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APHICIDAL EFFECTS OF ORGANIC FORMULATIONS AGAINST TOMATO APHID *MYZUS PERSICAE* (SULZER) TO OVERCOME CROP DAMAGE/DISEASES

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A B S T R A C T

Synthetic material against pests is now very common with a negative impact on environment due to which an alternative approaches are needed to reduce the harmful effects of chemicals. Botanicals are the most important source of natural constituents having potential to retard the growth of these pest, Aphids (*Myzus persicae*) are small sap-sucking insects belonging to Aphidoidea. This insect is marked as threatening enemy which attack on the members of *solanaceae* and cause numerous damages to the crop. It has ability to cause numerous other infection and induces fungal and viral attack on crop due to which yield decreases. Present experimental analysis was design to investigate the anti-aphid potential of plant parts in order to overcome the use chemical based pesticides, to control attack of disease due to aphid on crops. Current study was performed to synthesize biopesticides as insecticides {against *Solanum lycopersicum* L (*Lycopersicon esculentum* Miller)} aphid= *M. persicae*) by the fermentation of fresh leaves of *Azadirachta indica* L., *Nicotiana tabacum* L. and rhizomes of *Allium sativum* L. in the form of formulation 1 and A. sativum was replaced with Zingiber officinale L., for formulation 2. After assessing and anti-antiaphid activity, biopesticides were subjected to FTIR and HPLC analysis. Results showed that formulation1 reduced aphids after 24, 48, 72, 96 and 144 hrs (21.72±0.5, 20.19±0.2, 24.08±0.4, 28.78±0.4 and 34.32±0.2% pest reduction, respectively). Phytochemical analysis of both formulations showed that *A. sativum* had organosulfur compounds (11-35 mg/g of fresh *A. sativum*), arginine, oligosaccharides, flavonoids and selenium, while the pesticidal importance of *A. indica*, *N. tabacum* and *Z. officinale* was due to *azadirachtin*, *nicotine* and alpha-pinene, respectively. Outcomes of current work shows that plant-based pesticides are a better option for farming as an alternative to chemically syntheisized pesticide so this study proves to be environment friendly and biopesticides could be a good alternative to chemical pesticides to overcome insects which act as vector for various diseases.

Keywords: *Myzus persicae*, HPLC, FTIR, *Allium sativum* L, *Zingiber officinale* L., and *Solanum lycopersicum*.

INTRODUCTION

The material used by farmers or at domestic levels to kill plant pests, either from plant or microbial sources, are all termed pesticides. Naturally occurring plants and plant-based extracts have been widely used in agriculture to control this pest since ancient times. These substances are usually considered killer of the pest and are important to control these pests. In other words, pesticides are chemical material used in agriculture for a long time ago to assist the production of crops to enhance yield by offending and inhibiting pests. Pesticides are chemical substances that are used in agriculture to aid production and yield by repelling, preventing, and destroying pests. However, in recent

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years, due to the continuous use of synthetic pesticides in agricultural fields, there has been a great accumulation of pesticidal residues in the environment, which is responsible for many chronic illnesses. According to the report of the United Nations Environment Programme pesticides are the basic reason for poisoning approximately three million people and cause~200,000 deaths yearly around the world. Synthetic pesticides are extensively used to kill and stop pests, which have negative effects on human health and cause health hazards, which is why eco-friendly strategies require an hour as safer than chemical-based pesticides and are economically inexpensive .

Recently, a number of programs have been carried out to acknowledge the public about the increasingly ill effects of pesticides for the protection and production of food stuff along with their deteriorating effects on the surroundings. Biopesticides are suggested as the best alternative. Conventional pesticide-producing units have undergone a series of changes recently.[4] Therefore, these naturally occurring material have nontoxic effects, so the use of these plant extracts is a possible strategy to control insects. Furthermore, these can provide future control and eliminate the use of toxic chemically based pest controllers. Moreover, they also discourage pest resistance and could be termed eco-friendly because they are biodegradable and easily have an ill impact on useful insects too.[5] In recent studies, the main focus was to establish safer strategies for the management of insect pests than chemical pesticides.

Plant-based pesticides play an important role in all fields against many insects damaging fruit, such as bollworms, many jassids, aphid, whiteflies, leaf hoppers, thrips, and a number of pests. Some parts of plants have properties that discourage the feeding of predators and are repellant, and their seeds also have chemicals, which have inhibitory effects on the growth population of insect pests. A number of plant parts from Neem, Huing, Dhatoora, Eucalyptus, Tobacco and their solutions are also effective in controlling the sucking complex. Studies have been carried out for many years. Neem and nicotine are usually used as biopesticides and are under consideration, but a limited stock is available at the commercial level. Biopesticides are naturally derived material that are used for the betterment of plants and are considered key factors involved in the maintenance of disease-causing agents.[8] In comparison to chemical based pesticides biopesticides are widely accepted due to their low damaging effects on crops and are markedly beneficial for vegetable production. Moreover, plant-based material were the focus due to the negative impact of chemical-based pesticides on ecosystems. There is great pressure on experts to determine alternative ways to control pests and introduce eco-friendly strategies for pesticide synthesis.

