BIOLOGICAL CONTROL

Selectivity of Neem to *Trichogramma pretiosum* Riley and *Trichogrammatoidea annulata* De Santis (Hymenoptera: Trichogrammatidae)

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ABSTRACT - *Trichogramma pretiosum* Riley and *Trichogrammatoidea annulata* De Santis are commonly found in avocado and persimmon orchards in northern Parana state. However, their abundance depends on whether insecticides are used or not to control the key lepidopteran pests *Stenoma catenifer* (Wals.) (Lepidoptera: Elachistidae) and *Hypocala andremona* (Stoll) (Lepidoptera: Noctuidae), respectively. The aim of this work was to evaluate the effects of an aqueous neem seed extract (ANSE) at 15, 3 and 1.5%, and of an emulsifiable concentrate neem oil (ECNO) at 2.5, 0.5 and 0.25% on lifetime parameters of these trichogrammatids as a way of testing the feasibility of integrating the biological and chemical control methods. Chemicals were applied on *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs before or after parasitization (one, three or five days). ANSE was more deleterious to both parasitoid species than ECNO, regardless of the concentration and the time of application. The chemicals acted on a concentration and time dependent manner. Treating the host with neem before parasitism was less deleterious to wasp emergence, especially for *T. annulata*. Pre-treatments (24h) of the host eggs with ECNO at concentrations varying from 0.5% to 0.25% did not affect *T. pretiosum* longevity, but 2.5% reduced *T. annulata* survival. Feeding wasps with honey mixed with 0.25% ECNO negatively affected *T. annulata* survival.

KEY WORDS: Botanical insecticide, *Azadirachta indica*, egg parasitoid

Egg parasitoids of the genus *Trichogramma* are the most studied and successful taxa used in innundative releases against many economically import pests in the world. According to Hassan (1988) & Li (1994) these parasitoids were used in more than 30 million ha worldwide to control lepidopteran pests in agriculture and forestry.

The interest in *Trichogramma* in Brazil began in the early 80´s and an enormous amount of information in different areas of knowledge have been obtained thereafter. This knowledge led to the development of projects aiming to use trichogrammatids to control lepidopteran pests in several crops, including *Stenoma catenifer* (Wals.) (Lepidoptera: Elachistidae) on avocado (Parra & Zucchi 2004).

Indigenous *Trichogramma pretiosum* Riley and *Trichogrammatoidea annulata* De Santis have been found parasitizing eggs of *S. catenifer* on avocado (Hohmann & Meneguim 1993) and of *Hypocala andremona* (Stoll) (Lepidoptera: Noctuidae) on persimmon orchards (Hohmann & Lovato 2003), in the State of Parana, Brazil. These parasitoids were frequent and constant in the majority of the orchards evaluated in northern Parana, but their abundance depends on whether pesticides were used to control pests. Natural parasitism in pesticide-free avocado orchards can surpass 60% (Hohmann *et al* 2003).

The side effects of broad-spectrum insecticides on non-target organisms and the risks posed to the environment stress the need for ecologically sound pest management methods. One alternative that has proved effective to control various arthropod pests is the botanical insecticide neem, *Azadirachta indica* (Schmutterer & Singh 1995). These alternative methods are particularly suitable for more stable agroecosystems, which are characteristic of many small farms in Brazil.

Studies of the possible side effects of plant extracts on natural enemies have increased in the last years showing promising results (Raguraman & Singh 1999, Gonçalves-Gervásio & Vendramin 2004, Silva & Martinez 2004). According to Hohmann (2003, not publ.) the effects of botanical insecticides were evaluated in more than a hundred species of natural enemies, mainly hymenopterans, coleopterans, heteropterans and predaceous mites. However, their effects on life history parameters of biocontrol agents may vary according to its origin, formulation, concentration and the natural enemy itself, among other factors. Therefore,

it is important to consider each specific condition to determine the potential of botanical insecticides in IPM programs.

The aim of this study was to determine the effects of neem on life history parameters of *T. pretiosum* and *T. annulata* as a way of testing the feasibility of integrating the biological control agents with the botanical insecticide neem. The side effects of neem on the two trichogrammatids were assessed by using commercial emulsible oil and an aqueous seed extract. The results of this study may have important impact on future farmer's pest control strategies in organic managed avocados orchards.

