

1 **EFFICACY OF ESSENTIAL OILS AGAINST BLACK BEAN APHID**(*Aphis*
2 *fabae*Scopoli) **UNDER LABORATORY CONDITIONS**

5 **Abstract**

6 | The black bean aphid (*Aphis fabae*), poses a significant threat to cultivated crops, leading to
7 | food insecurity and hunger concerns. Essential oils from plants offer promising low-risk
8 | alternatives to synthetic chemicals for aphid control whose widespread use has raised serious
9 | environmental and health issues. To assess the efficacy of plant essential oils, an experiment
10 | was carried out at the Entomology Laboratory of Lamjung Campus following a completely
11 | randomized design with three replications using eight treatments including a control (distilled
12 | water and ethanol). The essential oils used were Neem (*Azadirachtaindica*), Eucalyptus
13 | (*Eucalyptus globulus*), Palma rosa (*Cymbopogon martini*), Citronella
14 | (*Cymbopogonwinterianus*), lemongrass (*Cymbopogoncitratus*), Juniperberry
15 | (*Juniperusrecurva*), Mint (*Menthaarvensis*) and control (distilled water and ethanol) at the
16 | concentration of 1% and 2% with the exposure time of 12, 24 and 48 hours for contact
17 | mortality and 2 μ L and 4 μ L with the exposure time of 12 and 24 hours for fumigation
18 | mortality. The result revealed that individual mortality rate increased with increasing oil
19 | concentration and exposure time. Lemongrass with 2% concentration resulted in the high
20 | mortality percentage (66.66%) whereas, Neem with 1% concentration demonstrated the
21 | lowest (23.3%) in contact assay. Similarly, in fumigation assay, the highest mortality
22 | percentage was exhibited by 4 μ L Lemongrass (100%) when compared to 2 μ L Neem and
23 | Juniper berry have lowest mortality percentage (73.33%) after the control. Therefore,
24 | Lemongrass with 2% concentration for contact assay and 4 μ L Lemongrass for fumigation
25 | assay was considerably more effective than other used essential oils. However, more research

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26 is necessary with locally available essential oils to assure the long-term effectiveness in field
27 and lab conditions.

28 **Keywords:** *Black bean aphid, Essential oils, Faba bean, Mortality.*

29

30 INTRODUCTION

31 Aphids being the most hostile pests for almost all cultivated crops, seriously diminish the
32 productivity of crops and cause spoilage by sucking the sap from the leaves as well as
33 delicate stems (Chalise & Dawadi, 2019). The annual average yield losses brought on by
34 aphids range from 30 to 50% (Alghamdi, 2018). Aphid's saliva includes non-enzymic,
35 reducing compounds, which, in the presence of oxidases, can mix with and inactivate
36 protective phytochemicals, including those generated in response to damage and transmitted
37 in the phloem sieve tube sap on which Aphid feed (Miles, 1999). The black bean aphid
38 (*Aphis fabae*) is a major pest of field beans, which are sown in the season of spring and the
39 data of field studies have revealed that yield losses by the black bean aphid infestations can
40 exceed 50% of the yield (Hansen et al., 2008). Black bean aphids directly damage the plants
41 by feeding on the phloem that results in severe reductions of plant development and
42 productivity (Pruter & Zebitz, 1991). In addition to direct plant injury, black bean aphid
43 infestation harms faba bean due to honeydew excretion on the leaves, which interferes with
44 physiological processes in the host plant (Hurej & Werf, 1993). These aphids can indirectly
45 damage by acting as vectors for plant viruses (Hogenhout et al., 2008). They transfer the bean
46 leaf rolling virus and the faba bean necrotic yellows virus (Casper, 1993). It has been long
47 since botanical insecticides have been regarded as acceptable alternatives for killing the
48 harmful insects because of their low retention in the environment, minimal toxicity to
49 mammals, better selectivity and the popularity (Ebrahimi et al., 2013). Numerous plant species'
50 essential oils have a variety of insecticidal, antifeedant, repellent, oviposition-deterrent,

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Comment [TF5]: Line 41-42: Check whether the honeydew excretion harbours pathogenic fungal diseases, in addition to interferences with physiological processes and vectors for virus diseases.

