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Toxic Effect of Malathion on Insect Pollinators Visiting Marigold Flowers

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ABSTRACT

The residual effect of malathion on insect pollinators i.e., honey bees, butterflies, syrphid flies and bumble bees, was observed in field and in semi-field experiments. For the study the recommended field dose of malathion (0.002gm/ml) was sprayed on a patch of marigold (*Calendula officinalis)*. Number of pollinators that visited the study patches before insecticide application (control) and after application (insecticide treatment) was recorded. The data was recorded till 10th day after the insecticide spray. Total number of insect pollinators was significantly higher before insecticide spray. There was drastic decline in the number of pollinators, especially of honey bees, after the insecticide treatment. However, it is concluded that malathion is highly toxic to insect pollinators and use of malathion should be minimized to protect the population of insect pollinators which provide important services to the pollination of agricultural crops.

Key Words: Pollination, Insect pollinators, malathion, honey bees.

INTRODUCTION

Farmers all over the globe depend on animal pollinators for the production of 35% of the total plant based food supply (Klein *et al*., 2007). Moreover, 60-90% of angiosperm reproduction is dependent upon them (Richards, 1986; Renner, 2006; Kremen *et al.,* 2007). Pollinators include bats, butterflies, birds, beetles, flies, and wasps, but 75% of all crop pollination services are held by bees globally (Buchmann & Nabhan, 1996). In North America bees pollinate more than 90 profitable crops (National Research Council, 2007). According to a review, value of crops which are pollinated by animals especially bee pollinators is about 217 billoin US dollars, worldwide. While in Pakistan it is only 1.59 billion US dollars (Irshad & Stephen, 2014).

Competition among plants often occurs for pollination services (Rathcke, 1983; Sargent & Ackerly, 2008). Plants can compete by exhibiting more attractive floral morphologies (Kevan & Baker, 1983; Giurfa *et al*., 1999; Dicks *et al*., 2002), or by providing more floral resources (Potts *et al.,* 2003, 2004). Honey bees have a lesser natural preference for particular floral colors (Lunau & Maier, 1995). They offer pollination services to numerous cultivated and wild species (Sharma & Abrol, 2005; Frankie *et al.,* 2009). Bee poisoning due to insecticides is a serious problem for beekeepers which occurs when they are exposed to insecticide treated flowering crops or weeds. During foraging about 99% bees are killed because of picking up the pesticides (Eckert & Shaw, 1960).

Bumble bees are elementary pollinators because many crops require bees to improve seed or fruit deposit and yield. In southern Britain, a severe turn down in the abundance of bumblebees occured due to the use of certain pesticides in the last thirty years. Butterflies also act as good bio-indicators of the health of an ecosystem and are highly sensitive to environmental change (Rosenberg *et al*., 1986). They are frequent diurnal visitors to flowers (Shepherd *et al.,* 2003).

Insecticides are normally used for insect pests but they also affect non-targeted organisms. During plant treatment insecticides could cause poisonous effects in the pollinators through physical touch. Additionally, polluted pollen and nectar of treated plants also cause negative impact on insect pollinators (Enan & Matsumura, 1992). Malathion is an organophosphate (EPA, 2012) which is a broad-spectrum insecticide used for a large variety of crops and flowering plants. The objective of the present study was to determine the residual effect of malathion on the survival, abundance and behavior of insect pollinators i.e., honey bee, bumble bee, syrphid fly and butterfly.

MATERIALS AND METHODS

Study Area

The study was carried out during May 2014 to May 2015 at Department of Botany, University of Sargodha and College of Agriculture, University of

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Sargodha. Study area was surrounded with various crops like wheat, mustard plant, maize and some other ornamental plants like marigold, roses etc. Patches having marigold were selected

Field Study

For the field study one patch of marigold "*Calendula officinalis*" (12 feet long and 5 feet wide) was selected at University College of Agriculture, University of Sargodha. The plants of marigold are very attractive and unique for their beautiful flower color. Large number of insect pollinators i,e., honey bee, bumble bee, syrphid, butter fly and other flies visits these flowers. Within selected floral patch we recorded different types of insect pollinators that visited marigold plants from 9-12 AM. Pollinators were identified on morphological basis.

