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Impact of neem and chinaberry fruit extracts on the pest/parasitoid *(Pieris rapae/Hyposoter ebeninus)* interactions

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Abstract

A new technique was performed to simulate exposure of the host *Pieris rapae* larvae to both botanical treatment and the parasitoid *Hyposoter eheninus* in different sequences. It was found that: host larvae subjected to both parasitoid and 1 % neem treatment showed significant or insignificant reductions both in pupal formation as well as adult emergence percentages when treatment preceded or followed parasitism, respectively. Both neem treatments revealed significant prolongation in the development of unparasitized and parasitized larvae (on average 4 to 5 days delay), whereas chinaberry caused significant prolongation only among parasitized larvae (on average 2.7 days delay in egg-larval duration). Fate of parasitism among untreated hosts was found to be faster than among neem-treated ones. Parasitism percentages among 1 and 0.5 % neem-treated third instar host larvae held 7 days before parasitism reached 3 and 2 times that achieved among those reared on untreated diet for the same period, respectively.

It was concluded that prolongation of the preferred target instars of the host, due to neem treatments, increased the chance for parasitism. Nevertheless, treatment with neem at the LC_{50} level exhibited a great reduction in parasitoid progeny. However, a lower concentration (LC_{25}) could reasonably potentiate parasitism without drastic losses in parasitoid emergence.

1 Introduction

Pieris rapae L. is a serious lepidopterous pest, attacking cabbage, cauliflower and many crucifers in Egypt. Protective measures using chemical control represent a great menace to human health, since the host plants are used in the human diet. For this reason and due to the pressing need to protect natural enemies, particularly the recently recorded, efficient endoparasitoid, *Hyposoter ebeninus* Grav. (Abbas and Hassanein, 1989), viable alternatives to conventional insecticides are, now, strongly advocated.

The wide-spectrum entomostatic neem derivatives have proven satisfactory entomocidal, repellent and/or deterrent effects against most pests attacking cabbage (Jacobson et al., 1978; Schauer and Schmutterer, 1980; El-Sayed, 1982 a, b; Matter and El-Borollosy, 1993; Matter et al., 1993). Reasonable safety of neem extracts to predaceous arthropods has been indicated in some research studies (Kaethner, 1991; Matter and El-Borollossy, 1993; Matter et al., 1993). However, information about the effect of neem or chinaberry on the host/parasitoid system is scarce. Therefore, it was convenient to explore their impact on the potential parasitoid *H. ebeninus* aiming to corroborate or disapprove their integration in a control program.

2 Material and methods

2.1 Host and parasitoid

Field samples of immature stages of *P. rapae* were collected from infested cabbage cultivations. The parasitoid pupae of *H. ebeninus* were easily distinguished through the host's translucent body wall as dark brown cocoons with 3–5 white strips (Abbas and Hassanein, 1989). Two separate colonies of both species were raised under laboratory conditions (25 ± 2 °C and 40–60 % R. H.) according to Matter (1993). The colonies were used for supplying the different experiments with the target stages. Mating of adults was accomplished in a large cage (2 × 1.5 × 1.8 m).

2.2 Plant extracts

Ethanol extracts of neem (*Azadirachta indica*) and chinaberry (*Melia azedarach*) fruits were obtained by extraction of the crushed kernels, using a Soxhlet apparatus and ethyl alcohol as solvent in a water bath until complete exhaustion. The solvent was then evaporated under reduced pressure, and the remains were used. The extract emulsions were prepared by mixing thoroughly 0.5 ml 1 % "superfilm" (emulsifier produced by Alexandria Corporation for Chemical Products, Alexandria, Egypt) with 5 ml of the oil, then completed with water to 100 ml to obtain a 5 % oil emulsion. Further dilutions were prepared by addition of the appropriate amounts of water.

2.3 Application methodology

The following experiments were oriented to evaluate the different effects on the host pest and its parasitoid.

To simulate field application, groups of pots planted with cabbage were used before head formation. They were sprayed, each, with the desired concentration of the proposed botanical extract emulsion to the point of run off. A small handsprayer (1,5 L capacity) was used to apply the various concentrations. Control groups were sprayed only with water and the emulsifier.