Neem and nicotine are mostly used as biopesticides; however, many other plants are under consideration for use as biopesticides. Furthermore, neem leaf extract has been reported as an antiinflammatory substance and is marked as an excellent plant due to its easy processing ability. Moreover, this plant has been reported as a solution to numerous problems, such as anti-feedent, anti-inflammatory, and insecticidal[10]. Neem extracts are also reported as powerful tools against the larvae of mosquitoes, aphid, and whiteflies. Some *A. indica* were found to be repellent against insects and were reported to eliminate the harmful effects caused by chemical pesticides. Presently, it has been evaluated through experimental investigations that extracts of neem had anti-feedant and repellent properties that declared the associations of organic fertilizers along with powder of neem leaves and that of boiler ash was seen to have a notable role in the improvement of the plant potential against attack of aphid.

Garlic (*A. sativum* L.) was also found to be the most effective plant-based bio-pesticide, as it has the ability to control seed-borne diseases due to having allicin.[12]There are various compounds that provide a specific smell and taste to garlic along with biological properties. It has been reported that garlic also holds insecticidal and fungicidal properties; it also has substances that would be poisonous to mites or ticks, along with substances that can kill nematodes and worms.[13] The findings of the study listed *A. sativum* as a useful plant pest controller, as it is naturally blessed by certain properties to kill fungi and pests and is among those substances that are found to be the best and most economical due to their nontoxic character; hence, they are safe for use in the field. However, garlic has the ability to kill insects, which is why its extracts are used as an insecticide and show remarkable toxicity to numerous pests at all life stages.

Chemical pesticides also play an important role in agriculture by compensating for the great losses of yield due to pests, but due to their immense usage over a broad spectrum, they have a negative impact on the environment; however, the latest articles showed a special link between the problems associated with human health and chemical pesticides.[14] Long-term applications of chemical-based sprays are linked with problems related to human health, such as reproductive issues, increased weight, diabetes, cancer and nervous disorders. Meanwhile, it has been recently reported that long-term usage of these pesticides is responsible for invoking resistance.[15] Some plants have certain substances because they are able to fight against devastating invaders, either insect pests or herbivores. These compounds are safe for humans and have no adverse effects on the environment, which are termed complex defense approaches. There is a variety of such plants, which are commonly used as an effective antifungal material while garlic and hot pepper are the two most commonly used. The compound responsible for the creation of defense is allicin, which has a vast range of biological activities and is produced in plants in response to tissue damage, which is further characterized by pest killing due to sulfur volatiles.16 Extracts from pesticide plants have always been thought to be an effect on the control of pests, and in comparison with other extracts, they are cost effective and safe for ecosystems. With the time, most of these chemicals had become extremely penetrating in the environment, in some cases, in the food that needs to be consumed as a result of their widespread repeated However, eventually these pesticides are extremely expensive and having a deteriorating effects to the environment.

Present study was designed to over come the threshhold of aphid on crops as these insects had great potential to causes numerous other disease. These are sap sucking insects due to which chances of fungal attack are more which increase mycotoxin production in fruits which is not good for human consuption. This insect is also responsible for wilting and necrosis which can affect yield greatly, and cause economic losses. These are called pathogenic insects due to their welcoming natur towards other crop damaging viruses.

MATERIAL AND METHODS

Work Place and Chemicals used: The present experimental study was carried out at PCSIR and IMBB, The University of Lahore, Lahore, Punjab, Pakistan. It has been confirmed that the experimental samples of plants, including the collection of plant material, are done by following the guidelines of national, and international legislation with appropriate permissions from authorities of The University of Lahore, Lahore, Punjab, Pakistan.

Aphid culture: A population of aphid, known to be resistant to synthetic insecticides, was collected from a commercial ornamental nursery, identified by an expert zoologist (entomologist) at the Department of Zoology, University of Punjab, Lahore, Punjab, Pakistan and maintained on tomato (*S. lycopersicum* L.) seedlings before it was used. Populations aphid were maintained in fine mesh insect cages of size 47.5 × 47.5 × 47.5 cm (Biopesticides Labs, PCSIR, Lahore). Plants were changed each week, and fresh plants infested with aphids were taken from the discarded plants. Insect cages were placed in controlled environment rooms set to 20 °C, 60% relative humidity and 16:8 (light: dark) hours (Blackman and Eastop, 2000).

