Journal of Scientific and Innovative Research 2013; 2 (5): 938-942

Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2013; 2(5): 938-942

Received: 08-09-2013
Accepted: 15-10-2013

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Bio-activity guided studies of Biocides and Biodyes from Jatropha (*Jatropha curcas* L.) seed oil

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Abstract

This study reports the characterization of biocides and bio-dyes from *Jatropha curcas* seed oil. The bio-pesticide potential of *J. curcas* seed oil was also evaluated against termites (*Odontotermes obesus*) and Cockroach (*Blattela germanica*). The bioassay study showed that Jatropha 10% oil caused 100% mortality in 48hrs and 72hrs against termite and cockroach, respectively. The LD50 was determined and found to be 0.64% and 1.24% for termine and cockroach respectively. The biobesticidal potential of the oil is statistically significant ($p < 0.05$) when compared with blank and solvent controls at all concentration tested. Furthermore, the biodye was synthesized by sulfonation and characterized using various spectrophotometric facilities. The intense FTIR peak at 1087 cm-1 confirmed the presence of -S=O which the sulfonation resulted the red biodye formation in this study. The UV/visible absorption at 680nm further revealed the formation of red dyes. Various physicochemical parameters were also evaluated in accordance with American standard testing method specifications.

Keywords: Anti-cockroach, Anti-termite, Biodye, Biopesticide.

Introduction

Plant-derived oils have been shown to possess various medicinal values.[1] *J. curcas* is used as traditional herbal medicines.[2] All parts of *J. curcas* have been used in traditional medicine and for veterinary purposes for a long time.[3] Recently, the substances responsible for anti-inflammatory effects have been isolated and characterized.[4]

Extracts from *J. curcas* seed and leaves showed a significant molluscicidal, insecticidal and fungicidal properties in various scientific reports.[6, 7] Phorbol esters have been suggested to be one of the toxic principles.[7] In addition to their acute toxic effects, they also have numerous sub-lethal effects, acting as larval growth inhibitors, anti-feedants, and repellents to a wide range of insects, mites and even nematodes, all of which are consistent with an octopaminergic mode-of-action.

The kernels of *J. curcas* contain between 0.03 and 3.4% of phorbol esters.[8] The methanol extract of the crude oil have been reported to have more active components with LD_{50} values of 0.004%.[9, 10] The crude oil from *J. curcas* has been formulated as an emulsifiable concentrate had contact toxicity to corn weevil *Callosubruchus chinensis* and bean weevil *Sitophilus zeamays* and deterred their oviposition on corn and sprayed mungbean.[7]

Besides, such plant seed oil after sulfonation can be converted into biodyes. It is used in textile industries, sugar industry, as a defoaming agent, as an emulsifier. In cosmetics it is used as humectants and as an emulsifier for oil bath. In agriculture, it is also used as organic manure, in textiles as surfactants and wetting agents, in paper industry for defoaming, in pharmaceuticals as undecylenate, in paints inks and as lubricants. It is also used extensively in dyeing and in finishing of cotton and linen. Hence, the present study describes testing Jatropha seed oil as biocide against, termites, and cockroach. In addition, synthesis and characterization of red dye from Jatropha seed oil was evaluated.

Materials and Methods

Sample preparation and Extraction

The Jatropha seed was cleaned, dried, and ground. The Oil was extracted using n-Hexane as solvent in Soxhlet extraction method.[9] The oil was collected and solvent recovered for further use. The oil was stored in the refrigerator for further experiments.

Biodye Synthesis and Characterization

20 mL of oil and 3ml of concentrated sulfuric acid (98%) mixture was warmed at 35°C. The reaction was allowed to completion with constant stirring.[10] After, the product was washed with hot distilled water and left to stand for 1hr. Then the sulfuric acid ester formed was finally neutralized with 2mL of 0.1M NaOH. The percentage of dye formed was estimated. Physicochemical analysis and spectroscopic studies using UV/Vis and Fourier Transforms Infrared Spectroscopy (FTIR) was carried out. The data were determined as per the prescribed methods and compared with American Standard Testing Method (ASTM). [11] The dye was tested on wooden material, paper and cloth.

Biopesticidal Value of Jatropha Seed Oil

Termite and cockroach were collected and acclimatized for about 24 h. Each batch of insects, held in a rigid polythene container with a mesh lid, was transferred to the test room maintained. A l0ml plastic beaker containing cotton wool soaked with l0ml water was inverted on the mesh to provide a water source for the insects. Then, modified bioassay method was employed to evaluate the biocidal activity of Jatropha oil of.[12] Hence, each time oil sample of 1, 2.5, 5 and 10% oil solutions in 20% of ethanol were made and 1ml of the solution was taken for testing. Food and/or water were given during the test period. After the insects had recovered, knockdown counts were recorded every 12hrs

Statistical analyses

The data collected were analyzed statistically using SPSS vers. 16 (SPSS Inc., Chicago, IL, USA) and Origin 6 and excel. One-Way Analysis of Variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) for mean comparison between values of the treatment was used. The data obtained was done in triplicates.

