International Journal of Fauna and Biological Studies
Available online at www.faunajournal.com

ISSN 2347-2677
www.faunajournal.com
IJFBS 2020; 7(4): 20-26
Received: 11-05-2020
Accepted: 13-06-2020

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Naturally occurring borational extractives against Bihar hairy caterpillar, *Diacrisia obliqua* walker (Lepidoptera: Arctiidae)

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Abstract
Laboratory trials were conducted to assess the insecticidal activities of naturally occurring indigenous extract and their formulations against *Diacrisia* (S*pilosoma* or *Spilarctia*) *obliqua* Walker. The experiment was done in three glass jar (23cm x 10cm) and replicated thrice by using each selected extractive per jar. For testing, the *Brassica juncea* ver. varuna leaves were used as food. *B. juncea* ver. varuna leaves were cut and sprayed the extracts of different concentrations for two minutes. The treated foods were kept in jar on moist filter paper. The treated leaf pieces were dried under clip and left under electric fan for an about half hour to make a dry film of the extracts on the leaves for each set of extract. Now, 24 hours starved laboratory reared third instars ten larvae were released in each treatment and each treatment replicated thrice. For control the leaf pieces were dipped in Benzene +emulsified water only. After 6, 12 and 24 hours, the data was collected on the larval mortality on treated food and mortality over control was recorded. The result indicated that alcoholic extracts of *A. maxicana* registered highest mortality (70.07%) to the larvae of *D. obliqua* when compared to other plant extracts showed as: *A. indica* (63.93%) and *B. frondosa* (63.90%), have registered encouraging (greater than 60% mortality) results having insecticidal properties. Consistently, remaining all these aforementioned plant extractives has revealed their insecticidal potential at various intervals.

Keywords: *Diacrisia obliqua*, Azadirachta indica, Butea frondosa insecticidal activity

1. Introduction
The Bihar hairy caterpillar *Diacrisia* (*Spilarctia* or *Spilosoma*) *obliqua* Walk. (Lepidoptera: Arctiidae) is most important insect-pest of mustard crops. *Diacrisia obliqua* Walker is a polyphagous pest of sporadic nature has been in regular occurrence, causing considerable damage to cruciferous vegetables and crop (Butani and Verma 1976, Butani *et al.*`977, and Chandel *et al.*2004) [1, 2, 3]. Major hosts include cruciferous, oilseeds, vegetables, groundnut, sunflower, cashew, castor, cucurbits, mulberry, pigeonpea, beans, jute, sweet potato and millets crop in abroad (Golob and Webley 1980, Chen and Chang, 1996,) [4, 5]. The caterpillars of *Spilarctia obliqua* feeds on the leaves of the plant and cause considerable damage to cruciferous crops and vegetables in our country (Tandon *et al.* 2004) [6] they occurs all parts of mustard and vegetables growing area of the country. The caterpillar's feeds on the leaves of the plant and cause considerable damage to mustard crop (Ahmed and Bhattacharya 1991, Chandel, *et al.* 2004, Dubey *et al.*2004) [7, 8, 9].
The insect-pests destroy more than one-third of the world's crop production and this heavy crop losses at both the International and National level can be successfully dealt with only through the intensive use of the pesticides (Zenno *et al* 1975) [10]. If pesticides are not used, the graph of the crop losses may rise to 50.0 per cent and even more in the developing countries. In India, in inflicts huge losses to early and late sown cauliflower and mustard. The pest is distractive in its larval stage (Sukhirun *et al.*, 2011) [11].
The synthetic insecticides are employed in the management of insect-pest on crops bearing direct hazardous adverse effects on humans, wildlife, aquatic life and the environment at large (Huen *et al.*, 2012; Koureas *et al.*, 2012) [12, 13]. However, there are concerns about the use of pesticides, because of their negative effects on the environment and human health (Yuan *et al.*, 2014) [14]. These pesticides are also expensive and out of reach to the poor farmers Wang *et al.*, 2014) [15]. Therefore, there is a need to develop alternative methods of pest management (Chowdhury. 2009) [16]. To solve this problem many synthetic pesticides were used. These chemicals have the ability to kill the target insect pest and non-target organism such as

