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In vitro Acaricidal Effect of *Jatropha curcas* Methanol Extract on *Rhipicephalus* (*Boophilus*) *decoloratus* larvae

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ABSTRACT

Tick infestation is considered a major problem because ticks cause widespread nuisance and losses, and are vectors of disease-causing agents, resulting in economic losses in livestock production. The use of chemical agents is the main stay in tick-control, though current research into viable plant extracts as alternatives are on the increase. This study evaluated the larvicidal effect of crude methanolic extract of *Jatropha curcas* seed oil extract on *Rhipicephalus (Boophilus) decoloratus* ticks. Various concentrations of 6.25, 12.5, 25 and 50 mg/ml were prepared from a stock (100 mg/ml) solution of the sample; control using 1% tween 80 and standard acaricide; Amitraz (positive control) were used. The mortalities were observed at time intervals of 6, 12, 18, 24 and 48 hours. The experiment was performed in triplicate. The extract had an LC_{50} and LC_{90} of 2.88mg/ml and 6.76mg/ml respectively, after 48h. Carbohydrates, cardiac glycosides, saponins, tannins, steroids, terpenes, alkaloids and flavonoids were detected as phytoconstituents, and the extract exhibited significant mean percentage mortality from the control across all the concentrations after 24h. The result confirms that *J. curcas* is a good alternative to be used as a larvicidal agent in the control of *Rhipicephalus (Boophilus) decoloratus*.

Keywords: Acaricidal; *Jatropha curcas*; larvae; *Rhipicephalus* (*Boophilus*) *decoloratus,*

INTRODUCTION

Ticks are obligatory blood-sucking arachnid arthropods infesting a wide variety of animals including mammals, birds, reptiles and amphibians. They are vectors of disease agents (Babesia, Cowdria, Anaplasma) causing anaemia, dermatitis, paralysis, otocariasis as well as loss of production (Schimdt and Roberts, 1989). Two families of ticks are of veterinary importance, Ixodidae (hard ticks) and Argasidae (soft ticks) (Sonenshine, 1991; Luqman *et* al., 2007). Ticks are the most important ectoparasites of cattle, causing economic losses in terms of reduced productivity, infertility, diseases and death (Rajput *et al*., 2006). Besides being haematophagous, they also inflict notable dermatitis, predispose their host to dermatophilosis and other arthropod-borne infestations such as myiasis (Mtshali *et al*., 2004).

Tick control by use of chemical acaricides, is fraught with various problems like residual effect in animals, environmental pollution and high cost, clearly demanding the need for alternative approaches (Ghosh *et al.*, 2007). Acaricide resistance have been reported with the use of the conventional chemicals (Muhammad *et al*., 2022). Even though many plant extracts with promising acaricidal effects have been reported in literature, the feasibility of many of these extracts for the control of ticks infesting animals in field conditions has not been adequately studied (Rechav and Hay, 1992). *J. curcas* seed extracts from previous studies have shown that the toxic phorbol esters are distributed in different parts of the plant and this includes seeds, leaves, stem, flower, buds, roots, bark, and wood but they are mainly concentrated in the seed kernel (Mbako *et al*., 2022).

MATERIALS AND METHODS

Identification and Preparation of *Jatropha curcas* Seeds

Jatropha curcas seeds were obtained from the National Research Institute for Chemical Technology, Zaria. The plant was identified at the Department of Biological Sciences, Ahmadu Bello University, Zaria and voucher specimen reference number (22873 was assigned). The seeds were de-shelled and pulverized to powdered form by using mortar and pestle and then stored in air-tight container before use.

Extraction

Five hundred grams (500 gm) of the seed powder was extracted using a Soxhlet apparatus. The methanol extract obtained was concentrated using a water bath to obtain an oily extract. The concentrate obtained was stored in a refrigerator till needed.

Phytochemical Screening

Phytochemical analysis of the plant extract was conducted to determine the presence of phyto-constituents (Trease and Evans, 2002).

Stock Preparation and Preparation of Different Concentrations

One gram of the concentrated seed extract of the pulverized seed of *J. curcas* was dissolved in 10 ml of 1% Tween 80 (as an emulsifying agent) and kept as stock (100 mg/ml) solution. This stock solution was used to prepare the desired concentrations through serial dilution to obtain concentrations of 50, 25, 12.5 and 6.25 mg/ml for use against *Rhipicephalus (Boophilus) decoloratus* larvae.

