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Insecticidal toxicity upto third trophic level: A case study of Cypermethrin and Bifenthrin

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Abstract
In tritrophic interactions organisms are linked with one another through food chains and food webs. An experiment was conducted in laboratory to observe the toxicity of cypermethrin and bifenthrin at three different concentrations against first instar of cabbage lopper and its predator lady bird beetle as compared to control in tritrophic interaction. Cypermethrin and bifenthrin were observed for its toxicity at lower, recommended and higher doses against cabbage looper and its predator lady bird beetle using leaf dip method in tritrophic interaction. For cypermethrin, cabbage looper mortality tests at lower, recommended, and higher doses showed that after two hours of treatment mortality was 40%, 73.33%, 73.33% respectively, while for lady bird beetle 46%, 66.67, 73.33% respectively. After four hour mortality for cabbage looper was 60%, 99.87%, 100% respectively, while for lady bird beetle 53.33%, 66.67%, 86.67% respectively. After six hours of tratment mortality was 66.67% while at recommended and high dose was 100% for cabbage looper, while for lady bird beetle moratliry at low dose was 66.67% while at recommended and high doses were 73.33% and 100% respectively. For bifenthrin, cabbage looper mortality tests at lower, recommended and higher showed that after two hours of treatment mortality was 66.67%, 73.33%, 40%, while for lady bird beetle was 60%, 73.33%, 46.67% respectively. After four hour mortality for cabbage looper was 80%, 100%, 60%, while for lady bird beetle 60%, 73.33%, 46.67% respectively. After six hour of treatment, mortality at lower dose was 66.67% while at recommended and higher doses was 100% for cabbage looper. After six hour mortality at low dose was 66.67% while 73.33%, 100% at recommended and high doses. The results showed that cypermethrin caused toxicity to cabbage looper at lower, recommended, and higher doses as compared to bifenthrin and control, nonetheless it also revealed more toxic to predator lady bird beetles as compared to bifenthrin and control. So, as conclusion it can be affirmed that cypermethrin caused early toxicity to insects and also upto third trophic level as compared to bifenthrin which rendered harmless at third trophic level.

Keywords: cypermethrin, bifenthrin, cabbage lopper, ladybird beetle, efficacy

Introduction
In an ecosystem all living organisms are linked with each other in food chains and food webs. If we consider simple food chain having upto third trophic level which is linked with first and second one. In these type of tritrophic interactions member of high trophic level evolve to increase consumption but member of lower trophic levels are forced to reduce the feeding of high trophic level [24]. Symbiotic relationships in alternate trophic levelsof food chain are very important feature of tritrophic interactions [21].

The plants belong to family crucifer and genus Brassica specially the plants of species *Brassica oleracea* which includes horticulture crops like cabbage (*Brassica oleracea* var. capitata), brussel sprouts (*Brassica oleracea* var. gemmifera), cauliflower (Brassica oleracea var. botrytis), chinese cabbage (*Brassica chinensis*), broccoli (Brassica oleraceavar. italica) having rich amount of magnesium, iron, phosphorous [9].Cabbage is very important horticultural crop growing more than 90 countries in an area of 2.073 million hectares. The largest cabbage producer in the world is china and grown all over the world an area 3.12 million hectare and production is 22.3 million ton per hectare. Various biotic and biotic factors responsible for the low yield of cabbage in Pakistan.

In biotic factors many insect pests attack on crops like *Hellulaundalis* Fab., *Pieris rapae* Linn., *Pieris brassicae* Linn., *Crocidolomiabinotalis* Zeller, diamond back moth (*Plutella xylostella)* Linn., mustard aphid (*Lipaphiserysimi* Kaltenbach), *Brevicoryne brassicae* Linn., *Athalia proxima*, *Aphis brassicae*., and *Phyllotreta brassicae* Goeze), *P. brassicae.* Among these the most dangerous insect pest is large cabbage white butterfly (*P. brassicae*) [22, 19, 37, 9].