beneficial insects. Many synthetic chemical insecticides can cause significant health problems and they can be harmful to the environment. They contaminated in soil and water resources and also they accumulate in food and many human systems and cause cancer (Guyton *et al.*, 2015) [17]. The exposure to these synthetic chemicals may cause many symptoms like dizziness, headache, nausea, vomiting, eye irritation, fatigue, muscular twitching, unconsciousness, brain and nervous disorders and immune systems failures (Mohapatra, 2001) [18] Botanical plant materials are currently recognized as biodegradable, systemic, eco-friendly and non-toxic to mammals and are thus considered as safe alternatives (Ali *et al.*1983, Pascual *et al.*1990, Sarup, and Srivastava 2001) [19, 20, 21]. Indeed, Secoy and Smith (1983) reported that pyrethrins, isolated from the dried flowers of *C. cinerariaefolium* Vis. possess insecticidal properties [22]. Insecticidal activities of certain neem, *Azadirachta indica* A. Juss extractives, products and derivatives on larvae of *E. vittella* was reported (Sharma *et.al.*1980, Raghuraman and Singh 1999, El and El, 2000, Gahukar, 2000) [23, 24, 25, 26]. Oil extracted from various materials was used in the tropics as a dressing for livestock to control blowfly. Biopotency of plant extracts were reported by Islam *et al.* 2009. Its high level of activity makes possible its commercialization as a mosquito larvicide (Lowery and Smirle 2000) [27].

To the best of our knowledge, there are no data available on use of such indigenous plant extractives and essential oils. Because of the significant difference in smell of the aerial parts of both plants, we have focused this study to use of certain indigenous plant extractives which are responsible for the insecticidal activities. However, in his study, plant extracts taken for study were not tested against third instars larvae of *S. obliqua.* Hence, the present studies were undertaken in the laboratory trials to study, the relative insecticidal efficacy of six plant extracts against third instar larvae of *Diacrisia obliqua* Walk.

2. Material and Method

2.1 Mass culturing of *Diacrisia obliqua* Walk.:

The larvae of *Diacrisia obliqua* Walker were obtained from the mustard experimental farmers' fields of Fattepur Dakshin village, Kanpur Nagar and maintained in the laboratory on natural diets. The collected larvae were kept for at least 5 days in the laboratory to check, whether or not, there are any other infections before using them for experiments. *Diacrisia obliqua* required for the study were mass reared on cruciferous leaves in the laboratory. The mass culturing was initiated by confining 10 larvae of *D. obliqua* in the plastic containers of 59x21x18cm having green mustard leaves which were covered with muslin cloth and secured tightly with rubber band. Mass culturing of *D. obliqua* larvae was done at 28 ±2 ºC temperature in the plastic container and observed daily.

2.2 Collection of Natural Plant Materials:

The natural plant materials used in the present investigation were collected mainly from cultivated fields of the farmers, wasteland and wild areas in the vicinity of Kanpur region. The collected six materials viz; *Aconitum ferox* Wall, *Adhatoda vesica Nees, Argemone maxicana* Linn., *Azadirachta indica* A. Juss, *Butea frondosa* Koen. and *Calotropis procera* Ait were used for their insecticidal efficacy against larvae of *D. obliqua.*

2.3 Powder Preparation and Extraction: Fresh collected plant materials like aerial parts and leaves etc.) Were washed with tap water and kept in the laboratory for 7 days for shadow air drying before making powder. Electric grinder was used to have coarse powder then these were passed through a 60-mesh sieve to get fine powder. Powders were kept in polythene bags at room temperature and properly sealed to prevent quality loss. For the extraction, Soxhlet Apparatus was used; about 20g powder of each category was extracted with 300ml of alcohol and distilled water). Extractions of each category of powder were done in about 12 hrs. After soxhlet extraction, the material was run on rotary evaporator. The extracts were concentrated on rotary evaporator by removing the excess solvent under vacuum. After evaporation of solvent the remaining extracted material was kept on water bath for removing remaining solvent from the extracts. The extracts were stored at 4 °C prior to application.