Material and Methods

Experiments. Two different sources of neem at three different concentrations were tested to evaluate their side effects on *T. pretiosum* and *T. annulata*. The aqueous neem seed extract (ANSE) was tested at 1.5, 3.0 and 15.0%, and the commercial product Dalneem® (0.5%), an emulsible concentrate of neem oil (ECNO) was tested at 2.5, 0.5 (concentration recommended by the industry) and 0.25%. Two other treatments were used for comparisons: deltamethrin (25 EC, 0.0075%) and water (control).

Parasitoid origin and colony maintenance. *Trichogramma pretiosum* and *T. annulata* were collected from *S. catenifer* eggs on avocado in Arapongas, PR, Brazil, in 2001, and used to initiate laboratory colonies by using parasitoid rearing units (8.5 x 2.5-cm glass shell vial) and UV-treated eggs of *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) as hosts. Parasitoid ($\sim F_{10}$) colonies were started at different days to provide eggs for the experiments. Honey was provided as a carbohydrate food source for adult parasitoids.

Aqueous neem seed extract (ANSE) preparation. Neem seeds were collected from *Azadirachta indica* trees cultivated at IAPAR´s Experimental Station in Paranavaí, PR. In order to prepare the stock concentration (T_1 = 15%), 30 g of neem seeds were soaked in 200 ml of distilled water during 24h, grinded in a blender and filtered. The stock concentration was further diluted in distilled water to obtain the remaining concentrations for testing (T2 = 3.0%; T3 = 1.5%).

Side effects of host egg pre-treatment with neem formulations on *T. pretiosum* and *T. annulata* biological parameters. The ANSE (15%; 3% and 1.5%) and the ECNO (2.5%; 0.5% and 0.25%) formulations were applied on cards containing approximately 100 UV-treated *A. kuehniella* eggs, using a manual sprayer device. One, three or five days later the egg cards were individually placed into the rearing units and exposed to three parasitoid females (< 6h old) for 24h. At the fifth day after exposing the wasps to the treated host egg cards, the number of parasitized eggs and adult emergence were recorded.

This experiment followed a completely randomized factorial design 2 x 5 x 3 (species x treatment x days before host eggs were treated) and was replicated five times.

Side effects of host egg post-treatment with neem formulations on *T. pretiosum* and *T. annulata* biological parameters. Fifteen cards containing approximately 500 UV-treated *A. kuehniella* eggs/treatment were offered to hundreds of *T. pretiosum* or *T. annulata* (< 6h old) females for 24h inside 1 L jars. Egg cards were then removed and one, three or five days later (egg-larva, pre-pupa and pupa stages of the parasitoid, respectively) (Cônsoli *et al* 1999) were sprayed either with ANSE or ECNO at the previously mentioned concentrations, and placed into the rearing units for further evaluation. Parasitoid emergence was determined indirectly considering the number of host eggs containing exit holes.

This experiment followed a completely randomized factorial design 5 x 3 (treatments x days after host eggs were treated).

Side effects of neem on *T. pretiosum* and *T. annulata* longevity. The side effect of neem oil on *T. pretiosum* and *T. annulata* longevity was evaluated in two experiments that followed a completely randomized block design. In the first experiment three concentrations of ECNO (2.5, 0.50 and 0.25%) and water (control) were sprayed 24h before the host eggs were parasitized. In a second test, newly emerged females were fed 0.25% ECNO either mixed with pure honey or water. In both cases, isolated females (15 per experiment; one female = one replicate) were exposed to 200 eggs of *A. kuehniella* eggs for 24h. All tests and rearing were conducted at controlled conditions (25 ± 2°C, 70 ± 20% RH, and 14L:10D photoperiod).

Statistical analysis. Treatment effects on host parasitization, longevity and adult emergence were subjected to ANOVA and means were compared using the Least Significant Difference *t*-test (Ferreira 2000). Data on the parasitization capacity and adult emergence were sqrt (x + 0.5) and arcsine transformed before analysis, respectively.

