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Jyothna B
Microbiology and Fermentation Technology Department, CSIR-Central Food Technological Research Institute, Mysore, Karnataka 570 020, India.

Rajaram V
Microbiology and Fermentation Technology Department, CSIR-Central Food Technological Research Institute, Mysore, Karnataka 570 020, India.

Manivannan S
Food Protectants & Infestation Control, CSIR-Central Food Technological Research Institute, Mysore, Karnataka 570 020, India.

Somashekar D
Microbiology and Fermentation Technology Department, CSIR-Central Food Technological Research Institute, Mysore, Karnataka 570 020, India.

Correspondence
Somashekar D
Microbiology and Fermentation Technology Department, CSIR-Central Food Technological Research Institute, Mysore, Karnataka 570 020, India.

Feeding deterrence and insecticidal activity of phorbol esters in *Jatropha curcas* seed oil against *Dinoderus minutus*. (Coleoptera: Bostrychidae)

Jyothna B, Rajaram V, Manivannan S, Somashekar D

Abstract
Dinoderus minutus (Coleoptera: Bostrychidae) is a serious pest of bamboos in the tropical regions of the world. The contact toxicity of *J. curcas* oil was evaluated against *D. minutus* using 20, 40, 50 and 60% concentration. The results showed that 60% *Jatropha* seed oil caused 100% mortality over 96 h exposure. The mortality of bamboo borers was due to bioactive compounds like phorbol esters present in *Jatropha* seed oil. The bamboo blocks (5x 3.5cm^2) were treated with 60 and 100% *Jatropha* seed oil to examine the feeding deterrence and mortality of *D. minutus.* The per cent reduction in the weight loss of bamboo blocks owing to *Jatropha* seed oil treatments at 60 and 100% concentrations in comparison to control were 15.69 and 9.80 percent respectively. The maximum wood protection against *D. minutus* was observed at 100% *Jatropha* seed oil concentration.

Keywords: *Jatropha curcas* oil, *Dinoderus minutus,* Phorbol ester, feeding deterrence

Introduction
The bamboo borer, *Dinoderus minutus* is a wood boring beetle that severely damages bamboo (*Bambusa* sp.) plants and bamboo products. These are problematic pests threatening agroforestry and urban environment. The adult beetles burrow into bamboo culms through wounds, cracks and cut ends, and make tunnels along the fibrovascular tissues of the culms. The damaged part of the culm becomes powdery, and the powder gets extruded from the beetle hole. Large populations of borers will leave numerous tunnels in the culm, leading to powdering and loss of strength of bamboo culms. Bamboos form the single most important item of forest produce used by the rural communities for cradle to the coffin in Asia and the Pacific [15]. Bamboo is a valuable forest resource in many countries of Asia. Over 1,200 bamboo species, belonging to 75 genera, are reported to occur in the world [20]. Insects that cause damage to felled culms and products are considered as serious pests in the Asian bamboo industry [21]. Bamboos are associated with the mainstay of rural life and thus form an integral part of entire South East India. It has a wide range of distribution forming an under storey in several forest types and the tropical moist deciduous and semi-evergreen regions of North Eastern parts [18]. Freshly felled bamboos are prone to attack by ghoon borers, mainly *D. minutus*. Roonwal and Chatterjee, reported that bamboo stacks when sprayed with 0.33% BHC in Kerosene, resulted in effective control of *D. minutus* [13]. The use of synthetic pyrethroid, deltamethrin was considered to be the effective means of control against *D. minutus* in United States and Europe [4].
Jatropha curcas is a non-edible oil seed plant widely cultivated in many tropical and subtropical countries as a hedge plant and also a source of biodiesel. It is commonly known as physic nut and grown as a biodiesel plant with seeds containing 30-40% oil. *Jatropha* grows wild in many parts of India, and it can grow in drought and infertile soil conditions. *Jatropha* oil has high saponification value and hence it is used in soap making industries. Extracts from *J. curcas* seeds and leaves have shown molluscicidal and insecticidal properties [7, 17, 19]. Phorbol esters are considered to be the most biologically active compounds present in various parts of *J. curcas* plant [3]. The *Jatropha* seed oil is not used for edible purpose due to the presence of antinutritional factors like phorbol esters. Considering the economic importance of *D. minutus* in bamboo and environmental risks involved with chemical pesticides, plant based biocidal compounds are preferred. The present study aimed to assess the insecticidal potential of *Jatropha* seed oil against the economically important pest *D. minutus* and also to examine the feeding deterrence of the pest when bamboo culms were treated with *Jatropha* oil.