2.4 Determination of the entomotoxic effects of the botanical extracts on unparasitized larvae

Six concentration levels were prepared for each botanical extract. They ranged from 0.0125 to 2 % at 1:2-fold dilutions. One hundred larvae (4 replicates of 25 each) were used per concentration and instar. The larvae were starved for 4 hours, then they were offered, after being dry, sprayed cabbage leaves from the correspondingly treated pots. Every other day, the leaves were replaced by others from the same source, while dead larvae

were counted and discarded daily till complete pupal formation. Cumulative percent mortalities were calculated at pupation and corrected in each case according to ABBOTT (1925). Slope values and LC levels were calculated according to Probit analyses (FINNEY, 1972).

2.5 Assessment of the indirect effects of the botanical extracts on parasitized larvae (larval treatment preceded or followed parasitism)

Forty-five third instar larvae (3 replicates, 15 each)/concentration/time-interval/treatment were used. The larvae were offered treated leaves as described. Neem extract was used at the LC_{50} (about 1 %) level, while chinaberry was used at the highest possible (from an economic point of view) concentration (2 %). The day of larval treatment was designated day "0". Parasitization were carried out on –1, –3, +1 and +3 days relative to the botanical treatment date. To ensure parasitism, the larvae were exposed to the parasitoid for 8 hours in separate vials at the rate of 3 larvae/3 newly mated female parasitoids (ABBAS and HASSANEIN, 1989). Percentages of pupal formation and adult emergence were calculated in each case. Reductions in parasitoid formation were calculated according to the equation:

$$1 - \frac{\text{No. of formed parasitoids in Tgp.}}{\text{No. of formed parasitiods in Cgp.}} \times \frac{\text{No. of Tested parasitized larvae in Cgp.}}{\text{No. of Tested parasitized larvae in Tgp.}} \times 100$$

2.6 Effects of botanical extracts on the development of the host and the parasitoid

Untreated parasitized or not parasitized third instar host larvae were used as controls. Parasitism was achieved, or not, one day after botanical treatment by subjecting 15 larvae to one mated female parasitoid for 24 h. In each case, 45 larvae (3 replicates of 15 larvae each) were used. Pupal formation was traced every other day. Pupae were isolated, singly in separate vials, till emergence of adults. The average duration of egg-larval stages of the parasitoid as well as larval stage duration of the pest was calculated in each case. The data were subjected to statistical analyses using F-test.

2.7 Fate of parasitism in treated and untreated host larvae

The method described by MATTER (1993) was adopted. Groups of third instar larvae (3 replicates/group and 30 larvae/replicate) were used. The 1st, 2nd, 3rd and 4th groups were bred on untreated cabbage leaves for 1, 3, 5 and 7 days respectively while the 6th, 7th and 8th groups were bred on treated cabbage leaves for the same periods, respectively.

At each time-interval, twenty survivors (per replicate) of the same age from treated as well as control groups were marked on their body, with one spot on the front (treated) or one spot on the back (control). Thereafter, they were placed in a 5 L glass vial with four newly mated female parasitoids for 12 h. During that exposure period, the parasitoid females were allowed free choice to select the desirable ovipositional site among hosts from both groups. Then, the larvae of each treatment group were transferred to their corresponding vial with the corresponding diet till maturation. Dead larvae were dissected under stereomicroscope to verify the presence of the parasitoid adopting (THOMS and WATSON, 1986). Parasitism and mortality percentages among parasitized and unparasitized groups were calculated in each case. Host/parasitoid ratio was calculated among emerged adults from neem-treated as well as from untreated larvae in each case. Botanical extracts used in this experiment were those showing significant retarded effects.

Table 1. Entomocidal effects of neem-ethyl extract on larvae treated in the 2nd, 3rd and 4th instar.

Aspect	Instars		
	2nd	3rd	4th
Slope	2.61	2.44	2.18
LC_{25}	0.44	0.54	0.53
LC_{50}	0.79	1.05	1.07

2.8. Direct effect of botanical extracts on adult parasitoid longevity

The extract suspension was added to the wasp's feeding solutions at the rate of 0.05, 0.1, or 0.2 ml per 10 ml of 5 % sucrose solution for 0.5, 1 % neem and 2 % chinaberry treatments, respectively. For the control group, the feeding solution comprised water and emulsifier only. For each treatment, five replicates (five newly emerged wasps/replicate) were used. Cotton pieces immersed in the prepared solutions were offered to the adults and replaced by new ones every other day from the same source. Living adults/replicate/treatment were counted every other day until all adults died.

