

# Antioxidant and Ecofriendly Ovicidal Activity of Medicinal plants Against Some Common Plant Pests

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**ABSTRACT:** Chemical and synthetic pesticides used for controlling various plant pest have resulted in the development of resistance to several classes of pesticides. In the present study, five medicinal plants were screened for their ovicidal activity against *Helicoverpa armigera*, *Spodopteralitura*, *Plutellaxylostella*, *Corcyracephalonica*. Hexane, Diethyl ether extract of plants was studied at 1% concentration. Effective plant extracts identified based on total antioxidant, flavonoid and phenolic contents and Maximum ovicidal activity was recorded in hexane extract of *Vitexagnuscastus* (96%) for *Spodoptera*, (93.5%) for *Plutella* and (90.5%) for *Corcyra* followed by hexane extract of *Ricinuscommunis* (92%) for *Corcyra* and *Pongamiapinnata* (84.5%) for *Corcyra*. Diethyl ether extract showed lower activity when compared to hexane extract. Further isolation and identification of bioactive principles responsible for the ovicidal activity is needed.

**KEYWORDS:** Plant pests, Ovicidal activity, Plant extracts, Hexane, Diethyl ether

## I. INTRODUCTION

Pest is a destructive insect or other animal that attacks crops, food, livestock, etc. The four plant pest that have been chosen for the study of the ovicidal activity through plant extracts are *Helicoverpa armigera*, *Spodoptera litura*, *Plutella xylostella*, *Corcyra cephalonica*. It is essential to control the growth of these pest at egg stage to prevent their damage caused to the crop production that too by using organic means. Pollinators like bees or natural predators like ladybirds may be susceptible to pesticides; pesticides may also be toxic to wildlife or to pets. If mishandled or misused, some pesticides can also be toxic to humans [1].

*Helicoverpa armigera*, the cotton bollworm is a moth, the larvae of which feed on a wide range of plants. It is a major pest in cotton and one of the most polyphagous and cosmopolitan pest species. It has multitudes of effects on various developmental stages of *Helicoverpa armigera* [2]. Oriental Leafworm Moth (*Spodopteralitura*) is a Noctuid moth, The larvae feed on a wide range of plants and has been recorded from over 40 mostly dicotyledonous plant families. The eggs are spherical and 0.6 mm in diameter [3]. The diamondback moth (*Plutellaxylostella*), sometimes called cabbage moth [4]. The eggs are oval and flattened, measuring 0.44 mm long and 0.26 mm wide. The larvae emerge from the eggs in about six days. The diamondback moth was the first insect found to have become resistant to biological control by the Bt toxin in the field [5]. The Rice Moth (*Corcyracephalonic*) is a pyraloid moth of the family Pyralidae. It is a pest of stored rice, millets and other cereal. Distribution: Cosmopolitan [6].

Plants used are *Vitexagnus-castus*, also Chaste Tree, Monk's Pepper, is a native of the Mediterranean region. In actual clinical trials, the fruit *Agni castifructus* was shown to relieve premenstrual syndrome (PMS) and especially breast swelling and pain, due to its dopaminergic effect. In alternative medicine, it is believed the berries are a tonic herb for both the male and female reproductive system [7]. Cytotoxic effects; Antibacterial activity [8]. *Pongamiapinnata*

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 4, April 2015

is a species of tree in the pea family, Fabaceae. It is commonly known as Karanj. Its oil is a source of biodiesel. Pharmacological Activities are Anti-inflammatory, Anti-diarrhoeal & Anti-ulcer Activity [9]. The seeds contain between 40% and 60% oil that is rich in triglycerides, mainly ricinolein. The seed also contains ricin, a water soluble toxin, which is also present in lower concentrations throughout the plant [10]. The pericarp of castor bean showed central nervous system effects in mice at low doses. *Ricinus communis* has cytotoxic effects [11]. *Azadirachta indica* is the most useful traditional medicinal plant in India. Biological activity of Neem are Anti-inflammatory; Antipyretic; hyperglycaemic; Antifungal, Antibacterial; antihelminthic [12]. Neem oil and the bark and leaf extracts have been therapeutically used as folk medicine [13]. *Annona muricata* is a species of the genus *Annona* of the custard apple tree family, Annonaceae, known mostly for its edible fruit. The fruit is usually called soursop due to its slightly acidic taste when ripe [14].

