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Toxicity bioassay of *Azadirachta indica, Allium sativum* and *Oscimum sanctum* on the larvae (fourth instars) of *Chilo-partellus*

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Abstract

The Present investigation was taken up to evaluate the toxic effect of aqueous and ethanolic extracts of *Azadirachta indica-leaves, Allium sativum-bulbs* and *Oscimum sanctum*-leaves against *C. partellus*, maize stem borer. The toxic effect of the plant extracts was carried out at five different concentrations, viz. 2.0%, 4.0%, 6.0%, 8.0% and 10% respectively. All the aqueous and ethanolic extracts showed high to moderate mortality. Complete mortality of larvae was recorded from ethanolic extract of *Azadirachta indica-leaves* giving 100 % percent average and corrected mortality followed by aqueous extract of *Azadirachta indica-leaves* giving 99.2 % of percent average and corrected mortality and than ethanolic extract of *Allium sativum-bulbs* giving 94.0% percent average and corrected mortality after 48 hours of treatment. The lowest mortality was recorded from aqueous extract of *O. sanctum* having 66.6% percent average and corrected mortality at 12 hours of treatment. The estimated LC50 calculated for total mortality for *Azadirachta indica-leaves* extracts which caused highest mortality were 86.1 mg/ml respectively. The results suggest that extract from *Azadirachta indica*-leaves and *Allium sativum-bulbs* may potentially be used for the management of *C.partellus.*

Keywords: *Azadirachta indica, Allium sativum* and *Oscimum sanctum*, Toxic, mortality

Introduction

The (Maize stem borer/ Stalked stem borer), *C. partellus* is a serious pest and a major constraint in the production of agricultural crops especially maize and sorghum throughout the world. This pest has been reported to cause heavy crop loss in the area of their outbreaks (Verkerk and Wright, 1996).Currently different kinds of preventive and curative control measures are practiced to get protection from this pest. Among those, chemical pesticides have been used for a long time, but have serious drawbacks (Sharaby, 1988), such as direct toxicity to beneficial insects, fishes and human (Goodland *et al*., 1985), pesticide induced resistance (Waiss *et al.*, 1981), health hazard (Bhaduri *et al.*, 1989) and increased environmental and social costs (Pimental *et al.* 1980). In many countries, efforts are being made to minimize the use of harmful insecticides through the use of indigenous plant products, implementation of IPM approaches, use of bio-degradable products (Khattach and Hameed, 1986). Botanical products are environmentally safe, less

hazardous, economic and easily available. Botanicals like Neem, Datura, tulsi, garlic, Durba, Eucalyptus etc. and many others may be grown by farmers with minimum expense and extracted by indigenous methods. These botanical materials can be used as an alternative to chemical pesticides. This will be very helpful in minimizing the undesirable side effects of synthetic pesticides. The present experiment was, therefore, undertaken to study the toxic effect of aqueous and ethanolic *Azadirachta indica-leaves, Allium sativum*-bulb sand *Oscimum sanctum-leaves* on the larvae (fourth instars) of *Chilo partellus.*

Materials and methods

Study Area

The proposed investigation was done in maize fields of Indore region. The following areas were selected in Indore region.

1. Mangliya
2. Mhow
3. Depalpur
4. Sanwer

Experimental plant:

Following plants were selected.
Azadirachta indica (Neem)
Allium sativum (Garlic)
Ocimum sanctum (Tulsi)

Collection of plant materials

Selected plant materials i.e. leaves of *Azadirachta indica* and *Oscimum sanctum* were collected from the botanical garden of Govt. Holkar science college in poly bags and brought to lab and their botanical identity was established and *Allium sativum* was brought market and it was also identified at the department of botany, Govt. Holkar science college, Indore (M.P.). The collected material was washed, shade dried under room temperature (27 ± 2^0C) and *Azadirachta indica* and *Oscimum sanctum* was powdered using electric blender and the paste of *Allium sativum* was made which were further processed for phytochemical analysis.

Experimental plant product used for extract

Following plant products were used for extract preparation
Azadirachta indica- Leaves
Allium sativum - Bulbs
Ocimum sanctum- Leaves

Soxhlet extraction

The ordinary method of extraction was not efficient to yield good amount of active principle of the plant material. To extract more active principle from all the plant materials, Soxhlet extraction given by Sharma and Gupta (2009) was used. The plant extract were prepared in two solvent one was distilled water and second was ethanol.

