Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J E Z S Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(1): 44-50

Received: 08-11-2016
Accepted: 09-12-2016

Krishnananda P Ingle
Biotechnology Centre,
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola,
Maharashtra, India

Amit G Deshmukh
Assistant Professor,
Nagarjuna Medicinal and Aromatic Plant Division,
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola,
Maharashtra, India

Dipika A Padole
Biotechnology Centre,
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola,
Maharashtra, India

Mahendra S Dudhare
Assistant Professor,
Vasantrao Naik College of Agril. Biotechnology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth,
Yavatmal, Maharashtra, India

Mangesh P Moharil
Biotechnology Centre,
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola,
Maharashtra, India

Khelurkar VC
Biotechnology Centre,
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola,
Maharashtra, India

Correspondence
Krishnananda P Ingle
PhD Research Scholar,
Biotechnology Centre, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola,
Maharashtra, India

Screening of insecticidal activity of *Jatropha Curcas* (L.) against diamond back moth and *Helicoverpa Armigera*

Krishnananda P Ingle, Amit G Deshmukh, Dipika A Padole, Mahendra S Dudhare, Mangesh P Moharil and Khelurkar VC

Abstract
In the present study extracts of leaf, bark, seed, seed coat and roots of *Jatropha curcas* were extracted using soxhlet extraction method with various solvents. Methanol was found to be the best extractant amongst all the solvents. In order to prescreen, the methanol and solvent extract were then subjected to check for insecticidal activity if any. The biological and phytochemical studies of *Jatropha curcas* were evaluated against third instar larvae of diamond back moth (*Plutella xylostella*) and *Helicoverpa armigera* by Leaf dip method. Antifeedant activity was observed in case of leaf extract against *Helicoverpa armigera* with total larval weight reduction of 36.27% after 24 hrs when they were fed on cotton leaf treated with the extract, but even after replacing the fresh cotton leaf the weight reduction was 42.08% (after 48 hrs) and 41.55% (after 72 hrs). The seed coat extract was most effective against *Plutella xylostella* and showed highest mortality 100% at 5% as compared to other extracts whereas in case of *Helicoverpa armigera* seed coat extract was most effective and showed highest mortality 10, 40 and 60% at 5, 10 and 15% concentration respectively. The results suggested that *Jatropha curcas* has good insecticidal/antifeedant activity therefore making it ideal potential option for incorporation into pest management program.

Keywords: Jatropha curcas, insecticidal activity, antifeedant activity, diamond back moth (Plutella xylostella), Helicoverpa armigera

Introduction
Jatropha curcas (Euphorbiaceae) has been considered as a “miracle tree”, and source of alternate fuel. The various plant extracts have been reported to have the insecticidal and anthelminthic activities on vectors of medical and veterinary interest or on agricultural or non-agricultural pests. Among the different extracts the phorbol esters fraction from the seed oil has been reported as a promising candidate for use as a plant derived protectant of a variety of crops, from the range of preharvest and postharvest insect pest. However, such extracts have not been widely used, despite the “boom” in the development of the crop in the tropics during recent years, and societal concerns about overuse of systemic and synthetic pesticides. The indiscriminate use of chemical pesticides in agriculture leads to adverse effects on public health also the issues of pesticide resistance, frequent pest outbreaks, emergence of new pests and pollution. In order to search an environmentally safe alternative, scientists considered (bio-pesticides) in the place of synthetic insecticides. Synthetic insecticides are replaced by bio-rational insecticide is universally acceptable and practicable approach worldwide. Since many years, plant products have been successfully exploited as insecticides, insect repellents and antifeedants. Recent plant protection researchers particularly revealed the importance of plant products that disrupt the normal insect growth and development [1]. Some of the plant derived materials are less toxic to mammals, may be more selective in action and may retard the development of resistance. Their main advantage is that they may be easily and cheaply produced by farmers and small scale industries as crude, or partially purified extracts. Since, last two decades considerable efforts have been directed for screening the plants with a view to develop new botanical insecticides as alternatives to the existing insecticides [2]. The diamondback moth (*Plutella xylostella*) is one of the most important vegetable insects [3-5] and they can develop resistant strains [6-8]. Similarly, *Jatropha curcas* possess pesticidal properties against *H. armigera and B. tabaci* and is environmentally safe and eco friendly that can be integrated in the management practices of agricultural pests.

