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Screening of methanolic extracts of *Euphorbia thymifolia* for insecticidal biopotency against larvae of *Abelmoschus esculentus* and *Glycine max* pests viz. *Earias fabia* and *Diacrisia oblique*

Rashmi Shrivastava and Jyotsana Mishra

Abstract
Laboratory experiments were conducted to evaluate the preliminary screening efficiency of methanolic extracts of *Euphorbia thymifolia* (*E. thymifolia*) plant materials against larvae of *Abelmoschus esculentus* and *Glycine max* pests viz. *Earias fabia* and *Diacrisia oblique.* The caterpillars were reared on healthy and clean leaves of both plants by keeping it in glass jars. One thousand insects were released in 1 kg of gram seeds capped with muslin cloth for ventilation. Culture were maintained in laboratory under controlled climatic conditions viz. temperature (28±2˚C), relative humidity (75±5% RH) and a photoperiod of 12: 12 (L:D) hrs in B.O.D. The crude extracts were tested in ten treatments and each treatment with three replications, along with control (methanol). Mortality of both larvae *Earias fabia* and *Diacrisia obliqua* were recorded after 24hr in the presence and absence of plant extracts, separately. Finally, LC_{50} value was calculated. The mortality of *Earias fabia* and *Diacrisia obliqua varied* with varied concentration of plant ex-tract. Methanolic crude extracts of *E. Thymifolia* was more effective against *Diacrisia oblique* pests having mortality (93.33%) than *Earias fabia* mortality (76.67 %) in compilation to standard neem oil (26.67, 16.67 % respectively). In conclusion, this botanical pesticide was effective in the control of *Earias fabia* and *Diacrisia oblique*, though they do not match the effectiveness of the synthetic pesticide. This botanical pesticide is affordable to low-income farmers. This natural pesticide has the potential for use in agriculture. In this paper, it has been suggested that botanical insecticides should prove most beneficial for farmers in developing countries and managing populations of *Earias fabia* and *Diacrisia oblique*.

Keywords: *Euphorbia thymifolia, Abelmoschus esculentus, Glycine max, Earias fabia*

Introduction
Plant based insecticides (PBI) have been used for many centuries [1, 2]. among limited resource farmers in developing countries to control insect pests of both field crops and stored produce, but their potential was initially limited and ignored. Nicotine, rotenone and pyrethrum were popular among the PBIs used to some extent for storage pests control and other pests in green houses [3]. Some plant species possess one or more useful properties such as repellence, antifeedant, fast knock down, flushing action, biodegradability, broad-spectrum of activity and ability to reduce insect resistance [4, 5]. Okra, *Abelmoschus esculentus* (L), commonly known as lady's finger is an important vegetable crop belongs to Malvaceae plant family cultivated in many regions of the world [6]. The exact origin of plant is unknown however many scientist reported it as an African and Asian originated vegetable [7]. It is very famous in sub-continent for its edible immature fresh green fruit consumed as vegetable [6], cooked in various ways like fried, grilled, boiled, steamed etc. It is known with various local names like Bhindi (Pakistan and India), Dherosh (Bangladesh) [8]. The green seed pods are utilized to make the pickle and seed extract also used to increase the consistency in soups, stews and sauces [6]. It is one of the cash crops of Pakistan including Sind Province cultivated in Kharif season [9]. The best season for okra growing is hot summer, it is sensitive to frost, however may be planted in late spring also [10]. Okra production is hampered caused by heavy infestations of insect pests, diseases and nematodes and damage the foliage, shoots, flowers and pods. It is prone to 72 insect pests from germination to harvest [11, 12], A total of 130 species of Earias were so far identified worldwide and found to attack many crops particularly under Malvaceae family [13, 14]. Ambekar *et al*. reported that two species of Earias namely E. vittella and E. insulana attack the shoot and fruit of okra in India [15].

