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Acute and chronic effects of neem on Myzocallis coryli (Homoptera: Aphididae)

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Abstract. Adults and immatures of the filbert aphid, *Myzocallis coryli* (Goetze), a major pest of hazelnuts worldwide, were exposed to foliage treated with different concentrations of a botanical insecticide, Margosan-O, derived from extract of neem seeds. Both mature and immature stages were highly sensitive to this compound. Acute toxicity causing immediate mortality of young nymphs, particularly at higher rates (50 ppm or more), and chronic effects resulting in reduced offspring production, lengthening of nymphal development time and reduced survival of subsequent generation were recorded. The offspring production of adult aphids fed on treated leaves was reduced in a dosage-dependent manner, nearly 50% at 2.5 ppm to over 80% at 62.5 ppm. However, no nymphal survival was recorded at 25 ppm or higher doses. A repellency effect was also recorded, but the feeding deterrence response was weak at best. Field trials also showed a marked reduction of aphid numbers on treated foliage, suggesting the effectiveness of neem against this major pest of hazelnuts.

1. Introduction

The filbert aphid, *Myzocallis coryli* (Goetze), is a serious pest of hazelnut, *Corylus avellana* (L.) in North America, Italy, Spain, and Turkey (AliNiazee, 1980, 1994). Currently, one or two sprays of organophosphate insecticides are applied annually to control this pest in North America. However, introduction of a highly effective parasitoid, *Trioxys pallidus* Haliday (Hymenoptera; Aphidiidae), from Europe to North America suppressed the aphid population in recent years (Messing and AliNiazee, 1989; AliNiazee, 1991). In spite of this successful biological control effort, in some orchards aphid populations may still require chemical control. A similar situation occurs in Turkey and Italy where occasional control measures are required for this pest.

Aphids and other homopteran pests are sensitive to seed extracts of the neem tree, *Azadirachta indica* A. Juss to varying levels (Schmutterer, 1985; Saxena, 1989; Isman *et al.*, 1990; Lowery *et al.*, 1993). Extracts of neem seed contain azadirachtin, a triterpenoid, which was found effective against more than 200 insect species. It is also effective against a wide variety of other organisms (Saxena, 1989; Schmutterer, 1990), and shows a great potential as an important source of botanical insecticides.

The neem extract is also an effective systemic insecticide (Larew *et al.*, 1985; Osman and Port, 1990; Naumann *et al.*, 1994). It has low mammalian toxicity (Jacobsen, 1989), degrades rapidly in the environment (Schmutterer, 1988), is relatively non-toxic to many natural enemies, has no known mutagenic effects, and development of resistance appears to be relatively slow (Saxena, 1989; Schmutterer, 1990). It causes various deleterious effects on insects including repellency, feeding and oviposition deterrence, and retardation of growth and development. Because of their selectivity and relatively minor impact on benficial arthropods, neem-based insecticides appear to be highly suitable for use in integrated pest management programmes (Schmutterer, 1990).

Reported here is a study dealing with the acute and chronic effects of a neem-based insecticide, Margosan-O, on *M. coryli* under laboratory and field conditions. Our goal was to assess the possibility of inclusion of neem-based insecticides in hazelnut pest management programmes under study in different parts of the world.

2. Materials and methods

2.1. Laboratory experiments

The aphids were reared in 30 ml clear plastic cups with lids that contain wet cotton swabs. Each cup had a 4 cm diameter leaf disc cut-out of freshly collected hazelnut leaves. Field collected adults were released in rearing cups and nymphs (< 24 h old) from these females were used in the experiments. Leaf discs were dipped in different aqueous concentrations of a neem seed extract formulated and commercially sold as Margosan-O for 60 s, and dried for 30 min before they were placed in experimental cups. A fine camel hair brush was used to transfer the aphids from rearing cups to treatments. The laboratory experiments were conducted at 21 ± 1°C, 70% RH, and L16:D8 photoperiod. Margosan-O was obtained from W.R. Grace & Co. (Columbia, Maryland, USA) as a 0.25% active ingredient of azadirachtin (AZA) in a liquid formulation, and different aqueous concentrations (2.5, 12.5, 25, 62.5, and 125 ppm) of this compound were tested. Leaf discs dipped in water alone were used as controls.

