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Extracts of *Jatropha curcas* L. exhibit significant insecticidal and grain protectant effects against maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae)

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Phytochemical composition of leaf extracts as well as biological effects of juice, leaf extracts and seed oil of *Jatropha curcas* against *Sitophilus zeamais* were examined. The study also investigated the inhibition of oviposition, progeny production and grain damage, insecticidal effects and mammalian toxicity of the extracts. Compared to other phytochemicals, the concentration of saponin and cardiac glycoside were higher in the leaf extract. All extracts of *J. curcas* (0 – 100 ppm) investigated showed a dose-dependent inhibition of oviposition, progeny production and promote significant ($P < 0.001$) insect mortality. Grains pre-treated with seed oil produced the highest result for all the parameters. The seed oil (100 ppm) produced 93% ($P < 0.001$) protection against grain damage by *Sitophilus zeamais*. Observable physical deformities were observed in rats administered with graded doses of the seed oil as opposed to other extracts. Administration of a single dose of the extracts produced significant ($P < 0.01$) elevation of serum level of alanine (ALT) and aspartic (AST) transaminases and alkaline phosphatase (ALP) in rats.

Key words: *Jatropha curcas*, *Sitophilus zeamais*, biopesticide, botanical insecticide, phytochemicals, antifeedant.

INTRODUCTION

Although, the bulk of calories consumed globally come from cereals, a considerable quantity of the world total cereal production is lost after harvest (Caliboso, 1983). Post-harvest losses of grains are higher in developing countries compared to developed countries. Adam and Schulten (1987) reported stored grains losses as high as 30% and an average of 8.7% during 3 to 6 months storage period in Nigeria. Pest infestation (insects, birds and rodents), microbial infection, change in moisture content, excessive temperature, poor handling and grain respiration have been implicated as responsible for grain losses in most developing countries (Caliboso, 1983).

However, insect pest infestation has been reported as the major cause of food grain losses in most developing countries (Talukder et al., 2004). Adam and Schulten (1987) and Longstaff (1991) particularly implicated *Sitophilus zeamais* as one of the common grain pests in tropical countries such as Nigeria.

The use of chemical pesticides in the control of storage pests have been the common practice since the 1950s. This, in most cases, involves fumigation with chemicals that kill all life stages of stored product insects in the storage structure. Disadvantages such as insect resistance has been reported for many of these chemical

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pesticides by Donahaye (2000) and Leelaja et al. (2007). Other associated problems with the use of chemical-based pesticides include issues with residual toxicity, environmental pollution, increased cost of chemicals, potential hazards to man and the increased demand for hygienic food supplies. These have stimulated a search for alternative means of storage-pests control.

The use of plant-based materials in protecting stored products is an old-age practice (Talukder, 2006 and Sahayaraj, 2008). Ancient Romans used false hellebore (*Veratrum album*) in the control of rodents while the Chinese were the first to discover the insecticidal activities of *Derris* species (Ahmed and Grainge, 1986). Indian farmers were reported to use extracts of *Azadiracta indica* in control of stored pests (Ahmed and Koppel, 1985) while extracts of *Ocimum suave* and *Eugenia aromatic* were reportedly used as grain protectants in Eastern Africa (Power, 1989). Botanical pesticides are biodegradable and are thus considered safer and more environmentally friendly. It is also believed that the botanical insecticides could replace expensive chemicals that are currently in use in many developing countries. Other factors such as availability, affordability, safety, and cost effectiveness have been cited as justifications for the use of botanical pesticides (Akou-edi, 1983; Isman, 1995; Pavela, 2007).

