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PHYTOTHERAPEUTIC ACTIVITY OF *EUPHORBIA CYPARISSIAS* EXTRACTS ON *IXODIDAE (ACARI)* FEMALE TICKS

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Abstract

Background: Given its numerous biologically active components, *Euphorbiaceae* has been found to be a large plant family and polyvalent with quite interesting therapeutic activity that can be studied.
Materials and Methods: The ixodicidal activity of *Euphorbia cyparissias* extracts was studied *in vitro* and *in vivo*. Tested concentrations were 10, 5, 2, 1, 0.5 and 0.25%.
Results: For the *in vitro* study, conducted on field-collected female specimens of *Dermacentor marginatus* and *Haemaphysalis punctata*, the efficacy results showed that the ticks died after exposure in the case of 10, 5, and 2% tincture concentrations. The effects appeared after 30 minutes and became more visible 120 minutes after each exposure. The statistical differences regarding the used concentrations were found to be: $F = 6.51$, $df = 5$, $P < 0.001$. The *in vivo* study of the efficacy of *E. cyparissias* concentrations was performed on 35 naturally infested sheep and on 30 bovines parasitized with *Ixodes ricinus*, sprayed with tincture and glycerinate dilutions (bovines) on days 0 and 7. The results revealed detrimental effects on the survivability of female ticks, the most prominent being the reduction of their movement capacity. In sheep *in vivo* efficiency observed within 24 hrs varied, between 1 and 23% for *D. marginatus* and between 7 and 27% for *H. punctata* and respectively between 2 and 53% after 24 hrs, for *I. ricinus*, comparable effects being also found 72 hrs after the second administration of *Euphorbia* extracts.
Conclusion: Extracts from *E. cyparissias* may be used, with results, as an ecologic alternative tick control management method, being a cheap solution, with a sizeable role in reducing the use of synthetic and/or other harming and resistance source ixodicidal conditionings.

Key words: bio-control; *Euphorbia; Acari: Ixodidae*; ruminants.

Introduction

Biotherapy has become a current topical issue in medicine. As a result, researchers bring new information in respect to the use of spontaneous flora from their countries, as well as other means to enrich the anti-parasite arsenal. Pursuits linked to parasite bio-control have diversified as new therapeutic alternatives are being studied (e.g. fungus, entomogenous nematodes, vegetal extracts, volatile oils, etc.) (Borges *et al.*, 2011; Chagas de Souza *et al.*, 2012; Kaaya *et al.*, 1996; Kaaya *et al.*, 2000; Jongejan and Uilenberg, 1994; Samish and Rehacek, 1999; Zahir and Rahuman, 2012).

Euphorbia cyparissias (common name - *Cypress spurge*) is easy to find and identify in Europe and Africa with Asia's spontaneous flora being an herbaceous to semi-wooden perennial plant 15-30 cm tall. It has many branched stems that are covered with numerous narrow leaves. The inconspicuous yellowish-green flowers grow in groups on the extremity of the main stem. All parts of the plant exude a white, milky sap when broken. Numerous components from extracts and latex of *Euphorbia*, mostly diterpenes: phorbolester (ingenole) euphorbone, piceatanole, aesculetine, jolkinol, hyperoside, kaempferol, acylphorbol, acylingenol etc. (Ahmad and Jassbi, 1999; Evanics *et al.*, 2001; Ferriera *et al.*, 1993) were identified.

In our previous study, we identified, within the inflorescence component of the plant thirteen compounds of which sesquiterpenoids is the dominant one: elemene (19.83%), beta-cariophyllene (3.31%) and its epoxidation compound, cariophyllene-oxide (0.58%). In lower concentrations, there were identified mono-terpenoids and aromatic compounds. Similar compounds were identified in the plants' strain, but in higher concentrations: elemene (40.73%), cariophyllene (7.2%) and its epoxide (0.97%), as well as selinene and guainene. In the root extract of *Euphorbia*, seven compounds have been identified, the highest concentration being also in favour of sesquiterpenes: elemene (64.49%), cariophyllene (7.2%) and its epoxide (1.53%), selinene (5.93%) and guainene (3.96%). Monoterpenes were also identified (Cristina *et al.*, 2008). Based on the toxicity of the main *E. cyparissias* flavonoids, it has been assumed, as proven for other acaricidal structures, that the effect was produced after the nervous system had been affected, consecutively to the acarians cuticle passing (Gerolt, 1969; Khater, 2012; Welling, 1977).

