Selective toxicity of diafenthiuron to non-target organisms: honey bees, coccinellids, chelonus, earthworms, silkworms and fish

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Abstract: Diafenthiuron, an insecticide widely used in the management of pests of cardamom and cotton, was assessed for its toxicity-effect on beneficials commonly found in these ecosystems. Diafenthiuron was found to be toxic to honey bees, the prime pollinators of crop plants. Diafenthiuron at the highest tested dose caused 40% mortality to the coccinelid grubs at 48 h after treatment so, diafenthiuron was found to be slightly harmful. Monocrotophos, on the other hand has been found to be a highly toxic pesticide. Diafenthiuron is moderately harmful to the adults of *Chelonus blackburni* L. The testing was done using the insecticide diafenthiuron. An insecticide-coated vial (scintillation) bioassay was performed. It was found that there was 86.67% mortality in 48 h, at the recommended dose. Diafenthiuron is highly toxic to the silkworm, killing more than 80% of the caterpillars in 24 h, at all the doses tested. Diafenthiuron, even in the highest dose tested, is non-toxic to the earthworm, *Perionyx excavatus*, which was found to have a 3.33% mortality. As far as fish are concerned, the common carp, *Cyprinus carpio* L., is found to be highly susceptible to diafenthiuron and even doses 10 times lower than the field dose can kill the fish within 6 h.

Key words: coccinellids, diafenthiuron, earthworm, fish, honey bees, selective toxicity, silkworm

Introduction

Diafenthiuron [1-tert-butyl-3-(2,6-di-isopropyl-4-phenoxyphenyl)thiourea], is the only non-organofluorine benzylurea insecticide/acaricide acting selectively on a group of insects and mites (Streibert *et al*. 1988) by inhibiting mitochondrial action and energy metabolism (Ruder and Kayser 1992). Diafenthiuron was very effective against chewing pests like *Plutella xylostella* L. (Ishaaya *et al*. 1993; Lingappa *et al*. 2004) and *Conogethes punctiferalis* Guenee (Stanley *et al*. 2009), and sucking pests like *Bemisia tabaci* (Gennadius) and *Amrasca biguttula biguttula* (Ishida) (Patel *et al.* 2006), *Trialeurodes vaporariarum* (Westwood) (Javed and Matthews 2002), *Myzus persicae* (Sulzer) and *Frankliniella schultzei* (Tyrbom) (Scarpellani 2000), *Sciothrips dorsalis* Hood, *Oligonychus coffeae* (Nietner) (UPASI 2005), and *Sciothrips cardamomi* Ramk. (Stanley 2007) in brinjal, tomato, tea, cardamom, and against many sucking pests in cotton (Kranthi *et al*. 2004). Among these crops diafenthiuron is used extensively in cotton and cardamom and also found compatible with other agrochemicals and microbial biocontrol agents (Stanley *et al*. 2010a). Pesticides used in pest management should be selective enough to kill the target pests with minimal disturbance to non-target organisms. The insecticide, diafenthiuron, having two modes of action, may not spare the non-target organisms because of its inhibitory action on mitochondrial ATP production and chitin synthesis inhibition (Rajabaskar 2003) and thus has to be studied.

Honey bees are important pollinators, without which pollination and fruit set is compromised. This is evident in cardamom, where only a 0.95% fruit set was observed in cardamom plants completely prevented from bee visits (Madhusoodanan and Dandin 1981). There is a chance of pesticide exposure as contact poisons during spraying, to the foraging bees, and as stomach poison when they take nectar or pollen. Predators and parasitoids play an important role in natural pest suppression. Elimination of these natural enemies is the major factor behind pest resurgence (Landis *et al.* 2000). Thus, natural enemies should be kept in mind during pesticide sprays. Multiple cropping is practiced in many places and mulberry plants are grown in fields adjacent to cotton since both grow well in black soil. This practice means spray drifts onto mulberry crops are possible. Earthworms also have a chance of exposure to pesticides, as the pesticides which are sprayed on the crops fall on the soil. The toxicity of insecticides has a greater impact on the aquatic environment as these insecticides enter the water via many pathways. There may be a direct application for pests and disease vectors, and there may be surface runoff from agricultural soils.

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These are ways the insecticide can enter water and affect the fish. For these reasons, the present investigation was undertaken to assess the safety of diafenthiuron to honey bees, coccinellids, chelonus, the earthworm, silkworm, and fish.