Insect resistance and contamination of the biosphere associated with large-scale use of synthetic pesticides have also partly necessitated the search for effective biodegradable pesticides with greater selectivity. This awareness has also created a worldwide interest in the discovery of newer insecticides that are non-phytotoxic, nontoxic to mammals, eco-friendly, less prone to pesticide resistance, relatively less expensive, and locally available (Heyde et al., 1984; Hermawan et al., 1997; Dayan et al., 2009). Conventionally, botanical pesticides are classified based on their physiologic activities on the insect (Jacobson, 1989). Many plant derivatives have to been reported to be toxic to stored product insects (Saxena et al., 1988; Obeng-Ofori and Reichmuth, 1997; Park et al., 2003; Isman, 2006; Dubey et al., 2007; Rajendran and Sriranjini, 2008) while others have showed antifeedant (Liu et al., 2002), grain protectant or insect repellent effects (Xie et al., 1995; Boeke et al., 2004; Koul et al., 2008). Saxena et al. (1986) and Asalawam and Adesiyani (2001) reported that some plant derivatives act as reproduction or insect growth inhibitors. These reports partly motivated the investigation of pesticidal effects of *J. curcas* extracts conducted in this study.

Jatropha curcas L. is a widely available tropical plant that often used for fencing by farmers. Nash (2005) reported the use of its seed oil as biofuel and its potential as a biopesticide. However, scientific evidence for the insecticidal effects of extracts of the plant against *Sitophilus zeamidis* or its antifeedant effects are lacking. This study investigated the phytochemistry of the plant, its insecticidal, antifeedant and reproduction-inhibitory of

J. curcas extracts against *S. zeamidis* as well as its mammalian toxicity were conducted.

MATERIALS AND METHODS

Plant material

Fresh leaves, seeds and juice of *Jatropha curcas* L. were collected around Nigerian National Petroleum Corporation (NNPC) depot, Yola, Adamawa State. Experts in Biological Sciences Department of the Federal University of Technology, Yola, carried out botanical identification. Leaves were dried under room temperature prior to pulverization. A mixture of 10% w/v of the ground sample and distilled water was prepared and left to stay overnight after which the suspension was filtered and the filtrate was kept as stock preparation of the leaf extract until when used. The seed oil was extracted from dried seed samples, pulverized by grinding with mortar and pestle. About 20 g of the pulverized sample was extracted with petroleum ether over a period of 10 minutes. The supernatant was filtered using Whatman No 1 filter paper and distilled as described by Adebowale and Adedire (2006). Oil yield was approximately 260 ml/kg plant sample. Serial dilution of the oil was carried out with distilled water to give appropriate dosages needed for the study. Juice from the *Jatropha curcas* was collected by tapping the stem with a knife. The juice was collected in a clean reagent bottle and serially diluted appropriately with distilled water to get various concentrations needed for the study. About 420 ml of juice was extracted from four plants and stored at -20°C until used.

Phytochemical screening of plant material

Phytochemical characterization of the leaf of *J. curcas* was carried out by screening for the presence tannin, phlobatanin, cardiac glycosides, anthraquinones, saponins, steroids, terpenoids and flavonoids. For tannin, 5 g of each portion of plant extract was stirred with 10 ml of distilled water and filtered as described by Trease and Evans (1998). Blue black, green, or blue-green precipitate formed following the addition of few drops of 5% ferric chloride was taken as evidence for the presence of tannins. Deposition of a red precipitate when aqueous solutions of leaf extract was boiled with 1% (v/v) HCl was taken as evidence for the presence of phlobatannin (Trease and Evans, 1998). Salkowski's test, as described by Sofowora (1993), was used to test for cardiac glycosides. Leaf extract (0.5 g) was dissolved in 2 ml of chloroform prior to the careful addition of 1% (v/v) H₂SO₄ to form a lower layer. A reddish-brown colour at the interface was taken as evidence for the cardiac glycoside. Borntrager's test was used for the detection of anthraquinones (Heyde et al., 1984). Plant material (5 g) was shaken with 10 ml benzene and filtered. Ammonia solution (5 ml, 10%) was added to the filtrate and a pink, red, or violet colour formed in the ammoniacal (lower) phase was recorded as an indication of the presence of free anthraquinones. Concentration of saponin, steroids and terpenoids were measured as described by Sofowora (1993). Plant materials (0.5 g) were mixed with acetic anhydride (2 ml) in the presence of concentrated H₂SO₄ (2 ml) to measure the concentration of steroids. For terpenoids, plant materials (0.5 g) re-suspended in distilled water was mixed with chloroform (2 ml) in the presence of concentrated H₂SO₄ (3 ml). Colour change in the presence of re-suspended plant materials and diluted ammonium solution (5 ml) was to estimate the concentration of flavonoids. For tannin, phlobatannin and cardiac glycoside, weight of precipitate formed per gram plant sample was recorded. The intensity of colour change observed for unknown samples were compared spectrophotometrically to the colour of standard solutions of known concentrations of saponin, terpenoids or flavonoids.