The extracts of *Jatropha curcas* bio assayed in the study, demonstrated significant larvicidal activities against *Cx. P. pipines* and such activities were dose dependent[9]. The larvicidal effect of different extract may be due to presence of flavonoids, saponins and glycosides and these phytochemical mostly present in the stem [10].

The present research is a biological and phytochemical study of *Jatropha curcas* on feeding, growth and metamorphosis against diamondback moth and *H. armigera.*

Materials and Methods

Collection of plant materials

The plant material leaf, bark, seed, roots and seed coat of *Jatropha curcas* were collected from the college of Forestry, Dr. Panjabrao Deshmukh Agricultural University, Akola. For extraction purpose, leaf, bark, seeds, seed coat and roots were used. All the plant parts were dried in hot air oven at 40 ^{0}C till they were completely dry. All the dried samples were powdered using mixer grinder, which in turn was used for further extraction using different solvents.

Preparation of the extract

Soxhlet extraction was carried out with Universal Extraction System (Buchi). Ten grams dried powder (leaf, bark, roots, seeds and seed coat) was taken in glass thimble and extracted with solvents such as methanol. The procedure was carried out for 10 cycles for each extract and the temperature was adjusted just below the boiling point of the respective solvents. Most of the solvent from each extract was evaporated by using the same instrument. Further drying of the extract was carried out at room temperature. The weight of each extract was also noted [11]. (Table 1).

Table 1: Yield of seed, bark, seed coat, leaves and roots extract with various solvents

S.N.	Name of solvent	Yield of extract%
		Soxhlet Extraction
Seed		
1	Methanol	20.8
2	Aqueous methanol (75%)	18.1
3	Acetone	13.5
4	Ethyl acetate	14.4
5	Hexane	18.0
Bark		
1	Methanol	11.1
2	Aqueous methanol (75%)	13.4
3	Acetone	11.0
4	Ethyl acetate	6.5
5	Hexane	7.2
Seed Coat		
1	Methanol	3.8
2	Aqueous methanol (75%)	3.4
3	Acetone	2.8
4	Ethyl acetate	3.0
5	Hexane	3.6
Leaves		
1	Methanol	12.1
2	Aqueous methanol (75%)	10.5
3	Acetone	11.0
4	Ethyl acetate	7.9
5	Hexane	8.3
Roots		
1	Methanol	13.5
2	Aqueous methanol (75%)	12.2
3	Acetone	10.6
4	Ethyl acetate	7.5
5	Hexane	6.8

Screening of insecticidal activity

Insect culture and maintenance

The larvae and pupae of *P. xylostella* were collected from cabbage and cauliflower field from outskirts of Akola and *Helicoverpa armigera* larvae were collected from horticulture field of Dr. PDKV, Akola both were reared in the laboratory under controlled conditions of temperature 250C ± 20C, 75 ± 5 per cent relative humidity and photo period of 13 hrs light: 11 hrs dark.

Preparation of sample extracts for bioassay

Accurately weighed 10 mg of extract and 1 ml of DMSO was added to it and vortexed so that all the extract was dissolved to form a clear solution of 1%. Similarly 5, 10, 15% concentration of the extracts were prepared for the final bioassay. For *P. xylostella* 5% extracts were used for the bioassay while for *H. armigera* 5, 10 and 15% concentrations of the extracts were used.

Insecticidal activity

The bioassay was carried out using cabbage leaf dip method[12]. Cabbage leaves were first washed with distilled water containing 0.1% Triton X 100 and air dried for about 1 hr. Cabbage leaf discs (5 cm diameter) were cut with a metal punch and then dipped in the test solution of extract prepared in Dimethyl Sulphoxide (DMSO) and methanol to facilitate uniform treatment of active ingredient for about 10 sec. The leaf discs were placed slanting for about 2 min over a blotting paper in a tray to drain excess solution for about 2 hrs at room temperature. Ten third instar larvae (6 hrs. starvation) were released on each disc in individual petri plate. Blotting paper was placed at the bottom of the petri plate. The plates were observed for 72 hrs for any insecticidal activity. The bioassay were conducted at temperature 25 ± 2 ^{0}C, relative humidity 75 ± 5%, dark and light regime of 13:11 hrs. Total 10 insect larvae were used for screening. The bioassay setup for *H. armigera* was similar to *Plutella xylostella* except for cotton leaf. Table 2 and table 3 and figure 1 and figure 2 showed the per cent mortality and per cent pupation of *P. xylostella* and *H. armigera* after treatment with extract.