3 Results and discussion

3.1 Effects of botanical extracts on unparasitized host larvae

The bioassay of neem ethyl extract using three larval instars of *P. rapae* showed that the slope values of mortality curves gradually decreased as larvae developed from 2nd to 4th instar (table 1). Probit analysis also evaluated higher susceptibilities of 2nd instar larvae to the botanical extract than either 3rd or 4th instar larvae.

The third instar larvae were selected as the target insects because the botanical agent is almost equi-toxic to both 3rd and 4th instar larvae and it also represents the preferable ovipositional site for the parasitoid. Attempts to use 2nd instar larvae or neem extract at the LC_{90} level were not successful because there were not enough survivors to evaluate the combined effects of both agents. Chinaberry showed low entomocidal effects and was, therefore, used at the highest possible concentration (2 %).

3.2 Indirect effect of botanical treatment on the parasitoid

The effects of different sequences of exposures of third instar *P. rapae* larvae to bioagents on the pupal formation and emergence percentages of the parasitoid are shown in table 2.

Pupal formation among untreated parasitized larvae was about 69 %. While successful formation of the parasitoid among 1 % neem-treated hosts was mainly dependent upon the date of treatment relative to the day of parasitism. When treatment of host larvae preceded parasitism by one or three days, the percentages of formed pupae were in both cases about half of those obtained

Table 2. Effect of neem and chinaberry-ethyl extracts on the pupation and emergence of *H. ebeninus* when treatment preceded or followed parasitism.

Parasitized on day "0" and treated on day	Mean number of formed parasitoid pupae ± S. E.	% pupation	Mean number of emerged parasitoids	% pupal mortality	% emerged parasitoids
			1 % neem		
–3	5.00 ± 1.0 bc (4–7)	33.33	2.67 ± 1.20 b (1–5)	46.6	17.8
–1	3.67 ± 0.88 c (2–5)	24.67	2.33 ± 0.67 b (1–3)	36.5	15.5
+1	10.00 ± 1.73 a (7–13)	66.67	9.0 ± 1.73 a (6–12)	10.0	60.0
+3	8.00 ± 1.15 ab (8–14)	53.33	7.0 ± 1.53 ab (5–10)	5.4	64.5
Parasitized and not treated (control)	10.33 ± 1.45 a (8–13)	68.87	9.67 ± 1.45 a (7–12)	5.4	64.5
			2 % chinaberry		
–3	7.33 ± 1.30 abc (6–10)	48.87	7.00 ± 1.00 a (6–9)	4.5	46.7
–1	9.00 ± 1.73 ab (6–12)	66.00	7.67 ± 2.03 a (4–11)	14.8	51.1
+1	11.00 ± 2.08 a (8–14)	73.33	11.00 ± 2.31 a (8–14)	0.0	73.3
+3	9.67 ± 0.88 a (8–11)	64.5	9.00 ± 1.15 a (7–11)	6.9	60
Parasitized and not treated (control)	10.33 ± 1.45 a (8–13)	68.87	9.67 ± 1.45 a (7–12)	5.4	64.5

Similar symbols within each column represent insignificant differences.

when parasitism preceded treatment by three or one day(s), respectively.

Statistical analysis of data showed that the mean numbers of formed pupae in neem-treated groups were only significantly less than that in the control group when exposure to neem treatment preceded parasitism, irrespective of the interval period between both exposures. Reasonable pupal mortalities (36.5–46.6 %) were evaluated only when treatment with 1 % neem preceded parasitism. Significantly high (72.4–76.0 %) or insignificantly low (6.9–27.6 %) reductions in adult emergence of the parasitoid, as compared with the corresponding controls, were revealed when neem treatment preceded or followed parasitism, respectively. Chinaberry treatment at the 2 % concentration level, caused no significant decrease or increase in pupal formation and adult emergence, as compared with control, respectively. Also, different sequential exposures showed no significant differences in either criteria.