Table 1. Phytochemical composition of *J. curcas* leaf extract.

Phytochemical	Concentration (mg/kg)
Alkaloid	23.5 ± 2.31
Tannins	75.9 ± 4.70
Phlobatannins	17.2 ± 6.10
Saponins	124.8 ± 10.52
Terpenoids	41.1 ± 2.41
Flavonoids	64.3 ± 1.13
Cardiac glycosides	131.5 ± 9.23
Anthraquinones	BD
Steroids	62.2 ± 3.32

Values are Mean ± SEM with n = 3. BD = Below detectable limit.

Insect culture and maintenance

Species of *Sitophilus zeamais* used for this study were obtained from field-infested grains in Jimeta, Yola, Nigeria and were identified by experts in Biological Sciences Department of the Federal University of Technology, Yola. Insects were cultured in the laboratory on clean uninfested kilner jars at fluctuating ambient temperature (23 to 27°C) and relative humidity (100 mmHg). The kilner jars were covered with muslin cloth held tightly in place for adequate aeration and to prevent entry or exit of insects. New generations of insects derived from this stock were cultured by infesting clean and uninfested grains with 10 pairs of adult insects to generate the required insect population for the study.

Determination of oviposition deterrent activities of *J. curcas*

Oviposition deterrent activities of the different plant preparations were assessed by coating maize grains with graded doses of the leaf extract, seed oil or juice (0 – 100 ppm) as described by Adedire and Lajide (1999). For each treatment, five treated grains and five untreated grains were arranged alternatively in ring-like manner in a petri-dish coated with paraffin wax (Adedire and Lajide, 1999). Two copulating pairs of insect samples were introduced into each covered petri dish. Eggs laid on each treated and untreated (control) grain seeds were enumerated with the aid of a magnifying lens prior to the demise of the female insects (14 days after treatment). F1 progeny population was determined at Day 42 of the experiment.

Determination of grain protectant activities of *J. curcas*

About 50 g of uninfested maize grains were treated with either leaf extract or seed oil or juice (0 – 100 ppm) of *J. curcas* in a conical flask. Uniform mixing was accomplished by manual agitation with the aid of a glass rod. The seeds were air-dried for 1 h before introducing five couples of adult *Sitophilus zeamais* into the conical flask. The seeds in the control flask were treated with distilled water instead of *J. curcas* oil, juice or leaf extract. Each flask was covered with muslin cloth held firmly in place with a rubber band to prevent escape of the insects or entry of some other insects while ensuring adequate aeration for a period of 12 weeks in an open laboratory kept at ambient temperature (30 ± 2°C) and relative humidity of 75 atmospheres. After twelve weeks, the number of damaged and unaffected seeds were counted and recorded. The numbers of

dead and live insects were also recorded.

Assessment of toxic effects of *J. curcas* extracts

Graded concentrations of the leaf extracts (0 – 100 mg per kg bw), juice and seed oil (0 – 100 µl per kg bw) of *J. curcas* were administered to different groups of Wistar albino rats of average weight of 130 g to assess the effect of a single administration of the different plant preparations. In addition to recording observable behavioural changes, plasma levels of alanine and aspartic transaminases as well as alkaline phosphatase were measured 48 h post administration. Commercially available kits from Randox Laboratories, UK were used for the assays according to the manufacturer's protocol.

