



Comparative studies on the Effectiveness of Pesticides for Aphid control in Cowpea

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Abstract

Considering the importance of safe food globally and organic farming, the effectiveness of two biopesticides (Neem seed kernel extract and spinosad) with a synthetic pesticide (chlorpyrifos) were evaluated against cowpea aphid, *Aphis craccivora* and its predator the ladybird beetle, *Micraspis discolor* (F.). The cowpea plant was sprayed with different pesticides when the aphid population reached at economic threshold level. Among the pesticides, Chlorpyrifos proved highly effective against aphids as compared to the rest of the pesticides. Furthermore, yield of cowpea was also higher in chlorpyrifos treatments. Thus, it is concluded that all the studied insecticides proved effective against the aphids but the toxicity studies of the insecticides was observed from maximum to minimum in the following order, Chlorpyrifos > Spinosad > Neem Seed Kernel Extract.

Keywords: *Aphis craccivora*, *Micraspis discolor*, Neem seed kernel extract, Spinosad and Chlorpyrifos.

Introduction

Aphids are an important group of insects with worldwide distribution. They are a truly interesting group of herbivorous insects and can affect plants directly or indirectly by feeding on the plant's sap¹. They have experienced some adaptations in relation to host plants so that many aphid taxa have biologically complex life cycles². *Aphis craccivora* Koch is associated with many host plants in the Leguminosae and also in many other plant families so that it attacks about 50 crops in 19 different plant families.

The cowpea aphid, *Aphis craccivora* (Koch) (Aphididae : Homoptera) is a widely distributed species of insect prevalent throughout India³. Aphids are economically important insects causing severe damage to a number of crop plants. Both nymphs and adults suck plant sap and cause serious damage right from the seedling to pod bearing stage. Cowpea aphids inject toxins into the plant while feeding; they most likely reduce mung bean vigour and yields⁴. Besides causing direct damage to the host by sucking the sap from various plant parts, they may lower the yield, quality and marketability of crops by transmitting plant viruses which result in early plant death and the production of an excess of honey dew⁵.

Aphids are attacked by parasitoids, predators and pathogens. In some instances, both larvae and adults of predators belonging to the family Coccinellidae (ladybird beetles) feeds on aphids. With regards to parasitoids, some species will pupate within or below the aphid cuticle forming mummies⁶. Adult wasps will emerge from the mummies and are free living. All species in the braconid subfamily Aphidiinae develop as endoparasitoids (inside) of aphids with one larva completing development in

each host. Some species of entomopathogenic fungi infest aphids through the cuticle eventually killing the host. Ladybirds are stronger, larger and usually more intelligent than the prey. A single predator may attack several hosts in a short period⁷.

The use of neem for controlling insect infestation has been known in rural communities in India for a long time. It is known to certain diverse array of biologically active principle of which *Azadiractin* is one of the know derivatives⁸. It has antifeedent, antiovipositional, growth disrupting and fecundity reducing properties of different insect and one suitable for inclusion in the IPM programmed⁹. Neem seeds contains only trace of *Azadiractin* but compounds like salanin and meliantriol etc., are also present which useful in the control of some insects. Neem products affect the metamorphosis of insects their fecundity and have on antifeedent effect¹⁰. The latest research neem has brought out into focus it's important in biological control of pests, nematodes and disease in agriculture.

Spinosad is a biologically derived insecticide produced by the actinomycete *Saccharopolyspora spinosa*, a bacterial organism isolated from soil. The mode of action of is that spinosad is a stomach and nerve poison. It act continuously on the motor neurons resulting in the paralysis of muscles and the insects dies from exhaustion. The advantage of using spinosad is that the feeding of aphids stops with in minutes and death occurs with in 48 hours¹¹. Growers should wait a minimum of two to three days to evaluate control. Spinosad is relatively fast active compared to that of neem extract.

Chlorpyrifos is an organochlorine pesticide which penetrates the intact skin and it affects the central nervous system producing sudden irregular movement of the body caused by

involuntary contraction of muscle¹². The advantage of using chlorpyrifos is that it is highly toxic to adult and the insect death occurs within 24 hours. But the disadvantages are undiluted chlorpyrifos is slowly and incompletely absorbed into the body. Stimulation of CNS is the major characteristic of chlorpyrifos poisoning resulting in vomiting, diarrhoea, convulsion and loss of consciousness¹³. Blindness has been documented for cows, sheep and pigs that grazed in the field sprayed with the compound.

