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Original Research

Application of Biocontrol Agents and Plants Extract Against Fungal Phytopathogens of Vegetable Crops

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Abstract:

The yield of vegetables is under threat due to climatic changes and resistance of pathogens against synthetic treatments and fertilizers. The soil-borne phytopathogens fungi and some climatic factors are responsible for decreasing the production of vegetables all over the world. In the present study, the vegetable plants showed symptoms of wilting, root-rot, stunted growth, chlorosis, blotch, etc. with soil collected from lower regions of Sindh, Pakistan. A total of twelve phytopathogen fungal genera *Aspergillus, Alternaria, Cladosporium, Curvularia, Nigrospora, Drechslera, Fusarium, Rhizoctonia, Macrophomina, Penicillium, Sclerotium,* and *Rhizopus* were isolated from the 80 samples of different vegetables including cabbage, Chilli, Cucumber, Okra and Tomato. But *Aspergillus, Fusarium, Macrophomina, Alternaria,* and *Rhizoctonia* were found dominant and recorded almost in all soil and root samples of vegetables. The infection was found due to several climatic factors including humid climate, the existence of moisture in the vegetable crop, the excess of water in the soil, transmission of infection by wind, gales and dust storms, packing, and the presence of moisture in storing units. For biological control and plant extract screening, two biocontrol fungal agents (*Trichoderma harzianum, Paecilomyces lilacinus*) and two plants leave extracts (*Striga hermonthica, Ocimum basilicum*) were applied in controlled lab conditions against the above-mentioned dominant phytopathogenic fungi. On the basis of this study, it is concluded that the leaves of *S. hermonthica* can be utilized as a biofertilizer in the soil. But it is necessary to need to extend this study and weed *S. hermonthica* should be studied furthermore before using it on large scale.

Keywords: biocontrol agents, phytopathogens, vegetables, plant extract

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Introduction

Vegetables are considered valuable for human health due to the presence of significant ingredients which help to repair and build up the body. They possess a rich number of mineral contents, vitamins and carbohydrates [1]. The marketing of vegetables is rapidly affected due to phytopathogenic fungi. It is threatened by some biotic factors and damaged by different soil-borne phytopathogens throughout vegetable-producing regions. The fungi are important and more affected phytopathogens which are responsible for several plant diseases and cause enormous losses of crop productivity in tropical and subtropical areas [2]. The major root-rot fungal soil-borne phytopathogens are reported in almost all crops and vegetables in Pakistan [3-6]. A high amount of vegetable varieties more than 63 vegetable species is cultivated in Pakistan during the winter and summer seasons [7]. Usman et al. [6] and Farzana [8] reported the different fungi from different areas of Sindh as well as Karachi and identified *Aspergillus flavus, Alternaria solani, Aspergillus fumigatus, Eurotium amstelodami, Aspergillus niger, Macrophomina phaseolina, Aspergillus oryzae, Rhizoctonia solani, Aspergillus terreus, Acremonium fusidioides, Cladosporium* sp., *Drechslera hawaiiensis, Ulocladium* sp., *Fusarium oxysporum, Penicillium commune* and *Trichoderma harzianum* from the soil of vegetable fields. The association of soil-borne phytopathogens fungi (root-rot) and nematode (root-knot) is damaging the roots of several vegetable crop fields including spinach, tomato, okra, brinjal and chilli. These vegetables are significantly infected by *Macrophomina phaseolina*, *Fusarium* sp., *Phytophthora* root-rot, *Rhizoctonia solani* and *Alternaria* spp. [3-5, 9].

