

1 **Bioassay on toxicity of plant extracts against**
2 ***Aphis craccivora* Koch (Hemiptera: Aphididae)**
3 **in French bean (*Phaseolus vulgaris* L.)**

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10 **ABSTRACT**
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The present experimental study was conducted under laboratory conditions in the department of Entomology, School of Agricultural Sciences and Rural Development, Nagaland University, Medziphema Campus during 2017 to 2018. For bioassay on the toxicity of plant extract on Aphids (*Aphis craccivora* Koch) in French beans, five botanicals were used namely, Neem (*Azadirachta indica*), Datura (*Datura stramonium*), Lantana (*Lantana camara*), Nigeria Eucalyptus (*Eucalyptus globules*) and Citronella (*Cymbopogon winterianus*) with one chemical i.e. Dimethoate 30 EC and control. The laboratory bioassay was done using dipping method and the LC 50 values were calculated using Probit analysis. The results from the Probit analysis at 24, 48 and 72 hours showed that the standard check Dimethoate 30 EC was the most toxic @ 0.01%. For botanicals, *L. camara* and *A. indica* extract @ 3% concentration, reported the highest mortality followed by *D. stramonium* extract @ 4%. In comparision *E. globules* and *C. winterianus* @ 5% reported the lowest mortality. Based on the study the order of toxicity of plant products based on Probit analysis are *A. indica* > *L. camera* > *D. stramonium* > *C. winterianus* > *E. globules*.

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13 *Keywords: French bean, Bioassay, Botanicals, Probit analysis.*
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15 **1. INTRODUCTION**

16 French bean (*Phaseolus vulgaris* L.) provides one of the most important sources of protein
17 [1] [2]. It is a great source of dietary fiber, two minerals, and vitamins [3] [4]. French beans
18 are delicate warm-season vegetables that cannot withstand frost, extreme heat, or rain. Its
19 seed does not germinate below 15° C and the most favourable soil temperature for its seed
20 germination ranges from 18 to 24° C. Green beans have been reported to contain 6.2 per
21 cent protein, 0.2 per cent fat, and 63 per cent carbohydrate [5]. The crop is attacked by
22 series insect pests during its life span. One of the major constraints in the production of

23 French beans, is the attack of various insect pests such as hadda beetle (*Epilachna*
24 *vigintioctopunctata*), the flea beetle (*Longitarsus belgaumensis*), aphid (*Smynthurodes*
25 *betae*), and the bean fly (*Ophiomyia phaseoli*) which cause considerable damage [6] [7] [8]
26 [9] [10]. Among them the sucking insect pests like, Aphid (*Aphis craccivora* Koch),
27 leafhopper (*Empoasca dolichi*), thrips (*Megalurothrips sjostedti* Trybom), whitefly (*Bemisia*
28 *tabaci* Gennadius) and mite (*Tetranychus urticae* Koch) are a common one.

29 In India, its cultivation is in 0.21 million ha with a production of 0.58 million MT and
30 productivity is 2.8 t per ha [11]. In Nagaland French bean is cultivated under an area of
31 17280 hectares with a yield of 22140 MT [12]. Studies have shown that essential oils are
32 readily biodegradable and less detrimental to non-target organisms as compared to synthetic
33 pesticides [13]. A variety of properties, including toxicity to the pest, repellence, anti-feedant,
34 and insect growth regulation activities against pests of agricultural value, are possessed by
35 botanical pesticides. More than 2500 plant species belonging to 235 families have been
36 found to possess the characteristics required for an ideal botanical insecticide.

37 2. MATERIAL AND METHODS

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39 The present experimental study was conducted in the laboratory of the Department of
40 Entomology, School of Agricultural Sciences and Rural Development, Nagaland University,
41 Medziphema Campus from 2017 to 2018. The experimental site is located at Medziphema in
42 the district of Chumukedima, Nagaland, India.

43 Five Botanicals were used for the experiment viz., Neem (*Azadirachta indica*), Datura
44 (*Datura stramonium*), Lantana (*Lantana camara*), Nigeria Eucalyptus (*Eucalyptus globules*)
45 and Citronella (*Cymbopogon winterianus*) with one chemical i.e. Dimethoate 30 EC and
46 control.

47 2.1 Plant extraction process

48 The extracts of the plant materials were prepared according to Singh [14] with modifications
49 using an automated Soxhlet extractor (SOCS PLUS SCS04 AS DLS). Acetone was used as
50 the solvent. Different plants used were collected from nearby local areas and then it was
51 dried under shade for 2 to 3 weeks. The dried plants were then crushed evenly using an
52 electric grinder. The crushed powder was sieved to obtain a fine powder.