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51 growth-regulating, and antivectorial effects on nuisance insects(Vinelina, Dima and Georgi,
52 2014). Essential oils are complicated, organic, volatile compounds with a distinct smell that
53 are produced by aromatic plants as secondary metabolites. They are often produced via steam
54 or hydro-distillation, which Arabs invented in the Middle Ages (Bakkali et al., 2008). The
55 majority of research concluded that essential oils have significant potential for pest
56 management, particularly in greenhouses and fields(Isman, 2000). Essential oils are very
57 bioactive and can be very effective insecticides. As a result, there should be a lot more
58 research done on them to help manage aphids (Tomova et al., 2005).

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59 MATERIALS AND METHODS

60 The experiment was carried out from March to April of 2023 in the entomology laboratory of
61 the Institute of Agriculture and Animal Science, Lamjung campus, which is in Sundarbazar,
62 the Lamjung district of Gandaki province. The site was located at an elevation of 610m above
63 sea level with latitude: 28.2847° N and longitude: 84.3637°E under humid sub-tropical
64 climate. The highest and lowest temperatures recorded were, ~~respectively~~, 28–30°C and 20–
65 22°C respectively. Apterous (wingless) black bean aphid was collected from the field of faba
66 bean located nearby Lamjung campus. The required essential oils were obtained from Herbs
67 Production and Processing Company Limited, Kathmandu. Two factorial Completely
68 Randomized Experimental Design was selected as the design of the research. Seven
69 treatments of essential oils namely: Neem, Eucalyptus, Palma rosa, Citronella, Lemongrass,
70 Juniper berry, Mint along with one control were used. Three replication per treatments were
71 done. The aphid mortality was assayed through two parameters during the experiment.

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72 a. Contact mortality assay (Leaf dip bioassay): Using ethanol as a non-polar solvent, each
73 treatment oil was dissolved before being utilized to investigate the direct contact effect of
74 essential oils on aphid species. There were two concentration levels 1% and 2% -prepared
75 for each treatment. In three replications, each individual faba bean leaf was immersed in

76 the oil solution of every treatment for about five minutes. The volatile solvent was then
77 allowed to evaporate by letting them dry for a while. Following treatment, the leaves were
78 placed in sterile Petri plates with damp filter paper within. In each petri dish, ten aphids
79 were released and given time to settle on the leaves that had been treated. Then the petri
80 plate was covered by muslin cloth so that aphids present inside the petri plate could not
81 escaped away. After 12 hours and 24 hours, the number of dead aphids was counted.
82 Using a little brush to gently strike the aphid and keeping an eye out for any movement of
83 its legs or antennae, one may identify dead aphids.

84
85 b. Fumigation mortality assay: Saturated filter paper was stored in three layers within sterile
86 9 cm petri dishes. At the bottom of the filter sheets was a tiny, rectangular (5×2 cm) filter
87 paper. Each essential oil was directly added to the rectangular piece of filter paper with a
88 micropipette, at concentrations of 2 µL and 4 µL. To avoid direct contact between the oils
89 and the experimental pest, ten aphids were then introduced onto the upper layer of the
90 petri dish. To keep the treated oils from leaking, parafilm was then used to enclose the
91 entire apparatus. Similar methods were described in Chalise & Dawadi, 2019.

92 Mortality of aphids was counted after 12hrs, 24hrs and 48hrs. Mortality of treated aphid was
93 then calculated by dividing the number of dead aphids in petri plate by total number of aphids
94 in petri plate and expressing it in terms of percentage. The collected data of aphid mortality
95 was processed with MS Excel 365 and analysis of variance was done using R- Stat software
96 (version 4.1.0). Mean comparison was done using Duncan's Multiple Range Test at 5% level
97 of significance.

