

EFFECTS OF INSECTICIDES ON NATURAL POPULATION OF HYMENOPTEROUS PARASITIDS IN ALFALFA (*Medicago sativa* L.) AGRO-ECOSYSTEM

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Injudicious application of insecticides can cause a sharp reduction in the population of several predatory insects and mites including parasitoids. The current study was designed to investigate the effect of four insecticides (i.e. bifenthrin, cypermethrin, deltamethrin and acetamiprid) against the natural population of hymenopterous parasitoids parasitizing various pests of Alfalfa. When used against the pests of Alfalfa, these insecticides also affected the natural population of hymenopterous parasitoids significantly, resulting into ecological imbalance and making the crop more susceptible to pest infestation. This study has proved that bifenthrin, cypermethrin, deltamethrin and acetamiprid can cause a sharp decline of the population of twelve different hymenopterous parasitoids families of (trichogrammatidae, mymaridae, aphelinidae, encyrtidae, eulophidae, pteromalidae, eupelmidae, ichneumonidae, braconidae, proctotrupidae, platygasteridae and scelionidae) with a detectable variation in their resistance to these insecticides. The comparison of means of survival percentage for different insecticides revealed that cypermethrin caused the lowest mortality (highest survival percentage) to parasitoids followed by deltamethrin, acetamiprid and bifenthrin to cause maximum mortality respectively (lowest survival percentage) to hymenopterous parasitoids. This would add further environmental concern to the natural population of several predatory insects as their viability will be significantly affected in different contaminative areas. Thus, such studies are also required to investigate the effects of other insecticides which have been extensively used for pest control.

Keywords: Alfalfa, hymenopterous parasitoids, Chemical control, Ecological imbalance, Resurgence.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is one of the most important perennial leguminous forage crops grown in the desert climate of the Kingdom of Saudi Arabia. Alfalfa and berseem clover (*Trifolium alexandrinum* L.) together make the backbone of forage agro-ecosystem (Al-Suhaibani, 2010). Alfalfa is often grown with barseem clover as mixed crop. Alfalfa can live generally from four to eight years and can be harvested many times. The Alfalfa plants have deep root system that helps to improve soil fertility by increasing fixed nitrogen and also protect soil erosion and alkalinity (Graves *et al.*, 1996). Alfalfa is a very common crop that is grown throughout the world as cattle feed widely. It has the highest yield potential and one of the highest feeding values of all adapted perennial forage legumes. It has high level of digestible protein that makes it an extremely valuable feed (Leghari *et al.*, 2000). Alfalfa can be used successfully in many types of livestock feeding programs for dairy cows, beef cattle, horses, goat, and sheep as pasture grazing, hay, silage and green chop (Al-Suhaibani, 2010). In poultry diets, dehydrated alfalfa and alfalfa leaf concentrates are used for pigmenting eggs and meat, because of their high carotenoids

content, which are efficient for colouring egg yolk and body lipids. Humans also eat alfalfa sprouts in salads and sandwiches. Dehydrated alfalfa leaf is commercially available as a dietary supplement in several forms, such as tablets, powders, tea etc. (Karimi *et al.*, 2013).

Like any other crop, the alfalfa crop is also afflicted by a variety of pests that include whiteflies, thrips, alfalfa weevil, aphids, leaf minors, dusky bugs, crickets, cutworm, armyworm and grasshoppers etc. which inflict damage to of Alfalfa crop at different stages (Shebl *et al.*, 2008). Natural enemies associated with these pests on alfalfa agro-ecosystem get attracted to maintain the ecological balance of pest populations (Wagan *et al.*, 2014, 2015; Hameed *et al.*, 2016, Natwick and Lopez, 2016). Among the natural enemies, the hymenopterous parasitoids of superfamilies chalcidoidea, ichneumonoidea, platygastroidea and proctroidea play a pivotal role to keep the population of pests under check (Abdel-Salam, 2016; Sathe and Chougale, 2014; Gupta, 1988).

Parasitic Hymenoptera forms one of the major groups of insects which have parasitoid mode of life style; they attack other insects for their survival. They attack a wide range of hosts on their egg, larval and adult stages. Parasitic

Hymenoptera are often a dominant factor regulating arthropod populations, and hence, significant components of most ecosystems (Stevens *et al.*, 2013).