The purpose of this research was to present the results obtained in two studies: one *in vitro* and one *in vivo*. In these experiments the ixodicidal and repellent activity of *E. cyparissias* tinctures and glycerinates were tested in different concentrations on naturally infested sheep and cattle, with a possible useful outcome. New data about the multiple therapeutic valences of this plant will complement the gathered knowledge about the ixodicidal activity of the compounds found in *Euphorbiaceae spp*.

Materials and methods

Tincture obtaining

Euphorbia cyparissias was collected from the Banat region, in Western Romania (the plant identification and authentication was made by comparing the collected specimens with a herbarium sample (voucher no. 41) from the collection of Vegetal Biology and Medicinal Plants Department from FVM Timisoara, Romania.

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Plant extracts were obtained according to the Romanian Pharmacopeia, Xth Ed. (1993) instructions at *Tincturae* or *Glicerolum* monograph. The whole plant was dried and sliced (1-1.5 cm) and was placed in a glass container for extraction in a 1:5 ratio (m/m) for 10 days in a 70° alcohol solution. The contents of the container were agitated 3-4 times/day and on the final day, the resulting extract was decanted and the remaining residue was pressed and both extracted liquids were homogenized and reunited and left to rest for an additional period of 6 days at 5-10 °C. Afterwards, the extract was filtered to obtain the mother tincture (20%).

The mother tincture, with initial concentration of 20%, was diluted gradually with 70° alcohol, obtaining the concentrations of 10, 5, 2, 1, 0.5 and respectively 0.25%. Similarly with the procedure presented for tinctures, glycerinates were obtained by diluting the 20% mother tincture with pure pharmaceutical glycerine (USP, purity 99.5%).

***In vitro* activity of tinctures**

Adult ticks were collected from naturally infested (heavily) free-grazing sheep. The identified species were *Dermacentor marginatus* and *Haemaphysalis punctata*. The species identification was done according to the adult tick morphological specific characteristics, respecting the usual definition keys used in our country (Feider, 1965; Babos, 1964; Estrada-Peña *et al.*, 2004). (figure 1).

Figure 1: Adult *Dermacentor marginatus* (left) and *Haemaphysalis punctata* (right) (original).

Figure 2: Adult *Ixodes ricinus* (original).

The following, 10, 5, 2, 1, 0.5, and 0.25% concentrations were tested respectively to the exposure time of 30, 60, 90 and 120 minutes. Tincture (4 ml) dampened filter sheets were put on Petri plates covering the whole surface, in order to uniformly humidify. The filter papers used were MN 640 de – ashless (Macherey-Nagel GmbH & Co. KG, Düren Germany), of 100 g/m^2 and 0.2 mm thickness with 1-2 µm retention capacity, used for very slow filtration and extremely fine precipitates.

Ticks were placed, 50 individuals / plate / concentration and also a Control group was formed, in the same conditions, but the filter sheet was dampened only with 70° alcohol. The tincture evaporation limit on the filter papers has been timed to be approximately 30 minutes.

Monitoring was accomplished with MOTIC SL-47 stereo-microscopic magnifier (Microscope Services Ltd.) (ob. 20 x 2.5), survivability being observed until tick exitus. The following parameters were taken into consideration: liveliness, movements of locomotory appendix, amplitude and frequency, the recovery from versostasys, aspect of the tarsal region, exitus, according to the key proposed in Table 1, the statistical evaluation moment was considered the stage before exitus (meaning: *versostasys* +).

Table 1: Tick survivability key used in the evaluation (50 ticks / plate / concentration)

Extensive movements		**Ortho or versostasys**	**Versostasys**	**Exitus**
Alert	Slow	Slow, difficult legs movement	Very slow legs movement	Total lack of movement
++++	+++	++	+	-

Legend:

++++ =very alert and extensive movements of ticks' majority.
+++ = the movements are diminished with 50% than the alert and extensive ones;
++ = the movements are diminished with 75% than the alert and extensive ones;
+ = the movements are only 5%, than the alert and extensive ones.

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***In vivo* activity of extracts**

a. Testing of tinctures on sheep

The study has been performed on 35 naturally infested sheep (five individuals / group, six treatment groups and one control). The identified species were *Dermacentor marginatus* and *Haemaphysalis punctata.* Treatment groups were sprayed with 50 to 100 ml of 10, 5, 2, 1, 0.5 and 0.25%, tincture dilution twice, on days 0 and 7. Control group was sprayed with 70° alcohol. Tincture was applied in each case on the sternal region, this region being considered the most intensely infested and easy to examine.

