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 mainstay 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 LC50 of 2.88mg/ml and LC90 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,
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):

\[
\text{Corrected mortality} (\%) = \frac{M_{\text{observed}} - M_{\text{control}}}{100 - M_{\text{control}}} \times 100
\]

Where; \(M_{\text{control}}\) = Mortality in control  
\(M_{\text{observed}}\) = 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 LCs50 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.

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 Aedide (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

<table>
<thead>
<tr>
<th>Constituent(s)</th>
<th>Test</th>
<th>Reference</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>Molisch</td>
<td>Trease and Evans, 2002</td>
<td>Reddish colour ring</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>Keller-Kiliani</td>
<td>&quot;</td>
<td>Purple, brown colouration</td>
<td>+</td>
</tr>
<tr>
<td>Steroids and triterpenes</td>
<td>Liebermann-Burchard</td>
<td>&quot;</td>
<td>Reddish colouration</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Froth Nazikyan</td>
<td>&quot;</td>
<td>Persistent (honeycomb) froth</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride</td>
<td>&quot;</td>
<td>Brownish blue ppt</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Meyer's</td>
<td>&quot;</td>
<td>Creamy white ppt</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Wagner's</td>
<td>&quot;</td>
<td>Reddish brown ppt</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Dragendoff's</td>
<td>&quot;</td>
<td>Orange, brown ppt</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Shinoda's</td>
<td>&quot;</td>
<td>Yellow colouration</td>
<td>-</td>
</tr>
<tr>
<td>&quot;</td>
<td>Sodium hydroxide</td>
<td>&quot;</td>
<td>Yellow colouration</td>
<td>-</td>
</tr>
<tr>
<td>Free Anthracene Derivative</td>
<td>Bontrager's</td>
<td>&quot;</td>
<td>Yellow colouration</td>
<td>-</td>
</tr>
<tr>
<td>Combined Anthracene Derivative</td>
<td>Modified Bontrager's</td>
<td>&quot;</td>
<td>Yellow colouration</td>
<td>-</td>
</tr>
</tbody>
</table>

+ 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

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

Means in the same row with superscripts are significantly different (p≤0.05).

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

<table>
<thead>
<tr>
<th>Treatment (Exposure) (Time (h))</th>
<th>Control (1% Tween 80)</th>
<th>6.25 (%)</th>
<th>12.5 (%)</th>
<th>25 (%)</th>
<th>50 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>4.09±1.06</td>
<td>8.27±2.67</td>
<td>12.73±1.24</td>
<td>58.79±9.95</td>
<td>63.41±4.82</td>
</tr>
<tr>
<td>12</td>
<td>5.57±0.74</td>
<td>9.13±3.18</td>
<td>23.42±2.86</td>
<td>86.70±4.57</td>
<td>85.13±5.66</td>
</tr>
<tr>
<td>18</td>
<td>5.57±0.74</td>
<td>17.24±3.74</td>
<td>35.81±6.60</td>
<td>93.70±3.80</td>
<td>92.79±3.18</td>
</tr>
<tr>
<td>24</td>
<td>5.57±0.74</td>
<td>29.15±1.26</td>
<td>47.54±8.15</td>
<td>95.50±3.38</td>
<td>97.19±2.06</td>
</tr>
<tr>
<td>48</td>
<td>6.27±0.23</td>
<td>66.26±1.47</td>
<td>76.85±4.89</td>
<td>97.35±1.61</td>
<td>100.0±0.00</td>
</tr>
</tbody>
</table>

Means in the same row with different superscripts are significantly different from the control (p≤0.05).

Table 4: Summary of the LC50 and LC90 of the Methanol Extract of *Jatropha curcas* Seed at Various Exposure Times

<table>
<thead>
<tr>
<th>Duration of Exposure (Time (h))</th>
<th>LC50 (mg/ml)</th>
<th>LC90 (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>21.38</td>
<td>245.47</td>
</tr>
<tr>
<td>12</td>
<td>17.38</td>
<td>169.82</td>
</tr>
<tr>
<td>18</td>
<td>15.13</td>
<td>131.83</td>
</tr>
<tr>
<td>24</td>
<td>13.18</td>
<td>104.71</td>
</tr>
<tr>
<td>48</td>
<td>2.88</td>
<td>6.76</td>
</tr>
</tbody>
</table>

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 Adejide (2006) where 92% inhibition effect was reported. It is speculated that suffocation by oil or lethal chemical poisoning due to Jatropha oil application prevented the adult emergence from the bruchid, C. maculatus. The acaridical 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 acaridical effects. This study was able to establish that Jatropha seed oil extract was concentration and time dependent in its action as an acaricide, exhibiting acaridical activity even at a low concentration of 6.25mg/ml with the acaridical 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 acaridical effect on the larvae of R. (Boophilus) decoloratus. The various concentrations of the extract exhibited acaridical 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 acaridical and other potential uses.

Acknowledgement
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Conflict of Interest
There authors have no conflict of interest to declare.

Authors’ Contribution
IBB, LI 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