Table 2: Screening of *Jatropha* extract for Insecticidal activity against *Plutella xylostella*

S.N.	Extract	Concentration%	Mortality%	Pupation%
1	Seed	5	80	20
2	Bark	5	40	20
3	Seed Coat	5	100	0
4	Leaves	5	80	20
5	Root	5	80	20
6	Control (DMSO)	-	0	0

(Total no of insects per extract = 10)

Table 3: Screening of *Jatropha* extract for Insecticidal activity against *Helicoverpa armigera*

SN	Extract	Concentration%	Mortality%	Pupation%
1	Root	**5**	**0**	**0**
		10	0	0
		15	10	0
2	Leaves	**5**	0	0
		10	0	0
		15	20	0
3	Seed Coat	**5**	10	0
		10	40	0
		15	60	0
4	Control (DMSO)	-	0	0

(Total No of insects per extract = 10)

Antifeedant activity

Feeding deterrence was so high that even after replacing the treated leaf with the fresh leaf after 24 hrs, the feeding was negligible. Therefore, it was decided to take an antifeedant assay of leaf extract at lower concentration (5%) to evaluate the no feeding and larval weight reduction. In the antifeedant activity assay the larvae were exposed to the cotton leaf treated with respective extract for 24 hrs and then the treated leaf was replaced with the fresh cotton leaf. After 48 hrs again the old leaves were replace with fresh leaves. The assay was taken for 72 hrs and total larval weight reduction after 24, 48 and 72 hrs were recorded. The experiment was designed statistically to confirm the significance of the findings. Table 4 and figure 3 showed the effect of different extracts on feeding and growth of *Helicoverpa armigera* at 5% concentration.

Table 4: Antifeedant activity of extract at 5% against *Helicoverpa armigera*

S.N.	Extract (Treatment)	Larval weight reduction		
		After 24 hrs	After 48 hrs	After 72 hrs
1	Leaves	36.27 (36.99)	42.08 (40.40)	41.55 (40.11)
2	Bark	12.02 (20.27)	13.10 (21.22)	11.61 (19.91)
3	Seed	14.33 (22.22)	22.33 (28.18)	23.06 (28.66)
4	Seed coat	16.85 (24.20)	23.91 (29.27)	25.56 (30.33)
5	Root	29.55 (32.90)	36.24(36.99)	43.20 (41.09)
	F test	Significant	Significant	Significant
	S.E.(M)	4.55	4.53	5.32
	S.E.(D)	6.44	6.40	7.52
	CD	13.53	13.45	15.80

Solvent extraction of potential extracts

Few amount (2 gm) of extract was taken and titrate with MeOH:H_20 (4:1) ratio and filtrated then the residue was separated and it was considered as fraction 1. The filtrate was then acidified with 2M H_2SO_4 and was extracted again with $CHCl_3$ for three times. Thus chloroform and aqueous acid layer get separated. Chloroform was dried and evaporated it was considered as fraction 2a and aqueous acid layer then basified with the NH_4OH_2 and was extracted again with chloroform and methanol for two times. Finally chloroform-methanol and aqueous basic layer get separated which were considered as fraction 2b and fraction 3 [13].

Solvent Extraction of crude extracts for partial purification

Since good bioactivity was found in case of seed coat, leaf and root extracts these were further purified using solvent extraction procedures[13]. After solvent extraction four fractions were obtained fraction 1 of residues (Fr 1), fraction 2 of chloroform (Fr 2a), fraction 3 of chloroform- methanol (Fr 2b) and fraction 4 of aqueous basic fractions (Fr 3). Since very less extract was obtained after drying of Fr 2a, the fractions Fr 2a and Fr 2b were combined and named further as Fraction 2. The maximum yield was obtained in case of seed coat (Fr 3) i.e.1296 mg while minimum yield was obtained in root (Fr 1) i.e. 312 mg (Table 5). Since the residue i.e. Fraction 1 is a neutral extract and contains mostly carbohydrates and mucilages. As most of the bioactivity has been shown in compounds like alkaloids, terpenoid, flavonoids and phenolics, this fraction 1 was not used for further screening of insecticidal and antimicrobial activity. Fraction 2 and fraction 3 were used for exploration of the mentioned bioactivity.