Cabbage butterfly (*Pieris brassicae* L.) is major insect pest of vegetable crops and attack on all the growth stages of cabbage such as flowering stage, vegetative and seedling etc also [16, 2, 10, 11, 28, 4]. There is a wide range of upto 83 host species plants belonging to family cruciferae. It is distributed from Asia to Himalaya mountains to north Africa across Europe [25, 26, 14]. *P. brassicae* found throughout the year in the field but most active period is mid February to October and this the time for growing of main vegetable crops included cabbage. In extreme cold conditions from November to February condition are adverse for the growth and development of this insect pest. The newly growing larvae of *P. brassicae* feed very actively on the leaves after that cover whole leaves and inflorescence cause complete defoliation of plant [39, 14, 11]. In cabbage and cauliflower the caterpillar of cabbage butterfly bores into the heads and cause economically damage [2,11, 28, 31]. 70-84sq.cm leaf area consume a single larvae almost eat all parts of the plant like pod, branches, leaves and seeds of vegetables mostly cabbage and cauliflower [35].

Many different synthetic insecticides are used to manage these insect pests[36]. The most easy and economical method used for controlling insect pest is chemical control that having adverse

effect on beneficial organisms. Predators play important role to manage the insect pest population [38]. One is the lady beetles feed on many soft bodied incest pest and aphids [27, 18]in which most effective female lady bird beetles [7]. Injudicious use of pesticides by the farmers having adverse effect on environment, human health and natural enemies in the field included lady bird beetles. Present study was conducted to find out the efficacy of Cypermethrin and Bifenthrin against cabbage first instar larvae and effect on the lady bird beetle.

Material and Methods

Some plants of cabbage were grown in Entomological research area of Department of Entomology University of Agriculture Faisalabad, Pakistan. In the month of February larvae of cabbage butterfly. After emergence of adult these were kept in a rearing a cage and upper of jar covered with muslin cloth for aeration and avoiding the escape butterflies. Relative humidity 65-70% and temperature 28-31^{0}C were present that is favorable temperature for growth and development of *P.brassicae.* A cotton wool soaked with sugar solution kept in cage food for adult and fresh cabbage leaves also kept for oviposition of adult females. To avoid the dryness of leaves their tips dip in the water. The leaves having eggs separated from the cage and were kept in small jars for hatching. We done our experiment on first instar of larvae and adult lady bird beetle. After round about 24 hour first instar emerge from the eggs and feed on small fresh leaves for 12 hour. Experiment consists of two phases in one phase we check the efficacy of Cypermethrin and Bifenthrin on cabbage first instar larvae after that we check the effect of these insecticides on adults of lady bird beetle. These beetles reared in the lab on aphid in rearing jars.

Bioassay

Bioassay of insecticides was done with leaf dip method. First of all made concentration of insecticides. Three concentration were made one is below the recommended dose and one is the high from recommended dose and third one was at recommended dose. Control treatment was also included in the experiment in which leaf dip with simple water. Concentrations were made with serial dilution method.

Table 1

Common name	Active ingredients	Recommended dose
Uni	Cypermethrin	250ml/acre
Tall star, Rocker	Bifenthrin	250ml/acre

Recommended concentration of both was 0.25ml/ 100ml of water and 0.12ml, 0.5ml were low and high concentration respectively. Concentrations were made in small beakers by using micropipette. Label all the beakers and concentrations according to each treatment. Use clean fresh petri dishes for experiment cabbage leaves were dip in insecticide solutions and kept in petri dishes. The experiment done in lab at temperature 28-31^{0}C and Relative humidity 65-70% conditions by using complete randomized design. One first instar larvae was allow to feed on these leaves on each petri dishes and recorded the data after 2hour, 4hour and 6hour. Insects which could not turn themselves after ten seconds by disturbing with fine brush were considered as dead. In second phase of experiment allow lady bird beetles to feed on larvae which feed on the insecticide treated leaves. Again check the mortality after 2 hour, 4 hour, and 6 hour.