Several researchers are involved in reducing the root-rot and root-knot disease management by nonchemical and environment-friendly strategies including organic amendment, natural fertilization, biological control, heat-based method, soil management and sanitation to improve the productivity of vegetables [10]. The use of microbes as a biocontrol vector is developing to control phytopathogens and does not have any adverse effect on the ecosystem or any other non-organisms [11, 12]. The biological control agent *Trichoderma harzianum* is highly utilized to suppress the growth of numerous soil-borne fungal phytopathogens [11, 13]. Another biocontrol agent *Paecilomyces* is also reported as an inhibitor against soil-infecting phytopathogens. In different previous investigations, it is reported that the extract of medicinal plants for soil amendment is a better choice with the conjugation of biocontrol agents i.e., *Pseudomonas aeruginosa* and *Paecilomyces lilacinus* are helpful in decreasing the activity of soil-borne phytopathogens [14]. Medicinal flora has been reported as a natural antimicrobial agent against diseases [15, 16]. The numerous plant extract materials used in ethnomedicine in remote and rural areas due to cheaper and easily accessible [17-21]. Several previous investigations have been reported on the antimicrobial and antifungal activity of *Annona squamosa, Ocimum basilicum* and *Cassia fistula* flowers and seeds along with some other medicinal plants [22-25]. The weed *Striga hermonthica* is also reported as an ethnomedicinal plant due to the use of traditional medicine. It is reported that no phytochemical studies have been previously done on plant material and extract; however, the plant material is highly beneficial against several diseases and infections [26, 27]. This weed is also reported as an antioxidant due to the presence of numerous properties of phenolic compounds including tannins anthocyanins, apigenin and luteolin [28-31].

The main domains of the study were, to explore the present status of occurrence (%) of phytopathogenic fungi in vegetable crops; to prevent the synthetic compounds and their hazards by replacing biological control and plant extracts and to find out biocontrol agents and plant extracts for the management of phytopathogenic fungi.

Material and Methods

Isolation of Soil-Borne Phytopathogens

A total of Eighty (80) samples were collected from different affected vegetable crops showing symptoms with soil from lower regions of Sindh, Pakistan. The root and soil samples were the culture in PDA (Potato Dextrose Agar) and CZA (Czapek's Agar) media. The isolates were incubated 5 to 6 d at 28ºC±2 in Dr. A.G. Lab of Aerobiology and Plant Pathology, Department of Botany, Federal Urdu University of Arts, Science & Technology, Karachi-Pakistan.

Collection and Screening Bioassay of Plant and Weed Material

The ethnomedicinal plants and weed (*Striga hermonthica*) were collected from field crops and public places. The plant materials were washed, and surface sterilization has been done with 1% sodium hypochlorite. After sterilization, plant materials were kept for drying at 40±2ºC in the oven. The plant parts and their material were grounded for powder extract. For screening of bioassay, a total of 16 g powder extract of the plant was soaked with the addition of 100 mL of distilling water. The plant exudation of bioactive compounds was placed for 24 h and filtered by standard Whatman No. 1 paper. The sieved extract was sterilized. The plants extract technique of Poison food against phytopathogenic fungi *Alternaria solani, Rhizoctonia solani, Aspergillus flavus, Fusarium* sp. and *Macrophomina phaseolina* was conducted in lab conditions. The material of plant extract at 2.2 mL of each stock solution (16% concentration) was transferred into PDA Petri plates. The filter paper disc (5 mm diam) of six days old, cultured fungi was transferred to the central part of Petri plates. There are three replicates randomized for one and all treatments. All Petri plates were placed in the incubator at 30±2ºC for six days. The final observations, mycelium and its radial growth were calculated and compared with the control.

Biocontrol Agents and Antagonistic Test

For antagonistic microorganism activity against phytopathogens was conducted in controlled lab conditions. Both antagonists and the pathogens were individually inoculated and placed at the opposite end of Petri dishes of 20 mL PDA medium. There are three Petri dishes that were utilized for one and all antagonists. The same number of controls was also placed with the alone pathogen at the center of the Petri plate. After the inoculation of antagonists and pathogens, the Petri dishes were placed for incubation at 30ºC for 6 days. The interaction was determined by the growth of the two interacting microorganisms. The diameter of the colony of an antagonist towards the pathogen was calculated. The diameter of the colony of the pathogen alone (control) and in combination (dual culture) were measured. The colony diameter (mm) and zone of inhibition (ZI) was calculated by the following formula:

$$\textbf{Inhibition}\ \% = \frac{\mathbf{Y - Z}}{\mathbf{Y}} \times \mathbf{100}$$

Where Y = Radial mycelial growth (mm) of control (pathogen alone); Z = Radial mycelial growth (mm) of pathogen and antagonist.