After 24 hours from the application, the adult ticks recovered from the sternal region were collected, counted and their behaviour was observed. The ticks were placed on separate Petri dishes for each concentration and the same procedure has been followed for the control group. For the identification of the long-lasting effect, a second application with the same quantity was performed after seven days from the first spraying, followed by the collection of the ticks, three days later.

b. Testing of glycerinated solutions on cattle

This study was accomplished in accordance to the WAAVP metodology, presented by Holdsworth *et al.* (2006). The activity of *Euphorbia* glycerinated solutions was tested through direct contact on 30 young Spotted Romanian calves, heavily parasited with *Ixodes ricinus* (figure 2), divided in five treatment groups (five individuals / group / concentration) and one control (sprayed only with 10% glycerine). After 24 hours from the applications, all ticks from the sternal region were collected, counted and their behaviour was observed in the laboratory using the same methodology as for sheep.

Statistics

All data was analyzed using *Graph Pad Prism* 5.0 (San Diego, USA). The data in different groups was compared by one way Anova with *Bonferroni* correction, to counteract the problem of multiple comparisons. Statistical differences were considered to be significant when $P < 0.05$, or lower.

Results

A. *In vitro* activity of tinctures

Table 2 presents the average evolution of *D. marginatus* and *H. punctata* females that survived the applications, representing the efficacy of the tincture concentrations in relationship to the exposure time. We have applied the tincture tests only on female ticks because we considered it to be sufficient, due to the fact that ixodides females are mostly responsible for the direct or indirect pathogenic activity. Males are feeding very little or not at all, and so, their pathogenic activity in this case is low or insignificant.

Table 2. Female ticks which survived to the contact with *Euphorbia tinctures* (average± D.S. for 5 tests/dosis)

Concentration %	Parasite's Species	Exposure (minutes)			
		30	60	90	120
10	*D. marginatus*	49(9.8±0,4)	47(9.4±0.8)	31(6.2±1.3)	6(1.2±0.8)
	H. punctata	50(10±0)	44(8.8±0.4)	27(5.4±1.1)	17(3.4±1.1)
5	*D. marginatus*	49(9.8±0,4)	46(9.2±0.4)	37(7.4±1.1)	14(2.8±0.8)
	H. punctata	50(10±0)	44(8.8±0.4)	31(6.2±1)	21(4.2±1.3)
2	*D. marginatus*	50(10±0)	49(9.8±0.4)	36(7.2±0.8)	24(4.8±1.6)
	H. punctata	50(10±0)	45(9.0±1)	32(6.4±2.1)	23(4.6±0.8)
1	*D. marginatus*	50(10±0)	49(9.8±0.4)	32(6.4±1.5)	24(4.8±1.7)
	H. punctata	50(10±0)	45(9.0±0.7)	33(6.6±1.1)	23(4.6±1.3)
0.50	*D. marginatus*	50(10±0)	50(10±0)	39(7.8±1.7)	26(5.2±1.9)
	H. punctata	50(10±0)	46(9.2±0.8)	35(7.0±1.2)	26(5.2±0.8)
0.25	*D. marginatus*	50(10±0)	50(10±0)	32(6.4±1.1)	30(6.0±0.7)
	H. punctata	50(10±0)	47(9.4±0.5)	36(7.2±0.8)	25(5.2±1.2)
Control	*D. marginatus*	50(10±0)	50(10±0)	50(10±0)	50(10±0)
	H. punctata	50(10±0)	50(10±0)	50(10±0)	50(10±0)

After 30 minutes from the contact with the tinctures, unrelated to the used concentrations, the majority of female ticks lost their liveliness and movement capacity and were not able to recover from versostasys.

After 60 minutes, the exposed ticks only moved when the applied concentrations were lower than 10%, but the great majority of the specimens only moved their legs slightly. The average number difference of surviving ticks, between 30 - 60 minutes, was only of 0.6 ($P < 0.1$).

After 90 minutes, the leg movements became very sparse, and the number of the ticks that showed any movement at all was much lower than before. Statistically, the average difference between the results at 30 and at 90 minutes was significantly higher: 3.28 ($P < 0.001$) and between 30 and 120 minutes it was of: 5.65 ($P < 0.001$). Between 60 and 90 minutes from the exposures, the average difference was also significant: 2.68 ($P < 0.001$), as well as the one noted between 60 and 120 minutes: 5.05 ($P < 0.001$).

