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RHIZOSPHERE FUNGI OF DIFFERENT VEGETABLES AND THEIR ANTAGONISTIC ACTIVITY AGAINST PATHOGENIC FUNGI OF BRINJAL AND SPINACH

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ABSTRACT

The yield of vegetables is reducing gradually every year due to the soil-borne pathogens (fungi and nematodes). Soil borne plant diseases cause significant damage to almost all crops particularly to the vegetables. Important plant soil-borne fungi which cause significant losses are *Pythium* spp., *Phytophthora blight*, *Fusarium oxysporium*, *Leveillula taurica*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Alternaria* spp. and some important nematodes were recorded for losses. During our survey conducted during July 2010 to December 2012 it was observed that in the vegetable crops of different areas of Sindh Province including Karachi (Malir, Sharafi Goth, Memon Goth and Gadap Town), Kunri, Mirpurkhas, Ghotaki, Tando Allahayar and Digri show heavy losses and several symptoms including wilting, stunted growth, chlorosis and blotch on vegetables crops. Diseased plant specimens were collected and brought to laboratory. The soil borne fungi *Rhizoctonia solani*, *Macrophomina phaseolina* and *Fusarium oxysporum* were isolated from the roots of brinjal and spinach plants collected from these areas. In antagonistic activity, four antagonistic fungi *Aspergillus flavus, A. niger, Penicillium commune* and *Trichoderma harzianum* isolated from the roots of vegetables were used against the above mentioned plant pathogenic fungi which successfully suppressed the activity of pathogenic fungi. In addition, it disclosed that *Aspergillus niger* was highly antagonistic towards *R. solani, M. phaseolina* and *F. oxysporum* as it showed a strong inhibitory effect on the growth and mycelial development.

Key-wortds: Fungi, vegetables, antagonistic activity, brinjal, spinach.

INTRODUCTION

Endophytic fungi can be transmitted from one generation to the next through tissue of host seed, vegetative propagules, horizontally, external to host tissues, by spores, carried by air, insects or small animals. These fungi based largely on pure culture of surface-sterilized stems and leaves, have revealed a broad diversity of fungal species existing subcuticularly or within the tissues of many kinds of healthy plants (Carroll, 1988). Fungal phytopathogens are the causes of many plant diseases and cause enormous losses of crop yields, especially in subtropical and tropical regions (Brimner and Boland, 2003).

Fungi are increasingly recognized as important mediators of interactions between plants and their competitors, seed dispersers, herbivores, and pathogens (Carroll, 1988; Clay, 1988; Chapela, 1989). Fungal endophytes have also been recognized as a repository of novel secondary metabolites, some of which have beneficial biological activities (Bills and Polishook, 1991). Use of antagonistic organisms against *Macrophomina* root rot has been well documented in several crops (Mukhopadhyay, 1987; Raguchander *et al*., 1995).

Most of fungal antagonistic have been used because of their high antifungal properties (Kayya and Okech, 1990). Biological control of plant pathogen by microorganism has been considered more natural and environmentally acceptable alternative to the existing chemical methods (Baker and Paulitz, 1996). Biological control has been developed as an alternative to synthetic fungicide treatment and considerable success has been achieved in utilizing antagonistic microorganism to control both pre-harvested and post-harvested diseases (Janisiewicz and Korsten, 2002).

Microorganisms as biological control agents have high potential to control plant pathogens and do not have any adverse effect on the environment (or) other non-target organisms (Yang et *al*., 2008). *Trichoderma* species are well known antagonistic fungi useful in controlling soil borne diseases (Howell and Stipanovic *et al*., 1983; Howell, 2003; Ha, 2010).

A biocontrol agent may act against pathogens by using one or more of the following mechanisms: competition, antibiosis, and parasitism well as activating host defense mechanisms (Papavizas and Lumsden, 1980). In fact, several fungi have been reported to be effective biocontrol agents of some fungi on Potato dextrose agar (PDA) medium. Among these are species of *Glioclodium* (Papavizas, 1985; Van den Boogert, 1996), *Trichoderma*

(Papavizas, 1985; Wilson *et al.*, 2008), *Penicillium* (Chand and Logan, 1984) and *Aspergillus* (Feng *et al.*, 2008) were frequently isolated from sclerotia of some fungi.

The present study focuses on evaluating the antagonistic activity of some fungi, isolated from the rhizospheres and rhizoplanes of some vegetables, against some pathogenic fungi including *Rhizoctonia solani*, *Macrophomina phaseolina* and *Fusarium oxysporum.*

MATERIALS AND METHODS

Isolation of Pathogens:

The infected plants of Brinjal and Spinach were collected from the different localities of Karachi *i.e.* Malir, Sharafi Goth, Memon Goth and Gadap Town during the period July 2010 to December 2012. The infected roots were placed in Petri dishes which contained Potato Dextrose agar medium and Czapek's agar medium after surface sterilization by 2 % Ca $(OCI)_2$ for 1 min. These Petri dishes were incubated for 5-6 days at 28°C for the isolation of fungi.