Phytochemical screening

Phytochemical screening was done in order to detect the presence of bioactive constituents such as alkaloids, tannins, saponins, phenols, glycosides, flavonoids and glycosides using the methods described by Sofowora (1978), Trease and Evans (1989).

Experimental insect and rearing

Maize stem borer; *Chilo partellus* (Swinhoe) was selected for the present investigation. A series of surveys were undertaken for the collection of larvae in the maize fields. The sampling was conducted two times at the vegetative and reproductive stage (tasseling and soft dough stages). In each stage, evaluation was conducted, each maize plant was dissected and the larvae of the pests per plant were counted and placed in wide-mounted jars. Rearing of larvae was done in accordance with Koul *et al.* (2013).

Toxicity bioassay

The toxic bioassay was carried on according to the procedures given by Sharma and Gupta (2009) then the percentages of corrected mortality were calculated by using Abbott's formula (Abbott, 1925).

Mc = (Mo－Mc / 100－Me) × 100
Where,
Mo = Observed mortality rate of treated larvae (%),
Me = Mortality rate of control (%)
Mc = Corrected mortality rate (%)

Based on the mortality of the test organisms recorded in these bioassays, LC_{50} was calculated along with their fiducial limits at 95 % confidence level by probit analysis using SPSS software package (Busvine, 1925). All data thus obtained was tabulated and graphically presented as per the required statistical methods

Results

The maximum percent average and corrected mortality was showed by *A. indica* (99.2%) followed by *A. sativum* (94.0%) at 48 hours of treatment and the minimum percent average and corrected mortality was showed by *O. sanctum* at 2.0 % concentration (66.6 %) at 12 hours of treatment.

The maximum percent average and corrected mortality was showed by *A. indica* (100 %) followed by *A. sativum* (96.2%) at 48 hours of treatment and the minimum percent average and corrected mortality was showed by *O. sanctum* at 2.0 % concentration (80.6 %) at 12 hours of treatment.

In Aqueous extract of *A. indica*, the highest mortality (99.2%) was recorded from the LC_{50} value (87.02 mg/ml) having χ^2 value of 11.64 and 95 % confidence limit (84.36 - 90.84) followed by *A. sativum* (94.0 %) having LC_{50} value (79.19 mg/ml), χ^2 value of 11.68 and 95 % confidence limit (75.22 - 87.18) and lowest mortality was showed by *O.sanctum* (66.6 %) havingLC_{50} value (58.13mg/ml), χ^2 value of 6.20 and 95 % confidence limit (56.49 - 69.91).

In Ethanolic extract of *A. indica*, the highest mortality (100 %) was recorded from the LC_{50} value (86.01mg/ml) having χ^2 value of 11.64 and 95 % confidence limit (84.36 - 90.84) followed by *A. sativum* having LC_{50} value (81.20 mg/ml), χ^2 value of 9.95 and 95 % confidence limit (78.82 - 88.38) and lowest mortality was showed by *O.sanctum* havingLC_{50} value (75.00 mg/ml), χ^2 value of 9.23 and 95 % confidence limit (71.92 - 84.08).

Data pertaining to the below tables indicate that the ethanolic extract of *A. indica* showed the maximum (100%) percentage of average and corrected mortality followed by aqueous extract of *A. Indica* both at 10 % concentration after 48 hours of treatment and than *A. Sativum* (96.2 %) at 10 % concentration after 48 hours of treatment and lowest percentage of average and corrected mortality was showed by aqueous extract *O. Sanctum* at 2.0 % concentration after 12 hours of treatment. Thus the above result reveals that as the concentration of plant extracts increases, percentage of average and corrected mortality also increases. Therefore in general, the toxic effects of different concentrations, irrespective of the extracts decrease with decrease in concentration from 10.0 % to 2.0 %.

Table 1: Mortality percentage of larvae (fourth instars) of *C. partellus* treated with different aqueous plant extracts.

