Neem Oil and Neem Oil Components Affect the Efficacy of Commercial Neem Insecticides

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A series of toxicity studies with pea aphid, *Acyrthosiphon pisum* (Harris) and the neem insecticides Margosan-O (MO), MO devoid of neem oil, Azatin, RH-9999, Azatin with 5% neem oil, RH-9999 with 5% neem oil, and neem oil (5%) were conducted. We found that addition of neem oil increased the efficacy of neem insecticides that did not contain the oil, while removal of neem oil from MO reduced its efficacy 62%. Neem oil was also extracted with methanol. When methanol-extracted neem oil was added to MO devoid of neem oil, its efficacy was still 30% lower than MO. Addition of canola oil gave a similar response. Six limonoids, nimbandiol, deacetylnimbin, 6-acetylnimbandiol, deacetylsalannin, nimbin, and salannin, and two unidentified chemicals, believed to be limonoids, were identified in neem oil. Our data indicate that neem oil and other oils increase the efficacy of neem insecticides, but a polar component(s) of neem oil also contributes to increased biological activity of neem insecticides.

Keywords: *Neem; oil; efficacy; insect control*

INTRODUCTION

Environmentally benign pesticides, which we define as pesticides that are selectively toxic, do not bioaccumulate, and exhibit relatively short persistence in the environment, are needed for modern integrated pest management programs. Neem insecticides appear to fit this definition because they have been shown to be selective (Saxena et al., 1984; Mansour et al., 1987; Kale et al., 1986; Rössner and Zebitz, 1987; Stark et al., 1992), have short persistence (Stark, unpublished results), and pose less negative impacts to ecosystems than conventional insecticides (Stark, 1992).

Interest in neem insecticides has grown over the past 10 years as more pesticides are lost due to environmental and food safety problems (Koul et al., 1990; Schmutterer, 1990; Ascher, 1993). Two neem insecticides are now commercially available in the United States (Margosan-O, W.R. Grace and Co., Columbia, MD, and Azatin, Agridyne Technologies Inc., Salt Lake City, UT) and have recently received an exemption from residue tolerance on food crops by the U.S. EPA. Several other neem products may soon be available. There are differences in formulation between the neem insecticides available in the United States. Margosan-O (MO) has a 0.25% azadirachtin content and 3–5% neem oil while Azatin has 3% azadirachtin, but no neem oil. Both MO and Azatin are emulsifiable concentrate formulations. RH-9999, another neem insecticide in the experimental stage, produced by Rohm and Haas Co. (Philadelphia, PA), is a wettable powder that contains chemically modified (hydroxylated) azadirachtin (20% AI) and no neem oil. Neemguard (W.R. Grace and Co., Columbia, MD) is a formulated neem oil product from neem seed kernels (90% AI) which has insecticidal activity for some species.

Stark and Rangus (1994) found that MO was toxic to the pea aphid, *Acyrthosiphon pisum* (Harris). MO interfered with molting and reduced longevity and fecundity in a dose-dependent manner. The study presented here was originally conducted to compare the toxicity of several neem insecticides to the pea aphid. We expected to find little or no difference in toxicity between three commercially developed neem insecticides that contained azadirachtin as the active ingredient. What we found was quite different and led us to examine the relationship between neem oil and the effectiveness of neem insecticides.

MATERIALS AND METHODS

Insects. Pea aphids were obtained from cultures maintained at Washington State University, Puyallup Research and Extension Center, Puyallup, WA.

Chemicals. The following neem insecticides were evaluated: Margosan-O (MO), an EC formulation which contains 0.25% azadirachtin and 3–5% neem oil, formulated neem oil (90% AI EC), MO without neem oil which was specially made for this study, and clarified neem oil, which is purified neem oil that is unformulated (all from W.R. Grace and Co., Columbia, MD), Azatin, an EC formulation with 3% azadirachtin and no neem oil (Agridyne Technologies Inc., Salt Lake City, UT), and RH-9999, a chemically modified (hydroxylated) azadirachtin (20% AI wp) which also contains no neem oil (Rohm and Haas Co., Philadelphia, PA). The azadirachtin content of chemical standards was quantitated throughout the study with the methods developed by Hull et al. (1993).