2.4 Preparation of Stock Solution and Insecticidal Formulations 50ml. extract in each case was taken into reagent bottles and 50ml. benzene was added in it to dissolve the constituents of the selected plant materials. The mouth of the bottles were stopper with airtight corks after which, these bottles containing the solutions were kept in refrigerator. The alcoholic extracts of Aforementioned were tested under laboratory against third instar larvae of *D. obliqua*, which is noxious insect pest of okra vegetables and crop. Three concentrations of plant extractives (0.5, 1.0, and 2.0 percent) were used for experiments on insecticidal tests in the laboratory conditions. The different concentrations of the herbal extracts were prepared from the stock solution using benzene as solvent and Triton X-100 as emulsifier. The level of solvent and emulsifier were kept constant.

2.5 Apparatus used for experiment: More than one hundred glass petridishes (15cm diameter) were used for the experiment, One hand compression poly sprayer and muslin cloth was required for covering the petri-dishes and ridges of plots either going or coming the larvae in the cruciferous leaves in the petridishes.

3. Experimental Protocol: Laboratory experiment was conducted to the insecticidal effect of six extractives viz; *Aconitum ferox* Wall, *Adhatoda vesica Nees, Argemone maxicana* Linn., *Azadirachta indica* A. Juss, *Butea frondosa* Koen. and *Calotropis procera* Ait against third instars larvae of *D. obliqua*, which are noxious insect pest of mustard vegetables and crop. For testing the insecticidal effect the mustard leaves were used as food against the third instar larvae of *D. obliqua* treated with different concentrations of six selected *extractives* insecticides. The treated foods were covered with muslin cloths. . Then third instar, 24 hours starved larvae of *D. obliqua* were released in each set of extract and one control was introduced under field conditions. For control set the leaves and fruits were sprayed with Benzene + emulsified water only. After 6hr, 12h and 24 hours of the release of larvae the data was collected on the number of larvae dead at each treated food set. Three replication of treatment were made. The insecticidal effect of each the plant extractives was judged by counting the mortality of larvae after 6, 12 and 24 hours and the larval mortality percentage were adjudged over control. All the values were calculated as per Abbott formula (Abbott 1925) [30].

Table 1: Mean mortality percentage of *Diacrisia obliqua* Walker in case of different combinations under laboratory conditions

Treatment (Plant extracts)	Con. (%)	Mean Mortality percent After					
		6 hrs.		12 hrs.		24 hrs.	
		T_1	T.B.V.$_1$	T_2	T.B.V.$_2$	T_3	T.B.V.$_3$
Aconitum ferox Wall	0.5	0.5	37.22	XX	48.84	56.7	59.00
Aconitum ferox Wall	1.0	41.15	43.3	50.85	60.1	70.07	88.4
Aconitum ferox Wall	2.0	54.09	67.1	63.44	80.0	77.70	95.4
Adhatoda vesica Nees	0.5	45.00	50.0	48.93	56.8	53.07	63.9
Adhatoda vesica Nees	0.1	0.1	46.92	53.4	50.93	60.3	59.21
Adhatoda vesica Nees	2.0	53.07	63.9	59.21	73.8	72.29	90.8
Argemone maxicana Linn.	0.5	45.00	50.0	54.99	67.1	70.07	88.4
Argemone maxicana Linn.	1.0	61.02	76.8	64.22	81.1	72.29	90.8
Argemone maxicana Linn.	2.0	67.86	85.8	72.29	90.8	83.85	98.9
Azadirachta indica A. Juss	0.5	48.84	56.7	59.00	73.5	63.93	80.7
Azadirachta indica A. Juss	1.0	59.00	73.5	67.84	85.8	81.14	97.6
Azadirachta indica A. Juss	2.0	61.92	77.8	81.14	97.6	90.00	100.0
Butea frondosa Koen.	0.5	37.14	36.5	48.93	56.8	63.93	80.7
Butea frondosa Koen.	1.0	39.41	39,9	52.84	63.3	70.07	88.4
Butea frondosa Koen.	2.0	61.92	77.8	81.14	97.6	90.00	100.0
Calotropis procera Ait.	0.5	39.23	40.0	41.14	43.3	45.00	50.0
Calotropis procera Ait.	1.0	55.00	50.0	54.78	66.7	61.22	76.8
Calotropis procera Ait.	2.0	48.84	56.7	59.00	73.5	67.86	85.8
Control	0.00	0,00	0.0	0.00			