The studies reported here were conducted by setting up four different experiments. In the first experiment, 10 young nymphs (1 day old) were exposed to hazelnut leaf discs treated with different concentrations of neem as described above, and the effects were determined by recording several life history measurements, including survival, developmental time, developmental abnormalities and offspring production. All observations were conducted at 2-day intervals until all nymphs had completed their development or died. These experiments were replicated four times (each replication containing four leaf discs) and compared with untreated controls. For the developmental studies, the nymphs reared on neem-treated leaf discs were

observed until they had reached the adult stage; nymphal mortality, length of nymphal stage, and quality (morphological abnormalities and reproductive ability) of the adults produced were recorded. The effect of earlier neem treatments on these adults was checked by randomly selecting four adults from each replication and following their offspring production for their entire adult life. These adults were fed on untreated foliage after removal from the treatment discs, thus any abnormalities found were attributable to the chronic effects. The developmental rate function (1/developmental time) for each treatment was calculated to determine the dosage-dependent response.

In an attempt to determine the differential response of different nymphal instars to neem, two more experiments were carried out. In one experiment (expt 2), similar numbers of mid-sized nymphs (8 days old) were released on neem-treated leaf discs and their life history and survival measurements followed until they became adults. The longevity and progeny production of adults derived from these experiments were also checked as reported in the first experiment. In the other experiment (expt 3), fully grown (third instar) nymphs were tested for their survival and development as detailed above in experiments 1 and 2.

In the fourth experiment, newly formed adults (< 24 h old) were used. One aphid was released on each leaf disc treated with different concentrations of Margosan-O and its survival and reproduction were checked on a daily basis until death. A total of four adults was used in each replication and each treatment was replicated four times. As the F_1 offspring were produced, four 1-day old nymphs were randomly selected from each replication and moved to newly treated (same concentration) leaf discs and their survival and developmental rates recorded on a daily basis until they reached the adult stage. As these individuals became adults (F_2), four 1 day old adults per replication were randomly selected and released on untreated foliage. Here we studied the inter- and intra-generation chronic effects for another generation.

The repellency tests were conducted in a choice test arena where treated and untreated leaf discs were provided for selection. All the laboratory experiments were conducted in small Petri dishes (9 cm diameter) with a wet filter paper at the bottom, and under laboratory conditions at $21 \pm 1^{\circ}C$ and a photoperiod of L16:D8 in Percival growth chambers. Three concentrations (25, 50 and 125 ppm) of neem were tested, along with a water-treated leaf disc. Ten young nymphs (< 24 h) were released on neem-treated leaf discs. After 24 h, the number of aphids found on the treated and untreated leaf discs were counted. Each treatment was replicated four times.

In the antifeeding test, 10 one-day-old nymphs (starved for 30 min) were released on treated (concentrations 25, 50 and 125 ppm) leaf discs and the aphid feeding behaviour was observed at 2, 4, 8, and 30 h post-treatment intervals. Water-treated leaf discs were used as controls. These experiments were replicated four times and Petri dishes were re-randomized after each count; the laboratory setup was similar to that described in the repellency tests.

2.2. *Field studies*

A block of 5-year-old hazelnut trees located near Corvallis, Oregon, USA was selected for this study. The trees were small (2–3 m in height) and had been maintained using standard horticultural practices except insecticides. Trees were sprayed using a power sprayer with a hand-gun at two rates, 25 and 50 ppm concentrations of Margosan-O in water, in the early morning hours of 18 June 1994. Control trees were sprayed with water. One day after spray application, four less-than-1-day-old adult aphids, reared in the greenhouse, were released in each of six clip cages (5.5 cm diameter) on each tree. The clip cages were fixed on leaves of treated and untreated trees. There was no native aphid population on these trees. Seven days later, the survival and production of offspring in treatments and control were recorded. At this time all adults were removed and the nymphal development was recorded for another 3 weeks at weekly intervals. The experiments were replicated four times and terminated after a month.

2.3. *Statistical analysis*

The data were subjected to ANOVA, and means compared using Tukey's multiple range test through the STATGRAPHIC computer program. Data were compared to identify statistically significant differences at $P \leqslant 0.05$ level. In experiments where data were collected at different time intervals (table 6), means of counts taken at each specific time (i.e. 2 h, 4 h, etc.) were compared separately.