Increasing tolerance to neem treatment among preparasitized larvae might be attributed to their feeding behaviour after parasitism. It was reported that some endoparasitoids decrease the daily food consumption of the host, particularly, at late stages of parasitoid development (Marchal Segault, 1975; Thompson 1982; Yasteslad, 1986; Matter, 1993). Consequently, with such reduced uptake, botanical dose might be below the threshold of effective entomocidal dose. Such sublethal doses cause some prolongation in development, but they do not prohibit parasitoid formation. On the other hand, when treatment preceded parasitism, the effect of the whole uptake dose of the effective botanical ingredient may interact positively with the other stress or (parasitism), resulting in the enhancement of premature host death and, consequently, in the the reduction of parasitoid formation.

3.3 Effect of neem and chinaberry extracts on the developmental rate of host and parasitoid

The ethanol extract of neem was used at the LC_{50} and LC_{25} (about 1 and 0.5 % concentration levels). Chinaberry extract was used at the 2 % concentration level. Treated and untreated third instar *P. rapae* larvae were subjected or not to the parasitoid as described. Figures 1 and 2 illustrate the percentage pupal formation, calculated, cumulatively, every other day, for unparasitized and parasitized larvae. It was markedly obvious that the rate of development of unparasitized as well as parasitized larvae was greatly affected by both neem treatments as compared with the corresponding controls. (80.0, 35.6, 64.4 and 68.9 % pupated in the control, 1 %, 0.5 % neem and 2 % chinaberry, groups, respectively). Though host larvae failed surviving treatments or achieving normal pupation were observed exhibiting much longer periods than those surviving treatment, the latter also showed various levels of growth retardation. Based on the total number of survivors, on day 22nd, 88.9 % of the untreated unparasitized larvae reached pupation compared with 56.3, 44.8 and 64.5 % in 1 %, 0.5 % neem and 2 % chinaberry groups, respectively. The remaining survivors in the control (11.1 %), 1 % neem (43.7 %), 0.5 % neem (55.2 %) and 2 % chinaberry (35.5 %) groups achieved pupation during the following four, eighteen, fourteen and eight days, respectively. The mean

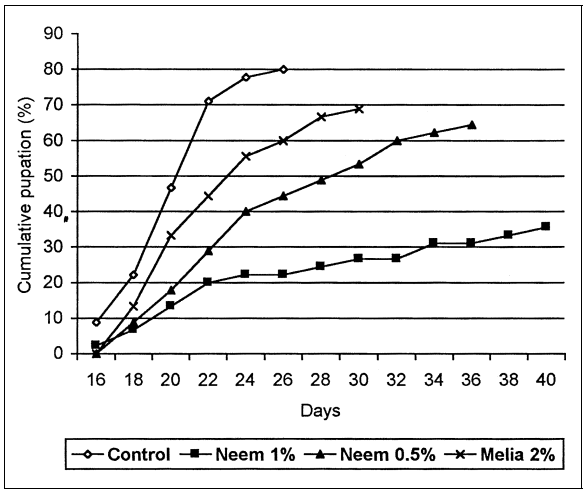

Fig. 1. Cumulative percent pupation of treated or not 3rd instar *P. rapae* larvae (based on the number of tested individuals).

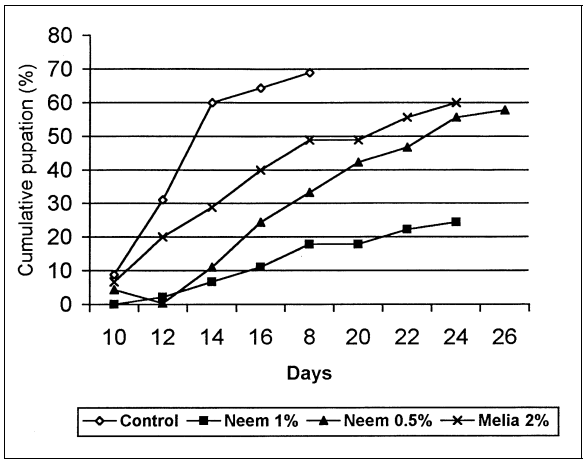

Fig. 2. Cumulative percent pupation of *H. ebenimus* as affected by host treatments with neem or chinaberry ethyl extracts (based on the number of tested individuals).

larval duration of survivors in the control, 1 % neem, 0.5 % neem and 2 % chinaberry were 20.33 ± 0.41, 24.00 ± 1.61, 24.69 ± 0.97 and 22.06 ± 0.62 days, respectively.