Experiment 1. Comparative Effects on Population Growth. In a previous study we determined that population growth of *A. pisum* reared on broad bean, *Vicia faba* L., which had been treated with 100 mg of azadirachtin/L was only 28% of controls (Stark and Rangus, 1994). Following the same procedures presented in Stark and Rangus, we tested MO, Azatin, and RH-9999 at the 100 mg of azadirachtin/L level. We used caged potted broad bean, *V. faba* L. var. Banner, and aphids. Broad bean plants were planted seven per pot (10 cm diameter) in potting medium. Plants and aphids were kept in an environmental chamber at 25 ± 0.5 °C and 78 ± 5% RH and a 16:8 light–dark regimen. When the plants were ca. 25 cm high, they were thinned to five per pot and sprayed to runoff with the equivalent of 100 mg of azadirachtin/L of water MO, RH-9999, or Azatin with a Thomas atomizer powered by

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Table 1. Final Population Density of *A. pisum* Exposed to Broad Bean Treated with Several Neem Insecticides at the Equivalent of 100 mg of Azadirachtin/L (Experiment 1)[a]

no. of aphids ± SD			
control	Azatin	MO	RH-9999
1392.25 ± 58.66a	654.75 ± 41.33b	232.00 ± 39.70c	1378.50 ± 56.51a

[a] ANOVA followed by lsd test. Means followed by the same letter are not significantly different. Based on four replicates.

Table 2. Toxicity of Neem Insecticides to Immature *A. pisum* Exposed as First Instars to Neem Insecticides at 100 mg of Azadirachtin/L (Experiment 2)[a]

% mortality ± SD			
control	Margosan-O	Azatin	RH-9999
2.0 ± 4.47c	90.0 ± 7.07a	68.0 ± 10.95b	8.0 ± 13.04c

[a] ANOVA followed by lsd test. Means followed by the same letter are not significantly different. Based on five replicates.

an air compressor set at 25 psi. Care was taken to ensure that both the upper and under surfaces of leaves were treated. When plants had dried, 30 young apterous adult female *A. pisum* (ca. 24–48 h old) were placed in the soil at the base of the plants. A Mylar cage was placed over the pots to contain the aphids. The caged plants were arranged in a completely randomized design on tables in the environmental chamber. Seven days after adult introduction, all aphids were removed and counted. This experiment was replicated four times.

Experiment 2. Effects on First Instars. We also determined that first instars of *A. pisum* were the most susceptible stage to MO (Stark and Rangus, 1994). As such, we tested the toxicity of MO, Azatin, and RH-9999 to immature *A. pisum* at the 100 mg of azadirachtin/L level. Plants were treated as in experiment 1, but after drying, one adult aphid was placed in each of 10 clip cages attached to the under surface of the leaves (total of 10 aphids per pot). Twenty-four hours after introduction of adults, all aphids were removed except for one first instar in each cage. This ensured that newly born aphids were exposed at birth to the treatments. Plants and aphids were kept in an environmental chamber at 25 °C and 78% RH. Previous data indicated that the adult stage was reached in 5 d under our conditions and that MO at the 100 mg of azadirachtin/L level killed most aphids before they reached the adult stage (Stark and Rangus, 1994). This test was replicated five times and was done to confirm the results of experiment 1 with the most vulnerable stage of *A. pisum*.

Experiment 3. Removal of Neem Oil from MO. On the basis of the results of experiments 1 and 2, we hypothesized that neem oil or a component of the oil, enhances the activity of neem insecticides. This hypothesis was tested in a third experiment. MO and MO devoid of neem oil were tested at the 100 mg of azadirachtin/L level and were compared as described in experiment 2 with first instars and clip cages. A solution of neem oil (5% AI) in water was also prepared. The oil solution was tested at the same concentration of neem oil contained in MO at the 100 mg of azadirachtin/L level.

Experiment 4. Addition of Neem Oil to Neem Insecticides. This study was conducted to determine whether the addition of neem oil to azatin and RH-9999 would increase the toxicty of these products. Azatin and RH-9999 were compared to Azatin and RH-9999 to which neem oil was added such that each product contained 5% neem oil prior to dilution for spray application. This study was conducted in the same manner as experiment 1 with adult aphids and final aphid density was the endpoint of interest. This test was replicated five times.

