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Toxic effects of ethanolic extracts synthesized from five different medicinal plant species of Kashmir valley against Rose aphid, *Macrosiphum rosae* (L.) (Hemiptera: Aphididae)

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ABSTRACT

Use of eco- friendly naturally occurring plant products in the form of ethanolic extracts (of five plant species viz. Artemisia absinthium, Euphorbia helioscopia, Iris germanica, Viola odorata and Mentha longifolia) for the management of Rose aphid, Macrosiphum rosae (L.) (Hemiptera: Aphididae) under laboratory conditions was assayed to reduce the dependence on the unwise use of synthetic pesticides against this insect pest species. The efficacy of the five extracts was studied via No choice bioassay employing leaf dip technique. Three different concentrations of 1%, 2% and 3% were used to check the efficiency of prepared plant extracts; 24, 48, 72, 96 and 120 hrs post treatment of M. rosae in the laboratory. For comparison purposes, leaves treated with distilled water served as Control. Aphid mortality data sets subjected to analysis of variance (One- way ANOVA) by Duncan's test at 5% revealed that mortality of rose aphid varied significantly at all the three treatment concentrations of five plant extracts in comparison to Control. Further, the ethanolic extract of E. helioscopia at 3% concentration performed better than the rest of ethanolic extracts in reducing the rose aphid populations, inflicting the highest mean aphid mortality percentage of 72.35% with least LC_{50} value of 1.65%. The observed order of aphid mortality inflicted by five ethanolic extracts was E. helioscopia > I. germanica > A. absinthium > V. odorata > M. longifolia. The Regression analysis of the mortality data gave Regression coefficient (R^2) close to 1 which was indicative of Positive correlation existing between X (concentration of extracts) and Y (mortality of aphids) variables. In other words, as the concentration of plant extracts is increased, the aphid mortality percentages also show a subsequent increase. The Chi square test of association further validated the existing relationship between concentration of extracts and aphid mortality as χ^2 value was significant at $p \leq 0.05$ for all the five extracts. The study demonstrates that the plant extracts represent a novel and cost effective approach with minimal residual effects and wide public acceptance against rose aphid, M. rosae.

Keywords: Artemisia absinthium, Euphorbia helioscopia, Iris germanica, Macrosiphum rosae, Mentha longifolia, No choice bioassay, One way ANOVA, Viola odorata

1.INTRODUCTION

The deciduous and coniferous ornamental shrubs characterize a valuable decorative component of the city areas. Rose plants belonging to Genus *Rosa* of family Rosaceae have a noteworthy facet in enhancing the beauty and grandeur of the adjoining landscape. Besides, being used exclusively for beauty and decoration, roses are used for extraction of attar for making fragrant mixtures. The true Bulgarian rose oil is used in 96% of all women's perfumes. The different varieties of roses provide base material to a number of industries viz. perfume, food, pharmaceutical, cosmetic etc (1). In global flower trade, roses occupy the topmost position in terms of average production and consumption and consequently they are grown commercially to meet the demand of cut blooms all over the world. Accordingly, their key use is for the trade (2) and thus constitutes the backbone of the lucrative floriculture industry of many global economies. However, roses are inhabited by numerous pests that pose many challenges and threats to Rose plants. Among them rose aphid, *M. rosae* cause considerable economic losses to growers particularly during spring and summer seasons. This sucking pest of rose crops is seldom found singly and can become extremely plentiful on roses, reducing their medicinal, decorative and economic value. High *M. rosae* infestation cause momentous damages like bent stems, weak foliage and early leaf fall. This aphid species is notorious as it disfigures the foliage, cripples the shoot and injures the bud and as a result vigour of plant is reduced and quality of flower deteriorates (3). Besides, the honeydew secretion by aphids facilitates the growth of molds on surface of leaves and flowers responsible for dip in photosynthetic ability and thus in the overall yield of the roses (4).