Earias vettelle has been identified as the major pest of okra also in other countries [16]. The name of E. *fabia* has been changed to E. vittella. Atwal reported that E. vitteila is widely distributed in North Africa, India, Pakistan and other countries and is a serious pest of okra and cotton [17]. Soybean, *Glycine max* (L.) Merrill is a fascinating crop with innumerable possibilities of not only improving agriculture, but also supporting industries. Soybean is a major source of edible oil (20%) and high quality protein (40%). It is a rich source of amino acids, vitamins and minerals. Soybean oil is used as a raw material in manufacturing antibiotics, paints, varnishes, adhesives, lubricants etc. Soybean meal is used as protein supplement in human diet, cattle and poultry feeds. Soybean is a major oil seed crop of world grown in an area of 91m ha with production of 204 mt and productivity of 2,233 kg/ha [18]. The crop is mainly cultivated in USA, China, Brazil, Argentina and India. Major soybean growing states in India are Madhya Pradesh, Maharashtra, Rajasthan, Karnataka, Uttar Pradesh, Andhra Pradesh and Gujarat[19]. The low productivity of soybean both at national and state level is attributed to abiotic and biotic stresses like drought, weeds, insect pests and diseases. Among these, insect pests often pose a serious threat to soybean production by increasing cost of cultivation and impairing quality of produce in many ways. *Diacrisia obliqua* (Bihar hairy caterpillar) is a polyphagous pest. It has been recorded feeding on as many as 33 host plants including oil seed crops. Among oil seed crops, the important ones are soyabean, groundnut, sesamum, linseed, safflower, castor, etc. This pest has been recorded in India as well as in other Asian countries such as Bornea, China and Japan. The pest makes its first appearance from its winter hibernation in March. Their caterpillars feed gregariously and voraciously on a variety of food plants. Having destroyed one field, they move in swarms to another field. There are 6 generations of this pest in a year. As the pest passes the first generation mostly on weeds, it should be destroyed in the weed itself before the pest multiplies and migrates to the cultivated crops. Application of pheromone system will help in forecasting its attack and intensity [20]. The aim of present study was evaluate the effect of methanolic extracts of *E. Thymifolia* plant materials against larvae of *Abelmoschus esculentus* and *Glycine max* pests viz. *Earias fabia* and *Diacrisia oblique.*

Materials and Methods

Plant material

The whole plant material of *E. Thymifolia* was collected from the Betwa riverine zone of Vidisha during rainy seasons of the year 2014 and washed thoroughly with distilled water. It was identified and authenticated by Taxonomist Dr. Sunil Dubey, Department of Botany, St. Mary P. G. College, Vidisha (M.P.). A voucher specimen was procured which was deposited in Department of Botany and Microbiology, St. Mary's P. G. College, Vidisha (M. P.) for future reference and the specimen voucher no. of *E. Thymifolia* is 104105.

Extraction Procedure

Defatting of plant material

Plant material of *E. Thymifolia* was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with n-Hexane using soxhlation method. The extraction was continued till the defatting of the material had taken place.

Extraction

100gm of dried plant material were exhaustively extracted With methanol as solvent using Soxhlet apparatus for 4 days. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts. Dried extract was collected in an air tight container and stored at 4°C for further analysis [21-24].

Rearing of testing insect in the laboratory

The research trial was conducted at the Rabindranath Tagore University, Chiklod Rd, Mendua, Bhopal, Madhya Pradesh 464551. Caterpillar of *Earias fabia* and *Diacrisia obliqua* were collected from the crops of Okra and Soybean crops, respectively from agricultural field of Vidisha (M.P.). The caterpillars were reared on healthy and clean leaves of both plants by keeping it in glass jars. One thousand insects were released in 1 kg of gram seeds capped with muslin cloth for ventilation. Culture were maintained in laboratory under controlled climatic conditions viz. temperature (28±2˚C), relative humidity (75±5% RH) and a photoperiod of 12: 12 (L:D) hrs in B.O.D.

Experimental biological assay methods

Caterpillar of *Earias fabia* and *Diacrisia obliqua* were exposed with various increasing concentrations of methanolic plant extracts from 10% to 100%. For this purpose, separate filter paper strips ($1cm^2$) were coated with different concentrations of plant extracts and were placed in the glass culture tubes and open ends were plugged with cotton balls. The coated filter paper strips were kept air-dried before application. Only solvent treated filter paper strips were used to set control. Ten adult insects were release in glass culture tubes and petrifies (10 cm Height × 4 cm diameter) for pesticidal activity, 10 different concentrations of plant extract purified fractions were applied and for each concentration, three replicates were set. Mortality of both larvae *Earias fabia* and *Diacrisia obliqua* were recorded after 24hr in the presence and absence of plant extracts, separately. Finally, LC_{50} value was calculated according to the methods of Finney 1971[25].

