

Original Research Article

The effects of aqueous root bark and seed extracts of *Azadirachta indica* and pyrethrin in the treatment of ticks (*hyalomma spp*) in vitro.

ABSTRACT

The acaricidal effect of the aqueous extracts of neem (*Azadirachta indica*) seed and root bark on tick (*Hyalomma spp*) was investigated. Adult *Hyalomma* ticks of both sexes were incubated in aqueous extracts of neem seed and root bark. The extracts were made in varying concentrations (100%, 50% and 25%) with sand and sawdust media. Survival rate of the ticks in the extracts were monitored daily by recording of the ratio of dead to live ticks. Phytochemical screening of the extracts was also determined. The results showed the presence of tannins, saponins, anthraquinones, phlobatanins and cardiac glycosides. Both extracts contained alkaloids, phlobatanins and cardiac glycosides. Tannin occurred in larger concentration in the root bark than in the seed whereas the seed contained larger quantities of anthraquinones and saponins than the root bark. The results further showed that the seed and root extracts of neem have acaricidal effect against the ticks through death of the ticks incubated in them. However, the root bark extract was more effective than the seed extract as it produced higher killing effects of the ticks incubated in it.

Therefore, the aqueous extracts of the root bark and seeds of *Azadirachta indica* can be used as commercial acaricide to control ticks and tick infestation.

Key words: *Azadirachta indica*; tick; root bark; seed.

1.INTRODUCTION.

Ticks are ectoparasites of vertebrates with vital roles in the transmission of many disease causing agents to man and livestock [1-3]. Production losses in livestock production and quality has been attributed to tick infestation [4] and coupled with the increased risk to life threatening diseases in humans [5]. The control of tick infestations and transmission of tick-based-diseases remains an important huddle to the livestock industry [6]. Until recently, the indiscriminate use of acaricides has led to the fast development of tick resistance, environmental toxicity [7-9] and the possible appearance of chemical residues in products of animal origins [10].

The most common acaricides used in the control of ticks include pyrethroids, carbaryl, fipronils and amitraz [11]. Plant extracts have been employed to control some species of ticks [12-14]. Some of the advantages of phytotherapies that justify their use are the synergistic effects of its constituents and the combination of mechanisms for substances acting on various molecular

targets among others [15, 16]. Alternative insecticides derived from compounds in plants can act on parasites by reducing their development, reproductive capacity and survival [17]. Experiments on neem plant have indicted some acaricide activities of its extracts [18-20]. The current knowledge on the composition of the extract and the doses required for the proper control of ticks are scarce [21].

Therefore, this study was designed to evaluate the efficacy of aqueous root bark and seed extracts of *Azadirachta indica* on ticks (*Hyalomma spp*).

2.MATERIALS AND METHODS.

2.1.Tick collection.

The ticks (*Hyalomma spp.*) used were collected from cattle and camels brought for slaughter in Maiduguri Metropolitan Abattoir, North Eastern Nigeria. The animals were brought for slaughter from different parts of Borno State and adjoining States. The ticks were collected from the different body parts of camels and cows of several breeds immediately after being slaughtered, placed directly into sample bottles covered with wire netting, and transported immediately to the laboratory for the study.

2.2.Collection of standard tick acaricide (pyrethrin).

Tick flea, a 5% pyrethrin based powder produced by Primose Veterinary (Victoria Island Lagos) was used as the standard acaricide for the study.

2.3.Collection and extraction of plant materials.

Fresh quantities of the root bark and seeds of neem (*Azadirachta indica*) were collected from the trees planted within the University of Maiduguri campus.

The fresh samples of the root bark of *Azadirachta indica* were dried under room temperature for about two weeks. The dried root barks were pulverized using pestle and mortar into coarse particles and then grounded finely with a mechanical grinder. Also, the fresh ripped seeds were separated from the seeds, washed and then shade dried. The seeds were cracked to remove the kernels.

250g of the powdered root bark extract and 350g of the grounded seed were each respectively extracted in 1000ml of distilled water with intermittent shaking at intervals of 6hours. The extract was filtered using What-man size 1 filter paper and concentrated in vacuo using vacuum rotary evaporator. The percentage yield was calculated as described by Omoja *et al.* [22] and the extracts were each kept in the refrigerator at 4°C for use in the study.