Table 1. Fungal disease isolation from roots and soil of vegetables.

Name of Fungi	Cabbage	Chilli	Cucumber	Okra	Tomato
Isolation from Roots					
Alternaria solani	Present	Present	Present	Present	Present
Aspergillus flavus	Present	Present	Present	Present	Present
A. niger	Present	Absent	Present	Present	Present
A. terreus	Absent	Present	Present	Present	Present
Cladosporium sp.	Present	Absent	Present	Present	Absent
Curvularia sp.	Absent	Absent	Absent	Present	Absent
Drechslera sp.	Absent	Present	Present	Present	Present
Fusarium sp.	Present	Present	Present	Present	Present
Macrophomina phaseolina	Absent	Present	Absent	Present	Absent
Nigrospora spherica	Absent	Present	Absent	Present	Absent
Penicillium sp.	Present	Present	Absent	Present	Absent
Rhizoctonia solani	Absent	Present	Absent	Present	Absent
Rhizopus sp.	Absent	Absent	Absent	Present	Present
Sclerotium rolfsii	Absent	Absent	Present	Absent	Absent
Isolation from Soil					
Alternaria solani	Present	Absent	Absent	Absent	Present
Aspergillus flavus	Present	Present	Present	Absent	Present
A. niger	Present	Present	Present	Present	Absent
A. terreus	Present	Present	Absent	Absent	Absent
Fusarium spp.	Present	Present	Present	Present	Present
Macrophomina phaseolina	Present	Present	Present	Present	Present
Penicillium sp.	Present	Present	Absent	Absent	Absent
Rhizoctonia solani	Present	Present	Present	Present	Present
Rhizopus sp.	Absent	Present	Present	Absent	Present

= Present = Absent

Identification of Fungi

The slides were examined for microscopic study by using 10 ×, 40 × and 100 × magnifications under the compound electron microscope to distinguish the hyphae, sporangiophore, sporangia, conidia, conidiophore and other physical characters. The species were confirmed by standard manuals of [32-35] and others by microscopic views.

Results and Discussion

In the present study, a total of twelve genera and fourteen different myco species were identified from roots and soil samples of different vegetables (Table 1). The three fungal species *Alternaria solani, Aspergillus flavus* and *Fusarium* sp. were more influenced as compared to other fungal species in root samples of cabbage, Chilli, Cucumber, Okra and Tomato. The Okra crop of vegetables was highly induced by soil-borne fungal phytopathogens. Almost all fungal species were observed in root samples of the Okra crop as compared to the other four vegetable crops. However, in soil samples, *Fusarium* sp., *Macrophomina phaseolina* and *Rhizoctonia solani* were more abundant as compared to other soil-borne fungi. In out of five vegetable crops, cabbage and chilli crop were highly affected due to the presence of soil-borne mycoflora.

Twelve different myco genera including *Cladosporium*, *Aspergillus*, *Curvularia*, *Nigrospora, Drechslera*, *Penicillium*, *Fusarium, Alternaria*, *Macrophomina, Rhizoctonia, Sclerotium* and *Rhizopus* were isolated from soil and roots of different vegetable crops including cabbage, chilli, cucumber, okra and tomato (Fig 1). The occurrence (%) of isolated fungi was calculated on the basis of infection and presence in samples. In this study, it is noted that the root samples were more infected as compared to the soil samples.

A total of 14 fungi species were isolated from root samples. The roots of the cabbage crop were more infected as compared to the other four crops. The maximum 57 and 58 mean occurrence % of *Aspergillus flavus* and *A. niger* were noted, respectively in root samples. However, the remaining phytopathogenic fungi infected the root samples in low abundance.

