African Journal of Biotechnology Vol. 9(7), pp. 1068-1073, 15 February, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

The effects of aqueous root extract of *Senna italica* subsp. *arachoides* on the feeding performance of *Hyalomma marginatum rufipes* adults

M. K. Thembo[1], S. R. Magano[2]* and L. J. Shai[3]

[1]Medical Research Council, Cape Town, South Africa.
[2]Department of Life and Consumer Sciences, University of South Africa, Florida Campus, Johannesburg, South Africa.
[3]Department of Biomedical Sciences, Tshwane University of Technology, Pretoria, South Africa.

Accepted 11 December, 2009

The effects of aqueous root extracts of *Senna italica* subsp*. arachoides* on the feeding performance of adults of *Hyalomma marginatum rufipes* in three consecutive infestations of rabbits were studied under laboratory conditions. Rabbits were divided into treatment group (n = 3), fed aqueous root extracts and rabbit pellets while the control group (n = 3) was fed with rabbit pellets and given distilled water only. Ticks that infested the treatment rabbits fed poorly, taking significantly longer feeding periods (P < 0.05) to acquire the bloodmeal, and having significantly reduced engorgement weights (P < 0.05) compared to those that fed on the control rabbits during the first and second infestations. These results suggest that aqueous root extracts of *S. italica* subsp. *arachoides* reduced the feeding performance of *H. marginatum rufipes* adults.

Key words: *Senna italica* subsp*. arachoides*, root extract, *Hyalomma marginatum rufipes* adults.

INTRODUCTION

It is well known that laboratory hosts such as guinea pigs and rabbits acquire some degree of anti-tick resistance when subjected to repeated tick infestations (Rechav et al., 1994). Ticks feeding on laboratory hosts that are resistant, typically spend extended periods on the hosts trying to complete the bloodmeal and have reduced weight of engorgement compared to those that feed on naïve hosts (Rechav and Dauth, 1987). Based on this understanding, many researchers believe that induction of host resistance to tick infestation is a promising tick control method that excludes the use of chemicals. Currently, chemicals such as organophosphates and formamides are largely relied upon as agents for tick control (Fletcher, 1984). Unfortunately, this approach to tick control is marred by numerous problems including environment pollution and the emergence of tick strains that are resistant to chemicals (Kunz and Kemp, 1994; Ware, 2000).

Despite the impressive amount of literature that exists on host resistance to tick infestation, knowledge on the effects of plant material ingested by animals on their resistance to tick infestation is still limited. Nonetheless, a few studies have been conducted in this regard. For instance, Sutherst et al. (1983) showed that resistance of cattle to *Rhipicephalus* (*Boophilus*) *microplus* increased when a high-grade supplement of lucern was given to these animals. Similar views were expressed by Rechav (1987) who reported that cattle in South Africa carried more ticks towards the end of winter, a period during which the nutritional value of grass is poor. Given the limited reports on the role of plants on host resistance to tick infestation, there may be more plants or plant products with the capacity to increase the resistance of hosts to tick infestation which have not yet been scientifically tested. Using an *in vitro* bioassay, Magano et al. (2008) recently demonstrated that *S. italica* subsp*. arachoides* has anti-tick properties. *Hyalomma marginatum rufipes* is a vector of *Theileria annulata* (Jongejan et al., 1983), a causative agent of theileriosis in cattle and Crimean-Congo Haemorrhagic fever virus to man (Swanepoel et al., 1983). Therefore, the effects of aqueous

*Corresponding author. E-mail: magansr@unisa.ac.za. Tel. +27 11 471-3649

Table 1. Mean (±SD) weight (kg) of rabbits and Mean (±SD) amount (ml) of distilled water and aqueous root mixture of *S. italica* subsp. *arachoides* ingested by rabbit s before and during the 1st, 2nd and 3rd infestations.