2.4.Processing of the sawdust and sand samples.

67 The sawdust was dried at room temperature for ten days. The sand sample collected was sharp
68 porous sand without dust. One hundred grams of the sand sample was sterilized at 100°C for 10
69 min to destroy all living microorganism that may be present in the sand.

70 2.5. Phytochemical screening.

71 Basic phytochemical screenings were carried out on the seed and root bark extracts using the
72 methods of Shellard [23] and Trease and Evans [24].

73 2.6. *In Vitro* acaricidal efficiency test.

74 The ticks collected were divided into several groups each of which were treated either with the
75 extract, the conventional acaricide or remained untreated as control as stated below. The ticks
76 were grouped into thirteen (13) groups for each extract. For each experiment, 30 ticks were
77 placed in the sample bottle containing either extract powder, acaricide or their mixture with the
78 media. The bottles were then covered with a wire netting to prevent the ticks from escaping. The
79 experimental groups were kept in the laboratory at room temperature. The experimental set-up
80 was monitored everyday to assess the survival of the ticks in each of the extract powder,
81 acaricide and their mixture with the various media. On each day, the number of dead ticks or live
82 ones were determined and recorded. Dead ticks were removed from the experimental group
83 during each observation period.

84 Group A control (Untreated).

85 Group B extract 100%

86 Group C extract 50% + sawdust 50%

87 Group D extract 25% + sawdust 75%

88 Group E extract 50% + sand 50%

89 Group G acaricide 100%

90 Group H acaricide 50% + sawdust 50%

91 Group I acaricide 25% + sawdust 75%

92 Group J acaricide 50% + sand 50%

93 Group K acaricide 25% + sand 75%

94 Group L sawdust 100%

95 Group M sand 100%

96 2.7. Statistical analysis.

Data obtained were presented as graph for the packed cell volume and as means in table for the parasite count. The data were analyzed using ANNOVA followed by the student t-test. The acceptable level of significance was $P < 0.05$.

3.RESULTS.

The results of the phytochemical constituents of the aqueous seed and root bark extracts of the plant is presented in Table 1. The results show that the aqueous root bark extract of the plant contained appreciable quantities of alkaloids, tannins, phlobatannins and cardiac glycosides, moderate quantities of saponins and a little quantities of cardiac glycosides. In comparison, the two extracts appeared to contain the same amounts of alkaloids and phlobatanins. However, the root bark had more tannin than the seed extract while the seed extract contained more anthraquinones and saponins than the root bark extract.

The results of incubating the ticks in 100% standard acaricide (5% pyrethrin), 100% root bark extract, and seed extract is shown in Figure 1. The results show that incubation in the 100% standard acaricide killed all the ticks after 4 days post incubation while the 100% root bark and 100% seed extract killed all the ticks after 8 days post incubation respectively. Also, incubation in 100% sawdust and 100% sand produced 100% lethality after 8 and 14 days post incubation respectively. Generally there was a gradual death from day 6 post incubation in the untreated control group during the 21 days period of experiment.

The results of incubating the ticks in 50% root bark extract, 50% seed extract, acaricide (5% pyrethrin), sawdust and sand is shown in Figure 2. The incubation in 50% acaricide combination with sawdust and sand respectively produced 100% lethality by 2 and 4 days post incubation respectively while the incubation in 50% root extract in combination with sawdust and sand produced 100% lethality on the ticks by day 9 post incubation. Also, the combination of 50% seed extract with sawdust and sand produced 100% lethality after 14 and 16 days post incubation.

The results of incubating the ticks in 25% root bark extract, 25% seed extract, acaricide (5% pyrethrin), sawdust and sand is shown in Figure 3. The results showed that the combination of 25% acaricide with sawdust or sand killed all the ticks after 4 days post incubation while the incubation in root bark extract with sawdust and sand respectively produced 100% lethality on the 21 and 13 days post incubation. The treatment in 25% seed extract in combination with sawdust and sand produced 100% lethality after 9 and 21 days respectively.

4.DISCUSSION.