Materials and Methods

The chemical used in the study is a formulated product of diafenthiuron (Diafenthiuron 50 WP) obtained from Mahamaya Agrisciences Pvt, Ltd. This new formulation is also compared with Pegasus® (Diafenthiuron 50 WP) of Syngenta. Another pesticide, monocrotophos, was also tested to make a comparison. The different insecticide concentrations used in the study were prepared immediately before use so as to minimise degradation of the chemical. The field recommended dose of diafenthiuron is 1.6 g · l^{-1} (800 mg a.i. · kg^{-1}). So, different treatments with two lower doses (0.8 and 1.2 g · l^{-1}) and two higher (2.4 and 3.2 g · l^{-1}) than the recommended dose, were used in the study, except for with the fish. The stock solutions of the tested chemical were made in distilled water, since the formulated product of insecticide which had an adjuvant to make it readily dissolved in water is used in the study. Mortality of the test organisms was taken as the endpoints in all the bioassays. The mortality counts were corrected for the control's mortality, if there was any mortality, and the mortality data of the new formulation of Diafenthiuron 50 WP tested in five different concentrations, were subjected to Probit analysis. This procedure was done to find the median lethal concentration using EPA Probit Analysis for LC/EC values version 1.5.

Honey bees

The effect of diafenthiuron was assessed using the contact toxicity method for honey bees with 1–3 days old worker Indian bees, *Apis cerana indica* Fab. Whatman No. 40 filter paper discs with an 8 cm diameter were made wet with 1 ml of different concentrations of insecticides dissolved in distilled water, and then the discs were allowed to dry. Shade-dried filter paper was placed in the bioassay container which had a perforated lid, and honey bees were released at a rate of 10 per container. The procedure was replicated thrice. After exposure for 1 h, the honey bees were allowed into big polythene bags. The bags had been perforated using pins. A 40% sucrose solution soaked in cotton wool which had been tied in a twine, was provided as feed for the bees. The mortality was observed after 3, 6, 12, and 24 h of treatment and corrected for the control mortality, if any.

Predator

The glass scintillation residue bioassay method as described by McCutchen and Plapp (1988) was adopted with slight modifications. The vials were evenly coated with 0.5 ml of different concentrations of insecticide formulations and dried for 15 min. Third instar grubs of *Menochilus sexmaculatus* Fab. were released at a rate of 5 per vial and covered with muslin cloth. The procedure was replicated thrice. After an hour of exposure, the grubs were taken and put in an uncontaminated container. Aphids were given as feed. The mortality was observed after 12, 24, and 48 h.

Parasitoid

The glass scintillation residue bioassay was used to find the contact toxicity of diafenthiuron to *Chelonus blackburni* Cameron adults, in the procedure as was described above for coccinellids. After 1 h of exposure, they were released into test tubes. A honey solution was given as feed. They were observed for mortality.

Silkworm

Bioassays were done as per Begum and Shivanandappa (2003) with slight modifications. Mulberry leaves (four leaves per replication) were plucked, surface sterilised, sprayed with the insecticide solution using an atomizer, and shade dried. After complete drying, the leaves were transferred to bioassay trays and ten pre-starved 4th instar *Bombyx mori* (Linnaeus) larvae (2 h) were released for each treatment and replicated four times. Mortality observations were taken at 6, 12, 24, and 48 h after treatment, and per cent mortality worked out.

Earthworms

Toxicity of diafenthiuron to earthworm, *Perionyx excavatus* Perrier was assessed as per Awaknar and Karabhantanal (2004) with modifications. Soil, sand, farmyard manure, and compost were used in fixed proportions (1 : 1 : 1 : 1) in large basins (40 × 13 cm). Different treatments of diafenthiuron concentrations were prepared and sprayed on the substrate in the basin. Calculations were done based on the surface area (1,000 cm^2). Fifteen minutes later, 20 earthworms of equal size were released on the surface. Each treatment was replicated three times. Water was sprayed on the surface using a hand sprayer at weekly intervals. Mortality observations were recorded 15 and 30 days after treatment.

Fish

Toxicity of diafenthiuron to fish was assessed using static bioassay as per Sprague (1970) and Agbon *et al.* (2002) according to the Organisation for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals. Aquarium tanks 30 × 45 × 60 cm with 120 l capacity were provided with artificial aeration facilities. Different diafenthiuron concentrations which are much lower than the field dose (Diafenthiuron 50 WP 0.8, 0.4, 0.2, 0.1 g · l^{-1}), were used as different treatments in 20 l of water. The control tanks were maintained without the test chemical. Each experiment was replicated four times. To each tank 20 carp, *Cyprinus carpio* L., of a similar size (~10 g), were released. The mortality was assessed at 6, 12, and 24 h.

