Insecticidal Effect of Neem Cake Extracts on Cabbage Pests, *Aphis gossypii* and *Pluetella xylostella*

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Abstract - In organic agriculture, choose of effective and cheap bio-pesticide is very important. The authors developed an insecticidal extract from neem cake, waste of neem oil from kernel, and applied as a bio-pesticide. Bio-pesticide neem cake extracts experiment on cabbage pest was carried out at Wonju Agricultural Technology and Extension Center from 11 March to 30 May 2003. There were six treatments with three replications, using completely randomized design. Treatments involved three and six sprays of synthetic pyrethroid pesticide cypermethrin 10 EC at the dilution rate of 2.2 mL L^{-1} of distilled water and four, five and six sprays of bio-pesticide neem at the dilution rate of 13.3 mL L^{-1} of distilled water, and untreated control. For each treatment, designated sprayings were done at 7 days interval. Pre-spray data showed that the plants in all the experimental plots were already infested with aphid (*Aphis gossypii*), and diamondback moth (*Pluetella xylostella*). The results indicated that all neem pesticide treatments were more effective in insecticidal activity than the untreated control and the chemical treatments in controlling aphids and diamondback moth. Among the three neem treatments, there were no significant differences between them.

Key words : azadirachtin, bio-pesticide, neem, aphid, diamondback moth

INTRODUCTION

Under agricultural conditions, partly polyphagous insect pests can attack several crops in one rotation, making intensive vegetable production unsustainable (Brader 1979; Binyason 1997).

Frequent use of synthetic insecticides during the last half century leads to a number of well-known problems. Some of these are destabilisation of the ecosystem and enhanced resistance to insecticides in pests (Dittrich *et al.* 1990; Magaro and Edelson 1990), contamination of food with toxic residues, and side effects on non-target insects and other organisms (Schmutterer 1995). In addition, increasing documentation of negative environmental and health impact of synthetic toxic insecticides and increasingly stringent government regulation of pesticides have resulted in renewed interests in the use, research and development of botanical pest management products (Ascher 1993).

The neem tree (*Azadirachta indica* A. Juss) and its products have the potential components for the control of insect pests (Lowery *et al.* 1993; Schmutterer 1995; Basedow *et al.* 2002) and, therefore, neem-based insecticides are being investigated as effective control agents for insect pest management in many countries, especially in India and Myanmar in where neem trees are abundant. Neem-based insecticides containing azadirachtin as an active component have played important roles in crop protection.

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Azadirachtin, a very complex tetranortriterpenoids, has been effectively used against more than 400 species of insects, including many key crop pests, and has proved to be one of the most promising plant ingredients for integrated pest management at the present time (Rembold 1989; Schmutterer 1990; Schmutterer 1995).

Cabbage is one of the most important vegetable crops in Korea. The diamondback moth (*Pluetella xylostella*) is one of the most economically important insect pests of cabbage and other related crucifer vegetables (Liang *et al.* 2003). Aphid (*Aphis gossypii*) is also very harmful insect in cabbage cultivation in Korea.

The objective of this study was to prepare the neem pesticide from waste of neem oil for cheap production and to determine the effects as bio-pesticide on control of the cabbage pests.

MATERIALS AND METHODS

1. Preparation of neem-based insecticide

Neem cake were used for methanol extraction. Neem seeds were dried in the shade at ambient temperature to avoid decomposition of the active principles and used for neem oil production. The waste from neem oil production is neem cake. The steps of neem cake pesticide production was explained in Fig. 1.

For the preparation of the plant material, methanol was used as the solvent for extraction and the crude extract was fractionated by solvent partition to remove the fatty esters.

The isolation and chromatographic techniques of azadirachtin were followed by the method of Warthen *et al.* (1984). Neem extract samples were prepared with methanol to 1% diluted sample and filtered by 0.2 μm membrane filter. Prepared sample was injected as 20 μL into C_{18} RP column (Waters PPS 839540, 250 × 4.6 mm, 5 μm), and eluted with acetonitrile buffer at the flow rate of 1 mL min^{-1} and the azadirachtin component was detected by UV detector at 214 nm with standard molecule.

The dilution of each insecticide was prepared separately by slowly adding the extract to a basket filled with appropriate amount of water while stirring consistently. The concentration of neem extract used in this study was adjusted within the range of 3.5-3.9 mg azadirachtin/liter.