Results and Discussion

Side effects of host egg pre-treatment with neem formulations on *T. pretiosum* and *T. annulata* biological parameters. The analysis of the isolated effect of ANSE on parasitism capacity of *T. pretiosum* and *T. annulata* revealed significant differences for species and treatments, but not for spraying time. However, there were significant interaction between the last factor and treatments (Table 1). Spraying *A. kuehniella* eggs with 15% ANSE or 0.0075% deltamethrin one, three or five days before their exposure to *T. pretiosum* females (pre-treatment) reduced parasitization significantly (74% to 84%, 86% to 95% and 77% to 93%), respectively as compared with control treatment (Table 1). A drastic negative effect on the number of *A. kuehniella* eggs parasitized by *T. pretiosum* was also reported by Gonçalves-Gervásio & Vendramin (2004) after application of 10% aqueous neem seed extract. The parasitism rates of wasps exposed to lower doses (3% and 1.5%) were not negatively affected.

The effect of the neem seed extract on parasitism capacity of *T. annulata* females was more severe as compared to *T.*

Table 1 Mean (± SE) of *Anagasta kuehniella* eggs parasitized by trichogrammatids during 24h after spraying the hosts with aqueous neem seed extract (ANSE) one, three or five days before parasitization (25 ± 2ºC, 70 ± 20% RH and 14L:10D photoperiod).

Treatment	*Trichogramma pretiosum*			*T. annulata*		
	1	3	5	1	3	5
ANSE 15%	2.8 ± 0.50 bAα	4.5 ± 1.20 bAα	3.2 ± 1.57 bAα	0.0 ± 0,00 cAα	0.0 ± 0,00 cAβ	4.0 ± 2.54 abAα
ANSE 3%	13.7 ± 2.04 aAα	9.6 ± 1.59 abAα	9.6 ± 4.07 aAα	2.3 ± 1.22 bcAβ	1.5 ± 1.54 bcAβ	3.7 ± 3.40 bAβ
ANSE 1.5%	13.0 ± 0.58 aAα	10.8 ± 2.95 abAα	17.6 ± 3.46 aAα	4.9 ± 2.27 bAβ	5.2 ± 2.48 abAα	4.1 ± 2.08 abAβ
Deltamethrin 0.0075%	2.4 ± 0.55 bAα	1.0 ± 0.32 cAα	1.0 ± 0.45 bAα	0.9 ± 0.61 bcAα	0.5 ± 0.27 bcAα	0.4 ± 0.16 bAα
Water	17.5 ± 2.50 aAα	16.7 ± 3.19 aAα	13.7 ± 1.73 aAα	16.3 ± 2.85 aAα	11.8 ± 3.05 aAα	10.3 ± 3.62 aAα

Character	DF	SS	MS	F-value	PR > F
Species (S)	1	44.075441	44.075441	42.010	0.0000
Treatments (T)	4	119.675903	29.918976	28.517	0.0000
Spraying day (SD)	2	0.785633	0.392817	0.374	0.6885
T x SD	8	15.922236	3.980559	3.794	0.0061

Means followed by the same lower case letter within columns or by the same upper case letter within rows (comparisons within species) or by a Greek letter (comparisons between species for the same day) were not significantly different (P = 0.05).

pretiosum, especially when the host eggs were sprayed 24h before the wasps had access to them. Under this condition all treatments differed from control and mortality reached up to 100% (Table 1). In the following period (three days) only the lower concentration (1.5%) was not harmful to *T. annulata* females and at five-day period only deltamethrin significantly reduced parasitism.

As occurred with ANSE data there were significant differences for species and for treatments, but not for insecticides application time when ECNO was tested. In addition significant interactions existed for insecticide and days and for the three factors (Table 2). Pre-treatment of *A. kuehniella* eggs with ECNO (2.5%) or deltamethrin (0.0075%) 24h after exposure to the wasps also reduced (over 85%) parasitism by *T. pretiosum*. The lower concentrations had no negative effect of parasitism rates (Table 2). The same trend was observed for the remaining time intervals. In contrast to the findings reported here, Rocha *et al* (unpubl.) mentioned reduction on parasitism when neem oil at 0.5 and 0.25% was tested on *T. pretiosum* on *A. kuehniella* eggs.