Results

Fig. 1, after two hour application of cyperrmethrin at recommended field dose rate at 0.25ml/100ml water caused mortality of 73.33%. Higher and lower doses than recommended at rate values 0.5ml, 0.12ml/100ml water caused mortality of 73.33% and 40% respectively. While in control mortality was 20%. After four hour application of Cypermethrin cause mortality at recommended field dose (0.25ml/100ml water) rate was 99.87%. At high and low dose rate values (0.5ml, 0.12ml/100ml water) from recommended dose rate cause mortality were 100% and 60% respectively. While in control mortality was 20%. After six hour mortality was 100% at recommended and high dose rate while at low dose rate mortality was 73.33% and control was 73.33%. Significant and non-significant differences are shown in Table 2.

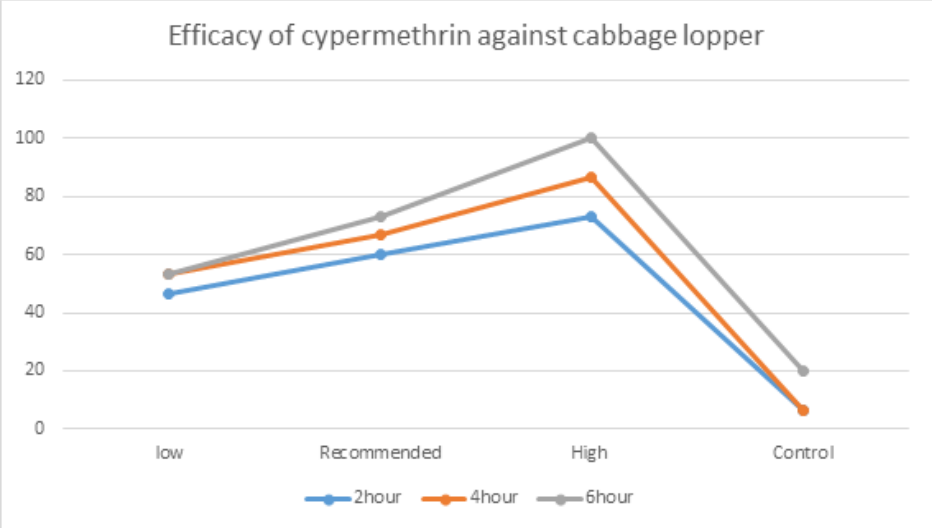

Fig 1: Toxicity of cypermethrin against cabbage looper at lower, recommended and higher doses. Data is percent mortality of cabbage looper neonates

Table 2: Cypermethrin mortality data analysis after 2, 4, 6 hours of application. ($P \leq 0.005$)

Insect/ Predator	Dose rate	After 2h of application	After 4h of application	After 6h of application
Cabbage looper	Low	(P=0.002, t=2.04, df=28)	(P=0.002, t=2.04, df=28)	(*P*=0.0003, t=2.07, df=21)
	Recommended	(P=0.002, t=2.04, df=28)	(P=0.0004, t=2.05, df=26)	(*P*=0.0001, t=2.14, df=14)
	High	(P=0.0004, t=2.04, df=28)	(P=0.00002, t=2.14, df=14)	(*P*=0.0001, t=2.14, df=14)
Lady bird beetle	Low	(P=0.0004, t=2.14, df=14)	(P=6.504, t=2.07,df=22)	(*P*=0.0004, t=2.05, df=26)
	Recommended	(P=0.0001, t=2.14, df=14)	(P=6.245, t=2.06,df=23)	(*P*=6.504, t=2.07, df=22)
	HIgh	(P=1.669, t=2.14, df=14)	(P=5.970, t=2.04,df=28)	(*P*=2.295, t=2.14, df=14)

Fig. 2, on the other hand cypermethrin was very toxic to important predator ladybird beetle. After two hour application of cypermethrin at recommended field dose at the rate of 0.25ml/100ml water caused mortality of 66.67%. Higher and lower doses than recommended at dose rate values of 0.5ml, 0.12ml/100ml water caused mortality of 73.33% and 46% respectively. While in control mortality was 0%. After four hour application of cypermethrin at recommended field dose rate of 0.25ml/100ml water caused mortality of 80%. Higher and lower doses than recommended at rate of 0.5ml, 0.12ml/100ml water caused mortality of 86.67% and 53.33% respectively. While in control mortality was 6.67%. After six hour mortality was 93.33%, 100% at recommended and high dose rate respectively while at low dose rate mortality was 53.33% and control was 27%. Significant and non-significant differences are shown in Table 2.

