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Effects of an azadirachtin-based formulation on the non-target muscoid fly parasitoid *Muscidifurax raptor* (Hymenoptera: Pteromalidae)

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ABSTRACT

Extracts from the Indian neem tree, *Azadirachta indica* (Meliaceae), have demonstrated high potential for the control of numerous insect pests. Azadirachtin [AZ], a tetranortriterpenoid compound, is considered the most important active principle contained in neem seed kernels. Although neem-based formulations are generally considered safe to beneficial insect species, adverse effects on hymenopteran parasitoids have been reported. The susceptibility of the muscoid fly pupal parasitoid *Muscidifurax raptor* to an azadirachtin-based insecticide was determined in laboratory bioassays. Parasitoid adults directly fed a diet containing the insecticide showed a slight reduction in their lifespan (25.1% and 15.7%, males and females, respectively) and reproduction rate (27.5%) at the highest concentration tested (20 μg [AZ]/ml). No significant effects were noticed at lower concentration. Interestingly, *M. raptor* males (LC_{50} = 43.0 μg [AZ]/ml) were more susceptible than females (LC_{50} = 94.7 μg [AZ]/ml). Immature parasitoids were able to develop and emerge successfully from house flies pre-treated at concentration levels that almost completely inhibited fly emergence (30 μg [AZ]/g). At this concentration level, longevity of emerging parasitoids was significantly reduced (20.6% and 17.6%, male and female, respectively), as was F1 progeny rate (35.9%), in comparison to the control.

Overall, the effects of azadirachtin on *M. raptor* are minimal compared to those in major livestock pests, thus the use of azadirachtin-based formulations can be considered compatible with integrated livestock pest management programs.

1. Introduction

The family Pteromalidae (Hymenoptera) includes important pupal parasitoids of muscoid flies. Among them, *Muscidifurax raptor* Girault and Sanders is considered to have high potential for the control of muscid pests affecting animal husbandry environments, such as the house fly, *Musca domestica* L., the hematophagous stable fly, *Stomoxys calcitrans* L. and species of *Fannia* (Legner and Olton, 1968; Rutz and Axtell, 1979). Some calliphorid and sarcophagid species are also listed among its hosts (Rueda and Axtell, 1985).

Presently, livestock pest management relies mainly on the intensive application of chemical insecticides (Mullen and Durden, 2002). This situation represents a severe threat for biological control agents and, more in general, for the environment. For these reasons, the search for alternative and environmentally "friendly" methods of pest control is encouraged (Crespo et al., 1998; Hogsette, 1999).

Among natural insecticides, extracts from the Indian neem tree, *Azadirachta indica* (Meliaceae), have demonstrated high potential for the biological control of different noxious insects (Isman, 2006). Azadirachtin [AZ], a tetranortriterpenoid compound, is considered the most important active principle contained in neem seed kernels. This triterpenoid shows variable effects on insect pests, including oviposition and feeding deterrence, growth regulation, fecundity and fitness reduction (Schmutterer, 1990). Although neem is considered generally safe to beneficials, there are reports of its adverse effects on parasitoids (Condor Golec, 2007).

The potential of neem against the house fly, the stable fly and other pests of livestock has been demonstrated (Miller and Chamberlain, 1989; Khan and Ahmed, 2000; Larramendy et al., 2004). However, no information on the susceptibility of their parasitoids to neem active principles have been reported so far. In addition, the susceptibility of *M. raptor* to insecticides is still little known (Scott et al., 1991; Geden et al., 1992).

The aim of this study was to determine the susceptibility of *M. raptor* to an azadirachtin-based insecticide. For this purpose, we determined whether this parasitoid is able to develop and emerge from azadirachtin-treated house flies. In addition, the effects on lifespan and reproduction have been studied both on adults directly exposed to the insecticidal formulation and on adults eclosing from azadirachtin-treated house flies.

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2. Materials and methods

2.1. Insects

House flies and parasitoids used in the bioassays were reared under our laboratory conditions (25 ± 1 °C and a photoperiod of L14:D10) at the Department of Plant Protection of the University of Sassari (Italy).

Musca domestica was maintained in our laboratories as described in Ruiu et al. (2006). Adult mating and reproduction was ensured by a diet based on a mixture of sucrose and milk powder (1:1), and water (provided separately), whereas immature stages were reared on a diet consisting of wheat bran (34%), milk powder (1%), benzoic acid (0.1%) and water (64.9%) (w/w).

Muscidifurax raptor adults were reared in plexiglass cages (30 × 30 × 30 cm) where water and honey drops were provided separately as a food source. House fly pupae (24–48 h-old) were periodically exposed to parasitization, and then incubated at 25 ± 1 °C to allow immature development until adult parasitoid emergence (Ruiu et al., 2007).

2.2. Bioassays

A neem-based emulsifiable concentrate (Oikos 25 Plus, Sipcam S.p.A., Italy) whose active ingredient is azadirachtin [AZ] (2.5%) was employed in all experiments. Bioassays were conducted by incorporating this insecticidal formulation into an artificial diet administered to insects. Distilled water was used to dilute stock solutions. General bioassay techniques and conditions were similar to those reported by Ruiu et al. (2007).