Similarly to ANSE, *T. annulata* females were more susceptible to ECNO than *T. pretiosum*. Reduction in parasitism capacity surpassed 95% when *T. annulata* females were pretreated (24h) with ECNO (2.5%) or deltamethrin

Table 2 Mean (± SE) of *Anagasta kuehniella* eggs parasitized by trichogrammatids during 24h after spraying the hosts with emulsible concentrate neem oil (ECNO) one, three or five days before parasitization (25 ± 2ºC, 70 ± 20% RH and 14L:10D photoperiod).

Treatment	*Trichogramma pretiosum*			*T. annulata*		
	1	3	5	1	3	5
ECNO 2.5%	1.2 ± 0.61 bAα	1.3 ± 0.84 bAα	6.0 ± 2.07 abAα	0.5 ± 0.28 bcAα	0.0 ± 0,00 cAα	1.1 ± 1.14 aAα
ECNO 0.5%	6.3 ± 2.02.68 abAα	10.3 ± 0.96 aAα	8.0 ± 1.18 aAα	10.9 ± 1.88 aAα	0.2 ± 0.26 bcBβ	4.6 ± 3.60 aBα
ECNO 0.25%	14.7 ± 3.25 aAα	10.6 ± 1.85 aAα	12.0 ± 4.08 aAα	9.6 ± 5.20 aAα	7.2 ± 1.72 abABα	1.9 ± 1.84 aBβ
Deltamethrin 0.0075%	1.5 ± 0.28 bAα	1.1 ± 0.66 bAα	2.04 ± 1.01 bAα	0.0 ± 0.00 cAα	2.6 ± 0.52 abcAα	0.0 ± 0.00 aAα
Water	10.4 ± 3.26 abAα	8.7 ± 0.70 abAα	9.24 ± 1.08 aAα	8.7 ± 2.93 abAα	15.5 ± 4,02 aAα	9.0 ± 2.99 aAα

Character	DF	SS	MS	F-value	PR > F
Species (S)	1	10.650673	10.650673	8.861	0.0035
Treatments (T)	4	102.817194	25.704298	21.384	0.0000
Spraying Day (SD)	2	5.165177	0.392817	2.149	0.1211
Tx SD	8	22.655972	2.831997	2.356	0.0217
SxTxSD	8	20.072075	2.509009	2.087	0.0421

Means followed by the same lower case letter within columns or by the same upper case letter within rows (comparisons within species) or by a Greek letter (comparisons between species for the same day) were not significantly different (P = 0.05).

(0.0075%) (Table 2). The lower concentrations of ECNO did not reduce parasitism significantly. The effect of the oil was even more drastic at the third day pre-tretment period, and at the fifth day after spraying, despite the drastic reduction on parasitism on most of the treatments, the differences were not significant.

Side effects of host egg post-treatment with neem formulations on *T. pretiosum* and *T. annulata* biological parameters. Post-treatment of the host eggs 24h after parasitism (egg-larval stage) with ANSE 15% or 3% reduced *T. pretiosum* emergence by 86% and 58%, respectively, in comparison with control treatment (Table 3). For the remaining developmental stages only the highest neem concentration significantly affected *T. pretiosum* emergence. This is an indicative that the egg-larval developmental period was the most susceptible stage when *T. pretiosum* was exposed to *A. kuehniella* eggs treated with ANSE (Table 3). Gonçalves-Gervásio & Vendramin (2004) reported also reduction on emergence of *T. pretiosum* on *A. kuehniella* eggs sprayed with 10% aqueous neem extract. However, the authors found the pupa as the most susceptible developmental stage. Differences due to parasitoid intrinsic characteristics and/or methodological differences may explain the contradictory results.