	Weight Before Infestation	1st Infestation		2nd Infestation		3rd Infestation	
		Weight	Volume	Weight	Volume	Weight	Volume
R1C	5.22 ± 0.14	5.43 ± 0.22	77.08 ± 11.90	5.51± 0.12	73.33 ± 14.08	5.49±0.04	82.68 ± 15.37
R2C	5.39 ± 0.11	5.57 ± 0.21	80.10 ± 12.51	5.51± 0.15	80.62 ± 15.52	5.40±0.02	79.83 ± 17.44
R3C	4.37 ± 0.39	4.97 ± 0.48	83.13 ± 11.10	Died	Died	Died	Died
R1T	5.32 ± 0.15	5.53 ± 0.18	68.12 ± 9.32	5.53 ± .14	62.18 ± 13.20	5.44±0.06	60.24 ± 12.20
R2T	4.91 ± 0.24	5.21 ± 0.21	71.01 ± 10.02	5.37± 0.08	59.22 ± 11.04	5.41±0.03	66.41± 10.61
R3T	4.87 ± 0.33	4.99 ± 0.13	64.03 ± 8.31	5.48 ± 0.73	60.31 ± 9.57	5.36±0.02	58.72 ± 13.90

R = rabbit, C = Control, T = Treatment.

root extracts of *Senna italica* subsp. *arachoides* ingested by rabbits on the feeding performance of *H. marginatum rufipes* adults was examined. *S. italica* subsp. *arachoides* commonly known as “Sebete” in Setswana, is widely used by the Botswana people of South Africa as a medicament for both animal and human conditions.

MATERIALS AND METHODS

Ticks

Unfed, 3 weeks old adult ticks of *H. marginatum rufipes*, from a pathogen free laboratory colony bred on rabbits were used in this study. The colony was established from ticks collected from Naauwpoort farm in Limpopo. Prior to and after infestation, these ticks were maintained in the laboratory at 25 ± 2°C and 75 ± 5% relative humidity (RH) in the Department of Biology at the University of Limpopo (Medunsa Campus). The relative humidity was attained by using saturated sodium chloride solution in glass humidity chambers as described by Winston and Bates (1960).

Preparation of plant extracts

S. italica subsp. *arachoides* was collected from Moruleng village of Rusternburg, North West Province in South Africa. The identification of the plant was confirmed by a plant taxonomist in the Department of Biology, University of Limpopo.

The dried roots of the plant were crushed into finer particles using an iron pestle and mortar. Extracts were prepared for each rabbit in the treatment group by adding 600 ml distilled water to 40 g of plant material. The crude mixture was left standing overnight prior to filtration using gauze with 1 x 1 mm perforations. To avoid stagnation, the crude mixture was freshly prepared on a daily basis and supplied to rabbits in the treatment group in the place of drinking water.

Hosts

Four months old Himalayan rabbits of both sexes bred in the Production Unit of the University of Limpopo (Medunsa Campus) were placed individually in the cages (56 X 56 X 38 cm) for the whole experimental period. These animals were kept at room temperature (21 - 25°C) and natural day/night regime. The cages had screened metal floors and stood on metal platforms to allow ventilation from underneath.

Experimental design

Six rabbits were divided into two groups of three rabbits each, the treatment and control groups. The number of rabbits used per group was determined after considering the numbers used in previous studies (Heller-Haupt et al., 1989; Rechav and Hay, 1992; Mulenga et al., 2000) and the guidelines of the Animal Ethics Committee of the Medunsa Campus of the University of Limpopo. The treatment group was fed rabbit pellets (Epol-Premier Food Industries, SA) and 600 ml of crude aqueous extracts of the roots of *S. italica* subsp. *arachoides* starting five days prior to the first infestation until the end of the experiment. The control group was treated the same except that 600 ml distilled water was supplied in the place of the aqueous plant mixture. The dosage of the aqueous root mixture of *S. italic* subsp. *arachoides* was determined by trial and error. The guiding principle here was to ensure that the treatment rabbits would ingest the aqueous root extracts of *S. italica* subsp. *arachoides* equally as those in the control group would ingest distilled water. In order to establish this, the amounts of distilled water and aqueous plant extract consumed by rabbits both in the treatment and control groups were, respectively, determined daily using a measuring cylinder (Table 1). Regular body weight measurements of all rabbits were taken in an attempt to establish the impact of the root extracts of *S. italica* subsp. *arachoides* on the treatment rabbits.