Hence in the present study three different pesticides namely bio-pesticide (neem seed kernel extract), microbial pesticide (spinosad) and chemical pesticide (chlorpyrifos) are used to study the effect of toxicity, feeding and reproduction of cowpea aphid, *Aphis craccivora* and its predator the ladybird beetle, *Micraspis discolor* (F.)

Material and Methods

Insect culture: A stock culture of *A. craccivora* was maintained on cowpea, *Vigna unguiculata* under laboratory conditions of 20.0±5.0 °C and 70.0±5.0 % R.H. and a photoperiod of 16 L: 8 D for several generations. In all experiments, the insects were put on fresh cowpea plants cultivated in small pots and enclosed individually in glass cylinders, the tops of which were covered with muslin held in place with rubber bands.

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 cowpea plant. The predator, *Micraspis discolor* were collected and reared in wooden cages. They were allowed for mating. After reproduction, they newly emerged larva was introduced on *Aphis craccivora* reared on different stages of cowpea leaves.

Preparation of Neem Seed Kernel Extract: The neem seed kernel was washed and dried in shady place in the laboratory and the dried seed kernel was ground into powder. The dried neem seed kernel powder was mixed with 200 ml of water to prepare a stock solution. The required concentration ranging from 0.25-2.0 % was prepared using distilled water.

Effect of Neem seed kernel extract on *Aphis craccivora*: Cowpea leaves were dipped in 1-6 % test solution for 1 minute containing different concentration, dried for 2 hours and allow 5 *Aphis craccivora* to feed. The leaf area consumed by the larvae is calculated. The number of larvae died in 24, 48 and 72 hours was calculated.

Effect of Spinosad and Chlorpyrifos on *Aphis craccivora*:
Direct Spray test: Take 3 leaf disc were placed in each Petri dishes. Allow 15 to 20 *Aphis craccivora* were placed on each disc. Spray different concentration test solution and dry for 1 hour. Adult mortality and the number of eggs laid per female were recorded.

Indirect Spray test: The mature leaves of cowpea were dipped in test solution for 5 sec and allow to dry for 1 Hr. Allow 20 *Aphis craccivora* to feed and adult mortality was calculated after 24 and 48 Hrs.

Results and Discussion

Table-1 shows the effect of Neem Seed Kernel Extract on adult survival and emergence rate of *Aphis craccivora*. The present study shows that high concentration of Neem reduces the percentage of adult emergence. The reduction in aphid numbers on the neem-treated plants was due to the antifeedant effect of neem which led to starvation and death of the aphids. The major component of neem seed kernel, azadirachtin is the chemical responsible for its antifeedant properties¹⁴. However, the effects of the treatment were not immediate, since the aphids were found actively moving on the leaves a few days after the application. Thus there was a delayed effect of neem on susceptible insects after application.

Table 2 and 3 shows the effect of spinosad and chlorpyrifos on adult survival and emergence rate of *Aphis craccivora*. High concentration of chlorpyrifos reduces the percentage of adult emergence when compared to that of neem extract and spinosad. High concentration of Spinosad reduces the percentage of adult emergence. Spinosad also reduced the moulting of larvae or pupae to adult but it is less toxic than chlorpyrifos. Spinosad is a microbial derived insecticide which activates the central nervous system of the aphids resulting in paralysis and death of *Aphis craccivora*. High concentration of chlorpyrifos reduces the percentage of adult emergence when compared to that of spinosad. Chlorpyrifos show highest toxicity when compared to that of spinosad.

Table 4 shows the survival, longevity and reproduction of *Aphis craccivora* adult exposed to cowpea leaves treated with neem seed kernel Extract. Higher concentration of neem seed kernel extract reduced longevity of adults and average number of moults compared to that of the lower concentration. The variable levels of control of aphids on cowpea indicate that the host plant influences the effectiveness of neem treatment.

Table 5 and 6 denotes the survival, longevity and reproduction of *Aphis craccivora* adult exposed to cowpea leaves treated with chlorpyrifos and spinosad respectively. Percentage of survival, longevity and reproduction of *Aphis craccivora* adult is higher in spinosad treated leaves compared to that of neem seed kernel extract. Chlorpyrifos show highest toxicity when compared to that of neem seed kernel extract and spinosad.

Table 7 and 8 shows the mean number of eggs laid from female and adult mortality of *Aphis craccivora* treated with chlorpyrifos and spinosad in direct spray and residual contact test. In Direct spray method, the adult mortality of *Aphis craccivora* is highest in chlorpyrifos treated leaves when compared to that of spinosad after 48 hrs. Thus the direct spray proved to be more effective than residual spray.