98 **RESULTS**

99 **1. Effect of treatments on the contact mortality of aphid**

100 Table 1: Effect of Essential oils on aphid mortality on contact assay

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				101	*,
Factor A	Contact mortality after treatment application			102	**
(Essential oils)	12hrs	24hrs	48hrs	103	and
Neem	10.00 ^c	15.00 ^d	31.66 ^{cd}	104	***
Eucalyptus	15.00 ^{bc}	18.33 ^{cd}	30.00 ^d	105	at
Palmarosa	25.00 ^a	35.00 ^b	55.00 ^a	106	P=0
Citronella	13.33 ^{bc}	23.33 ^c	43.33 ^b	107	.05,
Lemongrass	26.66 ^a	43.33 ^a	61.66 ^a	108	P=0
Juniper berry	16.66 ^b	18.33 ^{cd}	38.33 ^{bc}	109	.01
Mint	23.33 ^a	33.33 ^b	45.00 ^b	110	and
Control	1.66 ^d	6.66 ^e	8.33 ^e	111	P=0
CV%	17.73	13.13	15.19	112	.00
F-test	***	***	***	113	1
LSD	5.36	6.57	6.99	114	lev
SEM	2.97	4.28	5.82	115	el
Factor B	12hrs	24hrs	48hrs	116	of
(Concentration)				117	sig
1%	12.08 ^b	18.33 ^b	32.91 ^b	118	nifi
2%	20.83 ^a	30.00 ^a	45.41 ^a	119	can
CV%	17.73	13.13	15.19	120	t
F-test	***	***	***	121	res
LSD	2.68	3.28	3.49	122	pec
SEM	4.375	5.835	6.25	123	tive
Grand mean	16.45	24.16	39.167	124	ly.
P value	.	**	**		

125 The effect of treatments on mortality of aphid was found significant at $p < 0.001$ at 12, 24 and
 126 48hrs. At 12hrs, the highest mortality percentage was found in Lemongrass (26.66%) which
 127 was statistically par with Palmarosa and Mint and the lowest mortality rate was in control
 128 .The lowest aphid mortality after control was in Neem (10%) which was statistically par with
 129 Citronella and Eucalyptus. Similarly, at 24hrs, highest aphid mortality was seen in
 130 Lemongrass (43.33%) and lowest mortality was in Neem (15%) but Eucalyptus and Juniper
 131 berry were also significantly at par. In the 48hrs, similar result was observed where
 132 Lemongrass had the highest aphid mortality rate (61.66%), which was statistically similar
 133 with Palmarosa and Eucalyptus. The lowest mortality percentage was shown by Eucalyptus
 134 (30%) which was statistically par with Neem.

135 Table2: Interaction of essential oils and concentration on aphid mortality in contact assay

136

Essential oils	12hrs		24hrs		48hrs	
	1%	2%	1%	2%	1%	2%
Neem	6.66 ^{ghi}	13.33 ^{efg}	13.33 ^{gh}	16.66 ^{fg}	23.33 ^g	40.00 ^{de}
Eucalyptus	10.00 ^{fgh}	20.00 ^{cde}	10.00 ^{gh}	26.66 ^{de}	26.66 ^{fg}	33.33 ^{ef}
Palmarosa	23.33 ^{bcd}	26.66 ^{abc}	33.33 ^{cd}	36.66 ^{bc}	43.33 ^{cd}	66.66 ^a
Citronella	10.00 ^{fgh}	16.66 ^{def}	13.33 ^{gh}	33.33 ^{cd}	30.00 ^{fg}	56.66 ^b
Lemongrass	23.33 ^{bcd}	30.00 ^{ab}	33.33 ^{cd}	53.33 ^a	56.66 ^b	66.66 ^a
Juniper berry	10.00 ^{fgh}	23.33 ^{bcd}	13.33 ^{gh}	23.33 ^{ef}	33.33 ^{ef}	43.33 ^{cd}
Mint	13.33 ^{efg}	33.33 ^a	23.33 ^{ef}	43.33 ^b	40.00 ^{de}	50.00 ^{bc}
Control	0.00 ⁱ	3.33 ^{hi}	6.66 ^h	6.66 ^h	10.00 ^h	6.66 ^h