Statistical analysis

Results are expressed as mean ± S.E.M. Treatment groups were compared with each other or control using Students t-test. Data on toxicity were also subjected to analysis of variance (ANOVA) followed by Newman-Keuls post-hoc test. Groups of data were considered to be significantly different if P < 0.05. Analysis were performed using GraphPad Prism version 3.0.

RESULTS AND DISCUSSION

Phytochemical screening of *J. curcas*

Phytochemical screening of *J. curcas* showed that the plant had an abundance of saponin and cardiac glycoside (Table 1). The concentrations of tannin, flavonoids, and steroids were observed to be lower than that of saponin but higher than the concentrations observed for alkaloids, phlobatannins, and terpenoids. The concentrations of tannin, flavonoids, and steroids were in the range of 62.2 ± 3.3 to 75.9 ± 4.7 mg/kg to of plant material. The concentration of alkaloids, phlobatannins, and terpenoids was less than 50 mg/kg plant material while the concentration of anthraquinone was below detectable limits.

Data on the phytochemical composition of *J. curcas* provides an insight into the mechanism of actions of *J. curcas*. Phytochemicals, such as tannins, have been reported possess strong activities against several plant pathogens and pests (Mila et al., 1996). Karamanoli et al. (2011) reported that tannin exerts its action by a combination of mechanisms that include iron chelation and enzyme inhibition. Though the exact mechanism behind the observed actions of *J. curcas* is not yet known, the preponderance of tannin in its extract may suggest a role for phytochemical. The concentrations of saponins and cardiac glycosides in the extract of *J. curcas* were significantly higher compared to other phytochemicals. Chaieb (2010) extensively reviewed insecticidal effects of saponins, linking their insecticidal activity their interaction with cholesterol, which results in impaired ecdysteroid synthesis. Al-Rajhy et al. (2003) on the other hand reported evidence for the insecticidal effects of purified cardiac glycosides from *Digitalis*