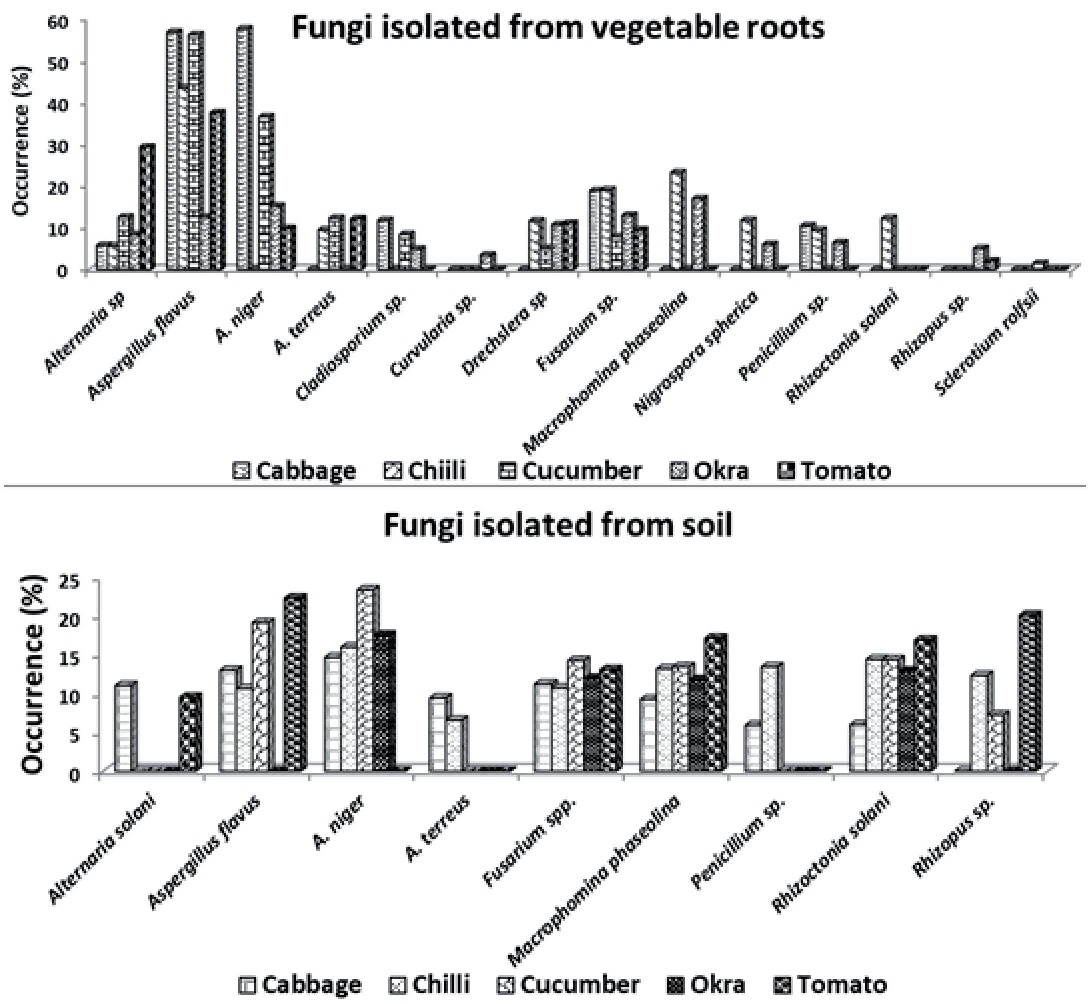

Fig. 1. Shows the occurrence % of fungi isolated from the soil and roots of various vegetable crops.

Table 2. Antagonistic fungal activity and the zone of inhibition (mm) against common soil-borne phytopathogen fungi with mean and standard error.

