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In vitro effect of extract of garlic's wood (*Gallesia integrifolia* Sprengel) Harms on engorged ticks and larvae of *Ripicephalus Boophilus microplus*

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Abstract. The cattle tick *Rhipicephalus Boophilus microplus* is responsible for high losses in cattle stocking in tropical area, the losses related to parasitism of this agent represents up to 80% of production losses, represented by spoliation, damage to the leather, hematozoa transmission, among others. The problem of increased of resistance to chemical ectoparasiticids has led to the search for new alternatives, among them there is the herbal medicine, in that it is more accessible, less polluting, suspected delay the resistance phenomenon. In this study, it sought to evaluate the efficacy of alcoholic extract of *Gallesia integrifolia* (garlic's wood) on tick control *R. B. microplus*. The test was carried out on engorged ticks and on larvae in impregnated papers *in vitro*. It was found that the hydroalcoholic extract tested at concentrations of 100, 50, 25, 10, 5, 2.5 and 1% was effective on mortality of adult ticks on 100% concentration from about 5% and on larvae, in 10% concentrations. And at 2.5%, the action on the mortality of larvae was considered satisfactory by the official regulatory criteria. These *in vitro* results are presented favorable regarding the candidacy of *G. integrifolia* extract for control of *R. B. microplus* in cattle.
Keywords: Herbal; Tick; Cattle; Efficacy test; *Gallesia integrifolia*; *Ripicephalus Boophilus microplus.*

Introduction

The use of herbal medicine for the control of parasitic diseases in animals has gained prominence due to the increase of the resistance phenomenon. The almost exclusive use of some chemical bases has contributed essentially to alternative search for the control of parasites (Morel et al., 2017), as *Rhipicephalus Boophilus microplus*, which has predatory, mechanical and toxic action on animals, especially in cattle. In addition, this tick is capable to transmit babesias and rickettsias (hematozoa) for cattle (Yessinou et al., 2018; Banumathi et al., 2017). A major problem of using synthetic acaricide in controlling these agents is the resistance phenomenon to these substances, which is described worldwide (Fouche et al., 2017; Khangembam et al., 2018; Webster et al., 2018). Parasitism by ticks are considered the main problem in cattle in the South America (Morel et al., 2017) causing very high losses in Brazil, around more than two billion dollars a year, according to Souza et al. (2017). It is believed that the use of herbal medicines can promote a slower development of resistance as well as being an important alternative in reducing economic and ecological impacts, since herbal medicines do not have the same impact on the environment than synthetic pesticides (Janadaree Bandara and Parakrama Karunaratne, 2017; Csordas et al. 2018; Webster et al., 2018). Considering also the issue related to residues in meat and milk and failure to comply with the range of drug grace period it appears that the use of herbal medicines is presented as a conditioning factor to the sustainability of animal production (Kumar et al., 2016; Fouche et al., 2017; Sharifi-Rad et al., 2017, Rosado-Aguilar et al 2017).

Gallesia integrifolia (Sprengel), known as garlic's wood, by exhaling smell like such, belongs to Phytolaccaceae family, is common in southeastern Brazil and is native of Brazil (Akisue et al., 1996, Arunachalam et al., 2017). Raimundo et al. (2017) reported the presence of *G. integrifolia* in Peru and Bolivia. Popularly, this plant is used to treat worm infections and bacterial infections in humans (Akisue et al., 1996, Arunachalam et al., 2016, Bacarin et al., 2016). The same authors above reported the presence of tannins and alkaloids and coumarins when performing extraction in this plant. Maia et al. (2013) report that essential oils with antimicrobial action can be extracted in high proportion in extracts of *G. integrifolia*, indicating that this plant has the potential to be explored. There are no reports in the

literature regarding the use of this plant in the control of mites, as ticks. The objective of this study was to test the effectiveness of hydro-alcoholic extract of *G. integrifolia* in tick control *R. B. microplus* in vitro.

Methods

This experimental test consisted at verify the efficacy of the hydroalcoholic extract of leaves of *Gallesia integrifolia* on engorged ticks of *Rhipicephalus Boophilus microplus* and on hatchability of the same tick larvae.