To control these insect pests, insecticides are the most powerful tool to regulate the pest populations under check (Gazzoni *et al.*, 1999). The most commonly used insecticides are broad spectrum neurotoxic chemicals which influence both target and non-target insects (Talebi *et al.*, 2008). Mostly, farmers prefer to use insecticides for quick results, as they most of the times, are not aware of the hazardous effects and recommended dosage of these insecticides which they are using against their target groups of insects (pests). This indiscriminate use of insecticides cause many problems like the insecticidal resistance in pest species, increase in pest control cost (Metcalf, 1980) and most importantly, affect the biodiversity of these tiny wasps under study (natural enemies). The reduction of natural enemies is caused due to no-selective usage of insecticides that leads to serious problems especially the resurgence of new pests and the eruption of secondary pests (Fernades *et al.*, 2010).

Commonly used insecticides are broad spectrum neurotoxic poisons which affect target as well as non-target insects (Talebi *et al.*, 2008). These wide spectrum insecticides pose potential threat to natural enemies (parasitoids) because of their common physiology with various pest species (Liu *et al.*, 2016). When the recommended concentrations of insecticides are used to control the insect pests they also affect the natural enemies (parasitoids) significantly. The density of parasitoids can be employed as an excellent indicator to know the side effects of insecticides (Bilibech *et al.*, 2015). The insecticides exert harmful effect on various life stages of parasitoids.

Pesticides affect the natural enemies in various ways, directly as well as indirectly, of which acute toxicity is most frequent (Ruberson and Roberts, 2004; Monsreal-Ceballos *et al.*, 2018), while some pesticides have been reported to cause sub-lethal effects by adversely affecting their biological attributes and minimize or eliminate their effectiveness (Roberson *et al.*, 2004).

Keeping in view the above fact, the present study was designed to know the impact of traditional insecticides sprayed on the natural population of hymenopterous parasitoids occurring in Alfalfa agro-ecosystem.

MATERIALS AND METHODS

Chemicals and solutions: Trials were performed with four wide ranging insecticides *viz.* cypermethrin, deltamethrin, bifenthrin and acetamiprid (Table 1) which are commonly used against various pests of Alfalfa in the region (Biondi *et al.*, 2013). These insecticides were purchased from market for conducting this study.

Selection and collection of hymenopterous parasitoids: To evaluate the population dynamics of hymenopterous parasitoids in response to selected insecticides field trials were carried out at Agriculture and Veterinary Experimental Research Station, College of Agriculture and Veterinary Sciences, Qassim University, Qassim, Saudi Arabia, between October and March, 2019. The collected hymenopterous parasitoids were arranged in a randomized complete block design. The Alfalfa crop for experiment was sown on 1st October, 2018 in specified five plots in a randomized complete block design and all the recommended doses of fertilizers, irrigation etc. were provided. The damage to crop through various pests was observed carefully as and when the damage crossed injury level then the recommended doses of insecticides were used to keep the damage under check.

Experimental protocol: The experiment was replicated thrice after every two months. The first dose of insecticides was given after four weeks from sowing *i.e.*, in November, 2018, second dose was given after another two months *i.e.*, in January, 2019 and the third dose was given again after two months *i.e.*, in March, 2019.

Five plots of 100 square meters were selected for the study. Out of these five plots, one plot was used as control where plain water was used. The formulations and doses of these insecticides were prepared in plain water as recommended. These insecticides were applied as foliar spray until runoff was observed on Alfalfa crop in every specified plot for each insecticide. The application was performed with a Knapsack sprayer (20 L).

After the spray of insecticide, each plot was surveyed after one day, two days, three days and 7 days to know the number of survived parasitoids and compared with the control plot. Due to very small size of these parasitoids, the dead ones cannot be collected in the field; consequently, the survived parasitoids were collected using sweep net and aspirator. The collected parasitoids were killed using fumes of ethyl acetate then they were preserved in 70% ethanol with a few drops of glycerin. Thereafter, these parasitoids were

Table 1. Tested insecticides against various pests of Alfalfa agro-ecosystem.