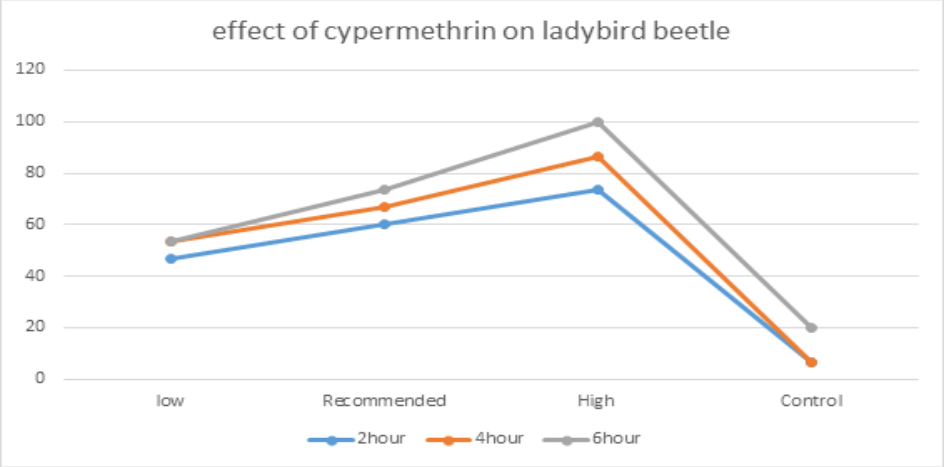

Fig 2: Toxicity of cypermethrin against lady bird beetle at lower, recommended and higher doses. Data is percent mortality of lady bird beetle neonates

Fig. 3, for cabbage looper, after two hour application of bifenthrin at recommended field dose 0.25ml/100ml water caused 66.67% mortality. Higher and lower than recommended dose of 0.5ml, 0.12ml/100ml water caused mortality of 73.33% and 40% respectively. While in control mortality was 13.33%. After four hour application of bifenthrin at recommended field dose 0.25ml/100ml water caused mortality of 80%. Higher and lower than recommended dose 0.5ml, 0.12ml/100ml water caused mortality of 100% and 60% respectively. While in control mortality was 20%. After six hour application of bifenthrin at recommended and high dose rate mortality was 100% while at low dose rate mortality was 66.67% and mortality in control was 26.67%. Significant and non-significant differences are shown in Table 3.

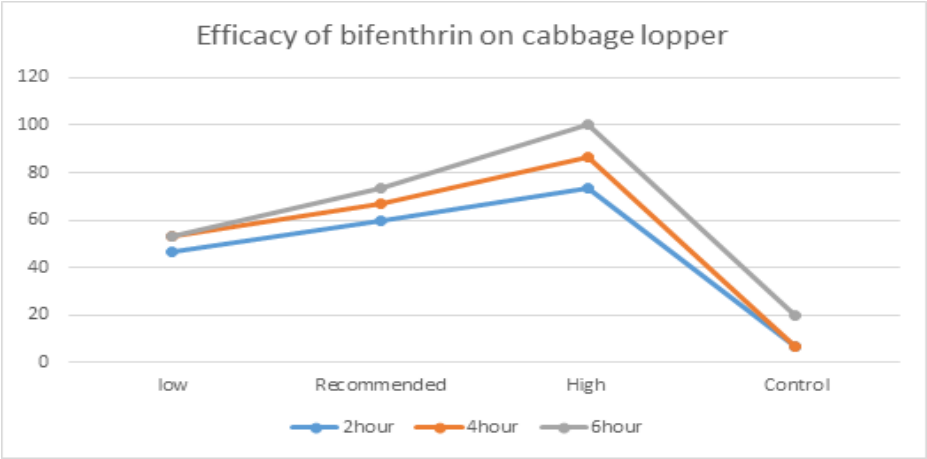

Fig 3: Toxicity of bifenthrin against cabbage looper at lower, recommended and higher doses. Data is percent mortality of cabbage looper neonates