Statistical analysis revealed insignificant and significant prolongation in the duration of larvae surviving the chinaberry and both neem treatments, respectively (F = 4.595, L. S. D. = 2.455).

The evaluated average duration of larvae surviving 0.5 % neem treatment was higher than that for larvae surviving 1 % neem treatment. This was, most probably, because a great number of slowly developed larvae could not survive the botanical treatment at its high concentration as well as at its lower concentration and were consequently not considered in the count.

Regarding parasitized larvae, survivors of parasitoid pupae were about 68.9, 24.4, 57.8 and 24.4 %, of the tested larvae in control, 1 % neem, 0.5 % neem and 2 % chinaberry treatment groups, respectively. About 87.1, 27.3, 19.2 and 44.8 % of survivors reached pupation fourteen days after parasitism in the same groups, respectively. The remaining survivors reached pupation during the periods form (14–18), (14–24), (14–24) days after parasitism, respectively. The means of egg-larval duration of the parasitoid in the control, 1 % neem, 0,5 % neem and chinaberry treatment groups were 13.23 ± 0.27, 17.62 ± 1.14, 18.3 ± 0.84 and 15.70 ± 0.82, respectively. Statistical analysis proved that all treatments caused significant prolongation in the egg-larval period of the parasitoid (F = 10.344, L. S. D. = 2.085).

Many authors report that azadrachtin, the most active ingredient in neem extracts, has phagorepellency, growth retardation, and insecticidal properties (REDFERN et al., 1980; GUJAR and MEHROTRA, 1983; ARNASON et al., 1985; OPENDER, 1985).

3.4 Fate of parasitism in neem-treated and untreated host larvae

According to ABBAS and HASSANEIN (1989) the 2nd and 3rd instar larvae of *P. rapae* are the preferable ovipositional sites for the parasitoid *H. ebeninus*. Previous experiments revealed that parasitized and unparasitized 3rd instar host larvae subjected to neem-treated diet exhibited various entomocidal and growth retardant effects.

In a trial to assess how far delayed development and growth of the preferable ovipositional instars or sizes of the host larvae would be advantageous for the parasitoid, the fate of parasitism in neem-treated and untreated 3rd instar *P. rapae* larvae was performed as described.

Tables 3 and 4 show parasitism percentages in neem-treated and untreated groups of 3rd instar host larvae when equal numbers of survivors from both groups were subjected to parasitism (free choice exposure) at 1, 3, 5 and 7 days post treatment.

Parasitism percentages among larvae surviving 1 or 0.5 % neem treatment for one day were similar to those obtained among untreated hosts of the same age in the corresponding control groups. This indicates that the parasitoid does not discriminate between treated and untreated hosts of almost the same size and instar. When parasitism followed exposure to either neem-treated or untreated diet by 3 or 5 days, parasitism percentages among treated larvae were generally higher than those evaluated for the corresponding untreated ones. Larvae treated with 1 or 0.5 % neem and held for 7 days before exposure to the parasitoid revealed about three or two times higher parasitism percentages than the corresponding values achieved among untreated larvae of the same age, respectively. This was, most probably because the treated larvae remained longer at the preferable ovipositional instar and size for the parasitoid. ABBAS and HASSANEIN (1989) considered that younger instars (2nd and 3rd) of *P. rapae* are the preferable oviposition targets for the parasitoid *H. ebeninus*. Some authors claim that the growth retardant properties of some biological agents allow more chances for parasitism because it extends the duration and consequently the exposure period of such target instars (WESELOH and ANDEREADIS, 1982; WEALNER et al., 1983; MATTER, 1993). Microscopical examination of dead larvae and pupae in neem-treated groups showed markedly higher mortalities among parasitized larvae than that among larvae which escaped parasitism within the same treatment groups.

Table 3. Parasitism (free choice) and mortality percentages among 1 % neem-treated and untreated growing larvae.

