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Mortality and predation efficiency of *Coleomegilla maculata lengi* Timb. (Col., Coccinellidae) following application of Neem extracts (*Azadirachta indica* A. Juss., Meliaceae)

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Abstract: The toxicity of two neem (*Azadirachta indica*) extracts and of the chemical insecticide malathion was evaluated on adults of the predacious coccinellid *C. maculata*. Bioassays were carried out with both neem formulations at 1, 5, 10, 20 and 50% concentration. The azadirachtin quantities contained in neem oil (v/v) and neem seed kernels (w/v) were 13.7 ppm AZA and 91.0 ppm AZA respectively. Malathion was tested at field rate (2.85 g (AI)/L). The mortality rate was evaluated in the laboratory after topical applications on the ventral side of the coccinellids. Adult mortality rate after 72 h was 100% following malathion treatments. Only one dose of neem oil (10%) resulted in significantly greater mortality than in the control group. No toxicity was observed after the treatments with the aqueous suspension of ground neem seeds. Predation efficiency of *C. maculata* was also evaluated after topical application of these three insecticides at sublethal doses. Fifteen min after treatments, adult coccinellids were presented 30 aphids for 24 h. The aqueous suspension of ground neem seeds caused a 50% reduction in the number of aphids consumed.

1 Introduction

Many parts of the neem tree, *Azadirachta indica* A. Juss. (Meliaceae), notably the fruits, possess interesting insecticidal properties for phytoprotection (KETKAR, 1976; JACOBSON, 1986; SAXENA, 1989). Several neem compounds, principally azadirachtin, are repellent or act as antifeedants for phytophagous insects (KETKAR, 1976; JACOBSON, 1986). They may also affect the reproduction and the development of insects by inhibiting oviposition and by interfering with larval molts (SCHMUTTERER, 1990). Neem extracts cause behavioral or physiological effects in more than 200 insect pest species (SCHMUTTERER et al., 1981; SAXENA, 1983; SAXENA, 1989; SCHMUTTERER, 1990). However, little information has been published on their effects on beneficial insects. Published studies indicate that unlike conventional insecticides, neem derivatives appear to have few negative effects on beneficial organisms and humans (SCHMUTTERER, 1990; SAXENA, 1989).

No adverse effects were observed in studies carried out on the impact of neem derivatives on eggs, larvae and pupae of parasitoids such as *Dieraetiella rapae*, *Aphidius cerasicola* and *Telenomus remus* (JOSHI et al., 1982; SCHAUER, 1983). Similar results were obtained with the Miridae *Curtorhinus lividipennis* Reut. and the spider *Lycosa pseudoannulata* (Boes. & Str.), two efficient predators of many rice pests (SAXENA et al., 1984; SAXENA, 1987). Neem seed extracts were considerably toxic toward a phytophagous mite, *Tetranychus cinnabarinus* Boisd., but not toward its predator *Phytoseiulus persimilis* A.-H. and the spider *Chiracanthium mildei* L. Koch (MANSOUR et al., 1987). Predatory coccinellids survived the application of a formulation containing a high (unspecified) neem oil content, while the targeted pest, the sorghum aphid *Melanaphis sacchari*, was successfully controlled with the neem extract (SRIVASTAVA and PARMAR, 1985). Neem-based extracts are effective as aphicides (SCHAUER, 1983; PATEL and SRIVASTAVA, 1989; LOWERY et al., 1993). Azadirachtin is also reported to interfere with the feeding and probing behavior of many aphid species (WEST and MORDUE, 1992).

The Coccinellidae are insect predators found in many crops of economic importance such as maize (WRIGHT and LAING, 1980; CODERRE and TOURNEUR, 1988), cereals (SHADE et al., 1970), leguminous plants (HODEK, 1973) and potatoes (BOITEAU, 1983). *Coleomegilla maculata* Timb. is a nearctic polyphagous species frequently found in many of these crops. It is an efficient predator of aphids, which constitute its principal food source (HODEK, 1973; CODERRE et al., 1987). It can also feed on eggs and larvae of many lepidopteran (CONRAD, 1959; WARREN and TADDIC, 1967; ANDOW and RISCH, 1985) and chrysomelid species (SHADE et al., 1970; HAZZARD and FERRO, 1991), as well as pollen from many plants (HODEK, 1973; CODERRE et al., 1987).