3. Results and discussion

3.1. *Laboratory studies*

Data (tables 1–3) show that *M. coryli* nymphs were highly susceptible to Margosan-O, a commercial formulation of neem

Table 1. Different life history parameters of M. coryli *exposed as young (< 24 h old) nymphs to leaves treated with different concentrations of neem insecticide Margosan-O under laboratory conditions at 21± 1°C and photoperiod L16 : D8*

	Parents			F_1 generation			
Treatments AZA conc. (ppm)	Survival to adult stage (%)	Nymphal development time (days)	Adult longevity (days)	Offspring produced per female	Survival to adult stage (%)	Nymphal development time (days)	Developmental rate[a]
Control	50.0 ± 9.1a	13.0 ± 0.0a	9.4 ± 1.0a	17.9 ± 2.7a	47.5 ± 7.5a	16.0 ± 0.4a	0.06
2.5	32.5 ± 13.1a	15.2 ± 0.9a	7.2 ± 1.1a	13.8 ± 1.8b	40.0 ± 3.0a	17.3 ± 0.4a	0.06
12.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Means ± SE followed by the same letter within a column are not significantly different ($P \leqslant 0.05$) from each other using Tukey's multiple range test.
[a]Reciprocal of number of days required for nymphal development.

seed extract. When 1 day old nymphs were released on leaf discs treated with 12.5 ppm or more of the neem insecticide, no survival was observed. In the 2.5 ppm treatment, minor effects were found on survival, developmental time, number of offspring produced, and the longevity of adults. However, none of these differences except the offspring production was significant ($P \leqslant 0.05$). All other treatments gave 100% mortality (table 1). Two days after release, nymphs began to die in increasing numbers, and by the 10th day, 100% mortality was observed in all treatments except at lower concentrations of 2.5 and 12.5 ppm (figure 1). The mortality in 2.5 ppm treatment did not change much between the 10th day and the 20th day, but in 12.5 ppm treatment the mortality increased to 100% by the 20th day and no adult formation occurred. The small but statistically significant difference recorded in progeny production between the controls and 2.5 ppm treatment may indicate a low level chronic effect of neem on this insect (table 1). When middle aged (8 days old) nymphs were exposed to leaf discs treated with different concentrations of neem, the survival and developmental rate declined with increasing dosages (table 2). There was a significant difference in the survival of nymphs to adult stage between the control and almost all neem treatments. The differences became noticeable 4–6 days after the beginning of treatment (figure 2). The regression of mean percent mortality with treatment dosage suggests that, in general, the survival decreased as dosage increased in a linear fashion ($r = 0.78$).

The longevity of the adults emerging from different treatments of middle aged nymphs was influenced by treatment rates; differences between the high rates (62.5 and 125 ppm) and low rates were significant ($P < 0.05$). In general, rates up to 25 ppm had little effect on the longevity of adults emerging from treatments. However, progeny production in F_1 was greatly affected by nymphal and/or adult feeding on treated foliage. There was no significant difference between the 2.5 ppm treatment and control, but in the remaining treatments offspring production was drastically reduced. The chronic effects were manifested in reduced survival of F_1 nymphs, even after they were moved to untreated foliage. Less than 10% of the nymphs survived in the 12.5 and 25 ppm treatments and none survived at the 62.5 and 125 ppm rates (table 2). Also, longer developmental time was required for nymphs produced by treated adults. In those treatments where last instars were tested, slightly higher rates of Margosan-O were used (table 3) and mortality was recorded on a daily basis for 1 week. Over 90% mortality was recorded in the 50 and 125 ppm rates within 4 days after treatment. The mortality reached 100% by day 6 and no adults emerged in these treatments. Only a few adults emerged in the 25 ppm treatment.

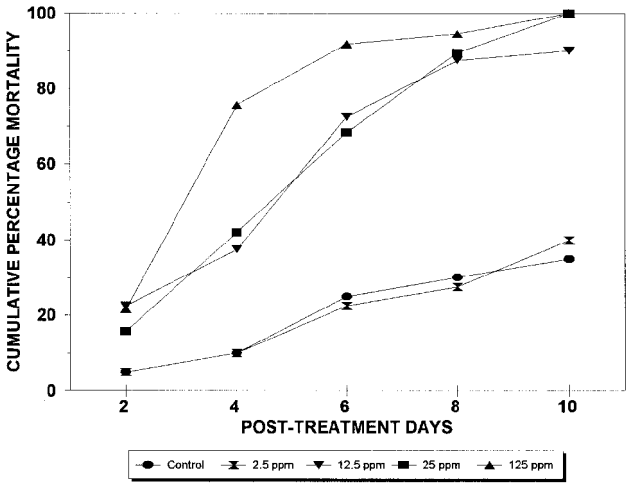

Figure 1. Mortality of M. coryli *(first instars) exposed to the neem-treated leaves.*