## II. MATERIALS AND METHODS

**Plant specimen and collection:** The fresh leaves were collected from swamy nursery in Karnataka. The plant leaves were identified using USDA website. The aqueous and alcoholic extracts were prepared and stored in a clean glass ware container until needed for analysis. The extracts were filtered using Whatmann filtered paper. *Vitex agnus castus* denoted as (Vx); *Pongamia pinnata* as (Po); *Ricinus communis* as (Ri); *Azadirachta indica* as (Ne); *Annona muricata* as (An).

**Phytochemical screening:** Chemical tests were carried out on the hexane and diethyl ether extract and on the powdered specimen using standard procedure to identify the constituents as described by [15].

**Antioxidant Activity Determination:** The total antioxidant capacity of the extract was determined with phosphomolybdenum, using  $\alpha$ -tocopherol as standard [23, 24]. An aliquot of 0.2 mL (containing 1.0 mg) of the extract was combined with 2.0 mL of the reagent (0.6 M sulfuric acid, 28.0 mM sodium phosphate and 4.0 mM ammonium molybdate). The blank solution was made by mixing 2.0 mL of the reagent solution with the appropriate volume of the same solvent used to dissolve the sample. The tubes were capped and incubated in water bath at 95 °C for a period of 90 minutes. The sample and blank were left on the shelf for half an hour to cool down to room temperature. The absorbance of the sample was measured at 695 nm. A tocopherol graph was plotted by using  $\alpha$ -tocopherol as standard and the total antioxidant activity of the plant extract was expressed as  $\mu$ g  $\alpha$ -tocopherol equivalent. The equation of the plotted graph is given as  $Y = 5.358x + 0.2427$  where, Y = Absorbance and X = Concentration [16].

**Total flavonoid content determination:** Quercetin was used as standard to determine the total flavonoid content of the plant extract. The dry extract (10 mg) was dissolved in 1.0 mL of 80% ethanol. An aliquot of 0.5 mL was taken out of it and added to a test tube containing 4.3 mL of 80% ethanol, 0.1 mL of 1 M potassium acetate and 0.1 mL of 10% aluminium nitrate. The mixture was incubated at room temperature for 40 minutes and then the absorbance measured at 415 nm. The total flavonoid content in the plant extract was expressed as  $\mu$ g quercetin equivalents by using standard quercetin graph. The equation of the graph established is  $Y = 0.0494x - 0.0026$  where, Y = Absorbance and X = Concentration [16].

**Total Phenolic Content Determination:** Total soluble phenolic content of the plant extract was determined with Folin-Ciocalteu reagent using pyrocatechol as standard. The amount of 25 mg of the dry extract was dissolved in 20 mL of distilled water and the total volume was transferred to an erlenmeyer flask. It was diluted to 46 mL by adding distilled water. One mL of Folin-Ciocalteu reagent was added to the extract solution in the flask and the mixture was shaken vigorously for 3 minutes, after which 3 mL of 2% sodium carbonate solution was added. The flask was covered with aluminium foil in order to protect the formed complex from light. The mixture was shaken occasionally at room temperature for 2 hours and then the absorbance was measured at 760 nm. A standard curve was made by using different dilutions of pyrocatechol and the total soluble phenolic content in the extract was expressed  $\mu$ g pyrocatechol equivalent. The Equation of Straight Line Graph is given as  $Y = 0.0533x - 0.0994$  where, Y = Absorbance and X = Concentration [16].

**Extraction of plant materials:** Plants collected were brought to the laboratory, washed with dechlorinated water, shade dried under room temperature and the plant materials were powdered individually using an electric blender. Each powdered plant material was sieved. The sieved plant powder extracted with hexane, diethyl ether, for a period of seventy two hours and then filtered. The crude extracts thus obtained were stored in bottles maintained at 4°C in a refrigerator. **Pest collection:** Egg stage of selected four plant pest was obtained from NBAIL (National Bureau Of

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 4, April 2015

Agriculturally Important Insects) and their genebank accession number are *Spodoptera litura*-NBAIR-MP-NOC-02; *Plutellaxylostella*- NBAIR-MP-PLU-01; *Corcyra cephalonica*- NBAIR-MP-PYR-01; *Helicoverpaarmigera*- NBAIR-MP-NOC-01