Simultaneous utilization of two control methods requires more than the independant evaluation of both botanical pesticides and natural enemies. Evaluation of the toxicity (direct effect) of neem extracts on *C. maculata* adults, as well as their effects on predation efficiency (sublethal effects), were evaluated. Results will be compared to those obtained with malathion. This insecticide is currently used in many crops of economic importance. Many predatory coccinellid species, includ-

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ing *C. maculata*, were very susceptible to the application of this conventional insecticide (Mok Yun and Ruppel, 1964; Croft and Brown, 1975; Roger et al., 1991). This study would allow the implementation of a sound integrated pest management program.

2 Material and methods

Adult coccinellids *C. maculata* used in the first three replicates were collected in maize fields in Saint-Hyacinthe (72°56′W, 45°39′N), Québec, Canada, during July 1989. The eight other replicates were carried out with coccinelids collected in October 1989 on hibernation sites near maize fields in Saint-Hyacinthe. The adults were placed in 375 ml glass containers and kept at 22°C, 65% RH with a photoperiod of 16:8 (L:D). They were maintained on a diet of clover pollen.

2.1 Effects on mortality

The oil was extracted from neem seeds collected in 1987 at Leguema, ca. 20 km NW of Bobo-Dioulasso (11°11′N, 4°18′W), Burkina Faso, West Africa. The neem oil was emulsified with water and a liquid detergent (Triton-X 1.5%). The neem seed kernels were ground and mixed with distilled water. Bioassays were carried out with both formulations at 1, 5, 10, 20 and 50% concentration. The azadirachtin quantities contained in neem oil (v/v) and need seed kernels (w/v) were 13.7 ppm AZA and 91.0 ppm AZA respectively. The azadirachtin content was determined using reverse phase gradient, high performance liquid chromatography (HPLC) as described by Isman et al. (1990). Malathion treatments (Malathion 50 [emulsifiable concentrate], American Cyanamid Co., Wayne, NJ) were done at the mean dose recommended by the Quebec Ministry of Agriculture (C.P.V.Q., 1987): i.e. 2.85 g (AI)/liter.

A 5 μl distilled water solution containing the desired insecticide quantity was applied on the ventral side of each coccinellid with a Pipetman P20 (Gilson). The coccinellids of the control groups were treated with the carrier only: distilled water for the first group and 98.5% distilled water with 1.5% liquid detergent (Triton-X 1.5%) for the second group. To decrease activity during insecticide applications, the beetles were maintained in concavities of aluminum plates deposited on an ice bed. After treatment, the beetles were placed on a filter paper covering the bottom of a Petri dish (9 cm diam.) along with a wet cotton ball and satiated with pollen. The Petri dishes were then placed back in growth chambers for observation.

For every treatment, the experimental design consisted of 11 replicates of six individuals. All treatments were carried out simultaneously and the replicates were separated by 1 week intervals. Mortality was assessed 72 h after treatment. The estimation of mortality was corrected according to Abbott's (1925) formula. Two-way analysis of variance followed by a multiple comparisons test (Fisher's protected; SAS Inst. Inc., 1985) were done to determine whether there were differences among the treatments as well as among the two seasons during which the beetles were collected.

2.2 Effects on predation efficiency

The coccinellids used came from the group collected in October 1989 on hibernation sites. These adults were treated with the two neem extracts at a 50% concentration: (1) neem oil containing 6.85 ppm AZA; and (2) neem seed kernels containing 45.5 ppm AZA. Malathion treatments were done at a LC_{20} of 0.07 g (AI)/L as estimated by Roger et al. (1994). The topical application of the three insecticides was carried out following the previously described method. Fifteen min after treatment the coccinellids were presented 30 pea aphid nymphs (*Acyrtosiphon pisum* Harris) in 375 cc glass containers. The aphids were mass-reared on vetch plants (*Vicia faba* var. *major* L.) in a growth chamber. In each container, a vetch leaf served as a plant support for the aphid nymphs. After 24 h contact with the nymphs, the surviving beetles were removed and the remaining number of nymphs were counted.