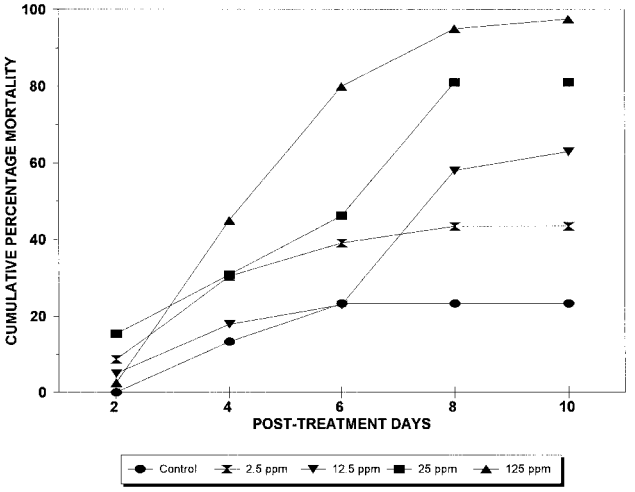

Figure 2. Mortality of M. coryli *(8 day old nymphs) exposed to the neem-treated leaves.*

Table 2. Effect of neem treatments on M. coryli *treated as middle aged (8 days old) nymphs under laboratory conditions*

				F_1 generation		
Treatment AZA conc. (ppm)	Survival to adult stage (%)	Adult longevity (days)	Offspring produced per female	Survival to adult (%)	Nymphal development time (days)	Developmental rate
Control	77.2 ± 8.0a	7.2 ± 1.0a	13.4 ± 0.5a	83.3 ± 2.6a	17.0 ± 0.5a	0.06
2.5	53.8 ± 7.5ab	10.5 ± 1.4a	12.3 ± 0.9a	77.7 ± 3.5a	20.4 ± 0.4b	0.05
12.5	34.6 ± 13.3bc	9.4 ± 2.5a	8.7 ± 0.9ab	9.9 ± 5.0b	19.6 ± 0.8b	0.05
25.0	19.0 ± 2.3c	6.0 ± 2.2a	5.8 ± 1.7b	4.8 ± 4.8b	20.8 ± 1.2b	0.05
62.5	8.3 ± 5.3c	4.0 ± 1.2ab	4.0 ± 0.0b	0.0	0.0	0.0
125.0	2.5 ± 5.0c	2.0 ± 0.0b	2.0 ± 0.0b	0.0	0.0	0.0

Means ± SE followed by the same letter within a column are not significantly different ($P \leqslant 0.05$) from each other using Tukey's multiple range test.

When adult aphids were exposed to treated leaves, no acute toxicity was observed, although adult longevity was slightly affected (table 4). However, offspring production, progeny survival and nymphal developmental times were greatly influenced. Significantly ($P \leqslant 0.05$) fewer offspring were produced in all treatments. At higher rates (25 ppm and above) no offspring survived (table 4). Even at the lower rate of 12.5 ppm the survival of nymphs was drastically reduced, developmental-time extended and offspring production reduced by nearly 60%. At the end of F_1, only five adults were found in the 12.5 ppm treatment while untreated controls had 285 adults.

The chronic effect of neem was investigated by transferring newly formed first generation adults (emerging from nymphs produced by the treated parents, both parents and F_1 nymphs maintained on treated foliage) to the untreated leaves and checking for the various life history parameters described earlier. Data (table 4) show that adult longevity, offspring production, F_2 nymphal survival and developmental times were adversely affected by neem treatments. Even though all stages in F_2 were fed on untreated foliage, the chronic effects of previous feeding on neem continue to unfold for the entire second generation. At the end of the experiment (30 days) there were a total of 137 aphids per adult in control versus 34.6 in 2.5 ppm and 0.2 in 12.5 ppm treatments. This appears to demonstrate long-term deleterious effects of neem on this species.

