STUDY ON THE COMPATIBILITY OF DIFFERENT CONCENTRATIONS OF NEEM SEED EXTRACT WITH *TRICHOGRAMMA CHILONIS* (Ishii)

Muhammad Hamayoon Khan[1], Abid Farid[2], Muhammad Naeem[3] and Fazal Syed[4]

Abstract

Study on the compatibility of different concentrations of neem seed extract with *Trichogramma* (Hymenoptera: Trichogrammatidae) was conducted at Nuclear Institute for Food and Agriculture (NIFA) Tarnab, Peshawar, during 2004. Among different concentrations of neem seed extract (4%, 2%, 1%, 0.5% and 0.25%), maximum mortality (68.29%) of *Trichogramma* was recorded with 4% neem seed extract and minimum (35.83%) with 0.25% neem seed extract. However the highest mortality (97.52%) of *Trichogramma* was recorded with Ripcard 0.5%. 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.

Introduction

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 (Hassan, 1992).

Non-selective use of pesticides is responsible for water pollution, soil degradation, insect resistance and resurgence, destruction of native flora and fauna. Some are responsible for ozone depletion and contribute to the greenhouse effect (Baloch and Haseeb, 1996).

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. (Shahid, 2003).

Biological control is the control of pests through other living organisms. There are numerous species, which are natural enemies of pests. Parasitoids and predators of agricultural pests reduce the population of their prey or host and help to limit damage caused by the pest. Parasitoids of the genus *Trichogramma* (Hymenoptera; Trichogrammatidae) are distributed worldwide and play important role as natural enemies of lepidopterous pests on a wide range of agricultural crops such as fruits, vegetables, cereals and forests. *Trichogramma* are small wasps that are egg parasitoids of lepidoptera. They parasitize the eggs of lepidopterous pests by laying eggs inside their eggs. Larvae hatch

1 Assistant Forest Entomologist, Pakistan Forest Institute, Peshawar
2 Principal Scientist, Nuclear Institute for Food and Agriculture (NIFA), Tarnab Peshawar
3 Department of Entomology, NWFP, Agricultural University Peshawar
4 Research Officer, Pakistan Oilseed Development Board, Tarnab, Peshawar

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 control pests on sugarcane, rice, cotton, soyabean, sugarbeet, vegetables and pine in at least 16 countries (Hassan, 1992).

Trichogramma chilonis (Ishii) (Hymenoptera: Trichogrammatidae) is the typical gregarious egg parasitoid widely distributed in Pakistan. According to Nagarkatti and Nagaraja (1979) *Trichogramma australicum* Girault and *T. confusum* vigiani are synonyms of *T. chilonis*. In Pakistan it has been recorded from *Chilo infuscatellus, C. partellus, Helicoverpa armigera, Bactra* sp., *Agrotis ipsilon, Autographa nigrisigna, Spodoptera litura, Acigona steniellus* and *Emmalocera depressella* (Hassan, 1993).

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 are 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*, 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. Meliantriol, salanin, and many other minor components of neem are also active in various ways. In insects, neem is most active as a feeding deterrent, but in various forms it also serves as a repellent, growth regulator, oviposition (egg deposition) suppressant, sterilant, or toxin (Tess and Weinzieril, 1989).

Very little work on the compatibility of neem seed extract with beneficial insects particularly side effects of neem on *Trichogramma chilonis* has been conducted in this part of the world. To observe the compatibility neem seed extract with *Trichogramma*, an experiment was conducted under laboratory conditions with the following objective to determine the effect of different concentrations of neem seed extract on the life history parameters of *Trichogramma*.

Materials and methods

A study on the compatibility of different concentrations of neem seed extract with *Trichogramma chilonis* was conducted at Nuclear Institute for Food and Agriculture (NIFA) Tarnab, Peshawar during 2004. The following methodology was adopted for the conduction of experiment:

1. Rearing of *Sitotroga cerealella* (Oliv)

Fresh eggs of *Sitotroga cerealella* were obtained from the culture maintained on wheat at the Entomology Laboratory at NIFA. Two gm of *S. cerealella* eggs were mixed in a glass jar (22x55 cm) containing ½ kg of wheat grains. The jars were kept at 25-30°C and 60-70% R.H. On emergence *S. cerealella* adults were collected and shifted to oviposition jars (14x15 cm) with 20-size mesh bras wire gauze at the bottom. The oviposition jars were placed on maize starch on plastic trays. Eggs were collected by sieving the starch by 50 and 70 size mesh. These eggs were used for further moth propagation and *Trichogramma* production in the experiment.

2. Rearing of *Trichogramma chilonis* (Ishii) on *S. cerealella* (Oliv)

The eggs of *S. cerealella* were glued on hard paper card (3x8 cm) and were put in glass jars (55x12 cm) having *T. chilonis* for parasitization. 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 for parasitizing host eggs.