Experiment 5. Topical Toxicity. To determine whether the increase in activity of neem insecticides by the addition of neem oil occurs only when the insecticides are applied to plants or whether this phenomenon occurs directly in insects we conducted topical toxicity studies with MO devoid of neem oil and compared these data to a previously developed data set

Table 3. Toxicity of Neem Insecticides to *A. pisum* Exposed as First Instars to Neem Insecticides at 100 mg of Azadirachtin/L or 5% Neem Oil (Experiment 3)[a]

% mortality ± SD			
control	Margosan-O	Margosan-O devoid of neem oil	neem oil
0c	92.5 ± 5.00a	35.0 ± 5.77b	0c

[a] ANOVA followed by lsd test. Means followed by the same letter are not significantly different. Based on four replicates.

with MO presented by Stark and Rangus (1994). The methods used for topical toxicity tests are described in Stark and Rangus (1994).

Experiment 6. Methanol Extraction of Neem Oil and Addition of Canola Oil. To determine whether a component of neem oil was responsible for the effects observed in the previous experiments or whether the oil itself imparted the increase in toxicity observed, we compared MO, MO devoid of neem oil, MO with methanol-extracted neem oil (5%), MO with canola oil (5%), and canola oil (5%). Canola oil was chosen because, like neem oil, it is a plant oil (derived from oil seed rape) and would therefore be a better comparison than insecticidal oils derived from petroleum. In this experiment, MO was made by adding clarified neem oil (5% by volume) to MO devoid of neem oil. MO with methanol-extracted neem oil was made by adding clarified neem oil (5% by volume) which had been previously extracted with HPLC grade methanol (six times, 1:1 by volume) to MO devoid of neem oil. Canola oil was added to MO devoid of neem oil such that the resultant product contained 5% oil. Canola oil (100%) was formulated with deionized–distilled water by adding 2.5 mL of oil, 0.2 mL Triton X-100, and 47.3 mL water. MO, MO devoid of neem oil, MO with methanol-extracted neem oil, and MO with canola oil were compared at the 100 mg of azadirachtin/L level as described in experiment 2 with first instar aphids and clip cages. Canola oil was tested at the same level of neem oil contained in MO at the 100 mg of azadirachtin/L level.

Experiment 7. Identification of the Polar Components in Neem Oil. Clarified neem oil used in experiment 6 was analyzed for the presence of chemical constituents. Clarified neem oil was diluted (1:10) with 90% methanol and passed through a C-18 solid phase extraction column (bond spec 18, J. T. Baker) and then injected into an HPLC for separation. The HPLC was operated using a 25 cm × 10 mm C-8 column (Supelco). The elution pattern was operated in a gradient using acetonitrile and water as eluents. The gradient started at 28% acetonitrile in water and increased to 95% acetonitrile in water over 65 min. The flow rate was 4.0 mL/min and the UV detector was set at 215 nm. Fractions from the HPLC were collected and then analyzed by a Finnigan mass spectrometer to identify the components. Identifications were made by comparing the spectra to an existing data bank as well as proposed structures from the literature. The neem oil sample was analyzed again after undergoing the methanol extraction process described in experiment 6.

Data Analysis. Data from all experiments were analyzed with one way analyses of variance (ANOVA) and means were separated by least significant difference (lsd) ($P < 0.05$) (SAS Institute, 1985) except for the topical tests involving MO devoid of neem oil. Here, the data did not meet the criteria of normality or equal variances and was therefore analyzed with Kruskal–Wallis one-way ANOVA on ranks followed by Student–Newmann–Keuls test for mean separation.

RESULTS

Experiment 1. Results of experiment 1 indicated that MO, Azatin, and RH-9999 did not affect aphid population growth in the same manner when the same concentration of azadirachtin was applied (Table 1). Aphid population increase was inhibited the greatest by MO, Azatin was the second most effective product, but was much less effective than MO. RH-9999 had virtually no effect on aphid population growth.

Table 4. Population Density of *A. pisum* Exposed to Several Neem Insecticides at a Rate of 100 mg of Azadirachtin/L (Experiment 4)[a]

no. of aphids ± SD				
control	Azatin	Azatin with neem oil	RH-9999	RH-9999 with neem oil
1437.75 ± 139.66a	625.25 ± 62.09c	281.75 ± 133.79d	1416.0 ± 21.34a	810.0 ± 101.99b

[a] ANOVA followed by lsd test. Means followed by the same letter are not significantly different. Based on four replicates.

