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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(4): 07-12

www.thepharmajournal.com
Received: 07-02-2022
Accepted: 10-03-2022

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Comparative *in vitro* evluation of deltamethrin and dichlorvos against chicken lice and mites

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Abstract
In vitro bioassay was carried out to determine the efficacy of deltamethrin (DMT) and dichlorvas (DCVP) against lice and mites through filter paper method. The lice and mite samples collected from the infested flocks were used for the *in vitro* bioassays. The different concentrations of DMT and DCVP with and without surfactant (soap oil) were applied against feather mites, shaft louse and wing louse. Mortality of ectoparasites was observed in time interval of 2, 5 and 30 minutes. The concentrations at the rate of 1000 ppm (0.1%), 400 (0.04%) ppm and 200 ppm (0.02%) of DMT and DCVP with surfactant for feather mites, shaft and wing louse treated groups caused 100% of mortality in 30 minutes post treatment respectively. The lethal concentrations LC_{50} values for DMT with surfactant were 250.03, 40.98 and 20.64 ppm and LC_{99} values were 5605.96, 1036.90 and 238.07 ppm, while for DCVP with surfactant LC_{50} values were 473.90, 100.00 and 134.79 ppm and LC_{99} values were 1413.51, 679.34 and 2089.65 ppm for feather mites, shaft louse and wing louse respectively in 30 minutes of lethal time interval. The study emphasized the role of surfactant in potentiating the efficacy of both deltamethrin and dichlorvos on account of lesser LC_{50} and LC_{99} values with surfactant against *Megninia ginglymura*, *Menopon gallinae* and *Lipeurus tropicalis* than without surfactant.

Keywords: Deltamethrin, dichlorvos, *Megninia ginglymura*, *Menopon gallinae* and *Lipeurus tropicalis*

Introduction
Chickens are easily infested with several types of ectoparasites including lice, mites, ticks, fleas and flies (Atkinson *et al.*, 2008) [1]. Ectoparasites of poultry play on important role in the transmission of certain pathogens which cause heavy economic losses to the poultry industry (Moller *et al*., 2009) [7]. Ectoparasitic infestation is a major impediment to commercial layer production in many parts of the world. Parasites are a threat not only to the health of poultry, but also to workers in the poultry industry (Windhorst, 2017) [9]. The occurrence and frequency of external parasite infestation has increased in the laying hens which are confined in cages that facilitates their easy spreading and rapid multiplication. Ectoparasites like lice, mites, bugs, fleas and ticks parasitize the commercial layers causing suboptimal egg production, ill health and suboptimal liveability.

Currently chemical insecticides are being used for control of ectoparasitic infestation in poultry all over the world. However, high dose of insecticide/acaricide, lack of reapplication and poor penetration of drugs render the control option often ineffective and hence, ectoparasitic problems remain a recurrent and perennial in poultry production worldwide, particularly in tropics (Khan *et al*., 2001) [4]. Although, ectoparasitic problems are a major impediment to commercial layer production in many parts of the world, the researchers have not bestowed much attention to ward off this menace. It is indeed the need of the hour to formulate a suitable control strategy to check ectoparasitic infestation in poultry.

Further, many insecticides are available in the market for control of ectoparasites. However, proven dosage and their efficacy against ectoparasites of poultry are not clear for controlling them in field condition. This has necessitated the study on the efficacy of selected chemical ectoparasiticides in different concentration with ideal time interval for reapplication, which is deemed to be necessary for sustainable control of ectoparasites. The study may not only help to improve the production performance, but also to minimize the environmental pollution, residues in eggs and meat and cost of control programme for ectoparasites in poultry. Therefore, the present study was undertaken with the objective to evaluate the *in vitro* insecticidal/ acaricidal potential of deltamethrin and dichlorvos in the control of ectoparasites in poultry.

Materials and Methods

Procurement of insecticides/acaricides was made from the following manufacturers.

1. Deltamethrin (DMT) 1.25% EC (Butox® Vet) from MSD Animal Health (Tarapur, Boisar, Palghar district, Maharashtra – 401 506), containing 12.5 mg DMT per millilitre.
2. Dichlorvos (DCVP) 76% EC (Nuvan®) from Insecticides (India) Limited (Chopanki, Bhiwadi, Rajasthan – 301 707) containing 83 mg DCVP per millilitre.
3. Soap oil (surfactant) from local market.

