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Full Length Research Paper

Screening of botanical extracts for the control of Artemisia rehan (*Artemisia absinthium* L.) Aphid (*Coloradoa absinthii Li*.) in green house and field condition

Mihiret Mekonnen[1], Begashaw Manahile[2] and Beemnet Mengesha[3]

[1,3]Wondo Genet Agricultural Research Center, EIAR, P.O.Box. 198, Shashemene, Ethiopia.
[2]Department of Forestry College of Forestry and Natural Resource, Hawassa University, P.O. Box 128, Shashemene, Ethiopia.

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***Artemisia absinthum* L. is a perennial aromatic and medicinal herb that belongs to the family of Asteraceae and the genus *Artemisia. A. absinthum* production is practically affected by aphid, which causes up to 90% infestation at the study site. The objective of the study was to screen effective botanicals for the control of aphid on *A. absinthum*. Crude extracts of *Pyrethrum*, *M. ferruginea*, *C. citrates*, *C. citrates + Pyrethrum and Pyrethrum + V. amygdalina* were investigated. Diazinon® 60EC was used as a standard check and untreated plants as control. Treatments were arranged in completely randomized design and randomized complete block design for greenhouse and field experiments, respectively. In greenhouse experiment, the botanicals (20% v/v) were sprayed 20 days after introducing aphids. However, in field experiment, botanicals were sprayed on established plants before aphid occurrence and continued at 15 day interval for five rounds. Significant variations were observed among most treatments. *Pyrethrum* extract showed the highest efficacy against the target aphid with 91.81 and 87.27% mortalities in greenhouse and field conditions respectively. *M. ferruginea* extract ranked second with 72.7% and 64.5% mortalities in greenhouse and field conditions, respectively. About 87.27- 91.81% aphid population can be reduced by application of *Pyrethrum* crude extract.**

Key word: *A. absinthium*, Aphid infestation, crude extract, mortality rate.

INTRODUCTION

Artemisia rehan *(Artemisia absinthum* L.) is a perennial aromatic and medicinal herb that belongs to the Asteraceae family and the genus *Artemisia.* It is commonly called wormwood, absinthe, absinthum, and madderwort (Simon *et al.,* 1984). It is an erect herb with 30-60 cm high. The plant is native to temperate regions of Eurasia, northern Africa and naturalized in North America. It can grow in both poor dry or deep rich soils. Naturally it grows on uncultivated, arid ground or rocky slopes, and at the edge of footpaths and fields. The plant can easily be cultivated in dry soils and also be planted under bright exposure in fertile, mid-weight soil. It can be propagated by vegetative cuttings taken in March or October in temperate climates or by seeds in nursery beds.

A. absinthium is widely cultivated, especially in the northern and central parts of Ethiopia for its aroma**.** The plant is widely cultivated in the Mediterranean and temperate world for the active ingredients used in the alcoholic drink called absinthe. In Ethiopia, the plant is also widely applied in rituals called "adbar" and in flavoring a locally distilled alcoholic drink called Areki. The plant is used as an ingredient in the spirit absinthe, and is used for flavoring in some other spirits and wines, including bitters, vermouth and pelinkovac. It is also used to spice mead (Tariku *et al.,* 2011).

It was also described that the dried leaves, flowering tops, and essential oil of the plant have traditionally been

*Corresponding author. E-mail: hanamihiret0@gmail.com

used as an anthelmintic, antiseptic, antispasmodic, carminative, sedative, stimulant, stomachic, and tonic. The plant has also been used to improve blood circulation as a cardiac stimulant, as a pain reliever for women during labor, and as an agent against tumors and cancers. Folk remedies call for the employment of this plant against colds, rheumatism, fevers, jaundice, diabetes, and arthritis. The plant is also recognized as a moth and insect repellent (Mulatu and Mekonnen, 2007).
However, Aphid feeding on *A. absinthium* causes poor growth, stunted plants or curled and distorted leaves. A pile of them on upper leaf surfaces may be the first sign for the arrival of these destructive insects. High incidence of the pest can sometimes cause complete loss of the severely infested plants often fail to grow at Wondo Genet Agricultural Research Center experimental field in Ethiopia. Aphids penetrate plant tissues primarily via the intracellular route and their impact on the host is thought to be largely due to a reduction of the photosynthesis process, withdrawal of plant sap and injection of saliva. In the process of sucking out plant juices, aphids - like mealy bugs and scale excrete sweetish, sticky "honeydew" that often turns a moldy black and may lure ants.

