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Research Article

PHRMACEUTICAL INVESTIGATION AND BIOPESTICIDAL ACTIVITY OF *JATROPHA CURCAS* L. SEED OIL ON DIGESTIVE ENZYMIC PROFILES OF *CNAPHALOCROCIS MEDINALIS* (RICE LEAF FOLDER) AND *HELICOVERPA ARMIGERA* (COTTON BOLL WORM)

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ABSTRACT

The present paper deals with biopesticidal activity of seed oil of *Jatropha curcas*.L. on Lepidopteran pests, *Cnaphalocrocis medinalis* (Lepidoptera:Crambidae) rice leaf folder and *Helicoverpa armigera* (Lepidoptera:Noctuidae) attacking musk melon crop. Methanol, Ethanol, Petroleum ether, Ethyl acetate, Benzene, Hexane and Water extracts in three different concentrations subjected on selected pests. *Jatropha curcas* oil affected the amylase and LDH activity. The study reveals that 500ppm concentration of methanol for larval mortality was the most suitable solvent. A detailed statistical analysis was also carried out for both field and laboratory studies.

KEYWORDS: *Jatropha curcas,* biopesticide , Lepidoptera , *Cnaphalocrocis medinalis, Helicoverpa armigera.*

INTRODUCTION

Population increase created a huge demand for food. Demand for food and feed has not always been able to keep up pace with the supply. Among several constraints of increasing agricultural productivity, insect pest was being recognized as one of the major limiting factors in many crops. Biopesticides generally affect only the target pest and closely related organisms, in contrast to broad spectrum conventional pesticides and largely avoide the pollution problems caused by conventional pesticides[1]. Plant families studied for biopesticidal activity are, Meliaceae, Rutaceae, Asteraceae, Labiateae, Piperaceae and Annonaceae[2,3]. A few commonly used botanicals are Annona[4], Neem[5], Nicotine[6]. Often the search for plant-derived chemicals as crop protectants (insecticides, antifeedants) often begins with the screening of plant extracts.

Jatropha curcas (L.) commonly known as physic nut is a medicinal plant and hedge crop[7]. Cultivation of this plant has gained prominence, mainly as a source of bio-fuel. Though extensive work was done on alternate uses of *Jatropha*, there is not much information available on its use as a pesticide. The seed cake remaining after oil extraction, known as seed meal is an excellent source of plant nutrients[8]. Phorbol esters (phorbol-12-myristate 13-acetate) have been identified as the major toxic principle in *Jatropha species*[9]. The persistent toxicity of *Jatropha curcas* seed extracts on *Sitophilus zeamaise* was reported by Ohazurike *et al*[10]. Toxicity of *Jatropha* seeds has been studied extensively in different animal models like goats, sheep, mice, rats, and fish when fed with phorbol ester containing feeds [11].

Experiments were conducted to assess the pesticidal activity of seed oil of *Jatropha curcas*, against the selected polyphagus pests attacking the crop plantsboth in laboratory and field. Pests selected for the present study were *Cnaphalocrocis medinalis* larvae (Lepidoptera:Crambidae) attacking rice crop and *Helicoverpa armigera* (Lepidoptera:Noctuidae) attacking musk melon crops. Biochemical investigations were carried out with special emphasis on amylase, protease and lactate dehydrogenase.

MATERIALS AND METHODS

Fruits of *Jatropha curcas* (Euphorbiaceae) were procured from Oil Technological Research Institute (OTRI), Anantapur, Andhra Pradesh, India. Seeds were separated, air dried and weighed. Oil was extracted from the seeds using Table expellor. When 10 kgs of seeds were used for the oil extraction, the clear oil obtained after filtration has weighed 2.429 kg. Seed oil was mixed with different solvents in 1:1 ratio. Solvents used in the present study are Methanol, Ethanol, Petroleum ether and Ethylacetate, Benzene, Hexane and Water. Solvent, oil layers after mixing thoroughly and reduced in a rotary evaporator of model Laborota 4000- efficient (Heidolph instrument). These extracts were transferred to amber colored sample bottles and stored in refrigerator at 4^{o}C. For testing 125ppm, 250ppm, 500ppm working stocks solutions of oil solvent extracts were prepared and stored[12].