3. Preparation of treatments

100g of crushed neem seed and 5g detergent were put in muslin cloth and put in 1 liter boiled water. The seed remained in the water for 24h which gave us 10% solution of neem seed extract. Different concentrations were made using the following formula:

V1C1 = V2C2
V1 = volume of water which is required
V2 = volume of known quantity
C1 = given concentration
C2 = required concentration

This way 0.25%, 0.5%, 1.0%, 2.0%, and 4.0% concentrations were made.

4. Effect of neem seed extract on *Trichogramma* pupae

To observe the effect of neem seed extract on *Trichogramma* pupae following concentrations of neem seed extract were used along with ripcard:

Different concentrations of neem seed extract including ripcard.

S.No.	Treatments
1	4% neem seed extract
2	2% neem seed extract
3	1% neem seed extract
4	0.5% neem seed extract
5	0.25% neem seed extract
6	0.5% Ripcard
7	0% (Control)

There were 9 replications of each treatment. Twenty to sixty fresh eggs of *Sitotroga cereallela* were glued on hard paper cards (1x8 cm) and (3x8 cm) and then exposed to parasitization in glass jar (8x12 cm) having *Trichogramma* adults for 24 hours. All the cards were placed in an incubator at 25^{o}C and 65% RH till complete pupation. Upon pupation the total numbers of pupae on each card were recorded. Each card with 30-50 parasites pupae was sprayed with one of the randomly chosen concentrations of neem seed extract or the insecticide mentioned above, and was considered a single replicate for that treatment. There were a total of seven treatments including five concentrations of neem seed extract and recommended dose of ripcard. After treatment, ten pupae were taken from each card and enclosed individually in plastic capsules. These capsules were observed daily to note their sexes, date of adult emergence and its mortality. The cards were then individually placed in a vial whose mouth was closed tightly with a piece of cloth and rubber band and placed in incubator till the death of all adults emerged from pupae. The total number of adults in each vial was 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 number of adults emerged on each card was determined. This gave us 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.

Parameters:

i. Percent Emergence of *Trichogramma*

Percent emergence of *Trichogramma* was calculated by using the formula:

$$\text{Percent adult emergence} = \frac{\text{No. of adult emerged}}{\text{Total No. of pupae}} \times 100$$

ii. Percent Mortality of *Trichogramma*
Percent mortality of *Trichogramma* was calculated by using the following formula:
Percent mortality = 100 – Percent adult emergence

iii. Longevity of *Trichogramma*
Longevity of *Trichogramma* was determined as:
Longevity = Date of death – Date of emergence

iv. Sex Ratio of *Trichogramma*

Males and females of *Trichogramma* were separated by examining them under microscope.

v. Emergence Time of *Trichogramma*
Emergence time of *Trichogramma* was determined as follows:
Emergence time = Date of emergence – Date of parasitization.

Completely Randomized design was used to analyze the data recorded on the compatibility of different concentrations of neem seed extract with *Trichogramma*.

Results and Discussion

The present studies were conducted at the Entomology division of NIFA to evaluate the compatibility of neem seed extract with *Trichogramma chilonis*. The results of the present experiment are presented in Table 1.

Effect of neem seed extract on the life history parameters of *Trichogramma*

Percent mortality

Data recorded on percent mortality of *Trichogramma* with different concentrations of neem seed extract are given in Table 1.

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%.

Statistical analysis of the data revealed 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 were significantly different from the control. The results are consistent with Innacone *et al*. (2003) who reported that neem employed at highest doses produced statistically significant effects on the mortality percentage.

Rao *et al*. (2002) found that cyhalothrin at 0.0025% caused 100% mortality of *Trichogramma*. Solayappan *et al*. (2001) reported that neemento (combination of neem seed extract, *Metha spicata* leaf extract and tobacco) when used on *Trichogramma*, resulted in the highest mortality.

Emergence time

Data regarding emergence time of *Trichogramma* with different concentrations of neem seed extract are given in Table 1.

Emergence time of *Trichogramma* with neem seed extract (4%, 2%, 1%, 0.5% and 0.25%) and ripcard was respectively, 9.01, 8.89, 8.87, 9.14, 9.10 and 9.75 days 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.

Table 1. Percent mortality, emergence time, longevity and sex ratio of *Trichogramma* after using different concentrations of neem seed extract and ripcard

S.No.	Treatments	Percent Mortality	Adult emergence time (days)	Longevity (days)	Sex ratio
1	4% neem seed extract	68.29 b	9.01 b	2.12 ab	0.22 a
2	2% neem seed extract	61.54 b	8.89 b	2.20 a	0.25 a
3	1% neem seed extract	59.61 b	8.87 b	2.30 a	0.26 a
4	0.5% neem seed extract	58.32 b	9.14 b	2.11 ab	0.25 a
5	0.25% neem seed extract	35.83 c	9.10 b	2.18 ab	0.25 a
6	0.5% Ripcard	97.52 a	9.75 a	1.95 b	0.25 a
7	0% (Control)	21.41 c	8.81 b	2.31 a	0.35 a

Means within a column followed by different letters are significantly different at 5% level of significance. ANOVA followed by LSD test.

