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Bioassay of some plant extracts against cowpea aphid, *Aphis craccivora* Koch

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Abstract
A laboratory bioassay was carried out to determine the LC_{50} and relative toxicity of three botanicals (leaf extracts) along with a check imidacloprid 17.8 SL and control to manage the effect of aphid, *Aphis craccivora* Koch in cowpea. Among the plant extracts tested *Ocimum sanctum* showed the lowest LC_{50} value (0.316) followed by *Ageratum conyzoides* (2.381) and *Lantana camara* (3.200) after 72 hours exposure period. Considering the relative toxicity of imidacloprid as unit value the comparison of relative toxicity revealed that *O. sanctum, A. conyzoides* and *L. camara* were 0.098, 0.013 and 0.010 times less toxic than imidacloprid after 72 hours exposure respectively. The order of relative toxicity was found in the following manner: *O. sanctum>A. conyzoides>L. camara.*

Keywords: Bioassay, *Aphis craccivora*, *O. sanctum*, *A. conyzoides*, *L. camara*, LC_{50} and relative toxicity

1. Introduction
The black cowpea aphid, *Aphis craccivora* Koch is a wide spread pest on cowpea and cause significant damage in India, the Philippines, tropical Africa and Latin America. The pest is polyphagous by nature affecting more than 15 different crops, mainly pertaining to the family Leguminosae (Souza *et al.* 2007) [8]. Heavy infestation leads to the stunting of plants and delay in the initiation of flowering as a result the young seedlings succumb to death, whereas the older plants show symptoms such as stunting, crinkling and curling of leaves, delayed flowering, shrivelling of pods and finally resulting in yield reduction. Besides these in several tropical regions, aphids are more important as agents in the transmission of viral diseases of cowpea than as direct plant feeders (Chalfant, 1976) [3]. For the management of aphids, farmers used to apply different types of chemical with repeated frequency in high dose and sometimes even with banned chemicals, that result endangered the sustainability of production system (Bhuyan *et al.* 2017) [2]. Realisation of negative consequences of chemical pesticides and the growing concerned over health and environment, a viable and sustainable alternatives other than chemical method of pest control is in search. The use of plant derivatives as an alternative to chemical insecticides has been studied throughout the world and found effective not only economically and ecologically safe, but also free from residual problems. Many of the botanicals have been explored having broad spectrum activity, so the focus should be on the encouragement of the use of botanicals to tackle problems associated with other insecticides. In the present investigation three different types of plant products were evaluated in the laboratory for their toxicity to control the effect of black cowpea aphid, *Aphis craccivora* in cowpea.

2. Materials and methods
2.1 Preparation of plant extracts
The present investigation using botanicals was carried out in the laboratory at Department of Entomology, Assam Agricultural University, Jorhat-13 during the year 2017-2018. For these three plants were selected based on the literature survey and also which are available indigenously. The plant parts (leaves) selected was collected from the nearby vicinity of Assam Agricultural University, Jorhat campus. Plant parts (leaves) were dried under shade and ground to fine powder. The powdered materials were sieved through 60 mesh sieve and extraction was done in soxhlet apparatus with methanol as the solvent. The details of the treatments are mentioned below:
T_1: Basil (*Ocimum sanctum*)
T_2: Goat weed (*Ageratum conyzoides*)

