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Repellent and Insecticidal Activity of *Mentha piperita* (L.) Plant Extracts Against Cabbage Aphid [*Brevicoryne brassicae* Linn. (Homoptera: Aphididae)]

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Abstract: Repellent and insecticidal activity of solvent extracts of *Mentha piperita* leaves were tested against cabbage aphid (*Brevicoryne brassicae*). Petroleum ether, acetone, ethanol and water extracts were prepared and tested at 1000, 500, 250, 125 and 62.6ppm concentrations. Repellent activity of plant extracts were tested by leaf disc bioassay method. Insecticidal activity of the plant extracts were tested by topical application method. Results showed that repellent activity of plant extracts were recorded above 50% in all the solvent extracts tested at 500 and 1000ppm concentration after 24hr exposure period. In general, repellent activity of the plant extracts were increased up to 48hr and it was decreased after 72hr post exposure period. Maximum insecticidal activity of 73.3% was observed in ethanol extract at 1000ppm concentration after 24hr exposure period. The insecticidal activity of plant extracts were increased from 24hr to 72hr post exposure period. The laboratory findings conclude that *Mentha piperita* plant extracts have both repellent and insecticidal activity against cabbage aphid (*Brevicoryne brassicae*).

Key words: Aphids · *Brevicoryne brassicae* · Cabbage · *Mentha piperita* · Plant Extracts · Repellent · Insecticidal

INTRODUCTION

In Ethiopia, area of cabbage cultivation, annual production and yields per hectare is increasing year by year. According to CSA [1], area of head cabbage cultivation is 1843ha/2004 and 2120 ha/2005 and annual production is 8577 ton/2004 and 14208 ton/2005 respectively. One of the constraints for the production of cabbage is mainly associated with wide range of pests which cause the damage from seedling to head formation stage. Cabbage aphid (*Brevicoryne brassicae*) belongs to the family Aphididae of the order Hemiptera is one of the serious plant sap sucking pests world wide [2] which is predominantly found in cabbage growing areas of Ethiopia. This pest primarily attack growing points of the host plants including tips, flowers, developing pods, leaves and eventually cover the whole plants at high density [3]. The direct damage caused by this pest is by sucking cell sap, secrete honey dew resulting in development of sooty mould on leaves and shoots and indirectly as a vector of 20 plant viral diseases in a large range of plants [4-8].

Chemical pesticides are extensively used for insect pest management. However, indiscriminate applications of chemical pesticide pollute the environment and pose serous threats to the non-target organisms including human beings. According to Bami [9], every year one million peoples are suffering from pesticide poisoning. To minimize or replace the use of dreadful chemical pesticides alternative pest management strategies are given importance in recent times. Botanicals are traditionally used for various purposes including pest control program which is given importance for recent scientific research and also considered as one of the alternatives for safe and sustainable insect pest management.

According to Dayane *et al*. [10], higher plants have been used from 4000 years to till today as a source of bioactive substances for therapeutic, agricultural and industrial purpose. Botanicals have many advantages over synthetic pesticides because of local availability and

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inexpensive method of pest control. The plants and their derivatives or extracts have been studied for different biological activities in economically important pests, assessing their toxic effect, lethal, antifeedant, repellent, fumigant, growth regulation and deterrent to oviposition [11]. Bio-potential of many species of plants have been studied by several workers globally against aphid species [12-17]. The insecticidal activity of essential oils isolated from *Mentha* species have been reported against rice weevil, *Sitophilus oryzae* and rice moth, *Corcyra cephalonica* [18] and larvicidal activity against dengue vector, *Aedes aegypti* [19]. In Ethiopia, available literature on biological activity of *Mentha piperita* plant leaves extract is limited. Therefore, to confirm repellent and insecticidal activity of solvent extracts of *Mentha piperita* leaves against cabbage aphid, *Brevicoryne brassicae* present study was conducted under laboratory condition.

MATERIALS AND METHODS

***Mentha piperita* Plant Collection and Extraction:** *Mentha piperita* plant leaves were collected from Samunaber, Gondar, Ethiopia. The leaves were cleaned thoroughly with tap water and shade dried to prevent chemical denaturation. After drying, leaves were powdered by using electric blender and sieved through kitchen strainer to obtain fine powder. Five conical flasks were taken; each filled with 20g of the powder and 100ml of petroleum ether, acetone, ethanol and water individually. The conical flask mouth was covered with aluminum foil and allowed for 12hr continuous agitation in a shaker. Then, liquid part was filtered by using Whatmann No.1 filter paper and allowed for solvent evaporation by keeping at 50°C in an oven. After solvent evaporation, residue was collected and different concentrations were prepared for subsequent experiment.