Infesting plants with aphids: A small number of *S. lycopersicum* L. plants were placed into insect cages in which *M. persicae* (aphid) was reared. Plants were placed close together so that aphids were able to colonize *S. lycopersicum* L. plants. As aphid population was stabled and completed their at least two generations, were further used for the infestation of the remaining *S. lycopersicum* L. plants by carefully placing aphid-infested leaves of the same plant species onto a previously uninfested plant. In this way, each plant was infested with approximately 10 mixed age aphids. Aphid populations were allowed to establish for two weeks before the start of the experiment.

Biopesticides formulation: Leaves of Azadirachta indica L. and Nicotiana tabacum L. and rhizomes of Allium sativum L. and Zingiber officinale L. were purchased from the local market of Lahore and identified by expert taxonomists at the Department of Botany, GC Women University Lahore, Punjab, Pakistan. One type specimen of each plant sample was also kept in the Departmental Herbarium with voucher specimen's No. 514 L and 515 L (for *A. indica* L. and *N. tabacum* L.) and 732R and 733R (for *A. sativum* L. and *Z. officinale* L.) with area, date, day and time of collection along with complete classification of plants.

Two different bio-pesticide formulations were prepared by fermenting leaves of *A. indica*, *N. tabacum* and water (01:08:500) at two locations (labeled Biopesticide Formulation 1 or BPF1 and Biopesticide Formulation 2 or BPF2). A fresh rhizome of *A. sativum* L was added to the above mixture to prepare BPF1, while *Z. officinale* was added to synthesize BPF2. The whole mixture was filtered through muslin cloth after 7 days of fermentation and was used for further analysis (Sisay *et al*., 2019).

Study design to evaluate the efficacy of bio-pesticides: *S. lycopersicum* L was grown in three plots; two plots were experimental {RBME1 (treated by formulation 1) and RBME2 (treated by formulation 2)}, while one was controlled {RBME3 (no treatment)} along with the synthesis of biopesticide formulations. When the aphid threshold reached the high population level, treatments (biopesticide) were sprayed. Five sprays were applied to both experimental plots and control plot was kept untreated, respectively, of the *S. lycopersicum* L crop by using a simple 1.0 liter gardener hand sprayer fitted with a hollow cone nozzle delivering a droplet size of 200–300 μm. Spray applications were made using three 3 bar pressures. The volume of bio-pesticide formulations were standardized at 600 l/Ha. Pretreatment observations were recorded before spraying, while posttreatment observations were recorded after 24, 48, 72, 96 and 144 h of biopesticides spraying, and their effects on the host crop were analyzed by comparing the population of aphid on pretreated (before applying bio-pesticide) and post treated (after the application of biopesticides) crops. The results were calculated by the following formula, and the average was taken as:

$$\text{Aphid Population (\%)} = \frac{\text{Number of aphid after Post treatment}}{\text{Number of aphid in Pretreatment}} \times 100$$

Qualitative Analysis of Flavonoid and Phenolic Contents: Total Flavonoid content (TFC): Flavonoid concentration was measured by calorimetric assay at 415 nm in which changed dilutions of quercetin (50 to 250 μg) were used as standards according to the modified protocol (Ahmed *et al*., 2018).

Total Phenolic content (TPC): The phenolic content was estimated by a spectrophotometric method with slight modifications21. Five different concentrations of formulations were prepared as stock solutions, Folin Ciocalteu reagent as oxidizing agent and gallic acid as a standard, and absorbance was measured at max= 760 nm. Whole experiment was run in triplicates.

FTIR Analysis: Fourier transform infrared spectroscopy (FTIR) is a remarkable tool to identify the types of chemical bonds or functional groups in compounds. However, the wavelength of light absorbed is the basic characteristic of the chemical bond, as seen in the annotated spectrum. Chemical bonds could be easily detected by interpreting the infrared absorption spectrum. To carry out this analysis, a prepared sample was loaded in an FTIR spectroscope (Shimadzu, IR Affinity 1, Japan) with a scan range from 400 to 4000 cm1and a resolution of 4 cm1.