The results of the phytochemical analysis of the root bark and seed extract of *Azadirichta indica* showed that both extracts have the same level of alkaloids. The root extract contained more

tannins than the seed extract while the seed extract contained more of saponins. It can be inferred from the results in Table 1 that these plant extracts contained copious amounts of phytochemicals acting synergistically and contributing to the observed acaricidal property.

In comparison, the two extracts appeared to have acaricidal effect that was manifested in a concentration dependent manner. Higher concentration of the extracts produced earlier killing of the ticks incubated in them than those produced by lower concentration of the extracts with either sand or sawdust. The results are in line with Giglioti *et al.* [9] that observed that the effectiveness of neem seed extract on *Rhipicephalus microplus* was concentration dependent. Also, the report of Ndumu *et al.* [25] observed that the efficacy of the neem seed extract on *Amblyomma variegatum* was dependent on the time of exposure to oil and quality of oil in the extract. The results also showed that at the 100% or 50% mixture with sand or sawdust acaricidal effect of the extracts (seed, and root bark) were comparable to those produced by the standard acaricide at the same level of mixture with the two media. However, the standard acaricide produced significantly ($p<0.05$) greater effect than the extracts at the 25% mixture with the media. The results suggest that the use of the extract at 25% concentration in combination with sand or sawdust could not produce acaricidal effects comparable to those caused by 25% mixture of the standard acaricide with sand or sawdust. The results from the present study were in agreement with reports of previous studies on ticks. Mohsin *et al.* [26] reported the anti-tick effect of *A. indica* against *Rhipicephalus microplus* which was time dependent. Other studies reported on the severity of *A. indica* extracts on *Rhipicephalus annulatus* reproduction and mortalities [27, 28]. The reports of Giglioti *et al.* [9] showed that the main toxic effects of neem seed extract on engorged female *Rhipicephalus microplus* was to inhibit its reproduction. The commercial neem seed oil (Nemm Azal F) has been shown to inhibit the reproduction of tick *Hyalomma anatolicum excavatum* [12] while Mwangi *et al.* [29] reported that the oil extracts from the leaves of *Ocimum suave* killed all the stages of *Rhipicephalus appendiculatus*.

At 100% concentration both extracts produced the same pattern of mortality and killed all the ticks incubated in them within 8 days. These effects appeared comparable to those produced by the acaricide. However, at the 50% combination with sawdust and sand, the root bark extract produced similar effect by causing 100% mortality by day 9 post incubation in both cases. On the other hand, 50% mixture of the seed extract produced a more gradual killing effect and the ticks survived up to 14 and 16 days post incubation in combination with sand and sawdust respectively. Therefore, the root bark extract appeared to produce earlier killing than the seed extract in mixture with either sawdust or sand. At 25% combination with sand or sawdust, the root bark produced a similar mortality pattern. However, 100% death was produced earlier (day 14) post incubation with sand than with sawdust (day 21) post incubation. In the case of the seed extract in 25% combination with sawdust or sand, the mortality pattern differed significantly while death was earlier (Day 9) with saw dust it was much more delayed with sand (day 21). Other plants extracts have been reported to be toxic to adult ticks [3]. The report of Cohen *et al.* [30] showed that other limonoids (nimbolide and epoxiazadiradione) in neem extracts appeared to

possess cytotoxic property and were proved to be toxic to parasites. It will be difficult to determine the exact effect of the different types of neem extracts in the control of livestock pests. This could be attributed to the complex nature of its constituents and their different mode of action which make it rather hard to explain the mechanism of action involved [9, 31]. The aqueous extracts were used in the study to justify the use in traditional medicine.

CONCLUSION: The results of the study showed that the aqueous root bark and seed extracts of *Azadirachta indica* have acaricidal effect on ticks (*Hyalomma spp*) through the death of the ticks incubated in them. The effect was manifested in a concentration dependent manner with higher concentrations of the extracts producing earlier killings of the ticks than those produced by lower concentrations. Therefore, the aqueous root bark and seed extract of *Azadirachta indica* can be a source of commercial acaricide to control tick infestation in livestock. Characterization and optimization of the extract is required.

References.