Statistical analysis

The results obtained in the present study were statistically analyzed with the med calc Software of PROBIT analysis [26]. The standard error, standard deviation and significant "P" values were also calculated during the bioassay experiments**.**

Results and Discussion

Bioassay for biopesticidal activity

For pest *Earias fabia*

The results as shown in Table 1 reported that in controlled group 1, 10 pests *Earias fabia* was kept in glass vials along with food stuffs of lady finger fruits in laboratory controlled conditions and after 24 hrs of observations, all insects were found to be alive. This experiment was performed in triplicates and results were found to be same. Similarly, 10 pests were kept in glass vials along with lady finger fruits and served as vehicle controlled group II with 1ml (100%) concentration of solvent methanol and noticed that when experiment repeated in triplicates only 1 pest was reported to be died out of 30 pests, after 24hrs of treatment of solvent which showed 3.33±0.58% mortality. These results suggest that vehicle solvent methanol may be effective solvent along with herbal extract. When the purified fraction of *E. Thymifolia* methanolic extract was applied on paper strip with

different concentration of extracts from 10% (50mg/ml) to 100% (500mg/ml) and kept in glass vials containing 10 pests of *Earias fabia* with food stuffs in each vials for 24hrs treatment. Mortality percentage of the extracts against pests *Earias fabia* was reported in the treated group III to XII with the increasing order of concentration viz.10% < 20% < 30% < 40% < 50% < 60% < 70% < 80% < 90%< 100% of extracts and the mortality percentage values were reported viz. 20.00±0.00%, 26.7±0.58%, 30.00±0.0%, 36.67±0.58%, 46.67±0.58%, 46.67±1.15%, 56.67±0.58%, 60.00±0.00%, 73.33±1.15%, 76.67±0.58% and 16.67±0.58%, respectively. LC_{50} value was reported at 67.48% concentration of purified fraction of the extract of *E. Thymifolia*. Finally the results were compared with the standard group XIII with 100% concentration of reference drug Neem Oil which was found to be very less effective with 16.67±0.58% mortality Fig 1.

Table 1: Biopesticidal activity of various concentrations of E. *Thymifolia* methanolic extracts and Standard Pesticide (Neem oil) on pest *Earias fabia.*

Experimental-group	Concentration	No. of Insect	Mortality				Std Deviation
			Trail 1	Trail 2	Trial 3	% mortality	
Control	0	10	1	0	0	3.33	0.58
Vehicle Control (Methanol)	1 ml	10	1	0	0	3.33	0.58
methanolic extract of *Euphorbia Thymifolia*	10% (100 mg/ml)	10	2	2	2	20.00	0.00
	20% (150 mg/ml)	10	2	3	3	26.67	0.58
	30% (200 mg/ml)	10	3	3	3	30.00	0.00
	40% (250 mg/ml)	10	3	4	4	36.67	0.58
	50% (300 mg/ml)	10	4	5	5	46.67	0.58
	60% (350 mg/ml)	10	4	4	6	46.67	1.15
	70% (400 mg/ml)	10	6	6	5	56.67	0.58
	80% (450 mg/ml)	10	6	6	6	60.00	0.00
	90% (500 mg/ml)	10	8	6	8	73.33	1.15
	100% (550 mg/ml)	10	8	7	8	76.67	0.58
Standard Neem Oil	100%	10	1	2	2	16.67	0.58

A **B** **C** **D**

E

Fig 1: Viability of pest *Earias fabia* under positive control (A) Viability of pest *Earias fabia* under vehicle control with methanol (B) Efficacy of 20%-30% concentration of *E. Thymifolia* methanolic extract on *Earias fabia* (C) Efficacy of 80%-100% concentration of *E. Thymifolia* methanolic extract on *Earias fabia* (D) Efficacy of Standard Neem oil concentration on *Earias fabia* (E) for pest *Diacrisia obliqua*