The eggs of *Cnaphalocrocis medinalis* (Lepidoptera:Crambidae) commonly known as rice leaf folder, were collected from the paddy fields in and around Anantapur fields. Rearing of *Cnaphalocrocis medinalis* was done placing the larvae in a growth chamber, incubating at 27 ±2^{o}C temperature in a 14L:10D photoperiod and 85% relative humidity. Larvae were provided with potted rice plants covered with mesh sleeves for feeding. Eggs of *Helicoverpa armigera* (Lepidoptera:Noctuidae) commonly known as cotton boll worm, were obtained from the Department of Entomology of Acharya N.G.Ranga Agricultural University, ANGRAU, Anantapur. These insects were reared after placing in growth chamber, maintained at 28 ± 2^{o}C temperature, 16L:8D photoperiod and 65±2% relative humidity. Larvae of *Helicoverpa armigera* was sufficiently provided with musk melon leaves.

Laboratory and field trials were conducted using 3rd instar larvae of selected pests which were responsible for major crop loss. Larvae were allowed to feed on the fresh leaves of respective crop plants, which were collected, dipped and dried in 125ppm, 250ppm and 500ppm working solvent extract solutions of seed oil. For field trails, rice field (*Oryza sativa*) and musk melon (*Cucumis melo*) field plots measuring 460 m^2 were selected located in Katiganikalava village in Anantapur mandal of Anantapur district, Andhra Pradesh, India. Above mentioned 3 extract solutions were applied after infestation. All experiments were repeated thrice. The entire study was conducted according to standard test methods[13].

Ten larvae of *Helicoverpa armigera* (third instar) that were dead, due to feeding on leaves dipped in selected test concentrations of oil extracts were collected. These larvae were then weighed and suspended in 0.15M NaCl solution along with methanol. Later, was homogenized using glass-Teflon homogenizer. This suspension was centrifuged at 10000g for 10min at 4^{0}C. The resulting supernatant was used for the enzyme assays. Protein concentration of extract was determined by the Bradford method[14]. Following the same procedure, insect extracts were prepared for all the remaining solvent extracts (ethanol, petroleum ether, benzene, hexane, water).

Amylase activities were determined based on the method of Bernfield [15] and as described by Ishaaya and Swirski[16]. Absorbance of the sample was measured at 550nm using UV-Visible spectrophotometer (model BL 198 Elico) against a blank in which the enzyme extract was replaced with sterile deionised water. Protease activities were determined as described by Snell and Snell [17] and optical density values were read at 600nm. Lactate dehydrogenase (LDH) activities were determined after 60 min of incubation, while the optical density values were measured at 440nm against blank.

Experimental data was statistically analyzed using Split plot design analysis. Data was analyzed using WindowStat version 8 (Indostat services, Hyderabad, India) statistical software with split plot analysis and two factorial RBD.[18]Means for laboratory enzyme studies were compared with Tukey's Honestly Significance Difference (Tukey's H.S.D) test at P=0.01 level of probability for significance of main effects and sub effects and their interactions[19].

RESULT

Changes in metabolism and physiology and decreases in the gut enzyme activity of oil-treated individuals may be expected to affect enzyme titers and activities.Among the tested solvent extracts in laboratory conditions, of seed oil the mean percent mortality of *Cnaphalocrocis medinalis* caterpillar under laboratory conditions resulted in significant effect of solvent type and concentration of extracts. Highest mean percent reduction in population over control was found with methanol (55.76 to 68.66%) followed by ethanol (50.78 to 65.95%) and petroleum ether (49.82 to 64.69%) at all three concentrations tested. (Tab.1). Statistically there was significant interaction effect between solvent extract and concentration i.e., the effect of methanol at 500 ppm (68.6%) was highest followed by ethanol (65.95%) and petroleum ether (64.69%). Under field conditions, highest mean percent reduction in population was observed with methanol at 500 ppm (70.3%) followed by ethanol (Fig.1). Except for benzene and aqueous extracts rest of the extracts at 250 ppm or 500 ppm resulted in more than 50% reduction in population.

