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RESEARCH ARTICLE

COMPARATIVE STUDY ON INSECTICIDAL ACTIVITY OF *JATROPHA CURCAS* OIL, SEED CAKE AND SEED CAKE EXTRACTS AGAINST *ANOPLOLEPIS GRACILIPES*

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ABSTRACT

The invasive yellow crazy ant, *Anoplolepis gracilipes* is commonly found in gardens and forests of tropical and subtropical locations. These ants are a problematic pest in both urban and rural environment because of their extreme foraging behavior. The control of these insect pests is by using synthetic pesticides. The use of plant derived compounds is now gaining importance, as they are found to be safe to the environment. The use of non-edible oil seeds of *Jatropha curcas* containing phorbol esters has been considered as a potential source of biopesticide. The present study evaluates the potential insecticidal activity of *Jatropha* seed oil and extracts from seed cake on *A. gracilipes*. The *Jatropha* seed oil (25, 50 and 100%), *Jatropha* seed cake (2, 4 and 6 g), *Jatropha* seed cake cold water extract and hot water extract (5, 10 and 20%) were evaluated for insecticidal activity by studying the mortality rates of the adult workers of *A. gracilipes.* The bioassay studies indicated that, 100% mortality was observed using *Jatropha* seed oil at 50 and 100% concentrations. The hot water and cold water extracts of *Jatropha* seed cake at 5, 10 and 20% levels exhibited increase in mortality with increasing concentrations and exposures. However, the ants were susceptible to the hot water extract than the cold water seed cake extract after 9 h exposure.

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INTRODUCTION

Jatropha curcas is a physic nut shrub within the family Euphorbiaceae (Carels, 2009). It has been considered as a "miracle tree" since seed oil can be used as an alternative fuel, a substitute for diesel oil (Heller, 1996; Kumar *et al*., 2008). Many parts of the plant have a wide range of applications in medicine for treating various ailments. The extracts of *Jatropha* oil were shown to have insecticidal activities against desert locusts (Bashir *et al*., 2013) and termites (Neelu Singh *et al*., 2008). Apart from this, the extracts from seeds and leaves of *Jatropha* have also been reported to exhibit nematicidal, fungicidal, molluscicidal (Solsoloy and Solsoloy, 1997; Liu *et al*., 1997) effects. Among these extracts, the main biocidal action has been ascribed to the phorbol ester (tetracyclic diterpenoid) fraction (Makkar *et al*., 1997; Devappa *et al*., 2011) from seed oil. Phorbol esters mimic the action of diacylglycerols, activator of protein kinase C (PKC) that interferes with different signal transduction pathways and other cellular metabolic activities (Bershadsky *et al*., 1990; Goel *et al*., 2007). However, there are no reports on the insecticidal potency of the bioactives from *Jatropha* against yellow crazy ants, which are generally known to have direct adverse effects on many animals, including vertebrates and invertebrates (Feare 1999; Hill *et al*., 2003). The yellow crazy ant, (*Anoplolepis gracilipes*) is one among the species listed in "100 of the World's Worst Invasive Alien Species" published by the Invasive Species Group (ISSG 2006). They are known to nest under leaf litter and on the branches of trees or holes in the ground. They are extremely competitive in foraging over every surface within its territory, including forests trees (Room 1975; O'Dowd *et al*., 1999). It grabbed the world's attention due to its deadly attacks on nesting birds in the Seychelles (Feare 1999; Hill *et al*., 2003) and on the endemic crabs of Christmas Island (Green *et al*., 1999; O'Dowd *et al*., 1999; O'Dowd *et al*., 2003). The control of ants generally involves bait application and chemical treatments in the form of use of synthetic insecticides.

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However, application of synthetic pesticides of systemic and non-systemic origins near human habitations would increase the chance of getting exposure to these pesticides. Nowadays, plant bioactive compounds are more preferred over synthetic compounds considering their adverse effects on the ecosystem. Hence, the objective of the present study was to examine the insecticidal activity of *Jatropha* seed oil and seed cake extracts against *A. gracilipes*.

MATERIALS AND METHODS

Jatropha curcas oil

The seeds of *J. curcas* were procured from the local market. The crude oil was extracted from *J. curcas* seeds using a hydraulic press. Dilution of the crude oil was carried out at three different concentrations (25, 50 and 100%) using hexane solvent (b.p.60°C). The *J.curcas* oil was tested for their insecticidal activity against ants at various concentrations with equal number of controls.

Jatropha curcas seed cake

After the extraction of oil from the seeds by hydraulic pressing, the seed cake was powdered and used for bioassay. The insecticidal activity of *J. curcas* seed cake on *A.gracilipes* was tested at 2, 4 and 6 g levels along with controls.