Table 5. Effects of Margosan-O and Margosan-O Devoid of Neem Oil on Longevity of Adult *A. pisum* after Topical Application (Experiment 5)

azadirachtin concn (mg/L)	longevity (days) ± SEM	
	Margosan-O[a]	Margosan-O devoid of neem oil[b]
100	10.43 ± 1.24c	16.23 ± 0.74b
80	12.00 ± 1.20bc	13.50 ± 0.95b
60	12.20 ± 1.32bc	15.38 ± 0.87b
40	13.40 ± 1.10bc	15.73 ± 0.95b
20	15.03 ± 1.10b	17.05 ± 0.94ab
10	16.00 ± 1.00b	15.07 ± 1.03b
0	23.10 ± 0.98a	18.98 ± 0.78a

[a] These data are from Stark and Rangus (1994). ANOVA followed by lsd test. Means followed by the same letter are not significantly different ($P > 0.05$). Based on three replicates. [b] Kruskal–Wallis one-way ANOVA on ranks followed by Student–Newman–Keuls test. Means followed by the same letter are not significantly different ($P > 0.05$). Based on four replicates.

Table 6. Effects of Margosan-O and Margosan-O Devoid of Neem Oil on Reproduction of Adult *A. pisum* after Topical Application (Experiment 5)

azadirachtin concn (mg/L)	no. of offspring ± SEM	
	Margosan-O[a]	Margosan-O devoid of neem oil[b]
100	22.87 ± 2.86d	75.53 ± 3.71b
80	31.33 ± 3.37cd	62.23 ± 4.71b
60	37.03 ± 4.36c	72.03 ± 4.00b
40	46.93 ± 4.98c	70.43 ± 4.97b
20	59.07 ± 4.24b	72.58 ± 5.57b
10	61.70 ± 3.65b	67.18 ± 5.72b
0	94.37 ± 3.73a	91.25 ± 3.66a

[a] These data are from Stark and Rangus (1994). ANOVA followed by lsd test. Means followed by the same letter are not significantly different ($P > 0.05$). Based on three replicates. [b] Kruskal–Wallis one way ANOVA on ranks followed by Student–Newman–Keuls test. Means followed by the same letter are not significantly different ($P > 0.05$). Based on four replicates.

Experiment 2. When MO, Azatin, and RH-9999 were compared against first instar *A. pisum*, MO was the most toxic product followed by Azatin while RH-9999 exhibited no toxicity (Table 2). MO was 1.3 times more toxic than Azatin.

Experiment 3. When MO, neem oil, and MO devoid of neem oil were compared, MO was again found to be the most effective product (Table 3). MO was 2.6 times more toxic than MO devoid of neem oil.

Neem oil caused no mortality of *A. pisum*. However, individuals in the MO devoid of neem oil treatment and the neem oil treatment were ca. 25% smaller than individuals in the controls.

Experiment 4. The addition of neem oil to Azatin and RH-9999 resulted in a significant increase in the effectiveness of each product (Table 4). Population growth of *A. pisum* exposed to Azatin was 43% of the control while population growth of aphids exposed to Azatin with neem oil was only 20% of the control group. Azatin with the addition of 5% neem oil was twice as toxic as Azatin alone.

The addition of neem oil to RH-9999 also increased its toxicity to *A. pisum* (Table 4). RH-9999 with 5% neem oil was 1.75 times more toxic than RH-9999 alone.

Experiment 5. MO was still more toxic than MO devoid of neem oil when applied topically (Tables 5 and 6). Although longevity was significantly reduced by all concentrations of MO devoid of neem oil compared to controls, reductions were much lower than in individuals exposed to MO. For example, at the equivalent of 100 mg of azadirachtin/L, the lifespan of *A. pisum* exposed to MO-treated broad bean was only 45% of the control group while the lifespan of *A. pisum* exposed to broad bean treated with MO devoid of neem oil was 85% of the control (Table 5). Production of offspring was also was significantly reduced by all concentrations of MO devoid of neem oil when compared to controls (Table 6). However, the reduction in offspring was much more pronounced in individuals exposed to MO. Individuals exposed to MO at the 100 mg of azadirachtin/L level, produced 76% fewer offspring compared to controls. Offspring production by individuals exposed to MO devoid of neem oil at the 100 mg of azadirachtin/L level was only 17% lower than controls. It is interesting to note that there was no dose response for MO devoid of neem oil.