Preparation of Stock and Working Concentration: The stock concentration of 1000ppm was prepared by mixing 100mg of residue in 2ml of acetone and make up to 100ml. To this stock solution, 2mg of detergent powder was added for emulsification purpose. From the stock solution, working concentration of 500, 250, 125 and 62.6ppm was prepared by serial dilution method before commencement of the experiment. All the concentrations were tested for their repellent and insecticidal activity against cabbage aphid (*Brevicoryne brassicae*).

Cabbage Aphid (*Brevicoryne brassicae*): Cabbage aphid (*Brevicoryne brassicae*) were collected from cabbage field, Bridge of Hope children village farm near University of Gondar. The aphids were brought to the laboratory along with infested leaves and subsequently fresh leaves were added for feeding. The cabbage leaves without infestation was also brought to the laboratory for leaf discs preparation to evaluate plant extracts.

Evaluation of Repellent Activity: Repellent activity of plant extracts were tested by the application of 62.5, 125, 250, 500 and 1000ppm concentration on 3cm circular leaf disc. In the petriplates, Whatmann No.1 filter paper soaked with water was added to maintain moisture content and also to prevent early drying of the leaf disc. One treated leaf disc was taken in each petriplate and 10 healthy aphids were introduced. The leaf disc treated with 2ml of acetone mixed with 100ml of water was used as negative control. The number of aphids attached to the treated leaf disc and out side was counted continuously for 72hr with 24hr interval from three replications. The percent repellent activity of plant extracts were calculated for every 24hr exposure period.

$$\text{Percent repellent activity} = \frac{\text{Number of aphids outside the leaf disc}}{\text{Total number of aphids introduced}} \times 100$$

Evaluation of Insecticidal Activity : Insecticidal activity of plant extracts were tested at 62.5, 125, 250, 500 and 1000ppm concentration. Thirty apterous (wingless) healthy aphids were separated from the culture and plant extracts were applied topically by using soft brush without causing physical damage. After the treatment, normal leaf disc was taken in three petriplate and 10 treated aphids were released. The leaf disc treated with 2ml of acetone mixed with 100ml of water was used as negative control. The number of dead aphids were counted and removed continuously up to 72hr with 24hr interval. The experiment for each concentration was replicated three times. The percent insecticidal activity of plant extracts were calculated for every 24hr.

$$\text{Percent insecticidal activity} = \frac{\text{Number of dead aphids}}{\text{Total number of aphids introduced}} \times 100$$

Statistical Analysis: Data collected from three replicates of each experiment was subjected to statistical analysis to calculate mean and standard deviation. The statistical significant difference on percent repellent and insecticidal activities of the plant extracts and concentrations tested were analyzed by using two way analysis of variance (ANOVA). All the statistical analyses were carried out by using Microsoft office excel 2007 program.

RESULTS

Repellent Activity of *M. piperita* Leaf Extracts on *B. brassicae* after 24hr Exposure Period: Table 1 revealed percent repellent activity of different solvent extracts of *M. piperita* leaves against cabbage aphid after 24hr exposure period. Repellent activity of 50% and above was observed in all the treatments at 500 and 1000ppm. At 1000ppm concentration, highest repellent activity of 83.3% was observed in water extract followed by ethanol extract (80%). At 250ppm concentration, maximum repellent activity of 63.3% was observed in ethanol extract followed by water extract (60%). The two way ANOVA results showed that within the sample ($F=9.45$) and within the concentration ($F=84.67$) repellent activity of the plant extracts was statistically significant ($p<0.05$). The interaction among the samples were statistically not significant ($F=1.63$; $p>0.05$).