. (T_1, T_2, T_3 = Treatments and TBV.$_1$, TBV.$_2$, TBV.$_3$= Transformed Back Values)
C.D. for the treatment combination means = 0.175

The analysis of variance in table 1 shows that the main effect of insecticide, concentrations and periods as well as "Control versus treated" in first order and periods, concentrations in second order interaction are more highly significant except the first order interaction "insecticide x concentration" and the Second order interaction" period x insecticide x concentration which is non-significant. The effect of control VS treatment is also significant, at 0.5ercent level of significance.

Table 2a: Mean mortality percentage of *Diacrisia obliqua* Walker in case of different combination under laboratory conditions

Treatment (Plant extracts)	Con. (%)	Mean Mortality percent After					
		6 hrs.		12 hrs.		24 hrs.	
		T_1	T.B.V.$_1$	T_2	T.B.V.$_2$	T_3	T.B.V.$_3$
Aconitum ferox Wall	0.5	37.22	XX	48.84	56.7	59.00	73.5
Adhatoda vesica Nees	0.5	45.00	50.0	48.93	56.8	53.07	63.9
Argemone maxicana Linn.	0.5	45.00	50.0	54.99	67.1	70.07	88.4
Azadirachta indica A. Juss	0.5	48.84	56.7	59.00	73.5	63.93	80.7
Butea frondosa Koen.	0.5	37.14	36.5	48.93	56.8	63.93	80.7
Calotropis procera Ait.	0.5	39.23	40.0	41.14	43.3	45.00	50.0
Control		0.00	00.0	0.00	00.0	21.14	13.4

(T_1, T_2, T_3 = Treatments and TBV.$_1$, TBV.$_2$, TBV.$_3$= Transformed Back Values)
C.D. for the treatment combination means = 0.147

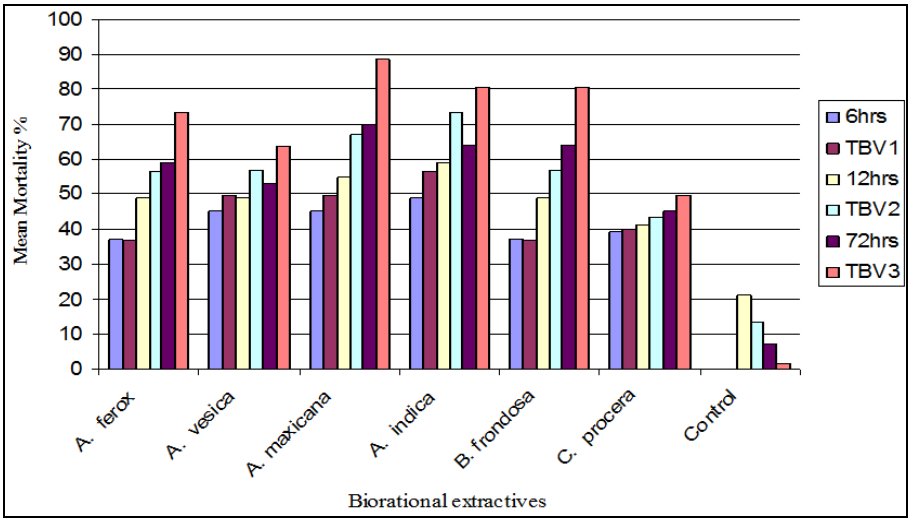

Fig 1: Mean mortality percentage of *Diacrisia obliqua* Walker in case of 5% combination under laboratory conditions

Table 2b: Mean mortality percentage of *Diacrisia obliqua* Walker in case of different combination under laboratory conditions