Experiment 6. The extraction of neem oil with methanol resulted in a reduction of activity, but the reduction was not as great as the complete removal of neem oil (MO devoid of neem oil) (Table 7). MO was much more toxic than MO devoid of neem oil, which was seen before in experiment 3. MO with methanol-extracted neem oil was less toxic than MO, but more toxic than MO devoid of neem oil. The addition of canola oil to MO devoid of neem oil resulted in activity similar to MO with methanol-extracted neem oil while canola oil alone did not cause mortality.

Experiment 7. HPLC/MS analysis of neem oil indicated that eight major chemicals were present (Figure 1). Six of these products, all limonoids, were identified and two remain unknown. The eight products were (A) nimbandiol, (B) deacetylnimbin, (C-1) 6-acetylnimbandiol, (C-2) deacetylsalannin, (D) unknown, (E) nimbin, (F) salannin, and (G) unknown (Chart 1). The two unidentified products are believed to be limonoids. Analysis of neem oil after methanol extraction and the methanol extract, revealed that all of these chemicals were completely removed from the oil (Table 8).

The percent of the eight chemicals in neem oil by weight and their ratio to azadirachtin in MO and RH-9999 is presented in Table 8. Salannin accounted for the greatest weight of the limonoids found in neem oil. Nimbin was the second most abundant chemcial followed by deacetylnimbin, unknown D, and unknown G. In MO, salannin was also the major limonoid, unknown D was second, and nimbandiol was third. Limonoids could not be detected in neem oil that had been extracted with methanol. The ratio of limonoids in RH-9999 was different than that found in MO. For example, five times more deacetylnimbin was present in RH-9999 than in MO and five times more nimbandiol was present in MO than in RH-9999. Salannin levels were about the same in both products.

Table 7. Toxicity of Neem Insecticides to *A. pisum* Exposed as First Instars to Margosan-O, Margosan-O Devoid of Neem Oil, Margosan-O with Methanol-Extracted Neem Oil (5%), and Margosan-O with Canola Oil (5%) All at the Equivalent of 100 mg of Azadirachtin/L and Canola Oil (5%) (Experiment 6)[a]

% mortality ± SD					
control	Margosan-O	Margosan-O devoid of neem oil	Margosan-O with methanol-extracted neem oil	Margosan-O with canola oil	canola oil
2.5 ± 5.0d	97.7 ± 5.0a	37.5 ± 5.0c	70.0 ± 8.16b	65.0 ± 12.9b	2.5 ± 5.0d

[a] ANOVA followed by lsd test. Means followed by the same letter are not significantly different. Based on four replicates.

