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

**Background &Justifications:**

Comment [Z1]: Almost well justified


**Abstract**

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

oils. However, more research is necessary with locally available essential oils to assure the long-term effectiveness in field and lab conditions.

What is the final fate of the study?

Comment [Z2]: Who will be the final out put user from the findings?

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



## INTRODUCTION

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

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

**MATERIALS AND METHODS**

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

a. Contact mortality assay (Leaf dip bioassay):Using ethanol as a non-polar solvent, each treatment oil was dissolved before being utilized to investigate the direct contact effect of essential oils on aphid species. There were two concentration levels 1% and 2% -prepared

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

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

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

**RESULTS**

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

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

| **Factor A (Essential oils)** | **Contact mortality after treatment application** | | |
|---|---|---|---|
| | **12hrs** | **24hrs** | **48hrs** |
| Neem | 10.00[c] | 15.00[d] | 31.66[cd] |
| Eucalyptus | 15.00[bc] | 18.33[cd] | 30.00[d] |
| Palmarosa | 25.00[a] | 35.00[b] | 55.00[a] |
| Citronella | 13.33[bc] | 23.33[c] | 43.33[b] |
| Lemongrass | 26.66[a] | 43.33[a] | 61.66[a] |
| Juniper berry | 16.66[b] | 18.33[cd] | 38.33[bc] |
| Mint | 23.33[a] | 33.33[b] | 45.00[b] |
| Control | 1.66[d] | 6.66[e] | 8.33[e] |
| CV% | 17.73 | 13.13 | 15.19 |
| F-test | *** | *** | *** |
| LSD | 5.36 | 6.57 | 6.99 |
| SEM | 2.97 | 4.28 | 5.82 |
| **Factor B (Concentration)** | **12hrs** | **24hrs** | **48hrs** |
| 1% | 12.08[b] | 18.33[b] | 32.91[b] |
| 2% | 20.83[a] | 30.00[a] | 45.41[a] |
| CV% | 17.73 | 13.13 | 15.19 |
| F-test | *** | *** | *** |
| LSD | 2.68 | 3.28 | 3.49 |
| SEM | 4.375 | 5.835 | 6.25 |
| Grand mean | 16.45 | 24.16 | 39.167 |

| | | | | |
|---|---|---|---|---|
| P value | . | ** | ** | *, ** |

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

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

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

| **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$^{h}$ |
| **LSD** | 7.59 | | 3.28 | | 9.89 | |
| **Grand mean** | 16.45 | | 24.16 | | 39.16 | |
| **CV%** | 17.73 | | 24.23 | | 15.19 | |

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

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

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

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 (concentration)** | **12hrs** | **24hrs** |
| **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** | *** | . |

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

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

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 | |

*, ** and *** at P=0.05, P=0.01 and P=0.001 level 0f significant respectively.

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

**DISCUSSION**

Tomova et al., (2005), Ebrahimi et al., (2013), Alghamdi, (2018) also reported that the action of essential oils on the aphid is dosage dependent that the individual mortality percentage

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

Significance of the statement:

**Comment [Z3]:** Please highlight the future directions, recommendations and limitations of the study

## CONCLUSION

*Aphis fabae*, the black bean aphid (Homoptera: Aphididae) is the most devastating pest of the faba bean. Synthetic pesticide shows negative impact on human health and ecosystem including pollution, pest resistance, biodiversity loss and toxicity to non-target organisms.

Botanical insecticides including essential oils, with their new, highly bioactive constituents, have the potential to be highly effective pesticides as they are non-toxic to beneficial fauna, are biodegradable, environmentally friendly, human health-safe, and residue-free. The research revealed that Lemongrass with higher concentration had the highest mortality percentage while the lowest mortality percentage was shown by Neem with lower concentration after control in both contact and fumigation process. Therefore, with proper validation, Lemongrass can be used as an alternative to synthetic insecticides for the control of black bean aphid. However, further research in field condition is highly recommended to test the derived result which allows broader application at the farmer's level.

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