T_3: Wild sage (*Lantana camara*)
T_4: Check Imidacloprid 17.8 SL
T_5: Control

2.2 Insect bioassay

For the determination of LC_{50} values, the stock solution of known strength of the botanical was prepared from standards and subsequent concentrations were prepared following flow chart. The botanicals were applied in the form of dry film, deposited on the inner surface of the petriplate. Thin and uniform film of treatments was prepared by taking 1 ml of insecticide solution in a petriplate and rotated till dryness. Toxicity of these films were determined against fourth instar nymph of *A. craccivora*. Twenty aphids were released into each petriplate, which served as one replication. Three replications of each concentration of the insecticide were maintained. Simultaneously one control set was also run. The plates were kept in an incubator at 28±2 °C for six hours. Then the aphids were transferred to battery jars (20cm×10cm diameter) containing flowers and pods of cowpea. The mouth of each jar was kept closed with a piece of muslin cloth held in position with the help of rubber bands. These jars were kept in incubator at 28±2 °C and after 24, 48 and 72 hours mortality counts were made. Percent aphid mortality in each treatment was worked out. The observed mortality was corrected if there were mortality in control by using Abbott's formula (1925) [1]. The dosages mortality data so obtained were subjected to Probit analysis to find out LC_{50} values. The relative toxicity of different insecticides was calculated by taking LC_{50} value of imidacloprid as unit.

The experimental data were subjected to 'Probit analysis' as described by Finney (1952) [4]. The median lethal concentration (LC_{50}) was obtained from the regression equation. The values for relative toxicity of botanicals were calculated as follows:

$$\text{Relative toxicity} = \frac{LC_{50} \text{ value of Imidacloprid}}{LC_{50} \text{ value of botanical}}$$

3. Result and Discussion

The data on mortality of *A. craccivora* revealed that *O. sanctum* @ 5.00 per cent caused highest mortality of 71.69, 79.24 and 85.55 per cent after 24, 48 and 72 hours whereas 8.00 per cent *A. conyzoides* caused mortality (67.10%) at 24 hours as well as at 48 hours (74.79%) and at 72 hours (81.10%). For *L. camara* leaf extract, mortality recorded was as 68.36, 74.79 and 82.16 per cent after 24, 48 and 72 hours at 10.00 per cent, respectively. The data on mortality of aphid was revealed that imidacloprid caused highest mortality in comparison to the botanicals with 73.14, 81.10 and 90.00 per cent after 24, 48 and 72 hours, respectively at 0.08 per cent. The mortality was increased steadily with increase in concentration as well as exposure period in all the botanical treatments (Table 1).

The regression equation, LC_{50} values, relative toxicity, fiducial limit and the order of toxicity using plant extracts and imidacloprid after 24, 48 and 72 hours are calculated. From that table it was found that the LC_{50} values of *O. sanctum, A. conyzoides, L. camara and imidacloprid* were 0.828, 4.664, 5.779 and 0.045 per cent respectively after 24 hours and 0.480,3.188,4.201 and 0.036 per cent respectively after 48 hours. For the 72 hours exposure period LC_{50} values were 0.316, 2.381, 3.200 and 0.031 per cent respectively (Table 2 and Fig 1).

The comparison of relative toxicity revealed that *O. sanctum* was 0.054, 0.075 and 0.098 times less toxic than imidacloprid when exposed for a period of 24, 48 and 72 hours, respectively. *A. conyzoides* was 0.009, 0.011 and 0.013 times less toxic than imidacloprid after 24, 48 and 72 hours exposure period whereas *L. camara* was 0.008, 0.009 and 0.010 times less toxic than imidacloprid when exposed for a period of 24, 48 and 72 hours, respectively.The order of toxicity with respect to LC_{50} was imidacloprid>*O. sanctum*>*A. conyzoides*>*L. camara* for the exposure period of 24, 48 and 72 hours, respectively (Table 2).

From the above bioassay study it was found that the aphid mortality was highest in *Ocimum sanctum* treatment with lowest LC_{50} value and highest relative toxicity for all the exposure period which are in conformity with the works of Sharma (2010) [6] who also reported that ether extract of leaves of *Ocimum sanctum* at 1% concentration resulted maximum mortality i.e 50% against short horned grasshopper, *Acrida exaltata*. Similar performance of *Ocimum sanctum* plant extract has also been reported by Singh *et al.* (2009) [7] against mosquitoes who suggested that at high concentration of *O. sanctum* leaf extract there was greater repellent activity.

From the order of toxicity with respect to LC_{50} values it was confirmed that the efficacy of *A. conyzoides* and *L. camara* are found to be less as compared to*O. sanctum* which are having high LC_{50} values than *O. sanctum*. This may also be in conformity with the results of Yanakanchi and Patil (2009) [9] who reported that *L. camara* leaf extract is not effective for management of diamond back moth, *Plutella xylostella* in cabbage and Onunkun (2012) [5] also reported that *A. conyzoides* leaf extract is not effective for the management of flea beetle in okra.

