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THE TOXIC EFFECT OF NEEM EXTRACT, SPINOSAD AND ENDOSULFAN ON THE GROWTH OF APHIDS AND ITS PREDATOR

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ABSTRACT

The present investigation was carried out to study the toxic effect of Neem seed kernel extract, spinosad and endosulfan comparitively. The chief volatile compound azardiractin present in Neem seed kernel extract appear to block the neurosecretory cells and resulting in disruption of development of eggs and preventing adult maturation. Spinosad reduces the moulting of larvae and pupae to adult, but it is less toxic than endosulfan. Our results clearly showed endosulfan contains highly toxic molecules when compare both natural (Neem) and bacterial (Spinosad). The present study positively suggests natural pesticide like neem extract is usually safe and beneficial for the environment.

INTRODUCTION

Aphids are economically important insects causing severe damage to a number of crop plants (Blackman and Eastop, 1984). Three species are commonly found on cotton, the cotton aphid, green peach aphid and cowpea aphid (Borror *et al.,* 1989). *Aphis gossypii* is an important pest throughout the world. The cotton aphid colonizes over 60 plant species including cucurbits, citrus and vegetables (Drees, 1993; Slosser *et al.,* 2001). Ladybirds (cocinellidae) are predators of phytophagous pests, such as aphids, diaspids, coccids, thirps and acaroids (Dixon, 1990; Ebert, 1997). These prey species are, however not equally suitable for growth, development and reproduction of ladybird, with suitable differing in relation to habitat,nutritional,requirement of the predator and the biochemical contents of their prey (Bond *et al*., 2004).

The primary active ingredient of most neem based pesticides is Azardiractin (AZ), a liminoid compound with excellent insecticidal activity against many phytophagous pests. Azardiractin has numerous effects on insects pest including insect growth regulation (IGR), feeding deterrence and reproduction inhibition (Mordue and Blackwell, 1993). Equally important, neem extract has minimal toxicity to nontarget, organisms such as parasitoids, predators, and pollinators (Isman, 1997) and it also degrades rapidly in the environment.

Spinosad is a mixture of spinosyns A and D derived from the naturally occurring soil actinomycete *Sacchar-opolyspora spinosa* (Sparks *et al.,* 1998). It has been classified as an environmentally and toxicologically reduced – risk insecticide (Copping and Menm, 2000; Cleaveland, 2001). Spinosad is both a nerve poison and a stomach poison, so it kills pests that it contacts and those that consume it on foliage they eat. Endosulfan is a highly toxic substance and carries the single word DANGER on the lable. It is used to control insects on food and non-food crops and also asa wood preservative. Exposure to endocsulfan happens mostly from eating contaminated food, but may also occur from skin contact, breathing contaminated air, or drinking contaminated water. Endosulfan affects the function of the central nervous system. Hence, in the present investigation an attempt has been made to study on the toxic effect of neem extract, Spinosad and Endosulfan on feeding and reproduction of Aphids, *Aphis gossypii* and its predator *Hippodamia convergens*.

MATERIALS AND METHODS

Laboratory culture

For culturing insects in the laboratory, the mature *Aphis gossypii* both alate and atperous forms were initially collected from the plant *Gossypium hirsutum* in and around the Bharathiar University Campus, Coimbatore. They were reared

in wooden cages covered with muslin cloth by giving *ad labium* of different stages of their host plant leaves. After settling down, the adult aphids were allowed to deposit their nymphs. The young first instar nymphs were removed from the culture colonies using a camel's hair brush and placed in separate cages. These young ones were cultured on different leaf stages of *Gossypium hirsutum*.

For culturing the predators, the adult male and female of *Hippodamia convergens* were collected and reared in wooden cages. They were allowed for mating. After reproduction, the newly emerged larvas were introduced on *Aphis gossypii* reared on different ages of *Gossypium hirsutum* leaves.