Table 9 and 10 shows the toxicity of different insecticides on three insecticides, chlorpyriphos showed highest toxicity on adult *Aphis craccivora* after 12 hours and 24 hours. Among the adult mortality after 24 hrs.

Table-1
Effect of Neem Seed Kernel extract on adult survival and emergence rate of *Aphis craccivora*

S.No.	Neem Seed Kernel Extract Treatment (%)	Percent Survival after Treatment			% Adult emergence
		8 hours	24 hours	48hours	
1.	Control	90.8 ^a	79.2 ^a	32.5 ^a	85.5 ^a
2.	0.5	90.7 ^{ab}	76.3 ^b	30.1 ^b	83.3 ^b
3.	1	90.1 ^b	71.2 ^c	29.7 ^c	80.4 ^c
4.	2	86.2 ^c	69.3 ^d	29.3 ^{cd}	79.2 ^d
5.	4	83.1 ^d	68.1 ^e	28.7 ^d	77.7 ^e
6.	6	82.3 ^e	63.4 ^f	26.6 ^e	76.7 ^f

Table-2
Effect of Spinosad on adult survival and emergence rate of *Aphis craccivora*

S.No.	Treatment (ppm) Spinosad	Percent Survival after Treatment			% Adult emergence
		8 hours	24hours	48hours	
1.	Control	90.8 ^a	79.2 ^a	32.5 ^a	85.5 ^a
2.	0.5	86.6 ^b	73.0 ^b	30.4 ^b	80.1 ^b
3.	1	81.5 ^c	70.1 ^c	24.6 ^c	79.4 ^c
4.	1.5	79.2 ^d	66.7 ^d	20.1 ^d	76.1 ^d
5.	2	78.4 ^e	59.1 ^e	19.7 ^e	62.3 ^e
6.	2.5	76.3 ^f	54.2 ^f	12.6 ^f	50.2 ^f

Table-3
Effect of Chlorpyriphos on adult survival and emergence rate of *Aphis craccivora*

S.No.	Treatment Chlorpyriphos	Percent Survival after Treatment			% Adult emergence
		8 hours	24hours	48hours	
1.	Control	90.8 ^a	79.2 ^a	32.5 ^a	85.5 ^a
2.	0.5	84.3 ^b	76.4 ^b	29.4 ^b	76.1 ^b
3.	1	76.4 ^c	72.1 ^c	26.4 ^c	67.8 ^c
4.	1.5	64.8 ^d	60.1 ^d	16.7 ^d	54.4 ^d
5.	2	59.4 ^e	53.2 ^e	11.2 ^e	43.7 ^e
6.	2.5	56.4 ^f	43.2 ^f	10.4 ^f	30.1 ^f

Table-4
Survival, longevity and reproduction of *Aphis craccivora* adult exposed to cowpea leaves treated with Neem seed kernel Extract

S.No.	Treatment (%) Neem seed kernel Extract	% Survival after treatment				Longevity (Days)	Avg No. of Molts
		1 day	2 days	3 days	4 days		
1.	Control	100 ^a	100 ^a	97.5 ^a	95.0 ^a	6.7 ^a	2.9 ^a
2.	0.5	97.9 ^b	89.0 ^b	76.3 ^b	10.1 ^b	5.7 ^b	2.7 ^{ab}
3.	1	94.2 ^c	86.7 ^c	53.4 ^c	0.7 ^c	5.3 ^c	2.3 ^{ab}
4.	2	90.3 ^d	82.5 ^d	44.8 ^d	0.5 ^{cd}	4.2 ^d	2 ^{ab}
5.	4	88.4 ^e	79.3 ^e	26.7 ^e	0.3 ^{cd}	3.1 ^e	1.9 ^b
6.	6	86.7 ^f	76.1 ^f	13.1 ^f	0.1 ^{cd}	2.6 ^f	1.9 ^b

Table-5
Survival, longevity and reproduction of *Aphis craccivora* adult exposed to cowpea leaves treated with Spinosad

S.No.	Treatment (ppm) Spinosad	% Survival after treatment				Longevity (Days)	Avg No. of Molts
		1 day	2 days	3days	4 days		
1.	Control	100 ^a	100 ^a	97.5 ^a	95.0 ^a	6.7 ^a	2.9 ^a
2.	0.5	96.3 ^b	87.3 ^b	60.4 ^b	8.0 ^b	4.2 ^b	2.3 ^b
3.	1	92.4 ^c	82.6 ^c	41.3 ^c	5.0 ^c	2.6 ^c	1.7 ^c
4.	1.5	88.3 ^d	74.4 ^d	34.7 ^d	0.0 ^d	2.0 ^d	0.2 ^d
5.	2	86.1 ^e	69.7 ^e	10.6 ^e	0.0 ^d	1.8 ^e	0.2 ^d
6.	2.5	80.2 ^f	67.3 ^f	8.3 ^f	0.0 ^d	1.3 ^f	0.2 ^d