Table 5: Solvent extraction of crude methanolic extracts

S.N.	Extract	Weight of extract			
		Dried Methanol (g)	Fraction 1 (Residues) Mg	Fraction 2 (Chloroform + Methanol) Mg	Fraction 3 (Aqueous basic) Mg
1	Root	2	312	545	1017
2	Leaf	2	562	637	798
3	Seed Coat	2.5	438	581	1296

Insecticidal activity screening of solvent extracted fractions

The chloroform methanol (Fraction 2) and aqueous basic fraction (Fraction 3) of root, leaf and seed coat methanol extract were tested for insecticidal activity against *Plutella xylostella* using leaf disc bioassay method. DMSO was used as control. Because of less quantity of fractions available the assay was carried out using only *P. xylostella*, (Table 6 and figure 4).

Table 6: Insect bioassay of fractions at 5% against *Plutella xylostella* (DBM)

S.N.	Extract	% Mortality	% Pupation
1	CM-Root	20	60
2	CM-Seed Coat	40	60
3	CM-Leaves	40	60
4	AB-Root	20	80
5	AB-Seed Coat	60	40
6	AB-Leaves	40	20
7	Control (DMSO) (DMSO)	0	0

(Total number of insects per treatment = 10)

CM: Chloroform methanol fraction AB: Aqueous basic fraction

Result and Discussion

Plant contains a wide variety of aromatic or saturated organic compounds. Hence, they are often extracted using solvents like methanol, acetone, chloroform and hexane. Some compounds are generally extracted using a particular solvent only e.g. Xanthozylines, Quassinoids and Phenones are particularly and specifically isolated using Methanol and hexane as an extractant (solvent). Ethanol is used for isolation of Polyacetylenes, Sterols, etc. Ether is used specifically for extraction of Coumarins and Fatty Acids. Eloff (1998) has ranked the solvents according to their extraction capacity and bioassay compatibility. Variety antimicrobial compounds have been isolated from the methanolic extracts [14].

The precise mode of extraction of naturally depends upon the texture and water content of the plant material i.e. the leaves, stem, bark, flowers and fruits. Initial screening of plant material for the assessment of bioactivity (prescreen) and purification of the bioactive compounds is done by processing the plant material by using a variety of methods like Soxhlet Extraction, Cold Percolation (Steeping), Distillation and Supercritical Fluid extraction for obtaining a crude extract.

It was observed that in general the extraction yields obtained with polar solvents such as methanol and aqueous methanol were more as compared to non-polar solvents such as ethyl acetate and hexane. In case of seeds the hexane has extracted the oil as *Jatropha* seed is rich source of oil. This is also reflected in the results. In almost all the methods amongst all the solvents methanol has given better yields except in few cases where acetone or hexane has given higher yields (Table 1). In almost all the cases soxhlet extraction with methanol (polarity index 5.1) has given better results as compared to other methods and solvents [15]. Amongst all the tissues, highest yield was obtained with seeds (20.8%), while lowest yield was obtained in seed coat (3.8%).

Different plant parts Viz. seed, bark, seed coat, leaves and roots were extracted using different solvent systems such as Methanol, Aqueous methanol, Acetone, Ethyl acetate and Hexane. Methanol was found to be best extractants amongst all in terms of percent yield of extract.

The methanolic extracts of seed, bark, seed coat, leaves and root were evaluated for insecticidal activity against *Plutella xylostella* using leaf disc bioassay method. Ten third instar larvae of *Plutella xylostella* were used for each concentration of extracts to assess the bioactivity (Table 3 and figure 1). All the extracts showed remarkable mortality ranging from 40-80%. The seed coat extract was most effective and showed highest mortality (100%) as compared to other extracts. Although, Sinchaisri *et al* (1991), did not recorded any mortality with *Jatropha* leaf extract against *P. xylostella*, 80% mortality was evident, observed with leaf extract in our results[16]. In leaf extract especially, non-feeding tendency was prominently observed.

Different extract of seed, bark, seed coat, leaves and root were used for insecticidal screening against *Plutella xylostella*. Amongst all, seed coat extract showed 100% mortality other extract showed least as compared to other and larvae also undergoes to pupation. The experiment was carried out in triplicate and total number of insects per extract was ten. DMSO used as a control. After 24 hrs feeding was observed and leaves were replaced by treated seed coat extract.