Video recordings of treated and control coccinellids were done to study the after treatment behavior of the coccinellid for 8 h. Global activity, searching and cleaning behavior were qualitatively evaluated.

The experimental design consisted of 20 replicates for each treatment. All treatments were tested at the same time and the replicates were separated by 1 week intervals. Each replicate included two control treatments with the carrier only. The first control group was treated with distilled water and the second with 98.5% distilled water and 1.5% liquid detergent (Triton-X 1.5%). The data obtained were analysed with a one-way analysis of variance followed by a multiple comparisons test (Fisher's protected; SAS Inst. Inc., 1985).

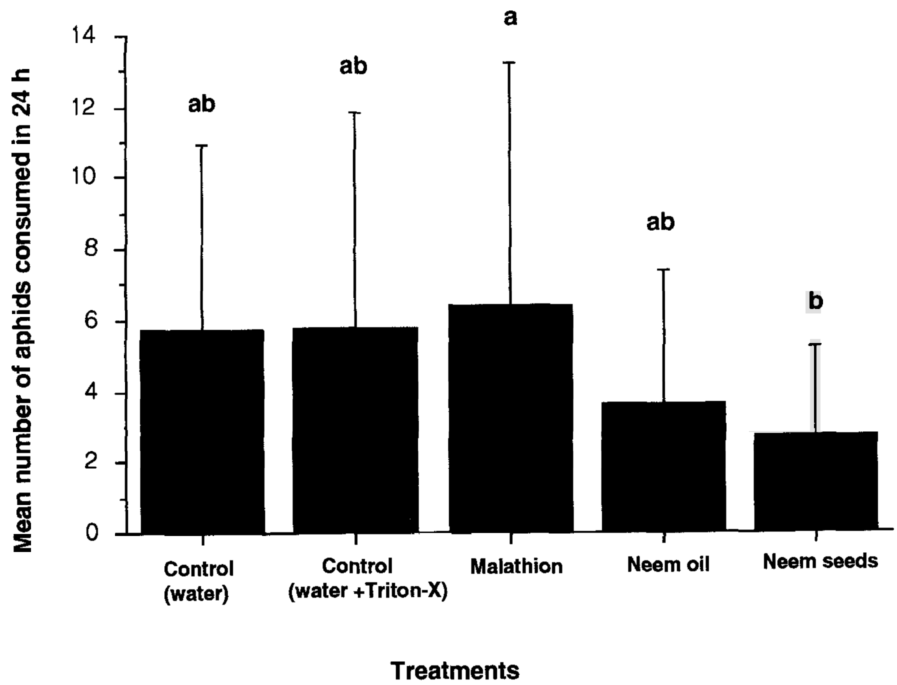
3 Results

3.1 Effects on mortality

Malathion caused 100% mortality 72 h after the treatment (table). No mortality occurred with the aqueous suspension of ground neem kernels. Neem oil, however, at a concentration of 0.014 g (AI)/L (= 10%), induced a mortality rate of 13.6%, which is significantly higher than that of the control group ($F = 49.72$, $df = 12$, LSD; $P = 0.0060$) (table). Surviving beetles were still active 1 month after treatments with both neem extracts. No difference was noted between the replicates carried out with beetles collected in the summer and the rep-

Mean percent mortality of adult C. maculata *72 h after treatments with neem seed oil (NSO), neem seed kernel (NSK) and a chemical insecticide. Vertically, treatments flanked with different letters are significantly different (Protected LSD; $P < 0.05$)*

Treatments	% Mean mortality	Standard error
NSO (1%)	1.5a	1.3
NSO (5%)	1.5a	1.8
NSO (10%)	13.6b	3.7
NSO (20%)	4.6ab	0.6
NSO (50%)	4.6ab	0.6
NSK (1%)	4.6ab	0.6
NSK (5%)	9.1ab	2.8
NSK (10%)	4.6ab	0.6
NSK (20%)	0.0a	0.0
NSK (50%)	0.0a	0.0
Malathion 50 EC 2.85 g (AI)/L	100c	0.0
Control (water)	0.0a	0.0
Control (water + Triton-X)	0.0a	0.0



