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Botanicals extracts for control of pea aphid (*Acyrthosiphon pisum*; Harris)

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Abstract
The pea aphids, *Acyrthosiphon pisum* (Harris) is an insect pest of economic importance in the production of pulses in Ethiopia. The economic yield losses in Field pea due to *A. pisum* was Calculated to be up to 230 kg/ha. Control methods include the application of insecticide and early planting. Reliance on synthetic chemicals to control pests has resulted in destruction of non-target organisms (parasitoids and predators) thereby affecting biological diversity. Garlic bulbs *(Allium sativum*), Endod (*Phytolacca dodecandra*) and Neem seeds (*Azadirachta indica*) grounded and prepared at 5 and 10% dilutions was tested for their effect on pea aphid under laboratory condition of Arsi University, School of Agriculture and Environmental Science. Both levels of Garlic and 5% Neem have induced mortality similar to Endosulfan 35% EC within 24 hours of treatment application. Levels of Endod dilutions performed much less as compared to other botanicals in 24 hours of application.

Keywords: Botanicals, Pea Aphids; *Acyrthosiphon pisum*; Garlic bulb *(Allium sativum*), Endod seed (*Phytolacca dodecandra*) Neem seed (*Azadirachta indica*), dilutions

1. Introduction
Field pea is (*Pisum sativa* L.) is the third most important pulse crop in Ethiopia, next to faba bean and chick pea in terms of both area coverage and total annual production. It is the source of food, feed and cash to the producers and also plays a significant role in soil fertility restoration through biological nitrogen fixation [1]. The pea aphids, *Acyrthosiphon pisum*, in the tribe Macrosiphini of the subfamily Aphidinae, exist as a number of biotypes and subspecies with different host plant ranges and preferences [2]. *A. pisum* Harris, is an insect pest of considerable economic importance in the production of field pea and lentil in many areas of Ethiopia. However, infestation is high in mid-altitude area (1800-2200m). Severe damage can occur to peas due to direct feeding and virus spread. Direct feeding on pea results in sap being removed from terminal leaves and the stem. Heavy infestations on pea can cause stunting, deformation, wilting and even death. Aphids can also feed on pods, causing them to curl, shrink and only partially fill [1]. Direct feeding therefore leads to yield loss and reductions in crop quality. [3] calculated economic losses in pea through a number of seasons due to *A. pisum*; with observed yield losses of up to 230 kg/ha. *A. pisum* is a vector of more than 30 plant virus diseases, including non-persistent viruses of beans, peas, beet, clover, cucurbits, Narcissus and Cruciferae. It transmits the persistent viruses Pea enation mosaic virus (PEMV) and leaf-roll virus (Pea seed-borne mosaic virus) [4]. It is an important vector of PEMV, which can cause considerable damage to peas, for example, levels of infection reached 20% in a survey in Canada [5]. It also transmits Pea top yellowing virus to beans and peas. In the UK, it transmits all three of the main viruses of beans: Bean leaf roll virus, Bean yellow mosaic virus and PEMV [6]. On peas and beans, *A. pisum* secretes honeydew from its siphunculi, which can coat plants, reducing photosynthetic efficiency and resulting in the growth of unsightly sooty moulds [7].
The recommended methods for control of pea aphids include the application of insecticide and early planting [8]. Given the conditions of climate and poverty in developing countries, the risks are many times higher than in industrialized countries [9]. Reliance on synthetic chemicals to control pests has also given rise to a number of problems such as destruction of beneficial non-target organisms (parasitoids and predators) thereby affecting the food chain and impacting on biological diversity.
There have also been cases of pests becoming tolerant to insecticides, resulting in the use of double and triple application rates [10]. In addition, due to other problems such as health hazards, undesirable side effects and environmental pollution caused by the continuous use of synthetic chemical pesticides [11], there is renewed interest in the application of botanical

pesticides for crop protection. Moreover, in field pea growing region of our country, most growers are subsistence farmer whose resource cannot warrant the use of synthetic insecticides against aphids. The use of such plant extracts to control pests is not a new innovation, as it has been widely used by small-scale subsistence farmers. The use of locally available plants in the control of pests is an ancient technology in many parts of the world [12]. Botanical pesticides are biodegradable [13] and their use in crop protection is a practical sustainable alternative. It maintains biological diversity of predators [14], 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 [15] for sustainable agriculture. Botanical pesticides are unique because they can be produced easily by farmers and small industries [12]. Therefore, this research is planned to determine how different botanicals are performing against pea aphids under laboratory condition and finally recommend effective rate of application of extracts for pea aphid management.