**Ovicidal activity:** The egg masses were counted with the aid of a hand lens (10x). Based on the preliminary screening, the plant extracts were further tested at a concentration of 10ppm. The numbers of eggs hatched in control and treated were recorded and percentage of ovicidal activity was calculated. The experiment was conducted at room temperature 30 ±2°C and 75 ±5 R.H. A total of five trials were carried out. The data obtained were subjected to angular transformation. The significant difference within various crude extracts were compared with Least significant difference (LSD) test to differentiate individual mean significant difference at 0.05% level [15].

$$\text{Percent ovicidal activity} = \frac{\% \text{ of eggs hatched in control} - \% \text{ of eggs hatched in treated}}{\% \text{ of eggs hatched in control}} \times 100$$

% of eggs hatched in control

The mortality was adjusted by Abbott's correction factor (Abbott, 1925).

**Statistical analysis:** The significance of treatments was found out by one way Analysis of Variance (ANOVA) and effective treatment was separated by Tukey's multiple range test.

### III. RESULTS AND DISCUSSION

**Table 1: Phytochemical analysis**

PLANT NAME	<i>Annonamuricata</i>		<i>Vitex-agnuscastus</i>		<i>Pongamiapinnata</i>		<i>Azadiracthaindica</i>		<i>Ricinuscommunis</i>	
	DEE	HEX	DEE	HEX	DEE	HEX	DEE	HEX	DEE	HEX
ALKALOIDS (WAGNERSTEST)	+	+	+	+	+	+	+	+	+	+
CARBOHYDRATES	+	+	+	+	+	+	+	+	+	+
MOLISCH TEST	+	+	+	+	+	+	+	+	+	+
FEHLINGS TEST	-	+	-	+	-	+	+	+	+	+
BENEDICTS TEST	+	+	+	+	+	+	+	+	+	+
TANNINS	+	+	+	+	+	+	+	+	+	+
FLAVONOIDS SHINODA TEST	-	-	-	-	+	+	-	-	-	-
PROTIENS BIURET TEST	+	+	+	+	+	+	+	+	+	+
AMINO ACIDS NINHYDRIN TEST	+	+	+	+	+	+	+	+	+	+
SAPONINS	-	-	-	-	-	-	-	-	-	+
PHYTOSTEROLS SALKOWSKI TEST	+	+	+	+	+	+	+	+	+	+
FIXED OILS SPOT TEST	-	-	-	-	-	-	-	-	-	-
GUMS & MUCILAGE	+	+	+	+	-	+	+	+	+	+
GLYCOSIDES BORNTRAGERS TEST	+	+	+	+	-	+	+	+	+	+

DEE-Diethyl ether ; HEX-Hexane

HEX extracts of plant showed better phytochemical activity analysis than DEE extracts.

The phytochemical analysis of the selected plants were performed and the results were tabulated according to the presence or absence of the phytochemicals in the leaves of the plants. '+' indicates the presence of the phytochemicals and '-' indicates the absence of the phytochemicals.

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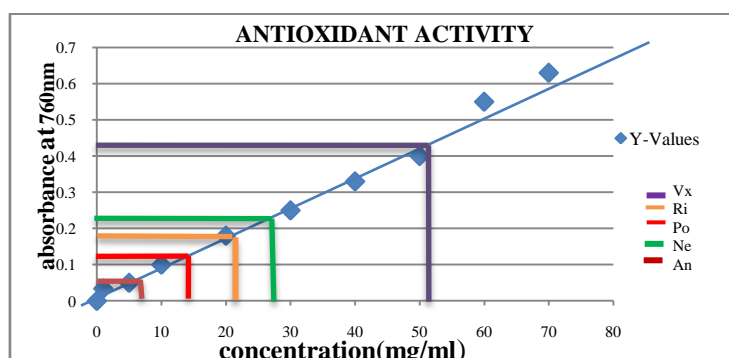
(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 4, April 2015

### QUANTITATIVE ANALYSIS IN PLANTS ANTIOXIDANT ACTIVITY

**Table 2: Antioxidant activity in plants**

Plants	X(concentration)mg/ml	Y(absorbance)nm
<i>Vitexagnuscastus</i>	54	0.4348
<i>Ricinuscommunis</i>	24	0.1824
<i>Pongamiapinnata</i>	16	0.1377
<i>Annonamuricata</i>	7	0.0661
<i>Azadirachtaindica</i>	28	0.2406



**Fig 1. Graph showing the antioxidant activity in plants**

The antioxidant activity was higher in *Vitexagnuscastus* than other plants. It possessed 54 mg/ml in it and *Azadirachtaindica* possessed lower antioxidant activity of 28 mg/ml.