2. Materials and Methods

All experiments in this study were conducted on the campus of CFTRI, Mysore in the laboratory environment. The study was conducted during the period of February to April.

Extraction of *J. curcas* seed oil

The seeds of *Jatropha* were procured from the local market. The crude oil was extracted from *Jatropha* seeds using a hydraulic press. Dilution of the crude oil was carried out using hexane solvent (b.p.63 °C). The *Jatropha* oil with various concentrations (20, 40, 50 and 60%) was tested for their insecticidal activity against bamboo borer along with untreated controls.

Test Insects

Infested bamboo logs were procured from local market. The adults of *D. minutus* were collected carefully from the infested bamboo logs using a soft paint brush, and they were transferred into a glass bottles containing small pieces of bamboo as the food source. They were acclimatized for 24 h in laboratory conditions before use.

Insecticidal bioassay experiments

Bioassays were performed to evaluate the insecticidal activity of *Jatropha* seed oil at 20, 40, 50 and 60% concentrations. Experiments were set up in Petri dishes (dia 9 cm) to assess the effect of contact toxicity of the *Jatropha* seed oil on *D. minutus*. Three replicates were maintained for each concentration, and 1 ml of each concentration was spread on Whatman No. 1 filter paper (dia 9 cm) placed in each Petri dish. The Petri dish with filter paper containing solvent and the other set with only filter paper served as controls for the bioassay experiment. After loading the different concentration of the seed oil, the filter papers were air dried at room temperature to evaporate the solvent. Fifteen active adults of *D. minutus* were then put into each Petri dishes. All the Petri dishes were held inside an incubator at 25±1°C. The percentage mortality of the adults was recorded at regular intervals till 96 h. The per cent mortality was calculated using the following formula:

$$\frac{\text{Number of dead insects}}{\text{Number of insects taken}} \times 100 = \text{Mortality \%}$$

Feeding deterrence bioassay

Test blocks of healthy non-infested, bamboo wood measuring 5x3.5cm^2 were prepared for insects as feed. The contact toxicity study of *Jatropha* oil against *D. minutus* at the lower concentration indicated that lesser mortality of the insects. Hence, the higher concentration of *Jatropha* seed oil was used for the feeding deterrence bioassay. The test pieces were dipped in 60% and 100% *Jatropha* seed oil for 12 h and were dried at room temperature for one hour. The treated test blocks were placed inside individual plastic boxes in batches. The untreated test blocks were used as the control. About 25 adults of *D. minutus* were introduced into each plastic box containing bamboo blocks and were held at rearing conditions of 25±1 °C. The data on the per cent weight loss of the treated and untreated blocks were recorded after 72 h. The data on the mortality adults of *D. minutus* over feeding was recorded at regular intervals of 6 h till 72 h.

Quantification of phorbol esters in *Jatropha* oil

5 g of *Jatropha* oil was weighed in a clean dried 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. The pooled methanol extracts from oil were concentrated by using a vacuum flash rotary evaporator at 65 °C. The phorbol esters in the oil were estimated using HPLC (Shimadzu-LC10A, Kyoto, Japan) as per the procedure described by Sharath *et al.* [16]. The phorbol ester content is expressed as equivalents to standard Phorbol-12 myristate-13-acetate.

Experimental design and data Analysis

Randomized Complete Block Design (RBCD) was used as the experimental design used for the toxicity and feeding studies in *D. minutus* since adult insects of different age groups were used. The data were analyzed using Analysis of variance (Anova) to assess the differences in mortality responses between the test concentrations, while the differences among the treatment means were compared by Duncan's Multiple Range Test at 5% level.