Repellent Activity of *M. piperita* Leaf Extracts on *B. brassicae* after 48hr Exposure Period: Table 2 showed repellent activity of *M. piperita* plant extracts tested against *B. brassicae* after 48hr exposure period. At 1000ppm concentration, maximum percent repellent activity of 90% was recorded in ethanol extract followed by 86.6% in petroleum ether extract. In this concentration all the solvent extracts tested was recorded above 80% repellent activity. At 500ppm concentration, 76.6% repellent activity was recorded in ethanol extract followed by water extract (73.3%). The remaining solvent extracts tested showed above 50% repellent activity. At 250ppm concentration, maximum repellent activity of 70% was observed in ethanol extract followed by water extract (66.6%). At 125ppm concentration, maximum percent repellent activity of 50% was observed only in water extract. The two way ANOVA results indicates statistically significant difference ($p<0.05$) within the sample ($F=8.36$) and within the concentrations ($F=82.08$) tested against cabbage aphids. The interaction among the samples were statistically found not significant ($F=1.78$; $p>0.05$).

Repellent Activity of *M. piperita* Leaf Extracts on *B. brassicae* after 72hr Exposure Period: Table 3 demonstrates repellent activity of *M. piperita* plant extracts tested against cabbage aphid after 72hr exposure period. At 1000ppm concentration, maximum repellent activity of 73.3% was recorded in ethanol extract. The repellent activity of petroleum ether extract (70%) was on par with water extract (70%). At 500ppm concentration, 66.6% repellent activity was recorded in water extract followed by ethanol (63.3%) and acetone (60%) extract. At 250ppm concentration, repellent activity of ethanol extract (60%) was on par with water extract (60%). The two way ANOVA results revealed statistically significant difference at 5% level within the solvent extracts ($F=3.36$) and within the concentration ($F=57.75$) tested. The interaction among the samples were statistically not significant ($F= 0.95$; $p>0.05$).

Insecticidal Activity of *M. piperita* Leaf Extracts on *B. brassicae* after 24hr Exposure Period: Table 4 depicts insecticidal activity of *M. piperita* plant extracts tested at different concentration against cabbage aphids after 24hr exposure period. At higher concentration (1000ppm), maximum mortality of 60% was observed in ethanol extract. At this concentration all the solvent extracts tested showed 50% and above mortality. At 500ppm concentration, maximum mortality of 46.6% was observed in water extract followed by petroleum ether (43.3%) and ethanol (40%). All the remaining concentrations percent mortality was less than 40%. The two way ANOVA results showed that insecticidal activity of plant extracts within the samples were statistically not significant ($F=2.69$; $p>0.05$) and within the concentrations statistically significant ($F=56.47$; $p<0.05$). The interaction among the samples were statistically not significant ($F=0.62$; $p>0.05$).

Insecticidal Activity of *M. piperita* Leaf Extracts on *B. brassicae* after 48hr Exposure Period: Table 5 showed insecticidal activity of *M. piperita* plant extract tested against cabbage aphids after 48hr exposure period. The maximum percentage mortality of 73.3% was recorded in ethanol extract followed by acetone (66.6%). All the solvent extracts tested at this concentration percent mortality rate were above 50%. At 500ppm concentration, maximum mortality of 66.6% was observed in ethanol extract followed by acetone extract (60%). At 250ppm concentration, acetone extract treatment showed 53.3% mortality. At 125ppm concentration, 50% mortality was recorded only in acetone extraction. The two way ANOVA indicates statistically significant ($p<0.05$) difference within the samples ($F=8.38$) and within the concentrations ($F=27.13$) tested against cabbage aphids. The interaction among the samples were statistically not significant ($F=1.64$; $p>0.05$).

Table 1: Repellent activity of *M. piperita* plant extracts against *B. brassicae* after 24hr exposure period

Extracts tested	Concentration in PPM				
	62.5	125	250	500	1000
Petroleum ether	13.3 ± 5.7	23.3 ± 5.7	40.0 ± 10.0	56.6 ± 5.7	76.6 ± 5.7
Acetone	26.6 ± 11.5	33.3 ± 5.7	40.0 ± 10.0	60.0 ± 10.0	70.0 ± 10.0
Ethanol	20.0 ± 10.0	40.0 ± 10.0	63.3 ± 5.7	73.3 ± 5.7	80.0 ± 10.0
Water	16.6 ± 5.7	43.3 ± 5.7	60.0 ± 10.0	70.0 ± 10.0	83.3 ± 5.7

Values are percentage mean ± standard deviation of three replications.