We observed that the survival rate was lower after 90 and 120 minutes after exposure. Significant differences between the average numbers were found in the readings at 90 and at 120 minutes: 2.36 ($P < 0.001$). That is why we considered the examination up to 120 minutes to be sufficient. In restrained space, ticks died after aproximately three hours from the exposures, especially in the case of the 10, 5 and 2% concentrations. Females which had been fed were much more resiliant than the unfed ones.

Analysing data, it can be ascertained that *in vitro* situations there are no significant differences on efficiency, related to tick species. In every situation, the ixodicidal effect appeared after 30 minutes from the exposure and manifested itself through loss of movement capacity in most cases. After this time, the effect became more obvious and after 120 minutes from the exposures, very few ticks showed any leg movement. Statistically, it was found that, there are differences regarding the concentration used ($F = 6.51$, $df = 5$, $P < 0.001$).

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B. *In vivo* activity of extracts

a. Testing of tinctures on sheep

The identified tick species were *Dermacentor marginatus* and *Haemaphysalis punctata*, with the first species being predominant. Following the behaviour of female ticks, it has been noted that their survivability and their movement capacity were reduced. Decrease of liveliness, as well as the incapacitation of ticks recovering from versostasys, with cuticle aspect changes (colour and consistency), in the tarsal area, the main place the active substances are absorbed in ticks, were observed compared with the Control group, and/or to the female ticks that attached to the animals after our applications. After 24 hours, the efficiency of the tincture *in vivo* varied between 1% and 23% for *D. marginatus* and for *H. punctata* between 7% and 27% (Table 3).

Table 3: Ticks collected from sheep treated with *E. cyparissias* tincture after 24 hours from the first application

Concentration %	Species	Ticks	Alive	Dead	Efficiency%
10	*D. marginatus*	304	236	68	23
	H. punctata	11	8	3	27
5	*D. marginatus*	332	297	35	11
	H. punctata	31	24	7	23
2	*D. marginatus*	448	376	72	16
	H. punctata	14	12	2	14
1	*D. marginatus*	389	339	50	13
	H. punctata	12	10	2	16
0.5	*D. marginatus*	381	362	19	5
	H. punctata	9	8	1	11
0.25	*D. marginatus*	282	278	4	1
	H. punctata	14	13	1	7
Control	*D. marginatus*	478	478	0	0
	H. punctata	213	213	0	0

From the data presented, it can be seen that there are no significant differences in mortality related to the extract concentration, and difference resulting from the number of ticks which were found attached to the sternal region. The unfed or partially fed female individuals were found more sensitive than the engorged ones, confirming our previous observation also made *in vitro*. On the second spraying, done after seven days, on sheep initially treated with 10, 5 and 2%, respectively tincture concentrations, no fed ticks were found, demonstrating the repellent activity of the tincture. The number of attached ticks was relatively low, beginning from 0 up to 25 unfed ticks, meaning that they weren't attached on the animals for a long time. At lower concentrations, of 1, 0.5 and 0.25%, the presence of almost completely fed female ticks (23-30) was observed, denoting an older presence on their hosts (Table 4).

Table 4. Ticks collected from sheep treated with *E. cyparissias tincture* at 72 hours after the second application

Concentration %	Species	Ticks
10	*D. marginatus*	3
	H. punctata	0
5	*D. marginatus*	9
	H. punctata	1
2	*D. marginatus*	25
	H. punctata	4
1	*D. marginatus*	20
	H. punctata	3
0.5	*D. marginatus*	32
	H. punctata	2
0.25	*D. marginatus*	38
	H. punctata	3
Control	*D. marginatus*	176
	H. punctata	47

After the second tincture application, the ixodides number was reduced, but in some situations, not proportionally with the used concentration. It was also observed that *H. punctata* seemed to be more sensitive to all tincture concentrations used, in comparison to *D. marginatus.* An "error" was observed concerning the 2% tincture concentration, probably due to a faulty spraying of the tincture. At first sight, the slow therapeutic action of *Euphorbia* extracts may be considered a disadvantage. It is noteworthy that, *in vivo*, the tincture effect is not spectacular or of a "*knock-down*" type, its action being slow, death occurring at a later stage, generally after 48-72 hours from the initial application.

b. Testing of glycerinated solutions on cattle

In all situations the survivability of ticks was altered, as well as the reduction of movement capacity, liveliness, incapacitation in recovering from versostasys, modification of the cuticle and tarsal area aspect (colour is whiter and the consistency is softer), compared to the control group or to the attached ticks, after our applications. Tick mortality after 24 hours, varied between 2% and 53%, the difference from a case to another resulting by the number of ticks found attached to the sternal region (Table 5).