Active ingredient	Trade name	Dosage	Mode of action	Target pests of Alfalfa
Bifenthrin 10%	Bytop	250ml/100L		Aphids, thrips and cutworms
Deltamethrin 10%	Delux Ultra	100ml/ hectare	Na ²⁺ channel	Aphids, cutworms and locust
Cypermethrin 10%	Asacyper	500ml/hectare		Aphids and cutworms
Acetamiprid 20%	Garastin	250ml/hectare	Nicotinic agonist	Aphids

identified using *Hymenoptera of World* (Goulet and Huber, 1993) and then counted family wise using stereoscopic zoom microscope [Their generic and specific identification is in progress, will be produced in the next paper].

Statistical analysis: The survival percentage of different families of parasitoids was counted for each insecticide. The mean and standard error were calculated using Microsoft Excel.

RESULTS

Effects of different insecticides on hymenopterous parasitoids: The parasitoids recorded in experimental Alfalfa crop are appended in Table 2. They belong to twelve families of the order Hymenoptera. Natural attack by various hymenopterous parasitoids on different pest species occurred before the application of insecticides, but that was not enough to control the pest population under check. When damage crossed the economic threshold level, four different insecticides were sprayed to control the pest population. These insecticides also affected the population of natural enemies (hymenopterous parasitoids) significantly. The survived parasitoids from designated plots of each insecticide were collected after one day, two days, three days and seven days of spray and compared with the control plot where no insecticide was used. The average number of parasitoids collected from each plot and their survival percentage were computed and provided in Table 3. The

results obtained clearly revealed the impact of insecticides on the natural prevalence of 12 families of hymenopterous parasitoids which were collected from Alfalfa agro-ecosystem. When the obtained data were compared with control plot as well as pre-spray number of parasitoids, it showed a significant difference in survival percentage of these important biological control agents. The tested insecticides caused considerable mortality of hymenopterous parasitoids.

Table 2. List of hymenopterous parasitoids families in Alfalfa agro-ecosystem

Superfamily	Family
Chalcidoidea	Trichogrammatidae
	Mymaridae
	Aphelinidae
	Encyrtidae
	Eulophidae
	Pteromalidae
	Eupelmidae
Ichneumonoidea	Ichneumonidae
	Braconidae
Proctotrupeoidea	Proctotrupidae
Platygastridae	Platygastridae
	Scelionidae

Effects of pyrethroids on parasitoids: Bifenthrin caused the least survival percentage (0%) to the members of the family

Table 3. Effect of different insecticides on the survival of various parasitoids.

Parasitoids family	Treatment	Control		Bifenthrin		Deltamethrin		Cypermethrin		Acetamiprid	
		Average no. of survived parasitoids	Survival percentage	Average no. of survived parasitoids	Survival percentage	Average no. of survived parasitoids	Survival percentage	Average no. of survived parasitoids	Survival percentage	Average no. of survived parasitoids	Survival percentage
Trichogrammatidae	Pre-spray	24.00	107.29	22.75	31.86	21.50	43.02	21.25	49.41	21.25	72.09
	Post-spray	25.75		7.25		9.25		10.50		15.50	
Mymaridae	Pre-spray	13.25	116.98	12.75	50.98	11.75	63.82	12.75	66.66	12.25	53.06
	Post-spray	15.50		6.50		9.75		8.50		6.50	
Aphelinidae	Pre-spray	9.75	120.51	8.75	31.42	8.25	78.78	9.50	76.31	8.50	26.47
	Post-spray	11.75		2.75		6.50		7.25		2.25	
Encyrtidae	Pre-spray	15.25	108.19	14.25	47.36	12.25	61.22	14.75	57.62	13.50	40.74
	Post-spray	16.50		6.75		7.50		8.50		5.50	
Eulophidae	Pre-spray	50.50	108.41	45.50	40.33	40.5	62.34	45.50	67.03	42.75	40.35
	Post-spray	54.75		18.25		25.25		30.50		17.25	
Pteromalidae	Pre-spray	37.75	103.97	33.50	39.55	30.75	52.84	37.25	51.67	36.75	31.29
	Post-spray	39.25		13.25		16.25		19.25		11.50	
Euplemidae	Pre-spray	9.00	119.44	7.75	12.90	6.75	40.74	8.75	48.57	7.75	0.00
	Post-spray	10.75		1.00		2.75		4.25		0.00	
Ichneumonidae	Pre-spray	7.25	117.24	6.50	0.00	5.75	0.00	6.75	33.33	6.75	0.00
	Post-spray	8.50		0.00		0		2.25		0.00	
Braconidae	Pre-spray	11.50	110.86	8.75	31.42	8.25	33.33	10.50	26.19	10.75	20.93
	Post-spray	12.75		2.75		2.75		2.75		2.25	
Proctotrupidae	Pre-spray	17.50	111.42	15.75	26.98	14.75	55.93	16.75	22.38	17.25	26.08
	Post-spray	19.50		4.25		8.25		3.75		4.50	
Platygastridae	Pre-spray	24.50	109.18	23.25	33.33	22.75	59.34	23.50	70.21	21.75	34.48
	Post-spray	26.75		7.75		13.50		16.50		7.50	
Scelionidae	Pre-spray	13.75	87.27	13.25	39.62	12.50	36.00	13.25	49.05	12.50	50.00
	Post-spray	12.00		5.25		4.50		6.50		6.25	
Mean±MSE of Survival Percentage			110.06±2.56		32.14±4.07		46.82±6.00		51.58±5.01		39.13±6.09