3. Results and Discussion

The insecticidal properties of *Jatropha* oil containing phorbol esters were evaluated against the adults of bamboo borer, *D. minutus* at four different concentrations *viz.*, 20, 40, 50 and 60%. Phorbol esters are the insecticidal compounds present in the *Jatropha* oil (Fig.1). The concentration of phorbol esters in *Jatropha* seed oil used in the experiment was 3815 µg/ml of oil. The insects were observed for mortality from 3 h; however, no death was evident till 12 h exposure indicating the tolerance of *D. minutus* adults at shorter exposures (Table 1). The results showed that the adults were more susceptible to increasing concentrations of *Jatropha* seed oil with long exposure time. 100% mortality of the adults was noticed at a concentration of 60% over 96 h exposure. No mortality was detected in the blank and solvent control till 24h. The toxicity of phorbol esters may be due to the interference with the normal functions of the metabolic activities of insects [5].

A significant increase in the mortality response was observed between all the treatments and untreated control from 24 h to 96h exposure ($p < 0.05$, Duncan multiple range test). The natural mortality of the adults was observed from 48 h exposure in the control as adults of random age groups were used for the bioassay experiments. After 36 h, a significant increase in mortality response was observed between concentrations tested (One way Anova (F (5, 12) =15.045, p=0.000). At a lower concentration of 20% *J. curcas* seed oil with the maximum exposure of 96 h, 52% mortality of the adults was recorded. The mortality response was highest at 96 h exposure irrespective of the treatment undertaken. High mortality with increasing concentration and exposure indicated that the adults of *D. minutus* were significantly susceptible to *Jatropha* seed oil from 24 h exposure onwards, One way ANOVA (F (5, 12=7.04, p=0.003)). The present results suggested that, 60% *J. curcas* seed oil concentration, recorded the maximum mortality in all the treatment periods, indicating the response of adults to *Jatropha* seed oil was concentration and time dependent. Among the different treatment periods, 60 h exposure showed highly significant differences between the treatments (Duncan Multiple range test, $p<0.005$). The insecticidal activity of *Jatropha* oil was also reported by Solsoloy & Solsoloy [17] and they have shown contact toxicity to corn weevil *Callosobruchus chinensis* and bean weevil *Sitophilus zeamais* and deterred their oviposition on corn and mung bean. The crude oil was found to be more efficient than the methanolic extract to control the sorghum pests stem borers [8]. Verma *et al.*, have shown termiticidal activity using Karanjin and phorbol esters of *Jatropha* oil [19]. Satyavati *et al.*, have shown that the hot and cold water extracts of *Jatropha* seed cake caused 100% mortality of termites after 7 days [14].