Days after treatment and before parasitism	Treatment	% Parasitism	% Mortality		Host/parasitoid ratio among emerged adults
			Host	parasitoid	
1	Neem	66.0	38.1	61.5	0.87
	No Neem	68.3	10.5	17.1	0.50
3	Neem	73.3	50.0	59.1	0.44
	No Neem	63.3	9.1	15.8	0.33
5	Neem	68.3	52.6	68.3	0.69
	No Neem	45.0	12.1	22.2	1.39
7	Neem	58.2	48.0	82.9	2.17
	No Neem	20.0	18.8	16.67	3.90

Table 4. Parasitism (free choice) and mortality percentages among 0.5 % neem-treated and untreated growing larvae.

Days after treatment and before parasitism	Treatment	% Parasitism	% Mortality		Host/parasitoid ratio among emerged adults
			Host	parasitoid	
1	Neem	63.3	27.3	28.9	0.592
	No Neem	60.0	4.2	13.9	0.741
3	Neem	66.7	25.0	32.5	0.556
	No Neem	55.0	11.1	18.2	0.885
5	Neem	70.0	27.8	26.2	0.420
	No Neem	50.0	6.7	20.0	1.170
7	Neem	48.3	32.3	31.0	1.050
	No Neem	26.7	11.4	25.0	3.226

Using 1 % neem, mortality percentages among parasitized larvae surpasses the corresponding percentages obtained among those escaped parasitism within the same treatment group at all time intervals. This indicates that parasitism potentiates the entomocidal effect of neem at this concentration level. Such potentiation was accentuated as the time elapsing between treatment and parasitism was increased.

In a trial to asses how far the botanical treatment affects the pest/parasitoid system, the ratios between emerged hosts and parasitoids from parasitized treated larvae were compared with the corresponding ratios among untreated parasitized larvae. The data show that host parasitoid ratios among emerged adults in 1 % neem-treated groups were about 1.74, 1.33, 0.50 and 0.56 times those among the corresponding untreated groups when treatments preceded parasitism by 1, 3, 5 and 7 days, respectively (table 3). The corresponding figures when neem was applied at the LC_{25} level (0.5 %) were 0.80, 0.63 and 0.33 for the same intervals, respectively (table 4).

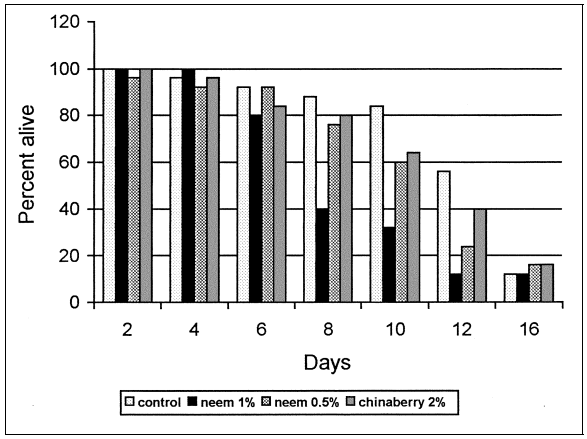

Fig. 3. Direct effects of botanical treatments on the longevity of the adult parasitoid.

3.5 Direct effect on the adult parasitoids

The data illustrated in fig. 3 showed that continuous exposure of the parasitoid adults to 1, 0.5 % neem- and 2 % chinaberry-treated diets did not affect their longevity before the 6th, 10th and 10th day, respectively. Feeding on the above diets for 12 days resulted in 78.6, 57.1 and 28.6 % reductions as compared with control, respectively. In another experiment, where exposure to 1 % neem-treated diets lasts 2 days only, a slight decrease (8–12 %) in the alive adults were evaluated as compared with the check after 10–16 days from emergence. This proved that neem-treated diet might affect the longevity of adults if it is the only food source over a long period of time which is not usual in nature.

In conclusion, with the foregoing results it could be mentioned that parasitization of neem-treated host larvae at both LC_{50} level, higher mortalities among parasitoid larvae and pupae were adversly inflicted on parasitoid production. When neem was applied at a moderate rate (LC_{25} level), increasing parasitism would occur without affecting the progeny of the parasitoid.

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