Identification of fungi:

Isolated fungi were identified using the standard manuals or refernces including Ellis (1971;1976), Barnett and Hunter (1972), Nelson *et al.* (1983), Singh *et al*., (1991) and Sutton (1980).

Preparation of inocula of antagonistic fungi:

Native potential fungal antagonists were isolated from the rhizospheres and rhizoplanes of various vegetables collected from fields. The affected roots of vegetable plants were detached and surface-sterilized with 2% sodium hypochlorite. Isolations were made from these vegetable plant roots in Potato Dextrose agar (PDA) and Czapex dox agar at room temperature (28 ± 2°C) for 5-6 days, to allow for the growth of all organisms. Isolations were also made from the respective field soils. Petri plates containing potato dextrose agar (PDA) were inoculated with the fungi (Odigie and Ikotun, 1982). Stock cultures of the isolates were maintained at 4° C in bottle slants for subsequent studies. Biocontrol agents including *T. harzianum*, *A. niger*, *A. flavus* and *P. commune* were obtained from the rhizospheres and rhizoplanes of vegetables. These fungi were multiplied on Potato Dextrose agar and Czapex dox agar for 5-6 days at 28°C.

Antagonism test:

In order to select some suitable antagonistic micro-organism against the pathogens were evaluated in the laboratory on PDA. Both antagonists and the pathogens were simultaneously inoculated at the opposite ends of the Petri dishes containing about 15 to 20ml PDA. Three Petri dishes were used for each antagonist and the same number was kept as control with the pathogen alone plated on one side of the Petri dish at the periphery. The inoculated Petri dishes were incubated at 30°C for 6 days and the final observations were recorded. The interaction was determined by the growth of the two interacting microorganisms. The colony diameter of the antagonist towards the pathogen was recorded. The colony diameter of the pathogen alone (control) and in combination (dual culture) were measured. Percentage decrease over the control was calculated by the following formula:

$$\text{Percent decrease over control} = \frac{\text{Average colony diameter} - \text{Average colony diameter of the pathogen against the antagonist in the treatment}}{\text{Average colony diameter of the pathogen in the treatment}}$$

Some of the antagonists caused inhibition at a noticeable distance whereas the others inhibited the pathogen on contact and continued to grow over the inhibited colony.

RESULT AND DISCUSSION

Fig.1. shows the effect of four antagonistic fungi on the colony growth of the three pathogenic fungi. *F. oxysporum* was best controlled by *A. flavus* and *T. harzianum* as indicated by colony inhibition. *R. solani* was highly inhibited by *A. flavus* and *P. commune*. On the other hand, *M. phaseolina* colony growth was highly suppressed by *P. commune* only.

Table 1. F- ratios derived from ANOVA for antagonist effect on different pathogen fungi based on analysis of colony diameters.

Source	F-ratio	P-value	$LSD_{0.05}$
All three Pathogen fungi	1.08	0.3530ns	0.87
Antagonist fungi (4 spp.)	4.30	.0146*	1.01
Pathogenic fungi × Antagonist fungi	6.07	.0006***	

F= F-ratio was obtained from ANOVA tables, **LSD**=Least significant difference at P=0.05

Table 1 shows the results of ANOVA for antagonist effect on different pathogenic fungi. Three fungal species including *F. oyxsporum, M. phaseolina* and *R. solani* showed significant differences and were inhibited by antagonist including *A. flavus, A. niger, P. commune* and *T. harzianum*. All four species proved as being antagonistic to at least one of the fungal pathogens.

All the isolates in the study inhibited the growth of *F. oxysporum, R. solani* and *M. phaseolina* to varying degree but significantly ($P < 0.05$) (see Fig. 1). Among these isolates, *A. flavus* and *P. commune* showed greater effectiveness as antagonists inhibiting the growth of the pathogen by 73% and 70% respectively. The least antagonist was *A. flavus* and *P. communes* which inhibited the growth of the pathogen by 61.33% and 57.6% respectively. *M. phaseolina* and *R. solani* were also inhibited by all the isolates used in the experiment as shown in Fig. 1.

Surprisingly the interaction of antagonists and the pathogenic fung was found to be highly significant ($P < 0.001$).