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LSD	7.59	3.28	9.89
Grand mean	16.45	24.16	39.16
CV%	17.73	24.23	15.19

137 *, ** and *** at P=0.05, P=0.01 and P=0.001 level of significant respectively.

138 The mortality rate had increased with exposure time and higher the concentration of Essential
139 oils from 1% to 2%, more was the mortality rate. From the interaction table, in 12hrs it was
140 found that Mint at 2% concentration revealed the highest mortality percentage (33.33%)
141 which was statistically par with Lemongrass (30%) and Palmarosa (26.66%) but Neem at 1%
142 concentration demonstrated lowest mortality (6.66%). In 24hrs, Lemongrass with 2%
143 concentration exhibited the highest mortality rate (53.33%) whereas lowest mortality was
144 shown by Neem, juniper berry and citronella at 1% concentration with 13.33% .Similarly, in
145 48hrs, Lemongrass with 2% mortality exhibited the highest mortality (66.66%) whereas
146 Neem with 1% mortality showed the lowest (23.33%).

147 **2. Effect of treatments on fumigation mortality of aphid**

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148 Table 3:Effect of Essential oils on aphid mortality in fumigation assay

Factor A (Essential oils)	Fumigation mortality after using treatments	
	12hrs	24hrs
Neem	71.66 ^c	75.00 ^d
Eucalyptus	81.66 ^{ab}	95.00 ^{ab}
Palmarosa	85.00 ^a	90.00 ^{bc}
Citronella	83.33 ^{ab}	96.66 ^{ab}
Lemongrass	78.33 ^{abc}	98.33 ^a
Juniper berry	76.66 ^{bc}	83.33 ^c
Mint	85.00 ^a	95.00 ^{ab}

Control	5.00 ^d	6.66 ^e
CV%	8.39	7.21
F-test	***	***
LSD	6.99	6.78
SEM	9.54	10.83
FactorB	12hrs	24hrs
(concentration)		
2µl	66.25 ^b	77.08 ^b
4 µl	75.41 ^a	82.91 ^a
CV%	8.39	7.21
F-test	***	**
LSD	3.49	3.39
SEM	4.58	2.91
Grand mean	70.83	80.00
P value	***	.

149 *, ** and *** at P=0.05, P=0.01 and P=0.001 level of significant respectively.

150 From the table, at 12hrs, the highest mortality percentage was shown by Palmarosa and Mint
151 with 85% but statistically, it was similar with Citronella, Eucalyptus and Lemongrass.
152 Mortality percentage in Lemongrass was highest (98.33%) at 24hrs which were significantly
153 par with Citronella, Eucalyptus and Mint. Neem at 12hrs (71.66%) and 24hrs (75%) showed
154 the lowest mortality percentage after control.

155

156

157 Table 4: Interaction of essential oils and concentration on aphid mortality in fumigation assay

Essential oils	12hrs		24hrs	
	2µL	4 µL	2 µL	4 µL
Neem	66.6 ^{ef}	76.6 ^{cd}	73.33 ^d	76.66 ^d
Eucalyptus	73.3 ^{de}	90.0 ^{ab}	93.3 ^{abc}	96.6 ^{ab}
Palmarosa	83.33 ^{bc}	86.6 ^{ab}	86.66 ^c	93.3 ^{abc}
Citronella	83.33 ^{bc}	83.3 ^{bc}	96.66 ^{ab}	96.6 ^{ab}
Lemongrass	63.33 ^f	93.33 ^a	96.66 ^{ab}	100.0 ^a
Juniper berry	70.0 ^{def}	83.3 ^{bc}	73.33 ^d	93.3 ^{abc}
Mint	83.33 ^{bc}	86.6 ^{ab}	90.00 ^{bc}	100.0 ^a
Control	6.6 ^g	3.3 ^g	6.66 ^e	6.66 ^e
CV	8.39		7.21	
LSD	7.29		9.59	
Grand mean	70.83		7.21	