Table-6
Survival, longevity and reproduction of *Aphis craccivora* adults exposed to cowpea leaves treated with Chlorpyrifos

S.No.	Treatment Chlorpyrifos	% Survival after treatment				Longevity (Days)	Avg No. of Molts
		1 day	2 days	3 days	4 days		
1.	Control	100 ^a	100 ^a	97.5 ^a	95.0 ^a	6.7 ^a	2.9 ^a
2.	0.5	83.4 ^b	64.3 ^b	34.6 ^b	9.6 ^b	5.3 ^b	1.7 ^b
3.	1	76.6 ^c	42.2 ^c	19.4 ^c	6.4 ^b ^c	2.4 ^c	1.3 ^{bc}
4.	1.5	74.7 ^d	36.4 ^d	12.6 ^c	0.0 ^c	1.7 ^d	0.1 ^c
5.	2	63.8 ^e	28.6 ^e	6.9 ^d	0.0 ^c	0.9 ^e	0.1 ^c
6.	2.5	65.4 ^f	20.1 ^f	6.4 ^{de}	0.0 ^c	0.9 ^e	0.1 ^c

Table-7
Mean number of eggs laid from female and adult mortality of *Aphis craccivora* treated with Spinosad in direct spray and residual contact test

S.No	Conc. Spinosad (ppm)	Mean eggs per Female				Adult Mortality%			
		Direct		Residual		Direct		Residual	
		24 hours	48 hours	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
1.	Control	3.9 ^a	7.2 ^a	2.8 ^a	6.0 ^a	0 ^f	4 ^d	0 ^f	0 ^f
2.	0.5	3.5 ^{ab}	6.9 ^b	2.6 ^{ab}	5.7 ^{ab}	8 ^e	10 ^{bc}	5 ^e	2 ^e
3.	1	3.4 ^{ab}	6.4 ^{bc}	2.4 ^{ab}	4.3 ^c	10 ^d	12 ^b	7 ^d	5 ^d
4.	1.5	3.0 ^b	6.1 ^{bc}	2.3 ^{ab}	4.1 ^{cd}	15 ^c	9 ^c	10 ^c	7 ^c
5.	2	2.9 ^{bc}	5.7 ^c	1.4 ^c	3.2 ^d	18 ^b	10 ^{bc}	14 ^b	10 ^b
6.	2.5	2.6 ^{bc}	5.3 ^{cd}	1.2 ^{cd}	3.1 ^{de}	20 ^a	15 ^a	16 ^a	13 ^a

Table-8
Mean number of eggs laid from female and adult mortality of *Aphis craccivora* treated with chlorpyrifos in direct spray and residual contact test

S.No.	Conc. Endosulfan (ppm)	Mean eggs per Female				Adult Mortality%			
		Direct		Residual		Direct		Residual	
		24 hours	48 hours	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
1.	Control	3.9 ^a	7.2 ^a	2.8 ^a	6.0 ^a	0 ^f	4 ^f	0 ^f	0 ^f
2.	0.5	3.1 ^b	6.5 ^b	2.4 ^{ab}	5.6 ^{ab}	10 ^e	15 ^e	8 ^e	6 ^e
3.	1	2.7 ^c	6.0 ^c	2.0 ^{ab}	4.0 ^b	14 ^d	18 ^c	10 ^d	8 ^d
4.	1.5	2.3 ^{cd}	5.8 ^{cd}	1.8 ^b	3.8 ^{bc}	16 ^c	20 ^b	12 ^c	10 ^c
5.	2	2.0 ^d	5.4 ^{cd}	1.6 ^{bc}	3.6 ^{bc}	20 ^b	16 ^d	15 ^b	12 ^b
6.	2.5	1.7 ^{de}	5.0 ^d	1.0 ^c	3.3 ^{bc}	25 ^a	21 ^a	18 ^a	14 ^a

Neem's efficacy to non-target and beneficial organisms has been documented in previous and recent literature^{15,16}. Many biologically active compounds can be extracted from neem, including triterpenoids, phenolic compounds, carotenoids, steroids, and ketones. The tetranortriterpenoid azadirachtin has received the most attention as a pesticide because it is relatively abundant in neem kernels and has shown biological activity on a wide range of insects¹⁷. Azadirachtin is actually a mixture of seven isomeric compounds labeled as azadirachtin-A to azadirachtin-G with azadirachtin-A being present in the highest quantity and azadirachtin-E regarded as the most effective insect growth regulator. Many other compounds have been isolated that shows antifeedant activity as well as growth regulating activity on insects¹⁸. This cocktail of compounds significantly reduces the chances of tolerance or resistance developing in any of the affected organisms. However, only

four of the compounds in neem have been shown to be highly effective in their activity as pesticides: azadirachtin, salannin, meliantriol, and nimbin¹⁹.