Treatment (Plant extracts)	Con. (%)	Mean Mortality percent After					
		6 hrs.		12 hrs.		24 hrs.	
		T_1	T.B.V.$_1$	T_2	T.B.V.$_2$	T_3	T.B.V.$_3$
Aconitum ferox Wall	1.0	41.15	43.3	50.85	60.1	70.07	88.4
Adhatoda vesica Nees	0.1	46.92	53.4	50.93	60.3	59.21	73.8
Argemone maxicana Linn.	1.0	61.02	76.8	64.22	81.1	72.29	90.8
Azadirachta indica A. Juss	1.0	59.00	73.5	67.84	85.8	81.14	97.6
Butea frondosa Koen.	1.0	39.41	39,9	52.84	63.3	70.07	88.4
Calotropis procera Ait.	1.0	55.00	50.0	54.78	66.7	61.22	76.8
Control		0.00	00.0	0.00	00.0	21.14	13.4

(T_1, T_2, T_3 = Treatments and TBV.$_1$, TBV.$_2$, TBV.$_3$= Transformed Back Values)

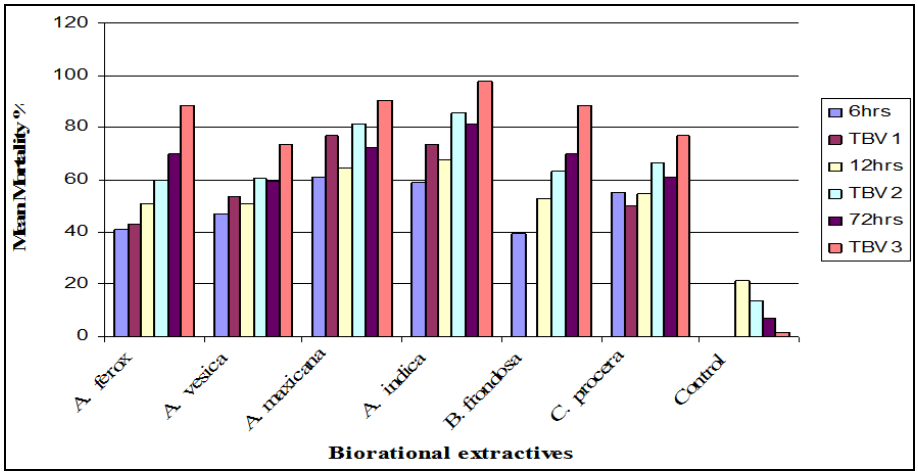

Fig 2: Mean mortality percentage of *Diacrisia obliqua* Walker in case of 1.0% combination under laboratory conditions

Table 2c: Mean mortality percentage of *Diacrisia obliqua* Walker in case of different combination under laboratory conditions

Treatment (Plant extracts)	Con. (%)	Mean Mortality percent After					
		6 hrs.		12 hrs.		24 hrs.	
		T_1	T.B.V.$_1$	T_2	T.B.V.$_2$	T_3	T.B.V.$_3$
Aconitum ferox Wall	2.0	54.09	67.1	63.44	80.0	77.70	95.4
Adhatoda vesica Nees	2.0	53.07	63.9	59.21	73.8	72.29	90.8
Argemone maxicana Linn.	2.0	67.86	85.8	72.29	90.8	83.85	98.9
Azadirachta indica A. Juss	2.0	61.92	77.8	81.14	97.6	90.00	100.0
Butea frondosa Koen.	2.0	43.07	46.6	59.21	73.8	75.00	93.3
Calotropis procera Ait.	2.0	48.84	56.7	59.00	73.5	67.86	85.8
Control		0.00	00.0	0.00	00.0	21.14	13.4

(T_1, T_2, T_3 = Treatments and TBV.$_1$, TBV.$_2$, TBV.$_3$= Transformed Back Values)