3.2. *Repellency and antifeeding effects*

Margosan-O at the concentrations tested (25, 50 and 125 ppm) showed moderate to low levels of repellency as measured by the dispersal of the aphids away from treated leaves in comparison to controls (table 5). The repellency began to show within 2 h after release (less than 60% of the aphids were found on the leaves in treatments vs more than 90% in controls) and continued for 24 h when the treatments were terminated. The percentage of aphids settling on treated leaves increased slightly with lower rates of Margosan-O, although the differences were not significant. Aphid mortality was also recorded particularly at higher rates within 24 h, suggesting a substantial level of acute toxicity of this compound to nymphs. The antifeedant effect was determined by observing feeding under a binocular microscope at different time intervals after aphids were released on treated foliage. Aphids were considered feeding only if mouthparts were inserted in the leaves and they were in the act of sucking nutrients from the leaf discs. Table 6 shows that a significant number of aphids were deterred from feeding on treated foliage 2 h after release, but these differences disappeared at 4 and 8 h and very little effect was noticed at 30 h, again only at higher rates. In other words, although aphids may not prefer to feed on treated foliage, they apparently will feed on treated foliage if that is the only source available. The antifeedent effect, therefore, appears to be very weak and may not be a significant component of overall effectiveness of this compound against this aphid.

Table 3. Mortality of last instar M. coryli *fed on neem-treated leaf discs under laboratory conditions*

Treatment AZA conc. (ppm)	Mean percent mortality after treatment	
	4 days	7 days
Control	3.3a	16.6a
25	57.5b	79.0b
50	90.9c	100.0c
125	91.4c	100.0c

Means followed by the same letter within a column are not significantly different ($P \leqslant 0.05$) from each other using Tukey's multiple range test.

3.3. *Field studies*

When the hazelnut trees were sprayed once and aphids were released in clip cages (within 24 h after spraying) the survival and offspring production was not affected for the first week. However, the differences became more noticeable after 2 weeks and very pronounced at 4 weeks after treatment. At the higher rate tested, nearly 90% reduction in total number of aphids occurred at the 4 week count (table 7). Although the effects were slow to manifest, they appear to confirm our laboratory findings. There were some noticeable differences between the two neem rates tested, but both rates were effective.

It is evident from data presented here (tables 1 – 4) that the neem preparation, Margosan-O, had a profound effect on the biology of filbert aphid. When young nymphs were exposed to treated foliage, survival to adult stage and production of nymphs by the surviving adults was reduced in a dose-dependent manner. Similarly, longevity, nymphal production and survival of

Table 4. Chronic effects of neem treatments on offspring of M. coryli *when adults were fed on treated foliage and nymphs reared on treated and untreated leaves under laboratory conditions*

Treatments AZA conc. (ppm)	F_1 on treated foliage				F_2 on untreated foliage			
	Parent longevity (days)	Offspring produced per female	Nymphal survival (%)	Nymphal development time (days)	Adult longevity (days)	Offspring produced per female	Nymphal survival (%)	Nymphal development time (days)
Control	14.4 ± 1.3a	20.6 ± 0.9a	85.9 ± 1.5a	17.0 ± 0.5a	15.3 ± 10.2a	18.8 ± 1.1a	67.5 ± 4.8a	17.4 ± 0.3a
2.5	11.5 ± 1.5ab	10.8 ± 2.0b	71.1 ± 2.5b	20 ± 4 ± 0.5b	9.6 ± 0.8b	15.6 ± 2.2a	40.0 ± 10.4b	15.3 ± 0.5b
12.5	9.3 ± 1.1b	8.8 ± 2.4b	3.3 ± 2.0c	24.8 ± 1.9c	4.4 ± 1.0c	6.8 ± 0.3b	12.9 ± 1.4c	14.8 ± 0.5b
25.0	9.5 ± 0.7ab	7.8 ± 0.7b	0.0	—				
62.5	9.9 ± 1.1ab	4.1 ± 0.9b	0.0	—				
125.0	12.3 ± 1.6ab	6.5 ± 1.6b	0.0	—				

Means ± SE followed by the same letter within a column are not significantly different ($P \leqslant 0.05$) from each other using Tukey's multiple range test.