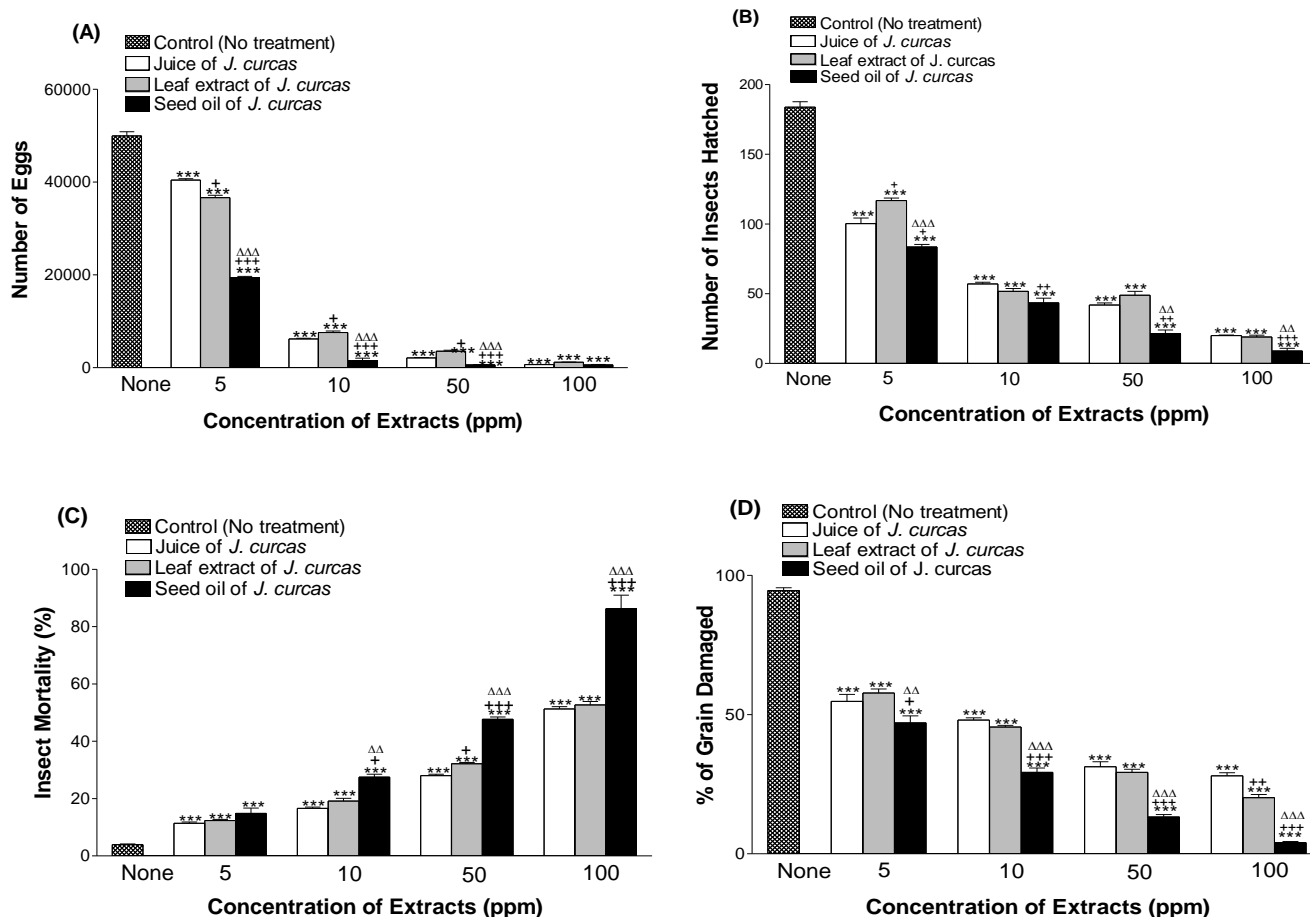


Figure 1. Effects of various extracts of *J. curcas* on (A) oviposition, (B) progeny production, (C) insect mortality and (D) grain damage by *Sitophilus zeamidis*. Values are Mean \pm SEM with $n=4$. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared to control. $^+P<0.05$, $^{++}P<0.01$, $^{+++}P<0.001$ compared to juice of *J. curcas*. $^{\Delta}P<0.05$, $^{\Delta\Delta}P<0.01$, $^{\Delta\Delta\Delta}P<0.001$ compared to leaf extract of *J. curcas*.

purpurea (digitoxin) and *Calotropis procera* against camel tick, *Hyalomma dromedarii*.

Grain protectant and insecticidal activities of *J. curcas* extracts

To assess grain protectant effects of *J. curcas*, different extracts of the plant were tested for their abilities to inhibit oviposition and hatching of *S. zeamidis* eggs. Figure 1A shows that pre-treatment of grains with graded doses of the various extracts of *J. curcas* significantly ($P < 0.001$) prevented oviposition by *S. zeamidis*. The observed effect followed a dose-dependent pattern with the lowest number of eggs laid on grains pre-treated with the highest concentration of plant extracts. At the concentration of 100 ppm, all the extracts almost completely inhibited oviposition by *S. zeamidis* ($>90\%$, $P < 0.001$). However, the inhibitory effects of the leaf extract and juice of *J. curcas* were reduced by 26.7 and 31.4% respectively at 50 ppm. No significant difference was

observed in the oviposition inhibitory effects of the seed oil at concentration ≥ 10 ppm while its effects were reduced by about 50% at 5 ppm ($P < 0.001$). The potency of other extracts were significantly lower at concentrations ≤ 10 ppm ($P < 0.001$, Figure 1A).