Dosage of insecticides/acaricides was calculated as given below for preparing different concentration of insecticides / acaricides to conduct *in vitro* and *in vivo* trials.

$$\text{Dilution factor} = \frac{\text{Concentration required in ppm / percent x Volume required}}{\text{Quantity of active ingredient / ml}}$$

Dose calculation of Deltamethrin, Synthetic pyrethroid was done as given under.

Commercial preparation of Deltamethrin - 1.25% EC which signifies 12.5 mg per ml. Hence following dilutions were made to obtain the desired concentration.

0.8 ml of Butox/lit of water = 100 ppm; 1.6 ml of Butox/lit of water = 200 ppm and 2.4 ml of Butox/lit of water = 300 ppm.

Dichlorvos, a Organophosphorus compound (OPC) was diluted according to the calculation given below. Commercial preparation of Dichlorvos which is 76% EC contains 83 mg per ml. Further dilution, as mentioned below, yields the following concentration.

0.12 ml of Nuvan/lit of water = 100 ppm; 0.24 ml of Nuvan/lit of water = 200 ppm; 0.36 ml Nuvan/lit of water = 300 ppm and 0.48 ml Nuvan/lit of water = 400 ppm.

In vitro bioassay, as per method described by Khater *et al.* (2013) [5] was carried out to determine the efficacy of DMT and DCVP against lice and mites. The lice and mite samples were collected from the infested flocks and used for the *in vitro* bioassays.

The concentration of insecticides/ acaricides used against different ectoparasites as follows:

Name of the ectoparasites	Concentration of insecticide/ acaricide tested	
	DMT (in ppm)	DCVP (in ppm)
Shaft Louse	50, 100, 200, 300 and 400	50, 100, 200 and 400
Wing Louse	50, 100 and 200	50, 100 and 200
Feather mite	100, 200, 300, 600 and 1000	200, 400, 600, 800 and 1000

*Each concentration was tested with and without surfactant soap oil (1 ml/lit) in three replicates.

The different concentrations of drug were prepared for a total volume of one litre of distilled water. Thirty number of lice (shaft and wing louse each) and 50 – 60 number of feather mites (the number of mites in a field was counted under microscope before exposing them to drugs) were used in each replicate of each concentration. The lice and mites were carefully transferred to petri dishes layered with filter paper (Whatman No. 1). Immediately after placing the lice and mite, different concentrations of insecticides/acaricides were sprayed using hand sprayer. In the control group, the ectoparasites were sprayed with distilled water. The petri dishes of both treatment and control groups were kept at room atmosphere. The mortality of lice and mite was observed at the time interval of 2, 5 and 30 minutes. The lice and mites that were unable to move with or without upend posture was considered as moribund or dead (Figure 1 to 6).

Fig 1: Wing louse on the insecticide impregnated filter paper

Fig 2: Wing louse died in response to the insecticide treatment – 4X

Fig 3: Shaft louse on the insecticide impregnated filter paper

Fig 4: Shaft louse died in response to the insecticide treatment – 4X

Fig 5: Feather mites on the insecticide impregnated filter paper

Fig 6: Feather mites died in response to the insecticide treatment – 4X

Results and Discussion

The mortality pattern of feather mites, shaft and wing louse at different concentrations at the rate of 1000 ppm (0.1%), 400 (0.04%) ppm and 200 ppm (0.02%) of DMT with surfactant respectively resulted in 100% mortality in 30 minutes post treatment (Table 1 & and Figure 7).

The LC_{50} values for DMT with surfactant were 250.03, 40.98 and 20.64 ppm, whereas without surfactant the LC_{50} values were 363.68, 61.02 and 7.90 ppm for feather mites, shaft and wing louse respectively in 30 minutes of lethal time intervals.

The LC_{99} values for DMT with surfactant were 5605.96, 1036.90 and 238.07 ppm, while without surfactant the LC_{99} values were 3268.74, 1428.12 and 1054.66 ppm for feather mites, shaft and wing louse respectively in 30 minutes post application (Table 2).

The different concentrations of DCVP with and without surfactant were treated against feather mites (200, 400, 600, 800 and 1000 ppm), shaft louse (50, 100, 200 and 400 ppm) and wing louse (50, 100 and 200 ppm) and mortality was noted in 2, 5 and 30 minutes post application. The concentrations at 1000 ppm (0.1%), 400 ppm (0.04%) and 200 ppm (0.02%) of DCVP with surfactant caused 100% of mortality in feather mites, shaft and wing louse treated groups in 30 minutes post treatment (Table 3 and Figure 8).