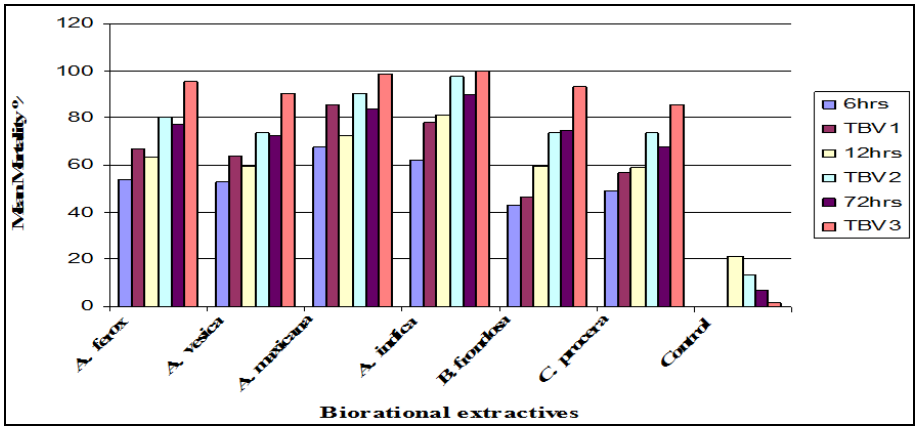

Fig 3: Mean mortality percentage of *Diacrisia obliqua* Walker in case of 2.0% combination under laboratory conditions

Table 3: Mean mortality% of *Dicricia obliqua* Walker in different periods irrespective of concentration in laboratory conditions

Treatment (Plant extracts)	Mean mortality percent after						Mean% Mortality	
	6 hrs.		12 hrs.		24 hrs.			
	T_1	TBV_1	T_2	TBV_2	T_3	TBV_3	G.T.	TBV
Aconitum ferox Wall.	44.45	49.0	54.37	66,18	68.92	87.1	5.91	67.3
Adhatoda vesica Nees.	48.33	55.8	53.02	62.8	61.52	77.3	54.29	65.9
Argemone maxicana Linn.	58.26	72.3	63.83	89.6	75.0	93.7	65.83	83.2
Azadirachta indica A. Juss.	56.68	68.7	69.33	87.5	78.35	95.9	68.08	86.1
Butea frondosa Koen.	39.78	69.7	53.66	64.9	69.63	87.9	57.72	71.5
Calotropis procera Ait.	44.25	48.7	51.64	61.5	58.02	72.0	51.30	60.9
Total	48.62	56.3	57.14	70.6	59.00	73.5	54.92	67.00
Control		00.0	0.00	00.0	21.14	13.4	7.04	1.5

(T_1, T_2, T_3 = Treatments and T.B.V.$_1$, T.B.V.$_2$, T.B.V.$_5$= Transformed Back values
C.D. for treatment x period means =0.078
C.D. for treatment means (plant extra =0.037
C.D. for treatment means (control) =0.162

The table 2 and Figure 2a reveals that toxicity of six bioactive plant extracts can be summarized based on their relative mortality and transformed back values as*: A. maxicana* (70.07) >*A. Indica* (63.93) >*B. frondosa* (63.90) >*A. Ferox* (59.00) >*A. Vesica* (53.07) >*C. Procera* (45.00) >control (21.14), respectively.

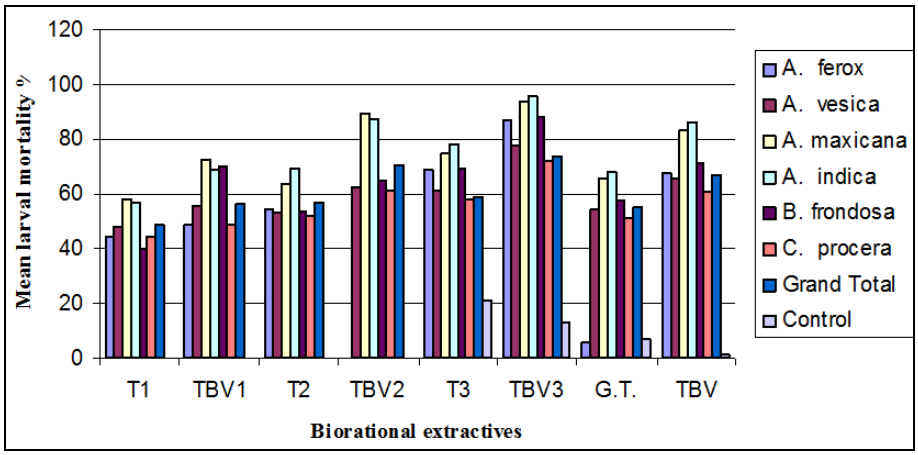

Fig 4: Mean mortality percentage of *D. obliqua in concentrations irrespective of periods under the laboratory*