Tick Collection

Engorged females of *Rhipicephalus* (*Boophilus*) *decoloratus* were collected from naturally infested cattle in Fulani herds Kaduna by hand picking and brought to the Entomology Laboratory of the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, where they were reared.

Rearing of Ticks

The engorged ticks were cleaned with 10% ethanol-soaked cotton wool and rinsed with distilled water. Each engorged female tick was placed in a sterile plain plastic tube with the open end plugged with cotton wool. The properly labeled plastic tubes were then maintained in an incubator in the laboratory at 28 ± 2°C and a relative humidity of 84 ± 5% as described by Mattioli and Cassama (1995); George *et al*., (2001) and Natala *et al*., (2002) to create a suitable condition until egg-laying was completed. After egg-laying, the dead female ticks were removed from the universal bottles and the eggs incubated under the same laboratory conditions for the eggs to hatch. The larvae that emerged from the eggs were used for the bioassay test after 14-20 days post hatching.

Evaluation of acaricidal effect of *Jatropha curcas*

Petri dishes (9cm in diameter) with one sheet of Whatman® filter paper of the same size placed inside each of the petri dishes were used for the experiment. One milliliter of 50, 25, 12.5 and 6.25 mg/ml of *J. curcas* seed extract and 1% tween 80 and Amitraz (negative and positive controls respectively), were evenly soaked (using syringe and needle) on different filter papers of each petri dish. Then 60 larval ticks were placed inside each petri dish with the help of a soft paint brush and then monitored for mortality at 6, 12, 18, 24 and 48 hours later.

Larvicidal Bioassay Groups

Group A was treated with 50 mg/ml of the extract; Group B with 25 mg/ml; Group C with 12.5 mg/ml; Group D with 6.25 mg/ml; Group E with 1% tween 80 while Group F were treated with Amitraz (1.4 ml/ liter of water according to manufacturer's specification). Each treatment (group) was carried out in triplicates. Larvae were considered dead when on being agitated, were unable to exhibit paddling of the legs and separated pedipalps. Corrected mortality was obtained using Abbot's formula for control (of mortality):

$$\text{Corrected mortality (\%)} = \frac{\text{Mobserved} - \text{Mcontrol}}{100 - \text{Mcontrol}} \times 100$$

Where; $M_{control}$ = Mortality in control

$M_{observed}$ = Observed treatment mortality

Data Analyses

Data from mortality was analyzed using ANOVA (Graph-pad prism version 5). $P < 0.05$ was statistically significant. Regression coefficient between the probit kill and log concentration of the extract was used to determine the LC_{50} following the toxicity bioassay (Finney, 1971). Analysis of variance (ANOVA) and Duncan multiple range tests (DMRT) (1955) were used to test for differences between levels of treatments and to separate means respectively.

RESULT

Table 1: shows results for phytochemical screening of crude methanol extract of *J. curcas*, the following phytochemicals were present; carbohydrates, cardiac glycosides, saponins, tannins, steroids and tannins, steroids and terpenes, alkaloids and flavonoids. Anthraquinones were not detected.

Table 2 shows corrected mean percentage mortality of *Rhipicephalus* (*Boophilus*) *decoloratus* larvae treated with *J. curcas* methanol seed extract at various exposure period, the exposure period was 48 hours and mortality record were taken at 6 hourly intervals. The corrected mortality is calculated from the control using abbot's formula. The various exposure time showed that at higher exposure time the mortality increased and the higher the concentration the higher the mortality.

Table 3 shows mean percentage mortality of *Rhipicephalus* (*Boophilus*) *decoloratus* larvae treated with *J. curcas* methanol seed extract to test the toxicity of the extract of the larvae at an exposure time of 48 hours. The result showed that the mortality was time and concentration dependent. At lowest concentration of 6.25mg/ml the mortality values were lower when compared to the highest concentration of 50mg/ml. The mortality rate increased with the increase in exposure time.

Table 4 shows Summary of the LC_{50} and LC_{90} of the methanol extract of *J. curcas* seed at various exposure times. From the table it can be deduced that the higher the concentration the lesser time it took to attain the LC_{50} and LC_{90} thus LC_{50} and LC_{90} and are indirectly proportional to the exposure time.