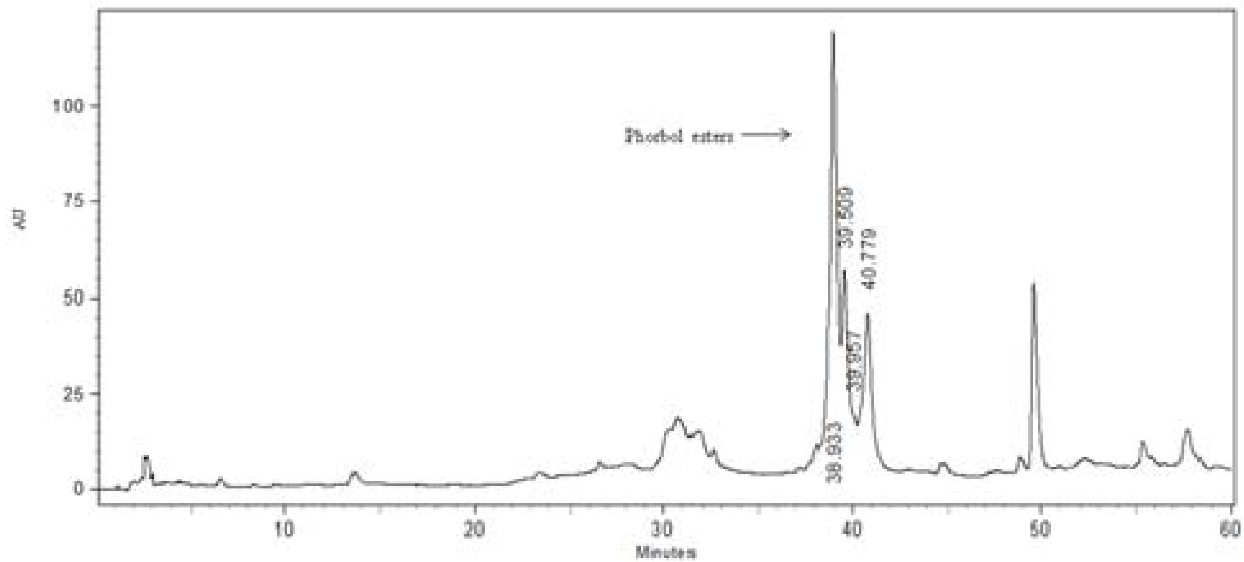

Fig 1: HPLC chromatogram of phorbol esters from *Jatropha curcas* seed oil.

Table 1: Mortality percentage of *D. minutus* with different concentrations of *Jatropha* seed oil.

Treatments	Mean mortality ± SE* over durations (h)						
	12	24	36	48	60	72	96
Control	0.00 ± 0[a]	0.00± 0[a]	0.00 ± 0[a]	11.11 ± 2.22[a]	13.33 ± 3.85[a]	13.33 ±0.00[a]	20.00 ± 3.85[a]
Hexane	0.00± 0[a]	0.00± 0[a]	4.76 ± 2.38[a]	11.11 ± 2.22[a]	24.44 ± 8.01[ab]	17.78 ±2.22[a]	20.00 ± 0.00[a]
20%#	0.00 ± 0[a]	2.222 ± 2.22[a]	4.44 ± 2.22[a]	22.22 ± 8.01[a]	48.89 ± 21.92[bc]	35.56 ±8.01[b]	51.11±5.88[a]
40%#	0.00 ± 0[a]	6.667± 0.00[ab]	24.44 ± 5.88[b]	44.44 ± 11.76[a]	73.33 ± 3.85[cd]	82.22 ±4.44[c]	86.67 ±3.85[c]
50%#	0.00 ±0[a]	8.89 ±2.22[ab]	37.78 ± 8.01[ab]	55.56 ± 4.44[ab]	75.56±2.22[cd]	86.67 ± 3.85[c]	97.78 ±2.22[d]
60%#	0.00± 0[a]	13.33 ± 3.85[b]	44.44 ± 5.88[b]	71.11 ± 2.22[b]	91.11 ±2.22[d]	95.56 ± 2.22[c]	100±0.00[d]
F value	---	7.04	15.045	15.771	9.790	76.082	121.00
P value	---	0.003	0.000	0.000	0.001	0.000	0.000

Concentration of *Jatropha* seed oil used (%); 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.

Feeding deterrence and mortality of *D. minutus* with *Jatropha* oil treated Bamboo culms

Two different concentrations of *Jatropha* seed oil 60% and 100% were tested against the *D. minutus* for their feeding behavior on bamboo blocks (5x3.5cm^2), based on the preliminary toxicity experiments. The weight loss in the bamboo blocks after 96 h was significantly higher in the control compared to the treatments at 60% and 100% concentrations of *Jatropha* seed oil. The mean weight loss was 0.255g in control, which indicates that control bamboo blocks suffered more damage by bamboo borer as compared to 0.04 and 0.025 post-treatment weights in 60 and 100% concentrations of *Jatropha* seed oil respectively (Table 2). The per cent reduction in the weight loss of bamboo blocks owing to *Jatropha* seed oil treatments at 60 and 100% concentrations in comparison to control were 15.69 and 9.80 percent respectively. The weight and the corresponding weight loss of the test bamboo blocks after treatments at 60 and 100% of *Jatropha* seed oil was highly significant, One way ANOVA (F (2, 3=33.462)), P<0.009 (Table 2). Similar type of study was carried out to observe the feeding activity behavior of termites on the *Jatropha* oil treated wood pieces of mango plant. The maximum wood protection against termites was at 20% *Jatropha* oil and the per cent weight loss of wood was 18% [10]. Bashir & Shafie reported that the antifeedant effect of *Jatropha* seed oils on the desert locust, *Schistocerca gregaria* [1].

Table 2: Efficacy of *Jatropha* seed oil treated bamboo blocks for feeding deterrence against *D. minutus*.