Name of the plants	Concentration (%)	No. of insect used in each concentration	No. of insect dead			Total no. of insects dead (Mean Value)	% of average and corrected mortality
			12h	24h	48h		
Azadirachta indica	2.0	90	76	80	84	80	88.8
	4.0		78	80	86	81	90.0
	6.0		82	84	88	85	94.4
	8.0		86	88	90	88	97.7
	10		88	90	90	89.3	99.2
	Control	30	0			0	
Allium sativum	2.0	90	72	74	76	74	82.2
	4.0		74	76	78	76	84.4
	6.0		76	78	80	78	86.6
	8.0		80	82	84	82	91.1
	10		82	84	88	84.6	94
	Control	30	0			0	
Oscimum sanctum	2.0	90	58	60	62	60	66.6
	4.0		60	62	64	62	68.8
	6.0		62	64	68	64.6	71.7
	8.0		64	68	70	67.3	74.7
	10		72	76	78	75.3	84.1
	Control	30	0			0	

Table 2: Mortality percentage of larvae (fourth instars) of *C. partellus* treated with different ethanolic plant extracts.

Name of the plants	Concentration (%)	No. of insect used in each concentration	No. of insect dead			Total no. of insects dead (Mean value)	% of average and corrected mortality
			12h	24h	48h		
Azadirachta indica	2.0	90	80	82	84	82.0	91.1
	4.0		80	84	86	83.3	92.5
	6.0		84	86	88	86.0	95.5
	8.0		86	86	90	87.3	97.0
	10		90	90	90	90.0	100
	Control	30	0			0	
Allium sativum	2.0	90	74	78	78	76.6	85.1
	4.0		76	80	82	79.3	88.1
	6.0		80	82	84	82.0	91.1
	8.0		82	86	86	84.6	94.0
	10		84	88	88	86.6	96.2
	Control	30	0			0	
Oscimum sanctum	2.0	90	72	72	74	72.6	80.6
	4.0		76	78	78	77.3	85.8
	6.0		76	80	82	79.3	88.1
	8.0		82	84	84	83.3	92.5
	10		84	86	86	85.3	94.7
	Control	30	0			0	

Table 3: χ^2 values, regression equations, LD $_{50}$ and 95 % confidence limits of aqueous extracts of *Azadirachta indica, Allium sativum and Oscimum sanctum* extracts against *C. partellus*, maize stem borer after 12, 24 and 48 hours of treatment.

Plant extracts	Hrs after treatment	X^2 values for heterogeneity	Regression equations	LC_{50} values	95 % confidence limit	
					Lower	Upper
Azadirachta indica	12h	14.268	Y=72.4+1.6X	77.09	75.67	88.33
	24h	15.843	Y=76+1.4X	81.11	78.74	90.06
	48h	11.644	Y=82.8+0.X	87.02	84.36	90.84
Allium sativum	12h	6.108	Y=69+1.3X	73.00	71.65	81.95
	24h	7.016	Y=71+1.3X	75.47	73.65	83.95
	48h	11.688	Y=72.2+1.5X	79.19	75.22	87.18
Oscimum sanctum	12h	6.206	Y=53.6+1.6X	58.13	56.49	69.91
	24h	9.091	Y=54.6+1.9X	61.32	58.15	73.85
	48h	9.454	Y=57+1.9X	63.66	60.67	76.13

χ^2- Goodness of fit. The tabulated value of χ^2having *df*=9 and is p<0.05.

Table 4: χ^2 values, regression equations, LD $_{50}$ and 95 % confidence limits of ethanolic extracts of *Azadirachta indica, Allium sativum and Oscimum sanctum* plant extracts against *C. partellus*, maize stem borer after 12, 24 and 48 hours of treatment.

Plant extracts	Hrs after treatment	X^2 values for heterogeneity	Regression equations	LC_{50} values	95 % confidence limit	
					Lower	Upper
Azadirachta indica	12h	12.857	Y=82.03+0.08X	21.45	18.74	29.26
	24h	8.411	Y=84.16+0.05X	83.10	81.92	89.28
	48h	11.644	Y=82.8+0.8X	86.01	84.36	90.84
Allium sativum	12h	7.239	Y=71.4+1.3X	76.05	74.05	84.35
	24h	10.389	Y=75+1.3X	79.13	77.65	87.95
	48h	9.958	Y=76.4+1.2X	81.20	78.82	88.38
Oscimum sanctum	12h	9.231	Y=69+1.5X	75.00	71.92	84.08
	24h	13.500	Y=69.8+1.7X	76.18	73.20	86.80
	48h	11.235	Y=71.8+1.5X	77.87	74.82	86.78

χ^2- Goodness of fit. The tabulated value of χ^2having *df*=9 and is p<0.05.