Pawar and Patel (1957) has recorded severe blighting under storage conditions even though John (1991) has recorded severe blighting of the stem and root of brinjal and spinach under field conditions. Majority of the fungi associated with spoilage were already reported to be seed-borne (George, 1992). Pathak *et al.,* (1981) has recorded hyphal parasitism by coiling, penetration, rupture of the host hyphae and ramification inside the host by antagonistic activity.

El-Mehalawy (2004) reported that these antifungal organisms may interact with sensitive cells involving the cellular membrane. As a result of this interaction, the membrane no larger serve as a selective restraining barrier and the specific permeability of the cell wall will be loosed.

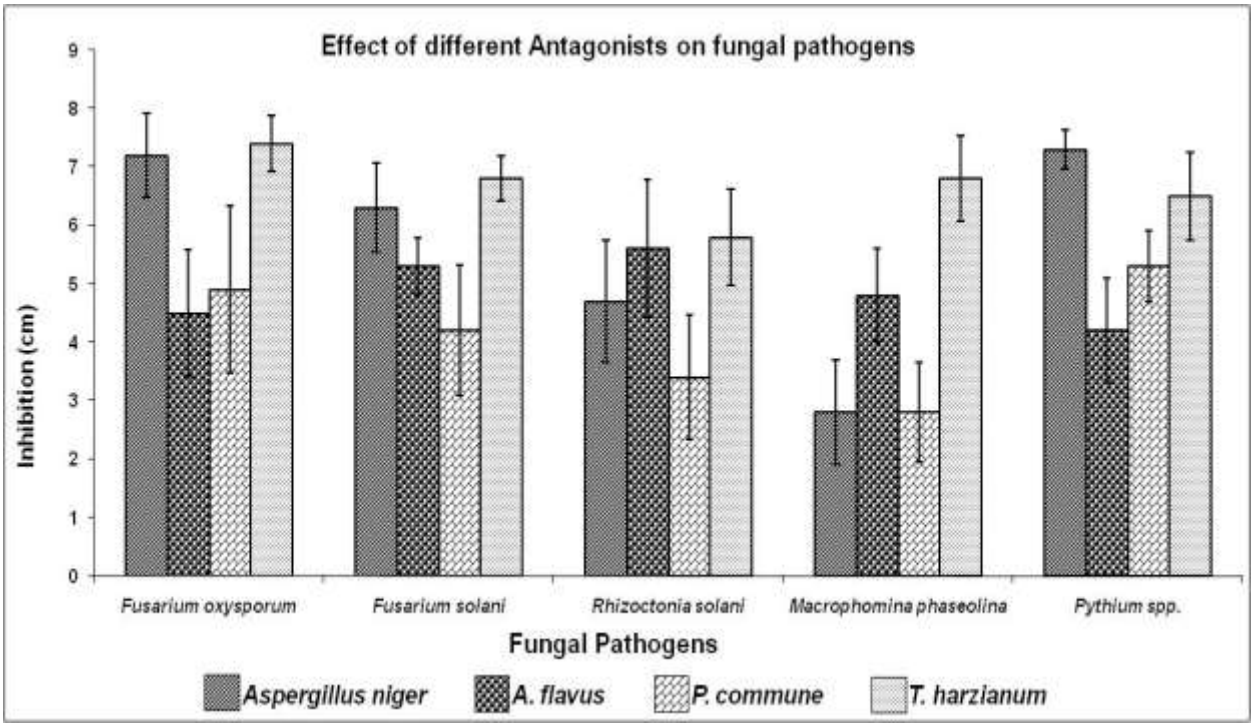

Fig. 1. The effect of different antagonists on growth inhibition of selected fungal pathogens at 6 days of culture. Inhibition implies reduction in colony diameter.

The result confirm the findings of Gokulapalan and Nair (1984); Gogoi and Roy (1993); Zhang and Wu (2011). *Aspergillus niger*, the antagonist also showed inhibitory activity against several other pathogenic fungi, including *Fusarium graminearum, Fusarium oxysporum, Fusarium decemcellulare, Alternaria alternata , Exserohilum turcium, cochliobolus heterostrophus, Rhizoctonia solani, Alternaria* sp. They reported that some *A.niger* and *A. terreus* etc. were found to be antagonistic fungi against *R. solani* by inhibiting the growth of several fungi when tested under in vitro. These results proves that some *Aspergillus* sp. have the capacity of inhibition and effected the growth of *F. oxysporum, M. phaseolina* and *R. solani* mycelium and sclerotial bodies. The mechanism of antagonistic behavior of Trichoderma has been elaborated by Howell (2003).

In the present scenario various reports are available on the potential use of biocontrol agents as replacements of agrochemicals (Schimizu *et al.,* 2000; Yang *et al.,* 2008).

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