2. Methodology

2.1 Source and Preparation of Botanicals

Botanical seed extracts was tested against pea Aphids (*Acyrthosiphon pisum;* Harris) under laboratory condition of School of Agriculture and Environmental Science; Arsi University. Two botanicals seeds, Neem (*Azadirachta indica*) and Endod (*Phytolacca dodecandra*) were collected from Melka worer Agricultural Research Centre and around school campus respectively. The collected matured Neem seeds was soaked in tape water and pressed by hand to remove sticky materials and seed coverings. After complete removal of seed coat and sticky materials, it was spread on flat wire mesh bed under room temperature for complete drying. The collected Endod fruits sets was directly spread on some wire mesh bed under similar condition for complete drying. The bulk of seed materials was stirred up two times a day for maintaining uniformity in losing moisture so that grinding could be done smoothly. In addition to these botanicals, the bulbs of non-germinated garlic (*Allium sativum*) was also obtained from local market and shell was manually removed before grinding.

All collected materials were grinded using electronic blender. The grinded seed materials were sieved using sets of sieves of different size fitted together and the sieved seed powder obtained on the finest sieve size and blended garlic bulbs were soaked in tap water that can completely moisten the powder and fully cover the blended garlic bulbs. Each botanicals was placed in two different bickers for making two levels of concentration for 24 hours after being sealed with par film and aluminum foil. The soaked botanicals was then transferred to pure muslin closes and squeezed manually by gradual addition of total amount of water to make 5 and 10% (botanical weight to water volume ratio including the amount added for soaking) concentration. Extraction was continued till clear water comes out of the botanical materials. Soon after extraction was completed, 1% of powdered soap was added to each botanical dilution for serving as spreading agent during spry application. Similar amount of powdered soap was added to tap water which serve as control during the experimentation.

2.2 Method of Application

A. Establishing pea aphid colony

Winged Pea aphids were collected from field planted pea and released to into insect free cage containing healthy field pea plants grown under greenhouse condition. After 48 hours of release, all adult aphids were removed from pea seedlings and newly born healthy pea aphids colony established in green house was used for laboratory testing. Maximum Care was taken in avoiding the establishment of parasitoids, predators and entomo-pathogens within the colony.

B. Conducting laboratory test

Young sets of Leaves from growing tips of Insect free field pea plants established under field condition was used for laboratory testing. Young leaves collected from these plants were sprayed by 2% clorox solution for surface disinfection and rinsed in tap water before releasing test insects. The cut tip of each young leaves was rolled with tap water soaked cotton ball to keep the cutting fresh after release of aphids. Five aphids of similar size collected from test colony were picked by moistened camel brush and released on each leaf cutting and each treatments was replicated four times to make total of 20 insects exposed to each treatment. Prior to placing released insects in to top perforated plastic Petridis, all botanical treatments (5 and 10% dilution of Neem, Garlic and Endod extracts), synthetic Chemical pesticide (Recommended rate of Endosulfan 35% EC) which was used as standard check and control treatment (tap water added 1% powdered soap) were sprayed by using atomized hand spryer separately on a try which was covered with filter paper. The rate of mortality was recorded every 24 hours for four consecutive days and fresh young leaves was also provided after every record for remaining life aphid population. The recorded average room temperature during experimentation was ranged from 20-25 C^o with relative humidity of below 30%. The obtained mortality values were squire root transformed before analysis and SAS software package was employed for statistical analysis. Least significance difference (LSD) range test ($p \leq 0.05$) was used to evaluate the significant difference observed between treatments.

3. Results

3.1 Mean Daily Mortality

A. Mortality after 24 hours of Treatment Application

Both levels of garlic dilutions and Neem 5% has induced aphids' mortality which was at per with Endosulfan 35% EC within 24 hours of application (Table 1). Though not significantly different, Endosulfan had caused 100% mortality followed by garlic 5% (93.3%), garlic 10% (80%) and Neem 5% (73.3%), Table 2. However, the lowest aphid mortality which was significantly different ($p<0.05$) from all botanicals applied in causing mortality within 24 hours of application was recorded in population treated with both dilution levels of Endod (1.22 and 1.44). Such lowest rate of mortality had been found to be similar to control (tap water + 1% soap) where no aphids were killed. Even though the aphicidal effect of remaining all botanicals were not significantly different from each other within 24 hours of application, the effect of Neem 10% (1.93) had been observed to be significantly different from standard check chemical (Endosulfan 35% EC) which induced complete death of all aphid populations treated while mortality recorded with other botanicals was at per ($P<0.05\%$) with standard check chemical pesticide used.

B. Mortality after 48 hours of Treatment Application

48 hours after treatment application, mortality was recorded at both levels of Neem and Endod, the highest mortality being at 10% dilution levels for both botanicals (26.7%) and the lowest was 20% and 13.3% for Endod and Neem 5% respectively (Table 2 & Fig.1). However, the mortality recorded was not significantly different from each other. No mortality was recorded in populations treated with both levels of garlic and control within second day of treatment application.