The trial was conducted at the Parasitology Laboratory of the Faculty Castelo Castle, Espírito Santo state. The engorged ticks were collected in a dairy production property in rural municipality of Castelo, Espírito Santo state, in which the animals had been treated against ticks for over 65 days with amitraz. Leaf samples were taken in small amount of some trees, they have been duly identified by botanist in the Federal University of Viçosa, Department of Plant Science, it was classified as *G. integrifolia*. The trees were located in a rural property, located in rural area in Castle, 20º42'61.40''S, longitude 41º13'4.45''W latitude, which has typical rainforest vegetation of Mata Atlântica e and climate classified Aw, tropical climate with a typical dry station according to the Koppen-Geiger classification, climate typical habitat of this plant. The design of the research was conducted in a way that was tested in the control group: a group treated with water, treated with hydroalcoholic solution at 70% and another was tested with a commercial formulation of cypermethrin (5g/100ml), chlorpyrifos (2.5g/100ml) and piperonyl butoxide (1.0g/100ml) at 20% concentration in water.

In the treated group, initially made a macerated of leaves garlic's wood and had remained the same for three days in amber glass at 70% alcohol solution in 20% of mass garlic's wood, so after that, it was made by dilution of this solution at 100, 50, 20, 10, 5, 2.5 and 1%.

The ticks immersion tests was performed after the selection of those engorged ticks, adaptated of Chagas et al. (2016). Assays were performed in triplicate with 10 ticks selected by plaque.

According to these calculations preconized of estimated reproduction (ER):

$$ER = \frac{\text{eggs weight x \% hatching x 20.000*}}{\text{weight of females}}$$

* Constant that indicates the number of eggs present in 1g of laying

And

Product efficacy: PE

$$PE = \frac{\text{ER (control) – ER (treatrd)}}{\text{ER (control)}} \times 100$$

It is recommended that the insecticide to be marketed and licensed must have minimum efficiency of 95% *in vitro* test (Chagas et al., 2016).

The larvae tests were conducted on paper impregnated BOD (27°C e RH> 80%) to induce the production of eggs and larvae. Larvae from 14 to 21 days were placed in impregnated papers, that were previously immersed in the solutions: the control group; a group treated with water, a group treated with hydroalcoholic solution at 70% and another was tested with a commercial formulation of cypermetrin (5g/100ml), chlorpyrifos (2.5g/100ml) and piperonyl butoxide (1g/100ml) at 20% concentration in water. For the treated group; in water-alcohol solution of extract of garlic's wood in dilutions 100, 50, 20, 10, 5, 2.5 and 1%, in three replications. Described as:

Mortality (Mort.):

$$Mort. = \frac{\text{\% mort. control - \% mort. treated x 100\%}}{\text{mort. control}}$$

$$Mort.mean\ (\%) = \frac{\text{mort. of repetition 1 + mort. of repetition 2 + mort. of repetition 3}}{3}$$

Results and discussion

It was found that the hydroalcoholic extract of *Gallesia integrifolia* (Sprengel) showed mortality of ticks *Rhipicephalus Boophilus microplus* at concentration from 5% (of 100%, 20% higher than the commercial formulation tested), Table 1.

These results are significant when comparing with the of Leonor *et al.* (2018), in which tested the efficacy of extract of *Ocotea elegans* on engorged ticks of *R. B. microplus* and it was obtained efficacy above 70% after 48 hours in the extract concentration of 10%, while the hydroalcoholic extract of *G. integrifolia* at 5% was able to kill 100% of the ticks after 48 hours. It is observed that *O. elegans*, showed meritorious results of efficacy *in vitro* tests. Extract of *Syzygium aromaticum* showed an average efficiency above 70% on ticks and *R. B. microplus* larvae in vitro, in hydroalcoholic (Ferreira et al., 2018). Extract and fractions of *Digitaria insularis* showed regular results of efficacy in larvae of *R. B. microplus* (Santos et al., 2018). It is verified, in several studies, many variations of the effectiveness of plant extracts on the cattle tick (Souza et al., 2017; Csordas et al. 2018). Vinterelle et al. (2017) verified 100% mortality of engorged females at the highest concentration (10%). Many other plant extracts were tested on larvae and ticks *R.B. microplus* and found efficacy that varied considerably and the techniques used to evaluate the effectiveness of these extracts are evaluated by standard techniques (Chagas et al., 2016).

G. integrifolia Showed high efficacy and acceptable and concentration of the extract to 2.5%,

greater than 95% (Table 2). At 5% extract concentration, the efficacy observed was 100%. This plant showed efficacy at low concentrations and it was presented as promising in control of *R. B. microplus*. It appears that several reports about resistance to acaricid products have increased alarmingly, when it comes to use of synthetic chemical formulations (Canevari et al., 2017, Singh et al., 2018). It is noted that even super dosages have not combated this parasite of cattle tick at field, and often, it is verified the use of these drugs in the treatment of animals in intervals each once shorter time and without the expected success (Singh et al., 2016; Rosado-Aguilar et al., 2017).