### FLAVONOID ACTIVITY

**Table 3: Flavonoid activity in plants**

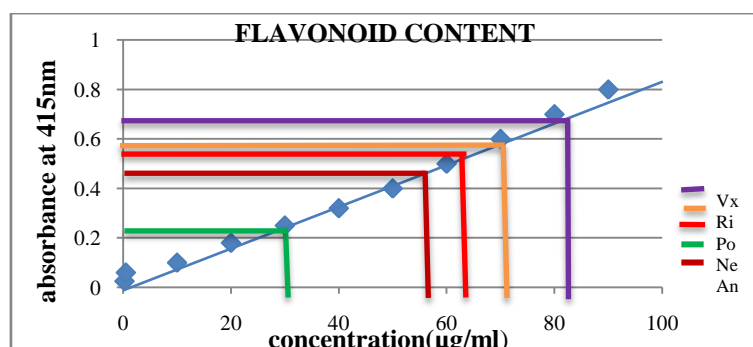
Plants	X(concentration) µg/ml	Y(absorbance at 415nm)
<i>Vitexagnuscastus</i>	82	0.6437
<i>Ricinuscommunis</i>	73	0.5708
<i>Pongamiapinnata</i>	66	0.5176
<i>Annonamuricata</i>	59	0.4806
<i>Azadirachtaindica</i>	30	0.2458

The plants were performed with Flavonoid activity at 415 nm and the absorbance was recorded

## International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 4, April 2015



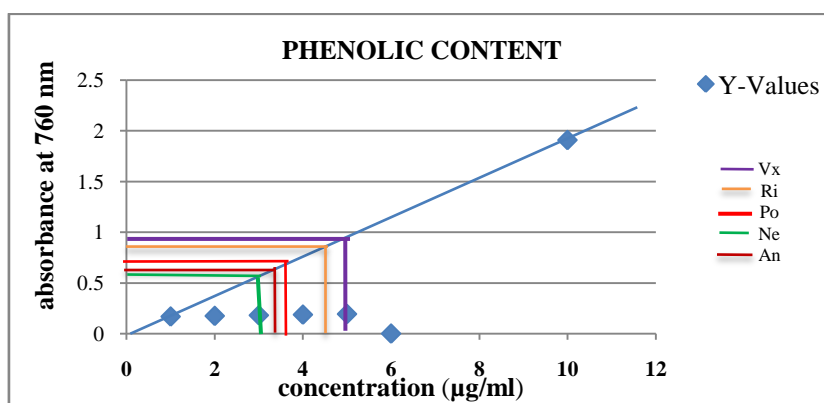
**Fig 2. Graph showing the flavonoid activity in plants**

The flavonoid content was higher in *Vitexagnuscastus* than other plants. It possessed 82 µg/ml flavonoid content in it and *Azadirachtaindica* possessed lower flavonoid activity of 30 µg/ml.

### PHELOLIC ACTIVITY

**Table 4: Phenolic activity in plants**

Plants	X(concentration) µg/ml	Y(absorbance at 760nm)
<i>Vitexagnuscastus</i>	5	0.9128
<i>Ricinuscommunis</i>	4.9	0.8157
<i>Pongamiapinnata</i>	3.5	0.6864
<i>Annonamuricata</i>	3.2	0.6278
<i>Azadirachtaindica</i>	3.0	0.6078



**Fig 3. Graph showing the phenolic activity in plants**

The phenolic content was higher in *Vitexagnuscastus* than other plants. It possessed 5 µg/ml phenolic content in it and *Azadirachtaindica* possessed lower phenolic activity of 3µg/ml.

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 4, April 2015

## OVICIDAL ACTIVITY

The ovicidal activity was performed by treating the collected eggs with the prepared extracts, Diethyl ether and Hexane extract and the eggs hatchment was calculated.