The aphid control measures employed throughout the world fundamentally include the use of chemical insecticides including chlorinated hydrocarbons, organophosphates and carbamates. However, the greatest obstacle in the use of these synthetic pesticides is their loss of efficacy caused by resistance development in insects particular in aphids due to their rapid reproductive capabilities (5, 6). Also, environmental aspects, like persistence of active compounds in soil, ground water and lakes as well as effects on non targets (7) have to be considered more conscientiously than in the past. Moreover, excessive use of chemical insecticides used against this aphid has resulted in secondary pest outbreak (8). It is therefore, obligatory to explore some alternative strategies in pest control in order to circumvent existing resistance and curtail the danger of new resistance.

In the last 20 years much attention has been devoted to natural pest control agents (9). Previous research have indicated the successful use of plant materials such as plant powders, spices, oils and extracts (10). Certain plant materials are toxic to insect pests and inhibit their reproduction (11, 12). One of the most important groups are the so- called "botanicals" i.e. active substances or mixtures of substances extracted from plants. These botanical insecticides are known for their low persistency in environment and little mammalian toxicity as compared to synthetic insecticides (13). These plant materials are inexpensive and naturally available in the surroundings.

A large number of research investigations have been carried involving many plant products possessing pesticidal properties for the control of various insect pests both in field and in laboratory conditions (14, 15). Many researchers (16, 17, 18, 19, 20) have used numerous medicinal plant extracts against different aphid species. This has resulted in renewed interest to pursue use of plant extracts for control of this aphid pest. The present study was conducted to check the lethal effects of selected plant species using their ethanolic extracts against the insect pest, *M. rosae* under laboratory conditions in order to minimize the hazards associated with chemical insecticides.

2. MATERIAL AND METHODS

2.1. Test insect culture: Random surveys were carried out in special rose growing locations of different ornamental gardens of Srinagar district viz. Nishat, Shalimar, Naseem bagh and Kashmir University Botanical garden for the collection of rose aphid. The collection of the rose aphids was done following the standard method (21). The *M. rosae* stock culture was established from collected apterous viviparous female adults in large glass rearing jars with their open end covered by muslin cloth to prevent their escape and to protect them from predators and parasites. This colony of aphids was maintained on fresh, apical, tender rose plant leaves in the Entomology laboratory of Department Of Zoology, University of Kashmir under natural conditions of temperature and humidity until the completion of laboratory bioassays.

2.2. Procurement of Therapeutic Plant Materials: Different plant materials viz. *A. absinthium, E. helioscopia, I. germanica, M. longifolia* and *V. odorata* were collected from different localities of Kashmir division. Voucher specimens were deposited in Kashmir University Herbarium (KASH). Table 1 shows the selected plant materials along with their scientific name, the part used for extract preparation and the area of collection.

2.3. Preparation of ethanolic plant extracts: The collected plant materials were first washed to remove any exterior contaminants and then shade dried. The completely dried plant materials were pulverized to fine powder with the help of an electric grinder. All the five powdered plant materials were extracted separately using Soxhlet apparatus using ethanol as solvent.

For preparation of ethanolic extracts of the selected plant materials, 100g of powdered plant materials of each plant species was extracted with 500 ml of ethanol in Soxhlet apparatus for 6- 8 hrs at 60^0C. The excess of ethanol was allowed to evaporate at reduced pressure of 22- 26 mm Hg in a Vacuum rotary evaporator at 55^0C (22 and 23). A stock solution of each plant material was prepared by dissolving the extracted materials in ethanol (1: 10 w/v). The subsequent serial concentrations of 1%, 2% and 3% were prepared from this stock solution by dilutions with the ethanol (v/v) to provide the necessary mortality inducing concentrations for each plant extract. These 3 concentrations of each plant extract were tested against rose aphid using No choice bioassay to calculate the mortality levels induced by these ethanolic plant extracts.