The methanolic extracts of root, leaves and seed coat were tested for insecticidal activity against *Helicoverpa armigera* using leaf dip bioassay method. Ten third instar larvae of *Helicoverpa armigera* were used for each concentration of extracts to assess the bioactivity. The seed coat extract was most effective and showed highest mortality 10, 40 and 60% at 5, 10 and 15% concentration respectively. However mortality was not observed with other extracts at low concentrations. The insecticidal activity of seed and seed cake has been reported in literature [9, 17, 18]. Arvinda *et al* 2009 reported 100% mortality at very low concentration as 500 ppm, however such strong insecticidal activity was not reported even at higher concentration of seed and seed coat extract in our studies [9]. The seed of the *Jatropha* plant is known to contain several toxic metabolites such as sterols and terpene alcohols which are known to have insecticidal property [19]. The phorbol esters found in the seeds were also responsible for the insecticidal activity [17]. Although 20% mortality was observed with leaf extract, this mortality may be attributed to starvation as a result of antifeedant effect, strong repellency was observed at all the concentration leaf extracts tested and even the larval size was found reduced.

The antifeedant effect was so high that even after replacing the treated leaf with the fresh leaf after 24 hrs, the feeding was negligible. Therefore it was decided to take an antifeedant assay of leaf extract at lower concentration (5%) to evaluate the no feeding and larval weight reduction. In the antifeedant assay the larvae were exposed to the cotton leaf treated with respective extract for 24 hrs and then the treated leaf was replaced with the fresh cotton leaf. After 48 hrs again the old leaves were replace with fresh leaves. The assay was taken for 72 hrs and total larval weight reduction after 24, 48 and 72 hrs were recorded. The experiment was designed statistically to confirm the significance of the findings (Table 4 and Figure 3). Overall most of the antifeedant activity was observed in leaves and also most of the larval weight reduction was also observed in leaves followed by root and seed coat whereas in case of bark and seed extract non feeding behavior was not prominent. This suggests the different mechanism of mortality in case of seed and seed coat extract and the larval death may not be on account of starvation.

In case of leaf extract the total larval weight reduction was 36.27% after 24 hrs when they were fed on cotton leaf treated with the extract, but even after replacing the fresh cotton leaf the weight reduction was 42.08% (after 48 hrs) and 41.55% (after 72 hrs). Similarly the root extract was also found to have good antifeedant activity with larval weight reduction ranging from 29.55% after 24 hrs to 43.20% after 72 hrs. The antifeedant activity was least in bark among all the tissue extract tested with larval weight reduction ranging from 12.02% (24 hrs), 13.10% (48 hrs) and 11. 61% (72 hrs). This indicates the tissue specific localization of bioactive molecules.

Since good bioactivity was found in case of seed coat, leaf and root extract, these were further purified using solvent extraction procedure. Maximum yield was obtained in Seed coat fraction in case of Fraction 3 (Table 5) followed by root and leaf.

The chloroform methanol (Fraction 2) and aqueous basic fraction (Fraction 3) of root, leaf and seed coat methanol extract were tested for insecticidal activity against *Plutella xylostella* using leaf dip bioassay method. Dimethylsulfoxide was used as control. Because of less quantity of fractions available the assay was carried out using only *P. xylostella*. Aqueous basic (Fraction 3) of seed coat extract was found to be have better insecticidal activity and has better insecticidal activity against *Plutella xylostella* (60%). Similarly the chloroform-methanol (Fraction 2) and aqueous basic (Fraction 3) of leaf extract was also found to have some mortality

indicating that the bioactive molecules are distributed between the two fractions (Figure 4)

Figure 4 indicate that aqueous basic (Fraction 3) of seed coat extract was found to be have better insecticidal activity and has better insecticidal activity against *Plutella xylostella* (60%). Similarly the chloroform-methanol (Fraction 2) and aqueous basic (Fraction 3) of leaf extract was also found to have unfit some mortality (40%) indicating that the bioactive molecules are distributed between the two fractions.