Table 1: Daily mean mortality of Pea Aphids (*Acyrthosiphon pisum;* Harris*)* as Affected by Different levels Botanicals, Chemical and water spry

S. no	Treatments	1DAT	2DAT	3DAT	4DAT
1	Endosulfan 35%	2.3452a (15)	0.7071b(0)	0.7071d(0)	0.7071c(0)
2	Garlic 5%	2.2892ab(14)	0.7071b(0)	0.8365dc (1)	0.7071c(0)
3	Garlic 10%	2.1706ab(12)	0.7071b(0)	0.8365dc (1)	0.7071c(0)
4	Neem 5%	1.9894ab(11)	1.1441ab(1)	1.0550bcd (2)	0.8365bc (1)
5	Neem 10%	1.9335b(10)	1.4029a(4)	0.8365dc (1)	0.7071c(0)
6	Endod 5%	1.2247c(3)	1.2735a(3)	1.7162a (8)	0.7071c(0)
7	Endod 10%	1.1441c(4)	1.4184a(4)	1.4029ab (6)	1.0953ab (2)
8	Control (Water Spray+1% soap)	0.9659c(1)	0.7071b(0)	1.2792ab (4)	1.2735a (3)
	LSD	0.3573	0.4573	0.5118	0.2849
	CV	13.92775	31.07134	32.35770	23.16488

Note: - Figure in parenthesis is non transformed value while others are squire root transformed values, figures with similar letters are not significantly different, DAT= Days after treatment application.

C. Mortality after 72 hours of Treatment Application

Highest mortality, which is significantly different from both levels of garlic and Neem 10% (0.84) was recorded in aphids treated with both levels of Endod (1.72 and 1.4 for 5% and 10% respectively) within third days of treatment application (Table 1). It was after 72 hours of treatment application that the highest mortality was recorded for Endod 5% (46%) and 10% (33%) within single day after treatment application. Similar to Endod, the highest mortality with in single day after application of treatment was recorded during third day of application for water mixed with 1% soap; control, (40%), Table 2, Fig. 1.

Table 2: Percent Mortality of Pea Aphids as affected by Botanicals

S. no	Treatments	1DAT	2DAT	3DAT	4DAT	Total
1	Endosulfan 35%	10.0a(100)	0.71b(0.0)	0.71c(0.0)	0.71b(0.0)	10.03a(100)
2	Garlic 5%	9.67a(93.3)	0.71b(0.0)	1.98bc(6.7)	0.71b(0.0)	10.03a(100)
3	Garlic 10%	8.69a(80.0)	0.71b(0.0)	1.98bc(6.7)	0.71b(0.0)	9.32ab(86.7)
4	Neem 5%	8.53a(73.3)	2.6ab(13.3)	1.98bc(6.7)	1.981ab (6.7)	10.03a(100)
5	Neem 10%	8.18a(66.7)	5.14a(26.7)	1.98bc(6.7)	0.71b(0.0)	10.03a(100)
6	Endod 5%	4.53b(20.0)	3.87ab(20.0)	6.84a(46.7)	0.71b(0.0)	9.27ab (86.7)
7	Endod 10%	4.48b(26.7)	4.34a(26.7)	5.75ab(33.00)	3.25ab(13.3)	10.03a(100)
8	Control (Water Spray+1% soap)	1.98b (6.7)	0.71b(0.0)	5.35ab(40.00)	3.866a(20.0)	8.06b(66.7)
9	LSD (p<0.05)	2.69	3.5	3.83	2.06	1.43

Note: - Figure in parenthesis is non transformed percentage value while others are squire root transformed value, figures with similar letters are not significantly different, DAT= Days after treatment application

D. Mortality after 96 hours of Treatment Application

After four days of treatment application, no mortality was recorded in Endod 5% and garlic 10% even though there are still 13.3% of live aphids in the treated population. Highest mortality in the fourth day of application which is at par with Endod 10% (1.10, 13.3%) was recorded in control (1.27, 20%). The least mortality recorded was observed in aphid population treated with Neem5% (0.84, 6.7%), Table 1 &2.