Expectedly, results obtained for number of eggs hatched showed that all the different plant preparations significantly prevented egg hatching in a concentration-dependent manner (Figure 1B). Grains pre-treated with the seed oil produced the lowest number of hatched eggs at all the concentrations tested ($P < 0.001$) compared to other extracts. No significant difference was observed in the effects of leaf extracts and *J. curcas* juice at concentrations >5 ppm. At 5 ppm, egg hatching reduced by 44.3%, 36.3% and 48.5% in grains pre-treated with the leaf extract, juice and seed oil of *J. curcas* respectively ($P < 0.001$, Figure 1B). At the highest concentrations of seed oil, 92.3% reduction in egg hatching was observed compared to 64.3 and 66.2% reduction observed in the presence of leaf extract and juice of the plant respectively. Results obtained in this

study revealed the superior actions of the seed oil compared to other extracts. Several studies have previously reported that plant parts, oil, extracts, and powder mixed with grain reduced insect oviposition, egg hatchability, postembryonic development, and progeny production (Saxena et al., 1986; Asawalam and Adesiyun, 2001; Schmidt et al., 1991). Though there is scientific evidence that plant derivative, including essential oils could inhibit progeny production by causing insect egg mortality (Obeng-Ofori, 1997), it is not yet clear if the reduction in progeny production observed in this study is caused by insect egg mortality. Other reports have shown that some plant extracts showed harmful effects on insect growth and development. These effects include from reduced larval, pupal and adult weight, prolonged larval and pupal period, reduced pupal recovery and impaired rate of adult emergence (Khanam et al., 1990; Boeke et al., 2004; Koul et al., 2008).

Significant ($P < 0.001$) insect mortality was observed in grains pre-treated with juice, seed oil and leaf extracts of *J. curcas* over a concentration range of 0 to 100 ppm (Figure 1C) for 42 days. Figure 1C shows that at the lowest concentration of 5 ppm, the effect observed for the three different extracts were not significantly different from each other. However, insect mortality increased with the concentration of extract. At concentrations >10 ppm, the seed oil showed significantly ($P < 0.001$) higher insecticidal effects compared to other extracts (Figure 1C). Mortality of 100% was recorded in grains pre-treated with the seed oil compared to 58.9% and 55.6% mortality observed for grains pre-treated with the leaf extract and juice of *J. curcas* respectively. Based on the results obtained in this study, estimated LC50 values for leaf extract, juice and seed oil of *J. curcas* was 78, 75 and 42 ppm respectively.

These insecticidal effects were similar to what has been previously reported for many other plant materials. For instance, Pascual-Villalobos and Robledo (1998) showed evidence for the actions of *Anabasis hispanica*, *Senecio lopezii*, *Bellardia trixago*, and *Asphodelus fistulosus* against *Tribolium castaneum*. Huang et al. (2000) reported the potency of essential oils from garlic, *Allium sativum*, as toxicant and fumigants against *Sitophilus zeamais* and *T. castaneum*. Essential oils from anise, cumin, eucalyptus, oregano, and rosemary have also been reported to exhibit potent insecticidal effects against *Tribolium confusum* and *Ephestia kuehniell* (Tunc, 2000).

In addition to aforementioned effects, extracts of *J. curcas* exhibited antifeedant and grain protectant effects with the seed oil producing the best results. Grain protectant effects were assessed by estimating percentage of damaged grain 42-days after pre-treatment with the various extracts. Pre-treatment of grains with 5 ppm of seed oil prevented grain damage by 53% ($P < 0.001$) compared to 43% ($P < 0.001$) and 40% ($P < 0.001$) observed for grains pre-treated with the same

concentration of leaf extract and juice of *J. curcas* respectively (Figure 1D). At the highest concentration of extracts tested, 93% ($P < 0.001$) of grains pre-treated with seed oil were protected from damage compared to 62% ($P < 0.001$) and 82% ($P < 0.001$) observed for grains pre-treated with leaf extract and juice of *J. curcas* respectively.

Previously, the root bark of *Dictamnus dasycarpus* has been reported to show significant feeding deterrence against two stored-product insects (Liu et al., 2002) while essential oil of *Artemisia annua* was found as a repellent against *Tribolium castaneum* and *Callosobruchus maculatus* (Tripathi et al., 2004). Oil extracted from *Azadirachta indica* and its kernel powder have also been previously linked with effective grain protection against stored grain insect pests like *Sitophilus oryzae*, *T. castaneum*, *Rhyzopertha dominica*, and *Callosobruchus chinensi* (Pereira and Wohlgenuth, 1982).