53 For extraction, 20 g of plant powder was weighed and transferred into thimbles and placed in
54 beakers. 80 ml of solvent (acetone) was added to the beakers. Then the beakers were
55 loaded in the extractor and boiled at 80 °C for 1 hour. After that, the temperature was

56 increased to recovery temperature at 160 °C and boiled for 30 minutes. The thimbles were
 57 rinsed 2 to 3 times. The beakers were taken out from the extractor and the thimbles were
 58 removed. After that the beakers were placed in a warm air oven at 100 °C for 20 to 30
 59 minutes to remove the leftover acetone. The beakers were then removed and placed in a
 60 desiccator and cooled at room temperature. After extraction, the final extract was kept as a
 61 crude extract solution (100 %) in glass bottles. The crude extract was then used for testing
 62 insecticidal activities against the Aphid pests of French beans in Laboratory conditions.

63 2.2 Laboratory Experiments

64 In the laboratory, the different concentration of plant extract was evaluated for their toxicity
 65 against sucking pests of French bean. The method adopted for the experiment was as
 66 follows:

67 **Table 1** Treatment details for bioassay in Aphids, *Aphis craccivora* Koch. (2017-18)

SI. No.	Treatments/ crude plant extracts	Part used	Concentration (%)
1	Neem (<i>Azadirachta indica</i>)	Leaf	2,4,6,8,10
2	Datura (<i>Datura stramonium</i>)	Leaf	2,4,6,8,10
3	Lantana (<i>Lantana camara</i>)	Leaf	2,4,6,8,10
4	Nigeria Eucalyptus (<i>Eucalyptus globules</i>)	Leaf	2,4,6,8,10
5	Citronella (<i>Cymbopogon winterianus</i>)	Leaf	2,4,6,8,10
6	Dimethoate 30 EC / Rogor	-	0.03,0.04,0.05,0.06,0.07
7	Control (water)	-	-

68 2.2.1 Bioassay on toxicity of plant extract on Aphids (*Aphis craccivora* Koch.) by 69 dipping method

70 The plant extracts emulsions of required concentrations were made by dilution with water
 71 and 1ml of triton X (0.1 %). The plant extracts were diluted to make 2, 4, 6, 8, and 10 %
 72 solutions. For comparison Dimethoate 30 EC @ 0.03, 0.04, 0.05, 0.06 and 0.07 % was used
 73 in Table 1. 10 Adult Aphids (*Aphis craccivora* Koch.) was dipped for 10 seconds in each
 74 concentration with 3 replications. After that, the insects were removed, air-dried, and kept for
 75 observation in Petri dishes containing fresh French bean leaves. Observation count was

76 taken before treatment and 24, 48, and 72 hours after treatment [15]. The mortality data
 77 were recorded for chemical and botanicals 24, 48, and 72 hours after treatment. Insects
 78 were observed regularly and those that did not move or react to mild touch were counted as
 79 dead. Insect mortality data was corrected by Abbott's formula [16]. The concentration
 80 mortality line was calculated using Probit analysis [17] in SPSS software with a log₁₀
 81 transformation of the concentrations. The results were expressed as concentration (%) per
 82 insect.

Plant extracts	Concentration (%)	Insect mortality rate (%)		
		24 HAT	48HAT	72HAT
1. <i>Eucalyptus globules</i>	2	10	20	40
	4	16.67	43.33	53.33
	6	26.67	56.67	63.33
	8	40	60	70
	10	60	83.33	83.33
2. <i>Lantana camara</i>	2	36.67	36.67	60
	4	46.67	56.67	63.33
	6	60.00	70	73.3
	8	76.67	80	86.67
	10	83.33	86.67	100
3. <i>Cymbopogon winterianus</i>	2	10	20	36.67
	4	20	33.33	60
	6	30	46.67	63.33
	8	40	50	73.3
	10	66.67	73.33	83.3
4. <i>Azadirachta indica</i>	2	6.67	26.67	46.67
	4	26.67	40	66.67
	6	30.00	63.33	86.67
	8	60.00	66.67	90
	10	63.33	83.33	96.67
5. <i>Datura stramonium</i>	2	40	56.67	93.33
	4	43.33	70	100
	6	63.33	76.67	100
	8	86.67	86.67	100
	10	86.67	96.67	100
6. Dimethoate 30 EC	0.03	43.33	83.33	86.67
	0.04	83.33	86.67	93.33
	0.05	96.67	100	100
	0.06	100	100	100
	0.07	100	100	100

83 Table 2 Mortality of Aphid (*Aphis craccivora*) at 24, 48 and 72 hours with plant
 84 extracts treatment

85 *HAT : Hours after Treatment

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Percent mortality in treatment – Percent mortality in control

91 Corrected percent mortality = ----- x 100%

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100 - Per cent mortality in control

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94 **3. RESULTS AND DISCUSSION**

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96 **3.1 Bioassay on toxicity of plant extract on Aphids (*Aphis craccivora* Koch) by** 97 **dipping method**

98 In the present study from Table 2, the mortality at 24, 48, and 72 hours due to direct toxicity
99 of plant extracts, *D. stramonium*, *L. camara* and Dimethoate 30 EC at different
100 concentrations shows variations in per cent mortality ranging from 0 to 100 %. From the
101 details presented in Table 3, *L. camara* and *A. indica* extract @ 3 % concentration reported
102 the highest mortality followed by *D. stramonium* extract @ 4 % at 24 hours after treatment.
103 While *E. globules* and *C. winterianus* @ 5 % reported the lowest mortality at 72 hours after
104 treatment. The results show that the per cent mortality increases with the increase and time
105 after treatment.