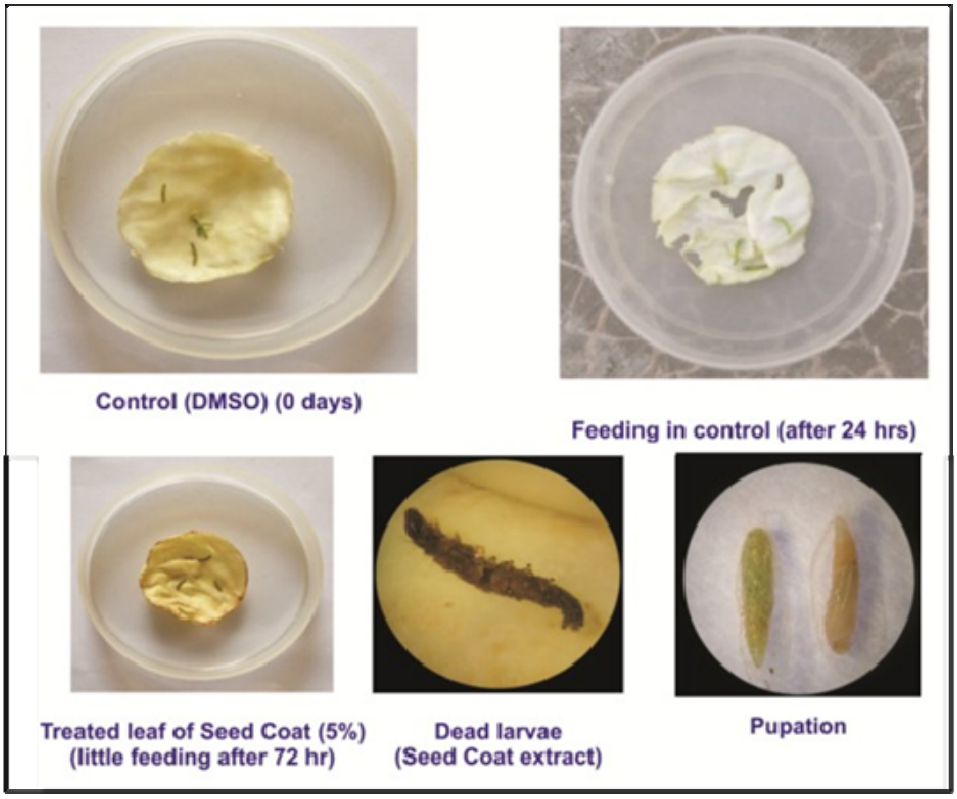

Fig 1: Insecticidal activity of seed coat extract against Diamond Back Moth (*Plutella xylostella*)

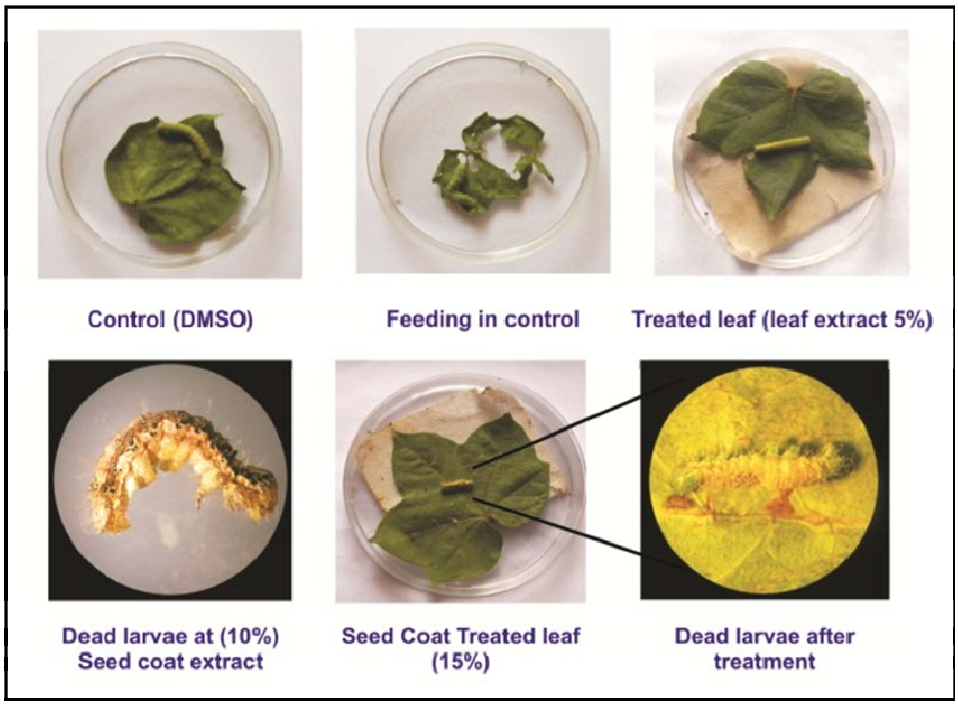

Fig 2: Insecticidal activity of Jatropha leaf extract against *Helicoverpa armigera*