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Table 5: Ticks collected from cattle treated with *E. cyparissias* glycerinates after 24 hours from the first application.

Concentration %	Species	Ticks	Alive	Dead	Efficiency%
10	*I. ricinus*	410	194	216	53
5	*I. ricinus*	299	297	112	37
2	*I. ricinus*	398	376	69	17
1	*I. ricinus*	459	339	44	10
0.5	*I. ricinus*	327	362	21	6
0.25	*I. ricinus*	385	278	8	2
Control	*I. ricinus*	366	478	0	0

The ixodicidal effect of glycerinates was the most visible and easy to monitor. The action was slow, tick death occurring at a later stage, in most cases after 24-48 hours from the applications and being linked to the concentration in every case. Results suggest that glycerinated solutions have a better efficiency in comparison to tinctures, probably due, to a better adhesion and an easier parasite cuticle penetration. From the efficiency point of view, the recommendable concentrations in our opinion are: 5% and 10% glycerinated solutions. On the second spraying with glycerinates, performed after seven days, on cattle subjected to the treatment with 10, 5, 2 and 1% concentrations, no fed ticks were recovered, the number of ixodides being low and all of them unfed. At lower concentrations (0.5 and 0.25%), we identified fed female ticks. After the second application, when ticks were harvested three days after the treatment, the numbers of collected ticks were linked to the concentration (Table 6).

Table 6: Ticks collected from cattle treated with *E. cyparissias* glycerinates after 72 hours after the second application.

Concentration %	Species	Ticks
10	*I. ricinus*	0
5	*I. ricinus*	2
2	*I. ricinus*	7
1	*I. ricinus*	13
0.5	*I. ricinus*	28
0.25	*I. ricinus*	43
Control	*I. ricinus*	201

No local and/or general side effects have been observed on the animals after the exposures to the plant extracts used by us in this experiment.

Discussion

The study of vegetal extracts in parasitology is an attractive and promising field of research. At the moment, there is yet little information about the acaricidal activity of *E. cyparissias* extracts. For example, similar *in vitro* testing on ticks was done by Iori *et al.*, (2005), who experimented the effect of *Melaleuca alternifolia* oily extract at different doses (4, 6, 8 and 10 µl) and different exposure periods, much like our own research (at 30, 60, 90 and 120 minutes) on *Ixodes ricinus* nymphs. The study revealed that the lethal dose, for more than 70% of the ticks was 8 µl, the mortality raising over 80%, when the 10 µl doses was used. The effect was linked to the exposure time, becoming singnificant after 90 minutes of exposure in this case. The presented results are comparable to those obtained by our collection in this experiment.

In vivo, the contact time between ticks and tincture is always much shorter (being an alcoholic solution it quickly evaporates, making the contact shorter). That is why, when interpreting the results, it is compulsory to take into account the way in which the ixodicidal - acaricidal contact was performed (meaning; *in vivo* or *in vitro* conditions). This fact must be analyzed closely from the perspective of making the right choice of excipient basis, from the point of view of a greater remanence, especially for the tarsal-cuticle area of the parasites, the contact area with highest therapeutic significance in this case, which is also proven by us.

For asserting with certainty that the *E. cyparissias* tincture can be currently used for the control of tick populations, we consider that the studies need to be extended, but for now, it is certain that the tincture can be used as an alternative, ecological method, for ticks population control, avoiding or postponing the resistance phenomenon occuring with the use of tipical acaricides. Maybe using oily or glycerinated extracts, a better efficiency can be achieved, as argued by our study, turning the recorded ixodifugal effect, into a certainly ixodicidal one.

Also an unforseen aspect, but important for future studies, is the specific effect of the plant extracts on: food conversion specific capacity, reproductive index and larvae hatching.

In this context, for example, in a research *in vitro* performed with alcoholic extracts from *Melia azedarach* in 0.25% concentration, a 100% mortality of *Boophilus microplus* larvae was determined, while in the case of *in vivo* experiments, the results were modest (although a significant reduction of satiated females was observed after treatments). Calves artificially infested with *B. microplus* were sprayed with 3 litres of aqueous extract (0.25%) and alcoholic extract from the plant, noticing a significant reduction ($P < 0.05$) of the number of fed ticks after 21 days from the treatment application (Borges *et al.*, 2003).