Anagasta kuehniella eggs treated with ECNO 2.5% or deltamethrin 0.0075% reduced *T. pretiosum* emergence in comparison with control treatment (Table 4). The same response was obtained with 2.5% and 0.5% concentrations for eggs treated three days after parasitization by *T. pretiosum*. Spraying the host eggs with neem oil at 2.5, 0.5 or 0.25%, three days (prepupal stage) after parasitization, resulted in much drastic effect on emergence than when the eggs were treated 24h (egg-larval stage) after parasitism (Table 4). However, there was no apparent differences between hosts treated 24h or five days after parasitism as well as on the effects of the compounds on the emergence for the later time (five days) period. The results indicated that 3-day-old (pre-pupal stage) parasitized eggs were more susceptible to neem oil. Contrasting results were reported by Raguraman & Singh (1999), who did not found influence of the neem seed oil extract (0.3 to 5%) on immature development and adult emergence of *T. chilonis* females exposed to post-treated *C. cephalonica* eggs. Similarly, Oliveira *et al* (2003) revealed no significant effect on emergence of *T. pretiosum* reared on *A. kuehniella* eggs when the commercial neem oil (1.25 to 2.0%) was used.

Effect of neem on *T. pretiosum* and *T. annulata* survival. Treating host eggs with ECNO at concentrations varying from 2.5% to 0.25%, 24h before exposing them to females, did not affect *T. pretiosum* longevity, but the highest concentration (2.5%) reduced *T. annulata* survival (Table 5). Oliveira *et al* (2003), however, reported significant reduction on longevity of *T. pretiosum* on *A. kuehniella* eggs even when concentrations

Table 3 Percentage of *Trichogramma pretiosum* emergence (x ± SE) from *Anagasta kuehniella* eggs treated with aqueous neem seed extract (ANSE) one, three or five days after parasitism (25 ± 2°C, 70 ± 20% RH and 14L:10D).

Treatment	Stages of development (days after treatment)		
	Egg-larva (1)	Prepupa (3)	Pupa (5)
ANSE 15%	9.9 ± 1.80 dB	75.1 ± 2.30 bA	79.1 ± 1.94 aA
ANSE 3%	39.9 ± 4.26 cB	82.0 ± 2.01 abA	76.6 ± 3.95 aA
ANSE 1.5%	78.8 ± 1.29 bB	87.1 ± 1.32 aA	84.6 ± 1.72 aAB
Deltamethrin 0.0075%	72.4 ± 2.75 bB	90.1 ± 0.91 aA	76.5 ± 3.09 aB
Water	89.3 ± 0.74 aA	87.5 ± 0.91 aA	82.8 ± 2.16 aA

Means followed by the same lower case letter within columns or by the same upper case letter within rows were not significantly different (P = 0.05).

Table 4 Percentage of *Trichogramma pretiosum* emergence (x ± SE) from *Anagasta kuehniella* eggs treated with emulsible concentrate neem oil (ECNO) one, three or five days after parasitism (25 ± 2°C, RH 70 ± 20% and 14L:10D).

Treatment	Stages of development (days after treatment)		
	Egg-larva (1)	Prepupa (3)	Pupa (5)
ECNO 2.5%	84.9 ± 1.91 aA	44.9 ± 3.24 cB	77.6 ± 3.09 aA
ECNO 0.5%	85.3 ± 2.34 aA	77.4 ± 2.53 bB	81.9 ± 1.90 aAB
ECNO 0.25%	89.9 ± 1.23 aA	83.9 ± 2.06 aA	85.0 ± 1.72 aA
Deltamethrin 0.0075%	86.3 ± 1.20 aA	82.1 ± 2.30 abA	79.8 ± 2.86 aA
Water	91.0 ± 1.27 aA	89.8 ± 1.92 aA	79.0 ± 3.90 aB

Means followed by the same lower case letter within columns or by the same upper case letter within rows were not significantly different (P = 0.05).

Table 5 Survival in days (x ± SE) of trichogrammatids emerged from *Anagasta kuehniella* eggs treated with emulsible concentrate neem oil (ECNO) 24h before parasitism (25 ± 2°C, 70 ± 20% RH and 14L:10D photoperiod).

Treatment	*Trichogramma pretiosum*	*T. annulata*
ECNO 2.5%	11.6 ± 0.69 a	7.6 ± 0.69 b
ECNO 0.5%	10.0 ± 0.72 a	9.8 ± 0.97 a
ECNO 0.25%	10.1 ± 0.81 a	9.9 ± 0.53 a
Water	7.4 ± 0.24 b	9.8 ± 0.70 a

Means followed by the same letter within columns were not significantly different (P = 0.05).

varying from 1.25% to 2% were tested.