The LC_{50} values for DCVP with surfactant were 473.90, 100.00 and 134.79 ppm, whereas without surfactant the LC_{50} values were 676.89, 141.42 and 159.69 ppm for feather mites, shaft and wing louse respectively in 30 minutes post application. The LC_{99} values for DCVP with surfactant were 1413.51, 679.34 and 2089.65 ppm, while without surfactant the LC_{99} values were 3144.60, 3506.17 and 3063.63 ppm for feather mites, shaft and wing louse respectively in 30 minutes post application (Table 4).

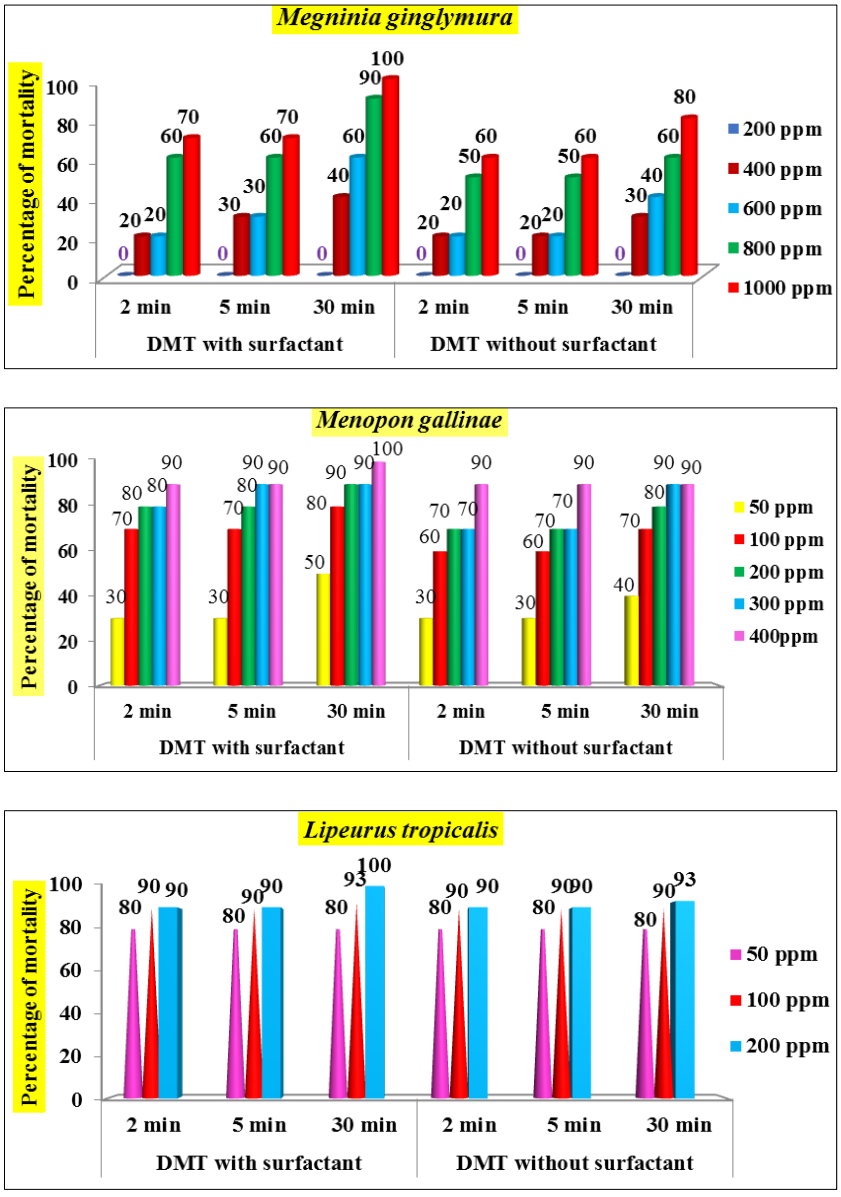

Fig 7: Percentage of mortality in deltamethrin treated with chicken mites and lice