Phytopathogens	Zone of Inhibition (ZI)			
	Paecilomyces lilacinus	*Trichoderma harzianum*	*Striga hermonthica*	*Ocimum basilicum*
Alternaria solani	A	0.7±0.15	B	1.4±0.34
Aspergillus flavus	B	1.1±0.17	A	0.9±0.11
Fusarium sp.	A	3.1±0.27	A	B
Macrophomina phaseolina	B	2.3±0.40	1.1±0.11	1.6±0.57
Rhizoctonia solani	B	1.9±0.55	1.3±0.46	1.3±0.47

A = Overgrowth, B = Growth stop

It was very interesting to investigate that only nine fungal species were recorded from the soil samples (rhizosphere and rhizoplane). The soil of tomato and chilli crops were significantly infected with a high occurrence percentage of fungal phytopathogens. The results indicated in the soil samples that *Aspergillus niger* and *A. flavus* were maximum 23 and 22 mean percentages of occurrence, respectively as compared to the other seven fungi. However, the presence of *Fusarium, Macrophomina* and *Rhizoctonia* in almost all vegetable crops in soil samples may become a cause of heavy loss after becoming multiplication in population. It may be some synthetic treatment or chemical fertilizers were applied in vegetable crop fields a few days before. Therefore, the populations of soil-borne phytopathogens become reduced.

For biocontrol and plant extract management strategy, an antagonistic test has been conducted in controlled incubator lab conditions for six days against five majors selected phytopathogenic fungi which were almost found in all root and soil samples of vegetable crops. In Table 2, it was observed that the antagonistic activity was significantly high in *Trichoderma harzianum* and *Ocimum basilicum*. The maximum zone of inhibition (mm) was 3.1 and 2.3 mean against the *Fusarium* sp. and *Macrophomina phaseolina*, respectively as compared to the other three major phytopathogenic fungi. However, it was interesting that weed *S. hermonthica* showed 1.3 and 1.1 mean activity against *Macrophomina phaseolina* and *Rhizoctonia solani* and suppressed its growth, respectively.

The inhibition (%) of the current study is presented in Fig. 2. It showed that the maximum 92 inhibition (%) was recorded in *T. harzianum* against *A. solani*. The total leading inhibition (%) was 65.55 to 92% recorded in *T. harzianum* as compared to biocontrol agent *P. lilacinus* which was the failure to show inhibition activity against any fungal pathogen. However, in plant extract, *Ocimum basilicum* indicates a maximum of 90 % inhibition against *A. flavus* and it leads to 82.22 to 90% inhibition percentage against almost all five selected fungal phytopathogens except *Fusarium* sp. It was interesting to observe that *S. hermonthica* also evaluated 85.55 to 88% inhibition against *Macrophomina phaseolina* and *Rhizoctonia solani* which are considered highly damaging soil-borne phytopathogens in different crops and plants.

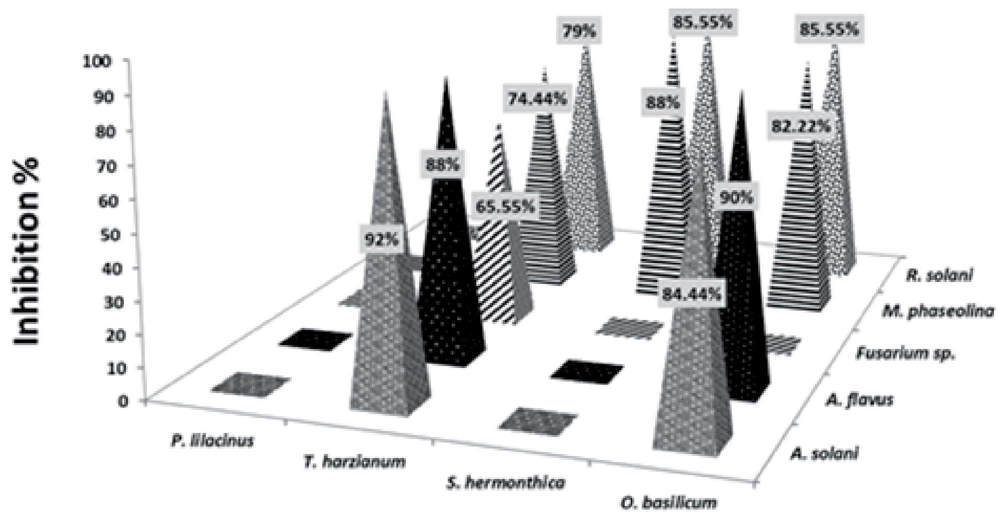

Fig. 2. shows the inhibition % of antagonistic fungi against some common soil-borne phytopathogenic fungi.