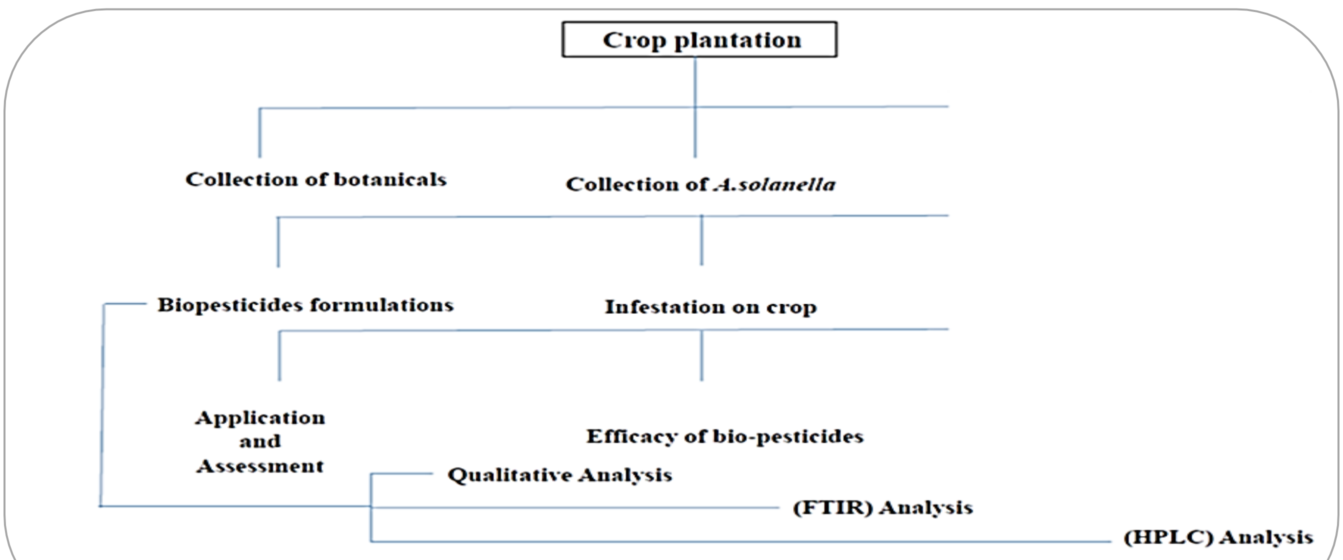

Figure 1 Schematic diagram for the representation of work summary

HPLC Analysis: For HPLC analysis, samples were prepared as per protocol (Ahmed *et al*., 2018).[18] Approximately 50 g dried extract of each formulation was re-dissolved in 500 mL of 80% ethanol, shaken at 25 °C for 24 hours and centrifuged at 10 °C for 10 minutes at 10000 rpm. The mixture was filtered using Whatman filter paper No. 41, and the filtrate was left at room temperature so that the solvent evaporated to dryness and re-suspended in one mL C2H5OH (80%). Then, the extract was filtered through 0.45 µm nylon 66 filter paper and injected 10 µL from this solution into the HPLC system {Perkin-Elmer model of HPLC with binary LC pump of 250, an LC 600 auto sampler, a UV/V with spectrometric detector of LC- 290, PV Nelson 900 series INTERFACE, Hewlett- Hewlett-Pakkrad 3394 Integration and a Bonda pak C-18 column (250*4.6 mm)}. The mobile phase was acetonitrile and methanol v/v (1:2); the flow rate was 1 mL/minute, the injection volume was 20 µL, and the aglycone content was measured using 95% quercetin dihydrate as a standard. The temperature was kept at 30°C with max = 350 nm.

STATISTICAL ANALYSIS

Data were analyzed statistically by applying one-way ANOVA, and the results are expressed as the mean ± SEM through Graph Pad.

RESULTS

Effect of Biopesticides on aphid: The findings of the current experiment suggested that BPF1 was very effective in controlling aphids, as the average reduction in pests was more pronounced in the crop treated with BPF1 than in the crop treated with BPF2 and control (Figure 2). After 24 h of treatment, the average reduction seen by BPF1 was 21.72±0.5%, followed by BPF2 and control (16.22± 0.3% and 3.52±0.1%, respectively). BPF1 was also effective in aphid reduction over time, with mean reductions after 48 h, 72 h, 96 h and 144 h (27.84±0.6, 33.68±1.2, 41. 32±1.3 and 42.92±1.4% reduction, respectively). After 48, 72, 96 and 144 hours, aphid reduction due to BPF2 (20.19±0.4, 24.08±0.5, 28.78±0.4 and 34.32±0.2% reduction, respectively) and control (7.04±0.3, 9.28±0.5, 10.12±0.1 and 15.76±0.6% reduction, respectively) was also increasing, but this reduction was still less than that in the BPF1-treated crop. BPF1, having garlic as an important constituent, was found to be very effective in controlling insects over BPF2. Moreover, reduction was also seen in BPF2compared with the control group, as an increase in the threshold of insects was seen after 72, 96 and 144 h (9.28 ±0.5, 10.12±0.1 and 15.76±0.6%, respectively) in the control group. The main reason for the higher reduction in the pest population was due to the repellent properties of garlic.

The most active ingredient among formulations was azadirachtin, along with some other important related triterpenoids, such as azadirachtin B, salannin and nimbin, and all of these secondary metabolites were due to neem plants in both formulations, which are responsible for disrupting the growth and development of insects and deteriorating their feeding (Khan *et al.*, 2020)·

However, the effectiveness of BPF1 was due to garlic (Waziri *et al*., 2015). This study suggested biopesticides as the best alternative to chemical pest killers (Damalas and Koutroubas, 2018).[20] Numerous studies have concluded that A.sativum has properties to repel insects. The active ingredient in garlic is allicin, which is a colorless liquid with a pungent smell because it has antibacterial and antifungal features. The most important point about garlic is that its active substance (*allicin*) imparts a defense mechanism against insect pests (Debra and Misheck, 2014).