2.2.1. Adult parasitoid feeding experiments

This bioassay group involved a dose–response experiment and a sub-lethal experiment. In both case adult parasitoids were tested by an ingestion bioassay.

In the dose–response bioassay, newly emerged adults of *M. raptor* (<24 h old) collected from the stock culture, were placed singly into plastic pots (3-cm in diameter and 3-cm high) and were provided for 5 days (food refreshed daily) with 30% sucrose solution mixed with the azadirachtin formulation. Food was administered by capillary tubes (5 μl) and the following azadirachtin [AZ] concentrations were assayed: 320, 160, 80, 40, 20, 10, 5 and 0 μg/ml. Males and females were tested separately. There were 20 males and 20 females for each concentration and the experiment was replicated four times, for a total of 80 adults per sex per treatment. Mortality was assessed after 5 days.

In the sub-lethal bioassay, newly emerged adults of *M. raptor* were paired (1 male and 1 female), and put in plastic pots (10-cm diameter × 10-cm high). They were fed daily for 5 days a treated diet as previously described, but in this case the azadirachtin concentrations assayed were 20, 10 and 0 μg/ml.

From the 6th day on, parasitoid pairs which had survived after treatment were maintained separately on a 30% sucrose solution and honey drops. Each pot had a window through which each female received 20 house fly pupae (24-h-old) daily until death occurred. Parasitoid mortality was assessed daily until the last one died.

After being exposed to parasitoids for 1 day, pupae were maintained at 25 °C until house fly or *M. raptor* adult emergence. The numbers of emerged parasitoids, corresponding to the numbers of puparia with an emerging hole, were recorded. Adult parasitoid emergence rate was then calculated taking into account the number of puparia with an emerging hole and the number of exposed pupae. The whole experiment was repeated twice with different batches of flies and parasitoids.

2.2.2. Immature house fly feeding experiments

Survival and reproductive performance of parasitoids developing on azadirachtin-treated house flies was studied through two different experiments.

The first experiment employed pupae obtained from 2nd instar house fly larvae reared on their normal medium containing the following azadirachtin concentrations: 30, 15, 5, 0 μg/g. For each concentration, five groups of five newly emerged and just mated *M. raptor* females were kept in plastic pots (10-cm diameter × 10-cm high) and provided for 3 h with 10 house fly pupae (24-h-old) developed from azadirachtin-treated larvae. Five additional groups of 10 pupae from the same treated larvae were not exposed to parasitization but incubated until fly emergence. Adult emergence rate was calculated as in the previous experiment.

The second experiment employed newly emerged *M. raptor* adults developed on house flies treated as in the previous experiments with azadirachtin at the following concentrations: 30, 5, 0 μg/g. Groups of 10 parasitoid adults for each concentration were paired (1 male and 1 female) and maintained on 30% sucrose solution and honey drops. Longevity and progeny emergence rate were determined according to the methodology used in the adult parasitoid feeding experiment, but in this case house fly pupae were provided only for 15 days.

Both experiments were repeated three times with different generations of flies and parasitoids.

2.3. Statistical analysis

Dose–response bioassay data for LC_{50} determinations were analyzed by the probit procedure (Finney, 1971). Differences were considered significant when 95% fiducial limits (FL) did not overlap.

Emergence, longevity and reproduction data were analyzed with one-way analyses of variance (ANOVA). When F tests were significant, means were separated using least significant differences (LSD) ($P \leqslant 0.05$). All percentage data were transformed to arcsine square root of proportion before analysis of variance.

Because variance analysis for each repetition of the same experiment gave similar results, data were combined for analysis and presentation. SAS software was used for analyses (SAS Institute, 1999).

3. Results

3.1. Adult parasitoid feeding experiments

Toxicity of azadirachtin insecticide to parasitoid adults was concentration-dependent, with males twice as susceptible (LC_{50} = 43.0 μg [AZ]/ml) than females (LC_{50} = 94.7 μg [AZ]/ml) (Table 1).

Longevity of *M. raptor* adults was significantly affected by dietary exposure to azadirachtin (Male: $F = 12.25$; $df = 2,117$; $P < 0.0001$) (Female: $F = 8.87$; $df = 2,117$; $P = 0.0003$), but only at 20 μg [AZ]/ml in comparison to the control (Table 2).

Similarly, direct feeding on a treated diet caused a reduction in female reproductive rate (Table 3). When females were exposed to

Table 1
Results of probit analysis of the concentration-mortality data for *Muscidifurax raptor* adults exposed to the azadirachtin-based formulation

	n[a]	Slope ± SEM	LC50 (95% FL) μg [AZ]/ml	χ^2	*df* (*P*)
Male	80	2.05 ± 0.27	43.0 (28.6–56.8)	55.09	1 (<0.0001)
Female	80	1.38 ± 0.24	94.7 (58.0–131.4)	33.64	1 (<0.0001)

[a] Number of insects assayed for each concentration.