Plant extracts of plant parts are beneficial for the soil because these organic materials ultimately provide nutrients to plants and improve crop yields [36]. Phytopathogens including bacteria, nematodes, viruses and fungi cause significant damage to plants resulting in yield reductions in infested crops [37]. Considerable development has been made in the use of organic matter as soil management against soil-borne plant pathogens. Organic amendments have produced positive effects on crop viability, biological activities, soil nutrients and physical conditions [38, 39]. In several studies, different species of *Trichoderma* are well-known biocontrol agents against soil-borne diseases [3, 11, 40, 41,]. However, *Ocimum basilicum* recently has been used successfully against soil-borne phytopathogen by Mazhar et al. [8], Farzana et al. [41].

The plant extract *Striga hermonthica* was first time used against phytopathogenic fungi. This plant is reported as a plant-parasitic weed due to allelopathic secretion in its roots. It is interesting that some biocontrol agents also had been used against this weed in several previous studies to suppress the growth of this weed. But in our study, the leaf extract of this weed was used against phytopathogenic fungi which were found effective against some fungal phytopathogens. In this study, *Striga hermonthica* weed was used for the first time against soil-borne phytopathogens. This weed is reported as a plant-parasitic weed due to allelopathic secretion in its roots in maize and sorghum crops. It is interesting that some biocontrol agents also have been used against this weed in several previous studies. Abdelgadir & Bushra [27] reported that the weed *Striga hermonthica* is famous for parasitizing cereal crops in Africa and is a cause of threat to food security. On but another side it is also recognized as a beneficial weed for the ethnomedicinal purpose such as antibacterial, pharmacological abortifacient effect, pneumonia and jaundice remedy, leprosy ulcer, dermatosis, trypanocidal effects, diabetes and anti-plasmodial in the local inhabitant of Africa. The roots of weed are also approved as an abortifacient [42,43], antimicrobial and anti-diabetic in different regions of Sudan and Africa [44]. According to Mahmoud [45], *Striga hermonthica* extract can be utilized for antimalarial activity. The extract of *Striga sulphurea* is already reported as antimicrobial activities against *Pseudomonas aeruginsoa, Staphylococcus aureus, Aspergillus niger, Escherichia coli* and *Candida albicans* [43, 31]. But in our study, the leaf extract of this weed was used against soil-borne phytopathogens which were found effective against some phytopathogens. In future, *S. hermonthica* and some other weeds can be applied for the control of diseases.

Conclusions

In this recent study, it was concluded that biocontrol agents with the combination of plant extracts can be utilized for the growth improvement of crops. In this scenario, two biocontrol fungi and two plant leave extracts were applied against fungal phytopathogens. The results indicated that the use of *Trichoderma harzianum* with *Ocimum basilicum* is better for the control of fungal phytopathogens. But it was also interesting to investigate that the weed *Striga hermonthica* which is usually considered plant parasitic weed was applied against fungal phytopathogens. It successfully suppressed the growth of two destructive fungi *Macrophomina phaseolina* and *Rhizoctonia solani.* This weed can be used as an antifungal and antimicrobial activity in future. Therefore, it needs furthermore phytochemical studies and trial experiments. On the basis of this study, it is concluded that the leaves of *S. hermonthica* can be utilized as a fertilizer for the soil. But it is necessary to extend this study and weed *S. hermonthica* should be studied furthermore before using it on a large scale.

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Conflict of Interest

The authors declare no conflict of interest.

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