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Efficacy of plant essential oils on black bean aphid (*Aphis fabae*) and cabbage aphid (*Brevicoryne brassicae*) under laboratory condition

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Abstract
The black bean aphid (*Aphis fabae*) and cabbage aphid (*Brevicoryne brassicae*) are the major pest of black bean and cabbage respectively and their control still depends on the use of insecticides. In recent years, application of essential oils derived from aromatic plants has been considered as potential low-risk alternatives. A lab experiment was therefore, performed to study the effects of different essential oils on two different aphid species, at two different concentrations and time duration. The oils used were Citronella (*Cymbopogon winterianus*), Eucalyptus (*Eucalyptus globulus*), French Basil (*Ocimum basilicum*), Juniper berry (*Juniperus recurva*), Lemon grass (*Cymbopogon citratus*), Mint (*Mentha arvensis*) and Palmarosa (*Cymbopogon martini*) at the concentration of 1% and 2% for contact mortality and 2µl and 4µl for fumigation mortality, with exposure time of 24 and 48 hours. The results revealed that individual mortality percentages generally increased with increasing oil concentrations and exposure time. Among the treatments, Citronella and Lemon grass resulted in highest contact mortality percentage, whereas, Eucalyptus and Juniper berry gave the lowest percentage in both the aphid species. In case of fumigation mortality, Eucalyptus and Juniper berry resulted in highest mortality percentage with Palmarosa giving the lowest percent, in both the aphid species.

Keywords: black bean aphid, cabbage aphid, essential oils, mortality

Introduction
Aphids (Homoptera: Aphididae) causes serious losses on crop by sucking the sap from the tender stems and leaves resulting into distortion of the shoots, stunted plants, reduced yield and spoiled crops. The cabbage aphid, *Brevicoryne brassicae* L. is found on brassica crops with worldwide distribution and severe damage and outbreaks. This aphid was identified for the first time in Europe and later on it has been reported worldwide in most countries with a temperate climate [1]. They prefer feeding on young leaves and result in significant yield losses in many Cruciferous crops. The cabbage aphid is also responsible for non-persistent mode of pathogen transmission in which the aphid picks up the virus by feeding on infected plants and transfers the pathogen to healthy plants by probing with its mouthparts or feeding [2].
Black bean aphid is widely distributed species that occurs particularly on beans, peas, beets, crucifers, cucurbits, potato, tobacco, tomato and many other host plants. It is also responsible for the transmission of over 40 plants viruses [2]. The plants are stunted by the removal of sap, the stems are distorted, harmful viruses are transmitted, and aphid residues may contaminate the crop.
Although many insecticides have been recommended for aphid control, new alternatives are being rapidly searched. This is because of resistance of aphids against several classes of insecticides and the fact that aphids often feed on the undersides of leaves, which are not usually treated by insecticidal application. Moreover, the hazardous effect of chemical insecticides on non-target pest and the environment is also a matter of great concern. However, currently, botanical insecticides (BIs) based on the insecticidal efficacy of secondary plant metabolites are considered a suitable alternative for plant protection against pests including aphids [3].

Bio pesticides made from essential oils are made of several compounds with multiple action mechanisms; they have various modes of implementation; contact, fumigation and repulsive. They are reported as highly effective in controlling several pest [4]. But there are very few study on control of aphid by essential oil, especially in Nepal. Thus, one needs to search highly selective and easily biodegradable pesticides to solve the problem of long-term toxicity to human beings and, on the other hand, solve the need of environmental friendly pesticides. Hence, this study was carried out to explore the efficacy of different plant essential oils on two species of aphid under laboratory condition.

Materials and Methods

Insect cultures

Pure culture of *Aphis fabae* and *Brevicoryne brassicae* was established under controlled condition at department of Plant Protection, Institute of Agriculture and Animal Science. The aphids were reared at 25 ± 2 °C and 55 ± 5% Relative Humidity, and a photoperiod of 16: 8 L: D in greenhouse.

Essential oils

The required essential oils were obtained from Jadibuti Association of Nepal- JABAN, Kathmandu. Air tight conditions were maintained till the oils were ready for use. 100% pure and certified oils were procured for the experiment.