Fecundity of *Aphis gossypii* and its predator

For studying the fecundity of *Aphis gossypii* on different staged host leaves, each day after moult to adult, the newly born nymphs were counted. The two thirds of the batches were recorded for 10 days, only the remaining one third was then continued until they die to obtain figures for total fecundity and for the reproductive period.

Preparation of pesticides

Neem seed kernel extract (NSKE)

Neem seed were collected, dried and made into powder.10g of powder and suspended in 100mL of distilled water which gives 10% extract. The water soluble portion was isolated in subjected to further dilution to obtain solution with concentration range 0f 0.5,1,2,4,6 % Different groups of aphids were subjected to NSKE treatment. Group A – Control; Group B – 0.5%; Group C –1 %; Group D – 2% Group E – 4%; Group F – 6%.

Spinosad

It's a common bacterial insecticide was used to treat other group of aphids. The commercial name of Spinosad is success.

Endosulfan

It's a common chemical insecticide was used to treat other group of aphids. The commercial name of Endosulfan is sicosulfan 35.

Effect of neem on *Aphis gossypii*

Gossypium hirsutum leaf discs (2.6 cm dia.) obtained from *Gossypium hirsutum* leaves of the same maturity were dipped in each NSKE solution for 1 minute. They were allowed to dry for 2 hr and offered for feeding to five Aphis *gossypii* placed in a petridish (9cm dia).Leaf discs dipped in distilled water were used as the untreated control. Leaf area (mm^2) consumed by the larvae and the number of larvae , that were dead after 12, 24, and 48, hours after introduction (HAI) were recorded. Larvae were provided with fresh treated *Gossypium hirsutum* leaf disc, when necessary. Adult emergence and developmental abnormalities if anywhere also recorded.

Effect of spinosad on *Aphis gossypii*

Biological activity of the bacterial insecticide Spinosad was determined by using a leaf disc bioassay. In the bioassay, adult *Aphis gossypii* were used. *Gossypium hirsutum* leaf disc were prepared by using a cork borer with a diameter of 3.8cm. For the *Aphis gossypii*, Spinosad treatment of 0.5 to 2.5ppmwas done. All leaf discs were dipped into solution containing different concentration of Spinosad for 5 sec. and then they were air dried for one hour. Control leaf disc were dipped into double distilled water. Afterwards the leaf disc were placed in a 16 well insect rearing plate and one *Aphis gossypii* adult was added per well and allowed to feed on the treated leaf disc for 24 hr. The experiment was recorded 3 times, 20 using adult for each experiment.

Effect of Endosulfan on *Aphis gossypii*

Direct spray test

Three leaf discs were placed in each Petri dishes (9cm dia) 15 to 20 *Aphis gossypii* were placed on each disc. Each leaf disc within petridish was assigned randomly to the 0.5, 1, 1.5, 2 or 2.5 ppm treatment. Gravid predator *Hippodamia convergens* was sprayed with the solution of 0.5, 1, 1.5,2 or 2.5 ppm Endosulfan. After one hour of drying at room temperature, five predator from each treatment were placed inside humidity boxes in an incubator at 26 ± 2ºC.75% RH, and a photoperiod of 16: S (L: D) hr. Adult mortality and the number of eggs laid per female were recorded after 24 and 48 hr.

Residual spray test

Fifteen to twenty *Aphis gossypii* were placed on each 15 cotton disc and 5 disc each sprayed with solution of either 0.5,1, 1.5, 2 or 2.5 ppm Endosulfan.After one hour of drying at room temperature, first leaf disc per treatment was placed on randomly in each of 5 petridishes. Five untreated predators were placed on each disc.

The petridishes were placed inside the humidity boxes in an incubator at 26 $\pm$ 2ºC. 75% RH and a photoperiod of 16: S (L; D) hr.Adult mortality and the number of eggs laid per female were recorded after 24 and 48 hr.