When different solvent extracts of the oil were tested for mean percent mortality of *Helicoverpa armigera* larvae over control, under laboratory conditions, highest mean percent reduction was at 500 ppm with methanol (85.94%), followed by ethanol (68.85%) and petroleum ether (66.14%) at all the three concentrations tested (Fig.2). Under field conditions, also highest mean percent reduction in population was observed with methanol and ethanol at all the three concentrations tested. Highest reduction being obsersved at 500 ppm concentration (70.26% with methanol and 67.50 % with ethanol) (Fig. 3)

Among the different enzymatic studies conducted, methanol extract was found to be most effective in reducing the amylase activity at all the three concentrations tested (1.08, 0.74, and 0.67×10^4 µg/mg/min) for larvae, compared to control (Fig.4). Among amylase activity studied on larva of *Helicoverpa armigera*, extract of methanol was found to be more effective in decreasing activity of amylase (2.16×10^4 µg/mg/min) compared to control (4.7×10^4 µg/mg/min). Protease activity of third instar larva, when studied with different solvent extracts of *Jatropha curcas* oil indicated (Tab.2) showed, significant effect of solvent type used among the three different concentrations tested. Highest percent mortality was found to be at 500 ppm methanol (0.57×10^4µg/mg/min). While ethyl acetate, benzene and aqueous extracts showed no significant decrease in protease activity when compared to rest of the solvents. When Lactate dehydrogenase activity of third instar larva was investigated, methanol and ethanol at 500 ppm were more effective to reduce LDH activity (4.42 and 4.48×10^4 µg/mg/min respectively) in larva under laboratory conditions (Fig.5).

DISCUSSION

Among all the solvent extracts tested, methanol, ethanol and petroleum ether extracts showed potent antifeedant and pesticidal activity which indicated that these extracts contained classes of compounds that can control different insects and they are exclusively the phorbol ester fraction of the oil. Methanol extract again was the potent preparation which is effective against all the pests. It was observed that all the pests were sensitive to125 ppm, 250 ppm showing moderate results and 500 ppm being the most effective in 48 hours i.e., LC_{100}=500ppm in 48 hours. The percent reductions in population with methanol extracts of oil at 500ppm are 85.94% on third instar larva of *Helicoverpa armigera* and 68.66% on third instar larva of *Cnaphalocrocis medinalis* after 48 hours of treatment. Corresponding control treatments alone showed no insect mortality. Phorbol esters in oil are enriched as hydrophobic molecules by methanol extraction[20]. Phorbol esters are known to directly activate protein kinase C [21]. This key enzyme of signalling cascades plays a critical role in maintaining the integrity of the insect surface. The phorbol esters are amphiphillic molecules and they are soluble in all the solvents up to certain extent which is responsible for the insecticidal activity of the oil. The toxic components of seeds are less soluble in the aromatic organic solvents like benzene and hence the results are poor. Generally as all phorbol esters are extracted with the oil fraction, oil extracts of solvents are more effective.