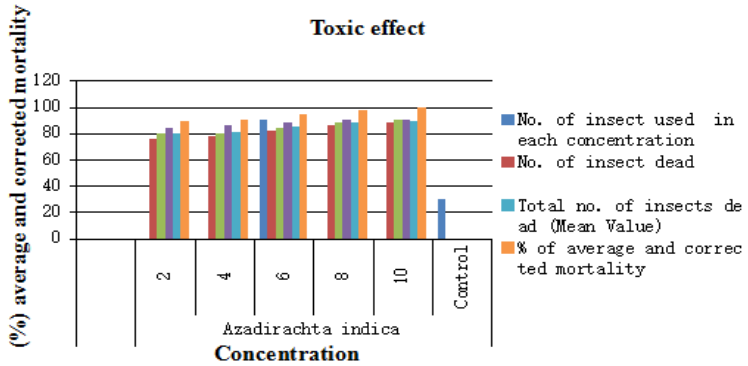

Figure 1: Mortality percentage of larvae (fourth instars) of *C. partellus* treated with aqueous extract of *A. Indica* at different concentrations.

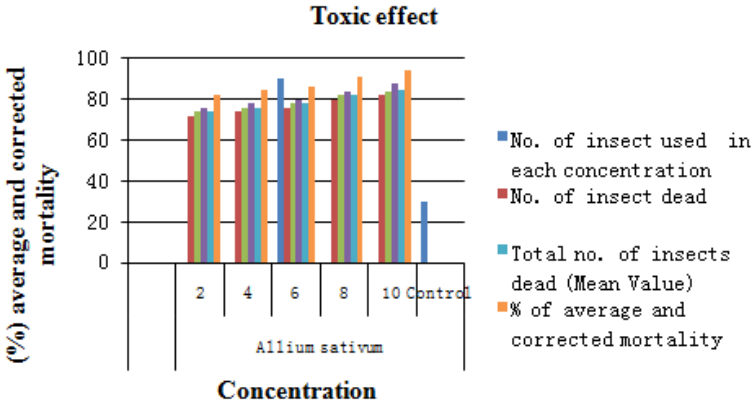

Figure 2: Mortality percentage of larvae (fourth instars) of *C. partellus* treated with aqueous extract of *A. Sativum* at different concentrations.

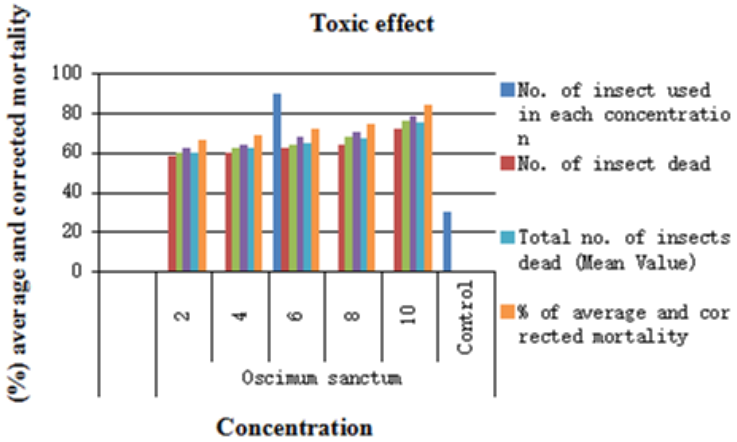

Figure 3: Mortality percentage of larvae (fourth instars) of *C. partellus* treated with aqueous extract of *O. sanctum* at different concentrations.

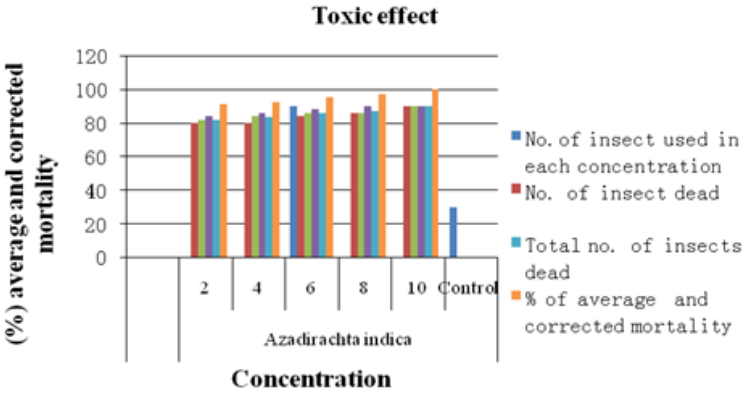

Figure 4: Mortality percentage of larvae (fourth instars) of *C. partellus* treated with ethanolic extract of *A. Indica* at different concentrations.