After recording the data of insect pollinators for three days, the marigold patch was sprayed with recommended field dose of malathion (i.e., 0.002gm/ml) to study the effect of malathion on the numbers of pollinators visiting the area i.e., honey bee, bumble bee, syrphid fly, butter fly and other flies. After treatment, data was recorded for consecutive ten days to check the residual effect of malathion on the number and activity of visitors.

Residual effect of Insecticide on Mortality Rate (Honey Bees)

To observe the effect of malathion on honey bee behavior and mortality rate, semi field experiment was conducted. For this purpose three pots were selected, one for control and two for insecticide treatment. In each set up one pot (30 cm^2) of marigold (height: 3 ft) containing 2-3 flowers was used. Each pot was covered with transparent plastic sheet and a piece of net was adjusted for proper ventilation. Plants of control pot were sprayed with water while remaining two pots were sprayed with recommended field dose of malathion (0.002gm/ml). The plants were allowed to dry for one hour. After that twenty honey bees were released in each pot. After releasing the bees the set up was closed properly. Data of mortality was recorded after 4 hour intervals till 24 hours.

Statistical Analyses

The Probit analysis was used to determine LT_{50} and LT_{95}. Minitab 14.3 was used to compute the Probit analysis. One-way ANOVA using SPSS 20 followed by Post Hoc test (Tukey test) was applied to compare the number of different types of insect pollinators. The results were considered significant at p value < 0.05.

RESULTS

Before the application of malathion, number of honey bees was higher than after the application of malathion. The residual effect of malathion remained in field till 10^{th} day after insecticidal spray (Table I). The malathion negatively affected the honey bees. It is depicted in the Table I that mean number of honey bees was 209 before the application of malathion. After the application of malathion number of honey bees on 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, $6^{th,}$, 7^{th}, 8^{th}, 9^{th} and 10^{th} day was 107,109,116,137,78,104,82,112,82,52, respectively. When we compared the number of honey bees and other insect pollinators during different days after malathion application, a significant difference was observed (Table II). The results of Multiple Comparison were significant and listed in Table 3. Significant difference was recorded among compared groups. It is evident from results of Multiple Comparisons that number of honey bees was significantly higher from all other insect pollinators however; all other insect pollinators do not differ significantly in terms of their abundance (Table III).

To record the effect of malathion on honey bees a semi-field experiment was performed. During the experiment no mortality was observed in the control group. However; in the experimental group, nine deaths were observed after 4 hours and this number increased upto 16 after 12 hours. After 20 hours all honey bees died (Fig., 1). The calculated LT_{50} and LT_{95} were 6.29 hours and 16.15 hours, respectively.

DISCUSSION

The decline in the population of valuable insects such as bees occurs due to widespread use of insecticides. An important role is played by bees in the food web and they also act as natural enemies of various insect pests (GCT, 2004). Due to the use of pesticides in agriculture the honey bees population is suffering badly (Maini *et al.,* 2010; Mullin *et al.,* 2010; Porrini *et al.,* 2003). In the UK, between 1995 and 2001, about 95 incidents of bee poisoning were recorded. Organophosphates contributed 42% of overall mortality while carbamates and pyrethroids caused 29% and 14% of mortality among pollinators (Fletcher & Barnett, 2003). Bee adversity is threatening worldwide food security because one third of global agricultural production depends on pollination by bees, especially that of honey bees (McGregor, 1976; Klein *et al*., 2007). Regardless of their importance honey bees are decreasing with disturbing speed

Table I: Effect of malathion on abundance of different groups of insect pollinators on plants.

