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Original Article

Acaricide resistance in *Boophilus microplus* ticks collected from two ecological Zones of Khyber Pakhtunkhwa, Pakistan

Resistência a acaricidas em carrapatos *Boophilus microplus* coletados em duas zonas ecológicas de Khyber Pakhtunkhwa, Paquistão

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Abstract

Boophilus microplus is a major cattle tick specie causing great economic loss to the dairy industry throughout the globe including Pakistan. Trichlorfon and Deltamethrin are used to control bovine ticks, and their sprays are also used in other pest control programs that exert pressure on ticks to gain resistance. This study is aimed to examine the resistance level of *Rhipiciphalus microplus* against trichlorfon and deltamethrin. The engorged ticks were collected from two ecological regions of Khyber Pakhtunkhwa, KPK Pakistan i.e., Swat & Dir (zone-1), and Charsadda & Nowshera (zone-2). Four concentrations of acaricides in two-fold and ten-fold ppm with three replicates for each were used in both bioassays. Egg hatch assay and adult immersion tests were used to assess the resistance status. The probit analysis of egg hatch assay showed the highest hatching percentage in zone 1 on both dilutions (67-76% on two-fold and 68-88% on ten-fold dilution) while lethal concentration (LC95) was found to be 2.187 ppm and discriminating dose (DD) as 4.374 ppm for trichlorfon. In zone 2, hatching percentage was 73-84 on two-fold and 72-91% on ten-fold dilution while LC95 was recorded as 0.599 ppm and DD as 1.198 ppm. The same parameters were studied for deltamethrin and in zone 1 the hatching percentage was found as 38-56% on two-fold dilution and 37-80% on ten-fold dilution while LC95 was recorded as 0.001 ppm and DD as 0.002 ppm. In zone 2, the hatchability was recorded as 42-58% on two-fold and 43-85% on ten-fold dilution. The values for LC95 was recorded as 0.001 ppm and DD as 0.002 ppm. Further, analysis of adult immersion test against trichlorfon revealed the values of LC50 as 2.85 ppm and LC95 as 4.71 ppm in zone 1 and in zone 2 as 3.14 ppm and 5.28 ppm, respectively. Similarly, LC50 and LC95 against deltamethrin was recorded as 0.79 ppm & 1.71 ppm in zone 1 and 0.45 ppm & 4.325 ppm in zone 2, respectively. Based on the findings of this study, the isolated *Rhipicephalus microplus* was found to be more resistant to the widely used acaricides i.e., trichlorfon than deltamethrin. In order to maintain the efficacy of acaricides at country level, the study recommends continuous monitoring of resistance.

Keywords: acaricides, resistance, ecological zones, deltamethrin, Khyber Pakhtunkhwa Pakistan, *Rhipicephalus microplus*, trichlorfon.

Resumo

Boophilus microplus é uma das principais espécies de carrapatos bovinos, causando grandes perdas econômicas para a indústria de laticínios em todo o mundo, incluindo o Paquistão. Triclorfon e deltametrina são usados no controle de carrapatos bovinos, e seus *sprays* também são usados em outros programas de controle de pragas que exercem pressão sobre os carrapatos para ganhar resistência. Este estudo tem como objetivo examinar o nível de resistência de *Rhipicephalus microplus* contra triclorfon e deltametrina. Os carrapatos ingurgitados foram coletados de duas regiões ecológicas de Khyber Pakhtunkhwa, KPK Paquistão, ou seja, Swat & Dir (Zona 1) e Charsadda & Nowshera (Zona 2). Quatro concentrações de acaricidas em duas e dez vezes ppm com três repetições para cada foram usadas em ambos os bioensaios. O ensaio de eclosão de ovos e testes de imersão em adultos foram usados para avaliar o *status* de resistência. A análise probit do ensaio de eclosão de ovos mostrou a maior porcentagem de eclosão na zona 1 em ambas as diluições (67% na diluição de duas vezes e 68,33% na diluição de dez vezes), enquanto a concentração letal (CL95) foi de 2,187 ppm e dose discriminante (DD) como 4,374 ppm para triclorfon. Na zona 2, a porcentagem de eclosão foi de 73 em duas vezes e 72% em dez vezes, enquanto LC95 foi registrado como 0,599 ppm e DD como 1,198 ppm. Os mesmos parâmetros foram estudados para a deltametrina, e na zona 1 a porcentagem de eclosão foi de 38% na diluição de duas vezes e 37% na diluição de dez vezes, enquanto CL95 foi registrado como 0,001 ppm e DD como 0,002 ppm. Na zona 2, a eclodibilidade foi registrada como 42% na diluição de duas vezes e 43% na diluição de dez vezes. Os valores para LC95 foram registrados como 0,001 ppm e DD como 0,002 ppm. Além disso, a análise do teste de imersão de adulto contra triclorfon revelou os valores de LC50 como 2,85 ppm e LC95 como 4,71 ppm na zona 1 e na zona 2 como 3,14 ppm e 5,28 ppm, respectivamente. Da mesma forma, LC50