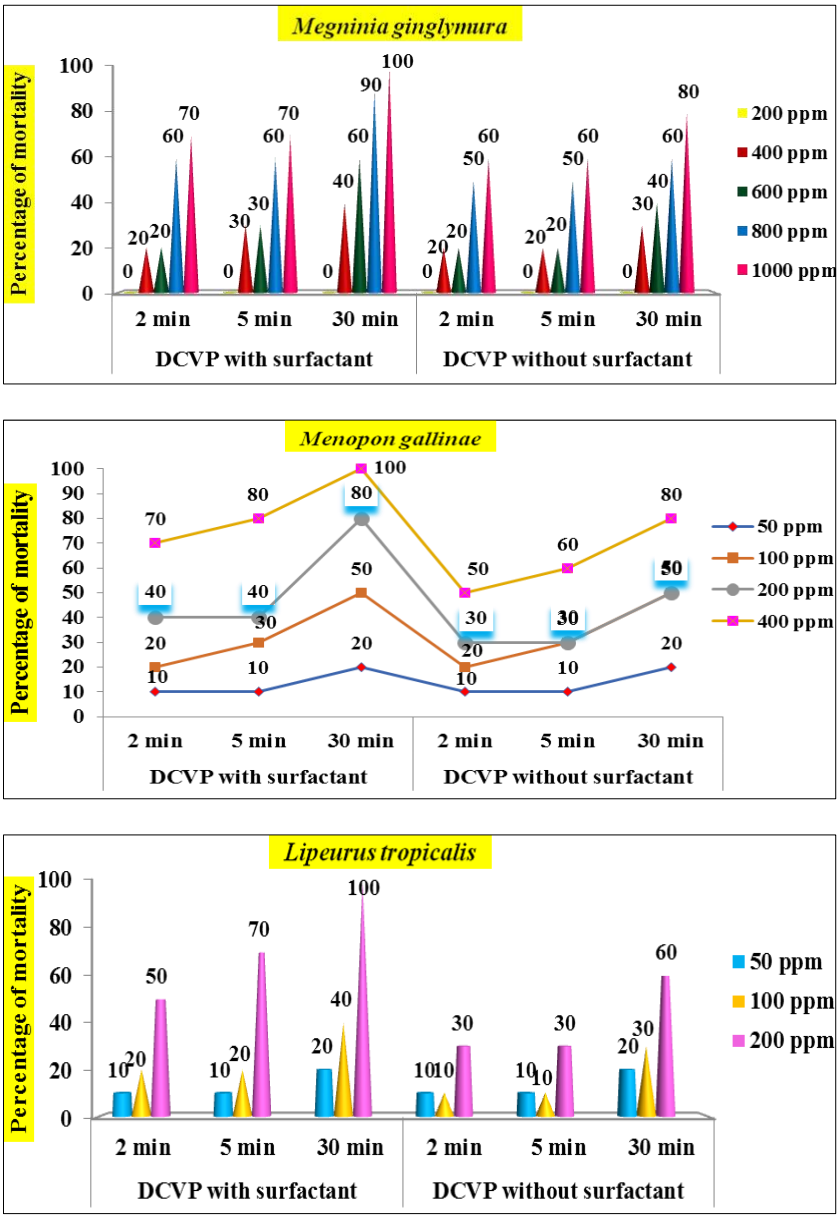

Fig 8: Percentage of mortality in dichlorvos treated with chicken mites and lice

Table 1: Percentage of mortality in chicken mites and lice with response to deltamethrin treatment

Name of the ectoparasites	Concentrations used (ppm / litre of water)	No. of mites exposed / concentration	DMT with surfactant			DMT without surfactant		
			2 min	5 min	30 min	2 min	5 min	30 min
			Average no. of dead	Average no. of dead	Average no. of dead	Average no. of dead	Average no. of dead	Average no. of dead
Feather mites	100	50	5 (10)	5 (10)	10 (20)	0 (0)	5 (10)	5 (10)
	200	50	10 (20)	20 (40)	25 (50)	5 (10)	5 (10)	10 (20)
	300	50	16 (33)	20 (40)	30 (60)	15 (30)	20 (40)	25 (50)
	600	50	19 (38)	25 (50)	35 (70)	16 (33)	30 (60)	30 (60)
	1000	50	40 (80)	45 (90)	50 (100)	33 (67)	40 (80)	45 (90)
	Control	50	0	0	0	0	0	0
Shaft louse	50	30	9 (30)	9 (30)	15 (50)	9 (30)	9 (30)	12 (40)
	100	30	21 (70)	21 (70)	24 (80)	18 (60)	18 (60)	21 (70)
	200	30	24 (80)	24 (80)	27 (90)	21 (70)	21 (70)	24 (80)
	300	30	24 (80)	27 (90)	27 (90)	21 (70)	21 (70)	27 (90)
	400	30	27 (90)	27 (90)	30 (100)	27 (90)	27 (90)	27 (90)
	Control	30	0	0	0	0	0	0
Wing louse	50	30	24 (80)	24 (80)	24 (80)	24 (80)	24 (80)	24 (80)
	100	30	27 (90)	27 (90)	28 (93)	27 (90)	27 (90)	27 (90)
	200	30	27 (90)	27 (90)	30 (100)	27 (90)	27 (90)	28 (93)
	Control	30	0	0	0	0	0	0