Longevity

Data about the longevity of *Trichogramma* with different concentrations of neem seed extract are given in Table 1.

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

Statistical analysis of the data revealed the longevity with control (2.31 days), 1% neem seed extract (2.30 days) and 2% neem seed extract (2.20 days) was significantly higher than ripcard (1.95 days). Longevity in control (2.31 days), 1% neem seed extract (2.30 days), 2% neem seed extract (2.20 days), 0.25% neem seed extract (2.18 days), 4% neem seed extract (2.12 days) and 0.5% neem seed extract (2.11 days) was non significantly different among each other.

Table 1 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*. (1992) who reported that longevity of braconid wasps, exposed to azadiractin was not significant. Akol *et al*. (2002) reported that Neemroc EC and neemros had no significant effect on longevity of adult wasps.

Sex ratio

Data about the sex ratio of *Trichogramma* with different concentrations of neem seed extract are given in Table 1.

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%.

Statistical analysis of the data revealed no significant differences in the sex ratio of *Trichogramma* among all the treatments. which means that neem seed extract had no effect on sex ratio. These findings are in agreement with the findings of Lyons *et al*. (2003) who reported that neem EC and azatin EC had no effect on the sex ratio of *Trichogramma chilonis*. Drescher *et al*. (1996) reported that neem Azal had no negative effect on the sex ratio.

References

Akol, A. M., S. Sithanantham, P. G. N. Njagi, A. Varela, and J. M. Mueke, 2002. Relative safety of sprays of two neem insecticides to *Diadegma mollipla* (Holmgren), a parasitoid of the diamonback moth: effects on adult longevity and foraging behaviour. Crop Prot. 21(9): 853-859.

Baloch, U. K. and M. Haseeb, 1996. Xenobiotics in Third World Agriculture Environment. In Environmental xenobiotics ML Richerdosn, Taylor and Francis, Caller Macmillian Publisher London, U.K. 286 pp.

Drescher, K., G. Madel, H. Kleeberg, and C. P. W. Zebitz, 1996. Effect of Neem Azal-T/S on the anthocorid predator *Orius majusculus* Reuter. Practice oriented results on use and production of neem ingredients and pheromones. Proceedings 5th Workshop Wetzlar, Germany. 124-126.

Hassan, S. A., 1992. Guideline of the side-effects of plant protection product on *Trichogramma chilonis*. In Guideline for testing the effect of pesticides on beneficial organisms, (ed) Hassan, S.A. IOBC/WPRS Bulletin XV(3), 18-39.

Hassan, S. A., 1993. The mass rearing and utilization of *Trichogramma* to control lepidopterous pests: Achievement and Lookout Pestic. Sci. 37(1): 387-391.

Iannacone, J. and G. Lamas, 2003. Toxicological effects of neem, rotenone and cartap over three microwasp parasitoids of agricultural pests in Peru. Boletin de Sanidad Vegetal, Plagas. 29(1): 123-142.

Lyons, D. B., B. V. Helson, R. S. Bourchier, G. C. Jones and J. W. McFarlane, 2003. Effects of azadirachtin-based insecticides on the egg parasitoid *Trichogramma* minutum (Hymenoptera: Trichogrammatidae). Canadian Entomologist. 135(5): 685-695.

Nagarkatti, S. and H. Nagaruja, 1979. The status of *T. chilonis* (Ishii) (Hynenoptera: Trichogrammatidae) Oriental Insect. 13(3): 115-118.

Rao, N. B. V. C., T. R. Goud and T. B. Gour, 2002. Toxicity of newer insecticides to egg parasitoid, *Trichogramma chilonis* ISHH. Jour. Res. ANGRAU. 30(2): 124-126.

Shahid, M., 2003. Principles of insect pest management. Higher Education Commission Islamabad, Pak. 30, 161-166.

Solayappan, A. R., M. Radham, T. Sujatha and R. W. A. Jesudasan, 2001. Toxicity of biopesticides to *Trichogramma chilonis* Ishii and *Chrysopa scelestis* Banks, the natural enemies of sugarcane pests. Indian Jour. Plant Prot. 29(1-2): 85-87.

Stark, J. D., T. T. Y. Wong, R. I. Vargas and R. K. Thalman, 1992. Survival, longevity, and reproduction of tephritid fruit fly parasitoids (Hymenoptera: Braconidae) reared from fruit flies exposed to azadirachtin. Jour. Econ. Entomol. 84(4): 1125-1129.

Tess Henn and Weinzeiri, 1989. Use of botanical insecticides for pests control. Journal of Botany. 10(9): 21-28.