Table 4: Mean mortality percentage of D. *obliqua* in concentrations irrespective of periods under the laboratory condition

Treatments extractives	Mean mortality percent after					
	6hrs.		12hrs.		24hrs.	
	T_1	TBV_1	T_2	TBV_2	T_3	TBV_3
0.5	42.07	43.0	50.30	59.2	59.16	73.7
1.0	50.91	60.2	56.91	60.2	69.00	87.2
2.0	54.80	64.8	65.71	83.1	77.78	95.5
Over All	48.62	56.3	57.14	70.6	59.00	73.5
Control	0.00	00.0	0.00	00.0	21.14	13.4

(T_1, T_2, T_3 = Treatments and T.B.V.$_1$, T.B.V.$_2$, T.B.V.$_5$= Transformed Back values

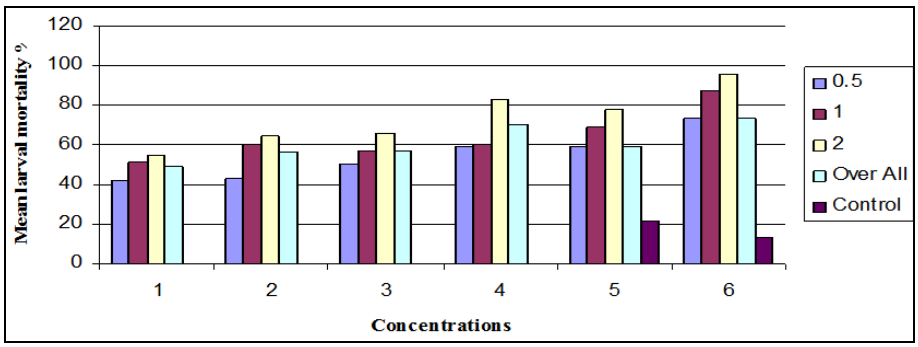

Fig 5: Mean mortality percentage of Dicricia oblique Walker in control irrespective of treatments under the laboratory condition

Table 3 and Figure 3 indicate that all the three concentration differed significantly to one another. The concentration 2.0 per cent is superior to concentration 1.0 and 0.5 per cent. It is observe that the difference in the percentage larvae of *Dicricia obliqua* Walker kill in concentration 2.0 per cent and 1.0 per cent is greater than the difference in concentration to kill the larvae in 1.0 per cent and 0.5 per cent in all the three periods. Similarly the difference in percentage mortality of larvae of *Dicricia obliqua* in 24 hours (84.6 per cent)) and 12 hours (83.39per cent) is greater than the difference in percentage mortality in the period of 12 hours (78.7 percent) and 6 hours (59.81 percent).

Table 5: Mean mortality percentage of *Dicricia obliqua* Walker in control irrespective of treatments

Treatments extractives	Mean mortality percent after						Mean% Mortality	
	6 hrs.		12 hrs.		24 hrs.			
	T_1	TBV_1	T_2	TBV_2	T_3	TBV_3	G.T.	TBV
Control	0.00	00.0	0.00	00.0	21.14	13.4	7.04	1.5
Extractives	48.62	56.3	57.14	70.6	59.00	73.5	54.92	67.0

4. Results and Discussion

The data depicted in text indicated that all the selected plant extractives have proved to more or less effective in controlling the *D. oblique* larvae under laboratory trials. The larval infestation of D. *oblique* infestation was recorded only at an early stage of crop growth and it was limited for a month only. The pooled mean data presented in Table-1-3 and figure 1-3 reveals that insecticidal biopotency of different plant based insecticidal treatments showed that *A. maxicana* emulsions in dry film registered highest *Spilarctia obliqua* larval mortality (70..07 percent) and placed at the top of the effectiveness. and placed at the top of the effectiveness. The data on larval mortality percentage of remaining plant extracts as; > *A. Indica* (63.93) >*B. frondosa* (63.90) >*A. Ferox* (59.00) >*A. Vesica* (53.07) >*C. Procera* (45.00) >control (21.14), respectively.