Table 2: Repellent activity of *M. piperita* plant extracts against *B. brassicae* after 48hr exposure period

Extracts tested	Concentration in PPM				
	62.5	125	250	500	1000
Petroleum ether	16.6 ± 5.7	30.0 ± 10.0	43.3 ± 5.7	60.0 ± 10.0	86.6 ± 5.7
Acetone	30.0 ± 10.0	40.0 ± 10.0	50.0 ± 10.0	66.6 ± 5.7	70.0 ± 10.0
Ethanol	23.3 ± 5.7	46.6 ± 5.7	70.0 ± 10.0	76.6 ± 5.7	90.0 ± 10.0
Water	23.3 ± 5.7	50.0 ± 10.0	66.6 ± 5.7	73.3 ± 15.2	83.3 ± 11.5

Values are percentage mean ± standard deviation of three replications.

Table 3: Repellent activity of *M. piperita* plant extracts against *B. brassicae* after 72hr exposure period

Extracts tested	Concentration in PPM				
	62.5	125	250	500	1000
Petroleum ether	13.3 ± 5.7	26.6 ± 11.5	50.0 ± 10.0	53.3 ± 5.7	70.0 ± 10.0
Acetone	23.3 ± 5.7	30.0 ± 10.0	40.0 ± 10.0	60.0 ± 10.0	66.6 ± 15.2
Ethanol	13.3 ± 5.7	40.0 ± 10.0	60.0 ± 10.0	63.3 ± 5.7	73.3 ± 5.7
Water	20.0 ± 10.0	43.3 ± 5.7	60.0 ± 10.0	66.6 ± 5.7	70.0 ± 17.3

Values are percentage mean ± standard deviation of three replications.

Table 4: Insecticidal activity of *M. piperita* plant extracts against *B. brassicae* after 24hr exposure period

Extracts tested	Concentration in PPM				
	62.5	125	250	500	1000
Petroleum ether	13.3 ± 5.7	16.6 ± 5.7	36.6 ± 5.7	43.3 ± 5.7	56.6 ± 5.7
Acetone	10.0 ± 0.0	13.3 ± 5.7	26.6 ± 5.7	36.6 ± 5.7	50.0 ± 5.7
Ethanol	16.6 ± 11.5	20.0 ± 10.0	33.3 ± 5.7	40.0 ± 10.0	60.0 ± 10.0
Water	13.3 ± 5.7	26.6 ± 11.5	33.3 ± 5.7	46.6 ± 5.7	50.0 ± 10.0

Values are percentage mean ± standard deviation of three replications.

Table 5: Insecticidal activity of *M. piperita* plant extracts against *B. brassicae* after 48hr exposure period

Extracts tested	Concentration in PPM				
	62.5	125	250	500	1000
Petroleum ether	30.0 ± 10.0	40.0 ± 10.0	46.6 ± 5.7	50.0 ± 10.0	60.0 ± 10.0
Acetone	46.6 ± 5.7	50.0 ± 0.0	53.3 ± 11.5	60.0 ± 10.0	66.6 ± 5.7
Ethanol	23.3 ± 5.7	30.0 ± 10.0	46.6 ± 20.8	66.6 ± 15.2	73.3 ± 11.5
Water	13.3 ± 5.7	26.6 ± 5.7	46.6 ± 5.7	46.6 ± 5.7	56.6 ± 5.7

Values are percentage mean ± standard deviation of three replications

Table 6: Insecticidal activity of *M. piperita* plant extracts against *B. brassicae* after 72hr exposure period

Extracts tested	Concentration in PPM				
	62.5	125	250	500	1000
Petroleum ether	53.3 ± 11.5	60.0 ± 10.0	63.3 ± 11.5	73.3 ± 11.5	76.6 ± 5.7
Acetone	50.0 ± 10.0	53.3 ± 5.7	66.6 ± 5.7	70.0 ± 10.0	80.0 ± 10.0
Ethanol	33.3 ± 5.7	46.6 ± 5.7	63.3 ± 11.5	80.0 ± 10.0	86.6 ± 11.5
Water	16.6 ± 5.7	40.0 ± 10.0	50.0 ± 10.0	53.3 ± 15.2	63.3 ± 15.2