Toxicity assessment of *J. curcas* extracts

Physical observation following a single administration of graded doses (10 – 100 mg/kg bw) of leaf extracts and juice of *J. curcas* to Wistar albino rats revealed no immediate or gradual adverse effects up to 48 h post-administration. However, rats administered with the seed oil showed observable restriction in movement within 24 h of administration. Observable deformity (paralysis of lower limbs) and death were also recorded in the same group 72 h after the administration of the seed oil.

Furthermore, effects of administration of graded doses (10 – 100 mg/kg bw) of the various extracts of the *J. curcas* on plasma levels of some biochemical markers of toxicity were assessed and reported in Table 2. No significant elevation in serum levels of aspartic transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were observed in animals administered with juice at a dose of 10 mg/kg bw. However, beyond this concentration, plasma levels of ALT, AST and ALP were significant ($P < 0.05$) elevated in all treated animals. The trend observed in animals treated with the leaf extracts and seed oil showed a significant increase in plasma levels of the enzymes at all the doses administered (Table 2).

These results showed that the seed oil is toxic to rodents at high concentrations. Elevated plasma levels of transaminases (AST and ALT) and alkaline phosphatase (ALP) indicated hepatotoxicity (Ojo et al., 2005). This suggests that in addition to acting as an insecticide, the seed oil could also be used as a rodenticide; offering a multi-faceted protection for stored grains. This could also partly be advantageous in pre-treating seeds for planting particularly in Africa and some other parts of the world where rodent destruction of planted seedlings has been observed as a major problem. However, the observed toxicity of *J. curcas* extracts in rat is also an indication of

Table 2. Effects of different extracts of *J. curcas* of serum levels of biochemical markers of hepatotoxicity in rats.

Groups	AST	ALT	ALP
Juice of <i>J. curcas</i>			
Control	11.0 ±0.58	23.3 ±0.42	14.0 ±0.82
10 mg/kg body weight	12.0 ±0.82	23.8 ±0.31	16.00 ±0.82
50 mg/kg body weight	17.3 ±2.06*	41.0 ±0.59**	20.3 ±0.85***
100 mg/kg body weight	22.8 ±0.95***	52.5 ±0.61***	30.0 ±0.82***
Leaf extract of <i>J. curcas</i>			
Control	11.0 ±0.58	23.3 ±0.42	14.0 ±0.82
10 mg/kg body weight	14.0 ±0.82*	31.3 ±0.33**	21.3 ±1.25***
50 mg/kg body weight	24.5 ±2.94***	44.3 ±0.48***	27.3 ±1.70***
100 mg/kg body weight	28.8 ±1.25***	63.7 ±1.45***	43.0 ±2.55***
Seed oil of <i>J. curcas</i>			
Control	11.0 ±0.58	23.3 ±0.42	14.0 ±0.82
10 mg/kg body weight	27.3 ±2.50***	46.3 ±0.36***	61.8 ±2.02***
50 mg/kg body weight	40.0 ±4.32***	55.5 ±1.24***	73.3 ±4.11***
100 mg/kg body weight	68.0 ±2.16***	110.2 ±5.31***	92.0 ±2.94***

AST=Aspartic transaminase, ALT = Alanine transaminase, ALP = Alkaline phosphatase. Values are Mean ± SEM with n =4. ***P < 0.001, **P < 0.01 and *P < 0.05 compared to control.

possible toxicity in humans.

Conclusion

Literature on the grain protectant and insecticidal activities of *J. curcas* extract is lacking and this study has opened an interesting line of research towards a novel use for *J. curcas* (most often considered for bio-fuel production). Future studies will be dedicated towards the structural characterization of the compound as well as studies of the biodegradation of its residues to examine its advantages over chemical pesticides that are currently in use.

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