Infestation procedure

The procedure of Rechav et al. (1994) was followed for the infestation of ticks on rabbits. The two groups of rabbits were subjected to three consecutive infestations with adults of *H. marginatum rufipes*. In each of the three infestations, each rabbit in the treatment and control groups was infested with 10 males and 10 females of *H. marginatum rufipes*. Males of *H. marginatum rufipes* were included in each infestation because mating is necessary for the females to complete the bloodmeal. A two week tick-free interval between successive infestations was allowed after the collection of the last engorged female tick. This was done to allow the rabbits to heal from the tick inflicted wounds prior to the subsequent infestation.

Analysis of data

The number of female ticks that successfully fed to engorgement, their engorgement weights and feeding period were recorded for both treatment and control groups. Data were presented as mean ± standard deviation. Significant differences between ticks that fed on rabbits in the treatment group and control groups were determined

Table 2. Mean number of engorged female ticks of *H. marginatum rufipes* recovered from each rabbit after repeated infestations.

Infestation	Number of ticks infested	Control		Treatment	
		Mean number of ticks collected	Range	Mean number of ticks collected	Range
1st	10♂ (10♀)	9.0 ± 1.0	9 - 10	8.7 ± 1.04	8 - 10
2nd	10♂ (10♀)	10 ± 0	10	9.7 ± 1.58	9 - 10
3rd	10♂ (10♀)	9.0 ± 1.41	8 - 10	8.3 ± 1.53	7 - 10

♂ = Male ticks, ♀ = female ticks

Table 3. Mean engorgement weights and feeding periods of *H. marginatum rufipes* following repeated infestations on rabbits.

Infestation	Mean weight (g)			Mean feeding period (days)		
	Control	Treatment	P value	Control	Treatment	P value
1st	1.007 ± 0.256	0.865 ± 0.335	P < 0.05	15.32 ± 3.52	19.81 ± 7.62	P < 0.05
	n = 28	n = 26		n = 28	n = 26	
2nd	0.754 ± 0.214	0.654 ± 0.216	P < 0.05	13.8 ± 2.79	17.68 ± 6.13	P < 0.05
	n = 25	n = 32		n = 25	n = 32	
3rd	0.650 ± 0.230	0.674 ± 0.241	P > 0.05	16.2 ± 4.99	16.81 ± 6.61	P > 0.05
	n = 19	n = 26		n = 19	n = 26	
	P < 0.05	P<0.05...		P < 0.05	P < 0.05	

using the student's t-test.

RESULTS

Tick yield, feeding period and drop off rhythms

The mean number of ticks recovered from rabbits in the control group (9.0 ± 1.0) in the first infestation was higher than that of ticks recovered from rabbits in the treatment group (8.7 ± 1.04). The difference was not significant (Table 2). The mean feeding periods of female ticks that fed on rabbits in the treatment group during the 1st and 2nd infestations were significantly longer (19.8 ± 7.6 days, t = 1.4, p < 0.05 for first infestation and 17.68 ± 6.13 days, t = 1.24, p < 0.05 for second infestation) than those of female ticks fed on control rabbits (15.32 ± 3.52 days in 1st infestation and 13.8 ± 2.79 days in 2nd infestation) (Table 3). The highest mean number of ticks that dropped off from rabbits in the control group were recorded on days 12 (n = 10) and 15 (n = 5) during the 1st and 2nd infestations, respectively (Figures 1 and 2). During the 3rd infestation, the drop off rhythm was somewhat even with the highest mean number of 2 ticks dropping per day. For ticks that fed on treatment rabbits, the highest drop off was recorded on days, 12 (n = 4 in 1st infestation, n = 6 in 2nd infestation), 13 and 14 (n = 4 in 3rd infestation) (Figure 3).