Table 2: LC_{50} and LC_{99} values of deltamethrin for *Megninia ginglymura*, *Menopon gallinae* and *Lipeurus tropicalis*

Concentrations used (ppm / litre of water)	No. of mites exposed / concentration	Lethal time interval (min)	LC_{50} ppm	95% CL		LC_{99} ppm	95% CL		$\chi 2$	df	Slope ± SE	SD (σ)
				LL	UL		LL	UL				
Megninia ginglymura												
100, 200, 300, 600 and 1000 (DMT with surfactant)	50	30	250.03	152.62	409.61	5605.96	3421.94	9183.92	0.258^{NS}	2	1.740 ± 0.109	0.575
100, 200, 300, 600 and 1000 (DMT without surfactant)	50	30	363.68	251.81	525.24	3268.74	2263.26	4720.90	0.247^{NS}	3	2.474 ± 0.081	0.404
Menopon gallinae												
50, 100, 200, 300 and 400 (DMT with surfactant)	30	30	40.98	22.69	74.02	1036.90	574.05	1872.95	0.833^{NS}	2	1.679 ± 0.131	0.595
50, 100, 200, 300 and 400 (DMT without surfactant)	30	30	61.02	35.56	104.70	1428.12	832.22	2450.73	0.947^{NS}	3	1.707 ± 0.120	0.586
Lipeurus tropicalis												
50, 100 and 200 (DMT with surfactant)	30	30	20.64	10.22	41.72	238.07	117.80	481.13	-	-	2.191± 0.156	0.456
50, 100 and 200 (DMT without surfactant)	30	30	7.90	2.29	27.33	1054.66	304.99	3647.05	0.947^{NS}	1	1.095 ± 0.275	0.913

NS - Non Significant (*P*>0.05); **'-'** The given concentration was not effective

Table 3: Percentage of mortality in chicken mites and lice with response to dichlorvos treatment

Name of the ectoparasites	Concentrations used (ppm / litre of water)	No. of mites exposed / concentration	DCVP with surfactant			DCVP without surfactant		
			2 min	5 min	30 min	2 min	5 min	30 min
			Average no. of dead	Average no. of dead	Average no. of dead	Average no. of dead	Average no. of dead	Average no. of dead
Feather mites	200	50	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	400	50	10 (20)	15 (30)	20 (40)	10 (20)	10 (20)	15 (30)
	600	50	10 (20)	15 (30)	30 (60)	10 (20)	10 (20)	20 (40)
	800	50	30 (60)	30 (60)	45 (90)	25 (50)	25 (50)	30 (60)
	1000	50	35 (70)	35 (70)	50 (100)	30 (60)	30 (60)	40 (80)
	Control	50	0	0	0	0	0	0
Shaft louse	50	30	3 (10)	3 (10)	6 (20)	3 (10)	3 (10)	6 (20)
	100	30	6 (20)	9 (30)	15 (50)	6 (20)	9 (30)	15 (50)
	200	30	12 (40)	12 (40)	24 (80)	9 (30)	9 (30)	15 (50)
	400	30	21 (70)	24 (80)	30 (100)	15 (50)	18 (60)	24 (80)
	50	30	3 (10)	3 (10)	6 (20)	3 (10)	3 (10)	6 (20)
	100	30	6 (20)	9 (30)	15 (50)	6 (20)	9 (30)	15 (50)
Wing louse	50	30	3 (10)	3 (10)	6 (20)	3 (10)	3 (10)	6 (20)
	100	30	6 (20)	6 (20)	12 (40)	3 (10)	3 (10)	9 (30)
	200	30	15 (50)	21 (70)	30 (100)	9 (30)	9 (30)	18 (60)
	Control	30	0	0	0	0	0	0

