $V_1C_1 = V_2C_2$, where, V_1 = volume of water which is required, V_2 = volume of known quantity, C_1 = given concentration, C_2 = required concentration. In this way 0.25, 0.5, 1.0, 2.0 and 4.0% concentrations were made. Likewise, 0.5% concentration of ripcard was prepared.

2.4. Effects of Different Concentrations of Neem Seed Extract on Pupae of *Trichogramma*

To observe the effects of neem seed *Azadiracta indica* (Sapindales; Meliaceae) on pupae of *Trichogramma*, extract of different concentrations 4, 2, 1, 0.5 and 0.25 were used alongwith standard pyrethroid pesticide ripcard 0.5%. There were 9 replications of each treatment. About sixty fresh eggs of *S. cerealella* were glued on hard paper cards (1×8 cm) and (3×8 cm) and then exposed to parasitization in glass jar (8×12 cm) having *Trichogramma* adults for 24 hours. All the cards were placed in an incubator at 25°C and 65% RH till complete pupation. Upon pupation the total numbers of pupae on each card were recorded. Each card with about 60 parasitoid pupae was sprayed with one of the randomly chosen concentrations of neem seed extract or the ripcard and considered a single replicate for that treatment. A total of seven treatments including five concentrations of neem seed extract and recommended dose of ripcard along with control were made. After treatment, ten pupae were taken from each card and enclosed individually in plastic capsules. These capsules were observed daily to note date of adult emergence, their sexes and mortality. The cards were then placed individually in a vial whose mouth was tightly closed with a piece of cloth and rubber band, and placed in an incubator till the death of all adults emerged from pupae. The total numbers of adults in each vial were recorded. By adding the number of adults emerged in each vial with the number of adults

emerged in its respective capsules (10 capsules from each card in a vial), the total numbers of adults emerged on each card were determined. This gave the total number of pupae on a card and the number of adults emerged from the card. The data were recorded on percent emergence, percent mortality, emergence time, longevity and sex ratio. Percent emergence of *Trichogramma* was calculated by using the formula:

$$\text{Adult emergence (\%)} = \frac{\text{No. of adult emerged}}{\text{Total No. of pupae}} \times 100$$

Adult mortality (%) of *Trichogramma* was calculated by using the formula: Percent mortality = 100 – adult emergence (%). Longevity of *Trichogramma* was determined as: Longevity = Date of death – Date of emergence. Males and females of *Trichogramma* were separated by examine them under microscope. Emergence time of *Trichogramma* was determined as:

Emergence time = Date of emergence – Date of parasitization. Completely randomized design was used to analyze the data recorded on the compatibility of insecticide and different concentrations of neem seed extract with *Trichogramma* by following Steel and Torrie [10].

3. Results and Discussion

The results of the present experiment on evaluation of the compatibility of neem seed extract and standard insecticide with *T. chilonis* are presented in Table 1.

Table 1. Percent mortality and developmental traits of *Trichogramma* after using different concentrations of neem seed extract and ripcard.

Treatments	Adult mortality (%)	Adult emergence (days)	Longevity (days)	Sex ratio (females %)
4% neem seed extract	68.29 b	9.01 b	2.12 ab	0.22 a
2% neem seed extract	61.54 b	8.89 b	2.20 a	25 a
1% neem seed extract	59.61 b	8.87 b	2.30 a	26 a
0.5% neem seed extract	58.32 b	9.14 b	2.11 ab	25 a
0.25% neem seed extract	35.83 c	9.10 b	2.18 ab	25 a
0.5% ripcard	97.52 a	9.75 a	1.95 b	25 a
Control	21.41 c	8.81 b	2.31 a	35 a

Means within a column followed by different letters are significantly different at $P \leq 0.05$.

3.1. Adult Mortality

The mortality (%) of *Trichogramma* on neem seed extract (4, 2, 1, 0.5 and 0.25%) and ripcard was respectively, 68.29, 61.54, 59.61, 58.32, 35.83 and 97.52% as compared to control where the mortality was 21.46%. Results showed that the mortality with ripcard (97.52) was significantly higher than all other treatments. Mortality with 4% neem seed extract (68.29%), 2% neem seed extract (61.54), 1% neem seed extract (59.61) and 0.5% neem seed extract (58.32) was non significantly different from each other. Similarly, mortality with 0.25% neem seed extract (35.83) and control (21.46) was significantly lower than all other treatments. Neem seed extract (0.25%) had a little effect on *Trichogramma*, while ripcard had a significant effect on percent mortality. Other concentrations of neem seed extract used were non significant but significantly different from the control. These results are consistent with Innacone *et al.* [11] who reported that neem employed at highest doses produced statistically significant effects on the mortality percentage. Rao *et al.* [12] found that cyhalothrin at 0.0025% caused 100% mortality of *Trichogramma*. Solayappan *et al.* [13] reported that neem (combination of neem seed extract, *Metha spicata* leaf extract and tobacco) when used on *Trichogramma*, resulted in the highest mortality.

3.2. Emergence Time

Emergence time (days) of *Trichogramma* after exposure to neem seed extract (4, 2, 1, 0.5 and 0.25%) and ripcard was 9.01, 8.89, 8.87, 9.14, 9.10 and 9.75, respectively as compared to control where the emergence time was 8.81 days. Statistical analysis of the data presented that the emergence time with ripcard (9.75 days) was significantly higher than all other treatments. Emergence time was non significantly different in all the rest of the treatments. Reasons for such results are not clear, however it is clear that emergence time is inversely proportional to the temperature, when temperature increases, it decreases and vice versa.

3.3. Longevity

Longevity (days) of *Trichogramma* with neem seed extract (4, 2, 1, 0.5 and 0.25%) and ripcard was 2.12, 2.20, 2.30, 2.11, 2.18 and 1.95 days, respectively as compared to control where the longevity was 2.31 days. Data revealed that the

longevity with control (2.31), 1% neem seed extract (2.30) and 2% neem seed extract (2.20) was significantly higher than ripcard (1.95 days). Longevity in control (2.31), 1% neem seed extract (2.30), 2% neem seed extract (2.20), 0.25% neem seed extract (2.18), 4% neem seed extract (2.12) and 0.5% neem seed extract (2.11 days) was non significantly different among each other. Table shows that ripcard had significant effect on longevity of *Trichogramma*, while other treatments did not affect the longevity significantly. The results roughly tally with Stark *et al.* [14] who reported that longevity of braconid wasps, exposed to azadiractin was not significant. Akol *et al.* [15] reported that Neemroc EC and Neemros had no significant effects on longevity of adult wasps.

3.4. Sex Ratio

It is clear that the females of *Trichogramma* with neem seed extract (4, 2, 1, 0.5, and 0.25%) and ripcard were respectively, 22, 25, 26, 25, 25 and 25% as compared to control where the females were 35%. Data revealed that there were no significant differences in the sex ratio of *Trichogramma* among all the treatments. This means that neem seed extract had no effect on sex ratio. These findings are in agreement with the findings of Lyons *et al.* [16] who reported that neem EC and azatin EC had no effect on the sex ratio of *Trichogramma chilonis*. Similarly, Drescher *et al.* [17] reported that neem had no negative effect on the sex ratio.

4. Conclusions

It is concluded from the present research findings that *Azadirachta indica* upto a concentration of 4% can be used in integrated pest management having little effect on adult emergence, adult longevity and sex ratio of *T. chilonis* as compared to control, whereas in the presence of standard insecticides (ripcard) these biological parameters of *T. chilonis* were adversely affected. Hence, the neem seed extract up to 4% concentration can be included in IPM to protect parasitoid *T. chilonis* as biological control agent.

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