The results as shown in Table 2 reported that in controlled group 1, 10 pests *Diacrisia obliqua* was kept in glass vials along with soybean leaves for feeding in laboratory controlled conditions and after 24 hrs of observations, all insects were found to be alive. This experiment was performed in triplicates and results were found to be same. Similarly, 10 pests were kept in glass vials along with soybean leaves and served as vehicle controlled group II with 1ml (100%) concentration of solvent methanol and noticed that when experiment repeated in triplicates only 1 pest was reported to be died out of 30 pests, after 24hrs of treatment of solvent which showed 0.33±0.58% mortality. These results suggest that vehicle solvent methanol may be effective solvent along with herbal extract. When the purified fraction of *E.*

Thymifolia methanolic extract was applied on paper strip with different concentration of extracts from 10% (50mg/ml) to 100% (500mg/ml) and kept in glass vials containing 10 pests of *Diacrisia obliqua* in each vials for 24hrs treatment. Mortality percentage of the extracts against pests were reported in the treated group III to XII with the increasing order of concentration viz.10% < 20% < 30% < 40% < 50% < 60% < 70% < 80% < 90 %< 100% of extracts and the mortality percentage values were reported viz. 23.33±0.58 < 30.00±0.00 < 36.67±0. 58 < 43.33±0.58 < 60.00±0.00 < 63. 33±0.58 < 73. 33±0.58< 83 .33±0.58< 86. 67 0.58< 93.33±0. 58< 26.67±0.58, respectively. LC_{50} value was reported at 49.13% concentration of purified fraction of the extract of *E. Thymifolia.* Finally the results were compared with the standard group XIII with 100% concentration of reference drug Neem Oil which was found to be very less effective with 26.67±0.58% mortality Fig. 2.

Table 2: Biopesticidal activity of various concentrations of *E. Thymifolia* methanolic extracts and Standard Pesticide (Neem oil) on pest *Diacrisia obliqua*.

Experimental-group	Concentration	No. of Insect	Mortality				Std Deviation
			Trail 1	Trail 2	Trial 3	% mortality	
Control	0	10	1	0	0	3.33	0.58
Vehicle Control (Methanol)	1 ml	10	1	0	0	3.33	0.58
Euphorbia Thymifolia methanolic extract	10% (100 mg/ml)	10	3	2	2	23.33	0.58
	20% (150 mg/ml)	10	3	3	3	30.00	0.00
	30% (200 mg/ml)	10	4	4	3	36.67	0.58
	40% (250 mg/ml)	10	5	4	4	43.33	0.58
	50% (300 mg/ml)	10	6	6	6	60.00	0.00
	60% (350 mg/ml)	10	7	6	6	63.33	0.58
	70% (400 mg/ml)	10	8	7	7	73.33	0.58
	80% (450 mg/ml)	10	9	8	8	83.33	0.58
	90% (500 mg/ml)	10	9	8	9	86.67	0.58
	100% (550 mg/ml)	10	10	9	9	93.33	0.58
Standard Neem Oil	100%	10	2	3	3	26.67	0.58

A **B** **C** **D**

Fig 2: Viability of pest *Diacrisia obliqua* under positive control (A) Viability of pest *Diacrisia obliqua* under vehicle control with methanol (B) Efficacy of 30%-40% concentration of *E. Thymifolia* methanolic extract on *Diacrisia obliqua* (C) Efficacy of 90%-100% concentration of *E. Thymifolia* methanolic extract on *Diacrisia obliqua* (D)

Bio-statistical Probit Analysis

Probit regression (Dose-Response analysis) of the bioassay results of plant extract's purified fraction of *E. Thymifolia* was performed with the help of Med Calc Statistical Software version 16.8.4 (2016) to assess the goodness of fit model, to calculate regression coefficient and to derive dose-response relationship in the bioassay.