Insect pollinator	No. of insect pollinators										
	Before insecticide application (Mean)	After insecticide application									
		Days									
		1	2	3	4	5	6	7	8	9	10
Honey bees	209	107	109	116	137	78	104	82	112	82	52
Syrphid fly	7	20	24	21	25	6	7	12	6	9	1
Bumble bees	2	13	12	2	1	5	2	3	5	2	0
Butter flies	20	23	26	25	6	29	1	7	7	2	2
Others	16	24	29	30	27	12	8	17	11	13	9
Total	254	187	200	194	196	130	122	121	141	108	64

Table II: Results of analysis of variance showing comparison of honey bees number with other groups of pollinators.

Difference	Sum of Squares	Df	Mean Square	F	P-Value
Between Groups	82118.11	4	20529.527	53.121	**P< 0.001**
Within Groups	19323.27	50	386.465		
Total	101441.4	54			

Table III: Multiple comparison test of relative abundance of different pollinators

Treatment	Factors	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Honey Bee	Honey bee					
	Syrphid fly	95.45455*	8.38251	<0.005	71.7337	119.1754
	Bumble bee	103.72727*	8.38251	<0.005	80.0064	127.4482
	Butterfly	94.54545*	8.38251	<0.005	70.8246	118.2663
	Others	90.18182*	8.38251	<0.005	66.4609	113.9027
Syrphid fly	Honey bee	-95.45455*	8.38251	<0.005	-119.1754	-71.7337
	Syrphid fly					
	Bumble bee	8.27273	8.38251	0.86	-15.4482	31.9936
	Butterfly	-0.90909	8.38251	1	-24.63	22.8118
	Others	-5.27273	8.38251	0.97	-28.9936	18.4482
Bumble Bee	Honey bee	-103.72727*	8.38251	<0.005	-127.4482	-80.0064
	Syrphid fly	-8.27273	8.38251	0.86	-31.9936	15.4482
	Bumble bee					
	Butterfly	-9.18182	8.38251	0.808	-32.9027	14.5391
	Others	-13.54545	8.38251	0.495	-37.2663	10.1754
Butterfly	Honey bee	-94.54545*	8.38251	<0.005	-118.2663	-70.8246
	Syrphid fly	0.90909	8.38251	1	-22.8118	24.63
	Bumble bee	9.18182	8.38251	0.808	-14.5391	32.9027
	Butterfly					
	Others	-4.36364	8.38251	0.985	-28.0845	19.3572
Others	Honey bee	-90.18182*	8.38251	<0.005	-113.9027	-66.4609
	Syrphid fly	5.27273	8.38251	0.97	-18.4482	28.9936
	Bumble bee	13.54545	8.38251	0.495	-10.1754	37.2663
	Butterfly	4.36364	8.38251	0.985	-19.3572	28.0845
	Others					

* The mean difference is significant at the 0.05 level.

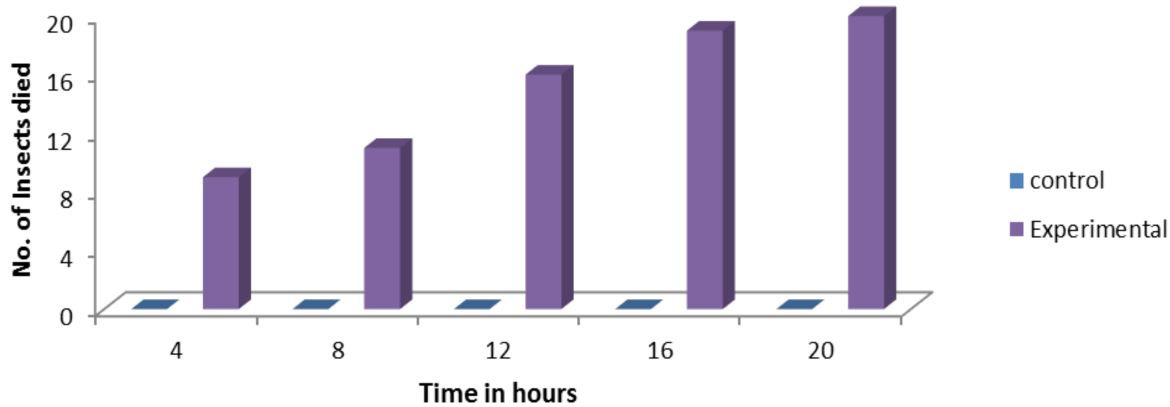

Fig., 1: Effect of malathion on mortality of honey bees.

due to pollution and pesticides (Riscu & Bura, 2013).