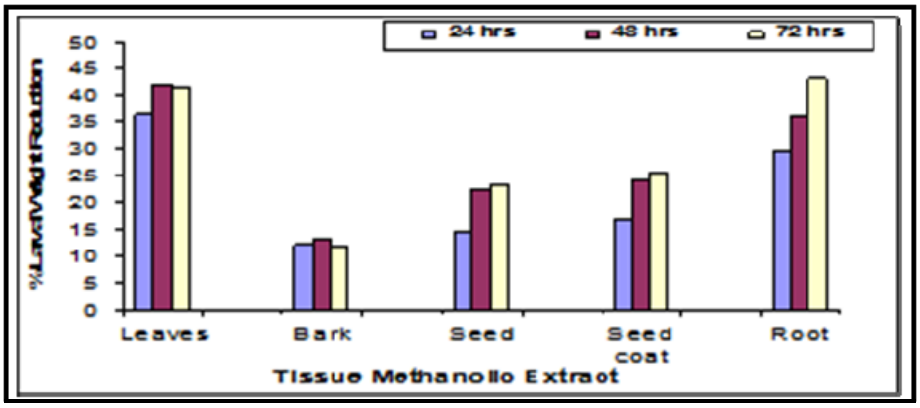

Fig 3: Antifeedant activity against *H. armigera* using different extracts

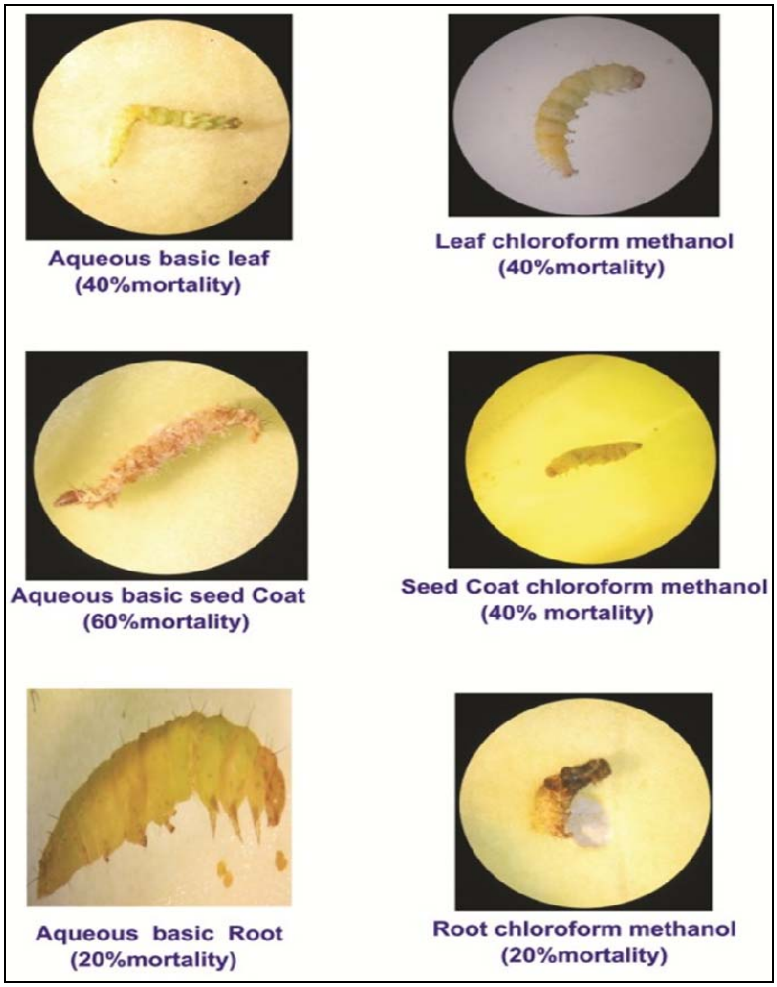

Fig 4: Insect bioassay of solvent extracted fractions against *Plutella xylostella* at 5% concentration

Conclusion

The present study revealed potential insecticidal/ antifeedant activity of the seed coat, leaf extracts of *Jatropha curcas.* Amongst the various solvents used for extraction, methanol was found to be the best extractant in terms of yields. Seed coat extract was found to have insecticidal activity against *Plutella xylostella and Helicoverpa armigera*, and leaf extract was found to have antifeedant activity. Extraction by soxhlet method was found to best in terms of yields. Overall, the study revealed that *Jatropha curcas* seed coat has very good potency against insect and leaf has good antifeedant activity and can be integrated in the management practices of agricultural pests.