Table 1. Egg weight mean in grams after 15 days, weight mean of ticks after 15 days and mortality (mean ± standard deviation) of engorged stick (%) by treatments with extracts garlic's wood (*Gallesia integrifolia* Sprengel) at concentrations of 100, 50, 20, 10, 5 2.5% and 1 and; association among chlorpyrifos and cypermethrin (concentration 20% in water), control groups with water and 70% alcohol

Repetition	Weight of Egg	Weight of ticks	Mortality (%)
Water	0,9911	2,2456	0,00 ± 0
Alcohol 70%	0,7925	2,2645	0,00 ± 0
Cypermetrin(0.5%)/clorpyiriphos (0.25%)	0,0271	2,2346	80,00 ± 4,50
Extract 1%	0,5796	2,2433	20,00 ± 1,20
Extract 2.5%	0,3247	2,5878	70,00 ± 3,90
Extract 5%	0,2687	2,2614	100,00 ± 0,00
Extract 10%	0,2040	2,2491	100,00 ± 0,00
Extract 25%	0,1499	2,2562	100,00 ± 0,00
Extract 50%	0,0112	2,2588	100,00 ± 0,00
Extract 100%	0,0101	2,2499	100,00 ± 0,00

Table 2. Estimated reproduction (ER) and product efficiency (PE)% by treatments with extracts garlic's wood (*Gallesia integrifolia* Sprengel) at concentrations of 100, 50, 20, 10, 5 2.5% and 1 and; association among chlorpyrifos and cypermethrin (concentration 20% in water), control groups with water and 70% alcohol.

Repetition	ER	PE (%)
Water	680,184.37	-
Alcohol 70%	62,280.57	66.24
Cypermetrin (0.5%)/clorpyriphos (0.25%)	8,327.88	95.90
Extract 1%	31,215.51	86.80
Extract 2.5%	8,0178.47	95.87
Extract 5%	3,253.43	97.25
Extract 10%	850.60	99.58
Extract 25%	551.21	100.00
Extract 50%	354.74	100.00
Extract 100%	245.54	100.00

Akisue et al. (1996) reports that this plant has high concentrations in your sheet of volatile substances of tannins group and alkaloids and coumarins. The phytochemical examination of this plant extract should be performed in order to identify potential acaricid molecules.

When observing the alcoholic extract effect of *G. integrifolia* in the impregnated papers tests (Table 3), it is showed that the extract presented influence on the hatching of larvae at all concentrations employed. It was found that the extract at concentrations of 2.5%, was able to inactivate, under volatile action, over 90% of the larvae. Extract concentrations up to 5% and up of this were able to inactivate 100% of the larvae. The larval stage is a life stage of the tick that initially has free life and that is why, most of these do not suffer the direct effect of acaricides used the field.

Table 3. Larval mortality (mean ± standard deviation) caused by the impregnation after 24 hours by treatments with extracts garlic's wood (*Gallesia integrifolia* Sprengel) at concentrations of 100, 50, 20, 10, 5 2.5% and 1 and; association among chlorpyrifos and cypermethrin (concentration 20% in water), control groups with water and 70% alcohol.

Repetition	Mortality (%)
Water	0.00 ± 0
Alcohol 70%	4.20 ± 0.12
Cypermetrin (0.5%)/clorpyriphos (0.25%)	96.71 ± 2.23
Extract 1%	39.55 ± 3.26
Extract 2.5%	90.16 ± 0.29
Extract 5%	96.33 ± 0.29
Extract 10%	100.00 ± 0
Extract 25%	100.00 ± 0
Extract 50%	100.00 ± 0
Extract 100%	100.00 ± 0

Conclusion

It was found that *Gallesia integrifolia* presented efficacy of 100% on adults and larvae of *Rhipicephalus Boophilus microplus* in hydroalcoholic extract, even in concentrations at 5%. The extract of *G. integrifolia* showed inhibitory effect on hatchability of larvae and survival of this. These results are promising and allows that investigations to be carried out for phytochemical identification of molecules present in the extract and to be possible the application in the field of phytotherapic.

References

ARUNACHALAM, K., ASCÊNCIO, S.D., RAIMUNDO, I.M.S., AGUIAR, R.W.S., SILVA, L.I., OLIVEIRA, R.G., BALOGUNA, S.O., MARTINS, D.T.O.,. *Gallesia integrifolia* (Spreng.) Harms: In vitro and in vivoantibacterial activities and mode of action. Journal of Ethnopharmacolology 184:128-137, 2016.