Results and Discussion

Oil Extraction and Dye Synthesis and Physicochemical Characterization

The oil from *Jatropha curcas* was extracted using organic solvent and further synthesis of dye were conducted. The calculated percentage of oil extracted per dry weight of seed using Soxhelt extraction technique by organic solvents has been reported to be 46-52 %.8 in this study the oil was extracted using n-hexane as a solvent and the yield was found to be 41%. The oil was subjected to concentrated nitric and sulfuric acid treatment and resulted the formation of yellow and red dye respectively (Fig. 1 A and B). The Dye was synthesized by sulfonation and nitration f the oil extracted and the yield was found to be 33% (Table 1). The physicochemical parameters were determined within American Standard Testing Method (ASTM) and confirmed that the Jatropha oil is a suitable substitute of the chemically synthesized dyes commonly used in various textile industries. The physico-chemical properties of Jatropha seed oil and the biodye synthesized is shown below in Table 1 and Table 2.

Table 1: Physical Properties of Sulfated Jatropha Oil

Parameters	Jatropha	
	Crude Oil	**Sulfated oil**
Melting point	-3 to-1^{o}C	-2^{o}C
Solubility	Soluble	Soluble
Specific Gravity	0.967	1.05
Refractive index(20.5^{o}C)	1.452	1.456
R_f(toulene:acetone):9:1	0.68	0.74
Yield(%)	41	33

*ASTM Specifications

Various Spectrophotometric and spectroscopic techniques were also implemented for characterization of the dye. Thus, the FTIR spectra in KBr pellets were recorded. The FTIR absorption spectra were measured in the 500-4000 cm-1 region. Broad and strong peak centered at 3300cm-1 indicates -O-H functional groups and –C–H, where C is not part of a benzene ring sharp, strong peak on the low side of 3000 cm-1, between about 2850 and 3000 cm-1 is –C=O. The -S=O stretching at 1069 cm-1 confirmed that Jatropha oil sulfonation was confirmed the formation of red dye through sulfonation reaction (Fig. 2).

Table 2: Chemical Properties of Sulfated Jatropha Oil

Parameters	Jatropha oil	
	Sulfated	Crude
Sulfonation degree	4.5%	-
pH of 10% solution	7.5	6.8
Cetane number (^oC)	50	51
Flash Point(^oC)	252 ^oC	258 ^oC
Saponification Value [mgKOH/g of Oil]	180	188
Iodine Value [mg I_2 /100mg of Oil]	84	90.8

*ASTM Specifications

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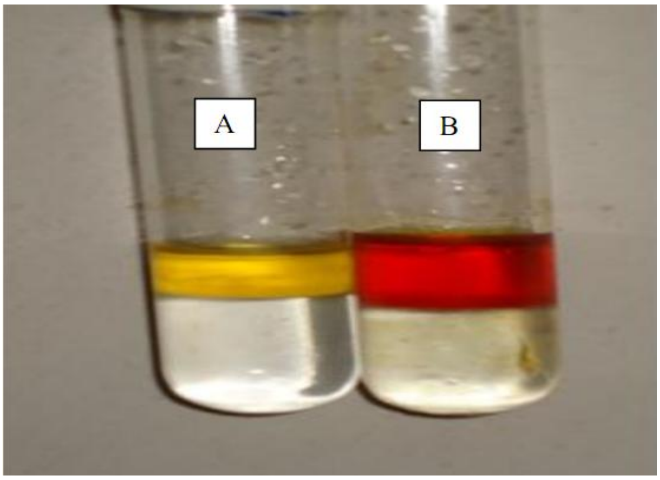

Figure 1: Color of Jatropha Oil in nitric acid (A) and sulfuric acid (B)

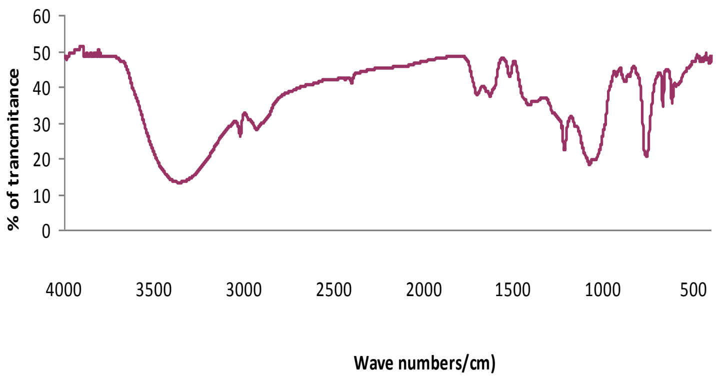

Figure 2: IR Spectrum of Sulfated Jatropha Oil for Synthesis of Dye

The UV-Vis spectroscopic measurement was also considered for characterization of the biodye synthesized. The new dyes synthesized was prepared in acetone solution and showed characteristic absorption maxima of 680 nm and molar absorptivity 229,300 (dm3mol-1cm-1) which showed the properties of red dyes (Figure 3). This further confirmed the synthesis of biodyes from Jatropha oil through sulfonation reactions. The study showed that the Jatropha oil can be used as a candidate raw material for the biosynthesis of dyes and insecticides for industries and agricultural sectors.