Spinosad's overall protective effect varies with pest species and life stage. Spinosad affects certain insect pests only in the adult stage, but can affect other pests at more than one life stage. The pests that are subject to very high rates of mortality as larvae, but not as adults, may gradually be controlled through sustained larval mortality²⁰. The mode of action of spinosoid insecticides is by neural mechanism. The spinosyns and spinosoids have a novel mode of action, primarily targeting binding sites on nicotinic acetylcholine receptors of the insect nervous system that are distinct from those at which other insecticides have their activity. Thus the present study reveals that Spinosad has high efficacy, a broad insect pest spectrum, low mammalian toxicity, and a good environmental profile.

Table-9
Toxicity of different Insecticides on adult *Aphis craccivora* after 12 hours

Insecticide	Treatment (%)	No. of Aphid		Mortality (%)	LC ₅₀	LC ₉₀
		Used	Died			
Neem Seed kernel Extract	0.5	20	5 ^d	25 ^d	2.086	5.134
	1	20	7 ^c	35 ^c		
	2	20	9 ^b	45 ^b		
	4	20	11 ^a	80 ^a		
Spinosad	0.5	20	6 ^d	30 ^d	0.339	-0.788
	1	20	8 ^c	40 ^c		
	2	20	11 ^b	55 ^b		
	2.5	20	12 ^a	89 ^a		
Chlorpyrifos	0.5	20	6 ^d	30 ^d	0.374	-0.663
	1	20	8 ^c	40 ^c		
	2	20	12 ^b	60 ^b		
	2.5	20	15 ^a	92 ^a		
Control	-	20	1	5.0	-	-

Table-10
Toxicity of different Insecticides on adult *Aphis craccivora* after 24 hours

Insecticide	Treatment (%)	No. of Aphid		Mortality (%)	LC ₅₀	LC ₉₀
		Used	Died			
Neem seed kernel Extract	0.5	20	5 ^d	25 ^d	1.856	1.52
	1	20	8 ^c	40 ^c		
	2	20	11 ^b	55 ^b		
	4	20	14 ^a	79 ^a		
Spinosad	0.5	20	8 ^d	40 ^d	0.497	-0.457
	1	20	11 ^c	53 ^c		
	2	20	14 ^b	70 ^b		
	2.5	20	16 ^a	86 ^a		
Chlorpyrifos	0.5	20	11 ^d	55 ^d	0.815	-0.357
	1	20	13 ^c	65 ^c		
	2	20	15 ^b	75 ^b		
	2.5	20	17 ^a	95 ^a		
Control	-	20	1	5.0	-	-

Conclusion

An assessment of toxicity studies of *Aphis craccivora* and its predator, *Micraspis discolor* based on different insecticides, Neem Seed Kernel Extract, chlorpyrifos and spinosad, reveals that the percentage of adult mortality was increased in chlorpyrifos treatment compared to that of spinosad treatment. This is due to the highly toxic nature of this insecticide, which stimulates the central nervous system of the aphids, bioaccumulate in its tissues resulting in ultimate death²¹. Thus, it is concluded that all the studied insecticides proved effective against the aphids but the toxicity studies of the insecticides was observed from maximum to minimum in the following order, Chlorpyrifos > Spinosad >Neem Seed Kernel Extract. Chlorpyrifos is a persistent organic pollutant which persists in the environment for extended periods of time, increasing the exposure risk of many non-target animals¹⁷. On the basis of present findings it could be concluded that azadirachtin based extracts may successfully be integrated, as a part of pest management programme. Azadirachtin and spinosad are easily degradable and ecofriendly and the treated crops are safe for human consumption. So the botanical insecticides neem and microbial pesticide, spinosad could be as alternatives to chemical insecticides for the control of *Aphis craccivora* to reduce the pesticide load in the environment. The results of the investigation would indicate a significant potential for these eco-friendly pesticides that could be used as an alternative to synthetic insecticides.

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