Engorgement weights

Repeated infestation by *H. marginatum rufipes* on rabbits in both the control and treatment groups resulted in a significant decline (P < 0.05) in the mean engorgement weights of ticks (Table 3). The mean engorgement weights of ticks fed on the control and treatment rabbits decreased by 24.42 and 21.1% in the second infestation, respectively. No further decline in the mean engorgement weight of ticks was recorded during the third infestation. The mean engorgement weights of ticks that fed on rabbits in the control group in the first and second infestations were significantly higher (P < 0.05) than those of ticks that fed on rabbits in the treatment group during these infestations. However, no significant differences in engorgement weight were recorded between ticks that fed on rabbits in the control group and the treatment group during the third infestation.

DISCUSSION

Data obtained from this study suggested that aqueous root extracts of *S. italica* subsp. *arachoides* reduced the feeding performance of *H. marginatum rufipes* adults. Exactly how the root extracts of *S. italica* subsp. *arachoides* interfered with the feeding process of *H. marginatum rufipes* cannot be accounted for in this study. However, there are two possible ways through which such interference might have occurred. The root extracts of *S. italica* subsp. *arachoides* might have strengthened the host immunological response to tick infestation. The occurrence of an immunological basis in host resistance

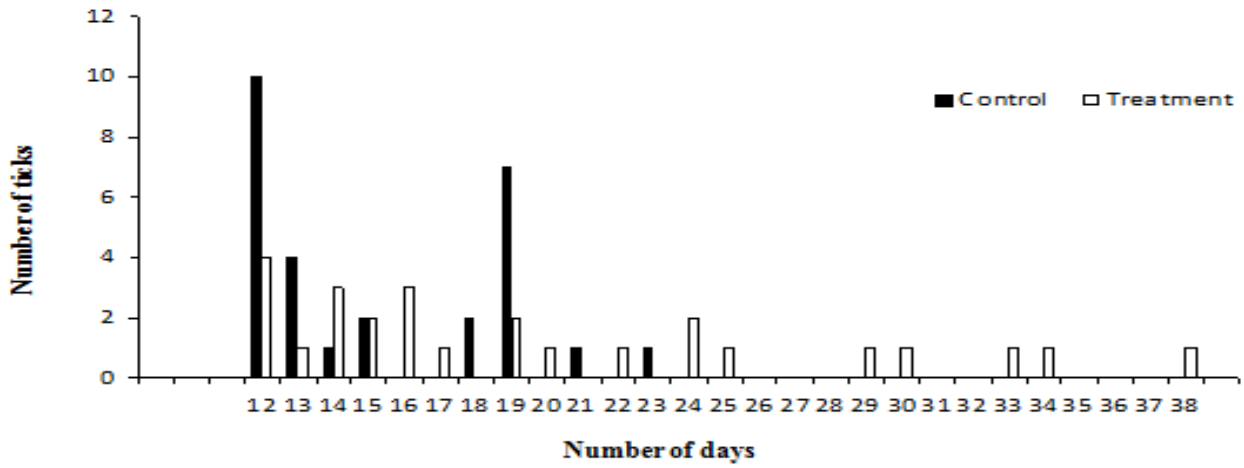

Figure 1. Drop off patterns of *H. marginatum rufipes* adult ticks that fed on rabbits during 1st infestation.