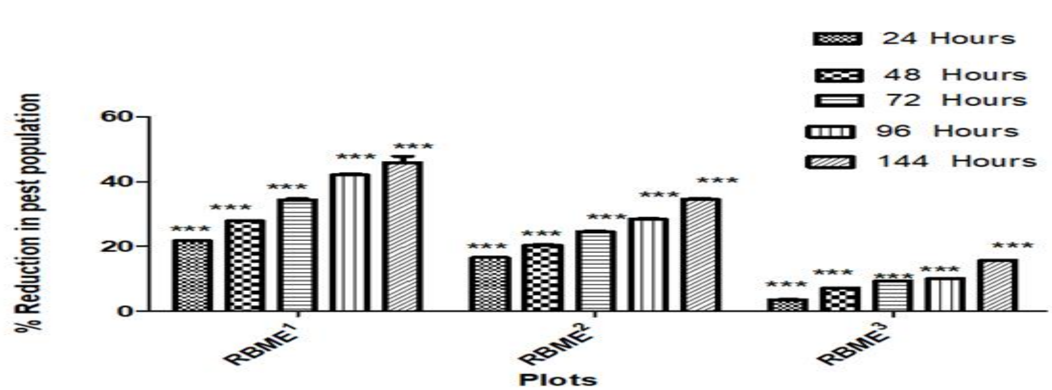

Figure 2 **Average population of aphid**

±= results had been obtained after triplicatte analysis of an individual plots *** shows highly significance results at P<0.0001 and at this value extremely significant insect reduction has been seen in all experimental plots w.r.t time duration. Moreover extremely significance pest has been shown in RBME1 and after 144 h of pesticide exposure.

Phytochemical estimation of Biopesticides: Quantitative estimation of total phenolic and flavonoids: Spectrophotometric quantification of secondary metabolites showed that the amount of total phenolic and flavonoids was higher in BPF1than in BPF2, which shows that the pest reduction potential of BPF1 might be due to the higher content of its secondary metabolites (Figure 3). Moreover, the amount of flavonoids was higher than that of phenolic, which led to the HPLC profiling of flavonoids in both formulations for further confirmation of targeted anti-aphid compounds. Phytochemical screening of biopesticides also showed good results, which were in favor of their anti-aphid characteristics, which were also in accordance with numerous studies.

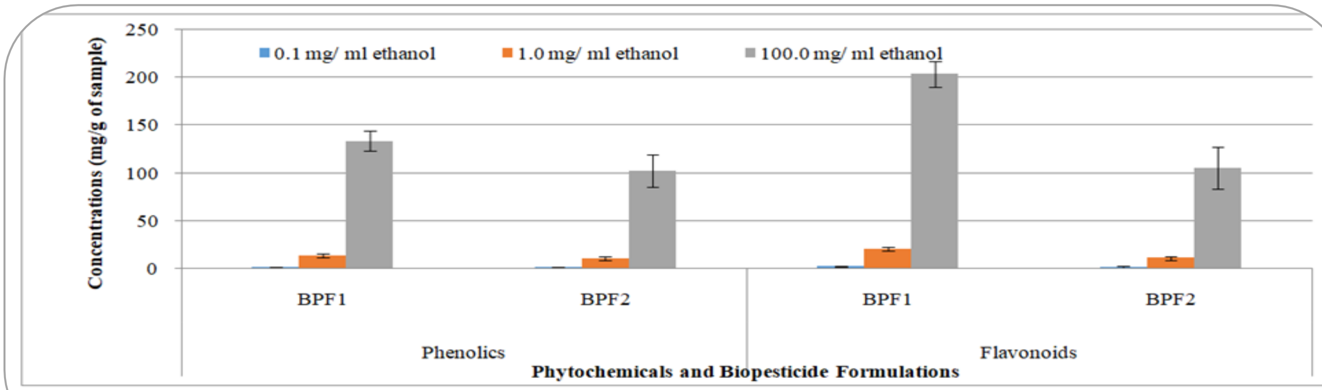

Figure 3 **Concentration of secondary metabolites in BPF[1] and BPF[2]**

FTIR analysis: Peak values of FTIR spectra confirmed the presence of alcohol, phenol, alkanes, alkyl halide, amino acids, carboxylic acid, aromatic and amines (Table 1) in both formulations of biopesticides, while Figure 4 and Figure 5 present the functional groups of BPF1 and BPF2, respectively.

HPLC profiling of Flavonoids: The concentration of flavonoids in both bio-pesticide formulations was confirmed through HPLC chromatograms. For this analysis, quercetin was used as a standard, and the obtained peaks of pesticides were compared with the standard. The peak shown in Figure 6 was seen at 2.570 mV, presenting the standard, while the peak was also seen at 2.6801 mV by BPF1 and at 2.8801 mV by BPF2, which shows that peaks by both formulations were in the range of quercetin standards (Figure 7, 8and Figure 9).