2. Field control experiments

Bio-pesticide neem cake extracts experiment on cabbage pest was performed at Wonju Agricultural Technology and Extension Center from 11 March to 30 May 2003.

There were six treatments with three replications, using completely randomized design. Each plot with a dimension of 1.4 m (w) × 2.7 m (L) consisted of two 2.7 m rows, each row contained five cabbages planted in the space of 45 cm (w) × 45 cm (L), and all plots were separated from one another by non planted border areas of 45cm.

Cabbage seedlings of 4 weeks old were transplanted and cultivated in the 18 plots and the treatments of insecticides were started when 30 days old after transplanting. For each treatment, sprays of insecticides were done at 7 days interval.

Chemical pesticide cypermethrin (commercial product) and neem extract were spread over the recommended field dose rate of 2.2 mL L^{-1} of water for cypermethrin and 13.3 mL L^{-1} of water for neem extract (azadirachtin 0.75%). Treatments were applied from 11 May using a backpack sprayer calibrated to deliver 20 gal per acre at 40 psi.

Applications of insecticides involved three sprays (T2) and six sprays (T3) in the treatments of chemical pesticide and four sprays (T4), five sprays (T5) and six sprays

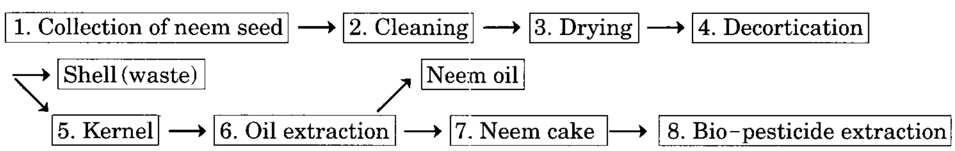

Fig. 1. Neem cake pesticide production steps.

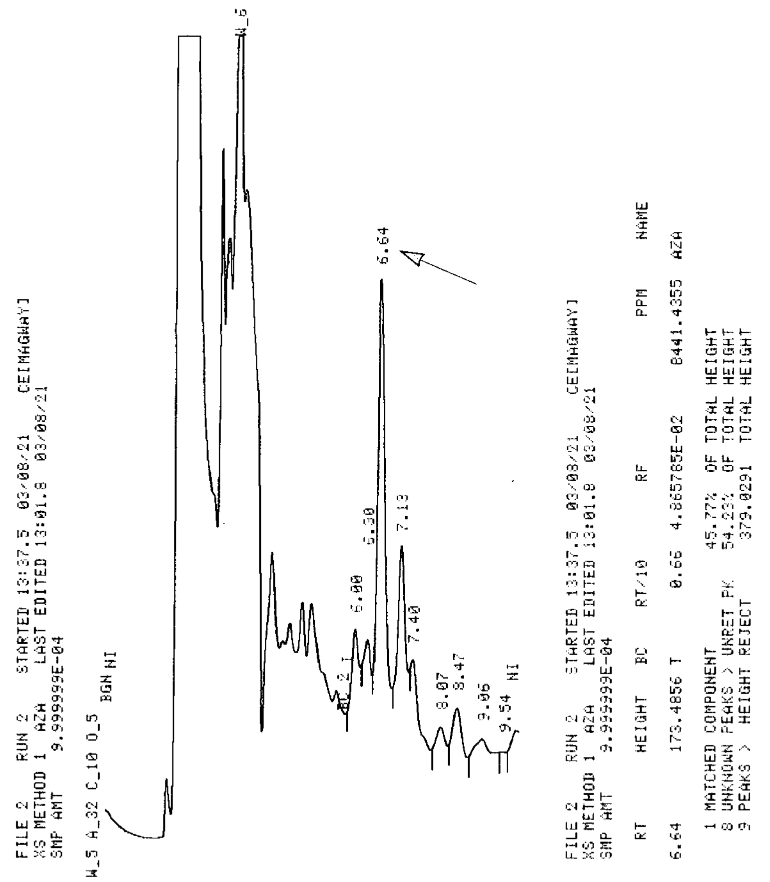

Fig. 2. HPLC chromatogram of neem cake extract. Neem extract sample was prepared with methanol as 1% diluted sample and filtered by 0.2 μm membrane filter. Prepared sample was injected as 20 μL into C_{18} RP column (Waters PPS 839540, 250 × 4.6 mm, ¢5 μm), eluted with acetonitrile buffer with the flow rate of 1 mL min^{-1} and the azadirachtin (arrow) was detected by UV detector at 214 nm.