Aphid populations vary widely throughout the year in response to seasonal factors and the attack of natural predators. For these reasons it is difficult to generalize about the need for intervention in controlling aphids. Because there are so many different kinds of aphids and because their life cycle and interactions with other insects in the ecosystem is complex, the methods of control may vary.

In spite of development of various modern synthetic insecticides, heavy losses of crops are recorded by the attack of aphids. In recent years, the use of pesticides, particularly of insecticides, has become very common. Excessive and indiscriminate use of these toxicants has unlimited hazards for human beings and every naturally growing population (Iqbal *et al*., 2007). Fumigation, spraying and dusting of pesticides liberate a fair volume of harmful vapors in the atmosphere and consequently create a certain degree of atmospheric pollution (Dheeraj *et al*., 2006). Some chemicals have posed serious problems to health and environmental safety, because of their high toxicity and prolonged persistence (Kulkarni and Joshi, 1998). Several control methods have been evolved for the control of aphids. These include cultural, physical, mechanical, biological, and chemical and host plant resistance. Mostly, the aphid populations are maintained below the economic injury level by combination of naturally occurring population regulating factors. But sometimes, the aphids can be extremely injurious if present in large number and chemicals have to be used for control (McIntyre *et al.*, 1989).

Farmers spray insecticides in their field indiscriminately. So, it causes resistance of the pest, destruction of beneficial organisms and environmental pollution (McIntyre *et al*., 1989). Control of aphids by any measure is a difficult task because of their rapid growth, mode of reproduction, polymorph nature and wide adaptation. So, it is necessary to find ecologically sound and environmentally safe control methods for aphid control. Botanical pesticides are comparatively less expensive; perform low toxicity, naturally available plant materials, less hazardous, biodegradable and also safe for beneficial organisms (Ahamed, 1984). Therefore, botanical control call for the employment of plant extracts containing insecticidal properties against aphids. Extracts from plant origin containing insecticidal properties are indigenously available and are considered comparatively safe for environment & public health. It has been reported that over 2000 plant species belonging to about 170 natural families are known to have insecticidal properties (Delvin and Zettel, 1999). Botanical pesticides are biodegradable and their use in crop protection is a practical sustainable alternative. It maintains biological diversity of predators (Buss and Park, 2002) and reduces environmental contamination and human health hazards. Research on the active ingredients, pesticide preparations, application rates and environmental impact of botanical pesticides are a prerequisite for sustainable agriculture (Dheeraj *et al.,* 2006). Botanical pesticides are unique because they can be produced easily by farmers and small industries (Radhakrishanan and Muraleedharan, 1993). This study is therefore, conducted to screen the best botanical extract for the control of aphid on *A. absinthium* as an alternative to pesticides for sustainable management of these pests of aromatic and medical importance as yet not been studied in Ethiopia.

MATERIALS AND METHODS

Greenhouse Experiment

The greenhouse experiment was conducted at Wondo Genet College of Forestry and Natural Resource (WGCFNR). Crude extracts of Pyrethrum, *Milletia ferruginea* L.*, Cymbopogon citrates (DS) stafes, pyrethrum + Cymbopogon citrates and Pyrethrum+ Vernonia amygdallina* were studied in greenhouse conditions. The chemical Diazinon® 60EC was used as a standard check with recommended dose and untreated plants as control. The experiment was arranged in a completely randomized design (CRD). A mixture of sterilized sandy clay loam soil, decomposed animal dung and sand with 2:1:1 ratio was autoclaved at 121^0C for 2 hr and filled into plastic pots (20cm ×15cm). Two Artemisia rehan seedlings were transplanted to each pot with three replications and were regularly watered. Then, approximately 100 aphids per pot were introduced on to three month old seedlings.
The pots were maintained in greenhouse at 26 ± 2 °C and 50–60% relative humidity.

The extraction technique used was a modification of Ruch's (2001). Healthy leaves were collected from selected plants and the excess water content was removed by keeping them under shade for few minutes till they become semi dried. Then, semi dried material (50 gm) of each plant was grounded using mortar and pastels and then dipped into 500 ml acetone for 48 hours for complete extraction of the active ingredients. After that, the grounded materials were filtered with the help of a very fine and clean piece of cheesecloth separately for every plant species. The extracts obtained were considered as stock and used to prepare desired test concentration (v/v) using tap water. The stock extracts were preserved in glass bottles in a refrigerator at 4ºC for further use. Then, the botanicals each at 20% concentration were sprayed on to infested plants after 20 days of introducing the aphids and continued at 15 day interval for five rounds. Data was recorded on aphid infestation and percent mortality rate.