Values are percentage mean ± standard deviation of three replications

Insecticidal Activity of *M. piperita* Leaf Extracts on *B. brassicae* after 72hr Exposure Period: Table 6 demonstrates insecticidal activity of *M. piperita* plant extracts tested against cabbage aphids after 72hr exposure period. At 1000ppm concentration, acetone and ethanol extract was observed with 80 and 86.6% mortality respectively. At 500ppm concentration, 80% mortality was observed in ethanol extract followed by petroleum ether (73.3%) and acetone (70%). At 250ppm concentration, except for water extract remaining all showed above 60% mortality. At 125ppm concentration, maximum mortality of 60% was recorded in petroleum ether extract. At 62.5ppm concentration, maximum mortality of 53.3% was recorded in petroleum ether extract followed by acetone (50%) extract. The two ANOVA results indicates statistically significant ($p<0.05$) difference within the concentrations ($F=27.35$) and within the samples (13.74) tested. The interaction within the samples were statistically not significant ($F=1.27$; $p>0.05$).

DISCUSSION

Botanicals are generally more compatible with the environmental components than synthetic pesticides in pest control program due to biodegradable nature, less toxic to non-target organisms and multiple modes of action. In view of these, efforts were undertaken for assessing repellent and insecticidal activities of *Mentha piperita* plant extracts against cabbage aphid. Repellent activity of the plant extracts results clearly demonstrates that at higher concentration and exposure period percent repellent activity was increased. This is because that the odour of aromatic plant, *Mentha piperita* may be repelled the insects to attach and feed on treated environment. It is clear that odour of plant extracts at lower concentration may not be sufficient to repel the insects. The solvent used for the extraction may also be plays an important role for repellent activity of plant extract. From the study it is evident that after 24hr exposure period at 1000ppm concentration, maximum repellent activity of 83.3% was observed in water extract followed by ethanol extract 80%.

After 48hr exposure period maximum repellant activity was observed in ethanol extract followed by water extract. After 72hr exposure period all the solvent extracts treated leaf discs repellent activity was decreased. It will indicate that volatile nature of the solvent extract may be decreased during the period of exposure thereby percent repellent activity was decreased. The result of present study is in corroborate with the report of Zaki *et al*. [13] they have observed different percentage mortality in petroleum ether extract of *Melia* and ethanol extract of neem against aphids. In addition, dissimilar mortalities were also observed in leaf discs treated with different solvents extracts. It may be associated with various phytochemicals present in the plant extract and their quantities. According to Sujana *et al*. [20], *Mentha piperita* plant leaves contain alkaloids, flavonoids, steroids, tannin and phenols. In general, phenolic compound may be interfering with the feeding of insects. Stevenson *et al*. [21] reported that development of *Spodoptera litura* was inhibited by a phenolic compound from the wild groundnut. Phenolics in plant tissues may change the rate of consumption of tissues by a geometrid caterpillar, *Epirrita autumnata*. From this, it is clear that phenolic compounds from the *Mentha* may not allow the insects to feed on treated enviroment.

Insecticidal activity of solvent extracts was varied irrespective of solvents used for extraction, exposure period and also concentration. Irrespective of solvents used for extraction maximum insect mortaltiy was observed in ethanol extract followed by acetone, petroleum ether and water. Water is least effective among the solvents used for extraction. The percent insecticideal activity was maximum at higher concentration compared to lower concentration. The percent mortaltiy was mainly associated with volatile nature of the essential oil persent in *Mentha* leaves. The increased mortality rate of the insect may be associated with any one of the possible reason. First reason, odour of the extract may enter through the spiracle and block the respiratory activity thereby mortality rate was increased due to the arrest of metabolic activity. Second possible reason, aphids are unable to settle in plant extract treated leaf disc due to starvation insect mortaltiy rate may be increased. According to Khani *et al*. [18], major chemical components in the pepper mint was menthol, isomenthone, limonene and cineole. Any one of the compound may also be toxic to aphids there by mortality rate was increased. Present study confirmed insecticidal and repellent activity of *M. piperita* plant extracts against cabbage aphids. Aqueous extract of the leaves was also proved to be effective in terms of repellent activity only. However, thorough field trials are necessary to confirm the laboratory findings before practice. Further, isolation, evaluation and characterization of the bioactive molecules may also be useful for the development of novel botanical pesticides. In conclusion, laboratory experiments proved that *Mentha piperita* plant extracts were effective against cabbage aphid, *Brevicoryne brassicae* in terms of

repellent and insecticidal activity. The plants were growing everywhere in Ethiopia without much agricultural input. The aqueous extracts of the plants were also effective and it can be useful for aphid management program after field confirmation.

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