Feeding *T. pretiosum* with honey mixed with ECNO 0.25% did not affect female's longevity when compared to those feeding on concentrated honey alone (Fig 1). In contrast, this mixture reduced longevity of *T. annulata* in 70%. Raguraman & Singh (1999) tested the effect of neem seed oil (0.3 to 5.0%) mixed with honey on *T. chilonis* longevity and did not find influence of the botanical insecticide on survival of males and females. The different response of the parasitoid species to the ingestion of honey mixed with neem oil may be due to intolerance to the botanical insecticide.

The effect of neem on biological parameters of parasitoids varied according to the species, product formulation, concentrations and time of application. *Trichogramma pretiosum* was less affected by the botanical insecticide than its counterpart. Comparisons on the effects of the two neem formulations (ANSE or ECNO) on parasitism capacity revealed that the aqueous neem extract was more deleterious than the emulsible neem oil, however, the differences were significant only for *T. pretiosum* ($P < 0.0005$). The same response was observed when the effect of both formulations on the emergence of *T. pretiosum* was compared ($P < 0.0000$).

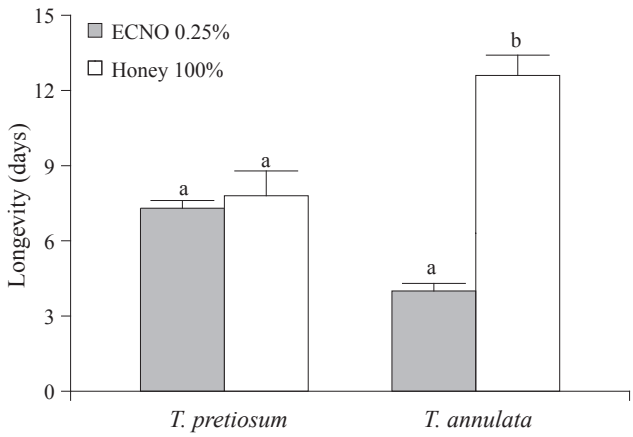

Fig 1 Survival in days (x ± SE) of species of *Trichogramma* fed on a mixture of emulsible concentrate neem oil (ECNO) during 24h (25 ± 2°C, RH 70 ± 20% and 14L:10D photoperiod). Means followed by the same letter were not significantly different (P = 0.05).

The reasons for more severe side effects of ANSE to the parasitoids were not clear, but could be related to the higher toxicity of the aqueous neem seed extract doses used as compared to the doses of the commercial oil, or due to higher concentration of azadirachtin in the aqueous solution. These hypotheses, however, need to be proved correct.

The time of spraying (pre or post-treatment) had different consequences to parasitoid survival. Treating the host eggs before exposing them to the females was more detrimental to parasitoid development and emergence than when the compounds were otherwise sprayed. Treating hosts after parasitization avoids the deterrent effects and mortality that the products may cause on adult wasps, thus affecting parasitism rates. Moreover, post-treatment of the host eggs affects less wasp emergence; this is more evident for *T. annulata*, either treated with ANSE or ECNO (Hohmann unpubl.). When spraying occurs after parasitism the immatures will be well protected inside the host eggs shell as it is well documented here and elsewhere (Raguraman & Singh 1999, Gonçalves-Gervásio & Vendramin 2004), except when the compounds were used at the highest concentrations.

The sensitivity of the parasitoid immatures, nonetheless, varies with its developmental stage and with neem formulation. The egg-first larval instar was the most affected stage when ANSE was used, whereas ECNO was more deleterious to prepupal stage.

Despite the data showed differences between the two egg parasitoid species, favoring *T. pretiosum,* additional studies are needed, using natural hosts under field conditions, to have a more reliable response of the side effects of the botanical insecticide on theses beneficial insects. The outcome of this study may contribute to a better understanding of the potentialities of using an integrated control approach, including egg parasitoids of the family Trichogrammatidae and neem, to control avocado and persimmon lepidopterous pests in organic managed systems.

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