ARUNACHALAM, K., BALOGUN, S.O., PAVAN, E., ALMEIDA, G.V.B., OLIVEIRA R.G., WAGNER T., CECHINEL FILHO, V., MARTINS, D.T.O. Chemical characterization, toxicology and mechanism of gastric antiulcer action of essential oil from *Gallesia integrifolia* (Spreng.) Harms in the *in vitro* and *in vivo* experimental models. Biomedicine & Pharmacotherapy 94:292-306, 2017.

AKISUE, M.K., AKISUE, G., OLIVEIRA, F. Caracterização farmacognóstica de pau d'alho *Gallesia integrifolia* (Spreng.) Hams. Revista Brasileira de Farmacognosia 1:166-182, 1986.

BACARIN, M.A., MARTINAZZO, E.G., CASSOL, D., FALQUETO, A.R., SILVA, D.M. Daytime variations of chlorophyll a fluorescence in pau d'alho seedlings. Revista Árvore, 40:1023-1030, 2016.

BANUMATHI, B., VASEEHARAN, B., RAJASEKAR, P., PRABHU, N.M., RAMASAMY, P., MURUGAN, K., CANALE, A., BENELLI, G. Exploitation of chemical, herbal and nanoformulated acaricides to control the cattle tick, *Rhipicephalus (Boophilus) microplus* – A review. Veterinary Parasitology 244:102-110, 2017.

CANEVARI, J.T., MANGOLD, A.J., GUGLIELMONE, A.A., NAVA, S. Population dynamics of the cattle tick *Rhipicephalus (Boophilus) microplus* in a subtropical subhumid region of Argentina for use in the design of control strategies. Medical and Veterinary Entomology 31:6-14, 2017.

CHAGAS, A.C.S., OLIVEIRA, M.C.S., GIGLIOTI, R., SANTANA, R.C.M., BIZZO, H.R., GAMA, P.E., CHAVES, F.C.M. Efficacy of 11 Brazilian essential oils on lethality of the cattle tick *Rhipicephalus (Boophilus) microplus*. Ticks and Tick-borne Disease. 7:427-432, 2016..

CSORDAS, B.G., CUNHA, R.C., GARCIA, M.V., SILVA, S.S., LEITE, F.L., ANDREOTTI, R. Molecular characterization of the recombinant protein RmLTIBmCG-LTB: Protective immunity against *Rhipicephalus (Boophilus) microplus*. Plos One 13:e0191596, 2018.

FERREIRA, F.M., DELMONTE, C.C., NOVATO, T.L.P., MONTEIRO, C.M.O., DAEMON, E., VILELA, F.M.P., AMARAL, MPH. Acaricidal activity of essential oil of *Syzygium aromaticum*, hydrolate and eugenol formulated or free on larvae and engorged females of *Rhipicephalus microplus*. Medical and Veterinary Entomology 32:41-47, 2018.

FOUCHE, G., SAKONG, B.M., ADENUBI, O.T., DZOYEM, J.P., NAIDOO, V., LEBOHO, T., WELLINGTON, K.W., ELOFF, J.N. Investigation of the acaricidal activity of the acetone and ethanol extracts of 12 South African plants against the adult ticks of *Rhipicephalus turanicus*. Microsc. Res. and tech. Onderstepoort Journal of Veterinary Research 84: e1-e6, 2017.

JANADAREE BANDARA, K.M.U., PARAKRAMA KARUNARATNE, S.H.P. 2017. Mechanisms of acaricide resistance in the cattle tick *Rhipicephalus (Boophilus) microplus* in Sri Lanka. Pesticid and Biochemistry Physiology 139:68-72, 2017

KHANGEMBAM, R., SINGH H., SHITANSHU, J., RATH, S., SINGH, N.K. Effect of synergists on ivermectin resistance in field populations of *Rhipicephalus (Boophilus) microplus* from Punjab districts, India. Ticks and Tick-borne Disease 9:682-686, 2018.

KUMAR, K.G.A., SHARMA, A.K., KUMAR, S., RAY, D.D., RAWAT, K.S., SRIVASTAVA, S., GHOSH, S. Comparative in vitro anti-tick efficacy of commercially available products and newly developed phyto-formulations against field collected and resistant tick lines of *Rhipicephalus (Boophilus) microplus*. Journal of Parasitology Diseases 40:1590-1596, 2016.

LEONOR, A.F., NASCIMENTO, M., LOPES, L.G., GIGLIOTIA, R., ALBUQUERQUE, R.D.D.G., SANTOS, M.G., FALCÃO, D.Q., NOGUEIRA, J.A.P., ROCHA, L., CHAGAS, A.C.S. First report of the effect of *Ocotea elegans* essential oil on *Rhipicephalus (Boophilus) microplus*. Veterinary Parasitology 252, 131-136, 2018.