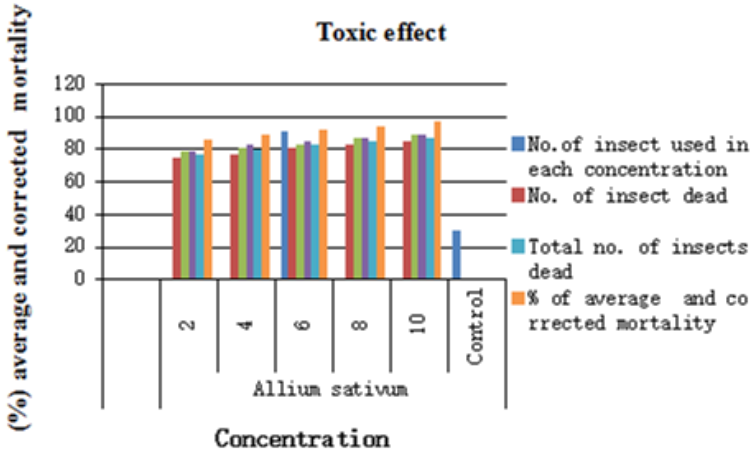

Figure 5: Mortality percentage of larvae (fourth instars) of *C. partellus* treated with ethanolic extract of *A. sativum* at different concentrations.

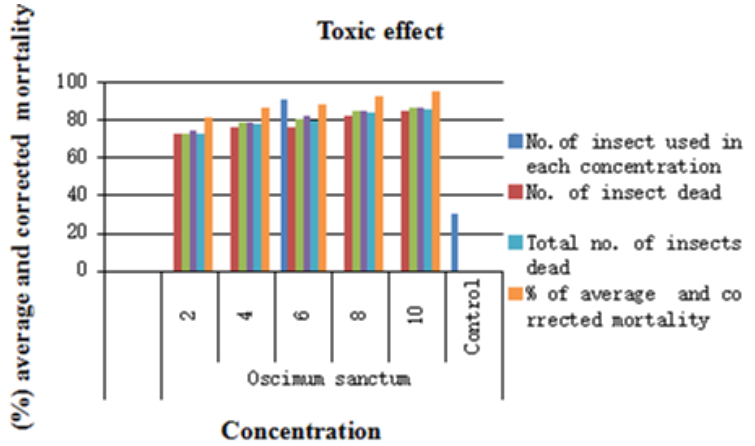

Figure 6: Mortality percentage of larvae (fourth instars) of *C. partellus* treated with ethanolic extract of O. *Sanctum* at different concentrations.

Discussion

The results of present study are summarized in table 1-4 and presented by graph 1-6.

The maximum percent average and corrected mortality was showed by *Azadirachta indica* (99.2%) followed by *Allium sativum* (94.0%) at 48 hours of duration and the minimum percent average and corrected mortality was showed by *Oscimum sanctum* at 2.0 % concentration (66.6 %) at 12 hours.

The maximum percent average and corrected mortality was showed by *Azadirachta indica* (100 %) followed by *Allium sativum* (96.2%) at 48 hours of duration and the minimum percent average and corrected mortality was showed by *Oscimum sanctum* at 2.0 % concentration (80.6 %) at 12 hours.

In Aqueous extract of *A. Indica*, the highest mortality (99.2%) was recorded from the LC_{50} value (87.02 mg/ml) having χ^2 value of 11.64 and 95 % confidence limit (84.36 - 90.84) followed by *A. Sativum* (94.0 %) having LC_{50} value (79.19 mg/ml), χ^2 value of 11.68 and 95 % confidence limit (75.22 - 87.18) and lowest mortality was showed by *O.sanctum* (66.6 %) havingLC_{50} value (58.13mg/ml), χ^2 value of 6.20 and 95 % confidence limit (56.49 - 69.91).