The visiting behavior of honey bees is adversely affected by the application of insecticide on flowering crops (Delaplane & Mayer, 2000). Strong repellents affect badly the visiting behavior of pollinators and deprive the flowers from pollinating benefits. Moderate repellents have mild effect on pollinators that resume their visiting activity after a short time (Delaplane & Mayer, 2000). It was recorded during the study that pollinators avoided such plants which were treated with insecticide. The mode of action is not fully understood, but visual, olfactory, gustatory and chemical cues may be involved (Ramirez *et al.,* 2005). Such repellent effects of insecticides on honey bee foraging have been reported several times (Pike *et al*., 1982; Shires *et al.,* 1984). In the present study we applied malathion on experimental patch. We found significant change in the behavior of pollinators. Under normal conditions they visit the selected patch frequently and spent more time but after application of insecticides they reduced their visiting activity (Vaidya *et al.,* 1996).

In semi field experiments malathion caused rapid effect on insect pollinators i.e., honey bee. Malathion is known to be highly toxic to honey bee workers when applied in fields (Arzone & Patetta 1987; Brar *et al*., 1992). We performed our experimental work on *Calendula officinalis* and observed 100% mortality after 20 hours of application while Suhail *et al*. (2001) found that application of malathion on cucumber resulted in 67% mortality of honey bees after 48 hours of application. Present study showed that after 24 hours of application residual toxicity was high but with the passage of time toxic effects decreased and were considerably reduced after 132 hours but according to Johansen & Mayer (1990), malathion is equally dangerous from 1 to 12 hours after spraying. Our findings are contradictory to Atkins & Anderson (1967), who found that most organophosphates (malathion) were highly toxic to bees but their effect disappear after 5 days. In the present study residual effect of malathion remained till 10th day. Sharma & Abrol (2014) found that morning application of insecticides (malathion) had some repellency effect on insect visitors including honey bees, which spent significantly less time on treated flowers than controls. Our finding is in accordance to him. Malathion affects the pollinators i.e., honey bees in different ways and causes mortality as noted in our observation. The observed effects might relate to acute, chronic or sub lethal intoxication, all contributing the honey bee death (Desneux *et al*., 2007).

CONCLUSION

It is concluded that malathion is highly toxic to insect pollinators and use of malathion should be minimized to protect the population of insect pollinators. In plantations and crops application of insecticides at flowering stage, should be avoided. However if this is essential, the application would be made under conditions that ensure the full protection of pollinators.

REFERENCES

Arzone, A. & Patetta, A., 1987. Research on the action of flucythrinate, propiconazol pyridafenthion and quinalphos on honeybees. *Apic. Mod*., **78**: 179-186.

Atkins, E. L. & Anderson, L. D., 1967. *Proceedings of XXIst International Congress of Apiculture*. University of Maryland, U.S.A. pp: 188-194.

Brar, H. S., Gatoria, G. S. & Jhaj, H. S., 1992. Field toxicity of insecticides recommended on American cotton, *Gossypium hirsutum* L., to honeybees, *Apis mellifera* L. *Indian. J. Ecol*., **19**: 183-186.

Buchmann, S. L. & Nabhan, G. P., 1996. *The forgotten pollinators*. Island Press/Shearwater Books, Washington, DC. ISBN 1-55963-353-0. 292pp.