MAIA, A.J., SCHWAN-ESTRADA, K.R.F., BOTELHO, R.V., JARDINETTI, V.A., FARIA, C.M.D.R., BATISTA, A.F., COSTA, W.F. Bud break and enzymatic activity in buds of grapevines cv. Ives treated with *Gallesia integrifolia* hydrolate. Acta Physiology Plantae 35:2727–2735, 2013.

MOREL, N., SIGNORINI, M.L., MANGOLD, A.J., GUGLIELMONE, A.A., NAVA, S. Strategic control of *Rhipicephalus (Boophilus) microplus* infestation on beef cattle grazed in *Panicum maximum* grasses in a subtropical semi-arid region of Argentina. Preventive Veterinary Medicine 144:179-183, 2017.

RAIMUNDO, K.F., BORTOLUCCI, W.C., SILVA, E.S., PEREIRA, A.F.B., SAKAI, O.A., PIAU, R., GONCALVES, J.E., LINDE, G.A., GAZIM, Z.C. Chemical composition of garlic wood ('*Gallesia integrifolia*') (Phytolaccaceae) volatile compounds and their activity on cattle tick. Australian Journal of Crop Sciece 11:1058-1067, 2017.

ROSADO-AGUILAR, J.A., RODRÍGUEZ-VIVAS, R.I., BORGES-ARGAEZ, R., ARJONA-CAMBRANES, K.A. Acaricidal activity of *Havardia albicans* and *Caesalpinia gaumeri* methanolic leaf extracts on *Rhipicephalus microplus* and its toxicity to laboratory animals. Experimental Applied Acarolology 71:345–354, 2017.

SANTOS, F.O., LIMA, H.G., ROSA, S.S.S., MERCÊS, N.B., SERRA, T.M., UZEDA, R.S., REIS, I.M.A., BOTURA, M.B., BRANCO, A., BATATINHA, M.J.M. In vitro acaricide and anticholinesterase activities of *Digitaria insularis* (*Poaceae*) against *Rhipicephalus (Boophilus) microplus*. Veterinary Parasitology 255:102-106, 2018.

Sharifi-Rad, J., Sureda, A., Tenore, G.C., Daglia, M., Sharifi-Rad, M., Valussi, M., Tundis, R., Sharifi-Rad, M., Loizzo, M.R., Ademiluyi, A.O., Sharifi-Rad, R., Ayatollahi, S.A., Iriti, M. Biological Activities of Essential Oils: From Plant Chemoecology to Traditional Healing Systems. Molecules 22:1-55, 2017.

in ticks *Rhipicephalus microplus* in Benin, West Africa. Tick and Tick-borne Disease 9:665-671, 2018.

SINGH, N.K., GOOLSBY, J.A., SHAPIRO-ILAN, D.I., MILLER, R.J., SETAMOU, M., PEREZ DE LEON, A.A. Effect of Immersion Time on Efficacy of Entomopathogenic Nematodes against Engorged Females of Cattle Fever Tick, *Rhipicephalus (=Boophilus) microplus*. Southwest. Entomology 43:19-28, 2018.

SOUZA, J.R.L., REMEDIO, R.N., ARNOSTI, A., ABREU, R.M.M., CAMARGO-MATHIAS, M.I. The effects of neem oil (*Azadirachta indica* A. JUSS) enriched with different concentrations of azadirachtin on the integument of semi-engorged *Rhipicephalus sanguineus* sensu lato (Acari: Ixodidae) females. Microscopy Research Technology 80:838–844, 2017.

VINTURELLE, R., MATTOS, C., MELONI, J., NOGUEIRA, J., NUNES, M.J., VAZ JR., I.S., ROCHA, L., LIONE, V., CASTRO, H.C., CHAGAS, E.F. In Vitro Evaluation of Essential Oils Derived from *Piper nigrum* (Piperaceae) and *Citrus limonum* (Rutaceae) against the Tick *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae). Biochemistry Research International ID 5342947:1-9, 2017.

WEBSTER, A., SOUZA, U.A., MARTINS, J.R., KLAFKE, G., RECK, J., SCHRANK, A. Comparative study between Larval Packet Test and Larval Immersion Test to assess the effect of *Metarhizium anisopliae* on *Rhipicephalus microplus* tick larvae. Experimental Applied Acarology 74:455-461, 2018.

YESSINOU, R.E., AKPO, Y., SIDICK, A., ADOLIGBE, C., YOUSSAO ABDOU KARIM, I., AKOGBETO, M., FAROUGOU, S. Evidence of multiple mechanisms of alphacypermethrin and deltamethrin resistance