In Ethanolic extract of *A. indica*, the highest mortality (100 %) was recorded from the

LC_{50} value (86.01mg/ml) having χ^2 value of 11.64 and 95 % confidence limit (84.36 - 90.84) followed by *A. sativum* having LC_{50} value (81.20 mg/ml), χ^2 value of 9.95 and 95 % confidence limit (78.82 - 88.38) and lowest mortality was showed by *O.sanctum* having LC_{50} value (75.00 mg/ml), χ^2 value of 9.23 and 95 % confidence limit (71.92 - 84.08).

Data pertaining to the below tables indicate that the ethanolic extract of A. Indica showed the maximum (100 %) percentage of average and corrected mortality followed by aqueous extract of *A. indica* both at 10 % concentration after 48 hours of treatment and than *A. sativum* (96.2 %) at 10 % concentration after 48 hours of treatment and lowest percentage of average and corrected mortality was showed by aqueous extract *O. sanctum* at 2.0 % concentration after 12 hours of duration. Thus the above result reveals that as the concentration of plant extracts increases, percentage of average and corrected mortality also increases. Therefore in general, the toxic effect of different concentrations, irrespective of the extracts decreases with decrease in concentration from 10.0 % to 2.0 %.

The high performances of ethanolic extracts of *Azadirachta indica* in this study confirm the views of Badam *et al*. (1987); Jeyasakthy *et al*. (2013) and Udeinya *et al.* (2008) that over 195 species of insects in West Africa, India, Myanmar etc are affected by aqueous and ethanolic neem extracts, and insects that have become resistant to synthetic pesticides are also controlled with these extracts. Further, water extract from fresh leaves gave a good control of stem borers in maize, *Chilo partellus*, when applied into the plant whorls in Mozambique (Segeren, 1993).

These results are in agreement with the findings elsewhere in which use of botanicals such as neem-based preparations have proven effective in the management of insect pests such diamondback moth, onion thrips, voles, etc (Curtis *et al*., 2002; Koschier *et al.*, 2002; Liang *et al*., 2003). The mechanisms of action for the various botanicals may be assumed to be on the basis of insecticidal activity (toxicity), repellence and anti-feedant. However, further studies are recommended to determine the mode(s) of action involved.

Garlic's pungent smell has been attributed to the presence of organo sulfur compounds such as allicin and diallyl disulfide (DADS) in the edible alliums (Bautista *et al*., 2005). It has been suggested that garlic's pungency contributes to its toxicity in weevils by disrupting regular respiratory events (Adedire and Ajayi, 2006).

Nchu *et al*. (2005), evaluating the toxic effects of extracts of *A. sativum* bulbs on adults of *Hyalomma* and *Rhipicephalus* sp. of ticks concluded that ethanolic extracts caused mortality of adult ticks after 24 h of exposure while dichloromethane extracts caused mortality in less than an hour. Ho *et al.* (1996) showed that the essential oil of garlic (*Allium sativum* L.) has insecticidal properties against *Tribolium castaneum* and *Sitophilus zeamais*. Garlic oil and its main component allyl disulphide also proved to be effective against *Blattella germanica.*

Oladimeji and Kannik (2010) found that *A. indica A. Juss* and *O. basilicum* L. leaf extract were not phytotoxic but increase in concentrations increased its effectiveness and its treated field yield was higher when compared to the treated synthetic pesticides as against *Podagrica spp.* Okigbo *et al*. (2010) found that *A. indica* leaf extract caused 100% mortality to culex larva species after 24 h at a concentration of 40% whereas, 100% mortality was seen at 50% concentration of *Ocimum gratissimum* L. after 24 h.

Surendra *et al*. (2010) Efficacy of natural plant products, Azadirachta *indica*, *Ocimum sanctum* and *Pongamia pinnata* in t he management of greater wax moth, *Galleria mellonella* L. under laboratory conditions and reported *Azadirachta indica*, the most toxic followed by *Ocimum sanctum.*

Basalingappa (2000) who reported the mean larval mortality ranging from 55.33 to 92.23 percent with Neem whereas, with Tulsi and Pongamia the larval mortality was ranged from 51.67 to 69.21 and 46.73 to 59.64 per cent respectively supports our findings.

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