(a)

(b)

Fig 4. (a) Treatment Of Eggs With Plant Extract (b) Counting Of Eggs

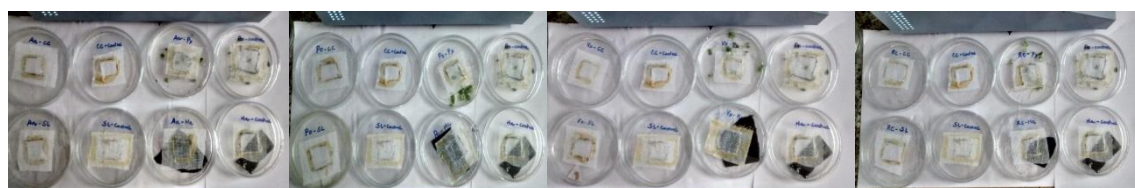


Fig 5. Different trails of plant pests against the plant extracts using solvents

Trials performed against the collected plant pests using the extracts for the ovicidal activity.

Table 5: Average eggs hatched for 5 trials

Plants Pest	Control		Vx		Po		Ri		Ne		An	
	Hex	Dee	Hex	Dee	Hex	Dee	Hex	Dee	Hex	Dee	Hex	Dee
Spodoptera	40	40	1.6	10.6	8.6	14.8	9.2	20.8	10.6	13.8	13.2	22.8
Plutella	40	40	2.6	12.4	11.4	17.4	7.6	23.4	15.2	21.2	19.6	25.4
Corcyra	40	40	3.8	13.6	6.2	19.8	3.2	18.6	9.2	21.8	14.8	21.6
Helicoverpa	40	40	9.6	19.6	14.8	20.6	13.2	19.6	18.4	18.6	18.6	23.8

Average eggs hatched are determined repeating procedure for five trials with Hexane (Hex) and Diethyl ether (Dee) extract for calculating the ovicidal activity.

Table 6: PercentOvicidal activity at 10ppm

Plants Pest	Control		Vx		Po		Ri		Ne		An	
	Hex	Dee	Hex	Dee	Hex	Dee	Hex	Dee	Hex	Dee	Hex	Dee
Spodoptera	0	0	96	73.5	78.5	63	77	48	73.5	65.5	67	43
Plutella	0	0	93.5	69	71.5	56.5	81	41.5	62	47	51	36.5
Corcyra	0	0	90.5	66	84.5	50.5	92	53.5	77	45.5	63	46
Helicoverpa	0	0	76	51	63	48.5	67	51	54	53.5	53.5	40.5

## International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

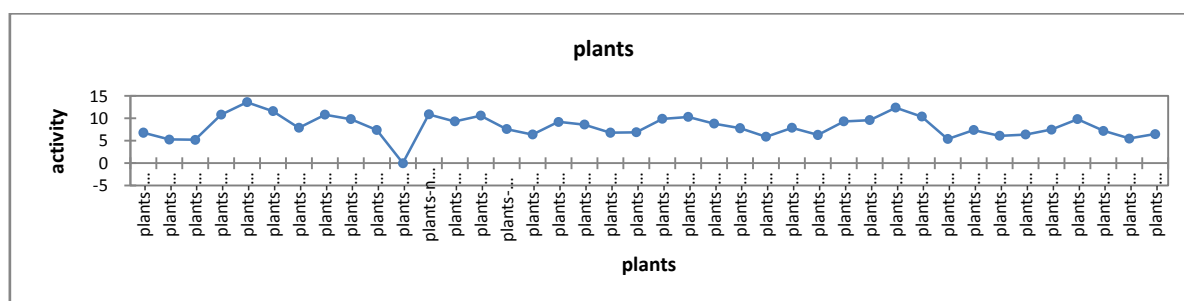
Vol. 4, Issue 4, April 2015

In Earlier studies *Vitexnegundo*(52.02%) showed maximum ovicidal activity against *Spodopteralitura* at concentration of 0.05% [20]. In this study, Maximum ovicidal activity of 76.74 and 67.39% was noticed in PONNEEM. Ovicidal activity at 10ppm is calculated for different plants with HEX & DEE extracts on selected insect. In which hex extracts showed best result than dee extracts specifically hex extracts of *Vitex*, *Pongamia*,*Ricinus*plants showed highest values.