- [1] Gonzalez J P, Camicas J-L, Cornet J-P, Wilson M L. Biological and clinical responses of West African sheep to Crimean-Congo haemorrhagic fever virus experimental infection. Res Virol. 1998; 149: 445–455.
- [2] Raoult D, Fournier P E, Fenollar F, Jensenius M, Prioe T, Caruso G, et al. Rickettsia africae, a tick-borne pathogen in travellers to Sub-Saharan Africa. N Engl J Med. 2001; 344: 1504–1510.
- [3] Nchu F, Magano S R, Eloff JN. In vitro investigation of the toxic effects of extracts of *Allium sativum* bulbs on adults of *Hyalomma marginatum rufipes* and *Rhipicephalus pulchellus*. J South Afri Vet Assc. 2005; 76(2): 99–103 (En.).
- [4] Mukhebi A W, Chamboko T, O’Callaghan C J, Peter T F, Kruska L, Medley GF., et al. An assessment of the economic impact of heart water (*Cowdria ruminantium* infection) and its control in Zimbabwe. Prev Vet Med. 1999;39: 173–189.
- [5] Parola P. Tick-borne rickettsial diseases: emerging risks in Europe. Comp Immunol Microbiol Infect Dis. 2004; 27: 297–304.
- [6] Biu A A, Gulani I A, Nkechi O P, Jajere S M, Yakaka W, Zango M K. et al. Efficacy of

- 200 *Azadirachta indica* a. Juss leaf aqueous extract against bacteria isolated from the guts of
- 201 ixodid ticks in Maiduguri, Nigeria. J Sci Multidiscipl Res. 2015; 7(1): 57-61.
- 202 [7] Santos ACG, Rodrigues OG, Araújo LVC, Santos SB, Guerra RMSNC, Feitosa MLT, et al.
- 203 Uso de Extrato de Nim no Controle de Acariase por *Myobia musculli* Schranck (*Acari:*
- 204 *Miobidae*) e *Myocoptes musculus* Koch (*Acari: Listrophoridae*) em camundongos (*Mus*
- 205 *musculus* var. *albina* L.). Neotrop. Entomol 2006; 35, 269–272.
- 206 [8] Albuquerque UP, Medeiros PM, Almeida ALS, Monteiro JM, Lins-Neto EMF, Melo JG. Et
- 207 al. Medicinal plants of the caatinga (Semi-arid) vegetation of NE Brazil: a quantitative
- 208 approach. J Ethnopharmacol. 2007; 114: 325–354.
- 209 [9] Giglioti R, Forimb MR, Oliveiraa HN, Chagasc ACS, Ferrezinid J, Britoe LG, et al. In vitro
- 210 acaricidal activity of neem (*Azadirachta indica*) seed extracts with known azadirachtin
- 211 concentrations against *Rhipicephalus microplus*. 2001; Vet Parasitol. 181; 309– 315.
- 212
- 213 [10] Paião JCV, Monteiro AC, Kronka S. Susceptibility of the cattle tick *Boophilus microplus*
- 214 (*Acari: Ixodidae*) to isolates of the fungus *Beauveria bassiana*. W J Microbiol
- 215 Biotechnol.2001; 17(3):245-251.
- 216 [11] Flávio ASP, Glyn MF, Andréa MA, Bruno RS, Maria ICM, Matias PJS, etal. Acaricidal
- 217 activity of ethanolic extract from aerial parts of *Tagetes patula* L. (*Asteraceae*) against
- 218 larvae and engorged adult females of *Rhipicephalus sanguineus* (Latreille, 1806). Par
- 219 Vect. 2012; 5:295 doi:10.1186/1756-3305-5-295.
- 220 [12] Abdel-Shafy S, Zayed AA. In vitro acaricidal effect of plant extract of neem seed oil
- 221 (*Azadirachta indica*) on egg, immature and adult stages of *Hyalomma anatolicum excavatum*
- 222 (*Ixodoidea: Ixodidae*). Vet. Parasitol. 2002; 106: 89–96.
- 223 [13] Carroll JF, Maradufu A, Warthen JDJ. An extract of *Commiphora erythraea*: a repellent and
- 224 toxicant against tick. Entomol Exp Appl. 1989; 53:111-116.