Table 3: Bifenthrin mortality data analysis after 2, 4, 6 hours of application. ($P \leq 0.005$)

Insect/ Predator	Dose rate	After 2h of application	After 4h of application	After 6h of application
Cabbage looper	Low	(P=0.106, t=2.05, df=25)	(P=0.0004, t=2.04, df=28)	(P=0.00006, t=2.07, df=22)
	Recommended	(P=0.002, t=2.05, df=25)	(P=0.0004, t=2.04, df=28)	(P=0.00002, t=2.14, df=14)
	High	(P=0.0004, t=2.05, df=26)	(P=2.952, t=2.14, df=14)	(P=0.00002, t=2.14, df=14)
Lady bird beetle	Low	(P=0.01, t=2.07, df=21)	(P=0.005, t=2.07,df=21)	(P=0.061, t=2.05, df=27)
	Recommended	(P=0.633, t=2.11, df=16)	(P=0.0003, t=2.07,df=21)	(P=0.002, t=2.04, df=28)
	High	(P=0.01, t=2.11, df=16)	(P=1.539, t=2.05,df=26)	(P=2.95, t=2.14, df=14)

Fig. 4, On the other hand bifenthrin was toxic to very important predator ladybird beetle. After two hour application of bifenthrin cause mortality at recommended field dose (0.25ml/100ml water) rate was 60%. At high and low dose rate values (0.5ml, 0.12ml/100ml water) from recommended dose rate cause mortality were 73.33% and 46.67% respectively. While in control mortality was 6.67%. After four hour application of bifenthrin cause mortality at recommended field dose (0.25ml/100ml water) rate was 66.67%. At high and low dose rate values (0.5ml, 0.12ml/100ml water) from recommended dose rate cause mortality were 86.67% and 53.33% respectively. While in control mortality was 6.67%. After six hour mortality was 73.33%, 100% at recommended and high dose rate respectively while at low dose rate mortality was 66.67% and control was 80%. Significant and non-significant differences are shown in Table 3.

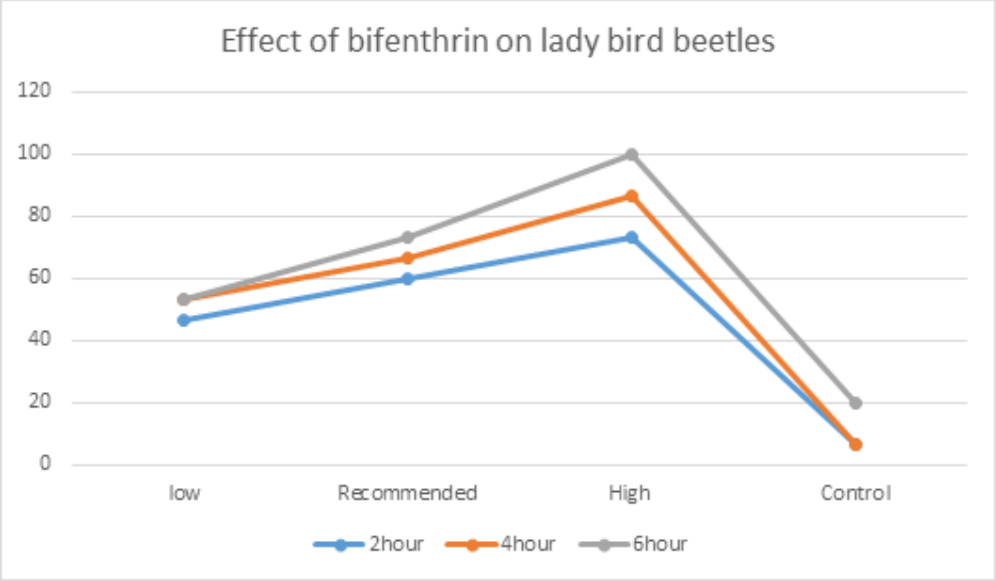

Fig 4: Toxicity of cypermethrin against lady bird beetle at lower, recommended and higher doses. Data is percent mortality of lady bird beetle neonates