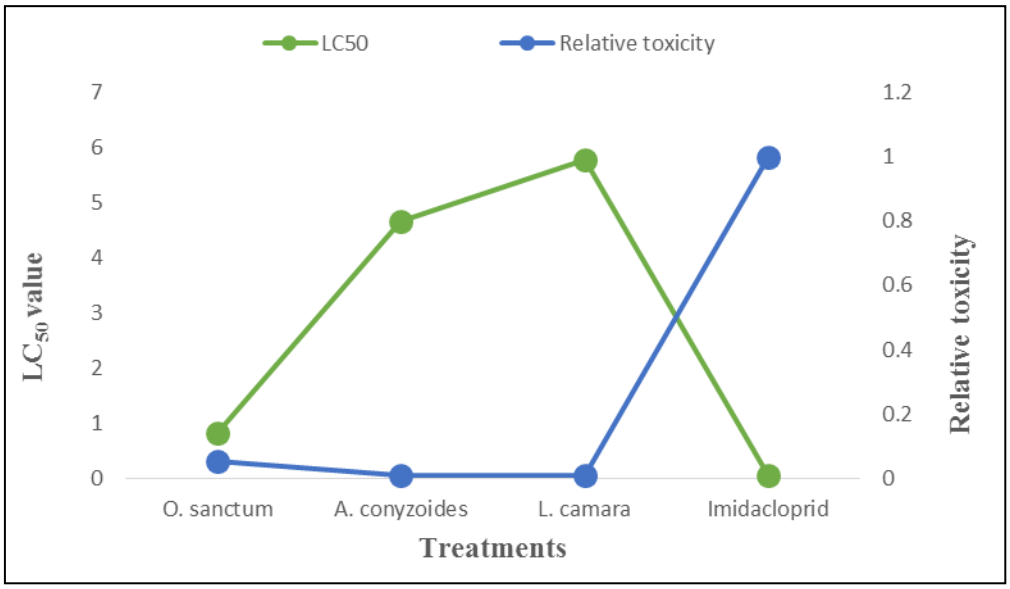
Table 1: Per cent mortality of *Aphis craccivora* caused by several botanicals and imidacloprid at different exposure period

Treatment	Concentration (%)	Post-treatment mortality		
		24h	48h	72h
O. sanctum	5.00	71.69(57.85)	79.24(62.89)	85.55(67.65)
	3.00	63.23(52.67)	69.59(56.53)	74.73(59.82)
	2.00	56.62(48.80)	62.97(52.51)	66.67(54.73)
	1.00	50.35(45.20)	56.09(48.49)	60.81(51.24)
	0.50	45.42(42.37)	48.99(44.42)	53.78(47.16)
	0.25	37.48(37.74)	42.95(40.94)	46.43(42.95)
	0.10	29.07(32.62)	34.81(36.15)	40.30(39.40)
S.Ed(±)		3.79	4.67	5.37
CD(P=0.05)		8.05	9.91	11.38
A. conyzoides	8.00	67.10 (54.99)	74.79 (59.86)	81.10 (64.23)
	7.00	60.99 (51.34)	68.34 (55.75)	73.26 (58.86)
	6.00	53.45 (46.97)	60.71 (51.18)	68.14 (55.63)