Research design

Completely Randomized Experimental Design (CRD) was chosen as the research design. Seven treatments of essential oils along with one control were used as treatments. Acetone was also included as control to counter the effect of acetone used to prepare oil solutions. Four replication per treatments were done.

Treatments details

List of treatment oils

S. No	Essential oils	Scientific name
T_1	Citronella	*Cymbopogon winterianus*
T_2	Eucalyptus	*Eucalyptus globulus*
T_3	French Basil	*Ocimum basilicum*
T_4	Juniper berry	*Juniperus recurva*
T_5	Lemon grass	*Cymbopogon citratus*
T_6	Mint	*Mentha arvensis*
T_7	Palmarosa	*Cymbopogon martini*
T_8	Control	Distilled water and acetone

Mortality measurement assay

During the experiment the mortality of the aphids was assayed through two parameters as follows:

Contact mortality assay (Leaf dip bioassay)

This methodology was performed to observe the direct contact effect of essential oils on the aphid species. Each treatment oils were dissolved in acetone as a non-polar solvent. Two level of concentration (1% and 2%) was prepared for each treatments. Host leaf of respective aphid species (i.e. cabbage leaf for Cabbage aphid and bean leaf for Black bean aphid) were cut at appropriate size and dipped in the oil solution for about 30 seconds. Then they were allowed to dry for some time to provide time for evaporation of the volatile solvent. Such treated leafs were kept in clean petri-dishes (9cm size) with a wet filter paper. Ten aphids were released in such petri-dishes and allowed to settle in the treated leafs. The number of dead aphids were counted after 24 and 48 hours. Dead aphids were identified by gently striking the pest with a small brush and observing any movements of legs or antennae.

Fumigation mortality assay

Above mentioned treatments were subjected for fumigation mortality assay in both aphid species for the same time duration in similar experimental setup. The mode of essential oil action was quite different. Four layers of saturated filter paper were kept in clean petri dishes (9 cm size). A small rectangular sized filter paper (5*2 cm) was placed at the bottom of those filter papers. Each essential oils were directly applied in that rectangular sheet of filter paper at concentration of 2 µl and 4 µl with the help of a micro-pipette. Then ten aphids were released in the top layer of the petri dish, preventing direct contact of the oils and the experimental pest. The entire set-up was then sealed by the help of Parafilms to prevent the escape of the treated oils. Mortality of aphids was counted after 24 and 48 hrs.by using above mentioned ways.

Statistical analysis

Mortality percentage of the treated aphids were expressed in terms of percentage of the total aphids treated (i.e. 10). After that, this observed values were compared with control to find out the corrected mortality percentages in accordance to Schneider-Orelli's formula. The parameters taken were statistically analyzed by Fischer test in two-factorial ANNOVA Complete Randomize Design in R – Stat 3.1.3 software package. The mean separation was performed by Duncan's Multiple Range Test at 0.05 probability level.

Results and Discussion

Table 1: Contact mortality for both species in each-treatments, in Lamjung, 2019

S.no	Treatments	Concentration	Contact mortality after treatment application			
			Cabbage Aphid		Black bean aphid	
			24 hours	48 hours	24 hours	48 hours
1.	Citronella	1%	89.47±12.2^{abc}	94.59±6.2^{ab}	83.74±6.2^{bcd}	82.61±6.7^{b}
2.	Citronella	2%	100±0.0^{a}	100±0.0^{a}	94.59±6.2^{ab}	97.1±5.8^{a}
3.	Lemon grass	1%	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}
4.	Lemon grass	2%	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}
5.	Mentha	1%	76.32±15.7^{cd}	78.38±8.8^{c}	72.97±6.2^{de}	79.71±5.8^{bc}
6.	Mentha	2%	94.74±6.1^{ab}	97.3±5.4^{a}	91.89±5.4^{abc}	94.2±6.7^{a}
7.	Palmarosa	1%	81.58±10.1^{bcd}	91.89±5.4^{ab}	78.38±9.4^{de}	82.61±6.7^{b}