2.4. No Choice Bioassay: To study the toxicity of different plant extracts, No choice test was followed (24). For this test, two leaves of same size were selected from the host plant and washed with tap water and then swapped by cotton pieces. Rose leaves were treated with prepared concentrations of plant extracts viz. 1%, 2% and 3% by leaf dip technique (25). Rose plant leaves treated with distilled water served as Control (Fig. 2). Both treated and untreated leaves were placed in separate Petri dishes above the filter paper lining its bottom. The petiole of each leaf was enclosed in cotton soaked with water to avoid the early drying of the leaves. 20 apterous adult rose aphids were released directly on these treated and control leaves with a fine camel hair brush at room temperature. The Petri dishes were covered with pieces of muslin cloth tightened by the rubber band Fig 1(A).

Each treatment had three replicates and each replicate included 20 rose aphids. The number of dead and alive aphids was counted 24, 48, 72, 96 and 120 hours post treatment to calculate the aphid mortality percentages. Mortality percentages so obtained in each case was corrected according to Abbott's formula (26).

Corrected mortality (%) = $[M_T - M_C / 100 - M_C] \times 100$

Where, M_T = Percent mortality of rose aphids in treatment

M_C = Percent mortality of rose aphid in control

Rose aphids were considered dead when no leg or antennal movements were observed Fig. 1(B).

2.5. Statistical analysis: The toxicity data was subjected to one- way ANOVA analysis using SPSS 16.0. Difference among treatment (means) were considered significant at $p \leq 0.05$ by using Duncan's test. The mean, Standard error, Regression equation, Lethal Concentration (LC_{50}) of the data sets for all the five plant extracts were calculated. The results were expressed in tabulated form as mortality percentages (± SE) and represented in graph format as well.

3.RESULTS

The toxicity of ethanolic extracts of five selected plant species acknowledged for their medicinal activity was investigated against the rose aphid. The results revealed that *E. helioscopia* was most potent against apterous *M. rosae* adults than the rest of plant extracts in terms of mean percent mortality. Its ethanolic extracts caused the highest mean percent mortality at all the three concentrations and attained the maximum mortality of 72.35% (±1.45) at 3% concentration. The analysis of variance (one- way ANOVA) indicated that *E. helioscopia* was significantly different from the rest of ethanolic plant extracts. At 3% concentration, *I. germanica* 62.00% (±3.38), *A. absinthium* 60.65% (±2.64) and *V. odorata* 56.35% (±4.09) caused moderate mortality levels which were not significantly different among themselves, however, mean percent mortality of *I. germanica* and *A. absinthium* were significantly different to mortality of *M. longifolia* which caused the lowest mean mortality percentage of 51.65% (±1.76). Further, the mean mortality percentages of *M. longifolia* and *V. odorata* were insignificant to each other at the same concentration.

At 2% concentration, the ethanolic extract of *E. helioscopia* again caused highest mortality in comparison to the rest of plant extracts. The one- way ANOVA results showed that *E. helioscopia* and *I. germanica* causing 55.00% (±4.36) and 54.65% (±3.48) mean mortality percentages do not differ significantly from each other and from the ethanolic extract of *A. absinthium* 50.00% (±3.05) and *V. odorata* 48.00% (±7.23) at the same concentration of 2%. The former two extracts (i.e. *E. helioscopia* and *I. germanica*) were, however, significantly different from *M. longifolia* in terms of mortality inflicted. Moreover, the mortality caused by *M. longifolia* at 2% was 39.00% (±5.03) that was statistically similar to *A. absinthium* and *V. odorata*.

At 1% concentration of ethanolic extracts, the maximum mortality of 49.35% (± 4.80) was achieved by *E. helioscopia* which was statistically different than *M. longifolia* that caused lowest mortality of 36.35% (± 3.28) but was insignificant to *A. absinthium*, *I. germanica* and *V. odorata*. The mortality rates of other three plants viz. *A. absinthium*, *I. germanica* and *V. odorata* 39.65% (± 2.60%) at 1% concentration does not differ significantly from *M. longifolia* at $p \leq 0.05$. All tested concentration of each of the five plant extract employed for the control of this phytophagous insect pest were found to be significantly different than the untreated Control (adult rose aphids on distilled water treated rose plant leaves).