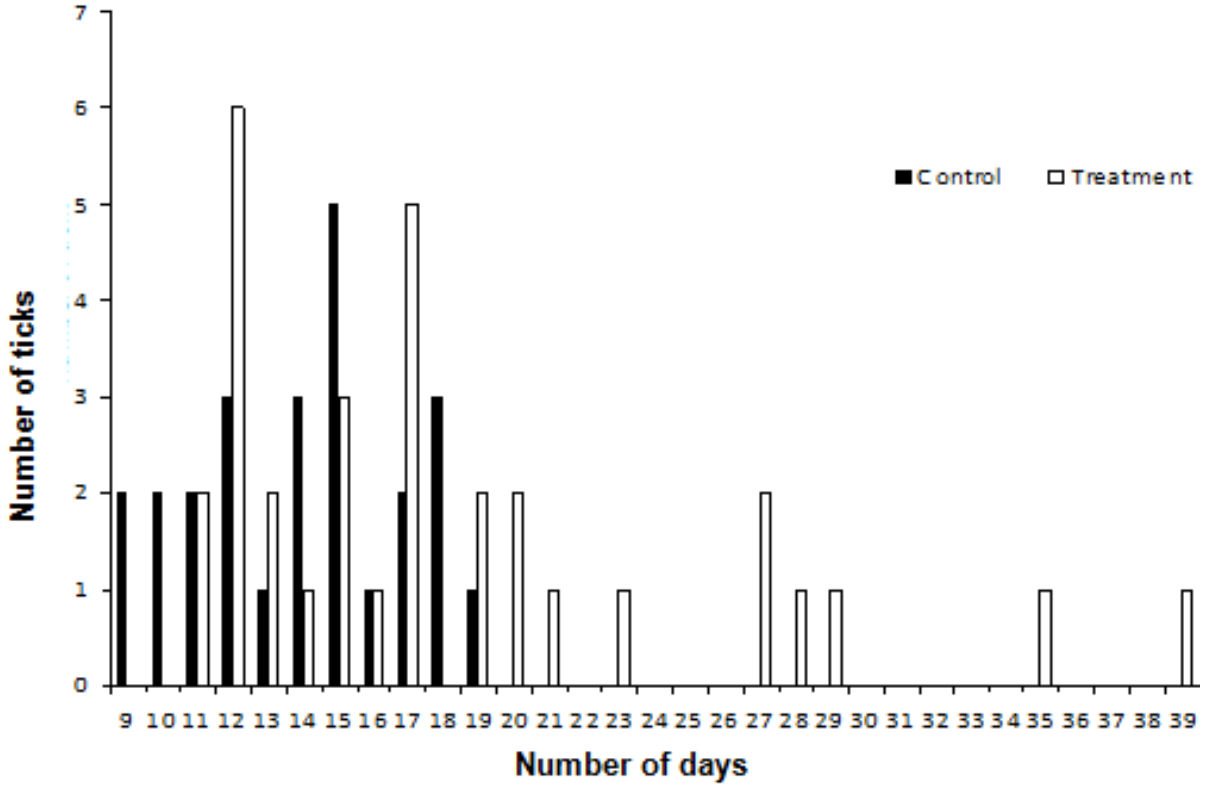

Figure 2. Drop off patterns of *H. marginatum rufipes* adult ticks that fed on rabbits during 2nd infestation.

to tick infestation is a well known phenomenon (Allen, 1973; Wikel and Allen, 1976; Wikel, 1999). Alternatively, some compound(s) in the root extracts of *S. italica* subsp. *arachoides* might have directly interfered with the tick feeding process once ingested with blood by the tick.

Magano et al. (2008) showed that the root extracts of *S. italica* subsp *arachoides* contain, 1, 2 –benzenedicarboxylic acid dibutyl ester, 1, 8 -dihydroxy- 3 -methylanthraquinone, 1 ,2 benzenedicarboxylic acid bis (2 -ethylhexyl) ester and hexadecanoid acid. Of these compounds, it is 1, 8 -dihydroxy- 3 -methylanthraquinone and hexadecanoid acid that are implicated to have anti-arthropod properties (Vencl and Morton, 1998). To determine whether these compounds interfere with the tick feeding process is a matter of further research. The safety of these compounds on rabbits should also be