Results

Diafenthiuron 3.2 g · l^{-1} caused 36.67% mortality at 6 h, while monocrotophos registered 43.75% mortality, in Indian bees. Pegasus® 1.6 g · l^{-1} and diafenthiuron 1.2 g · l^{-1} caused 40% mortality at 24 h (Table 1). The 24 h median lethal concentration (LC_{50}) of diafenthiuron to *A. cerana indica* was 1.63 g · l^{-1}. In the case of coccinellids, 1 day after treatment, the mortality of the grubs was found to be 33.33 and 26.67% when using diafenthiuron at 3.2 and 2.4 g · l^{-1}. But diafenthiuron at low doses of 1.2 and 0.8 g · l^{-1} recorded only a 6.67% grub mortality. The 24 and 48 h LC_{50} of diafenthiuron was found to be 4.71 and 3.84 g · l^{-1}, respectively. Monocrotophos was found to be very toxic, recording up to 93.99% of grub mortality in 48 h (Table 2). The highest dose of diafenthiuron (3.2 g · l^{-1}) caused about 40% mortality to *Chelonus* in about 6 h, whereas it was 26.67% in the standard check Pegasus® 1.6 g · l^{-1}. At 12 h, diafenthiuron at 3.2 g · l^{-1} registered 90% mortality to wasps, whereas in monocrotophos it was 100% (Table 2). The mortality reached 100% in wasps exposed to diafenthiuron at 3.2 g · l^{-1}, in about a day, revealing very high toxicity.

Studies conducted to test the toxicity of diafenthiuron on the silk worm, *B. mori* revealed that the chemical was highly toxic with a 24 h LC_{50} of 0.36 g · l^{-1}. A day after application of diafenthiuron at 2.4 and 3.2 g · l^{-1} caused 95 and 100% mortality, respectively. At the end of the second day, all the treatments recorded 100% mortality of silkworms (Table 3). But in the case of earthworms, diafenthi-

Table 1. Toxicity of Diafenthiuron 50 WP to Indian bees, *Apis cerana indica*

Treatments	Corrected mortality [%]			
	1 h	6 h	12 h	24 h
T_1 – Diafenthiuron 50 WP 0.8 g · l^{-1}	3.33	3.45	6.90	20.69
T_2 – Diafenthiuron 50 WP 1.2 g · l^{-1}	10.00	10.34	24.14	37.93
T_3 – Diafenthiuron 50 WP 1.6 g · l^{-1}	10.34	10.82	32.22	50.06
T_4 – Diafenthiuron 50 WP 2.4 g · l^{-1}	11.11	19.54	42.53	65.52
T_5 – Diafenthiuron 50 WP 3.2 g · l^{-1}	30.00	34.49	55.18	79.31
T_6 – Diafenthiuron 50 WP (Pegasus®) 2.0 g · l^{-1}	10.00	13.80	27.59	50.06
T_7 – Monocrotophos 36 WSC 2 ml · l^{-1}	37.50	41.81	54.74	80.60
T_8 – Untreated check (water)	–	–	–	–
Median lethal concentration (LC_{50}) for Diafenthiuron 50 WP	4.63	3.69	2.56	1.63

Data are the average percent mortality, n = 3

Table 2. Contact toxicity of diafenthiuron 50 WP to *Menochilus sexmaculatus* and *Chelonus blackburni*

Treatments	Corrected mortality [%]					
	M. sexmaculatus grubs			*Ch. blackburni* adults		
	12 h	24 h	48 h	12 h	24 h	48 h
T_1 – Diafenthiuron 50 WP 0.8 g · l^{-1}	0.00	6.67	0.00	14.28	28.57	40.74
T_2 – Diafenthiuron 50 WP 1.2 g · l^{-1}	3.33	6.67	3.85	28.57	57.14	77.78
T_3 – Diafenthiuron 50 WP 1.6 g · l^{-1}	10.00	13.33	11.54	57.14	67.86	85.19
T_4 – Diafenthiuron 50 WP 2.4 g · l^{-1}	16.67	26.67	23.07	71.42	85.72	100.00
T_5 – Diafenthiuron 50 WP 3.2 g · l^{-1}	23.33	33.33	38.46	89.29	100.00	100.00
T_6 – Diafenthiuron 50 WP (Pegasus®) 2.0 g · l^{-1}	33.33	36.67	36.15	64.29	71.42	96.30
T_7 – Monocrotophos 36 WSC 2 ml · l^{-1}	66.67	80.00	92.30	100.00	100.00	100.00
T_8 – Untreated check (water)	–	–	–	–	–	–
Median lethal concentration (LC_{50}) for Diafenthiuron 50 WP	4.71	4.25	3.84	1.57	1.14	0.89