Further, the chi square test of association was carried out on the mortality data and the results revealed a relationship between the two variables i.e. mortality of rose aphids and concentration of ethanolic plant extracts were associated. The chi square value for all five extracts was significant at $p \leq 0.05$ (Table 2). The regression equation for the ethanolic extracts of five plant species along with the regression coefficient (R^2) shows a positive correlation existing between variable Y (mortality) and variable X (concentration) as the value of R^2 was close to 1 (Table 2). Thus, a linear relation exists between the two variables and as such increase in concentration of plant extract caused a consequent increase in mortality of rose aphids in each of the three replicate (Fig. 4). The LC_{50} value for *E. helioscopia* was lowest among the five tested plant extracts while that of *M. longifolia* was the highest (Table 2). Therefore, the observed order of relative toxicity of different ethanolic plant extracts against *M. rosae* in the increasing order for the three concentratios was *Mentha* < *Viola* < *Artemisia* < *Iris* < *Euphorbia* as depicted in Fig.3.

4.DISCUSSION

Results of the experiments of the present study carried out under laboratory conditions indicated that different plant extracts had distinguished effect on the reduction of aphid influx. Traditionally, the use of synthetic insecticides was employed for the control of aphids. But the increasing resistance of aphids to insecticides provides the necessary impetus to look for the more effective alternative which includes the use of plant extracts to overcome this pest species. The use of plant extracts is more acceptable than the conventional pesticides because of their reputation in being less hazardous to humans and other non target organisms (27). A large number of extracts obtained from plants belonging to variety of families contain compounds possessing insecticidal activity, repellent action, antifeedant effects, insect growth regulations, toxicity to nematodes, mites and other agricultural pests and also antibacterial, antifungal, antiviral properties against pathogens (28, 29 & 30).

Among the various tested plant extracts, ethanolic extract of *E. helioscopia* recorded the highest mean percent mortality as well as the corrected mortality at 1%, 2% and 3% concentrations that were significantly different from the other plants whereas the *M. longifolia* achieved lowest mean percent mortality and corrected mortality percentages at the same concentrations. The remaining extracts showed intermediary lethal effect in terms of mean percent mortality and the corrected mortality percentage. The lowest LC_{50} values were obtained for the *E. helioscopia* among the ethanolic plant extracts. All the treatment concentrations caused significant higher mortality when compared to untreated (Control) check. However, none of the treatments caused 100% mortality among rose aphids throughout the study period. One of the plausible reasons for this may be that, since, the mortality of rose aphids is a direct function of concentration of the extract, so 100% mortality might be accomplished by increasing the treatment concentrations. The present results depict an increase in aphid mortality with increase in concentration of the extract. Usually higher concentrations of plant extracts inflict mortality either by inhibiting feeding or reducing digestibility or inhibiting growth. Lower concentration of the extract may not be sufficient for killing the killing the insect pest, however, it may sometimes induce deformity (31).

Toxicity and protectant properties of the plant extracts may be attributed to the presence of number of secondary metabolites such as alkaloids, flavonoids, terpenoids and quinines. The fact that these compounds are more soluble in ethanol explains higher mortality levels of ethanolic extract. The ascending order of toxicity of ethanolic plant extract can be represented as *Mentha* < *Viola* < *Artemisia* < *Iris* < *Euphorbia.* The mortality of aphids may be credited to contact toxicity or due to the initiation of some unknown physiological changes (32). One of the plausible reasons of the mortality among insect pests may be the suffocation caused by blockage of spiracles and hence the oxygen supply by the inhalation of toxic odours emitted from these extracts. Alternatively, refusal of food (host plant leaves) treated with plant extracts may be responsible for their death of starvation.