8.	Palmarosa	2%	94.74±6.1^{ab}	97.3±5.4^{a}	94.59±6.2^{ab}	94.2±6.7^{a}
9.	French basil	1%	26.32±12.2^{fgh}	67.57±8.8^{d}	43.24±5.4^{g}	44.93±5.8^{e}
10.	French basil	2%	73.68±18.3^{de}	86.49±5.4^{bc}	72.97±1.1^{de}	71.01±11.6^{cd}
11.	Eucalyptus	1%	15.79±8.6^{h}	18.92±6.3^{g}	56.76±1.2^{f}	50.72±5.8^{e}
12.	Eucalyptus	2%	36.84±8.6^{f}	45.95±12.4^{e}	81.08±6.2^{cde}	82.61±6.7^{b}
13.	Juniper berry	1%	21.18±6.2^{gh}	29.73±6.2^{f}	43.24±1.6^{g}	50.72±11.1^{e}
14.	Juniper berry	2%	36.84±10.1^{fg}	51.35±6.2^{e}	70.27±5.4^{e}	71.01±6.7^{cd}

As seen from the result table 1, it was clearly evident that mean mortality percentage increased with the increasing concentration and duration of treatment application. In all treatments, 2% conc. resulted in higher mortality at duration of 48 hrs after treatment application. Complete mortality of cabbage aphid was seen in Lemongrass (1% and 2%) and Citronella (2%) within 24 hrs but statistically this treatments were at par with Citronella (1%), Mentha (2%) and Palmarosa (2%). Very low mortality percentage was reported in Eucalyptus (1%) and Juniper berry (1%) which were also statistically inferior. In case of Black bean aphid, complete mortality was reported only by Lemon grass (1% and 2%). But statistically it was similar with Citronella (1%), Mentha (2%) and Palmarosa (2%). Lowest mortality was seen in Eucalyptus (1%) and Juniper berry (1%).

Table 2: Fumigation mortality for both aphids in each treatment in Lamjung, 2019

S. no	Treatments	Concentration	Fumigation mortality after treatment application			
			Cabbage Aphid		Black bean aphid	
			24 hours	48 hours	24 hours	48 hours
1.	Citronella	2µl	12±10.2^{gh}	35.21±16.9^{e}	31.59±6.1^{fg}	56.52±12.5^{e}
2.	Citronella	4µl	54.67±5.3^{cd}	63.38±10.7^{cd}	55.11±5.1^{cd}	83.69±18.8^{abc}
3.	Lemon grass	2µl	25.33±15.3^{fg}	60.56±11.2^{d}	10.53±6.1^{h}	59.23±13.6^{de}
4.	Lemon grass	4µl	52±6.1^{d}	85.92±10.7^{ab}	63.16±18.2^{def}	78.26±12.5^{bcd}
5.	Mentha	2µl	17.33±5.3^{gh}	38.03±10.5^{e}	36.84±12.2^{ef}	56.52±15.3^{e}
6.	Mentha	4µl	43.07±8.6^{de}	83.1±13.5^{abc}	42.11±10.5^{def}	83.69±6.2^{abc}
7.	Palmarosa	2µl	6.667±5.3^{h}	26.76±11.2^{e}	21.05±6.1^{gh}	48.36±5.4^{e}
8.	Palmarosa	4µl	36±0.0^{ef}	63.38±10.7^{cd}	42.11±6.1^{def}	67.39±8.8^{cde}
9.	French basil	2µl	12±10.2^{gh}	35.21±16.9^{e}	47.37±0.0^{de}	67.39±0.0^{cde}
10.	French basil	4µl	68±15.4^{bc}	83.1±16.5^{abc}	63.01±6.2^{bc}	83.69±6.2^{abc}
11.	Eucalyptus	2µl	94.67±10.6^{a}	100±0.0^{a}	94.74±6.1^{a}	100±0.0^{a}
12.	Eucalyptus	4µl	97.33±5.3^{a}	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}
13.	Juniper berry	2µl	22.67±10.2^{fg}	23.94±16.9^{e}	55.26±27.6^{cd}	67.39±15.3^{cde}
14.	Juniper berry	4µl	76±13.6^{b}	77.46±15.5^{bcd}	73.68±6.1^{b}	94.56±6.2^{ab}

As seen from the result table 2, it was clearly evident that mean mortality percentage increased with the increasing concentration and duration of treatment application. In all treatments, 4µl conc. resulted in higher mortality at duration of 48 hrs after treatment application. Complete mortality of cabbage aphid was seen in Eucalyptus (2µl and 4µl) after 48 hrs but statistically this-treatments were at par with Mentha (4µl), Lemon grass (4µl) and French basil (4µl) only after 48 hrs of treatments. Very low mortality percentage was reported in Palmarosa (2µl) and Mentha (2µl) which were also statistically inferior.