**Table7: IC<sub>50</sub> values of the plants**

Plants Pest	Control		Vx		Po		Ri		Ne		An	
	Hex	Dee	Hex	Dee	Hex	Dee	Hex	Dee	Hex	Dee	Hex	Dee
<i>Spodoptera</i>	0	0	5.2	6.8	6.3	7.8	6.4	10.4	6.8	7.6	7.4	11.6
<i>Plutella</i>	0	0	5.3	7.2	6.9	8.8	6.1	12.4	8.6	10.6	9.8	13.6
<i>Corcyra</i>	0	0	5.5	7.5	5.9	9.9	5.4	9.3	6.4	10.9	7.9	10.8
<i>Helicoverpa</i>	0	0	6.5	9.8	7.9	10.3	7.4	9.8	9.2	9.3	9.3	12.3

The maximum activity at 10ppm was exhibited by hexane extract of *Vitexagnuscastus* ic<sub>50</sub> (5.2 ) against *Spodopteralitura*,*Plutellaxylostella*ic<sub>50</sub>( 5.3 ),*Corcyra cephalonica* ic<sub>50</sub>(5.5 ) followed by *Ricinuscommunis* ic<sub>50</sub>( 5.4 ), against *Corcyra cephalonica* respectively and *Pongamiapinnata* ic<sub>50</sub>(5.9) against *Corcyra cephalonica* . Diethyl ether extracts showed lower ovicidal activity when compared to hexane extracts.



**Fig 3. Graph showing thecomparison of IC50 values using XLSTAT 2015**

Mean chart of IC50 values are determined using xlstat 2015 for calculating highest & lowest values using the Hex and Dee extracts for various plants determined against control. The hatchability of *S. litura*eggs was directly proportional to the concentration of plant extract.

**Table 8: Analysis of variance**

Source	DF	Sum of squares	Mean squares	F	Pr> F
Model	39	246.384	6.318	1.404	0.596
Error	1	4.500	4.500		
Corrected Total	40	250.884			
<i>Computed against model Y=Mean(Y)</i>					

By using the ANOVA software, analysis of variance, Degrees of freedom, Probability factor were determined.

## International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 4, April 2015

**Table 8: LS Means using XLSTAT 2015**

Category	LS means	Groups
an deepl	13.600	A
Rideepl	12.400	A
an deesp	11.600	A
ne dee cc	10.900	A
an dee cc	10.800	A
an hex ha	10.800	A
ne deepl	10.600	A
Rideesp	10.400	A
Podee ha	10.300	A
Podee cc	9.900	A
Vxdee ha	9.800	A
an hex pl	9.800	A
Ridee ha	9.600	A
ne dee ha	9.300	A
Ridee cc	9.300	A
ne hex ha	9.200	A
Podeepl	8.800	A
ne hex pl	8.600	A
an hex cc	7.900	A
po hex ha	7.900	A
Podeesp	7.800	A
ne deesp	7.600	A
Vxdee cc	7.500	A
an hex sp	7.400	A
ri hex ha	7.400	A
Vxdeepl	7.200	A
po hex pl	6.900	A
Vxdeesp	6.800	A
ne hex sp	6.800	A
vx hex ha	6.500	A
ri hex sp	6.400	A
ne hex cc	6.400	A
po hex sp	6.300	A
ri hex pl	6.100	A
po hex cc	5.900	A
vx hex cc	5.500	A
ri hex cc	5.400	A
Vx hex pl	5.300	A
Vx hex sp	5.200	A
control	0.000	A



# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 4, April 2015

The LS (Least square) means was calculated by using ANOVA Statistics 2015 using Tukey's multiple range test.

## IV. CONCLUSION

Chemical and synthetic pesticides used for controlling various plant pests have resulted in the development of resistance to several classes of pesticides. Ovicidal activity of plant extracts is important to control the pest at eggs stage itself thereby preventing the damage caused by other stages. Five plant extracts were been tested for the ovicidal activity against *Spodopteralitura*, *Plutellaxylostella*, *Corcyra cephalonica*, *Helicoverpaarmigera*. In this study clearly it was observed that all the treatments exhibited ovicidal activity against plant pests and the maximum activity at 10ppm was exhibited by hexane extract of *Vitex-agnuscastus* IC<sub>50</sub> (5.2 ) against *Spodopteralitura*, *Plutellaxylostella* IC<sub>50</sub> (5.3), *Corcyra cephalonica* IC<sub>50</sub>(5.5 ) followed by *Ricinuscommunis* IC<sub>50</sub>(5.4), against *Corcyra cephalonica* respectively and *Pongamiapinnata* IC<sub>50</sub>(5.9) against *Corcyra cephalonica*. Diethyl ether extracts showed lower ovicidal activity when compared to hexane extracts.

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