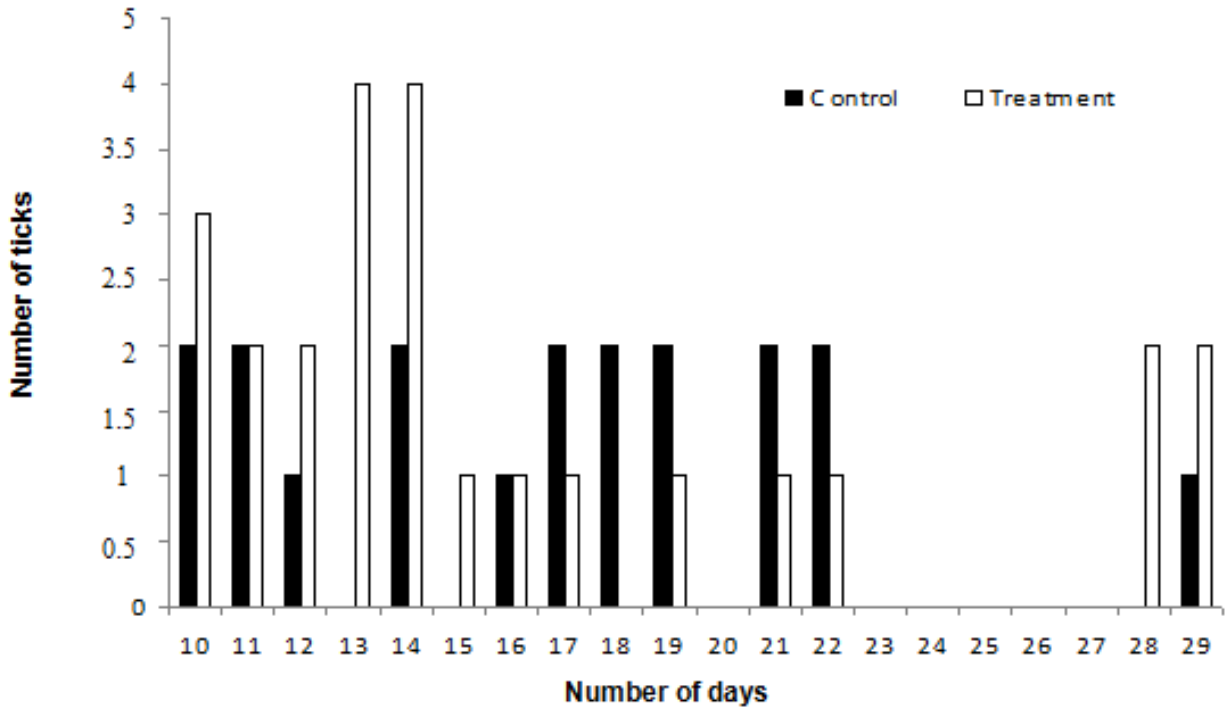

Figure 3. Drop off patterns of *H. marginatum rufipes* adult ticks that fed on rabbits during 3rd infestation.

studied. Body weight measurements taken in this study indicated that the root extracts of *S. italica* subsp. *arachoides* did not induce weight loss in rabbits at least for the duration of the study. The cause of death of rabbit RC3 cannot be accounted for in this study since this rabbit was fed normal rabbit pellets and distilled water only.

However, it is not clear why there was no further decline in the mean engorgement weight of females of *H. marginatum rufipes* during the third infestation of the treatment group. Similar anomalous observations in which the mean engorgement weight recovered following a decline were made by Fivas and Norval (1989) and Dipeolu et al. (1992).

In summary, our study demonstrated that the root extracts of *S. italica* subsp. *arachoides* reduced the feeding performance of *H. marginatum rufipes* adults. However, there is still much to be learned about the way the extracts of *S. italic* subsp. *arachoides* impact ticks and their safety on host animals. More importantly, future studies involving different host types need to be conducted in order to further clarify the effects of *S. italica* subsp. *arachoides* on the feeding performance of ticks.

ACKNOWLEDGEMENTS

We are indebted to Prof M. Pillay for the help in preparing the manuscript. The University of Limpopo is appreciated for allowing us space to conduct this study.