ichneumonidae but maximum survival percentage (50.98%) for the family mymaridae (Table 3; Fig. 1).

Deltamethrin also affected the family ichneumonidae to cause 0% survival while it was recorded to affect maximum (82.97%) survival percentage for family mymaridae (Table 3; Fig. 1).

In case of cypermethrin, the minimum (22.38%) survival percentage was recorded for the family proctotrupidae and maximum (76.31%) was recorded for the family aphelinidae (Table 3; Fig. 1).

Effects of neonicotinoid on parasitoids: Acetamiprid caused the least (0%) survival percentage to families eupelmidae and ichneumonidae while maximum (72.09%) survival percentage was recorded for the family trichogrammatidae (Table 3; Fig. 1).

Among the four insecticides used in the study, the means of survival percentage revealed that bifenthrin is the most toxic that caused 32.14% survival of parasitoids followed by acetamiprid which caused 39.13% survival of parasitoids then deltamethrin (46.82%) and cypermethrin (51.58%).

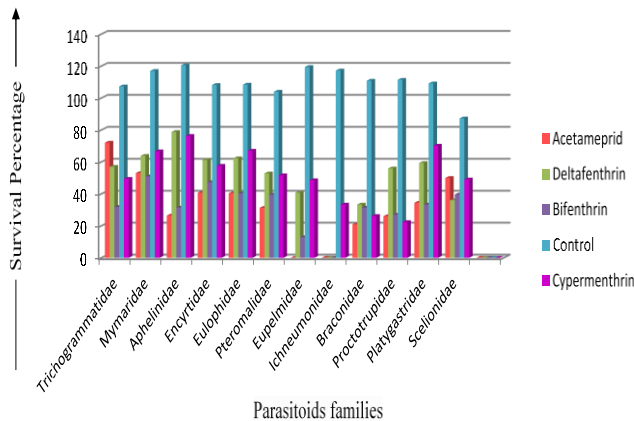


Figure 1. Survival Percentage of parasitoids in response to various insecticides.

DISCUSSION

The insecticides-treated plots were found to show declines in natural enemies abundance, which ultimately caused low parasitism rates. This indicates that natural enemies are vulnerable non-target organisms to the insecticides applications (Bommarco *et al.*, 2011; Chaplin-Kramer, 2011; Bengochea *et al.*, 2012; Smaili *et al.*, 2014; Arfan *et al.*, 2018). The tested insecticides showed the significant variation on the survival of various families of hymenopterous parasitoids attacking the pests in Alfalfa agro-ecosystem. In effective Integrated Pest Management (IPM), both insecticides and natural enemies are the most important tools, when natural enemy complex is ineffective, insecticides provide the alternative to control the pest population below economic injury level while on the other

hand, when natural enemy complex is highly effective, insecticides usage is unnecessary (Ruberson *et al.*, 1998).

Neurotoxicity of insecticides is the main cause of pests and parasitoids mortality. The insecticides used in the present study belong to the pyrethroids and neonicotinoids categories. Pyrethroids act on sodium channels while neonicotinoids act as acetylcholine agonist (Fernandes *et al.*, 2010). The toxic substances of insecticides penetrate in non-target organisms and spread in the body through blood circulation (Jepson, 1989) and cause acute, sub-acute and chronic effects on insects physiologically and biochemically (Fernandes *et al.*, 2010) that leads to affect the developmental delay, fecundity, fertility and longevity (Fernandes *et al.*, 2008) and growth synchrony of the host (Cônoli *et al.*, 1998) that ultimately affect the parasitism performance.