Toxicity Test

The cotton leaves were dipped for 5 seconds in suspension of NSKE, Spinosad and Endosulfan respectively and then dried for one hour. The control leaves were dipped similarly in distilled water. Separate experiments were conducted for aphid adults and nymphs. Group of 5 adult aphids or 10 second instar nymphs were transferred to each treated cotton leaves. Each cotton leaf with the aphids was placed in a petridish. Mortality of both adults and nymphs were recorded daily for 7 days. Present survival and longevity of adults and nymphs, average offspring per adult and average molts per nymph were calculated for each cotton leaves. Each treatment was replicates five times.

RESULTS

Table 1 provides the biological parameters of *Aphis gossypii* reared on different aged leaves viz., Bolls, young, mature and senescent leaves. *Aphis gossypii* reared on young showed better life history, parametes such as incubation period, total larval duration.

Table 2 provides application of neem seed kernel extract at 0.5% to 6% to *Gossypium hirsutum* leaves which did not significantly reduction to the survival was noticed after the treatment of 6 % treatment at 8 hr and 24 hr but not at 48 hrs post treatment.

Table 3 provides application Spinosad at 0.5 to 2.5 ppm to *Gossypium hirsutum* leaves did not significantly reduce the

Table 1: Life history of *Aphis gossypii* fed on *Gossypium hersutum*

Larval duration	Group A	Group B	Group C	Group D
I	1.91 ± 0.065	2.18 ± 0.19a^{ns}	3.39 ± 0.175**	2.48 ± 0.27*
II	2.54 ± 0.081	1.826 ± 0.077**	4.41 ± 0.288**	3.22 ± 0.193**
III	4.33 ± 0.080	3.863 ± 0.077**	5.38 ± 0.249**	4.33 ± 0.238^{ns}
IV	3.183 ± 0.130	2.316 ± 0.248**	3.633 ± 0.330**	2.88 ± 0.116**
Adult longevity	13.466 ± 0.236	11.81 ± 0.589**	8.426 ± 0.307**	10.626 ± 0.313**
Fecundity	9.25 ± 15.0	866.6 ± 26.539^{ns}	529 ± 36.290**	742.33 ± 37.434**

Values are expressed means ± SD (n = 20) statistical significance was calculated by one way ANOVA by using Dunnett multi-range test. Different alphabet in the superscript in a row denotes significance at 5% Level. p < 0.01** = Significance at 1%level; p < 0.05* = Significance at 1% level; p > 0.05 = Not significant

Table 2: Effect of neem seed kernal extract on adult survival and emergence rate of *Aphis gossypii*

Percentage of survival after treatment	Control Group A	Group B	Group C	Group D	Group E	Group F
8h	90.5 ± 0.264	90.43 ± 0.30 ^{ns}	89.7 ± 0.40ns	86.3 ± 1.05**	82.866 ± 1.464**	82.733 ± 1.401**
24h	77.8 ± 1.735	76 ± 1.572^{ns}	72.86 ± 1.49**	69.26 ± 0.85**	66.66 ± 1.290**	64.33 ± 0.86**
48h	32.46 ± 2.15	32.4 ± 2.20 ^{ns}	29.43 ± 0.731^{ns}	27.633 ± 1.52	28 ± 1.57*	27.3 ± 0.66**
Percentage of adult emergence	383.966 ± 1.72	83.566 ± 1.617 ^{ns}	81.73 ± 1.5^{ns}	78.8 ± 1.57**	76.63 ± 1.36**	73.8 ± 3.15**

Values are expressed means ± SD (n = 20) statistical significance was calculated by one way ANOVA by using Dunnett multi-range test. Different alphabet in the superscript in a row denotes significance at 5% Level. p < 0.01** = Significance at 1%level; p < 0.05* = Significance at 1% level; p > 0.05 = Not significant

Table 3: Effect of Spinosad on adult survival and emergence rate of *Aphis gossypii*