Table 1. FTIR peak values of bio-pesticide formulations

Functional groups in Biopesticides formulations	**Peak values by BPF[1]**	**Peak values by BPF[2]**
Alkenes(C=C) cm^{-1}	1665.37	1653.92
(C=C) stretch of aromaticcm^{-1}	1604.37 , 1587.03, 1517.55 and 1437.43	-
C-H alkane cm^{-1}	2979.59	2931.79
Nitro (NO_2strech)cm^{-1}	1375.59 and 1318.51	-
Alkyl & Aryl halides C-F stretch cm^{-1}	1217.56, 1220.91 and 1166-91	1291.34, 1240.89 and 1214.84
C-H alkynes cm^{-1}	3265.67	3272.45

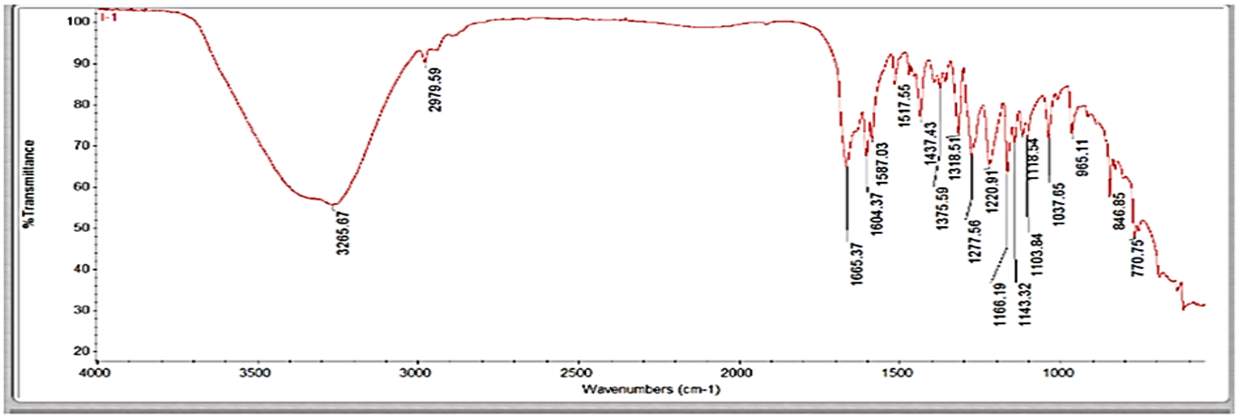

Figure 4 FTIR Spectrum of BPF[1]

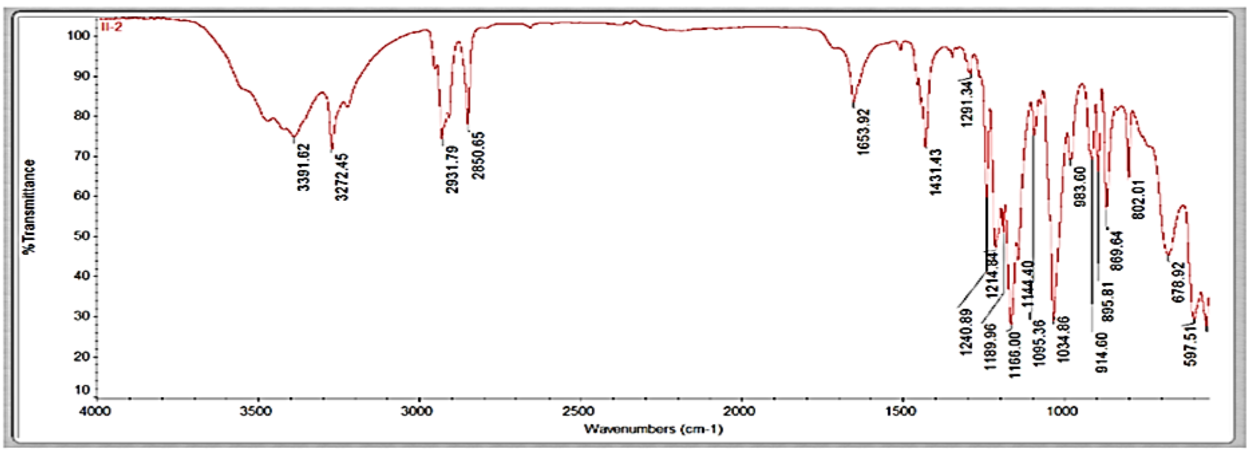

Figure 5 FTIR Spectrum of BPF[2]