Cold and hot water extracts of *J.curcas* seed cake

Cold water extracts (5, 10 and 20%) were prepared by dispensing 5, 10 and 20 g of dried seed cake in 100 ml of distilled water and kept in the refrigerator overnight with intermittent shaking. The cold water extracts were then filtered through Whatman No.1 filter paper. Hot water extracts (5, 10 and 20%) were prepared by adding 5, 10 and 20 g in 100 ml of distilled water respectively and were boiled for 20 min with intermittent mixing and the extract was filtered through Whatman No.1 filter paper.

Test insects

Adult worker ants of *Anoplolepis gracilipes* were collected from the tree nests on the campus of Central Food Technological Research Institute, Mysore, Karnataka, India. Prior to the bioassay experiments, the worker ants were held inside glass bottles of 2 L capacity at ambient experimental conditions (i.e at 25–27 °C) for 24 h. The collected adult ants were anaesthetized using a small ball of cotton with 1 ml of diethyl ether for 30 sec. The required number of ants was then tapped into the Petri dishes in batches for carrying out bioassays.

Contact toxicity studies

Bioassays were performed to evaluate the insecticidal activity of *J. curcas* oil (25, 50 and 100%), seed cake powder (2, 4 and 6g), cold and hot aqueous extracts (5, 10 and 20%) of seed cake. Experiments were set up in Petri dishes (9 cm dia) to assess the contact toxicity of *J.curcas* seed oil and seed cake extracts on the adults of yellow crazy ants. Three replicates were maintained for each concentration, and 1 ml of the test sample/extract of each concentration was loaded onto filter paper (9 cm) discs. The treated filter paper discs were placed at the bottom of each Petri dish. The Petri dish containing filter paper without any extract/solvent and a Petri dish with filter paper treated with solvent served as controls for *Jatropha* oil bioassay, while the filter paper treated with distilled water only was used as control for cold and hot aqueous extracts. The filter discs were air dried at room temperature to evaporate the solvent. Ten ants were then sorted using a soft bird feather into each Petri dish. The Petri plates with covers were then placed in an incubator at 25±1°C. Data for mortality was recorded every 3 h up to 12 h, and the end point mortality was taken after 18 h. For studying differences between groups, data were analyzed by student t-test or analysis of variance (ANOVA). The experimental data were statistically analyzed by Duncan's new multiple range test to determine significant differences in each treatment ($p<0.05$).

Estimation of phorbol esters

5 g of *Jatropha* oil was taken in a conical flask of 150 ml capacity, and 20 ml of HPLC grade methanol was added and kept in an orbital shaker at 250 rpm for 10 min. The upper methanol phase was taken by using a separating funnel. For residual oil, 20 ml of methanol was added, and the process was repeated 4 times and the extracts were pooled. 20 g of seed cake was added to cold water and hot water for extraction as mentioned above. 5 ml of filtered extract was taken, and 100 ml of methanol was added (HPLC grade) and was kept on an orbital shaker at 250 rpm for 1 h. 20 ml of methanol was added to a flask containing 5 g of *Jatropha* seed cake sample and kept for shaking in an orbital shaker at 250 rpm for 10 min. The extract was filtered through Whatman No. 1 filter paper, and the filtrate was collected. The extraction process was repeated four times. The pooled methanol extracts from oil, aqueous extract and seed cake were concentrated by using a vacuum flash rotary evaporator at 65°C. The phorbol esters in the oil, aqueous extracts and seed cake were estimated using HPLC (Shimadzu-LC10A, Kyoto, Japan) as per the procedure described by Sharath *et al*. (2004).

RESULTS

The *Jatropha* seed oil, cake and cake extracts were analyzed for the presence of phorbol esters. Phorbol esters are the polycyclic compounds present in the *Jatropha* oil, seedcake and seedcake extracts (Fig.1). The quantity of phorbol esters present in the seed oil was 3815 µg/ml oil; seed cake was 770 µg/g seed cake; hot water extract was 47.5 µg/ml and cold water extract was 41.5 µg/ml. The insecticidal properties of extracts of *J. curcas* obtained from the seeds were tested against the adults of *A. gracilipes*. The mortality of the adults observed followed a dose -dependent pattern over individual exposures. At 100% concentration of *J. curcas* seed oil, 96.7% mortality was observed in the treated ants over 18 h exposure (Table 1). All the tested extracts induced mortality of the adults from 6^{th} h onwards; however, a significant difference in mortality response between the individual concentrations was observed only in *Jatropha* seedcake extract.

