Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(6): 582-587
Received: 07-09-2019
Accepted: 09-10-2019

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Safety evaluation of certain insecticides on the mealybug predator, *Cryptolaemus montrouzieri* Mulsant by dry film method

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Abstract
Among safety evaluation of different insecticides on life stages of *C. montrouzieri,* by dry film method, acephate was highly toxic while, buprofezin and neem oil were least toxic to all the stages of the predator after 72 h of application.

Keywords: Safety evaluation, *C. montrouzieri,* dry film method

Introduction

Among the predators of mealybugs, the Australian lady beetle, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) has been reported to be a general predator of mealybugs at all stages of its development. Both the stages of the predator, that is larva and adult are voracious feeders of all the stages of mealybug. It is commonly referred to as mealybug destroyer. It has been employed as the possible solution for combating the menace of the pest around the world.

It is native to Australia and was introduced to California for the control of citrus mealybug. Following the success, the beetle was introduced to India in 1898 by H.O. Newport to control the coffee green scale (Mayne 1953) [9]. Though, the predator did not establish on green scale, it was responsible in checking mealybug in coffee growing zones (Chacko *et al.* 1978; Mani & Krishnamoorthy 1997) [3, 8].

The biological suppression of mealybugs through this potent predator in India was well documented (Rao *et al.* 1971; Babu & Azam 1989) [11, 2]. In other countries, *C. montrouzieri* has proved effective as it is evident from the study of Smith & Armitage (1920) [14] that succeeded in keeping the destructive mealybugs in California under control by large scale multiplication of beetles. It has played a major role in the control of different sucking pests especially mealybugs (Mani & Krishnamoorthy 2008; Shylesha *et al.* 2011) [7, 12].

In order to achieve the pest control at higher level as well as safety to the consumers, integration of chemical insecticides and bio-agents have been followed as IPM strategies. However, in most cases, use of chemical insecticides along with bio-agents exhibited mortality of the predatory stages. So, there is a need to search for selective chemicals which are less toxic to grubs and adults of the predator.

Materials and Methods

Laboratory multiplication of mealybug, *Maconellicoccus hirsutus*

The mass production of mealybugs was done on medium sized ripened red pumpkin (*Cucurbita maxima* Duch.) under laboratory conditions at 25 ± 5 °C temperature and 75 ± 5 % relative humidity as suggested by Chacko *et al.* (1978) [3] and Singh (1978) [3].

Maintenance of mealybug culture

Initial culture was obtained from National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, India. Just ripened red pumpkins with ridges and grooves and bearing a small stalk were selected for easy handling. They were washed with water to remove dust on them. In order to prevent rotting, the pumpkins were treated with 0.1 % carbendazim 50 WP (1 g/l (Babu & David 1999) [1]. Wounds on the pumpkins were plugged with paraffin wax. The egg sacs of mealybugs from which the eggs have just started hatching were placed on pumpkins. The inoculation was done at regular intervals to ensure regular supply of all the stages of mealybugs throughout the study period. The inoculated pumpkins were kept in wooden cages (30 × 30 × 33 cm) with sliding glass in the front and cloth on either sides as suggested by Padmaja *et al.* (1995) and Katke & Balikai (2008).

Care was taken to close all cracks and crevices with wax to prevent the escape of early instars. Fully matured mealybugs developed within 30 to 40 days.

Multiplication of the predator *C. montrouzieri*

The method adopted by Chacko *et al.* (1978) [3] and Singh (1978) [3] was followed for rearing the predator after sufficient development of mealybugs on pumpkins. About 8-10 pairs of predators were released into the cage. The beetles, besides feeding on the mealybugs, laid their eggs singly or in groups inside the ovisacs of mealybugs. Full grown larvae pupated on pumpkins or corner of the rearing cage. The first beetle emerged within 30 days from the date of exposure of mealybugs to the beetle, the emerging adults were used for further studies. The beetles were provided with enough number of preys during the study period.