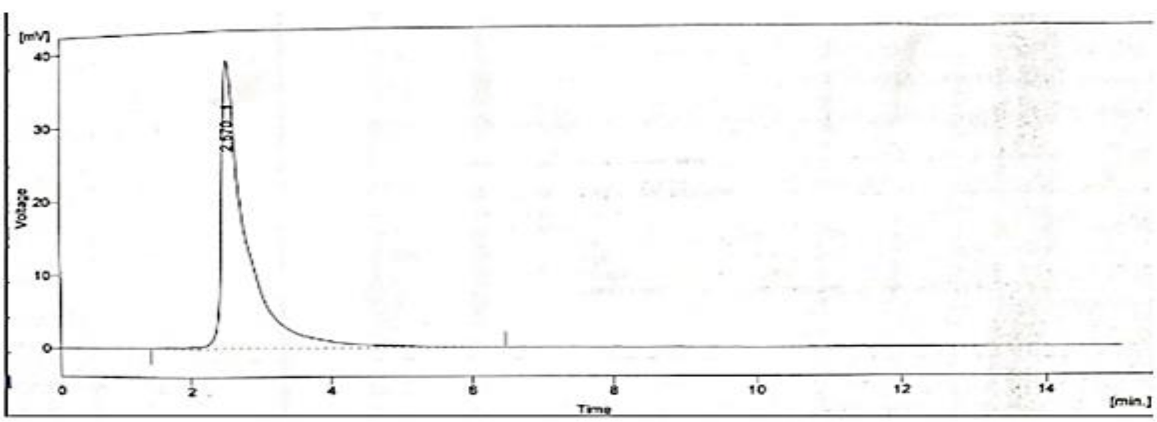

Figure 7 HPLC chromatogra of flavonoid (quercetin) at 2.570mV in standard

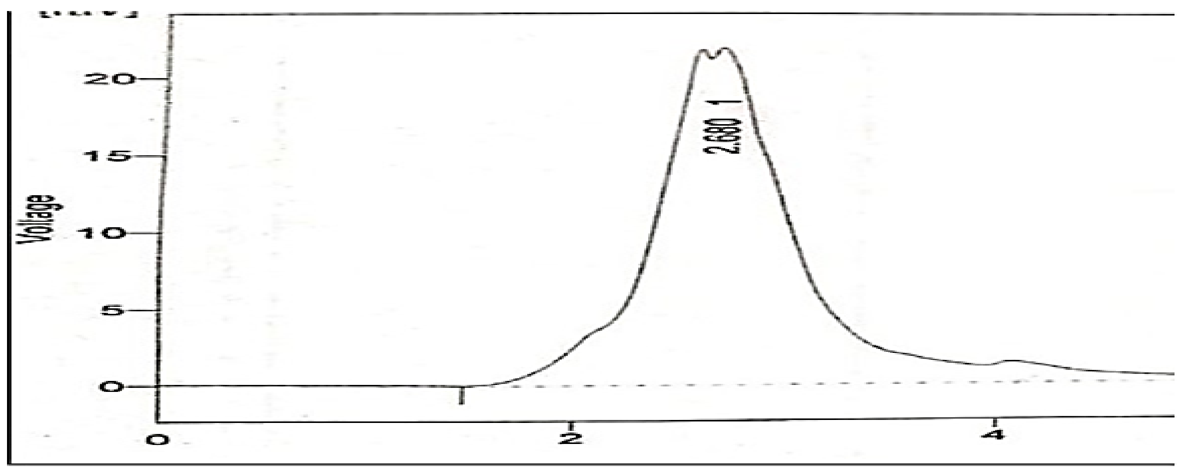

Figure 8 HPLC chromatogra of flavonoid (quercetin) at 2.6801 mV in BF1

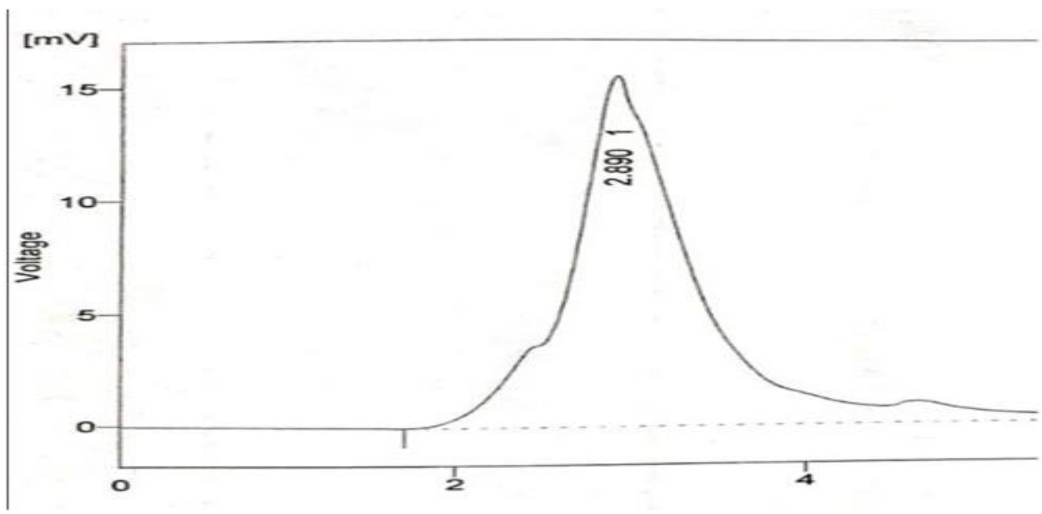

Figure 9 HPLC chromatogra of flavonoid (quercetin) at 2.890 mV in BF2

DISCUSSION

BPF1, having garlic as an important constituent, was found to be very effective in controlling insects over BPF2. Moreover, a reduction was also seen in BPF2 compared with the control group, as an increase in the threshold of insects was seen after 72, 96 and 144 h (9.28 ±0.5, 10.12±0.1 and 15.76±0.6%, respectively) in the control group. The main reason for the higher reduction in the pest population was due to the repellent properties of garlic. The findings of the study are closely match with the study reported by [5], who reported that neem, garlic, and extracts of some other plants provide strength to control pests. This pest reduction was also attributed to the modified biochemical composition of *S. lycopersicum* L. after the biopesticides sprays.

Positive results of biopesticides treated crop compared with the control group were due to the active ingredient and outstanding secondary metabolites found in the plant extracts. Flavonoids and phenolic compounds, which are naturally present in plants, act as antioxidants and antimicrobials and are good to use as repellents to various pests. The pesticide characteristics of plants are undoubtedly due to the chemical constituents present in them. Presently, a number of investigations have been carried out and concluded that plants possess a number of insecticide substances and that the extracts of plants have great control over pests, which are responsible for causing serious damage to crops or stored grains. The study conducted by[11] were in close accordance with the present study, as they found neem-based biological pesticides to have outstanding control against aphids. The most active ingredient among formulations was azadirachtin, along with some other important related triterpenoids, such as *azadirachtin* B, *salannin* and *nimbin*, and all of these secondary metabolites were due to neem plants in both formulations, which are responsible for disrupting the growth and development of insects and deteriorating their feeding.

However, the effectiveness of BPF1 was due to garlic.[22] This study suggested biopesticides as the best alternative to chemical pest killers.[23] Numerous studies have concluded that A. sativum has properties to repel insects. The active ingredient in garlic is allicin, which is a colorless liquid with a pungent smell because it has antibacterial and antifungal features. The most important point about garlic is that its active substance (*allicin*) imparts a defense mechanism against insect pests.[24] However, neem is also considered an eco-friendly insecticide, pesticide and agrochemical, as it contains the most important bioactive phytochemicals flavonoids, tannins, saponins, phenolic compounds and many mores.