Percentage of survival after treatment	Control Group A	Group B	Group C	Group D	Group E	Group F
8h	91.566 ± 0.72	85.4 ± 1.20**	82.43 ± 1.55**	77.96 ± 1.5b**	77.96 ± 1.40**	76.53 ± 1.76**
24h	73.53 ± 0.902	73.56 ± 2.49^{ns}	72.43 ± 2.08^{ns}	65.83 ± 2.13c**	58.33 ± 1.61**	55.13 ± 1.02**
48h	33.8 ± 1.13	31.96 ± 1.401^{ns}	25.26 ± 0.76d**	22.46 ± 2.12**	19.1 ± 0.55	13.66 ± 0.929**
Percentage of adult emergence	85.33 ± 0.960	80.133 ± 0.750**	77.7 ± 1.572d**	76.066 ± 1.0050**	62.633 ± 1.429**	50.2 ± 1.10**

Values are expressed means ± SD (n = 20) statistical significance was calculated by one way ANOVA by using Dunnett multi-range test. Different alphabet in the superscript in a row denotes significance at 5% Level. p < 0.01** = Significance at 1%level; p < 0.05* = Significance at 1% level; p > 0.05 = Not significant

Table 4: Effect of Endosulfan on adult survival and emergence rate of *Aphis gossypii*

Percentage of survival after treatment	Control Group A	Group B	Group C	Group D	Group E	Group F
8h	90.3 ± 0.62	83.53 ± 092**	75.8 ± 1.12**	63.9 ± 2.00**	57.96 ± 1.56**	54.76 ± 1.45
24h	98 ± 1.37	76.5 ± 0.75ns	73.8 ± 1.61*	61.4 ± 1.60**	54.63 ± 1.56**	43.36 ± 1.06**
48h	31.63 ± 0.96	28.433 ± 1.06**	25.4 ± 0.916**	15.56 ± 1.26**	10.53 ± 0.83	10.13 ± 0.30**
Percentage of adult emergence	84.56 ± 0.86	75.36 ± 1.02**	66.76 ± 1.002**	53.6 ± 0.91**	42.5 ± 1.25**	29.5 ± 0.79**

Values are expressed means ± SD (n = 20) statistical significance was calculated by one way ANOVA by using Dunnett multi-range test. Different alphabet in the superscript in a row denotes significance at 5% Level. p < 0.01** = Significance at 1%level; p < 0.05* = Significance at 1% level; p > 0.05 = Not significant

Table 5: Toxicity of different insecticide on adult *Aphis gossypii* after 12 hr

Insecticide	Treatment (%)	No. of Aphid Used	Died	Mortality (%)	LC_{50} (g/cm)	LC_{90} (g/cm)
Neem seed	0.5	20	5	25	2.086	5.134
kernel	1	20	7	35		
extract	2	20	9	45		
	4	20	11	80		
Spinosad	0.5	20	6	30	0.339	-0.788
	1	20	8	40		
	2	20	11	55		
	2.5	20	12	89		
Endosulfan	0.5	20	6	30	0.374	-0.663
	1	20	8	40		
	2	20	12	60		
	2.5	20	15	90		
Control	-	20	1	5.0		

Values are expressed means ± SD (n = 20) statistical significance was calculated by one way ANOVA by using Dunnett multi-range test.

Table 6: Toxicity of different insecticide on adult *Aphis gossypii* after 24 hr

Insecticide	Treatment (%)	No. of Aphid Used	Died	Mortality (%)	LC_{50} (g/cm)	LC_{90} (g/cm)
Neem seed	0.5	20	5	25	1.856	1.52
kernel	1	20	8	40		
extract	2	20	11	55		
	4	20	14	79		
Spinosad	0.5	20	8	40	0.497	-0.457
	1	20	11	53		
	2	20	14	70		
	2.5	20	16	86		
Endosulfan	0.5	20	11	55	0.815	-0.357
	1	20	13	65		
	2	20	15	75		
	2.5	20	17	95		
Control	-	20	1	5.0		

Values are expressed means ± SD (n = 20) statistical significance was calculated by one way ANOVA by using Dunnett multi-range test.

survival of adults during the experiment. However there was a small but significant reduction in survival caused by the 2.5 ppm treatment at 8hrs and 24hrs but not at 48hr post treatment. The percentage of adult emergence was decrease at 2.5ppm

treatment compare than the 0.5ppm treatment. The adult survival and emergence rate of *Aphis gossypii* was decreased in 2.5 ppm treatment compare than the 0.5 ppm treatment. Percentage of adult mortality was decrease in spinosad treatment when compare than the neem seed kernel extract treatment.