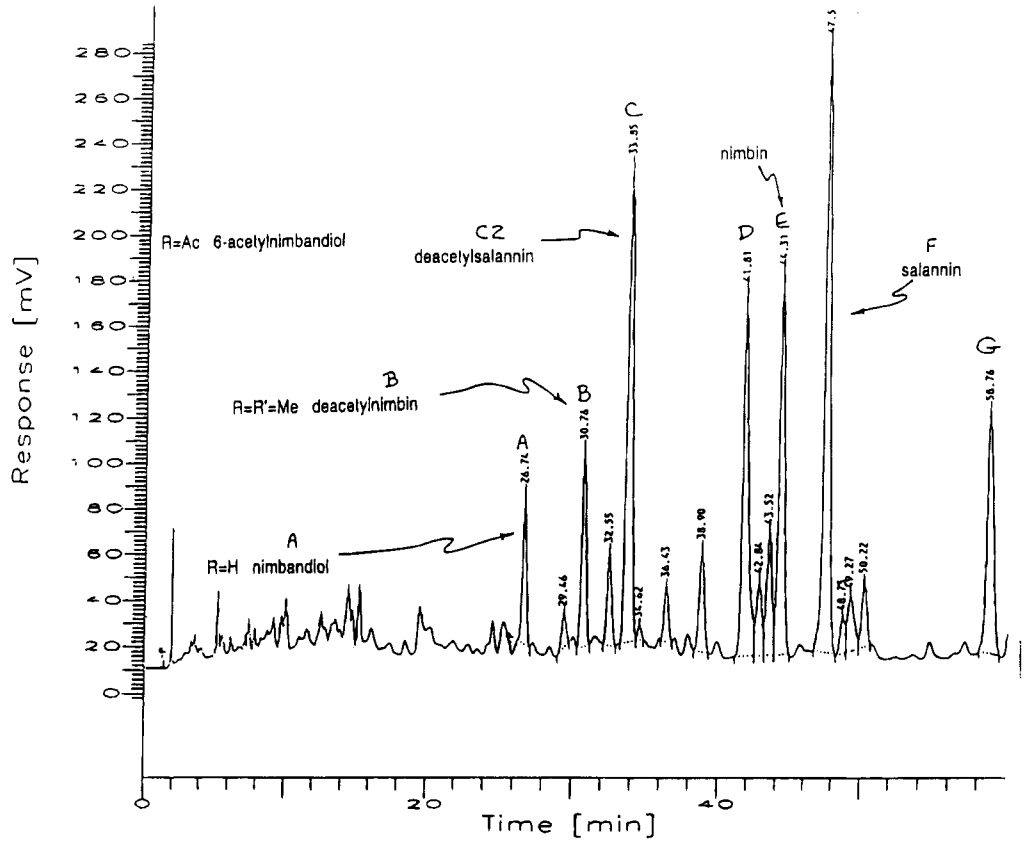


Figure 1. 1. HPLC chromatogram of neem oil prior to extraction with methanol.

DISCUSSION

Results of this study clearly show that neem insecticides are not equal in terms of pea aphid control. When applied at equivalent rates of azadirachtin, MO was significantly more toxic than the other neem insecticides. Azatin was the second most toxic product while RH-9999 was virtually nontoxic. The reason that RH-9999 was not toxic to *A. pisum* may have to do with the fact that azadirachtin is hydroxylated in this product. Hydroxylated azadirachtin may not penetrate plant tissue or insect cuticle as readily as unhydroxylated azadirachtin. Other reasons for differences in toxicity might have to do with formulation and the content of other limonoids. RH-9999 is a wettable powder while MO and Azatin, the more effective products, are emulsifiable concentrates. Also, the ratios of limonoids were different between RH-9999 and MO which might influence efficacy.

The removal of neem oil from MO greatly reduced its toxicity while adding neem oil to Azatin and RH-9999 resulted in increased toxicity. Thus, neem oil and/or a component of the oil influenced insecticidal activity of azadirachtin, the active ingredient in these neem insecticides.

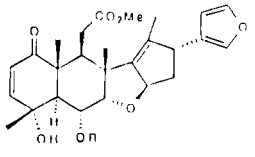
When we topically applied MO and MO devoid of neem oil, we found that the lack of oil greatly reduced the efficacy of MO. By eliminating the host plant in this experiment we showed that the phenomenon we observed with neem oil also occurred when aphids were directly exposed. Thus, we showed that the increase in activity of neem insecticides by the addition of neem

Table 8. Weight of Liminoids and Their Ratio to Azadirachtin in Various Neem Materials

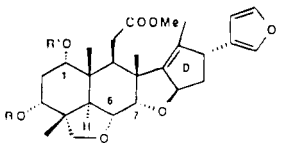
	wt % of limonoids (ratio limonoids/azadirachtin)							
neem material	azadirachtin	nimbandiol	deacetylnimbin	6-acetylnimbandiol	unknown d	nimbin	salannin	unknown G
neem oil	0.01	0.27 (27)	1.7 (170)	0.06 (6)	1.6 (160)	2.2 (220)	4.5 (450)	1.3 (130)
neem oil extracted with methanol	ND[a]	ND	ND	ND	ND	ND	ND	ND
Margosan-O	0.25	2.4 (9.6)	1.4 (5.6)	1.1 (4.4)	6.3 (25.2)	1 (4)	7.3 (29.2)	0.2 (0.8)
RH-9999	20.0	0.48 (0.02)	7.0 (0.35)	0.3 (0.01)	4.2 (0.21)	3.2 (0.16)	7.4 (0.37)	0.7 (0.04)

[a] ND = <0.01% wt %.

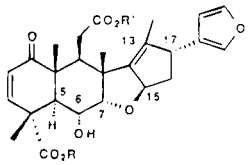
Chart 1



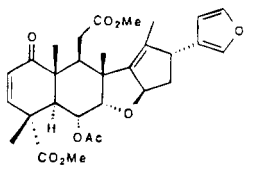
A R=H nimbandiol



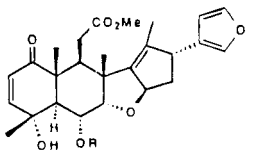
C2 Deacetylsalannin



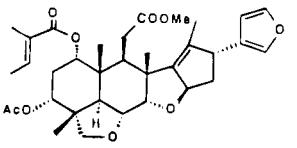
B R=R'=Me deacetylnimbin



E Nimbin



C1 R=Ac 6=acetylnimbandiol



F Salannin

oil occurs both directly in insects as well as when the insecticide is applied to plants.