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Table 1: Effect of different solvent extracts of *Jatropha curcas* (oil) on third instar larva of *Cnaphalocrocis medinalis* under laboratory conditions

Extacts	Concentration (ppm) percent of reduction in population over control		
	125	250	500
Methanol	68.3(55.76)	81.6(64.80)	86.6(68.66)
Hexane	55.0(47.87)	60.0(50.78)	66.6(54.78)
Ethanol	60.0(50.78)	78.3(62.78)	83.3(65.95)
Petroleum ether	58.3(49.82)	55.0(47.87)	81.6(64.69)
Ethyl acetate	48.3(44.03)	55.0(47.87)	61.6(51.75)
Benzene	50.0(44.99)	56.6(48.83)	63.3(52.77)
Aqueous extract	43.3(41.16)	46.6(43.08)	51.6(45.95)
	SEM	CD (P=0.05)	Significance
Solvent extracts	1.08	4.66	**
Concentrations	0.74	2.91	*

Values in parthenthesis are ARC SIN transformed values * and NS indicates significant (P=0.05) and non significant respectively.

Table 2: Effect of different solvent extracts of *Jatropha curcas* (oil) on protease activity of third instar larva of *Helicoverpa armigera* under laborat conditions

Extracts	Concentration (ppm) Protease activity ($\times 10^4$ µg/mg/min)		
	125	250	500
Methanol	2.45	2.39	1.45
Hexane	11.68	8.18	4.75
Ethanol	11.14	7.36	3.18
Petroleum ether	11.33	7.57	3.69
Ethyl acetate	12.59	10.94	9.59
Benzene	12.43	11.39	10.02
Aqueous extract	12.24	10.65	8.95
Control	13.07	12.84	12.79
	SEM	CD (P=0.05)	Significance
Solvent extracts	0.023	0.05	**
Concentrations	0.040	0.15	**

**indicates significant at P=0.01(Tukeys HSD Test)

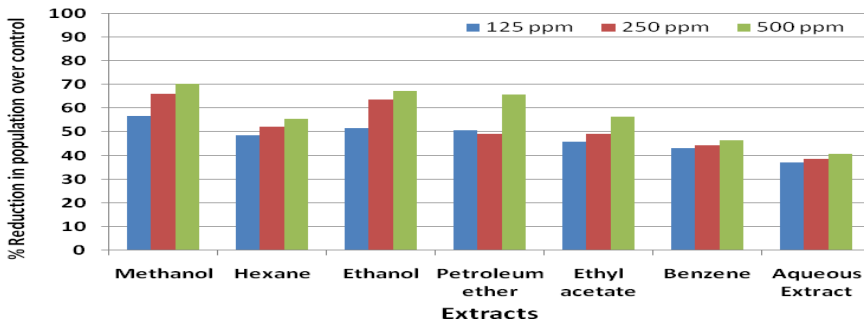

Fig 1: Effect of different solvent extracts of *Jatropha curcas* (oil) on *Cnaphalocrocis medinalis* under field conditions.

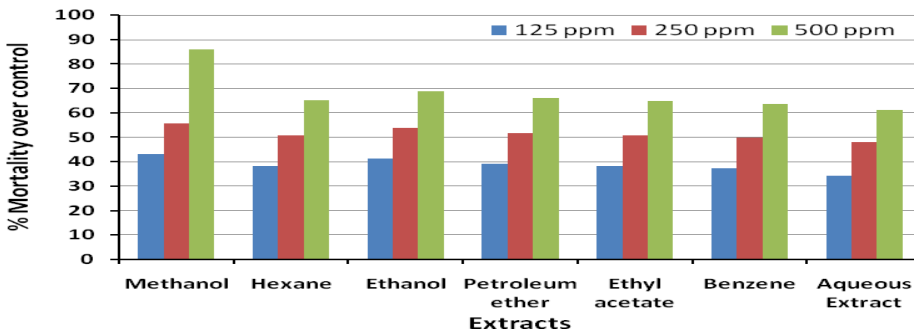

Fig 2: Effect of different solvent extracts of *Jatropha curcas* (oil) on third instar larva of *Helicoverpa armigera* under lab conditions