Table 4 provides application of Endosulfan at 0.5 to 0.25 ppm to *Gossypium hirsutum* leaves did not significantly reduce the survival of adults during the experiment. However there was a small but significant reduction in survival caused by the 0.25ppm treatment at 8 hr and 24 hr but not at 48 hr pest treatment. The percentage of adult emergence was decrease in 2.5 ppm of treatment. Among the percentage of adult emergence of survival had been gradually reduced after the treatment of spinosad, neem and Endosulfan.

Table 5 provides toxicity of different insecticides on adult *Aphis gossypii* after 12 hrs. Spinosad and Endosulfan were taken in the concentration of 0.5 to 0.25 ppm. Neem seed kernel extract showed toxicity at 10% only whereas, Spinosad and Endosulfan showed highest toxicity even at 2.5 ppm.

Table 6 provides toxicity of different insecticides on adult *Aphis gossypii* after 24 hrs.Spinosad and Endosulfan were taken in the concentration of 0.5 to 0.25 ppm. Neem seed kernel extract showed toxicity at 10% only whereas, Spinosad and Endosulfan showed highest toxicity even at 2.5ppm.

DISCUSSION

The aphid, *Aphis gossypii*, is one of the most common species in cotton family (Heneberry *et al.,* 2000) and is usually found on the abaxial surface of leaves, feeding on the phloem (Carter and Godfrey, 1999). As a result of feeding plant yield is reduced. In recent years, interest in developing natural insecticides has increased because of day packs in the use of synthetic insecticides. For example, environmental pollution, development of insecticides resistance, insecticides induced resurgence of insect pests, and adverse effects on non-target organism. The primary active ingredient of most neem based pesticide is Azadiractin, a limonoid compound with excellent insecticidal activity against many phytophagous pests (Fry, 1982).

Azardiractin has numerous effects on insect pest, including insect growth regulation (1GR), feeding and reproduction (Mordue and Blackwell, 1998). Equally important neem extract has minimal toxicity to non target organisms such as parasitoids, predators and pollinators (Lowery *et al.,* 1995) and degrades rapidly in the environment (Isman, 1997). Neem extracts are usually safe for beneficial organism, such as bees, predators and parasitoids, mammals and for the environment.

Spinosad is a mixture of tetracyclic macrocide, Spinosyn A and D, were produced during the fermentation and it may be considered as a bio insecticide (Copping and Menm 2000). Spinosad is a highly toxic to Lepidoptera, Diptera and some coleoptera and has a unique mode of action involving the postsynaptic nicotinic acetylcholine and GABA (Gama Amino Buteric Acid) receptors. The present study showed that spinosad was very effective in the control *Aphis gossypii*. The Endosulfan proves to be highly toxic to adult aphids and even at low concentration the impact of Endosulfan (Research Council Canada 1975). The mortality of aphids is low. Further endosulfan known to be highly toxic against Diptera and is registered for control of leaf mining dipterans pest of crop in many countries. Endosulfan is toxic when compare to conventional chemical insecticide.

The present study concludes that the toxicity of the insecticides was observed from maximum to minimum in the following order. Endosulfan > Spinosad > Neem seed kernel extract. These results further confirm the chemical pesticide Endosulfan contains highly toxic molecules when compare to both natural (Neem) and Bacterial pesticide (Spinosad). The natural pesticide like neem extracts is usually safe and beneficial organisms such as the bees, predators and parasitoids, mammals and for the environment.

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