Some oils are known to increase insecticidal activity (Sun, 1968). It is generally believed that the increase in efficacy caused by oils is mediated by increasing penetration into crops and insects and by increasing coverage and persistance (Anderson et al., 1986; de Licastro et al., 1983; Treacy et al., 1986, 1991). Therefore, the increase in toxicity caused by oils is not considered synergism, which is a situation where the effect of two compounds with similar modes of action, applied together or consecutively, is greater than would be expected from the sum of the individual effects (Bliss, 1939). Another term, potentiation, is used to describe a situation where synergism occurs, but the two compounds have different modes of action. Sun and Johnson (1972) used the term quasisynergism for cases of increased cuticular penetration in insects and went on to define "total synergism" as the product of enzymatic inhibiton and synergism due to an increase in penetration.

An example of an oil increasing the efficacy of an insecticide was shown by Sun (1968). He found that carbaryl was much more toxic to the house fly, *Musca domestica* L., when it was applied with kerosene than with acetone. Anderson et al. (1986) showed that sun spray oil acted as a potentiator of avermectin in *Spodoptera eridania* (Cramer), but not in *Heliothis virescens* (F.) after these insects ingested insecticide-treated Sieva bean, *Phaseolus lunatus* L., foliage.

Mineral oil synergized the toxicity of cyfluthrin compared to water in field tests with the boll weevil, *Anthonomus grandis grandis* Boheman, feeding on insecticide-treated cotton (Treacy et al., 1991). Paraffinic oils increased penetration of insecticides in the conenose bug, *Triatoma infestants* (de Licastro et al., 1983). However, a study by Southwick et al. (1983) showed just the opposite in cotton. The addition of cotton seed oil and soy bean oil to permethrin actually slowed penetration into cotton leaves compared to an aqueous formulation.

There may be other factors responsible for the increases in efficacy of insecticides in the presence of oils such as increased persistence on crops (Salt and Ford, 1984; Hesler and Plapp, 1986).

Xie and Isman (in press) found that tall oil, a product of the pulp wood industry, enhanced the activity of azadirachtin in the variegated cutworm, *Peridroma saucia*. These authors concluded that tall oil worked by increasing cuticular penetration of azadirachtin.

In our study, the substitution of canola oil for neem oil in MO resulted in a 33% loss of toxicity. The substitution of neem oil extracted with methanol resulted in a 30% loss of toxicity, but complete removal of oil from MO resulted in a 62% loss in toxicity. So the addition of oils increased the toxicity of MO, but something that was removed from the oil during the methanol extraction process resulted in a 30% loss in toxicity. Neem oil alone did not kill *A. pisum* within seven days after exposure, but individuals were much smaller than controls indicating biological activity of the oil.

Therefore, two processes are obviously at work. Oils increase toxicity, probably by increasing penetration, and a polar component(s) in neem oil also increases the toxicity of MO.

Mass spectral evaluations of neem oil before and after methanol extraction revealed the presence of six limonoids and two unknown compounds. We hypothesize that one or more of these products is responsible for 30% of the toxicity of MO. The importance of this finding cannot be determined until further work is done.

For future studies the following questions should be addressed:

1. Does the observed enhancement by neem oil occur only in the pea aphid or is it manifested in other aphid species and other pest and beneficial species?
2. Does neem oil or canola oil actually increase penetration of azadirachtin or is some other mechanism involved? Toxicodynamic studies with MO and MO devoid of neem oil should be conducted to prove whether greater penetration of azadirachtin into plants and insects is occurring.
3. What component(s) of neem oil is (are) responsible for the enhanced activity we observed?

Results of this study may have implications for the development and use of neem insecticides in the future. Neem oil is phytotoxic to some crops and thus the decision to eliminate it from a commercial insecticide may be very prudent depending upon the crops that are targeted for registration. However, our results show that addition of neem oil to neem insecticides increases their efficiacy at least with the pea aphid. If the same thing occurs with other pest species, addition of neem oil to future neem insecticide formulations may result in better control.

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