Various anti-insecticidal effects were evaluated. The results showed that 10% Jatropha oil in a neutral solvent DMSO, all the worker termites were killed within 48hrs (Figure 4). However, Jatropha oil concentrations of 1% killed the termite workers in a span of 108 hrs. In 1% and 10% concentration of oils, the worker cockroach was killed within 180hrs and 72hrs, respectively (Figure 5). The LD50 was determined and found to be 0.64% and 1.24% for termine and cockroach respectively. The biopesticidal potential of the oil is statistically significant ($p < 0.05$) at all tests. This is in agreement with the findings in various scientific reports.[7]

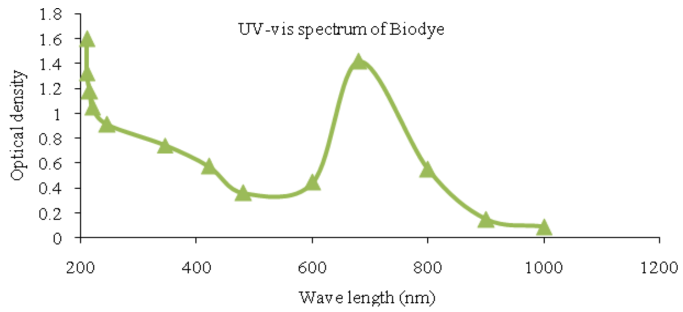

Figure 3: UV/visible spectrum of Dye Synthesized in Acetone Solution

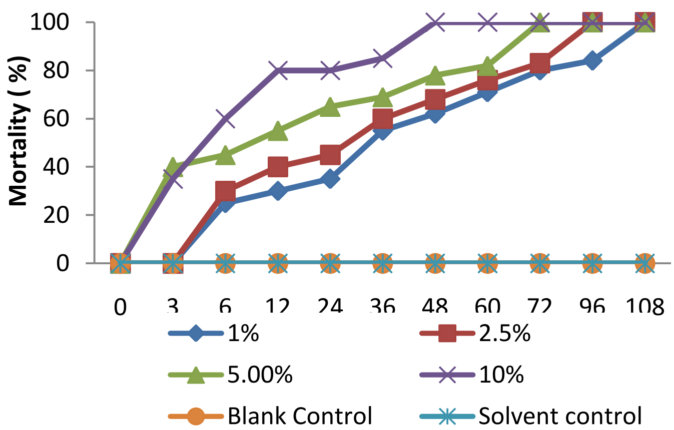

Figure 4: Mortality of termite workers vs. time with different concentration of Jatropha

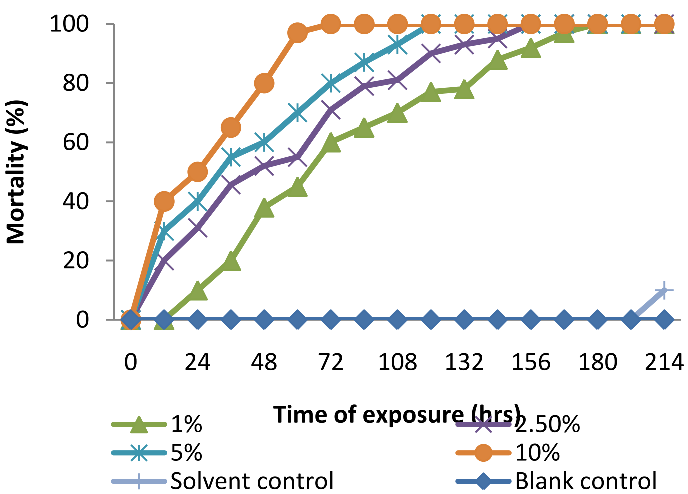

Figure 5: Mortality of Cockroach vs. time with different concentration of Jatropha oil

From the present study, it can be concluded that oil can be used as an alternative in pest management programs against many pathogens like termites and cockroach. Sulfonation of Jatropha oil gave significant biodye formation which can be used for textile industries. It was observed that the biodye found to stain the white cotton in the study. Further studies would still be required for better understanding the chemistry of the biodye. The researchers believe that field level studies will be needed to further validate biopesticidal potential of the seed oil.

Acknowledgments

The authors are grateful to Arba Minch University research directorate office for finance and using of laboratory of Chemistry and Biology Departments for analysis. In addition, they thank Addis Ababa University, Chemistry Department for FTIR analysis.

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