106 Based on the per cent mortality, the concentration mortality line was calculated using Probit
107 analysis. The details of the Probit analysis for 24, 48, and 72 hours are presented in Table 3.
108 The results from the Probit analysis at 24, 48, and 72 hours showed that the standard check
109 Dimethoate 30 EC was the most toxic @ 0.01 %. For the plant products, at 24 hours *D.*
110 *stramonium* and *L. camara* were the most toxic at low concentrations followed by *A. indica*.
111 The LC 50 values at 24 hours were *E. globules* 10 %, *L. camara* 4 %, *C. winterianus* 9 %, *A.*
112 *indica* 8 %, *D. stramonium* 4 %, and Dimethoate 30 EC 0.01 %. Comparable results were
113 obtained 48 hours after treatment where *D. stramonium* showed the lowest concentration
114 mortality value among botanicals @ 1 % (NS), where significant results could not be found.
115 Therefore at 48 hours after treatment, the most toxic at the lowest concentration was

116 observed in *L. camara* @ 4 %. The LC 50 values for 48 hours after treatment were *L.*
 117 *camara* 4 %, *A. indica* 5 %, *E. globules* 6 %, *C. winterianus* 8 % and *D. stramonium* 1 %
 118 (NS). However, at 72 hours, *L. camara* and *A. indica* was the most toxic at the lowest LC50
 119 value followed by *C. winterianus* and *E. globules*. While in *D. stramonium* and Dimethoate
 120 30 EC meaningful result could not be found. The LC50 values at 72 hours were *E. globules*
 121 5 %, *L. camara* 3%, *C. winterianus* 5 %, *A. indica* 3 %, *D. stramonium* 0 % (Non-significant)
 122 and Dimethoate 30 EC 0 % (Non-significant). Based on the study the order of toxicity of
 123 plant products based on Probit analysis was *A. indica* > *L. camara* > *D. stramonium* > *C.*
 124 *winterianus* > *E. globules*.

125 **Table 3 Probit analysis for toxicity at 24, 48 and 72 hours of plant extracts against**
 126 **Aphid, *Aphis craccivora***

Name of extract	LC50 (%)	95% fiducial limit	Slope ± SE	Goodness of fit chi squared
A. At 24 hours				
Eucalyptus	10	6.992-72.465	2.48±0.99	0.62
Lantana	4	0.984-6.808	1.94±0.77	0.02
Citronella	9	6.506-36.286	2.57±0.98	0.81
Neem	8	5.879-16.416	2.96±1.02	0.68
Datura	4	1.205-5.726	2.05±0.77	1.49
Dimethoate	0.01	0.031-0.02	6.53±2.04	0.72
B. At 48 hours				
Eucalyptus	6	6.021-3.878	2.36±0.84	0.53
Lantana	4	1.099-6.205	1.94±0.77	0.02
Citronella	8	5.146-39.221	2.05±0.84	0.53
Neem	5	5.466-3.187	2.20±0.80	0.39
Datura	1(NS)	-	1.58±0.77	0.40
Dimethoate	0.02(NS)	-	2.46±1.76	0.35
C. At 72 hours				
Eucalyptus	5	0.272-26.679	1.53±0.76	0.20
Lantana	3	0.000-4.765	1.52±0.76	1.19
Citronella	5	0.890-11.604	1.64±0.76	0.18
Neem	3	0.381-4.616	1.94±0.78	0.16
Datura	0(NS)	-	0.43±0.90	0.08
Dimethoate	0(NS)	-	1.60±1.66	0.11

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*NS : Non significant

129 **4. CONCLUSION**

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131 On bioassay of Aphids (*Aphis craccivora*) the most effective treatment was observed
132 in Dimethoate 30 EC / Rogor (0.01 %) followed by Botanicals Neem (3 %) and Lantana (3
133 %) followed by Datura (4 %) and the least effective treatment was seen in Eucalyptus (5 %)
134 and Citronella (5 %). Based on the study the order of toxicity of plant products based on
135 Probit analysis was *A. indica* > *L. camara* > *D. stramonium* > *C. winterianus* > *E. globules*.

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