Treatment	Weight of bamboo blocks (g)	Prefeeding weight of bamboo+ oil (g)	Post Feeding weight (g)	Weight Loss(g)
Control	12.07± 1.909	12.07±1.909	11.82± 1.859	0.255± 0.049
60%#	12.13 ± 1.548	12.81± 1.463	12.77± 1.477	0.040± 0.014
100%#	12.47± 0.806	13.25± 0.855	13.22± 0.850	0.025± 0.007

Concentration of *Jatropha* seed oil used (%)

The mortality of *D. minutus* upon feeding exposure to *Jatropha* seed oil (60 and 100%) treated bamboo blocks showed a dose-time dependent response. The bamboo blocks impregnated with 60 and 100% *Jatropha* seed oil concentrations, induced mortality in the adults of *D. minutus* from 6h onwards when compared with control blocks. No significant increase in mortality was observed at 60% concentration over 6, 12 and 24 h. The test concentration of 60% *Jatropha* seed oil resulted in 52% mortality over 72 h exposures while 100% concentration resulted in 46% mortality at initial treatment period of 6 h itself. The mortality response of *D. minutus* upon exposure to 60 and 100% *Jatropha* seed oil treated bamboo blocks was highly significant after the initial treatment period of 6h (F (2,5=253.5)), p=0.000 (Table 3). The mortality response of *D. minutus* at 100% concentration of *Jatropha* seed oil treated bamboo blocks showed a linear increase.

Large quantities of culms are destroyed each year by insect borers, although the extent of loss has not yet been assessed. It is evident that at the storage yards, stacks with immature culms are the starting points for attack and the bamboo often gets converted to dust. Hence, control of ghoon borers at the initial stages is highly essential to limit the extent of damage caused by them. However, dip treatment of infested bamboo logs are mostly preferred, earlier reports have highlighted that fumigation of infested bamboo to sulfuryl fluoride at a rate of 30-50 g/m^3 gave maximum protection to timber over 24 h exposure [6]. Remadevi *et al*., reported the potential use of phosphine for the prevention of damage to felled bamboo and bamboo handicrafts against the bamboo borer [12]. The present study has shown the suitability of *Jatropha* seed oil for the control of bamboo borer, *D. minutus* at storage.

Botanical pesticides are an excellent alternative to synthetic pesticides as a means to reduce negative impacts on the human health and the environment [9]. One possible means to reduce the high consumption of synthetic insecticides is the application of botanical insecticides, generally considered to be environmentally and medically safe [2, 11]. From the present study, it can be concluded that, the *Jatropha* seed oil containing phorbol esters can be considered as an alternative to synthetic pesticides or can be a complimentary to other prophylactic measures for the effective management of bamboo borer, *D. minutus* more efficiently at longer exposures. The study also enlightens the importance of antifeeding activity of bamboo borer over exposure to *Jatropha* seed oil treated bamboo blocks. However, further field-oriented studies would be required to test their bio-efficacy as potential alternatives for large scale applications.

Table 3: Mortality percentage of *D. minutus* after feeding bamboo blocks treated with *Jatropha* seed oil.

Treatments	Mean mortality ± SE* over duration (h)						
	6	12	24	36	48	60	72
Control	0.00 ± 0^a	0.00±0^a	0.00 ± 0^a	2.00 ±2.00^a	8.00 ± 4.00^a	10.0 ± 2.00^a	10.0 ± 2.00^a
60%$^\#$	2.0±2.00^a	4.00± 4.00^a	14.0 ±6.00^a	22.0 ± 18.00^a	40.0± 16.00^{ab}	50.0 ± 22.00^{ab}	52.0±20.00^{ab}
100%$^\#$	46.0 ±2.00^b	58.0± 2.00^b	72.0 ± 4.00^b	74.0 ± 2.00^b	80.0± 4.00^b	88.0 ± 4.00^b	90.0 ±2.00^b
F value	253.5	157.4	84.08	12.482	13.556	9.056	11.775
P Value	0.00	0.001	0.002	0.035	0.031	0.054	0.038

$^\#$ Concentration of *Jatropha* seed oil used (%); 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.

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