Dry film method

In this method larvae and adult predators were kept in a Petri dish and topically sprayed on the larvae with respective insecticidal spray fluid by using hand atomiser. The treated larvae were transferred in Petri dishes and provided with mealybugs along with their food. The same methodology was followed for adults. Observations on mortality of larvae and adults were recorded at 24, 48 and 72 h after treatment and calculated overall mean per cent mortality in each treatment.

Statistical analysis

The average percentage mortality of grubs and adults was worked out for each treatment and the data were subjected to statistical analysis as per Panse and Sukhatme (1985) [10].

Table 1: Details of insecticides and dosage used

Treatment	Trade name	Dosage	Source
Acetamiprid 20 SP	Prize	0.20 g/l	Jai Shree Rasayan Udyog Ltd., Delhi
Acephate 75 SP	Tremor	1.50 g/l	Biostadt India Limited, Mumbai
Imidacloprid 200 SL	Confidor	0.25 ml/l	Bayer Crop Sciences, Thane
Thiamethoxam 25 WDG	Sitara	0.20 g/l	Jai Shree Rasayan Udyog Ltd., Delhi
Dichlorvos 76 EC	Nuvan	1.00 ml/l	Insecticides (India) Limited, Agra
Profenophos 50 EC	Profex	2.00 ml/l	Nagarjuna Agrichem Limited, Hyderabad
Neem oil 0.5%	Neemark	5.00 ml/l	West Coast Herbochem Limited, Mumbai
Dimethoate 30% EC	Rogarin	2.00 ml/l	Insecticides (India) Limited, Agra
Buprofezin 25 SC	Addvant	1.50 ml/l	Sumitomo Chemical India Pvt., Ltd., Gujarat
Flonicamid 50 WG	Ulala	0.30 g/l	United Phosphorus Limited, Mumbai
Untreated control	-	-	-

Results and Discussion

Evaluation of toxicity of insecticides on first instar larvae

The results on safety of different insecticides against 1st instar larvae of *C. montrouzieri* at 72 h after the application (Table 2) revealed that acephate recorded the significantly higher mortality of (100.00%) of the grubs and proved to be most toxic followed by dimethoate (93.33%), imidacloprid (81.66%) followed by acetamiprid (71.67%), profenophos (70.00%) and are said to be on par with each other. The remaining treatments *viz.,* thiamethoxam, dichlorvos, flonicamid, buprofezin, neem oil recorded 61.67, 40.00, 11.67, 8.33 and 0.00% of mortality, respectively. However, no mortality was observed in untreated control, neem oil and was found to be safest to the predator (Fig. 1). A similar trend was followed at 24 and 48 h after the application.

Evaluation of toxicity of insecticides on second instar larvae

The results of the present study indicates that the highest percentage mortality was observed with acephate (90.00%) followed by dimethoate (85.00%), imidacloprid (75.00%), acetamiprid (71.00%), profenophos (55.00%), thiamethoxam (53.33%), dichlorvos (35.00%), flonicamid (8.33%), buprofezin (3.33%), neem oil (0.00%) and untreated control (0.00%). The same trend was followed at 24 and 48 h represented in Table 3. Neem oil was found to be safest while, acephate was most toxic to the predator (Fig. 2).

Evaluation of toxicity of insecticides on third instar grub

The results of the present study indicates that the highest per cent of mortality was observed in acephate (91.67%) followed by dimethoate (83.33%), imidacloprid (71.67%), acetamiprid (53.33%), thiamethoxam (53.33%), profenophos (50.00%), dichlorvos (43.33%), flonicamid (13.33%), buprofezin (0.00%), neem oil (0.00%) and untreated control (0.00%). The same trend was followed at 24 and 48 h as represented in Table 4. Neem oil and buprofezin was found to be safest while, acephate was most toxic to the predator (Fig. 3).

Evaluation of toxicity of insecticides on fourth instar grub

The present studies revealed that acephate was significantly more toxic after 72 h of application of insecticides with a percentage mortality of 91.67 followed by dimethoate (83.33%). The remaining treatments *viz.,* acetamaprid (48.33%), profenophos (45.00%), thiamethoxam (41.67%) and imidacloprid (40.00%) were on par with each other and are moderately toxic (Fig. 4). While flonicamid (15.00%) was least toxic. Buprofezin and neem oil were found to be non-toxic with no mortality as represented in Table 5.