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e LC95 contra deltametrina foram registrados como 0,79 ppm e 1,71 ppm na zona 1 e 0,45 ppm e 4,325 ppm na zona 2, respectivamente. Com base nos achados deste estudo, o isolado *Rhipicephalus microplus* mostrou-se mais resistente ao acaricida amplamente utilizado, ou seja, triclorfon do que deltametrina. Para manter a eficácia dos acaricidas em nível nacional, o estudo recomenda o monitoramento contínuo da resistência.

Palavras-chave: acaricidas, resistência, zonas ecológicas, deltametrina, Khyber Pakhtunkhwa Paquistão, *Rhipicephalus microplus*, triclorfon.

1. Introduction

Pakistan is an agricultural country, located in tropical and subtropical zones, Due to diversity in climatic conditions, different numbers of tick species of various genera are found in different localities of livestock (Durrani and Kamal 2008). Khyber Pakhtunkhwa is Pakistan's third most populous and economic province, although geologically it is the smallest of the four provinces. Arthropods are one of the most common vectors of pathogenic diseases, which may appear as epidemics or pandemics in humans and animals (Benelli, 2016). Among arthropods, ticks are the second most notorious vectors (Goddard 2016). Ticks are obligate hematophagous parasites that can transmit a large number of pathogenic diseases to humans and animals. Livestock population is heavily affected by ticks in terms of concealed damage, blood loss, toxicity, and production losses such as skin, hide, milk, and meat (Sajid et al. 2009). FAO (Food and agricultural organization) of the UN (United Nations) estimated a global annual loss of US$ 14-19 billion in the food industry due to tick invasion (Jabbar et al. 2015). It is estimated that every engorged female is accountable for the loss of 8.9 mL of everyday milk production and 1 g of body weight (Rodríguez-Vivas et al., 2014).

Boophilus microplus known as the blue tick parasite is the tropical cattle tick and an important parasite in livestock throughout the world. *Boophilus* also known as *Rhiphicephalus* (Murrell and Barker 2003) having quite small mouthparts and produce a considerable amount of sticky material (Cement) that helps in attachment to the host. *Rhipicephalus microplus* is solely accountable for annual loss through irritation, reduction in milk/meat production often causes the death of an animal and spread pathogenic diseases like babesiosis, theileriosis and anaplasmosis (de Castro, 1997, Alim et al., 2012). Furthermore, *Rhipicephalus* acts as a vector for numerous bacterial and viral diseases such as (Crimean-Congo hemorrhagic fever virus, tick-borne encephalitis virus), bacteria (*Rickettsia* spp). The management of tick-borne diseases is focused on the control measures of vectors. The major strategy employed for tick management is the repeated application of acaricides however the risk associated with this control measure is the development of drug resistance.

A large number of vector control program has been started to overcome these devastating effects. For this purpose, a large number of acaricides are used to control tick infestation. Chemical control is considered to be a reliable and instant way to overcome these losses. The major groups of acaricides such as organochlorines, organophosphates, carbamates, formamidines, pyrethroids, and macrocyclic lactones are widely used in vector control programs (Higa et al. 2015). All these chemicals are also used as insecticides, pesticides, and acaricides in many other vector control programs. So, the repeated and irregular use of these chemical groups directed to the expansion of acaricides resistance in the tick populace (Abbas et al. 2014).

Acaricides are easily available and frequently used by farmers against infested ticks in animals without considering proper dosage and essential requirements before using acaricides which leads to the development of resistance in the ticks (FAO, 2004; Nandi et al., 2018; Adehan et al., 2016). In a country like Pakistan, organophosphate (trichlorfon) and other synthetic pyrethroids (deltamethrin) are mostly used. However, they also cause increase resistance in several ectoparasites, which increases the need for proper diagnosis and monitoring of tick resistance in Pakistan. Resistance has been observed against one of the organophosphate compounds named "diazinon" in Indian samples of *Rhiphicephalus microplus* (Kumar et al., 2011). Moreover, synthetic chemicals like pyrethroids are easily available commercially in India. Deltamethrin and cypermethrin are the two most used chemicals all over the country. They are not only used against agricultural pests but also to control the spread of mosquitoes (Ansari and Razdan, 2003; Sharma et al., 2012).