Delaplane, K. S. & Mayer, D. F., 2000. *Crop pollination by bees*. 10th Edition. CABI Publishing, Wallingford, UK and New York, USA. ISBN 0-85199-448-2.

Desneux, N., Decourtye, A. & Delpuech, J. M., 2007. The sub-lethal effects of pesticides on beneficial arthropods. *Annu. Rev. Entomol.,* **52**: 81-106.

Dicks, L. V., Corbet, S. A. & Pywell, R. F., 2002. Compartmentalization in plant-insect flower visitor webs. *J. Anim. Ecol.*, **71**: 32–43.

Eckert, J. E. & Shaw, F. R., 1960. *Beekeeping*. 15th Edition. The Macmillan Co., New York, U.S. 536pp.

Enan, E. & Matsumura, F., 1992. Specific-inhibition of calcineurin by type-II synthetic pyrethroid insecticides. *Biochem. Pharmacol*., **43**: 1777–1784.

Environmental Protection Agency. 2012. Pesticide News story: The EPA publishes amended Azinphos-Methyl cancellation order allowing use of existing stocks through September 2012- 2013. www.epa.gov/pesticides/chemicalsearch.

Fletcher, M. & Barnett, L., 2003. Bee pesticide poisoning incidents in the United Kingdom. *B. Insectol*., **56**: 141-145.

Frankie, G.W., Rizzardi, M., Vinson, S .B. & Griswold, T. L., 2009. Decline in bee diversity and abundance from 1972–2004 on a flowering leguminous tree, *Andira inermis*, in Costa Rica at the interface of disturbed dry forest and the urban environment. *J. Kans. Entomol. Soc*., **82**: 1-20.

Game & Wildlife Conservation Trust. 2004. *Sussex study: 34 years of change in farmland wildlife*. 182pp. http://www.gct.org.uk/text03.asp?

Giurfa, M., Dafni, A. & Neal, P. R., 1999. Floral symmetry and its role in plant-pollinator systems. *Int. J. Plant Sci.*, **160**: 41–50.

Irshad, M. and Stephen, E., 2014. Pollination, pollinated and pollinators interaction in pakistan. *J. Bioresource Manage.,* **1**: 19-25.

Johansen, C. A. & Mayer, D. F., 1990. *Pollinator protection: a bee and pesticide handbook*. Cheshire, CT. Wicwas Press, USA. 212pp.

Kevan, P. G. & Baker, H. G. 1983. Insects as flower visitors and pollinators. *Annu. Rev. Entomol*., **28**: 407–453.

Klein, A. M., Vaissiere, B., Cane, J. H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C. & Tscharntke, T., 2007. Importance of crop pollinators in changing landscapes for world crops. *Proc .R. Soc. Lond[Biol].,* **274**: 303-313.

Kremen, C., Williams, N. M., Aizen, M. A., Gemmill-Herren, B., LeBuhn, G., Minckley, R., Packer, L., Potts, S.G., Roulston, T., Steffan-Dewenter, I., Vazquez, D. P., Winfree, R., Adams, L., Crone, E. E., Greenleaf, S. S., Keitt, T. H., Klein, A. M., Regetz, J. & Ricketts, T. H., 2007. Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. *Ecol Lett.,* **10**: 299–314.

Lunau, K. & Maier, E. J., 1995. Innate color preferences of flower visitors. *J. Comp. Physiol. A.,* **177**: 1–19.

Maini, S., Medrzycki, P. & Porrini, C., 2010. The puzzle of honey bee losses: a brief review. *B. Insectol***., 63**: 153-160.

McGregor S. E., 1976. Insect pollination of cultivated crop plants. U.S.D.A. Agriculture Handbook. 496: 93–98.

Mullin, C. A., Frazier, M., Frazier, J. L., Ashcraft, S., Simonds, R., VanEngelsdorp, D. & Pettis, J. S., 2010. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. *PLoS ONE*, **5**: e9754.