Data are the average percent mortality, n = 3

Table 3. Toxicity of diafenthiuron to the silk worm, *Bombyx mori*

Treatments	Corrected mortality [%]			
	6 h	12 h	24 h	48 h
T_1 – Diafenthiuron 50 WP 0.8 g · l^{-1}	0.00	26.31	78.95	100.00
T_2 – Diafenthiuron 50 WP 1.2 g · l^{-1}	5.13	26.31	89.47	100.00
T_3 – Diafenthiuron 50 WP 1.6 g · l^{-1}	5.13	34.21	94.74	100.00
T_4 – Diafenthiuron 50 WP 2.4 g · l^{-1}	10.26	42.11	94.74	100.00
T_5 – Diafenthiuron 50 WP 3.2 g · l^{-1}	12.82	52.63	100.0	100.00
T_6 – Diafenthiuron 50 WP (Pegasus®) 2.0 g · l^{-1}	7.69	42.11	89.47	100.00
T_7 – Monocrotophos 2 ml · l^{-1}	7.69	47.37	78.95	100.00
T_8 – Untreated check (water)	0.00	0.00	0.00	0.00
Median lethal concentration (LC_{50}) for Diafenthiuron 50 WP	22.38	3.30	0.36	–

Data are the average percent mortality, n = 4 uron at all the tested concentrations were found to be the least toxic. A mortality of only 3.33% was found with the highest dose of diafenthiuron tested. However, diafenthiuron is highly toxic to fish and all the fish died irrespective of the diafenthiuron concentrations tested at 12 h.

Discussion

Extensive use of synthetic chemicals may result in the destruction of non-target organisms directly or where the chemicals get transported. So safety studies must to be carried out to know the selectivity of the chemical. The insecticidal effects on non-target organisms are categorised as per Nasreen *et al.* (2000) as harmless (< 50% mortality), slightly harmful (50–79% mortality), moderately harmful (80–89% mortality), and harmful (> 90% mortality) when tested at the field recommended dose. This classification is used by the International Organization for Biological Control, West Palaearctic Regional Section (IOBC/WPRS) working group, to assess the insecticidal effects on non-target organisms (Hassan 1989).

Pollination is as essential as the protection of crops from pest damage, for better crop production. These two aspects of crop production appear to be antagonistic but they can, and have to, go hand-in-hand (Adlakha and Sharma 1976). Diafenthiuron was found to be slightly harmful to Indian bees, *A. cerana indica*, in laboratory conditions (Stanley *et al*. 2009) but non-toxic in field conditions (Stanley *et al*. 2010b). This may be due to the low oral toxicity and strong translaminar activity of diafenthiuron. Diafenthiuron was reported as relatively safe to *Apis mellifera* L. (Perveen *et al*. 2000).

In the present study, diafenthiuron was not found toxic to the coccinellid predators, *M. sexmaculatus*. Diafenthiuron, at the field recommended dose of 1.6 g · l^{-1}, caused only 13.33% mortality at 24 h. Diafenthiuron was reported to be safer to other parasitoids and predators (Zuhua and Shusheng 1998; Abdelgader 2000; Toress *et al*. 2002). It was safer to the adults of coccinellids, mirids, spiders (www.kingtaichem.com), and *Chrysoperla carnea* Stephens (Preetha *et al*. 2009), but toxic to immature *Anthocoris* and mirids (Veire *et al*. 2002). Diafenthiuron was found to reduce the predatory potential of *Podisus nigispinus* (Dallus) on *Alabama argillacea* (Hübner) (Torres *et al*. 2002). In the present investigation, diafenthiuron was found highly toxic to *C. blackburni* by contact, causing 86.67% mortality at 48 h, at the recommended dose. But diafenthiuron was reported to cause minimal mortality to both the grubs and adults of *Encarsia formosa* Gahan and *Eretmocerus eremicus* Rose and Zolnerowich in the field (Javed and Matthews 2002).

Diafenthiuron was found to be highly toxic to silkworms and should not be used for the management of mulberry pests. Lower doses of diafenthiuron were tried for fish because the chemical is not sprayed in water resources but may get into water by runoff from sprayed fields in low concentrations. Diafenthiuron was found to be harmful to *C. carpio*, even when used in a much lower dose than the field recommended dose. This finding was in confirmation with the reports of Syngenta which stated 0.0038 mg · l^{-1} of diafenthiuron as the median lethal concentration (LC_{50}) at 96 h to carp, 0.0007 mg · l^{-1} to rainbow trout, and 0.0013 mg · l^{-1} to bluegill sunfish (www.syngenta.co.th/pegasus.pdf). After 30 days, earthworms are not affected by diafenthiuron and a negligible mortality was found even when using the highest dose of diafenthiuron tested.

Conclusions

Diafenthiuron, an important insecticide used extensively for the management of insect pests, is found to be slightly harmful to Indian bees, *A. cerana indica.* It is not harmful to the predators, *M. sexmaculatus*, but moderately harmful to *C. blackburni*, and highly toxic to the silkworm, *B. mori*, and to the fish, *C. carpio.* So, diafenthiuron should not be recommended for use in nearby areas of sericulture and pisiculture. Diafenthiuron is not harmful to earthworms, *P. excavatus.* Since it is not harmful to most predators and earthworms it can be used in pest management programmes.

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