Trichlorfon and Deltamethrin are the two main chemicals that are widely used to control ticks in bovine. Tick resistance has been developed due to the frequent and intensive use of these acaricides. To achieve an efficient tick control program, it is required to detect early resistance in the ticks' population in field. These effective measures can help in developing further control strategies related to tick-borne diseases. Therefore, the main objective of the current study was to detect early resistance in ticks against trichlorfon and deltamethrin to understand their efficacy against the control of *Rhiphicephalus microplus.*

2. Materials and Methods

The study area included two ecological zones of Khyber Pakhtunkhwa (Pakistan), i.e. zone one consists of district Swat and Dir, whereas zone two consists of Charsadda and Nowshera. The ecological zones with dense livestock populations were selected based on climate variation to make the data more reliable and representative as shown in (Figure 1). Ticks were randomly collected from various body parts (ears, brisket, withers, knees, udder in case of female and testes in males along with the perennial region and tail) from infested bovine with the help of forceps without injuring their mouthpart by using convenient sampling technique as described by (Ali et al., 2013). The collected ticks were transported in pre-labeled falcon tubes with a perforated lid to the Entomology Laboratory, Department

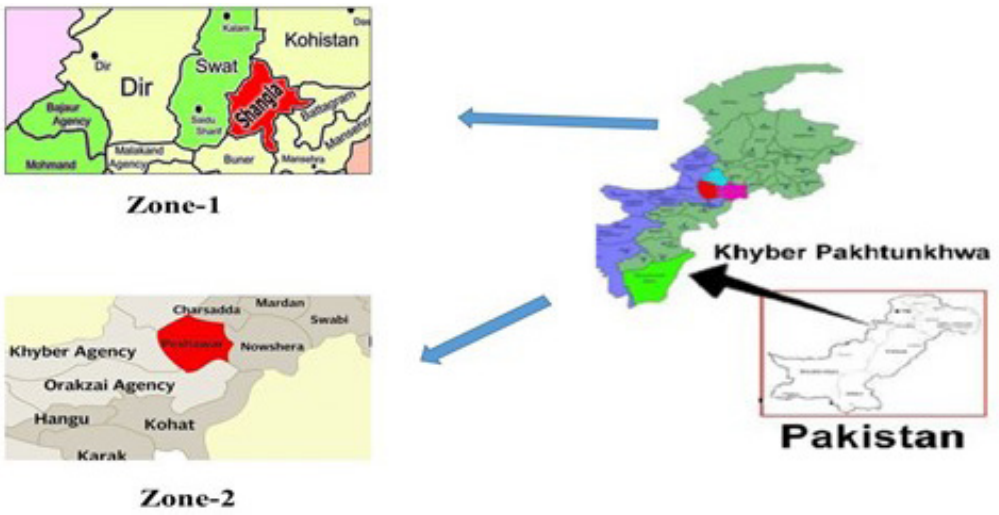

Figure 1. Map of ecological zones i.e. zone 1 and zone 2.

of Parasitology, UVAS Lahore, and identified up to genus level based on their morphological characteristics using morphology key by Walker (2003).

2.1. Preparation of acaricides dilutions

The acaricides resistance pattern of *R. microplus* was evaluated for organophosphate (Trichlorfon), pyrethroids (Deltamethrin). The different dilutions of commercial acaricides were prepared by using serial two-fold and ten-fold dilutions. For the experimental bioassay, various concentrations for egg hatch assay and adult immersion assay; two-fold and tenfold serial dilutions for trichlorfon 1500, 750, 375,187.5 and 1500, 150, 15, 1.5 ppm, while for deltamethrin two-fold and tenfold serial dilution 250, 125, 62.5, 31.25 and 250 25, 2.5, 0.25 ppm were used. Stock concentration for deltamethrin 2.5%W/V, trichlorfon 1.5mg/ml were planned from the stock solution in refined water and tested adjacent to the adult's engorged female ticks and eggs of *R. microplus*. To check the status of resistance in ticks, the following acaricides bioassays are recommended by FAO (2004) were performed.

2.2. Protocol for egg hatchability test/egg immersion

Egg hatchability assay was performed by using the methodology of (Haque et al., 2014) with slight changes. The hatching and inhibition of the hatching percentage were determined. LC95%, and DD% Discriminating dose, data were evaluated by using the Probit analysis method.

Live engorged female *Rhipicephalus microplus* ticks were washed by using PBS solution and dried by using a paper towel for hatching the eggs, the ticks were placed in a tube covered with a muslin cloth and placed in an incubator at 29°C (± 1°C) and 80-85% humidity for 14-20 days. The different dilutions of commercial acaricides were prepared by using serial two-fold dilutions and ten-fold dilutions. After hatching, approximately 150 eggs in each replicate were counted and placed in a glass tube; the eggs were immersed or exposed for 5 min. in 1 ml acaricides solution. The control group was treated with distilled water and each treatment contained three replicates. After complete evaporation of the test solution, eggs were incubated with a temperature of 29°C (± 1°C) and 80-85% humidity level for fourteen days. The results were recorded according to the following parameters (Haque et al., 2014).

