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Medicinal plants rhizosphere exploration for the presence of potential biocontrol fungi

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Abstract
During the present study, different fungi including *Paecilomyces sp*. from the rhizosphere of two medicinal plants i.e. *Aloe vera* (Ghee kuwar) and *Punica granatum* (Pomegranate) were isolated. For further biocontrol studies, *Paecilomyces sp*. which produced the highest (9 mm) and the lowest (2.7 mm) inhibition zone against *Fusarium solani* and *Helminthosporium sp*, respectively in dual culture tests was used. In screen house study the application of conidial concentration of *Paecilomyces sp.* against Fusarium wilt of chilli significantly reduced the disease. The highest disease reduction (90.1%) was recorded in the treatment when the chilli seedling roots were prior dipped in the conidial concentration of (1×10^4 conidia ml^{-1}) of *Paecilomyces sp.* Application of *Paecilomyces sp.* also positively affected the yield and the biomass i.e. fresh and dry shoot weight, fresh and dry root weight and plant height of chilli plants.

Keywords: Rhizosphere, *Paecilomyces sp*, *Fusarium solani*, medicinal plants, *Aloe vera*, *Punica granatum* L

1. Introduction
Medicinal plants constitute a large portion of the flora which provide raw materials for cosmetic and pharmaceutical industries i.e. *Punica granatum* L. (Pomegranate). It is medicinal fruit specie that belongs to family *Punicaceae*. It is a rich source of protein, carbohydrate, antioxidant, minerals, and vitamin A, B and C. It is also useful in treating tuberculosis, diarrhoea, acidity, leprosy patients, fever and pain in the abdomen. Similarly *Aloe vera* (Ghee kuwar) is another medicinal plant species that belongs to *Liliaceae* family. *Aloe vera* gel is a traditional medicine for inducing wound healing, anti-cancer, and anti-viral agent. *Aloe vera* makes arbuscular mycorrhizal associations, a linkage that helps plant in getting nutrients from the soil [13]. Due to huge colonization capacity and nitrogen fixation, the colonization of microorganism in the rhizosphere of medicinal plants is more as compared to non-rhizosphere soil and endosymbiotic linkages are established after entering the rhizosphere soil [18]. It stimulates the exudation of plant growth promoting substances e.g. kinetins, vitamins and gibberellins, that help in increasing the quality and quantity of the crops.
The major source of biological diversity is present in soil which is important for the ecosystem and rhizosphere [9]. The microorganisms are active in this area [19] involving huge community of microbes that influence plant development. The community structure of these microorganisms is influenced by specie of plant present, soil structure, land preparation, interacting microbes accompanied with effects of environment [16]. Microbes are added to rhizosphere for increasing soil aggregation, to suppress pathogenic microorganisms enhancing plant development [12].
Soil microorganisms establish relationship between plants and soil [11]. Fungi e.g. *Penicillium oxalicum*, *Trichoderma viride*, and bacteria e.g. *Bacillus subtilis* are regarded as useful microorganisms, *Rhizobacteria* species of *Trichoderma* and *Gliocladium* and some other sterile fungi have been investigated to enhance growth of plants [3]. Provision of nutrients to plants is enhanced due to the activities of rhizosphere microorganisms [2]. Biological control can be defined as the utilization of useful microbes, e.g. fungi or bacteria that stop pathogenic microbes to attack plants [6]. Biological control is an effective way to reduce the losses due to pathogens. Plant growth as a result of PGPR is stated to be due to fixing nitrogen, nutrients solubilization, enhancing activities of mycorrhiza, balancing ethylene within roots, production

of phytohormones while lowering heavy metals [17]. Biocontrol agents colonize and prevent disease causing organisms to attack plants. When mycorrhizal fungi colonize roots, they enhance the quality and quantity of yields in many crops due to more access to nutrients. The activities of arbuscular mycorrhizal fungi end pressure in plants due to allelopathy, resulting in development of crop with good yield [4]. For enhancing growth in plants, small amount of hormones are important which are produced by rhizosphere microbes [10]. Management of rhizosphere microorganisms positively affect the yield and quality of crops. Research is continuous throughout the world to utilize these microorganisms to increase agricultural productivity. Despite the valuable service of rhizosphere microorganisms to agriculture, this area still remains less-discovered. More research is needed to understand how these microorganisms can be used to control diseases of field crops.