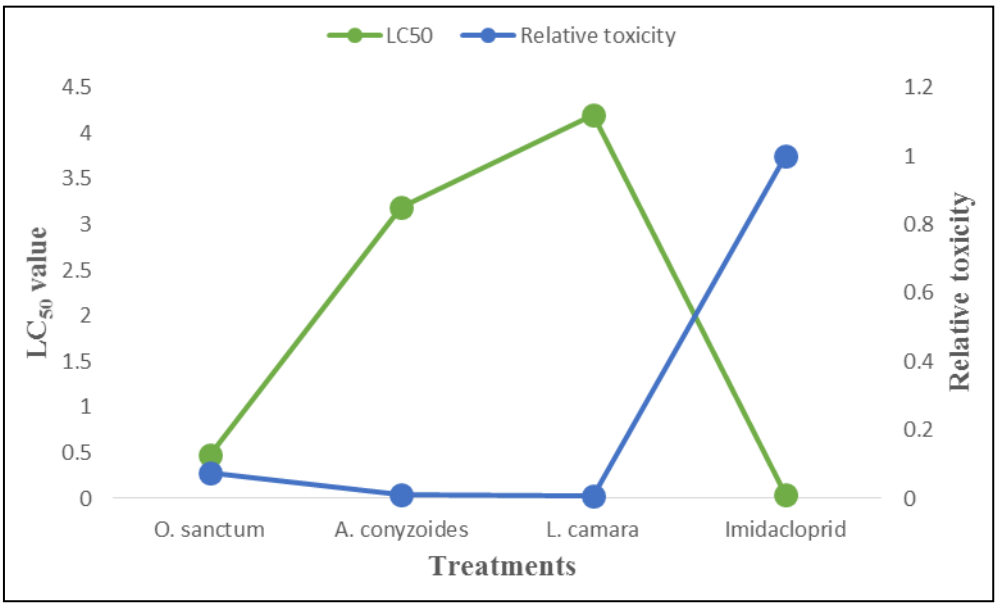
	5.00	45.45 (42.38)	53.11 (46.78)	59.26 (50.33)
	3.00	37.55 (37.79)	44.97 (42.11)	50.52 (45.29)
	2.00	30.86 (33.74)	38.86 (38.56)	45.43 (42.37)
	1.00	25.53 (30.34)	31.60 (34.20)	36.01 (36.87)
S.Ed(±)		4.25	4.38	4.71
CD(P=0.05)		9.02	9.29	9.99
L. camara	10.00	68. 36 (55.77)	74.79 (59.86)	82.16 (65.01)
	9.00	61.96 (51.91)	69.59 (56.53)	74.73 (59.82)
	8.00	55.47 (48.14)	61.86 (51.86)	66.67 (54.73)
	7.00	49.36 (44.63)	54.16 (47.38)	60.19 (50.87)
	5.00	40.51 (39.52)	45.95 (42.67)	52.84 (46.62)
	2.50	31.24(33.98)	38.84(38.55)	45.43(42.37)
	1.50	27.89(31.87)	33.60(35.42)	37.01(37.47)
S.Ed(±)		3.56	4.61	5.30
CD(P=0.05)		7.55	9.78	11.24
Imidacloprid	0.08	73.14 (58.75)	81.10 (64.23)	90.00(71.56)
	0.07	65.83 (54.19)	72.93 (58.64)	81.10 (64.23)
	0.06	57.49 (49.28)	64.12 (53.20)	71.35(57.63)
	0.05	50.35 (45.20)	57.25 (49.16)	63.91(53.07)
	0.04	42.27 (40.55)	48.93 (44.38)	54.71(47.70)
	0.03	35.51 (36.57)	41.93 (40.35)	47.47(43.54)
	0.02	27.89(31.87)	34.78(35.95)	39.30(38.82)
	Control	1.66 (7.40)	3.33 (10.51)	5.00 (12.92)
S.Ed(±)		4.16	3.62	3.68
CD(P=0.05)		8.83	7.67	7.81

Table 2: Estimated LC_{50} value, regression equation, heterogenety ($\chi 2$), fiducial limit and order of relative toxicity for three botanicals and imidacloprid at 24, 48 and 72 HAT.