(T6) in the treatments of neem extract and sprays with water alone (T1) were used as control.

3. Data analysis

Insect counts were done before the start of each spray. All 10 cabbages per plot were examined carefully for insect survival on each of 7 days and mortality was determined by the cessation of movement of insects. All data consisted of the average counts of three replications for each experimental unit and were analysed by means of one-way analysis of variance procedure (SAS Institute 2001).

RESULTS AND DISCUSSION

1. HPLC analysis

Azadirachtin was identified by HPLC techniques (Warthen *et al.* 1984) (Fig. 2). The main active ingredient of the neem preparations was azadirachtin A (Aza A), an antihormone for insects (Schmutterer 1995). The Aza A-content of the neem extract was confirmed by HPLC and diluted to 0.75% concentration with deionized water for standard solution in this study.

2. Aphids-*Aphis gossypii*

In untreated control (T1), the population of aphids on the day of 1st spray (1st week) was 161 and the numbers of aphids increased steadily to the maximum of 5,649 on the day of 7th spray and thereafter they decreased to 556.9 (Table 1).

In chemical treatment (T2), the population of aphids on the day of 1st spray was 1666.7, but after one spray, the populations dropped to 158.7. After 2nd spray, the population dropped further to 51. After the final 3rd spray, the population increased slowly to 145.3 and reached to the maximum of 4,383 and it declined again to 7.5 at the end of this experiment (Table 1). In the treatments of 6 times of chemical cypermethrin (T3), there was a slight decrease in population from 403 to 52 during the first week but the populations increased gradually despite of continuous sprays of 5 times.

On the other hand, there was a significant decrease in average number of pests in neem treatment (T4, T5 and T6). In the experiment of T4, the population before 1st spray was 583.7 and the populations dropped to zero level after 4th spray and climbed up to 96.7 when stopped to spray. Also, in the experiment of T5 and T6, there was similar tendency, that is, initial populations of 148.7 and 202 dropped to zero level after 4th and 5th sprays, respectively. As shown in the experiment of T4, T5 and T6, there was no pest when applied neem extract continuously (Table 1).

As shown in Table 1, there was a significant difference in population between the neem treatments (T4, T5 and T6) and the chemical treatment (T2 and T3) even after 4 sprays of insecticides.

In the population built-up trend, the population builds up continuously in the control plots whereas, in the chemical plots, the population drops for a certain period when treated with chemical after which it builds up again even with the continuous 7-day interval applications. But, in the neem plots, the populations drop at the first neem application and with the subsequent sprays, the populations dropped to zero level, showing that neem pesticide was very effective in preventing and controlling the population build-up.

In comparing the effect of neem pesticide with that of chemical pesticide cypermethrin, it was found that neem pesticide was significantly better than cypermethrin in all the spray frequencies. In fact, cypermethrin at this application rate was not better than the control and showed that it was not effective against *Aphis gossypii*.

As shown in the experiments of T4, T5 and T6, there was no significant difference in population among the treatments of neem extract.

3. Diamondback moth – *Plutella xylostella*

In control (T1), there was a low population of 2.0 on the day of 1st spray and population built up gradually and reached to the maximum of 128.3 at the end of the experiment (Table 2).

In experiments of the chemical treatments (T2 and T3), the initial populations of T2 and T3 were 5.0 and 5.7 and the populations decreased to 3.7 and 3.0 after the 1st spray, respectively. However, when sprays were

Table 1. Effect of botanical and chemical pesticides on the population of aphid (*Aphis gossypii*) infested on winter cabbage

Treatment	Average number of pest per acre before each spray							
	1st spray	2nd spray	3rd spray	4th spray	5th spray	6th spray	7th spray	8th spray
T1 Control	(161.0a)	(240.3a)	(308.0b)	(551.0b)	(537.7b)	(779.7b)	(5649.3b)	(556.9b)
T2 Cyper 3 sprays	1666.7a	158.7a	51.0b	(145.3b)	(782.0b)	(3233.3b)	(4383.3b)	(7.5b)
T3 Cyper 6 sprays	403.3a	52.0a	158.0b	167.7b	929.0b	1418.3b	(2570.0b)	(196.2b)
T4 Neem 4 sprays	583.7a	95.0a	13.3a	17.7a	(0a)	(0a)	(96.7a)	(2.3a)
T5 Neem 5 sprays	148.7a	100.3a	2.7a	12.7a	0a	(0a)	(7.3a)	(0a)
T6 Neem 6 sprays	202.0a	94.7a	34.7b	6.0a	0a	0a	(0a)	(0a)

*Data are means of three replicates; *Number in column means of aphid body number in acre; *Data in parenthesis indicate the numbers of pest without treatments of pesticides; *In each column, means followed by the same letter indicate no significant differences in mortality at $p = 0.05$ (LSD, SAS Institute 2001) among treatment levels.