REFERENCES

Allen JR (1973). Tick resistance: basophils in skin reactions of resistant guinea-pigs. Int. J. Parasitol. 3: 195.

Dipeolu OO, Mongi AO, Essuman S, Amoo AO, Ndungu JN (1992). Studies on naturally acquired immunity to African ticks. II Observations on cattle exposed to *Rhipicephalus appendiculatus* under varying periods of repeated infestations. Vet. Parasitol. 41: 293-320.

Fivas BH, Norval A (1989). Observations on successive infestations of the rabbit host by the tick *Rhipicephalus appendiculatus* and *R. zambeziensis* (Acari: Ixodidae). Exp. Appl. Acarol. 1: 267-279.

Fletcher WA (1984). A guide to practical cattle tick control in southern Africa. Intervet (Pty) Ltd. Isando

Heller-Haupt A, Varma MGR, Trinder PKF, Rechav Y (1989). Nymphal ticks as an antigen source for immunization of animals against adults of African *Amblyomma* ticks. Med. Sci. Res. 17: 661-663.

Jongejan F, Morzaria SP, Mustafa OEH, Latif AA (1983). Infection rates of Theileria annulata in the salivary glands of the tick *Hyalomma marginatum rufipes*. Vet. Parasitol. 13: 121-126.

Kunz SE, Kemp DH (1994). Insecticides and acaricides: resistance and environmental impact. Rev. Sci. Technol. OIE 13: 1249-1286.

Magano SR, Thembo KM, Ndlovu SM, Makhubela NFH (2008). The anti-tick properties of the root extracts of *Senn italic* subsp. *arachoides*. Afr. J. Biotechnol. 7: 476-481.

Mulenga A, Sugimoto C, Ohashi K, Onuma M (2000). Characterization of an 84kDa protein inducing an immediate hypersensitivity reaction in rabbits sensitized to *Haemaphysalis longicornis* ticks. BBA-Mol. Dis. 1501: 219-226.

Rechav Y (1987). Resistance of Brahman and Hereford cattle to African ticks with reference to gamma globulin and blood composition. Exp. Appl. Acarol 3: 219-232.

Rechav Y, Dauth J (1987). Development of resistance in rabbits to immature stages of ixodid tick *Rhipicephalus appendiculatus*. Med. Vet. Entomol.11: 177-183.

Rechav Y, Hay L (1992). The effects of nutritional status of rabbits and sheep on their resistance to the ticks *Rhipicephalus evertsi evertsi* and *Rhipicephalus appendiculatus*. Exp. Appl. Acarol. 15: 171-179.

Rechav Y, Magano SR and Fielden LJ (1994). The effects of tick numbers and intervals between infestations on the resistance acquired by guinea-pigs to adults of *Rhipicephalus evertsi evertsi*: Exp. Appl. Acarol. 18: 735-745.
Sutherst RW, Kerr JD, Maywald GF, Stegeman DA (1983). The effect of season and nutrition on the resistance of cattle to the tick *Boophilus microplus*. Aust. J. Agric. Res. 34: 329-339.
Swanepoel R, Struthers JK, Shepherd AJ, McGillivray GM, Nel MJ, Jupp PG (1983). Crimean-Congo haemorrhagic fever in South Africa. Am. J. Trop. Med. Hyg. 32: 1407-1415
Vencl FV, Morton TC (1998). The shield defense of the sumac flea beetle, *Blepharida rhois* (Chrysomelidae: alticinae). Chemoecology, 8: 25-32.
Ware GW **(**2000). The Pesticide Book, 5th edn. Thomson Publications, California
Wikel SK (1999). Tick modulation of host immunity: an important factor in pathogen transmission. Int. J. Parasitol. 29: 851-859.
Wikel SK, Allen JR (1976). Acquired resistance to ticks. Effects of cyclophosphomide on resistance. Immunol. 30: 479-484.
Winston PW, Bates DH (1960). Saturated solutions for the control of humidity in biological research. Ecology 41: 232-236.