Phytochemical screening of biopesticides were also is support of these organic pesticides to be used for agriculture, which were in favor of their anti-aphid characteristics, which were also in accordance with numerous studies. Flavonoids are important substances for the protection of plants against pests and fungus.[26] It has been suggested that flavonoids and isoflavonoids are the key components in protecting the plant against insects and fungi. The findings of experts reported that rutin and quercetin-3-glucoside inhibit the growth of pests with potential antifungal behavior against many other pathogenic fungi. Furthermore, quercetin/azadirachtin-based insecticides are considered safe and efficient insecticides.[27] Moreover, it was found that two polyphenolic flavonoids (flavanone naringenin and flavonol quercetin) were used as insecticides of the pea aphid.

This insect was targetted for present study because this is resposible for yield loses and can increase the chances of viral attack due to which numerous chemicals would be required for getting better production. For organic farming plant based material are recommended to be used as pesticide while *A. indica*, *S. tabacum* and *A. sativum* are already reported in preveous study to be used as antiaphid.

CONCLUSION

It was concluded that plant-based pest killers were the outstanding approach toward the IPM. This experiment was designed to control the population of aphid, as chemical sprays are becoming the cause of widespread chronic diseases to overcome them, the need of an hour is to promote organic farming. Although a combination of plant extracts were prepared as biopesticide formulations, they have proven to control aphid population of tomato crops.

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Rehana Badar	:	Performed all of the above work at IMBB and PCSIR as a part of her MS thesis and provided financial support for the above work and drafted the manuscript.
Asma Ahmed	:	Supervised the whole work at IMBB, provided all the technical and moral supports
Mehmooda Munazir	:	Collected and identified the plants as an expert botanist that was used for bio-pesticide formulations
Noman Khalique	:	Identified and supervised the maintenance of the insect population during the whole study and provided English editing services for this manuscript
Hafsa Waheed	:	Helped in methodology
Shehryar Munawar	:	Performed FTIR analysis
Shamma Firdous	:	Finalize manuscript
Hira Basheer	:	Helped in graph making