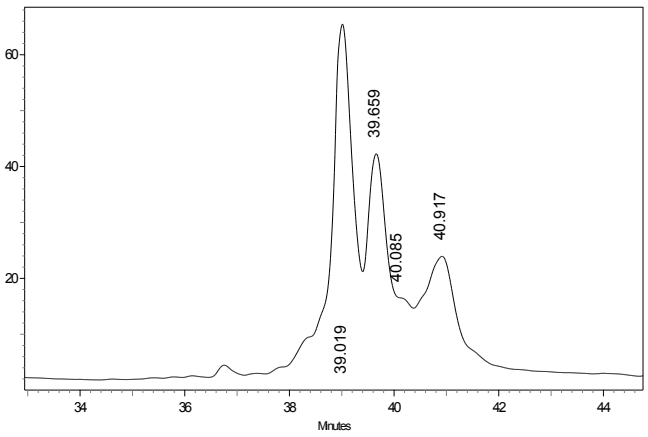

Fig. 1. HPLC chromatogram of phorbol esters from *Jatropha curcas* seed oil

Table 1. Mortality percentage of *A. gracilipes* with different concentrations of *Jatropha* seed oil

Treatment	Duration (h)				
	3	6	9	12	18
Control	$0.00 \pm 0.0_a$	0.00 ± 0.0^a	0.00 ± 0.0^a	0.00 ± 0.0^a	0.00 ± 0.0^a
Hexane	0.00 ± 0.0^b	0.00 ± 0.0^a	0.00 ± 0.0^a	0.00 ± 0.0^a	0.00 ± 0.0^a
25 %	0.00 ± 0.0^c	0.00 ± 0.0^a	6.67 ± 5.77^a	10.00 ± 0.0^a	66.67 ± 5.77^b
50%	0.00 ± 0.0^d	3.33 ± 5.77^a	6.67 ± 5.77^a	43.33 ± 20.81^b	90.00 ± 10.0^c
100%	0.00 ± 0.0^e	10.00 ± 10.0^a	40.00 ± 10.0^b	73.33 ± 5.77^c	96.66 ± 5.77^c
F-Value	--	2.125	25.20	33.321	203.70
P value	--	0.152	0.00	00.000	0.00

The means followed by the same letter in the same column are not significantly different at (P=0.05) according to Duncan multiple range test.
SE±= Standard Error, F-value, P value for One way Anova Test.

Table 2. Mortality percentage of *A. gracilipes* with different concentrations of *Jatropha* seed cake

Treatment	Duration (h)				
	3	6	9	12	18
Control	$0.00 \pm 0.0_a$	0.00 ± 0.0^a	0.00 ± 0.0^a	6.67 ± 5.77^a	16.67 ± 5.77^a
2 g	0.00 ± 0.0^b	20.00 ± 0.0^b	50.00 ± 10.00^b	76.67 ± 5.77^b	100.00 ± 0.00^b
4 g	0.00 ± 0.0^c	20.00 ± 0.00^c	56.67 ± 15.27^b	76.67 ± 5.77^b	100.00 ± 0.00^b
6 g	0.00 ± 0.0^d	30.00 ± 0.00^d	66.67 ± 5.77^b	93.33 ± 5.77^c	100.00 ± 0.00^b
F-Value	----	-----	28.848	134.00	625.00
P value	----	----	0.000	0.00	0.00

The means followed by the same letter in the same column are not significantly different at (P=0.05) according to Duncan multiple range test.
SE±= Standard Error, F-value, P value for One way Anova Test.

Table 3. Mortality percentage of *A. gracilipes* with different concentrations of hot water extract of *Jatropha* seed cake

Treatment	Duration (h)				
	3	6	9	12	18
Control	0.00 ± 0.0^a	0.00 ± 0.0^a	0.00 ± 0.0^a	0.00 ± 0.00	00.00 ± 0.00^a
Distilled H_20	0.00 ± 0.0^b	0.00 ± 0.0^a	00.00 ± 00.00^a	0.00 ± 0.00^a	00.00 ± 0.00^a
5 g	0.00 ± 0.0^c	0.00 ± 0.00^a	3.33 ± 10.00^a	46.67 ± 5.77^b	60.00 ± 0.00^b
10 g	0.00 ± 0.0^d	3.33 ± 5.77^a	6.67 ± 5.77^a	60.0 ± 3.33^c	83.33 ± 6.67^c
20 g	0.00 ± 0.0^e	6.67 ± 5.77^a	20.00 ± 5.77^b	66.67 ± 3.33^d	90.00 ± 0.00^c
F-Value	-----	2.00	6.200	237.00	218.125
P value	0.171	0.009	0.00	0.000	0.000

The means followed by the same letter in the same column are not significantly different at (P=0.05) according to Duncan multiple range test.
SE±= Standard Error; F-value, P value for One way Anova Test.