The infestation percentage (incidence) was calculated using the formula of Kondo (2004).

$$\text{Percentage incidence (PI)} = \frac{\text{Number of infested plants}}{\text{Total plant observed}} \times 100$$

And percent mortality was calculated as:

$$\text{Percent Mortality} = \frac{\text{Dead insect}}{\text{Total plant treated}} \times 100$$

Field Experiment

The experiment was conducted at Wondo Genet Agricultural Research Center experimental field, Ethiopia. It is located at 7^0 192´ N latitude and 38^0 382´ E longitudes with an altitude of 1780m above sea level. The site receives a mean annual rainfall of 1000mm with minimum and maximum temperatures of 10 and 30 ^{0}c, respectively. The soil is sandy clay loam with an average pH of 7.2. The experiment was laid out in randomized complete block design (RCBD) with three replications. A plot size of 3 x 3 m and 60 x 60 cm spacing between plants was used. Spacing between plots and blocks were 1 m and 1.5 m, respectively. As for greenhouse experiment, crude extracts of Pyrethrum, *Milletia ferruginea* L., *Cymbopogon citrates (DS) stafes* and *pyrethrum + Cymbopogon citrates* were studied. Diazinon® 60EC was used as a standard check and untreated plants as control. The botanicals each at 20% concentration were sprayed starting before aphid occurrence and continued at 15 days interval for five rounds. Data was recorded on fresh leaf yield (kg/ha), dry leaf yield (kg/plot, fresh stem wt. (kg/plot), essential oil yield (%), aphid infestation percentage and mortality rate (%). The infestation percentage and Percent mortality rate were calculated by the above formula.

Essential oil content was determined on a dry weight basis from 250 g of composite leaves harvested from three middle rows of a plot. Laboratory analyses were performed at Wondo Genet Agricultural Research Center. Essential oil yield was determined by hydro-distillation (Guenther, 1972). Experimental data was statistically analyzed using analysis of variance (ANOVA) with the help of SAS statistical software (PROC GLM, 2002). Difference between means was assessed using Duncan's multiple range tests at $P < 0.05$.

RESULTS

Greenhouse Experiment

All botanical extracts showed a varied degree of efficacy over the control in greenhouse conditions (Table 1). However, *Pyrethrum* extract showed the best efficacy (91.81%) against artemisia aphids. Whereas *M. fuerruginea* and *pyrethrum + C. citrates* extracts ranked second and third with mortality rate of 72.7% and 67.26%, respectively. The least efficacy (40.92%) was recorded in *Pyrethrum + V. amygdalina*. The standard chemical Diazinon® 60EC showed the best efficacy compared to all botanical extracts (Table 1).

Field Experiment

Among investigated botanicals, the maximum efficacy (87.27%) was recorded in *Pyrethrum* followed by *M. fuerruginea* (64.0%) in field conditions (Table 2). Significant results were also observed in *C.citratus and Pyrethrum + C. citrates* which showed average mortalities of 40.6% and 50.5%, respectively. The maximum result was observed in the standard chemical Diazinon® 60EC with 94.54 % efficacy. However, all botanical extracts reduced the level of aphid infestation compared to the control. It was observed that, when efficacies of the extracts increase, the target aphid population becomes significantly reduced. This study confirmed that *Pyrethrum* and Diazinon® 60EC significantly reduced aphid population on *A. absinthum* in field conditions. Diazinon® 60EC showed the highest reduction (1.0%) followed by *Pyrethrum* (2.33%). The highest essential oil yield (24.43 g) was obtained from *Pyrethrum* treated plots followed by Diazinon® 60EC (21.45 g) and *M. fuerruginea* (20.46 g) treated plots (Table 2). The lower essential oil yield from Diazinon® 60EC treated plots can be due the toxic effects of Diazinon® 60EC to the plant. On the other hand, significantly lowest essential oil yield (11.08 g) was obtained from untreated plots (control). Relatively lower and virtually equal essential oil yield was

Table 1. Efficacy of botanical extracts for the control of Aphid on *A. absinthum* in green house condition.