References

1. Saxena RC. Botanical pest control, in: Dhaliwal G.S., Heinrichs (Eds.), Critical issues in insect pest management, Commonwealth Publisher, New Delhi, India, 1998, 155-179.
2. Shadia E, Aziz AE, El-Din AAE. Insecticidal activity of some wild plant extracts against cotton leaf warm Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae). Pak. J. Biologi. Sci. 2007; 10(13):2192-2197.
3. Bonnmaison L. Insect pests and their control. Ann. Rev. Entomol. 1965; 10:233-256.
4. Areekul S. The diamondback moth, a serious problem of growing cruciferous vegetables in Thailand. Kasitkorn

1966; 39(3):257-262. (In Thai).
5. Talekar NS, Yang HC, Lee ST, Chen BS, Sun LY. Annotated bibliography of diamondback moth. Tropical Vegetable Information Service, AVRDC, Taiwan. 1985, 469.
6. Miyata T, Saito T, Noppun V. Studies on the mechanism of diamondback moth resistance to insecticides In diamondback moth management Ed. by N. S. Talekar and T. D. Griggs, AVDRC, Taiwan, 1986, 347-358.
7. Miyata T, Sinchaisri N, Sayampol B, Rushtapakornchai W, Vattanatangum A. Insecticide resistance patterns. Report meeting of the joint research project "Insect toxicological studies on resistance to insecticides and integrated control of diamondback moth, March 19-21, 1988, at Rama Garden Hotel, Bangkok, Thailand. 1988, 10-19.
8. Rushtapakornchai W, Vattanatangum A. Present status of insecticidal control of diamondback moth, Plutella xylostella, in Thailand. In Griggs, T.D., ea., Diamondback moth management proceedings of the first international workshop, Tainan, Taiwan, 11-15 March 1985. Asian Vegetable Research and Development Center, Shanhua, Taiwan. 1986, 307-312.
9. Aravinda A, Muralidhara D, Devann N. Bioefficacy of Jatropha curcas L. (Euphorbiaceae) on Helicoverpa armigera Hub. and Bemisia tabaci Genn. Current Biotica. 2009; 2(4):429-438.
10. Olayemi IK, Busari J, Adeniyi KA, Ukubuiwe AC. Comparative Larvicidal Efficacy of Leaf and Stem Extract of Jatropha Curcas against Culex Pipiens Pipiens. Malaya J of Biosciences. 2014; 1(2):104-108.
11. Harborne JB. Phytochemical methods. Chapman and Hall Ltd.London, 1973, 49-58.
12. Tabashnik BE, Cushing NL, Finson N. Leaf residue vs tropical bioassay for assessing resistance in the DBM. (Lepidoptera: Plutellidae). FAO. Pl. Prot. Bull. 1987, 1991; 35:11-14.
13. Harborne JB. Phytochemical Methods: A guide to modern techniques of plant analysis. Edition: 3, Springer. Germany, 1998.
14. Eloff JN. Which extractant should be used for screening and isolation of antimicrobial components from plants. J Ethnopharmacol. 1998; 60:1-8.
15. Shen J, Shao X. A comparison of accelerated solvent extraction, Soxhlet extraction, and ultrasonic-assisted extraction for analysis of terpenoids and sterols in tobacco. Anal Bioanal Chem. 2005; 383:1003-1008.
16. Sinchaisri N, Roongsook D, Chungsamarnyarrt N. Insecticidal activity of plant crude extract on Diamond Back Moth larvae. Kasetsart J (Nat. Sci. Suppl.) 25:106-110.
17. Orwa CA Mutua, Kindt R, Jamnadass R, Anthony S. Agroforestree Database: a tree reference and selection guide version 4.0, 2009.
18. Adebowale KO, Adedire CO. Chemical composition and insecticidal properties of the underutilized Jatropha curcas seed oil. African Journal of Biotechnology. 2006; 5(10):901-906.
19. Adolf W, Opferkuch HJ, Hecker E. Irritant phorbol derivatives fro four Jatropha species. Phytochemistry. 1984; 23:129-132.