Many researchers have tested the medicinal and ornamental plant extracts towards different aphid species and has reported the antifeedant, repellent and toxic effect of some plants against different genera of Homopteran insect pests including *M. rosae, Aphis gossypii, A. punicae, A. craccivora, Dactynotus carthami, Uroleucon sinchi, U. carthami, Lipaphis erysimi, Brevicoryne brassicae* and *Myzus pericae* (33, 34, 35, 36, 37, 38 & 39). All these support the implementation of plant extracts is quite promising in reducing the aphid influx.

5.CONCLUSIONS

Various laboratory bioassays conducted to determine the toxicity of ethanolic plant extracts revealed that the highest mortality was caused by ethanolic *E. helioscopia* at all the three concentrations (1%, 2% and 3%) with mean percent mortality 49.35%,, 55.00% and 72.35% respectively and the lowest LC_{50} value of 1.65% followed by *I. germanica* at the same concentrations with mean percent mortality of 46.65%, 54.65% and 62.00% respectively and LC_{50} of 2.07%. The lethal effects of *A. absinthium* and *V. odorata* were reported to be reasonably moderate ranging from 39.65% to 60.65% and LC_{50} values of 2.40% and 2.83% respectively. The lowest mean percent mortality was inflicted by *M. longifolia* as 36.35%, 44.65% and 51.65% at 1%, 2% and 3% concentration respectively with LC_{50} value of 3.65%. The aqueous extracts showed the same trend for all the tested plant materials. The rose aphids present on rose leaves treated with water served as control. The toxicity of ethanolic plant extracts in the ascending order is recorded as *Mentha* < *Viola* < *Artemisia* < *Iris* < *Euphorbia*.

Roses are well acclimatized in Jammu and Kashmir because of its suitable agro climatic conditions which can permit its large scale production and rose products produced in the state are at par with the international standards. However, roses are attacked by a number of pests which degrade the quality of both flowers as well as of its products. Among the various insect pests, *M. rosae* is the most damaging to roses depending on the economic losses it has incurred. The use of chemical insecticides for the control of this aphid species is widespread. But the high reproductive capacity of aphids has led to the development of resistance among this sucking pest of roses. Further, the use of synthetic insecticides is associated with a number of adverse effects such as environmental hazards, toxic residues and eliminations of natural enemies, pollinators and non target organisms. The use of eco friendly naturally occurring plant extracts against rose aphids are much safer to humans and environment than conventional pesticides with minimal residual effects; no resistance is developed besides being cheap resulting in good selectivity and wide public acceptance. Therefore, the use of plant extracts has been recommended even more as a suitable alternative of plant protection with minimum negative risks.

The study thus demonstrates that all the plant extract treatments were significantly superior over control and as a result represent a novel and cost effective approach with minimal residual effects and extensive approval against rose aphid, *M. rosae*. The LC_{50} of plant extracts (particularly ethanolic *E. helioscopia* extract) provide an interesting opportunity of preparing biopesticide from the extract of this plant for the use in pest management strategies against rose aphids infesting rose plants in urban environments. To understand the exact mode of action of the active compounds of these extracts and to know their effects on non target organisms, further studies are needed.

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Table 1. List of plant materials collected from different areas of Kashmir valley.

S. No.	Plant specimen collected	Common (Vernacular) name	Family	Area of collection	Extracted part	KASH Voucher No.
1	*Artemisia absinthium*	Wormwood (Tethwan)	Asteraceae	Nagam & Zabarwan Hills	Whole plant	2414
2	*Euphorbia helioscopia*	Milkweed plant (Guri gassa)	Euphorbiaceae	Pallar	Whole plant	2413
3	*Iris germanica*	German iris (Mazar mund)	Iridaceae	Zabarwan Hills	Aerial portions	2415
4	*Mentha longifolia*	Horse mint (Vena)	Lamiaceae	Zabarwan Hills	Stem + Leaves	2417
5	*Viola odorata*	Sweet violet (Banafsha)	Violaceae	Kashmir University campus	Leaves	2416