- 225 [14] Arnosti A, Brienza PD, Furquim KCS, Chierice GO, Neto SC, Bechara GH. Effects of
226 *Ricinus communis* oil esters on salivary glands of *Rhipicephalus sanguineus* (Latreille,
227 1806) (Acari: Ixodidae). Exp Par. 2011; 127:569-574.
- 228 [15] Dantas-Torres F, Figueredo LA, Faustino MAG. Ectoparasitos de cães provenientes de
229 alguns municípios da região metropolitana do Recife, Pernambuco, Brasil. Rev Bras Parasitol
230 Vet. 2004; 13(4):151-154.
- 231 [16] Prette N, Monteiro AC, Garcia MV, Soares VE. Patogenicidade de isolados de *Beauveria*
232 *bassiana* para ovos, larvas e ninfas ingurgitadas de *Rhipicephalus sanguineus*. Ciênc Rural
233 .2005; 35(4):855-861.
- 234 [17] Habluetzel A, Carnevali F, Lucantoni L, Grana L, Attili AR, Archilei F et al. Impact of the
235 botanical insecticide Neem Azal on survival and reproduction of the biting louse *Damalinia*
236 *limbata* on angora goats. Vet Parasitol. 2007; 144: 328–337.
- 237
- 238 [18] Kalakumar B, Kumar HSA, Kumar BA, Reddy KS. Evaluation of custard seed oil and neem
239 oil as acaricides. Vet Parasitol. 2000; 14:171–172.
- 240 [19] Benavides OE, Hernández MG, Romero NA, Castro AH, Rodríguez BJL. Evaluación
241 preliminar de extractos del Neem (*Azadirachta indica*), como alternativa para el control de
242 la garrapata del Ganado *Boophilus microplus* (Acari: Ixodidae). Rev. Colom. Entomol.
243 2001; 27:1–8.
- 244 [20] Srivastava R, Ghosh S, Mandal DB, Azhahianambi P, Singhal PS, Pandey NN. Efficacy of
245 *Azadirachta indica* extracts against *Boophilus microplus*. Parasitol. Res. 2008; 104: 149–
246 153.
- 247 [21] Jonsson NN, Piper EK. Integrated control programs for ticks on cattle. UQ Printery,
248 Australia, 2007; pp. 135–136.
- 249 [22] Omoja VU, Anaga AO, Obidike IR, Ihedioha TE, Umeakuana PU, Mhomga LI, etal. The
250 effects of combination of methanolic leaf extract of *Azadirachta indica* and diminazene

- 251 diaceturate in the treatment of experimental *Trypanosoma brucei brucei* infection in rats. Asian
252 Pac J Trop Med. 2001; 337-341.
- 253 [23] Shellard EJ. Practical plant Methods. Medical Publishing Company Limited London. 1957;
254 Pp. 30-35.
- 255 [24] Trease GE, Evans WC. (1978). Textbook of Pharmacology. 11th edition, Bulliere Trindal
256 London.
- 257 [25] Ndumu, PA, George JB, Choudhury MK. Short communication: toxicity of neem seed oil
258 (*Azadiracta indica*) against the larvae of *Amblyomma variegatum* a three-host tick in cattle.
259 Phytother. Res. 1999; 13: 532–534.
- 260 [26] Mohsin N, Sojid MS, Zulfiqar A, Muhammad W, Tanveer A, Abid H, et al. Anti-tick
261 activity of leaves of *Azadirachta indica*, *Dalbergia sisso* and *Morusaiba* against
262 *Rhipicephalus microplus* (Acari: ixodidae). Acta Parasitol Glob. 2015; 6(1):60-64.
- 263 [27] Kaposhi CKM. The role of natural products in integrated tick management in Africa. Insect
264 Sci Appl. 1992; 13(4): 595-598.
- 265
- 266 [28] Williams LAD. Adverse effect of extracts of *Artocarpus altilis* park and *Azadirachta indica*
267 (A. Juss) on the reproductive physiology of the adult female tick, *Boophilus microplus*
268 (canset). Invert Reprod Dev. 1993; 23(2-3): 159-164.
- 269 [29] Mwangi EN, Essuman S, Kaaya GP, Nyandat E, Munyinyi D, Kimondo MG. Repellence
270 of the tick *Rhipicephalus appendiculatus* by the grass *Melinis minutiflora*. Trop Ani Healt
271 Prod. 1995; 27(4): 211-216.
- 272 [30] Cohen E, Quistad GB, Jefferies PR, Casida JE. Nimbolide is the principal cytotoxic
273 component of neem-seed insecticide preparations. Pestic. Sci. 1996; 48: 135–140.
- 274 [31] Mossini SAG, Kemmelmeier C. A árvore Nim (*Azadirachta indica* A. Juss): múltiplos usos.
275 Acta Farmacol. 2005; 24: 139–148.
- 276

Table 1. Phytochemical analysis of the aqueous root bark and seed extracts of *Azadirachta indica*.