Table 2. Effect of botanical and chemical pesticides on the population of diamondback moth (*Plutella xylostella*) infested on winter cabbage

Treatment	Average number of pest per acre before each spray							
	1st spray	2nd spray	3rd spray	4th spray	5th spray	6th spray	7th spray	8th spray
T1 Control	(2.0a)	(14.3b)	(31.7b)	(45.7c)	(108.7c)	(96.7b)	(127.7d)	(128.3b)
T2 Cyper 3 sprays	6.4a	8.2a	8.0b	5.3b	(22.0b)	(33.3b)	(52.8b)	(99.4b)
T3 Cyper 6 sprays	5.7a	3.0a	7.7a	4.7a	16.0b	23.3b	(40.3c)	(120.0b)
T4 Neem 4 sprays	4.3a	1.0a	1.0a	0a	(0.3a)	(3.0a)	(27.3b)	(21.7a)
T5 Neem 5 sprays	201.3a	0.7a	4.7a	2.0a	0.7a	(1.3a)	(2.0a)	(41.0a)
T6 Neem 6 sprays	4.3a	1.0a	2.0a	7.0b	0a	0a	(0a)	(30.7a)

*Data are means of three replicates; *Number in column means of diamondback moth body number in acre; *Data in parenthesis indicate the numbers of pest without treatments of pesticides; *In each column, means followed by the same letter indicate no significant differences in mortality at $p = 0.05$ (LSD, SAS Institute 2001) among treatment levels.

stopped, the populations of T2 and T3 began to increase steadily and reached to 109.0 and 120.0 at the end of experiment, respectively (Table 2).

In the treatments of neem extract (T4 and T6), there were low initial populations of 4.3 before the 1st spray and the populations maintained low level after the 2 or 3 sprays but there was no pest in the treatments of neem extract 3 or 4 times. However, the pests started to rear again when stopped application of neem extract, so that, the numbers of pests of T4 and T6 increased to 21.7 and 30.7 at the end of the experiment, respectively. In the experiment of T5, there was a high initial population of 201.3 but the number of pest decreased to 0.7 even after the 1st spray. In this experiment, the pest was not eradicated completely by the consequent application of neem extract. The number of pest began to increase again immediately after the stop of application and increased to 41.0 whin 3 weeks.

There was no significant difference in initial populations between all treatments at the beginning of the experiments, but, after the first spray, there was a significant difference between the treatments of chemical and the control and also between the treatments of neem extract and the control. After the second sprays, there was no significant difference between the treatments of chemical and the control, but there was a significant difference between chemical and neem treatments (Table 2, column 5).

The effects of neem pesticide on *P. xylostella* were not always significantly different. However, it has been proved that biological activity of neem pesticide cannot be solely judged by the content of azadirachtin and neem extracts contain numerous other chemicals such as aflatoxins, salannin and nimbandiol that also have insecticidal activities (Walter 1999). In addition, differences in extraction process, formulations of solvent and adjuvant can dramatically influence toxicity and biological activity (Isman 1999; Walter 1999).

From the results of this study, it would be concluded that neem pesticide is more effective against the *P. xylostella*, whereas the chemical cypermethrin has no effect at this particular dilution rate.

CONCLUSIONS

In conclusion, this experiment shows that the neem cake, waste of neem oil, has enough insecticidal effect. The botanical neem pesticide (azadirachtin 0.75%), which extracted from neem cake, can effectively prevent and control *Aphis gossypii* and *Plutella xylostella* which attack the cabbage plants. The use of neem kernel was led us to make cheap and useful bio-pesticide.

In fact, it can be concluded that neem is a better pesticide than the synthetic pyrethroid cypermethrin since it is not only effective for the control of cabbage pests but is not harmful to the beneficial insects and is environment friendly.

The only drawback is that, for the neem pesticide to be effective, it must be sprayed at regular intervals for at least four times during the cropping season in order to prevent the pests from re-infestation.

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