Treatment	Regression Equation	Heterogenety χ^2	LC_{50} (%)	Fiducial limit	Relative Toxicity	Order of Toxicity
			24 hour			
O. sanctum	Y=0.051+ 0.623 X	16.678	0.828	0.673 1.015	0.054	I
A. conyzoides	Y=0.795+ 1.190 X	33.347	4.664	4.011 5.528	0.009	II
L. camara	Y=0.930+ 1.221 X	29.965	5.779	5.028 6.698	0.008	III
Imidacloprid	Y=2.641+ 1.964 X	24.944	0.045	0.048 0.054	1.000	-
			48 hour			
O. sanctum	Y=0.211+ 0.661 X	26.780	0.480	0.359 0.617	0.075	I
A. conyzoides	Y=0.600+ 1.191 X	33.102	3.188	2.702 3.703	0.011	II
L. camara	Y=0.752+ 1.206 X	45.657	4.201	3.425 4.998	0.009	III
Imidacloprid	Y=2.892+ 2.010 X	28.020	0.036	0.033 0.040	1.000	-
			72 hour			
O. sanctum	Y=0.347+ 0..692 X	42.266	0.316	0.205 0.437	0.098	I
A. conyzoides	Y=0.468+ 1.242 X	40.290	2.381	1.916 2.823	0.013	II
L. camara	Y=0.643+ 1.273 X	59.637	3.200	2.411 3.919	0.010	III
Imidacloprid	Y=3.485+ 2.306 X	39.458	0.031	0.027 0.034	1.000	-



(a)



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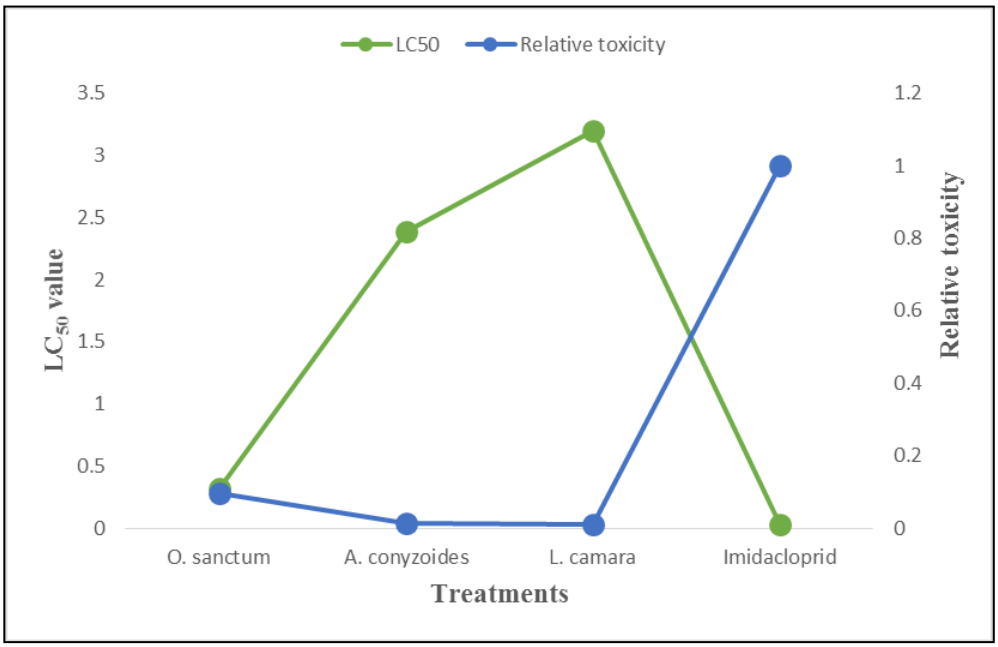

(c)

Fig 1: LC_{50} values and relative toxicity of three botanicals against aphid, *Aphis craccivora* at 24 hours (a), 48 hours (b) and 72 hours (c) exposure period

4. Conclusion

It may be concluded that the botanicals used has direct toxic effect to aphids. Among the tested extracts *O. sanctum* showed the highest toxic effect which was followed by *A.conyzoides* and *L. camara* leaf extract and most importantly these plants were available throughout India, so farmers can easily incorporate these for the management of aphids in field condition. Finally, it can be concluded that these initial bioassay tests of the present experiment will be helpful to identify the potentials of botanical products in pest management however further investigation is needed to confirm the findings before releasing this as new technology.

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