Evaluation of toxicity of insecticides on adult stages

The results of the present study indicates that the highest per cent of mortality was observed in acephate (93.33%) followed by dimethoate (86.67%), imidacloprid (81.67%), acetamiprid (75.00%), profenophos (68.33%), thiamethoxam (43.33), dichlorvos (43.00%), flonicamid (20.00%), Buprofezin (0.00%), neem oil (0.00%) and untreated control (0.00%). Same trend was followed at 24 and 48 h represented in the Table (6). Buprofezin and neem oil were found to be safest while, acephate was most toxic to the predator (Fig 5).

The results of the present study are in close agreement with Sundari (1999) [15] who revealed that acephate was found to be highly toxic compounds while neem oil showed the lowest toxicity. While, Halikatti *et al.* (2014) [4] reported that acephate, imidacloprid was found to be highly toxic. Whereas, neem oil and buprofezin were found to be non - toxic. The toxicity of acephate against *C. montrouzieri* was in conformity with the findings of Kulkarni (2000) [5]. Mali *et al.* (2008) [6] reported that azadirachtin was absolutely safe botanical insecticide to the predator.

Table 2: Evaluation of toxicity of different insecticides against first instar larvae of *Cryptolaemus montrouzieri* by dry film method

Treatments	Per cent mortality (hours after treatment)		
	24 h	48 h	72 h
Acetamiprid 20% SP @ 0.2 g/l	55.00 47.86)	66.67 (54.76)	71.67 (57.98)
Acephate 75 SP @ 1.50 g/l	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Imidacloprid 200 SL @ 0.25 ml/l	76.70 (61.44)	80.00 (65.63)	81.66 (66.73)
Thiamethoxam 25 WDG @ 0.20 g/l	50.00 (44.98)	56.67 (48.87)	61.67 (51.83)
Dichlorvos 76% EC @ 1 ml/l	33.30 (35.20)	36.67 (37.21)	40.00 (39.17)
Profenophos 50% EC @ 2 ml/l	59.20 (50.31)	63.33 (52.90)	70.00 (56.97)
Neem oil 0.5% @ 5.00 ml/l	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Dimethoate 30% EC @ 2 ml/l	90.00 (73.26)	90.00 (73.26)	93.33 (77.69)
Buprofezin 25% EC @ 2 ml/l	5.00 (0.00)	6.67 (10.57)	8.33 (13.64)
Flonicamid 50 WG @ 0.30 g/l	100.00 (90.00)	10.00 (16.71)	11.67 (18.06)
Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S.E.M. ±	2.33	2.84	2.87
C.D. at 5%	6.63	8.08	8.17

Figures in the parentheses are angular transformed values

Table 3: Evaluation of toxicity of different insecticides against second instar larvae of *Cryptolaemus montrouzieri* by dry film method

Treatments	Per cent mortality (hours after treatment)		
	24 h	48 h	72 h
Acetamiprid 20% SP @ 0.2 g/l	55.00 (47.86)	65.00 (53.86)	71.67 (57.87)
Acephate 75 SP @ 1.50 g/l	88.33 (72.15)	90.00 (73.26)	90.00 (73.26)
Imidacloprid 200 SL @ 0.25 ml/l	70.00 (56.76)	71.67 (57.98)	75.00 (60.12)
Thiamethoxam 25 WDG @ 0.20 g/l	41.67 (40.17)	45.00 (42.09)	53.33 (46.09)
Dichlorvos 76% EC @ 1 ml/l	23.33 (28.77)	28.33 (32.09)	35.00 (36.07)
Profenophos 50% EC @ 2 ml/l	46.67 (43.06)	53.33 (46.90)	55.00 (47.86)
Neem oil 0.5% @ 5.00 ml/l	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Dimethoate 30% EC @ 2 ml/l	80.00 (64.00)	83.33 (68.08)	85.00 (69.19)
Buprofezin 25% EC @ 2 ml/l	0.00 (0.00)	1.67 (3.07)	3.33 (6.14)
Flonicamid 50 WG @ 0.30 g/l	5.00 (9.21)	6.67 (12.28)	8.33 (13.64)
Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S.E.M. ±	2.16	7.33	2.70
C.D. at 5%	6.13	3.65	7.68