Probit analysis of *E. Thymifolia* on insect *Earias fabia*

From the bioassay results of *E. Thymifolia* methanolic extracts against *Earias fabia*, 13 samples of 130 sample sizes were uploaded with increasing variable concentration of doses and have observed that 30.85% cases were only positive and rest of the 69.15% cases were reported negative. The goodness of fit model was assessed by Chi-square test, Cox and Snell R^2 regression and Nagelkerke R^2 regression analysis. The difference between -2 Log Likelihood score for two models has Chi-square distribution. The difference between-2 Log Likelihood values (null model: 166.403 and full model: 134.385) has Chi-square value 32.018 at degree of freedom=1, which is significant at P< 0.0001 (99%) level. This indicates that range of concentration of plant *E. Thymifolia* methanolic extract have significant effect on mortality of insect *Earias fabia*. Value of Cox and Snell R^2 regression 0.2183 which is below 1 and high enough value of Nagelkerke[98] R^2 regression 0.3024 indicates the significant effect of extract concentration. Wald statistics is a ratio of regression coefficient divided by square of standard error. The regression coefficient value 0.020 is higher than the standard error 0.0038 resulting high value of Wald statistics, which is significant at P< 0.0001, indicating significant effect of plant extract with increased concentration. When the obtained results were uploaded in dose response table at 95% Confidence interval levels, a sigmoid graph was obtained between 0 to 100% concentration levels as shown in Table 3 and Fig 3. The probability of 0.50, the dose required is calculated to be 67.48 with upper limit of 85.13 and lower limit 55.40 of dose concentration.

Table 3: Probit analysis of *E. Thymifolia* methanolic extract against insect *Earias fabia*

Dose variable	Concentration
Sample size	130
Positive cases	44 (33.85%)
Negative cases	86 (66.15%)

Overall Model Fit

Null model -2 Log Likelihood	166.403
Full model -2 Log Likelihood	134.385
Chi-squared	32.018
DF	1
Significance level	P < 0.0001
Cox & Snell R^2	0.2183
Nagelkerke R^2	0.3024

Coefficients and Standard Errors

Variable	Coefficient	Std. Error	Wald	P
dose	0.020528	0.0038809	27.9800	< 0.0001
Constant	-1.38528	0.23051	36.1171	< 0.0001

Dose-Response table

Probability	Dose	95% Confidence interval	
		Lower limit	Higher limit
0.10	5.05	-24.80	20.95
0.20	26.48	6.71	39.00
0.25	34.62	17.97	46.56
0.50	67.48	55.40	85.13
0.75	100.33	83.20	133.32
0.80	108.48	89.58	145.77
0.90	129.91	106.01	178.91
0.95	147.60	119.35	206.51
0.97	162.95	130.82	230.54
0.98	167.52	134.22	237.70
0.99	180.80	144.09	258.55

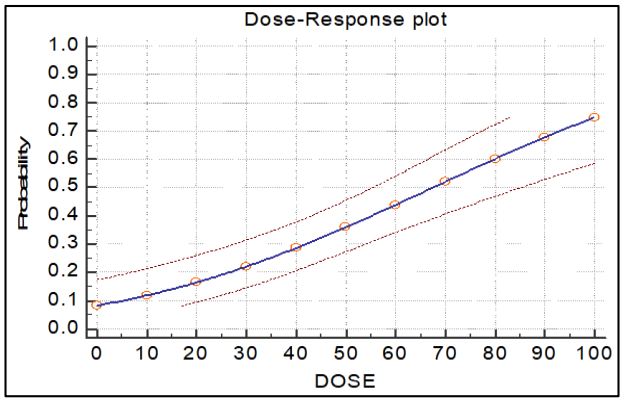

Fig 3: Dose response plot of probability and dose in concentration (at 0.5 Probit, dose concentration is 67.48% i.e. 385.6 mg/ml of *E. Thymifolia* methanolic extract against insect *Earias fabia*.