In case of Black bean aphid, complete mortality was reported only Eucalyptus (2µl and 4µl). But statistically it was similar with Mentha (4µl), Palmarosa (4µl), Citronella (4µl) and Juniper berry (4µl) after treatment for 48 hours. Lowest mortality was seen in Mentha (2µl), Palmarosa (2µl), Citronella (2µl) and Palmarosa (2µl).

Discussion

Bio pesticides made from essential oils are an interesting class of pesticides since, being made of several compounds with multiple action mechanisms; they have various modes of implementation; contact, fumigation and repulsive. They have been found highly effective in controlling pest. Under present investigation the essential oils showed varying toxic effects against both aphid and found statistically superior results over control.

Efficacy of essential oils in contact mortality

Higher aphid mortality with increasing duration and concentration of aphid was observed in this experiment. Similar, toxicity of different essential oils on aphid species has been seen by various researches [5, 6, 7, 8]. 100% insect mortality was observed only by the application of lemongrass (both concentration) and citronella (higher concentration) for cabbage aphid and only by lemongrass (both concentration) for black aphid. This higher efficacy of such certain oil as compared to other oils can be attributed to the presence of higher amount of effective monoterpenes. Not 100% mortality but statistically superior mortality was exhibited by higher concentration of mentha and palmarosa oil. Most of the oils have active insecticidal components in the form of monoterpenes, such as limonene, citronellal and geraniol that provide defense against aphids [9]. When such components interact with the integument of insects, it may affect digestive and neurological enzymes [10]. Similar, components could be the major composition of the oils of Citronella, Lemon grass and Palmarosa which explains their superior mortality rates. 96.57% mortality of *Myzus persicae* and *Aulacorthum solani* by the application of 1% essential oil solution of lemongrass was reported by Gorski and Tomezak [11]. When the constituent of such oils were studied it was found that Geranio, limonene, citronellal and citronellol were the major constituents. Application of lemongrass oil recorded 100% mortality of Foxglove aphid and 92.72% mortality of cabbage aphid. Sampson *et al.* (2005) [12] reported higher mortality of

Lipapahis pseudo brassicae by the action of Mentha oil and attributed it to the presence of insecticidal components like linalool, menthol and menthone [13].

Efficacy of essential oils in fumigation mortality

Results of increasing aphid mortality with increasing oil concentration and treatment application was observed in case of fumigation. Eucalyptus oil that demonstrated statistically inferior result in case of contact mortality resulted 100% mortality (death of all 10 aphids). This may be due to the volatile nature of monoterpenes making them more useful as insect fumigants. Vapour phase of the oil showed increase in the contents of oxygenated monoterpenes with better lipophilic properties that support better penetration of the insect cuticle [14]. 100% fumigation mortality of rose-grain aphid was reported by Klingauf *et al*. (1983). Insecticidal effect of eucalyptus oil was reported due to the major presence of 1, 8-cineole & α-pinene. 85.50% mortality of *Myzus persicae* was observed by the application of 10 µl ml $^{-1}$ dose of eucalyptus oil [15].

Mortality effect of higher concentration of menthol and French basil against wheat aphid and fennel aphid may be due to the presence of monoterpenes like isomenthone, linalool and menthol [16]. 69.11% mortality of *Lipapahis pseudo brassicae* resulted by the application of 1 µl ml $^{-1}$ dose of French basil oil. When analyzed the major constituents of these oils was found to be ocimene, isomenthone, pulegone and eugenol [13].

Summary and Conclusion

The given research was conducted to evaluate the efficacy of plant extracted essential oils on the mortality of two aphid species under laboratory conditions. The statistical analysis revealed that in case of fumigation mortality, higher efficacy was shown by eucalyptus oil at both concentrations. Since lower concentration is more economical so it can be considered for application in enclosed conditions like greenhouse. Similarly, in case of contact mortality lemon grass and citronella oil at higher concentrations proved to be statistically superior and such results can be used in field applications. Mentha oil of higher concentration can be proved useful in both conditions. Further research to test the derived results in field condition is highly recommended.

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