No.	Treatments	Insect infestation (%)	Mortality rate (%)
1	*Pyrethrum*	3.00[f]	91.81[b]
2	*C. citrates*	18.33[c]	50.00[e]
3	*M. fuerruginea*	10.00[e]	72.70[c]
4	*pyrethrum + C. citrates*	12.00[d]	67.26[d]
5	*Pyrethrum + V. amygdallina*	21.66[b]	40.92[f]
6	Diazinon® 60EC (standard check)	2.33[f]	93.63[a]
7	Control (untreated check)	36.66[a]	0.0
	CV	8.56	10.32
	LSD	1.98	10.22

Means with the same letter within the same column are not statistically different *(p < 0.05*)

Table 2. Efficacy of different botanicals for the control of Aphid on *A. absinthum* in field condition.

Treatment	FLWPP (g)	FLWPH (g)	DLWPP(g)	EOY(g)	PI (%)	MR (%)
Pyrethrum	30[a]	270.00 [a]	26.66 [a]	24.43 [a]	2.33[e]	87.27[b]
M. fuerruginea	26.33[a]	236.77[a]	23.4 [a]	20.46 [b]	6.44[c]	64.00[d]
C. citrates	20.44[b]	183.73[b]	18.16[b]	13.99[c]	10.00[b]	40.6[e]
Pyrethrum + C. citratus	20.66[b]	186.13[b]	18.36[b]	13.87[c]	15.00	50.5[c]
Diazinon® 60EC	29[a]	261.13 [a]	25.79 [a]	21.45 [b]	1.00[f]	94.54[a]
Control (untreated plants)	16.9[b]	152.23[b]	15.02[b]	11.08[d]	40.00[a]	0.0
CV	9.5	9.54	9.5	18.2	1..	11.29
LSD	4.13	37.32	3.67	5.95	2.07	11.29

Means with the same letter within the same column are not statistically different *(p< 0.05).* Were; FLWPP- fresh leaf weight per plant, FLWPH- fresh leaf weight per hectare, DLWPP- dry leaf weight per plant, EOY- essential oil yie/ld, PI- Pest infestation (%), MR- Mortality rate (%).

obtained in *C. citrates* and *Pyrethrum + C. citratus* treated plots (Table 2). Generally, fresh leaf weight and essential oil yield were higher in treated plants than untreated ones. The aphid infestation levels were high on untreated plants with decreased essential oil yield. There was a clear negative correlation between the level of aphid infestation and essential oil yield. After spraying the number of aphids decreased in all treated plots while it significantly increased in untreated plot. Regarding fresh leaf yield, Pyrethrum had similar effects with chemical Diazinon® 60EC.

DISCUSSION

The results of this study showed that populations of aphids on treated plots were significantly different (P < 0.05) from untreated plots (control). The reduced number of aphids on treated plots could be due to extracts' repellent, toxic and antifeedant effects since they contain essential oils and alkaloids constituents with pesticidal properties (Manenzhe *et al.,* 2004). The highest essential oil yield was recorded in pyrethrum treated plots than the standard pesticide, Diazinon® 60EC. The lower essential oil yield under Diazinon® 60EC treated plants may be due to its toxic and residual effects on the plant. On the other hand, the lowest essential oil yield was recorded in untreated plots. The highest essential oil yield obtained from pyrethrum treated plots indicates that it is an effective botanical for the control aphids on *A. absinthum.* Generally, application of pyrethrum extract resulted in better yield than other treatments in all parameters measured, fresh leaf yield, dry leaf yield, fresh stem weight and essential oil yield. Previous studies showed

that Pyrethrum is a broad-spectrum insecticide used to control caterpillars (Cox, 2002). The effectiveness of pyrethrum flower powder water extract for the control of worker and soldier termites was reported with 93.75% efficac (Tierto, 1994). Anonymous (1977) also reported that application of tobacco leaf around plants killed or repelled aphids, flea beetles and thrips. Selase and Getu (2009) also stated that application of *C. ambrosoides* leaf powder at higher rate (15 g/150 g) on haricot bean weevils resulted in 100% mortality. Similarly, application of *C. citrates* essential oil on maize weevil showed increased mortality rate when applied at 0.7 ml/50g (Odeyemi, 1993). Likewise, Araya (2007) found that fresh *C. citratus* essential oil exhibited high (85-100%) acricidal activity. Shiberu (2013) also stated the aqua water extract *of C. cinerariaefolium* was the best treatment against *B. fusca* with 75% mortality rate after second and third day of application.

CONCLUSION

As confirmed by this study, all investigated botanical extracts showed insecticidal activity to control aphid. However, pyrethrum crude extract gave best result to control aphid compared to other extracts. About 87.27% aphid population can be reduced by applying 20% of its extract under field conditions. However, extract of this plant should be tested at varying and higher concentrations to get the maximum aphid control on *A. absinthum*

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