Many plants can protect themselves against insects by producing their own chemical defenses that are insecticidal (Chandel, 2017) [31]. The consideration for the use of extracts of plants origin is that they are easily biodegradable, effective on some pests and considered safe in pest control operations as they minimize pesticide residues, ensure safety of the consumers of the treated grains and the environment. Further, the production of organic extracts of plant origin for pest control may be easier and less expensive than the synthesis of some complex chemical. They possess many of the attributes of an ideal biological control agent, including broad host range, high virulence, host seeking capability, ease of mass production, recycling ability, non- hazardous to environment, etc. (Thangapandian *et al.* 2011) [32].

Neem Pesticide is a natural product, absolutely non-toxic, 100% biodegradable and environmentally friendly in nature (Attri, 1975) [36]. If required, it can be mixed with other synthetic pesticides. Gradually, the ratio of Neem content in the mixture can be increased and synthetics reduced till you reach a stage where synthetics become redundant. Neem consists of several compounds hence development of resistance is impossible. Neem does not destroy natural enemies of pests thereby allowing these natural enemies to keep a check on the pest population (Joshi *et al.* 1984 and Lowery and Smirle 2000) [37, 38]. Neem also has a systemic action and seedlings can absorb and accumulate the neem compounds to make the whole plant pest resistant. Neem is harmless to non-target and beneficial organisms like pollinators, honey bees, mammals and other vertebrates. Neem has a broad spectrum of action active on many species of pests.

Our findings are in conformity with the findings of Rao *et al.* (1990) tested fifteen naturally occurring indigenous plant materials as antifeedant against the larvae of *H. vigintioctopunctata* Fabr. Petroleum ether extract at 0.5 to 1.00 per cent and aqueous extract at 1.00 to 5.00 per cent of the test plants and *Annona squamosa, Argemone maxicana, Calotropis gigantica, R. communis* were gave cent-percent leaf protection. [33]. Chitra *et al.* (1993) evaluated insecticidal efficacy of certain plant products against *H. Vigintioctopunctata, A. gossypii and L. orbonalis* and compare with monocrotophos and endosulfan. 1.0 per cent Petroleum ether leaves extract of *Argemone maxicana* (76.18 per cent) was superior 0.04 per cent monocrotophos (78.82 per cent) and 0.07 per cent endosulfan (75.46 per cent) followed by *A. indica* leaves extract 0.1 per cent neknool 0.1 per cent showed 71.75 per cent and 67.61 per cent mortality, respectively [34]. Pandey and Raju, (2003) tested different eco-friendly insecticides against *Plutella xylostella* larvae among them NSKE (neem seed kernel extract) 2.00 per cent gave considerable mortality to the second instar larvae of *Plutella xylostella* by using leaf dip method [35].

5. Conclusion

Conclusively, the present investigation revealed that *Argemone maxicana* Linn extractives registered highest mortality (70.07%) to the 3rd instars larvae of *Diacrisia obliqua* Walker followed by *Azadirachta indica* A. Juss (63.93) >*Butea frondosa* Koen (63.90) >*Aconitum ferox* Wall (59.00) >*Adhatoda vesica Nees* (53.07) >*Calotropis procera* Ait (45.00) >control (21.14), respectively whereas *C. Procera* leaves extract was proved least toxic showing 45.00 per cent larval mortality.

6. Acknowledgement

The authors are thankful to Principal, D.B.S. College, and Kanpur for providing the necessary facilities. Prof. N.D. Pandey, farmer Head, Division of Entomology, C S Azad University of Agriculture and Technology, Kanpur for rendering their support and help for the completion of this work.

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