Figures in the parentheses are angular transformed values

Table 4: Evaluation of toxicity of different insecticides against third instar larvae of *Cryptolaemus montrouzieri* by dry film method

Treatments	Per cent mortality (hours after treatment)		
	24 h	48 h	72 h
Acetamiprid 20% SP @ 0.2 g/l	46.67 (43.06)	53.33 (46.09)	53.33 (46.09)
Acephate 75 SP @ 1.50 g/l	81.67 (64.76)	91.67 (74.61)	91.67 (74.61)
Imidacloprid 200 SL @ 0.25 ml/l	36.67 (37.21)	71.67 (58.43)	71.67 (58.43)
Thiamethoxam 25 WDG @ 0.20 g/l	45.00 (42.09)	53.33 (46.09)	53.33 (46.09)
Dichlorvos 76% EC @ 1 ml/l	26.67 (30.98)	38.33 (38.17)	43.33 (41.13)
Profenophos 50% EC @ 2 ml/l	50.00 (44.98)	50.00 (44.98)	50.00 (44.98)
Neem oil 0.5% @ 5.00 ml/l	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Dimethoate 30% EC @ 2 ml/l	80.00 (63.90)	81.67 (65.01)	83.33 (66.12)
Buprofezin 25% EC @ 2 ml/l	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Flonicamid 50 WG @ 0.30 g/l	5.00 (9.21)	11.67 (18.06)	13.33 (19.42)
Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S.E.M. ±	1.84	2.23	2.18
C.D. at 5%	5.23	6.33	6.19

Figures in the parentheses are angular transformed values

Table 5: Evaluation of toxicity of different insecticides against fourth instar larvae of *Cryptolaemus montrouzieri* by dry film method

Treatments	Per cent mortality (hours after treatment)		
	24 h	48 h	72 h
Acetamiprid 20% SP @ 0.2 g/l	36.67 (39.22)	41.67 (40.13)	48.33 (44.02)
Acephate 75 SP @ 1.50 g/l	90.00 (73.26)	91.67 (74.61)	91.67 (74.61)
Imidacloprid 200 SL @ 0.25 ml/l	30.00 (32.99)	35.00 (36.21)	40.00 (39.17)
Thiamethoxam 25 WDG @ 0.20 g/l	36.67 (37.21)	38.33 (38.21)	41.67 (35.71)
Dichlorvos 76% EC @ 1 ml/l	16.67 (21.88)	20.00 (26.31)	23.33 (28.52)
Profenophos 50% EC @ 2 ml/l	36.67 (37.02)	43.33 (41.05)	45.00 (42.05)
Neem oil 0.5% @ 5.00 ml/l	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Dimethoate 30% EC @ 2 ml/l	75.00 (60.08)	81.67 (65.01)	83.33 (66.36)
Buprofezin 25% EC @ 2 ml/l	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Flonicamid 50 WG @ 0.30 g/l	8.33 (13.64)	11.67 (16.35)	15.00 (22.49)

Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S.E.M. ±	2.64	2.36	3.04
C.D. at 5%	7.41	6.71	6.11

Figures in the parentheses are angular transformed values

Table 6: Evaluation of toxicity of different insecticides against adult stages of *Cryptolaemus montrouzieri* by dry film method