Phytochemical test	Root bark extract	Seed extract
Alkaloids	+++	+++
Tannins	+++	++
phlobatanins	+++	+++
anthraquinones	+	++
saponins	+	++
Steroids	+++	+++
Cardiac glycosides	++	+

+++ = very positive, ++ = moderately Positive, + = mild positive.

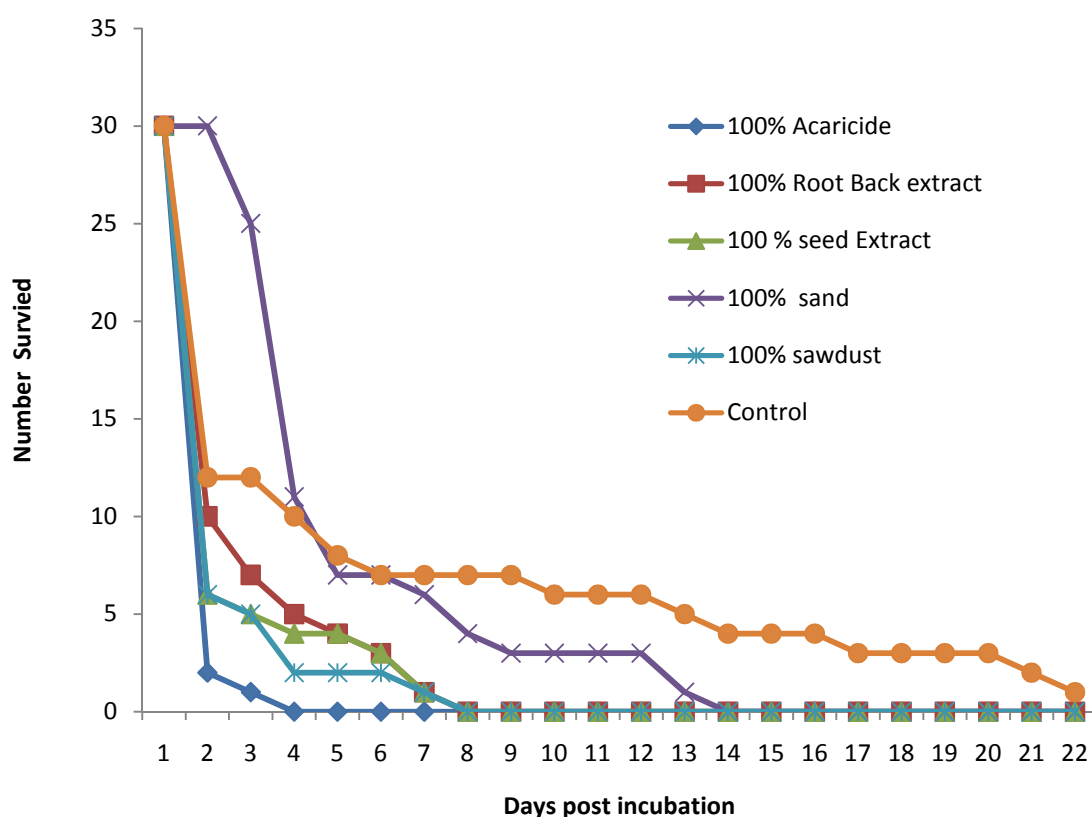
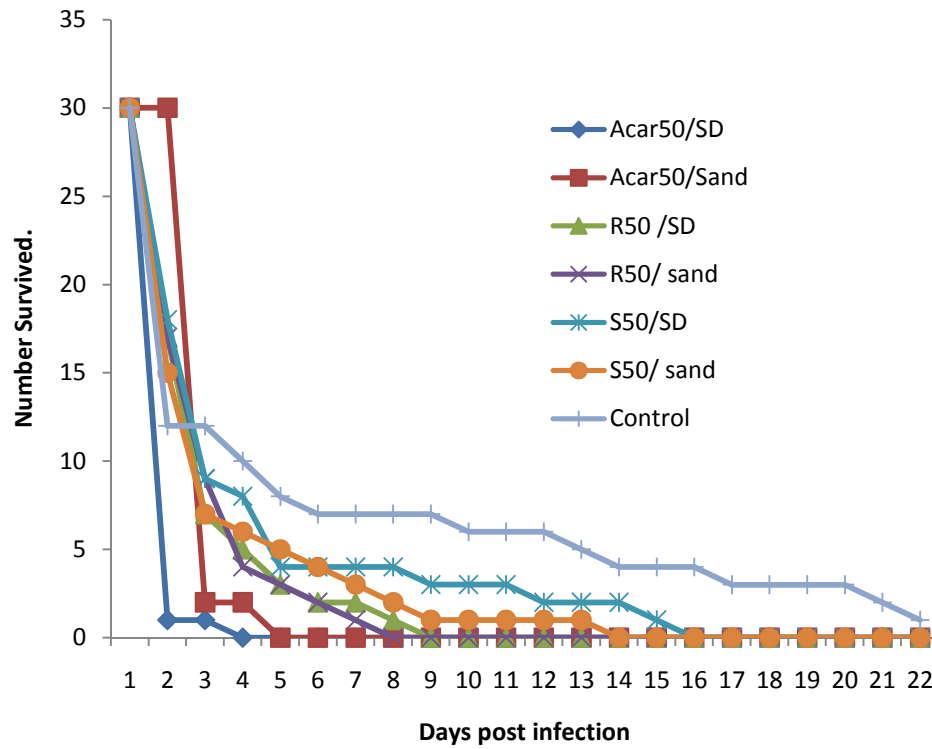