Other studies made on Tswana, Brahman and Siemmenthal cattle, naturally infested with ticks, treated with *Azadirachta indica* seeds 5% watery extract, sprayed in doses of 5g /kg.bw^{-1} (a very high quantity) have led only to a lower ectoparasite number, in comparrison to the control group, but not to the total elimination of the population (Webb and David, 2002).

From the present study with *Euphorbia cyparissias* extracts on different ectoparasites, it has been certainly proven that its efficiency was directly linked to its concentration, time of contact and dose. The obtained results are more significant therapeutically-wise, in our opinion, especially for the soft tick species, where the observed efficiency of *E. cyparissias* extracts was higher, in comparisson to the results obtained for ixodides in this study (Cristina *et al.*, 2010).

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In our study, in all situations, data regarding the efficiency of tincture concentrations on ticks *in vivo*, under field conditions were clearly inferior to the ones obtained *in vitro*, where the survivability of the ticks was much smaller. The results obtained, as well as ones reported by other authors, suggest that the efficiency of the plant extracts was different, depending on the chosen plant, type of extract, concentration, time and type of exposure as well as on the evolution stage of the parasite. (Borges *et al.*, 2011; Chagas de Souza *et al.*, 2012; Ismail et al., 2002; Kaaya *et al.*, 1996; Madzimure *et al.*, 2011).

Conclusions

Tinctures from *E. cyparissias* may be used, with noticeable results, as an ecological alternative to tick control management, being a cheap solution, with a sizeable role in reducing the use of synthetic or other harmful and resistance generating source ixodicidal conditionings.

Euphorbia cyparissias tinctures have a significant acaricidal effect, even in lower concentrations (1%), but only *in vitro*, being dependent on the exposure time ($P < 0.001$) and the used doses ($P < 0.001$).

In the *in vivo* conditions there are no significant differences resulting from the use of *E. cyparissias* tinctures regarding the ixodicidal / ixodifugal effects, the observed effect being mainly repellent and only low or moderately ixodicidal. The glycerinated solutions compared to the tinctures have shown a stronger ixodicidal effect at a concentration of 5 and 10% and an ixodifugal effect at 2%.