Equation 1:

$$Hatching\ \% = \frac{\text{No of hatched larvae}}{\text{Total number of incubated eggs}}$$

Equation 2:

$$Percent\ hatching\ inhibition\ (PHI)\% = \frac{\text{Hatching percentage of the control group} - \text{Hatching in treated group}}{\text{Hatching percentage of the control group}} \times 100$$

2.3. Protocol for adult immersion test

Live engorged *Rhipiciphalus microplus* female ticks were washed with PBS and wiped dry, weighed separately and divided into two groups each group containing 46 ticks, with 12 each adults ticks in three replicates for two and 10 fold serial dilutions. After weighing the tick, each group was dipped or immersed in acaricides solution of different concentrations. The different dilutions of commercial acaricides were prepared by using serial two-fold and ten-fold dilutions. The control group was treated with distill water for 5 minutes. The ticks were dried and placed in a tube covered with muslin cloth and was placed in an incubator with a temperature of 29°C (± 1°C) and 80-85% humidity level for fourteen days. The individual ticks were weighed in separate tubes, held in the same conditions until hatching was estimated visually (Kumar et al., 2011). The following parameters were compared;

I. Mortality rate was recorded up to two weeks after treatment
II. The masses of the eggs laid by the ticks under examination were documented.

Equation 3:

$$RI\ (Reproductive\ index) = \frac{\text{EW}(\text{weight egg mass})}{\text{FEW}(\text{female engorged weight}}$$

Equation 4:
IO% (Inhibition of oviposition)
Equation 5:

$$IO\% = \frac{\text{Reproductive Index Control - Treated Reproductive Index}}{\text{Control Reproductive Index}} \times 100$$

2.4. Statistical analyses

Dose–response data were analyzed by probit method in SPSS. The lethal dose concentration LC50 ppm, LC95 ppm and Discriminating dose DD values of trichlorfon and deltamethrin were determined by applying regression equation analysis to the probit transformed data of mortality. Discriminating dose (DD) was determined as 2x LC95. Probit analysis commonly use in toxicology in order to quantify the relative toxicities of chemicals to living organisms.

3. Results

3.1. Egg immersion test

The result of Egg immersion test showed overall high resistance in *Rhipicephalus microplus* against trichlorfon while deltamethrin was found effective against ticks. After treatment, The probit analysis of egg hatch assay showed the highest hatching percentage in zone 1 on both dilutions (67-76% on two-fold and 68-88% on ten-fold dilution) (Figure 2), while lethal concentration (LC95) was found to be 2.187 ppm and discriminating dose (DD) as 4.374 ppm for trichlorfon. In zone 2, hatching percentage was 73-84 on two-fold and 72-91% on ten-fold dilution (Figure 3), while LC95 was recorded as 0.599 ppm and DD as 1.198 ppm.

The same parameters were studied for deltamethrin and in zone 1 the hatching percentage was found as 38-56% on two-fold dilution and 37-80% on ten-fold dilution as shown in (Figure 4), while LC95 was recorded as 0.001 ppm and DD as 0.002 ppm. In zone 2, the hatchability was recorded as 42-58% on two-fold and 43-85% on ten-fold dilution as shown in (Figure 5). The values for LC95 were recorded as 0.001 ppm and DD as 0.002 ppm. While the slope of hatchability (95% CL), Y-intercept (95% CL), R^2, and P-Value is represented in (Table 1).

3.2. Adult immersion test

After treatment the results of adult immersion assay test showed, the mortality percentage of *Rhipicephalus*

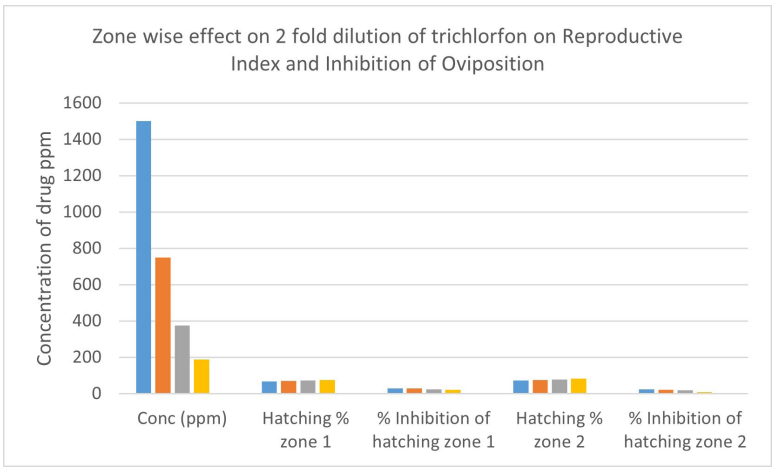

Figure 2. Zone wise effect of 2 fold dilution on egg hatch assay against trichlorfon on reproductive index and inhibition of oviposition.