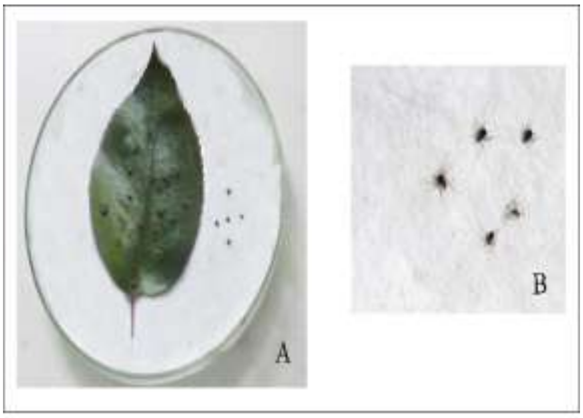

Fig. 1A. No Choice Bioassay for determining Toxicity of Plant Extracts. B. Dead apterous adults of *M. rosae*

Fig. 2. Control

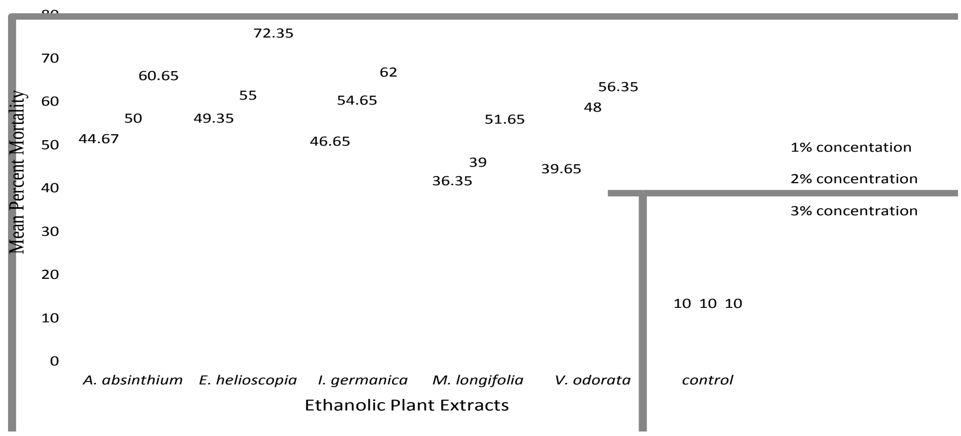

Fig.3. Graph representing Mean Percent Mortality of Ethanolic Plant Extracts at three different concentrations.

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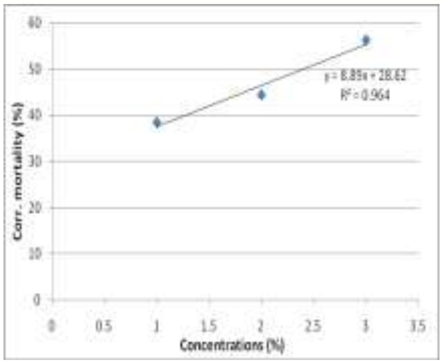
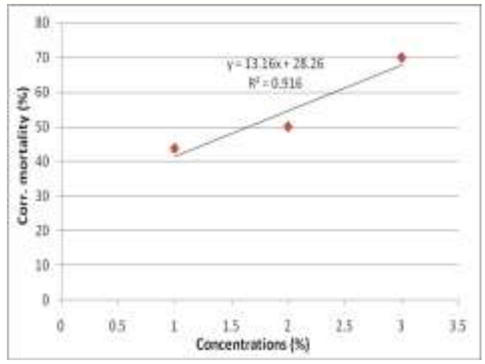
Table 2. Mean percent mortality caused by ethanolic plant extracts at three different concentrations against *M. rosae* in vitro

*** Mean of 20 adult aphids/ replication/ treatment; figures in parenthesis are standard error; means followed by same letters in each column are not significantly different by Duncan's test at 5%.**