*The number within parenthesis denotes percentage of mortality

Table 4: LC_{50} and LC_{99} values of dichlorvos for *Megninia ginglymura*, *Menopon gallinae* and *Lipeurus tropicalis*

Concentrations used (ppm / litre of water)	No. of mites exposed / concentration	Lethal time interval (min)	LC_{50} ppm	95% CL		LC_{99} ppm	95% CL		$\chi 2$	df	Slope ± SE	SD (σ)
				LL	UL		LL	UL				
Megninia ginglymura												
200, 400, 600, 800 and 1000 (DCVP with surfactant)	50	30	473.90	386.16	581.57	1413.51	1151.82	1734.65	0.017*	1	4.953 ± 0.045	0.202
200, 400, 600, 800 and 1000 (DCVP without surfactant)	50	30	626.89	480.13	818.51	3144.60	2408.43	4105.79	0.012*	2	3.356 ± 0.059	0.298
Menopon gallinae												
50, 100, 200 and 400 (DCVP with surfactant)	30	30	100.00	68.56	145.86	679.34	465.76	990.86	0.923^{NS}	1	2.796 ± 0.084	0.358
50, 100, 200 and 400 (DCVP without surfactant)	30	30	141.42	80.45	248.60	3506.17	1994.52	6163.49	0.344^{NS}	2	1.677 ± 0.125	0.596
Lipeurus tropicalis												
50, 100 and 200 (DCVP with surfactant)	30	30	134.79	77.27	235.11	2089.65	1197.96	3645.08	-	-	1.954 ± 0.123	0.512
50, 100 and 200 (DCVP without surfactant)	30	30	159.69	87.48	291.48	3063.63	1678.38	5592.19	0.390^{NS}	1	1.819 ± 0.133	0.550

NS - Non Significant (*P*>0.05); *Significant (*P*<0.05); **'-'** The given concentration was not effective

The results obtained in the present study indicated that 100 percent mortality of *Megninia ginglymura*, *Menopon gallinae* and *Lipeurus tropicalis* reached 30 min post treatment with 0.1%, 0.04% and 0.02% of DMT and DCVP with surfactant respectively. In contrast, 100% mortality of *Argas persicus* larvae in two minutes after exposure to 0.05% DMT and the LC_{50} and LC_{99} values, 33.20 and 61.82 mg/L (Khater *et al*., 2013) [5]. The lethal time and concentrations of drugs may be variable for different species of ectoparasites as they differ in their susceptibility. In a trial involving a ectoparasite and deltamethrin, the LC_{99} values were 1.62 to 5.8 µg of deltamethrin for *Haematobia* spp. when exposed to 30 minutes to two hours (Anbarasi, 2021) [1].

Usage of surfactants such as commercial shampoo, commercial powdered soap, formulated tobacco liquid soap, commercial liquid soap along with water produced desired results in the control of ectoparasites in poultry (Ismi *et al*. 2019)[3]. Endorsing the potentiating effect of surfactants, significantly higher number of ectoprasites including three species of lice such as *Menopon gallinae, Menacanthus stramineus, Lipeurus caponis* and two species of mites such as *Ornithonyssus bursa* and *Megninia* sp. were dislodged in surfactant mixed application than without in the current study.

In addition, DMT was shown to be highly efficient against the vector of bluetongue virus and DMT is a pyrethroid with strong insecticidal activity, photo stability, low mammalian toxicity and acts on the insect's central nervous system to give a rapid knockdown (Mehlhorn *et al.*, 2011; Dubey *et al*., 2011) [6, 2]. Dichlorvos expressed more efficacious against the mite of *Ornithonyssus, sylviarum*, tick *Argas persicus* and lice *Lipeurus caponis* (Yazwinski *et al.*, 2005; Sindhu *et al*., 2017) [10, 8]. The effectiveness of deltamethrin and dichlorvos in the present study was in agreement with the reports by above mentioned authors.

It is concluded that the present study ascertained a comprehensive and effective dosage of deltamethrin and dichlorvos against *Megninia ginglymura*, *Menopon gallinae* and *Lipeurus tropicalis* by *in vitro* trial evaluations for the control of ectoparasites in poultry. It also paves the way for reduction in insecticide residues as addition of surfactant minimizes the dosage of insecticide/acaricide that was well evidenced in the present report.

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