*Mean number of aphids (*Acyrthosiphon pisum*) consumed in 24 h by* C. maculata *after treatments with two neem extracts and a chemical insecticide. Doses: Malathion; 0.07 g (AI)/L(= LC_{20}), neem extracts; 0.455 g (AI)/L (= LC_{50}). Different letters indicate a significant difference between the products (Protected LSD; $P < 0.05$)*

licates carried out with beetles collected in the fall ($F = 1.86$, $df = 1$, $P = 0.1750$).

For each pesticide, significant toxicity differences were obtained between doses. *C. maculata* was significantly more susceptible to a neem oil application at the 0.014 g (AI)/L (=10%) dose as compared to the 0.001 (=1%)r and 0.007 g (AI)/L (=5%)r doses originating from the same formulation ($F = 49.72$, $df = 12$, LSD; $P = 0.0140$) (table).

3.2 Effects on predation efficiency

Compared to Malathion at CL_{20}, the aqueous suspension of ground neem kernels caused a significant reduction in the number of aphids consumed after 24 h ($F = 1.99$, $df = 4$, LSD; $P = 0.0197$) (fig.). Furthermore, aphid consumption was reduced by 50% as compared to both control groups (ANOVA; $F = 2.00$, $df = 4$, LSD; $P = 0.0686$). Neem oil at a 0.455 g (AI)/L (=50%) concentration (v/v) and Malathion at the estimated CL_{20} did not significantly affect the mean daily aphid consumption rate ($F = 2.00$, $df = 4$, LSD test; $P > 0.1901$) (fig.).

Immediately after malathion treatments, the insects were overactive and tremors and convulsions were observed. Coccinellids treated with both neem formulations displayed an intensive cleaning activity for several hours.

4 Discussion

In most studies involving pests, the neem doses usually applied vary between 0.1 and 5% concentration (SAXENA, 1989; SCHMUTTERER, 1990). At these doses, we did not observe any impact on *C. maculata* adults. However, at higher doses (≥10%), our results showed that *C. maculata* is susceptible to neem extracts (table, fig.) which contradict the results obtained by SRIVASTAVA and PARMAR (1985) on predatory coccinellids and the general tendency observed for natural enemies (SCHMUTTERER, 1990). Neem extracts therefore affect not only many pests species (SAXENA, 1989; SCHMUTTERER, 1990), but also the predator *C. maculata*. Intensive grooming bouts were observed for more than 8 h after the treatments with the aqueous suspension of ground seeds (ROGER et al., 1991, 1994). Time allotted to grooming has probably reduced the time spent searching for prey.

C. maculata is usually very susceptible to chemical insecticide applications (KEEVER et al., 1977; COATS et al., 1979; LECRONE and SMILOWITZ, 1980; CANTELO, 1986; ROGER et al., 1991). In our study, *C. maculata* was more susceptible to malathion than to neem extracts. Malathion is extremely toxic towards *C. maculata* adults since no beetle survived 72 h after the field rate treatment (table). Moreover, the effect of Malathion was very rapid: over 80% of the mortality occurred 15 min after the treatment and all individuals were dead after 2 h. MOK YUN and RUPPEL (1964) obtained similar results in the laboratory on oat plants; 72 h after the application of Malathion, 97% of the coccinellids *C. maculata* were eliminated.

Neem extracts exerted two types of effects, namely on mortality and on predation efficiency. Our results cast a doubt upon the reputation of their non-toxicity towards entomophagous coccinellids. Consequently, natural insecticides, as any synthetic pesticides, should be used with caution in IPM programs (CROFT, 1990). Evaluation of mortality is a standard procedure. However, evaluation of the impact of pesticides on predation efficiency is an important but often overlooked component. More research is necessary to specify the nature of the effects caused by the neem derivatives on *C. maculata* or on natural enemies likely to be used jointly with these products.

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