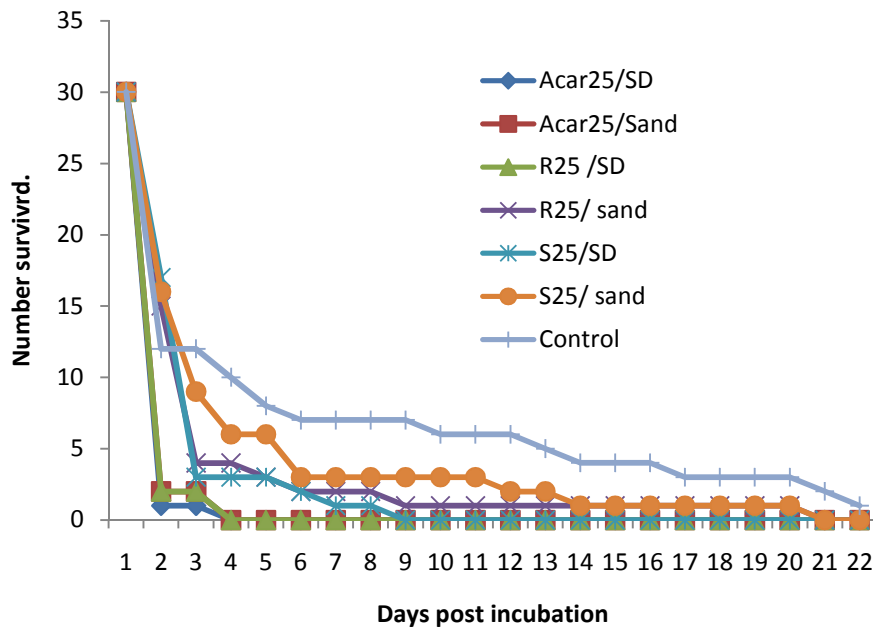
Figure 1. The survival of ticks incubated in 100% root bark, seed extract, and acaricide.



285

286 Acar50/SD = 50% acaricide +50% sawdust,

287 Figure 2. The survival of ticks incubated in 50% root bark, seed extract, and acaricide.



288

289 Figure 3. The survival of ticks incubated in 25% root bark, seed extract, and acaricide.