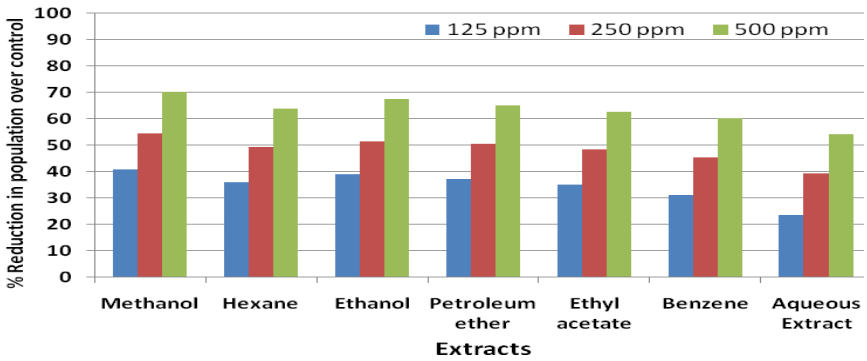

Fig 3: Effect of different solvent extracts of *Jatropha curcas* (oil) on third instar larva of *Helicoverpa armigera* under field conditions

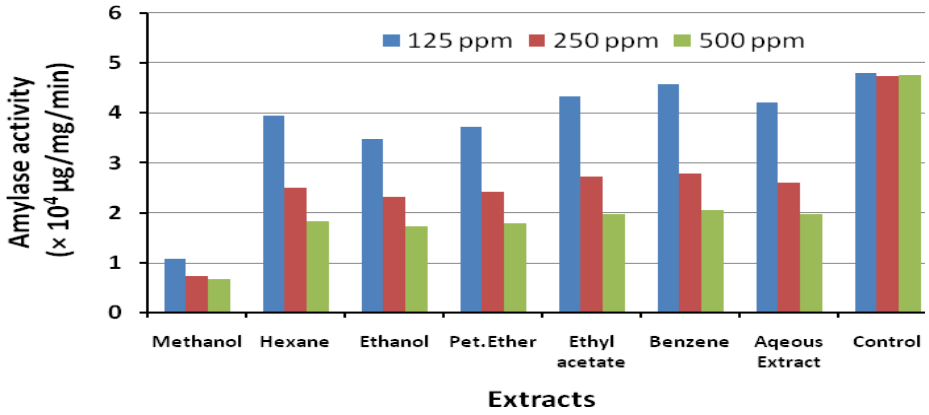

Fig 4: Effect of different solvent extracts of *Jatropha curcas* (oil) on amylase activity of third instar larva of *Helicoverpa armigera* under laboratory conditions

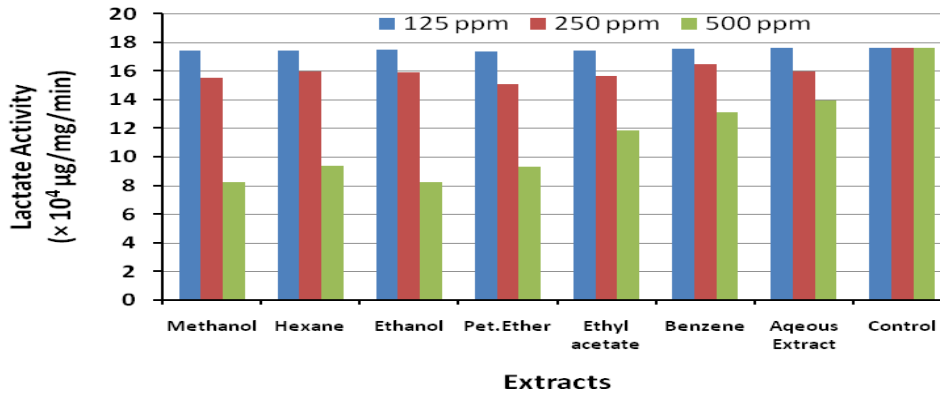

Fig 5: Effect of different solvent extracts of *Jatropha curcas* (oil) on lactate dehydrogenase of third instar larva of *Helicoverpa armigera* under laboratory conditions

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