Treatments	**Per cent mortality (hours after treatment)**		
	24 h	**48 h**	**72 h**
Acetamiprid 20% SP @ 0.2 g/l	53.33 (46.09)	61.67 (51.75)	75.00 (60.08)
Acephate 75 SP @ 1.50 g/l	86.67 (68.83)	91.67 (74.61)	93.33 (77.69)
Imidacloprid 200 SL @ 0.25 ml/l	66.67 (54.76)	73.33 (58.98)	81.67 (64.76)
Thiamethoxam 25 WDG @ 0.20 g/l	30.00 (33.19)	41.67 (40.17)	43.33 (41.13)
Dichlorvos 76% EC @ 1 ml/l	31.67 (34.20)	40.00 (39.17)	43.33 (41.33)
Profenophos 50% EC @ 2 ml/l	60.00 (50.79)	65.00 (53.86)	68.33 (55.76)
Neem oil 0.5% @ 5.00 ml/l	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Dimethoate 30% EC @ 2 ml/l	80.00 (63.65)	85.00 (67.47)	86.67 (68.83)
Buprofezin 25% EC @ 2 ml/l	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Flonicamid 50 WG @ 0.30 g/l	11.67 (16.35)	20.00 (26.06)	20.00 (26.06)
Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S.E.M. ±	1.94	1.67	1.74
C.D. at 5%	5.51	4.76	4.95

Figures in the parentheses are angular transformed values

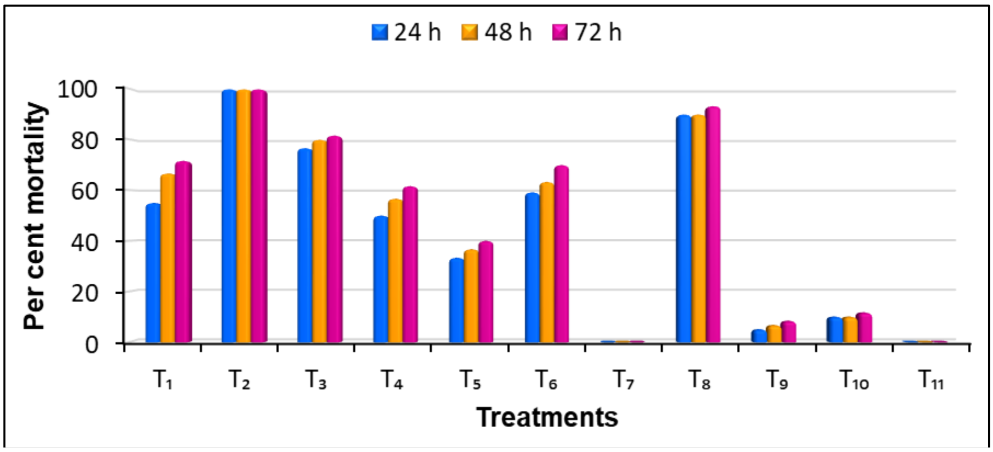

Fig 1: Evaluation of toxicity of different insecticides on first instar larvae of *C. montrouzieri* by dry film method

T1: Acetamiprid 20% SP @ 0.2 g/lit
T2: Acephate 75 SP @ 1.50 g/lit
T3: Imidacloprid 200 SL @ 0.25 ml/ lit
T4: Thiamethoxam 25 WDG @ 0.20 g/ lit
T5: Dichlorvos 76% EC @ 1 ml/lit
T6: Profenophos 50% EC @ 2 ml/lit
T7: Neem oil 0.5% @ 5.00 ml/lit
T8: Dimethoate 30% EC @ 2 ml/lit
T9: Buprofezin 25% EC @ 2 ml/lit
T10: Flonicamid 50 WG @ 0.30 g/lit
T11: Untreated control