DISCUSSION

In this study, 500g of *J. curcas* seeds were extracted using methanol as solvent giving a percentage yield of 39.27%; this agrees with reports of Gubitz *et al*, (1999) who obtained 39.7%, though fell short of 66.4%, recorded by Adebowale and Adedire (2006). The phytochemical screening in this study revealed the presence of carbohydrates, cardiac glycosides, saponins, tannins, steroids and triterpenes, alkaloids, and flavonoids. This shows that the seeds are as rich in phytochemicals as are other parts of the plant as reported by Igbinosa *et al*. (2009) on stem bark extract of *J.* curcas. However, Abdulhamid *et al.* (2013) did not isolate tannins, flavonoids, and phenols from *J. curcas* seed oil in a similar work done in Kebbi, Nigeria. This could be due to variation in geographical location, soil type, method of

extraction and solvent system used which has been shown to affect plant metabolites (Ekpendu *et al*., 2000). Terpenes, alkaloids, tannins, flavonoids, and phenolic compounds are the most effective and important bioactive compounds of plants as insecticide (Mann, 2012) and can be used to control ticks (Dimri and Sharma, 2004).

Table 1: Result of Qualitative Phytochemical Screening of *Jatropha curcas* Methanol Seed Extract

Constituent(s)	Test	Reference	Observation	Inference
Carbohydrates	Molisch	Trease and Evans,2002	Reddish colour ring	+
Cardiac glycosides	Keller-kiliani	"	Purple, brown colouration	+
Steroids and triterpenes	Liebermann-Burchard	"	Reddish colouration	+
Saponins	Frothing	"	Persistent (honeycomb) froth	+
Tannins	Ferric chloride	"	Brownish blue ppt	+
Alkaloids	Meyer's	"	Creamy white ppt	+
"	Wagner's	"	Reddish brown ppt	+
"	Draggendoff's	"	Orange, brown ppt	+
Flavonoids	Shinoda's	"	Red colouration	+
"	Sodiun hydroxide	"	Yellow colouration	+
Free Anthracene Derivative	Bontrager's	"	Yellow colouration	-
Combined Anthracene Derivative	Modified Bontrager's	"	Yellow colouration	-

+ Test substance present; - Test substance absent.

Table 2: Corrected Mean Percentage Mortality of *Rhipicephalus* (*Boophilus*) *decoloratus* Larvae treated with *Jatropha curcas* Methanol Seed Extract at Various Exposure Periods

Exposure Time (h)	Concentration of extract (mg/ml)			
	6.25 (%)	12.5 (%)	25 (%)	50 (%)
6	4.36±1.87	9.01±1.15	57.03±5.51^{a}	61.85±2.94^{a}
12	3.77±1.96	18.90±1.80^{a}	85.92±2.66^{a}	84.25±3.20^{a}
18	12.36±2.24	32.02±3.67^{a}	93.33±2.27^{a}	92.36±1.96^{a}
24	24.97±1.00^{a}	44.46±4.45^{a}	95.23±2.06^{a}	97.02±1.40^{a}
48	64.00±0.85^{a}	75.30±2.56^{a}	97.17±0.92^{a}	100.0 ±0.00^{a}

Means in the same row with superscripts are significantly different ($p \leq 0.05$).

Table 3: Mean Percentage Mortality of *Rhipicephalus* (*Boophilus*) *decoloratus* Larvae treated with *Jatropha curcas* Methanol Seed Extract

Treatment (Exposure) Time (h)	Control (1% Tween 80)	Concentration of Extract (mg/ml)			
		6.25 (%)	12.5 (%)	25 (%)	50 (%)
6	4.09±1.06^{a}	8.27±2.67^{a}	12.73±1.24^{a}	58.79±9.95^{b}	63.41±4.82^{c}
12	5.57±0.74^{a}	9.13±3.18^{a}	23.42±2.86^{b}	86.70±4.57^{c}	85.13±5.66^{d}
18	5.57±0.74^{a}	17.24±3.74^{a}	35.81±6.60^{b}	93.70±3.80^{c}	92.79±3.18^{d}
24	5.57±0.74^{a}	29.15±1.26^{b}	47.54±8.15^{c}	95.50±3.38^{d}	97.19±2.06^{e}
48	6.27±0.23^{a}	66.26±1.47^{b}	76.85±4.89^{c}	97.35±1.61^{d}	100±0.00^{e}

Means in the same row with different superscripts are significantly different from the control ($p \leq 0.05$)

Table 4: Summary of the LC_{50} and LC_{90} of the Methanol Extract of *Jatroha curcas* Seed at Various Exposure Times

Duration of Exposure Time(h)	LC_{50}(mg/ml)	LC_{90}(mg/ml)
6	21.38	245.47
12	17.38	169.82
18	15.13	131.83
24	13.18	104.71
48	2.88	6.76