National Research Council. 2007. The Status of Pollinators in North America. National Academies Press, Washington, D.C. ISBN: 978-0-309-10289-6.

Pike, K. S., Mayer, D. F., Glezer, M. & Kious, R., 1982. Effect of permethrin on mortality and foraging behaviour of honeybees in sweet corn. *Environ. Entomol*., **2**: 951-953.

Porrini, C., Sabatini, A. G., Girotti, S., Ghini, S., Medrzycki, P., Grillenzoni, F., Bortolotti, L., Gattavecchia, E. & Celli, G., 2003. Honey bee products as monitors of the environmental contamination. *Apiacta*, **38**: 63-70.

Potts, S.G., Vulliamy, B., Roberts, S., O'Toole, C., Dafni, A., Neeman, G. & Willmer, P. G., 2004. Nectar resource diversity organises flower-visitor community structure. *Entomol. Exp. Appl.,* **113**: 103–107.

Potts, S. G., Petanidou, T., Roberts, S., O'Toole, C., Hulbert, A. & Willemer, P., 2006. Plant pollinator biodiversity and pollination services in a complex Mediterranean landscape. *Biol. Cons.,* **129**: 519-529.

Ramirez, R. R., Chaufaux, J. & Pham-Delegue, M. H., 2005. Effects of Cry1Ab protoxin, deltamethrin and imidacloprid on the foraging activity and the learning performances of the honeybee, *Apis mellifera*: a comparative approach. *Apidologie*, **36**: 601-611.

Rathcke, B., 1983. *Pollination Biology*. Academic Press, New York. pp: 305-329.

Renner, S. S., 2006. *Plant-pollinator interactions: from specialization to generalization*. The University of Chicago Press, United States. pp: 123-144.

Richards, A. J., 1986. *Plant Breeding Systems*. George Allen & Unwin, London. 529pp.

Riscu, A. & Bura., M., 2013. The Impact of Pesticides on Honey Bees and Hence on Humans. *J. Anim. Sci. Biotechnol.***,** **46**: 272-276.

Rosenberg, D. M., Danks, H. V. & Lehmkul, D. M., 1986. Importance of insects in environmental impact assessment. *Environ. Manag.,* **10**: 773-783.

Sargent, R. D. & Ackerly, D. D., 2008. Plant-pollinator interactions and the assembly of plant communities. *Trends Ecol. Evol*., **23**: 123–130.

Sharma, D. & Abrol, D. P., 2014. Effect of insecticides on foraging behaviour and pollination role of *Apis mellifera* L. (Hymenoptera: Apidae) on toria (*Brassica campestris* var. toria) crop. *Egypt. J. Biol.*, **16**: 79-86.

Sharma. D. & Abrol, D. P., 2005. Contact toxicity of some insecticides to honeybee, *Apis mellifera* L. and *Apis cerana* F. *J. Asia Pac. Entomol*., **8**: 113-115.

Shepherd, M. D., Buchmann, S. L., Vaughan, M. & Black, S. H., 2003. *Pollinator Conservation Handbook*. 1st Edition. OR, Xerces Society, Portland. 145pp.

Shires, S. W., Leblance, J., Debray, P., Forbes, S. & Louveaux, J., 1984. Field experiments on the effects of a new pyrethroid insecticide W.L.-85871 on bees foraging on artificial aphid honeydew on winter wheat. *Pestic. Sci.,* **15**: 543-552.

Suhail, A., Abdin, Z., Iqbal, J., Waseem, U., Shahid, R. N. & Haq, I., 2001. Insecticidal mortality and pollination role of honeybees (*Apis mellifera* L.) on cucumber (*Cucumis sativus* L.) crop. *Int. J. Agr. Biol*., **3**: 501-502.

Vaidya, D. N., Kumar, S. & Metha, P. K., 1996. Repellency of some insecticides to *Apis mellifera* L. foragers on treated bloom of sarson, *Brassica campestris* L. var. brown sarson. *Ann. Biol*., **12**: 134–138.

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