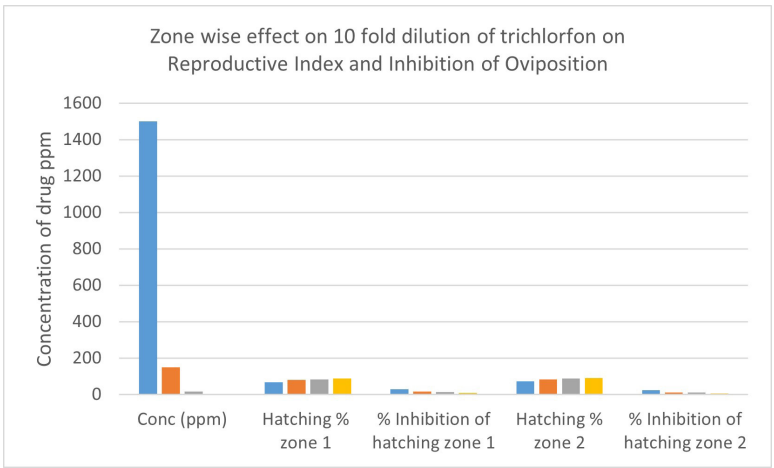

Figure 3. Zone wise effect of 10 fold dilution on egg hatch assay against trichlorfon on reproductive index and inhibition of oviposition.

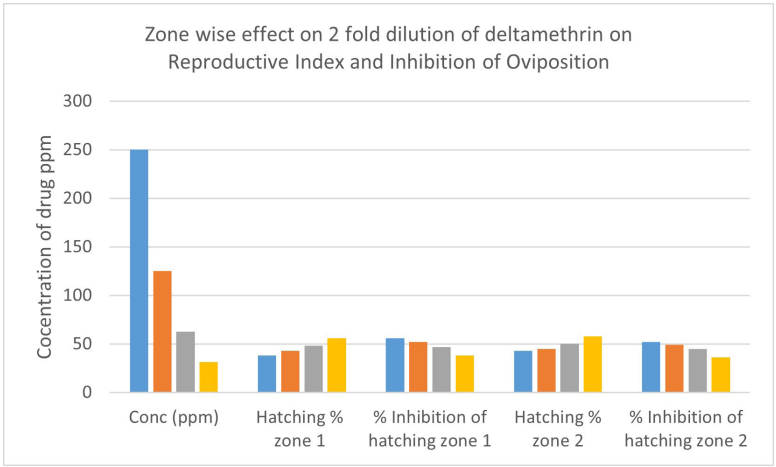

Figure 4. Zone wise effect of 2 fold dilution on egg hatch assay against deltamethrin on reproductive index and inhibition of oviposition.

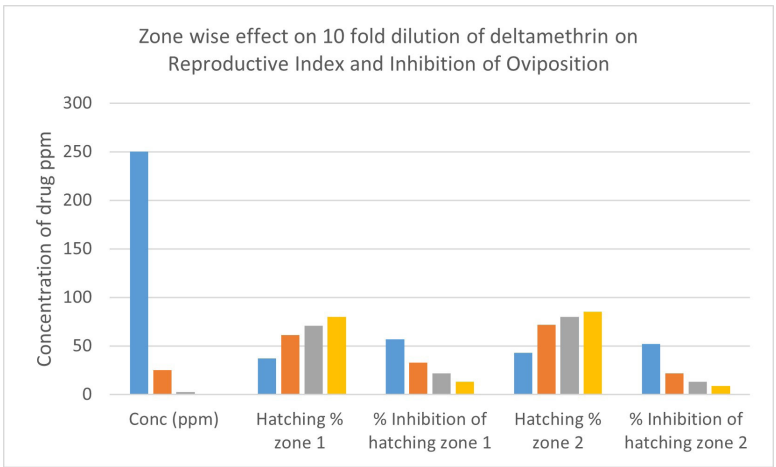

Figure 5. Zone wise effect of 10 fold dilution on egg hatch assay against deltamethrin on reproductive index and inhibition of oviposition.

Table 1. Probit analysis of eggs hatch assay to Trichlorfon and Deltamethrin for zone 1 and zone 2 on eggs of *R. microplus*.