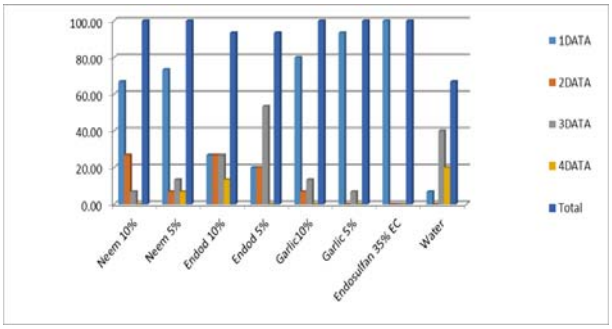

Fig 1: Percent Mortality of pea Aphids as affected by Botanicals

3.2 Mean Adult Longevity

100% mortality within 24 hours of treatment application had been recorded only in Endosulfan 35% EC treated aphid population, Table 2. However, the lowest longevity recorded within 24 hours of botanical extract application (7, 20 and 27% for both levels of garlic and Neem 5% respectively) was not statistically different from standard check, Table 2. The highest longevity after 24 hours of treatment application was recorded in 1% soap dilution spray (93.3%) and Endod 5% and 10% application (80% and 74% respectively). The total percent longevity analysis (Table 2) indicated that the highest survival of aphids after four days treatment application, which was significantly different from all treatments except for Garlic10% and Endod5% (14.3%) was recorded in control population where 44.3% of 1% soap sprayed aphids' population survived. On the other hand, the lowest longevity recorded after four days of treatment application for Garlic10%, Endod5% and Both levels of Neem was not significantly (P>0.05) different from standard check chemical pesticide which induced 100% mortality soon after application.

3.3 Phytotoxicity

Both levels of Endod dilutions had produced bleaching effect to the normal light green colour of field pea leaf cuttings after 24 hours of application. The remaining all applied botanicals, standard check chemicals (Endosulfan 35% EC) and powdered soap added water spray did not produce any change to the natural colour of field pea leaf cutting even after prolonged (four days) days of application.

4. Discussion

The result of this study indicates that all levels botanicals tested except that of Endod 5% and Garlic 10%, had shown sufficient aphicidal effect as both levels has produced more mortality which was significantly different from control population (1% soap spry) after four days of application. Application of soap as spry for managing aphid was done by

different scholars and reported to be not effective [16]. These botanicals had also been found to produce 100% aphids mortality after four days of treatment application. However, all botanicals had been failed to produce 100% mortality within 24 hours of application like that of Commercial insecticide used for this experiment. Never the less, the observed mortality of aphids within 24 hours of treatment application due to both levels of garlic dilution and Neem5% was found to be as effective as commercial pesticide used. This may indicates that the lowest delusion of garlic and Neem extract test (5%) could be used as appropriate dosage of application for pea aphid control. Reported 1.5% concentration of Garlic bulb has induced less mortality to peach aphids and recommended for farther evaluation of the concentration [17]. The result of experiment conducted by [18] also proved Neem seed extract used at the rate of 50gm/Lt and 75gm/Lt induced reduced aphids population by 80.5% and 79.9% respectively from count made before treatment application. Both levels of Endod could not produce mortality which is significantly different from soap 1% spry during individual four dates after treatment application. The highest level of Endod concentration tested (10%) had produced cumulative effect of 100% kill. The efficacy Endod and other botanicals against onion trips was evaluated and concluded Endod to be effective in reducing trips population without having negative effect on crop[19]. However, from the very beginning this study, both levels of applied Endod had produced phytotoxicity to applied pea plant leaf with 24 hours of application. Therefore, lower dose and frequency of application for this particular botanical against different field pea pests is recommended. All aphids dead within 24 hours for Neem seed and Garlic bulb extract application and Endosulfan spry were found attached to the leaf at almost the spot they were applied while those few dead insects found on Endod treated leaves were observed away from leaves and found dead in the Petridis. This may also suggests Endod do have some repellant effect on aphids while other botanicals like Endosulfan do have direct killing potential.

The highest mortality recorded through four days experimentation was due to Garlic, Neem and commercial insecticide (Endosulfan 35CE) and ranged from 66%-100% which was generally recorded within 24 houses of treatment application. This indicates that these botanicals are efficient enough to be utilized during critical time of aphid infestation to gain economic advantage of their cheapness and early reduced pest population for gaining the yield advantage of timely protected field pea plants. Most plants species which are used in phytomedicine contains ingredients which inhibit the development of insect, hinder their feeding (antifeedants) or act as repellents and confusants [20, 21]. The residual effect of all botanicals could not be studied with this experiment though there is indication that effect of garlic may not last longer as the result indicated they only induced fast mortality and the remaining 14.3% of population remained unaffected for even 10% garlic application. Though the intension of this experiment was to study the efficacy of different levels of botanical on field pea aphid, field study could not be done for there was faller of natural infestation pea aphids for the last two consecutive cropping season (2011/2012 and 2012/2013) near areas of Asela School of Agriculture and environmental Science farm station.

5. Conclusion

As a conclusive remark, all botanicals tested had been proved to have capacity of killing field pea aphids under laboratory condition. Moreover, even lowest doses (below 5%) of application shall be tested for economizing the utilization of botanical extracts as the applied 5% of dilution of some of the extracts were found to be effective in causing significantly different rate of mortality to treated aphids population. Moreover, the field efficacy of this botanicals shall be evaluated as there could be potentially negative or positive interaction of botanical extracts with existing physical environment.

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7. References

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