Table 4. Mortality percentage of *A. gracilipes* with different concentrations of cold water extract of *Jatropha* seed cake

Treatment	Duration (h)				
	3	6	9	12	18
Control	0.00 ± 0.0^a	0.00 ± 0.0^a	0.00 ± 0.0^a	0.00 ± 0.00^a	0.00 ± 0.00^a
Distilled H_20	0.00 ± 0.0^b	0.00 ± 0.0^a	0.00 ± 00.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
5 g	0.00 ± 0.0^c	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	3.33 ± 5.77^a
10 g	0.00 ± 0.0^d	3.33 ± 5.77^a	6.67 ± 5.77^{ab}	16.67 ± 11.54^b	33.33 ± 15.27^b
20 g	0.00 ± 0.0^e	6.67 ± 5.77^a	13.33 ± 11.54^b	36.67 ± 15.27^b	70.00 ± 26.45^b
F-value	----	2.00	3.200	10.773	14.534
P-value	-----	0.171	0.062	0.001	0.000

The means followed by the same letter in the same column are not significantly different at (P=0.05) according to Duncan multiple range test.
SE±= Standard Error; F-value, P value for One way Anova Test.

Table 5. Paired t-test between hot water extract and cold water extract of *Jatropha* seed cake

Treatments	Paired Differences					
	Mean ± SEm	95 % Confidence interval of the Difference		t	df	Sig. (2 tailed)
		lower	Upper			
9 h HWE -9h CWE	2.00 ±2.79	-3.993	7.993	0.716	14	0.486
12 h HWE-12h CWE	24.00 ±5.67	11.831	36.168	4.230	14	0.001
18h HWE-18 h CWE	25.33 ± 7.16	9.971	40.695	3.537	14	0.003

*HWE- Hot water extract; CWE- Cold water extract.

All the treatments were on par at the end of 6 h, except for *Jatropha* seedcake, which showed differences in their toxicity response (P=0.05, Duncan multiple range test) between 2g (20 %), 4g (20 %) and 6g (30 %) concentrations in comparison to the control (Table 2). At a low level of 2g seed cake powder, 100 % mortality of ants was observed in the *J.curcas* seed cake treatment over 18 h (Table 2), on the other hand, a significant increase in mortality response was noticed between the test concentrations (i.e. 5g, 10g and 20 g) after 12 h hot water seed cake extract treatment (Table 3). The ants responded well to all the concentrations of aqueous *J.curcas* seedcake extract from 6 h exposure (p=0.05, Duncan multiple range test) in comparison to control. Verma *et al*. (2011) have reported that the phorbol esters caused100% mortality of termites, *O. obesus* over 12 h exposure.

The insecticidal activities of *J.curcas* oil, seed cake, hot water and cold water seed cake extracts tested were evaluated against *A. gracilipes* adults by direct contact application method. It is evident that, among the cold and hot water extracts, hot water extract gave better mortality results in ants irrespective of the exposure period (Table 3 and 4). Increase in toxicity with increasing concentration and exposure indicated that ants were significantly susceptible to the hot water extract than the cold water seedcake extract after 9h (m= 2.00; SD=10.8; t=0.716; df= 14; P= 0.486), 12 hrs (m= 24.00; SD=21.9; t=4.23; df= 14; P= 0.01) and 18 hrs (m= 25.33; SD=27.7; t=3.53; df= 14; P= 0.003) of treatment (Table 5).

DISCUSSION

Sharma *et al*. (2011) confirmed that cold water extracts were more effective than the hot water extracts for termites. Cold water extracts of neem cake caused 100% mortality against termites at 72 h. Our results suggested that, 100 % concentration showed highest mortality (96.7 %) in *Jatropha* oil while *Jatropha* seed cake powder recorded maximum mortality (100%) at 2g concentration over 18 h exposure. Solsoloy and Solsoloy (1997) reported that the crude oil from *J.curcas* had better contact toxicity to corn weevil, *Callosobruchus chinensis* and bean weevil, *Sitophilus zeamais*. After 18 h, 20 g seed cake extracts resulted in 90 and 70 per cent mortalities in hot and cold water extracts respectively. Apart from contact toxicity, *J. curcas* extracts may also invariably interrupt the migration of colonies entering human habitations. The active components of botanicals having insecticidal activity have a high potential for the preparation of biopesticidal formulations. *A. gracilipes* are considered as the problematic pests in both urban and rural environment due to their high foraging behavior. The present study results demonstrate that, yellow crazy ant, *A. gracilipes* responded well to all the extracts of *J. curcas,* while the seed oil was highly effective among them implicating the potential use of *J. curcas* extracts for control of this species. Further studies could explore the commercial exploitation of plant derived bio actives of non edible *J. curcas* seed oil for insect control in an environmental safe manner.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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