Acaricides and Location	The slope of hatchability (95% CL)	*Y*-intercept (95% CL)	R^2	p-value	LC_{95} (ppm) (95% CL)	DD (%)
Trichlorfon Zone 1	-0.748±0.311	1.517±2.133	0.25	0.000	2.187	4.374
Trichlorfon Zone 2	-0.641±0.205	1.243±1.858	0.24	0.000	0.599	1.198
Deltamethrin Zone 1	-0.519 ±0.364	0.261±0.395	0.82	0.000	0.001	0.002
Deltamethrin Zone 2	-0.525±0.370	0.305±0.440	0.83	0.000	0.001	0.002

[CL]Confidence interval. [LC]Lethal concentration. [DD]Discriminating Dose. [SE]Standard Error. [RI]Reproductive index.

microplus against Trichlorfon in zone 1 on two and tenfold dilutions were (7-58%) while for deltamethrin it remained (25-83%), the egg mass laid by *Rhipicephalus microplus* for trichlorfon was 0.08-0.38 gram while for deltamethrin were (0.0-0.03 gram) which showed susceptibility as per criteria of FAO (2004). Similarly, for trichlorfon the Reproductive index was (0.19-0.37), inhibition of oviposition (52.72-5.54) (Figure 6), while for deltamethrin was (0.00-0.24) and (81-16) as describe in (Figure 7), were also recorded to assess the resistance status of *Rhipicephalus microplus*. Further, analysis of adult immersion test against trichlorfon revealed the values of the lethal dose concentration LC50 as 2.85 ppm and LC95 as 4.71 ppm in zone 1, the Slope ± SE and R^2 value were presented in (Table 2).

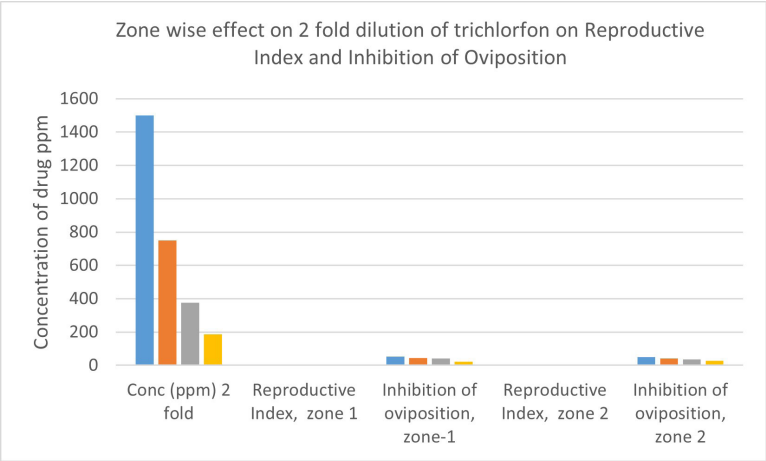

Figure 6. Zone wise effect of adult immersion 2 fold dilution of trichlorfon on reproductive index and inhibition of oviposition.

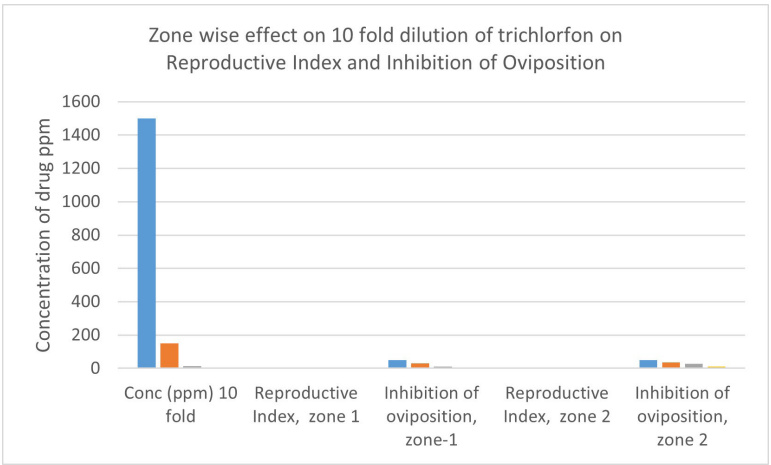

Figure 7. Zone wise effect of adult immersion 2 fold dilution of deltamethrin on reproductive index and inhibition of oviposition.

Table 2. Probit anaylsis of adult imersion test to Trichlorfon and Deltamethrin in zone 1 and zone 2 on *Rhipicephalus microplus*.