Redfren *et al*., (1982) reported that some phytochemicals act as toxicants which generally kill different life stages of insects while various other phytochemicals interfere with growth and metamorphosis. In this study, *Jatropha curcas* seed extract exhibited acaricidal activity against *R. (Boophilus) decoloratus* larvae in a concentration and time dependent manner. This

toxicity could be attributed to the presence of many chemical ingredients such as alkaloids, steroids, flavonoids, saponins, tannins and triterpenes. Alkaloids are known to possess medicinal and pesticidal properties (Fatnassi, *et al.,* 2014). These compounds have been reported in all parts of the plant but are more abundant in its seeds (Haas and Mittelbach, 2000). However, several works have shown that the major factor responsible for *J. curcas* toxicity is the high concentration of phorbol esters (tetracyclic diterpenoids) in the seeds (Goel *et al*., 2007). Saponins are not easily degraded during extraction as they remain hydrophilic and may have contributed to the toxicity of the crude methanol extracts (Rug and Ruppel, 2000). Saponins exhibit surfactant property by foaming (soap-like), as well as altering the permeability of cell walls and hence exert toxicity on all organized tissues (Moyo *et al.,* 2012). The anti-ovipositional and ovicidal effects of *J. curcas* against *Callosobruchus maculatus* was earlier demonstrated by Adebowale and Adedire (2006) where 92% inhibition effect was reported. It is speculated that suffocation and / or lethal chemical poisoning due to Jatropha oil application prevented the adult emergence from the bruchid, *C. maculatus*. The acaricidal properties exhibited by *J. curcas* seed extract was less efficacious than that of Amitraz (Amitik®) a standard synthetic acaricide recommended by WHO, which killed larval ticks of *Rhipicephalus (Boophilus decoloratus*) at lower concentration when compared to the extract. The results obtained by use of the extract was satisfactory in exhibiting acaricidal effects. This study was able to establish that Jatropha seed oil extract was concentration and time dependent in its action as an acaricide, exhibiting acaricidal activity even at a low concentration of 6.25mg/ml with the acaricidal activity increasing with concentration and time.

Conclusion

The crude methanol extract of *J. curcas* seed extract contains many bioactive chemical compounds that exhibit very good in-vitro acaricidal effect on the larvae of *R.* (*Boophilus*) *decoloratus*. The various concentrations of the extract exhibited acaricidal activity at a concentration dependent gradient and the level of significance increased with time increase. The result reported here opens the possibility for further investigation for its potential as standard acaricide and isolation and purification of crude methanol extract of *J. curcas* for acaricidal and other potential uses.

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Conflict of Interest

There authors have no conflict of interest to declare.

Authors' Contribution

IBB, LJ and GFI conducted the research, collected samples, and wrote manuscript. GBDJ and CN analysed the data and reviewed the manuscript. SMM, APK and MST provided laboratory assistance. All authors have read and approved the manuscript.

REFERENCES

Abdulhamid, A., Fakai, I. M., Sani, I., Warra, A. A., Bello, F. and Nuhu, B. G. (2013). Extraction, physicochemical characterization and phytochemical screening of *Jatropha curcas* L. seed oil. *Journal of Natural Products and Plant Resources*, 3 (5): 26-30.

Adebowale, K. O. and Adedire, C. O. (2006). Chemical composition and insecticidal properties of the underutilized *Jatropha curcas* seed oil. *African Journal of Biotechnology,* 5 (10): 901-906.

Dimri, M. and Sharma, C. (2004). Effects of sarcoptic mange and its control with oil of Cedrus deodara, Pongamia glabra, *Jatropha curcas* and benzyl benzoate, both with and without ascorbic acid on growing sheep: epidemiology; assessment of clinical, haematological, cell-mediated humoral immune responses and pathology. *Journal of Veterinary Medicine* Series A, 51: 71–78.

Ekpendu, T.O., Anyogo, P., Ityough, D. and Akpa, F. (2000). Nigerian ethnomedicine and medicinal plant flora: The Benue experience - Part 3, *Nigerian Journal of Natural Products and Medicine* 04: 13-22.

Fatnassi, B., Khouja, M. L. and El Ferchichi, O. H. (2014). Larvicidal efficacy of *Jatrophacurcas* L. (Euphorbiaceae) leaf and seed aqueous extract against *Culex pipiens* L. *African Journal of Biotechnology*, 13(26): 2641-2647.

Finney, D. J. (1971). Probit analysis. Cambridge Univ. Press, Cambridge, 333 pp.