The results obtained for the effects of insecticides on various hymenopterous families of parasitoids are consistent with the previous studies (Dalci *et al.*, 2011; Prabhaker *et al.*, 2007; Monsreal-Ceballos, 2018). Among the three pyrethroids (bifenthrin, deltamethrin and cypermethrin) tested in the study to effectively control the various pests of Alfalfa agro-ecosystem, by comparing the mean of survival percentage, bifenthrin was found more toxic to parasitoids. Ichneumonids showed the least resistance while mymarids were found to be more resistant to bifenthrin. Similar trend for bifenthrin susceptibility was observed by Algar, 2015 for a platygastid wasp, *Telenomus* sp. in cocoa agro-ecosystem; Prabhaker *et al.*, 2007 reported the toxic effects of bifenthrin in laboratory on one of aphelinids, *Aphytis melinus* and *Harmonia axyridis*, a coccinellid predator was also adversely affected by this insecticide (Galvan *et al.*, 2006). Deltamethrin followed the same trend of bifenthrin in causing the mortality to natural enemies. The obtained data shows that it caused the least survival to ichneumonids and the most to mymarids. The toxic effects of deltamethrin recorded on the adults of mymarid wasp, *Anagrus nilaparvatae* (Melin *et al.*, 2012), also on egg parasitoids, *Trichogramma oleae*, *T. cacoeciae* and *T. bourarachae* (Bilibech *et al.*, 2015), platygastid egg parasitoid, *Trissolcus grandis* (Saber *et al.*, 2005; El Wakeil *et al.*, 2013) support our findings. Our results for cypermethrin were similar to those of Dalci *et al.* (2011) and Jander *et al.* (2004), who studied the effect of cypermethrin on tiny hymenopterous wasps, *Trichogramma pretiosum* and *Trichogramma atopovirilia* and *Aphidius uzbekistanicus*, respectively. The less survival percentage was recorded for ichneumonids and maximum for mymarids in cypermethrin treated plot.

Acetamiprid is one of the important neonicotinoids introduced in the past few decades, which has been shown to have high efficacy on hymenopterous parasitoids as given by Ruberson *et al.* (2004) and Naranjo and Akey (2005) for parasitoids of whiteflies (*Bemisia tabaci*), *Eretmocerus* and *Encarsia*. In our

study, it afflicted most damage to individuals of the family Eupelmidae; consequently, least survival for them. Trichogrammatidae was found to be the most resistant family against acetamiprid.

The comparison of means of survival percentage for different insecticides revealed that cypermethrin caused the lowest mortality (highest survival percentage) to parasitoids followed by deltamethrin, acetamiprid and bifenthrin to cause maximum mortality respectively (lowest survival percentage) to parasitoids.

The extensive and non-selective usages of insecticides often promote the insect resistance development and resurgence of the pest (Fernandes *et al.*, 2010; Liu *et al.*, 2016). Therefore, it is very important to use the selective insecticides with high efficacy to the target pest and minimum impact on natural enemies and on the ecosystem. From the present study, it is very clear that insecticides with lower impact on natural enemies are the most appropriate to use in the successful IPM, though the impact of insecticides on biological control agents is complex and it requires systematic study to understand the sub-lethal effects on the physiology, biology and behavior of different parasitoids as well as other natural enemies.

Conclusion: This study has proved that bifenthrin, cypermethrin, deltamethrin and acetamiprid can cause a sharp decline of the population of twelve different hymenopterous parasitoids families (trichogrammatidae, mymaridae, aphelinidae, encyrtidae, eulophidae, pteromalidae, eupelmidae, ichneumonidae, braconidae, proctotrupidae, platygastriidae and scelionidae) with a detectable variation in their resistance to these insecticides. Therefore, while choosing the wide spectrum insecticides to control target pests, extra care must be taken to avoid disturbing the natural population of natural enemies. Biodiversity as well as viability of many predatory insects including hymenopterous parasitoids might be directly affected by extensive and misuse of many insecticides. Such studies are also required to investigate the effects of other insecticides which have been extensively used for pest control.

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