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References

1. Ahmad, V.U., and Jassbi, A.R. (1999). New diterpenoids from *Euphorbia teheranica.* J. Nat. Prod. **62:** 1016-1018.
2. Babos, S. (1964). Die Zeckenfauna Mitteleuropas. Akademiai Kiado, Budapest.
3. Barci, L.A.G. (1997). Biological control of the cattle *Boophilus microplus* (*Acari: Ixodidae*) in Brazil. Arq Ins Biol Sao Paulo **64:** 95-101.
4. Borges, F., Miranda, L., Sousa, D., Barbosa, A.L., and Silva, C. (2011). Perspectivas para o uso de extratos de plantas para o controle do carrapato de bovinos *Rhipicephalus (Boophilus) microplus.* Rev Bras Par Vet, **20(2):** 89-96.
5. Borges, L.M.F., Ferri, P.H., Silva, W.J., Silva, C., and Silva, J.G. (2003). *In vitro* efficacy of extracts of *Melia azedarach* against the tick *Boophilus microplus*. Med Vet Entomol, **17:** 228-231.
6. Chagas de Souza, A.C., de Barros, L.D., Cotinguiba, F., Furlan, M., Giglioti, R., Oliveira de Sena, M.C., and Bizzo, H.R (2012). *In vitro* efficacy of plant extracts and synthesized substances on *Rhipicephalus (Boophilus) microplus* (*Acari: Ixodidae*). Par Res, **110(1):** 295-303.
7. Cristina, R.T., Cosoroabă, J., Trif, A., Pârvu, D., Hădărugă, N., Dumitrescu, E., Argherie, D., and Costescu, C. (2008). Investigation on *Cypress spurge* (*Euphorbia cyparissias L.*) and its activity in the veterinary therapeutics. International Conference of cellular and tissue comparative pathology, July 3-5, 2008, Cluj Napoca. *Bull* USAMV-CN, **65(1-2):** 358-363.
8. Cristina, R.T., Muselin, F., Dumitrescu, E., and Petrovici, S. (2010). *In vitro Euphorbia cyparissias L.* extract's activity against poultry argasides. Savremena poljoprivreda **59(1-2):** 132-137.
9. Estrada-Peña, A., Bouattour, A., Camicas, J.L., and Walker, A.R. (2004). Ticks of domestic animals in the Mediterranean region. A guide to identification of species. ICTTD.
10. Evanics, F., Hohmann, J., Redei, D., Vasas, A., Gunther, G., and Dombi, G. (2001). New diterpene polyesters isolated from Hungarian *Euphorbia* species. Acta Pharm Hung, **71(3):** 289-292
11. Feider, Z. (1965). Fauna R.P.R. *Arachnida*. Vol. 2, Ed. Acad. R.P.R., Bucuresti (in Romanian).
12. Ferriera, M., Lobo, J.U., and Wyler, A.M. (1993). Triterpenes of *Euphorbia mellifera.* Fitoterapia, **64:** 377.
13. Gerolt, P. (1969). Mode of entry of contact insecticides. J Insect Physiol, **15(4):** 563-580.
14. Holdsworth, P.A., Kemp, D., Green, P., Peter, R.J., De Bruin, C., Jonsson, N.N., Letonja, T., Rehbein, S., and Vercruysse, J. (2006). World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the efficacy of acaricides against ticks (*Ixodidae*) on ruminants. Vet Parasitol, **136(1):** 29-43.
15. Iori, A., Grazioli, D., Gentile, E., Marano, G., and Salvatore, G. (2005). Acaricidal properties of essential oil of *Meleuca alternifolia Cheel* (tea tree oil) against nymphs of *Ixodes ricinus.* Vet Parasitol **129:** 173-176.
16. Ismail, M.H., Ketavan, C., and Solomon, G. (2002). Toxic effect of Etiopian neem oil on larvae of cattle tick, *Rhipicephalus pulchellus*. Kasetsart J - Nat Sci **36(1):** 18-22.
17. Jongejan, F., and Uilenberg, G. (1994). Ticks and control methods. Rev Sci tech Off Int Epiz **13(4):** 1201-1226.
18. Kaaya, G.P., Samish, M., and Itamar, G. (2000). Laboratory evaluation of pathogenicity of entomogenous nematodes to African tick species. Ann. N.Y. Acad. Sci. **916:** 303-308.
19. Kaaya, G.P., Mwangi, E.N., and Ouna, E. (1996). Prospects for biological control of livestock ticks, *Rhipicephalus appendiculatus* and *Amblyomma variegatum* with the entomogenous fungi, *Beauveria bassiana* and *Metarhizium anisopliae*. J Invertebr Pathol **67:** 15-20.
20. Khater, H.F. (2012). Ecosmart Biorational Insecticides: Alternative Insect Control Strategies, Insecticides - Advances in Integrated Pest Management, Dr. Farzana Perveen (Ed.), ISBN: 978-953-307-780-2, InTech, http://cdn.intechopen.com/pdfs/25668/InTech-Ecosmart_biorational_insecticides_alternative_insect_control_strategies.pdf
21. Madzimure, J., Nyahangare, E.T., Hamudikuwan, D.H., Hove, T., Stevenson, P.C., Belmain, S.R., and Mvumi, B.M. (2011). Acaricidal efficacy against cattle ticks and acute oral toxicity of *Lippia javanica (Burm F.)* Spreng. Trop An Health Pro, **43:** 481-489.
22. Samish, M., and Rehacek, J. (1999). Pathogens and predators of ticks and their potential in biological control. Ann Rev Entomol, **44:** 159-182.
23. Webb, E.C., and David, M. (2002). The efficacy of neem seed extract *(Azadirachta indica)* to control tick infestation in Tswana, Simmentaler and Brahman cattle. S Afr J Anim Sci **32(1):** 2002: 1-6.
24. Welling, W. (1977). Dynamic Aspects of Insect-Insecticide Interactions. Ann Rev Entomol, **22:** 53-78.
25. Zahir, A.A., and Rahuman, A.A. (2012). Evaluation of different extracts and synthesised silver nanoparticles from leaves of *Euphorbia prostrata* against *Haemaphysalis bispinosa* and *Hippobosca maculata*. Vet Parasitol **187(3-4):** 511-520.
26. *** Farmacopeea Romana (1993). Editia a X-a, Editura Medicala, Bucuresti (in Romanian).