Probit analysis of *E. Thymifolia* on insect *Diacrisia obliqua.*

From the bioassay results of *E. Thymifolia* methanolic extracts against *Diacrisia obliqua*, 13 samples of 130 sample sizes were uploaded with increasing variable concentration of doses and have observed that 44.62% cases were only positive and rest of the 55.38% cases were reported negative. In the overall goodness of fit model, difference between -2 Log Likelihood score for two models has Chi-square distribution. The difference between -2 Log Likelihood values (null model: 178.708 and full model: 129.727) has Chi-square value 48.980 at degree of freedom=1, which is significant at P< 0.0001 (99%) level. This indicates that range of concentration of plant *E. Thymifolia* methanolic extract have significant effect on mortality of insect *Diacrisia obliqua.* Value of Cox & Snell R^2 regression = 0.3139 which is below 1 and high enough value of Nagelkerke R^2 regression= 0.4202 indicates the significant effect of extract concentration. Wald statistics is a ratio of regression coefficient divided by square of standard error and the regression coefficient value is 0.025 which is higher than the standard error value 0.0040 resulting high value of Wald statistics, which is significant at P< 0.0001, indicating significant effect of plant extract of *E. Thymifolia* with increased concentration against insect *Diacrisia obliqua*. When the obtained results were uploaded in dose response table at 95% confidence interval levels, a graph was obtained between 0 to 100% concentration levels as shown in Table 4 and Fig. 4 The probability at 0.50level, the dose required is calculated to be 49.13 with upper limit of 59.87 and lower limit 39.42 of dose concentration.

Table 4: Probit analysis of *E. Thymifolia* methanolic extract against insect *Diacrisia obliqua*

Sample size	130
Positive cases	58 (44.62%)
Negative cases	72 (55.38%)

Overall Model Fit

Null model -2 Log Likelihood	178.708
Full model -2 Log Likelihood	129.727
Chi-squared	48.980
Degree of Freedom	1
Significance level	P < 0.0001
Cox and Snell R^2	0.3139
Nagelkerke R^2	0.4202

Coefficients and Standard Errors

Variable	Coefficient	Std. Error	Wald	P
Dose	0.025762	0.0040572	40.3194	< 0.0001
Constant	-1.26563	0.22037	32.9850	< 0.0001

Dose-Response table

Probability	Dose	95% Confidence interval	
		Lower limit	Higher limit
0.05	-14.72	-43.32	1.53
0.10	-0.61	-23.58	12.96
0.20	16.45	-0.24	27.35
0.25	22.94	8.32	33.12
0.50	49.13	39.42	59.87
0.75	75.31	63.94	93.21
0.80	81.79	69.43	102.05
0.90	98.87	83.43	125.79
0.95	112.97	94.68	145.69
0.97	125.21	104.33	163.08
0.98	128.84	107.18	168.27
0.99	139.41	115.45	183.38

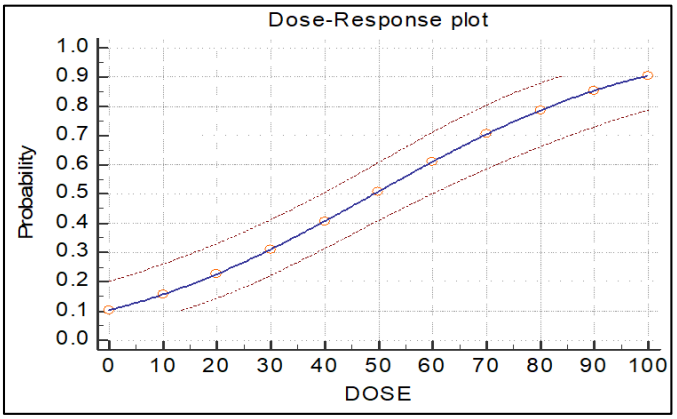

Fig 4: Dose response plot of probability and dose in concentration (at 0.5 Probit, dose concentration is 49.13% i.e. 294.78mg/ml of *E. Thymifolia* methanolic extract against *Diacrisia obliqua*.

Conclusion

This present study concluded that methanolic solvents extracts of *E. Thymifolia* possess toxic principles with insecticidal effect and could be potential larvae of *Abelmoschus esculentus* and *Glycine max* protectants against *Earias fabia* and *Diacrisia oblique.* Methanolic solvents extracts of *E. Thymifolia* showed the highest toxic effect against *Diacrisia oblique.* Therefore, whole plant extracts of *E. Thymifolia* may be recommended as cheap, easily available at farm level, eco-friendly with low mammalian toxicity and a good alternative to synthetic insecticides.

Conflict of interest

The authors declare no conflict of interest.

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