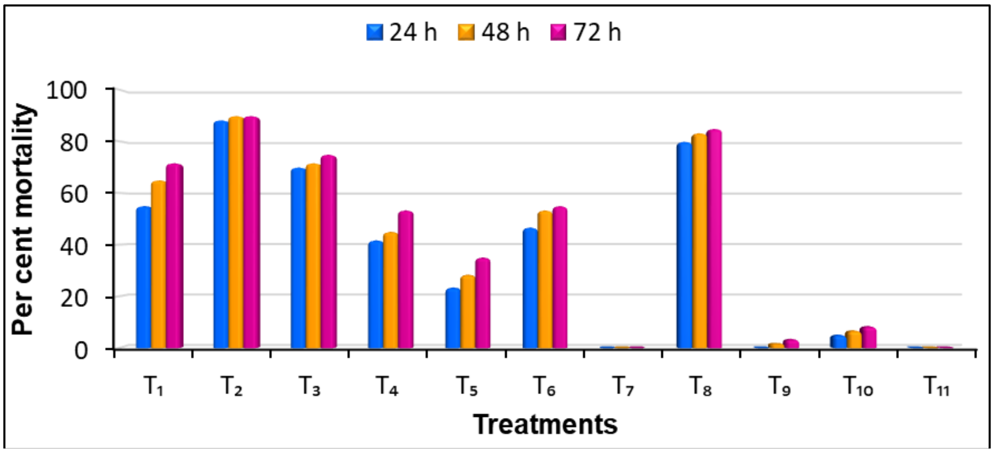

Fig 2: Evaluation of toxicity of different insecticides on Second instar of *C. montrouzieri* by dry film method

T1: Acetamiprid 20% SP @ 0.2 g/lit
T2: Acephate 75 SP @ 1.50 g/lit
T3: Imidacloprid 200 SL @ 0.25 ml/ lit
T4: Thiamethoxam 25 WDG @ 0.20 g/ lit
T5: Dichlorvos 76% EC @ 1 ml/lit
T6: Profenophos 50% EC @ 2 ml/lit
T7: Neem oil 0.5% @ 5.00 ml/lit
T8: Dimethoate 30% EC @ 2 ml/lit
T9: Buprofezin 25% EC @ 2 ml/lit
T10: Flonicamid 50 WG @ 0.30 g/lit
T11: Untreated control

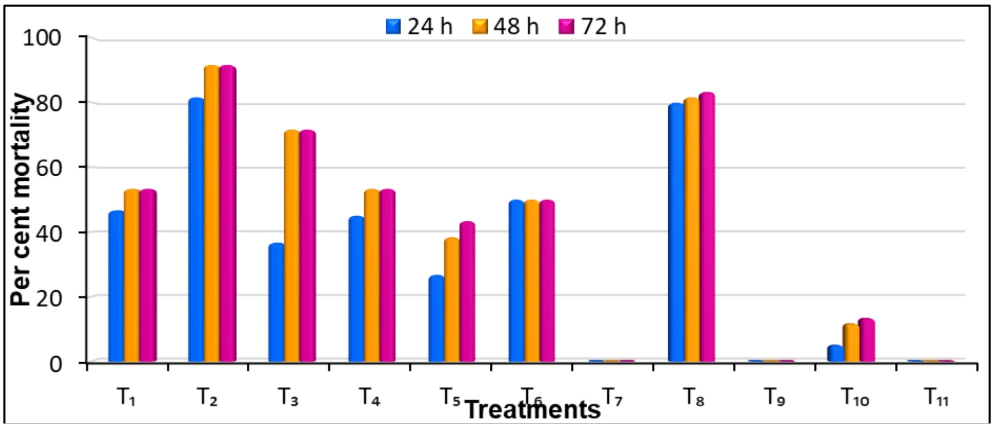

Fig 3: Evaluation of toxicity of different insecticides on third instar larvae of *C. montrouzieri* by dry film method

T1: Acetamiprid 20% SP @ 0.2 g/lit
T2: Acephate 75 SP @ 1.50 g/lit
T3: Imidacloprid 200 SL @ 0.25 ml/ lit
T4: Thiamethoxam 25 WDG @ 0.20 g/ lit
T5: Dichlorvos 76% EC @ 1 ml/lit
T6: Profenophos 50% EC @ 2 ml/lit
T7: Neem oil 0.5% @ 5.00 ml/lit
T8: Dimethoate 30% EC @ 2 ml/lit
T9: Buprofezin 25% EC @ 2 ml/lit
T10: Flonicamid 50 WG @ 0.30 g/lit
T11: Untreated control