158 *, ** and *** at P=0.05, P=0.01 and P=0.001 level of significant respectively.

159 The mortality rate had increased with exposure time and concentration of Essential oils from
160 1 µl to 2 µl. From the interaction table, in 12hrs, it was observed that Lemongrass with 4µl
161 concentration showed the highest mortality (93.33%) and in 24hrs, highest mortality was
162 observed in Lemongrass with 4 µl concentration (100%) whereas lowest mortality was shown
163 by 2 µl Neem and Juniper berry with 73.33%.

164 **DISCUSSION**

165 Tomova et al., (2005), Ebrahimi et al., (2013), and Alghamdi, (2018) also reported that the
166 action of essential oils on the aphid is dosage dependent that the individual mortality
167 percentage increased with increasing oil concentration. Hence, lemongrass was effective
168 against black bean aphid in both contact and fumigation assay which was in agreement with

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169 Shahid Nisar et al., (2023) reported that the best result with the highest mortality of aphid
170 was shown by lemongrass with 80% . Chalise & Dawadi, (2019) also reported that the
171 highest contact mortality percentage had resulted in Lemongrass. Ricci et al. discovered that
172 lemongrass oil could efficiently repel *Diuraphisnoxia* (Mordvilko), a Russian wheat aphid.
173 Zhang et al. (2016) reported that with LC₅₀ value of 169.6 mg/L in 24 hours after treatment,
174 Lemongrass essential oil showed high toxicity against *Aphis citricola*. The various bioactive
175 cyclic and acyclic terpene constituents present in the oil of Lemongrass attributed to its
176 insecticidal property (Eden et al. 2020). The primary contributor for the insecticidal property
177 of Lemongrass oil is citral which is a mixture of geranial and neral (Eden et al., 2020). Citral
178 can regulate cell proliferation by interacting with intracellular oxygen radicals and oxidative
179 stress. Furthermore, citral and other components may act on neuroreceptors, obstruct signal
180 transduction, and induce hormonal imbalance, membrane damage, and cytotoxicity in the
181 host (Feroz, 2020). In addition to its major constituent, minor component such as germacrene
182 D (2.24%), caryophyllene (0.57%) and caryophyllene oxide (0.58%) in limited quantity
183 exhibit substantial insecticidal and repellent activities (Talbi et al., 2020). Lemongrass has the
184 ability to block the function of several neurotransmitters, including acetylcholine esterase and
185 octopamine (Singh et al., 2017).

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186 CONCLUSION

187 *Aphis fabae*, the black bean aphid (Homoptera: Aphididae) is the most devastating pest of the
188 faba bean. Synthetic pesticide shows negative impact on human health and ecosystem
189 including pollution, pest resistance, biodiversity loss and toxicity to non-target organisms.
190 Botanical insecticides including essential oils, with their new, highly bioactive constituents,
191 have the potential to be highly effective pesticides as they are non-toxic to beneficial fauna,
192 are biodegradable, environmentally friendly, human health-safe, and residue-free. The
193 research revealed that Lemongrass with higher concentration had the highest mortality

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194 percentage while the lowest mortality percentage was shown by Neem with lower
195 concentration after control in both contact and fumigation process. Therefore, with proper
196 validation, Lemongrass can be used as an alternative to synthetic insecticides for the control
197 of black bean aphid. However, further research in field condition is highly recommended to
198 test the derived result which allows broader application at the farmer's level.

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