Table 5. Movement of M. coryli *nymphs exposed to neem-treated leaves, 24 h after release, under laboratory conditions*

Treatment, AZA conc. (ppm)	Aphids on treated leaves (%)	Aphids away from treated leaves (%)	Mortality (%)
Control	87.5 ± 2.5a	10.0 ± 0.0a	2.5 ± 2.5a
25	50.0 ± 4.1b	42.5 ± 8.5b	7.5 ± 4.8ab
50	52.5 ± 10.3b	30.0 ± 4.1b	17.5 ± 7.5ab
125	32.5 ± 8.5b	35.0 ± 2.9b	32.5 ± 9.5b

Means ± SE followed by the same letter within a column are not significantly different ($P \leqslant 0.05$) from each other using Tukey's multiple range test.

Table 6. Antifeeding effects of neem insecticide on nymphs of M. coryli *2, 4, 8, and 30 h after release*

Treatment AZA conc. (ppm)	Percent non-feeding aphids			
	2 h	4 h	8 h	30 h
Control	2.5 ± 2.5a	2.5 ± 2.5a	7.5 ± 2.5a	5.0 ± 2.9a
25	15.0 ± 2.9b	22.5 ± 8.5b	12.5 ± 2.5a	10.0 ± 4.1ab
50	15.0 ± 2.9b	20.0 ± 0.0ab	12.5 ± 4.8a	17.5 ± 4.8ab
125	22.5 ± 2.5b	17.5 ± 2.5ab	15.0 ± 2.9a	22.5 ± 2.5b

Means ± SE followed by the same letter within a column are not significantly different ($P \leqslant 0.05$) from each other using Tukey's multiple range test.

Table 7. Field evaluation of neem on development and reproduction of M. coryli*, Corvallis, Oregon, USA, 1994*

Treatment AZA conc. (ppm)	Mean no. aphids per clip-cage at post-treatment intervals			
	1 week	2 weeks	3 weeks	4 weeks
Untreated	12.0a	10.8a	25.5a	184.5a
25	14.8a	11.5a	20.0a	64.0b
50	15.8a	5.8a	4.0a	19.8b

Means followed by the same letter within a column are not significantly different ($P \leqslant 0.05$) from each other using Tukey's multiple range test.

F_2 was greatly affected when adults and nymphal stages were exposed to neem-treated foliage, suggesting the susceptibility of all aphid stages to neem. Chronic effects such as prolongation of nymphal period and reduction in offspring production were seen at lower rates and high acute toxicity to nymphs was recorded at higher rates.

In other homopteran insects, a moderate to strong influence on fecundity was observed. In *M. coryli*, this appears to be the major effect as well. Prolongation of nymphal period and a dosage-dependent mortality of nymphal stages was also noticed in other insects (Schmutterer, 1987). In the pea aphid, *Acyrthosiphon pisum* (Harris), when reared on plants treated with 20 ppm of purified neem extract, a drastic reduction in nymphal production and extended adult longevity were evident (Schauer, 1984). Other species of aphids including the green peach aphid, *Myzus persicae* (Sulzer), currant-lettuce aphid, *Nasonovia ribisnigri* (Mosley) and strawberry aphid, *Chaetosiphon fragaefolii* (Cockerell) were also susceptible to neem both under laboratory and field conditions (Lowery *et al.*, 1993). The contact toxicity of neem did not contribute significantly to the reduction in the numbers of these three aphid species and the host plant had a major contributing effect on mortality (Lowery *et al.*, 1993). The deleterious effects of neem on offspring production has been recorded in insects other than aphids including stored product pests. In a recent study, Xie *et al.* (1995) showed that the offspring production in *Cryptolestes ferrugineus* (Stephens) and *Tribolium castaneum* (Herbst) was negatively affected by neem treatments and that the response was proportional to the concentration of AZA in the solution.

Repellency and antifeedant effects of neem have been recorded against many insects including aphids (Schmutterer, 1985, 1990; Zahnder and Warthen, 1988; Jacobson, 1989; Saxena, 1989; Lee *et al.*, 1991). However, the response was variable. With *M. coryli*, this response appears to be weak.

4. Conclusion

In summary, our data show that the neem preparation Margosan-O was highly effective against *M. coryli*. The long-term deleterious effects on longevity and fecundity of adult aphids, reduced nymphal survival and prolonged nymphal period at concentrations as low as 12.5 ppm were highly impressive. Neem showed a moderate degree of repellency against adult aphids, but low levels of antifeedant property. The data also demonstrated efficacy under field conditions. It is clear from the results of this study and a recent report (AliNiazee *et al.*, 1997) that neem insecticides deserve serious consideration for inclusion in hazelnut IPM programmes as a more selective approach.

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