Discussion

Different botanicals have been used against cabbage lopper in the recent times. From neem trees active ingredient azadrachtin which is effective against many insect pest of different vegetables, fruits and crops was used. It can affect the insect in different ways like insect growth regulators, antifeedent, oviposition deterrent, sterlient. Insecticides derived from neem affect the oviposition of homopteran, lepidopteran, coleopteran and dipteran pests [29, 30, 34, 33, 23]. Cypermethrin, bifenthrin and azadrachtin tested against two aphid species *Lipaphis erysimi* (Katenbach), *Myzus persicae* (Sulzer). Result concluded that cypermethrin was most toxic and bifenthrin showed moderate toxicity against these species of aphid [40]. Cypermethrin was toxic to L. *erysimi* [15]. In another study, the effects of dimethoate and neemarin on the life cycle of cabbage butterfly were observed. Number of eggs were found lees on deltamethrin as compared to neemarin and dimethoate. Reproductivity rate also reduced but most susceptible stage against insecticides was found to be pre-pupal stage [17]. Cypermethrin increased the activity of acid and alkaline phosphatases in *Tribolium castenium* while bifenthrin did not show the significant difference [1]. In Bangladesh, a study on the effects of various insecticides on the population of the lady bird beetle was conducted on country bean agroecosystem. Cypermethrin and esfenvalerate caused high mortality but Emamectin benzoate, Neem oil fresh, Cartap showed low mortality of lady bird beetles. Deltamethrin also caused more than twenty five percent mortality of lady bird beetles [20]. Application of broad spectrum pesticides caused reduction of carbids and many other predatory organisms [5]. Spinosad and indoxacarb were found safer to polycoloured Asian lady bird beetle (*Harmoniaaxyridis*) which is used widely in IPM [8].

When fenitrothion was applied on predator *Menochilus sexmaculatus* F. caused 80% moratlity of both adult and larvae at the dose 0.002% and 30-40% mortality at dose rate 0.005% [13]. Ripcord and Cymbush caused low mortality of lady bird beetle [12]. Eight plant extracts *Azadirachta indica* A. *Juss*, *Meliaazedarach* Linn., *Lantana camara* L. Moldenke., *Neriumindicum* Mill., *Cannabis sativa* Linn., *Eucalyptus* sp., *Ricinuscommunis* Linn. And *Solanum nigrum* Linn. Were used against *P. brassicae*. *A. indica* and *M. azedarach* repelled more than ninty percent larvae, while *M. azedarach, N. indicum* and

A. indica caused high mortality of larvae of cabbage butterfly but *R. communis* was found less toxic [32]. Besides, feeding deterrent was caused of extract from *M. azedarach* and *A. indica* for *Plutella xylostella* (Linn.) [6]. After 15days of application endosulfan, annonin and spinosad killed more than 90% larvae of cabbage butterfly. In field conditions Bifenthrin, diafentheuron, imidacloprid and dimethoate checked against mustard aphid and ladybird beetles and syrphid fly. All insecticides effective against aphid and also toxic to these predators. Imidacloprid and dimethoate safe as compared to other insecticides [3].

Conclusion

Both insecticides are effective against cabbage lopper but cypermethrin was more effective as compared to bifenthrin. Both insecticides are toxic to important predator lady bird beetles but cypermethrin more toxic as compared to Bifenthrin. So, there is need to investigated further insecticides and find out safe insecticides for beneficial organisms. After that these insecticides used in IPM for insect pest management of crops.

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