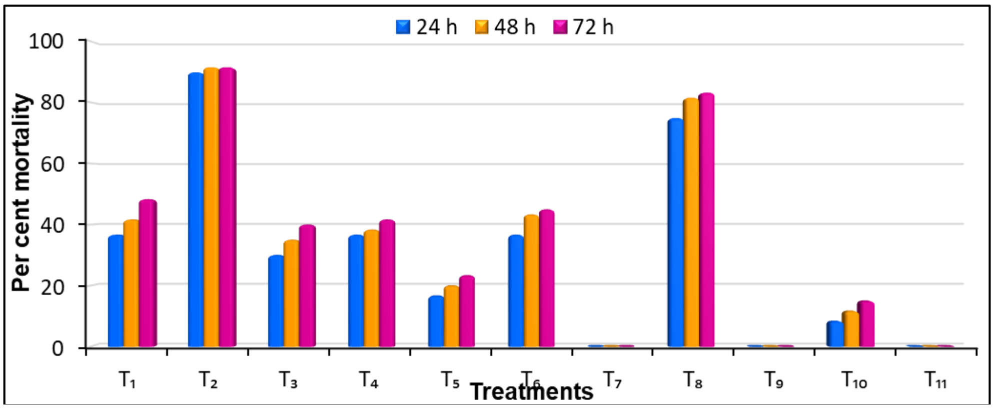

Fig 4: Evaluation of toxicity of different insecticides on fourth instar larvae of *C. montrouzieri* by dry film method

T1: Acetamiprid 20% SP @ 0.2 g/lit
T2: Acephate 75 SP @ 1.50 g/lit
T3: Imidacloprid 200 SL @ 0.25 ml/ lit
T4: Thiamethoxam 25 WDG @ 0.20 g/ lit
T5: Dichlorvos 76% EC @ 1 ml/lit`
T6: Profenophos 50% EC @ 2 ml/lit
T7: Neem oil 0.5% @ 5.00 ml/lit
T8: Dimethoate 30% EC @ 2 ml/lit
T9: Buprofezin 25% EC @ 2 ml/lit
T10: Flonicamid 50 WG @ 0.30 g/lit
T11: Untreated control

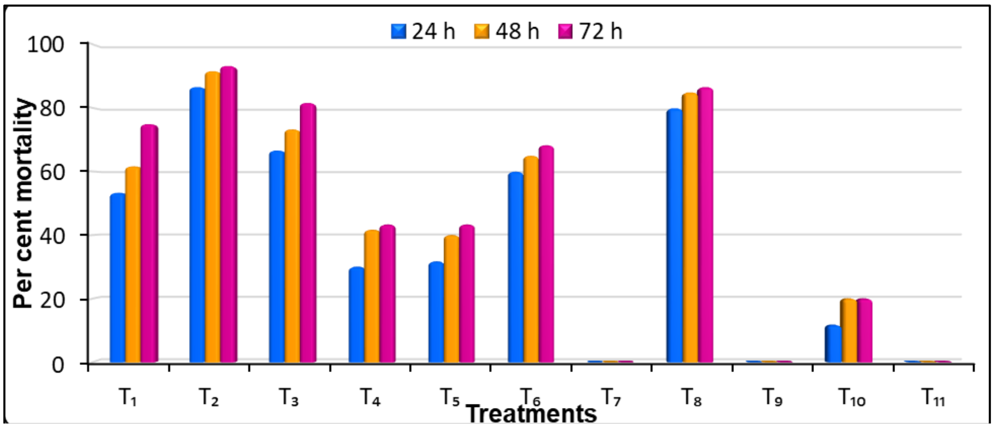

Fig 5: Evaluation of toxicity of different insecticides on adults of *C. montrouzieri* by dry film method

T1: Acetamiprid 20% SP @ 0.2 g/lit
T2: Acephate 75 SP @ 1.50 g/lit
T3: Imidacloprid 200 SL @ 0.25 ml/ lit
T4: Thiamethoxam 25 WDG @ 0.20 g/ lit
T5: Dichlorvos 76% EC @ 1 ml/lit
T6: Profenophos 50% EC @ 2 ml/lit
T7: Neem oil 0.5% @ 5.00 ml/lit
T8: Dimethoate 30% EC @ 2 ml/lit
T9: Buprofezin 25% EC @ 2 ml/lit
T10: Flonicamid 50 WG @ 0.30 g/lit
T11: Untreated control

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