Acaricides and Location	Variables	Slope ± SE	R^2	LC_{50} (ppm) (95% CL)	LC_{95} (ppm) (95% CL)
Trichlorfon Zone 1	**Mortality**	0.885±0.209	0.67	2.85	4.71
	Egg Mass	-9.234 ±0.000	0.50		
	RI	-9.770± 0.000	0.19		
	%IO	0.028± 0.023	0.19		
Trichlorfon Zone 2	**Mortality**	0.768±0.206	0.67	3.14	5.28
	Egg Mass	-7.480±0.0042	0.34		
	RI	0.000± 0.000	0.11		
	%IO	0.094± 0.085	0.16		
Deltamethrin zone 1	**Mortality**	0.363±0.123	0.61	0.79	1.71
	Egg Mass	-0.001± 0.000148	0.67		
	RI	-0.00294±0.000146	0.40		
	%IO	0.083996±0.041702	0.40		
Deltamethrin zone 2	**Mortality**	0.424±0.123	0.64	0.45	4.32
	Egg Mass	-0.000331± 0.000222	0.27		
	RI	-000108±0.000225	0.03		
	%IO	0.005479±0.063862	0.00		

[CL]Confidence interval. [LC]Lethal concentration. [DD]Discriminating Dose. [SE]Standard Error. [RI]Reproductive index. [IO]Inhibition of oviposition.

In zone 2 after treatment the mortality percentage of *Rhipicephalus microplus* against trichlorfon on two and tenfold dilutions were (7-57%) while for deltamethrin remained (25-75%), the egg mass laid by *R. boophilus microplus* for trichlorfon was 0.11-0.23 gram while for deltamethrin were (0.02-0.08 gram). Similarly, the Reproductive index for trichlorfon was (0.12-0.35%), inhibition of oviposition (50.78-12.19%) (Figure 8), and for deltamethrin were (0.00-0.19%) and inhibition of oviposition was (85-36%) as describe in (Figure 9), were recorded to check the resistance status of *R. boophilus microplus*. LC50 and LC95 against deltamethrin was recorded as 0.79 ppm & 1.71 ppm in zone 1 and 0.45 ppm & 4.325 ppm in zone 2, respectively. While the Slope ± SE and R^2 value were presented in (Table 2).

4. Discussion

Swat and Dir (Zone 1) and Charsadda & Nowshera (Zone 2) were chosen for the present research study because of their distinct climatic circumstances, like temperature, latitude, and rainfall. *Rhipicephalus (Boophilus) microplus* was tested for resistance to two commonly used acaricides from Pakistan's KPK province: Deltamethrin and Trichlorfon. To meet the objectives of the research study, experimental bioassays were used to determine the resistance level of ticks randomly collected from buffaloes and cattle. To produce precise and dependable findings, the egg hatch assay and adult immersion test were carried out with great care in both zones.

The slope of egg hatchability 95% CL against trichlorfon in zones 1 and 2 indicated the values (0.748 -0.311) and (0.641 -0.205) under identical conditions. The recorded Y-intercept value from zones 1 and 2 was (1.517 -2.133) and (1.243 -1.858). Keeping in view the study results, this large discrepancy can be attributed to bringing changes in the climate, particularly the temperature, between the two regions. Results of the study showed that the slope value could be used to forecast whether resistance was already present in the field population, or was just beginning to emerge. According to the findings of the

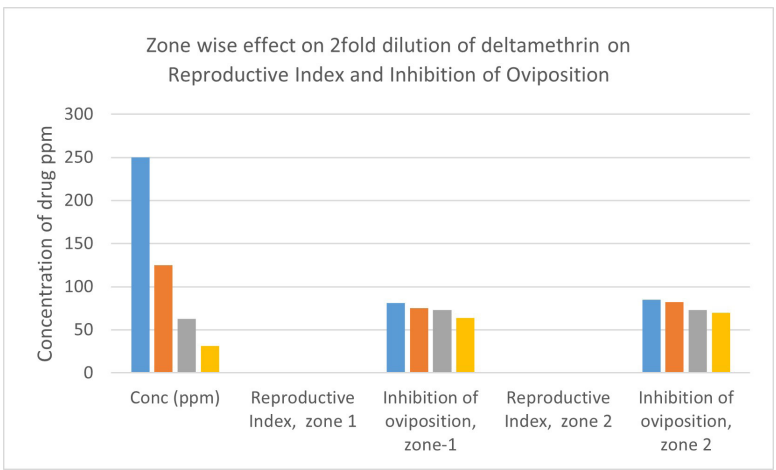

Figure 8. Zone wise effect of adult immersion 10 fold dilution of trichlorfon on reproductive index and inhibition of oviposition.