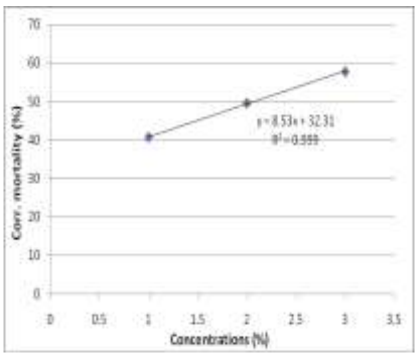
Plant species	Treatment (%)	Aphid mortality (Mean)	Mean percent mortality (± SE)	Corr. mortality (%)	95% confidence limit		Regression equation	Regression coefficient (R^2)	Chi square (χ^2)	LC_{50}
					Lower	upper				
Artemisia absinthium	1	8.93	44.65 (±4.37)[bc]	38.50	5.171	12.695	Y= 8.89x + 28.62	0.964	3.20	2.40
	2	10.00	50.00 (±3.05)[bc]	44.44	7.371	12.629				
	3	12.13	60.65 (±3.38)[c]	56.28	9.222	15.044				
Euphorbia helioscopia	1	9.87	49.35 (±4.80)[c]	43.72	5.279	14.003	Y= 13.16x+ 28.26	0.916	3.52	1.65
	2	11.00	55.00 (±4.36)[c]	50.00	7.249	14.751				
	3	14.47	72.35 (±1.45)[d]	70.05	13.216	15.717				
Iris germanica	1	9.33	46.65 (±2.18)[bc]	40.72	7.452	11.214	Y= 8.53x + 32.31	0.999	3.28	2.07
	2	10.93	54.65 (±3.48)[c]	49.61	7.938	13.928				
	3	12.40	62.00 (±2.64)[c]	57.78	10.123	14.676				
Mentha longifolia	1	7.27	36.35 (±3.28)[b]	29.28	4.441	10.091	Y= 8.5x + 18.92	0.875	2.66	3.65
	2	7.80	39.00 (±5.03)[b]	32.22	3.468	12.131				
	3	10.33	51.65 (±1.76)[b]	46.28	8.815	11.851				
Viola odorata	1	7.93	39.65 (±2.60)[bc]	32.94	5.693	10.173	Y= 9.28x + 23.66	1	2.88	2.83
	2	9.60	48.00 (±7.23)[bc]	42.22	3.374	15.825				
	3	11.27	56.35 (±4.09)[bc]	51.50	7.742	14.791				
Control		2.2	10.00 (±0.33)[a]	-	1.779	2.353				



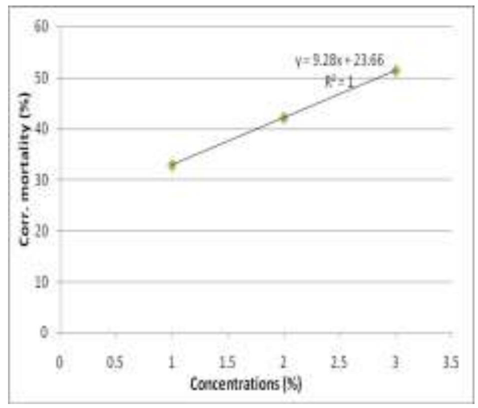
i. ***A. absinthium***



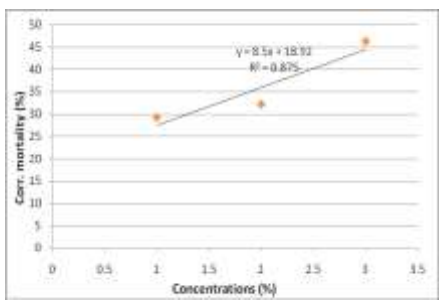
ii. ***E. helioscopia***



iii. ***I. germanica***



iv. ***V. odorata***



v. ***M. longifolia***

Fig. 4. Graphs showing Regression Equation for Ethanolic extract of five selected medicinal plants.