George, B. D. J., Ogbogu, V. C., Adekeye, J. O., Kwaga, J. P. K. and Ajanusi, O. J. (2001). Sequential histopathology of the skin of two breeds of sheep to the bites of *Amblyomma variegatum*. *Nigerian Journal of Parasitology,* 22: 149-156.

Ghosh, S., Azhahianambi, P. and Yadav, M. P. (2007). Upcoming and future strategies of tick control: a review. *Journal of Vector Borne Diseases,* 44: 79-89.

Goel, G., Makkar, H. P. S., Francis, G. and Becker, K. (2007). Phorbol esters: Structure, biological activity, biological activity and toxicity in animals. *International Journal of Toxicology,* 26: 279-288.

Gubitz, G. M., Mittelbach, M. and Trabi M. (1999). Exploitation of the tropical oil seed plant *Jatropha curcas* Linn. *Bioresource Technology*, 67: 73-82.

Haas, W. and Mittelbach, M. (2000). Detoxification experiments with seed oil from *Jatropha curcas* L. *Industrial Crops and Products*, 12:111-118.

Igbinosa, O. O., Igbinosa E. O., Aiyegoro, O. A. (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). *African Journal Pharmacy and Pharmacology*, 3(2): 58-62.

Luqman, T. O., Mohammed, A., Ulrike, S. and Jabbar, S. A. (2007). A survey of ticks (Acari:Ixodidae) on cattle, sheep and goats in the Dohuk Governorate, Iraq. *ParasitologyResearch*, 101(2): 179–181.

Mann, A. (2012). Phytochemical constituents and antimicrobial and grain protectant activities of clove basil (*Ocimumgratissimum* L.) grown in Nigeria. *International Journal of Plant Research*, 2(1): 51-58.

Mattioli, R. C. and Cassama, M. (1995). Comparison of Lifecycle in female ticks collected on N'dama and

Zebu cattle. *Tropical Animal Health and Production*, 27: 150-154.

Mbao, J., Clever, K. and Jerekias G. (2022). Quantification of Phorbol-12-myristate 13-acetate in Jatropha seed oil and cake at different stages of fruit maturity. *International Journal of Environmental Studies*, 79(1):88-97.

Moyo, B, Masika, P. J. and Muchenje, V. (2012). Antimicrobial activities of *Moringa oleifera* Lam extract. *African Journal of Biotechnology,* 11: 34-42.

Muhammad, K. O., Nabila, I., Abdulaziz, A., Alam, Z. K., Itabajara, V. J., Tetsuya, T. and Abid, A. (2022). Acaricide resistance in ticks: diagnosis, mechanization and mitigation. *Front Cell Infect Microbiol*, 12: 941831 doi:10.3389/fcimb.2022.941831

Mtshali, M.S., De Waal, D. T. and Mbati, P. A. A. (2004). Sero-epidemiological survey of blood parasites in cattle in the northeastern Free State, South Africa. *Onderstepoort Journal of Veterinary Research*, (71): 67–75.

Natala, A. J., Agyei, A. D. and Awumbila, B. (2002). Oviposition performance of adult *Amblyomma variegatum* (Fabr. 1794) female ticks under different relative humidity conditions. *Bulletin of Animal Health and Production in Africa*, 50: 252-258.

Rajput, Z. I., Hu, S., Chen, W., Arijo, A. G. and Xiao, C. (2006); Review: Importance of ticks and their chemical and immunological control in livestock. *Journal of Zhejiang University Science B*, 7(11): 912-921.

Rechav, Y. and Hay, L. (1992). The effects of nutritional status of rabbits and sheep on their resistance to the ticks *Rhipicephalus evertsi evertsi* and *R. appendiculatus. Experimental and Applied Acarology*, 15: 171–179.

Redfren, R. E., Kelly, T. J., Borkovec, A. B. and Hayes, D. K. (1982). Ecdysteroid titers and moulting abberations in last stages *Oncopeltus* nymphs treated with growth regulators. *Pesticide Biochemistry and Physiology,* 18: 351-356.

Rug, M. and Ruppel, A. (2000). Toxic activities of the plant *Jatropha curcas* against intermediate snail hosts and larvae of Schistosomes. *Tropical Medical International Health*, 5: 423-430.

Schimdt, G. D. and Roberts, L. S. (1989). Foundation of Parasitology: Fourth Edition. St. Louis: Times Mirror/ Mosby College Publishing.

Sonenshine, D. E. (1991). *Biology of Ticks*, New York: Oxford University Press.

Trease, G. E. and Evans, W. C. (2002). Pharmacognosy. 15th Edn. Saunders, pp. 214-393.