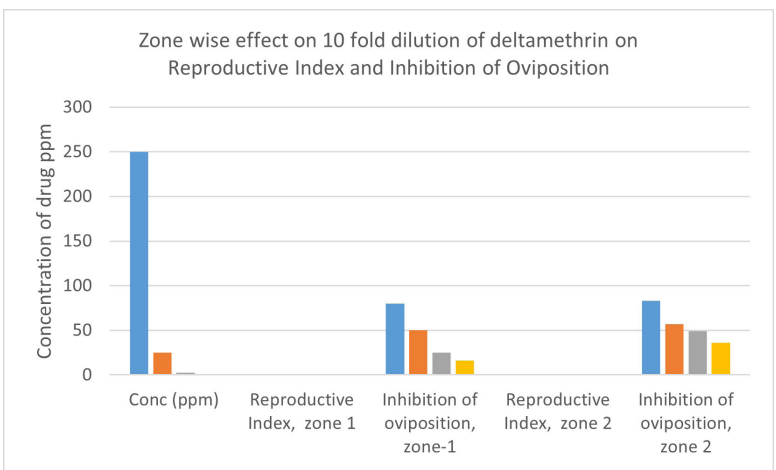

Figure 9. Zone wise effect of adult immersion 10 fold dilution of deltamethrin on reproductive index and inhibition of oviposition.

present research study, similar findings were identified by (Nandi et al., 2018).

In the same way, the statistical analysis of the egg hatch assay against deltamethrin showed the highest slope of hatchability, i.e. (-0.519 0.364) and (-0.525 -0.370) with 95 percent CL from zones 1 and 2, while the Y-intercept from zones 1 and 2 was (0.261, 0.395) and (0.305, 0.440) with inconsistent R^2 values LC95, DD percent, and P-value described as the highest form. The drop in trichlorfon values in zone 1 and 2 can be attributed to environmental variables such as high mountains, and heavy rainfall. The above findings are in agreement with those of Davey et al. (1989).

Deltamethrin from Zones 1 and 2 showed the highest LC95 and DD percent against both acaricides in the current investigation, with probit analysis of the egg hatch assay providing an accurate estimate. Results can fluctuate because of factors like average yearly rainfall, temperature, and the frequency with which trichlorfon is administered. Haque et al. (2014), examined the effect of commonly used acaricides (trichlorfon, cypermethrin, deltamethrin, and flumethrin) on *Rhipicephalus microplus* eggs obtained from Ludhiana, India, an egg hatch test was utilized to determine the effectiveness of the acaricides (EHA). Despite extensive research on the efficiency of acaricides against the various life stages of *R. microplus*, no studies had been carried out to investigate the effect of acaricides on the eggs of this species of carnivore. As a result, we investigated the in-vitro resistance status of commercially available acaricides (trichlorfon and deltamethrin) in the current study and discovered that ticks developed less resistance to deltamethrin and higher resistance to trichlorfon due to the latter's frequent and extensive use.

Commercially available trichlorfon and deltamethrin were employed in various dilutions against *Rhipicephalus (Boophilus) microplus* in the Adult immersion bioassay to check for resistance developed in response to the acaricides treatment. Trichlorfon and deltamethrin were tested in-vitro on engorged female ticks to check the number of oviposition inhibited, the reproductive index, LC50, LC95 as well as the mortality percentage. Same study was conducted by Gupta et al. (2016). The comparison of mortality rate for trichlorfon and deltamethrin in zone 1 and zone 2 were the lowest mortality against trichlorfon on two and tenfold dilutions i.e. (7-58 percent), while the highest mortality rate was (25-83 percent) against deltamethrin . Similarly in zone 2 on twofold and tenfold dilutions for trichlorfon were (7-57 percent) and for deltamethrin remains (25-75 percent) respectively. Lopez-Arias et al. (2014) concluded that extreme climatic variables, such as humidity, rainfall, and the amount of CO_2 in the surrounding media, contributed to the wide range of outcomes for calculating trichlorfon against *Rhipicephalus microplus*. *Rhipicephalus (Boophilus) microplus* adult female ticks had greater percentages of LC50 and LC95 based on the statistical analysis of the adult immersion test against trichlorfon. When comparing trichlorfon to deltamethrin, the greatest LC50 and LC95 values were 2.85 and 4.71 ppm and 3.14 and 5.28 ppm, respectively. There were considerable differences in results between the two ecological zones, which supported our hypothesis that each had its own unique set of environmental conditions. According to Sagar et al. 2020, similar results were found in Malaysia when researchers examined the acaricides activity of the cow tick *Rhipicephalus (Boophilus) microplus*. As per findings of previous studies, there is a dire need to monitor acaricides' resistance for strategic deployment of accessible acaricides and their long-term survival in the field.

5. Conclusion

The current study revealed that trichlorfon is the least effective agent against *Rhipicephalus microplus* and could provide edge in successful ticks' management program. Based on the results of the study, it has been investigated that a higher degree of resistance was found to trichlorfon than deltamethrin as a result of irregular usage in veterinary practices. In the light of these results it is highly recommended that resistance to trichlorfon should be closely monitored. In addition, it is also noteworthy to use such product in strictly prescribed ways.

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