Chilli (*Capsicum annum* L.) is an important vegetable crop of Pakistan. It has many medicinal and economical values. It is used in meals for pungency and red colour. It is an excellent source of phosphorus and vitamin A, B, C, and E. Chilli crop contributed 1.5 per cent share in the GDP of Pakistan and earned Rs. 1.127 billion through export of red chilli powder during 2003-2004. This reveals the potential of chilli crop. However, its production has declined from 86.5 thousand tons in 1994-95 to 55.8 thousand tons during 2003-2004 (Pakistan Horticulture Development and Export Board, PHDEB). This decline in yield is due to a number of factors including poor quality seed, mal-cultural practices and diseases.

One of the most economically damaging diseases of many vegetables including chilli is Fusarium wilt caused by *Fusarium solani*. The characteristic symptoms of the disease are wilting, chlorosis and stunted growth [9] and finally causes plant death. The disease is difficult to control due to many reasons e.g. the pathogen is soil inhabiting and polyphagus in nature. The most economical and eco-friendly method is the use of resistant cultivars but new races of pathogens break the resistance of the crop. Application of chemicals for controlling diseases is neither economical nor environmental friendly [16]. The difficulty in controlling such diseases has stimulated researchers to explore biological control agents. The present study was conducted to explore the rhizosphere of two medicinal plants i.e. *Aloe vera* and *Punica granatum* L. for the presence of potential biocontrol fungi and to check the biocontrol effect *in vitro* and *in vivo* against plant pathogens.

2. Materials and Methods

In present study two medicinal plants (*Aloe vera* and *Punica granatum* L.) were obtained from Botanical Garden, University of Peshawar. The samples having rhizosphere soil surrounding their roots were processed after 24 hours of sampling at the Plant Pathology Laboratory, the University of Agriculture Peshawar.

Wet sieving and decanting technique for the isolation of rhizosphere fungi

The procedure described by Daniels and Skipper [5] for the isolation of rhizosphere fungi was followed. In this technique 100 g of soil from each of the two samples was put into a 2 L container and 1.5 L water was added and suspension was made. Four replications of the samples were made. The suspension was gently mixed to make free the spores present in the soil and the roots. It takes 35 to 40 seconds for the heavy particles to settle down. Standard sieves of different sizes were used to get the desired spores e.g. 250 µm, 100 µm and 53 µm. The supernatant were decanted through these sieves. The sievings retained on the different sieves were washed into separate test tubes for further observations and purification by sucrose centrifugation.

Sucrose centrifugation for the isolation of rhizosphere fungi

Centrifugation process was done after resuspending sievings in 50 percent sucrose solution to further purify the spores in the sievings. The sievings were transferred to centrifuge tubes of 10 ml volume and centrifugation was done at 2000 rpm for 5 min. Supernatants were removed with care without disturbing the pellet. The soil particles were suspended in chilled 50 percent sucrose, the contents were mixed with a spatula and the samples were centrifuged quickly at 2000 rpm for 1 min. The supernatant were sieved through the small mesh sieve and the spores held on the sieve were carefully rinsed with tap water and the spores were washed and collected into a petri plate.

The spore suspension was added to petri dishes plated with about 10 ml potato dextrose agar medium (PDA), containing streptomycin. Each dilution was replicated three times and the plates were kept in incubator for 7 days at 25 ± 2 ºC. After spore germination, small plugs from the fungal cultures were transferred to other plates containing PDA medium for similar fungal colony growth to identify the spore easily.

In vitro screening of fungal isolates for their antifungal activities

To observe the biocontrol potential of the *Paecilomyces sp*, dual culture method was used. *Paecilomyces sp*. and the available pathogens in plant pathology lab, the University of Agriculture Peshawar, were allowed to grow in the same petri dish. *Paecilomyces sp*. plug was put on one side and that of the pathogen on other side of the petri dish. The inoculated petri plates were observed for the formation of inhibition zones or overgrowth otherwise.

Investigating the biocontrol potential of *Paecilomyces sp.* in screen house

Due to the highest zone of inhibition (9 mm) formed by *Paecilomyces sp.* against *Fusarium solani in vitro*, screen house experiment was conducted against chilli wilt disease caused by *Fusarium solani*. Local variety of chilli (*Capsicum annum* L.) as selected for this purpose. Spore suspension of biocontrol agent (*Paecilomyces sp*.) and the pathogen (*Fusarium solani*) were prepared and the number of spores was measured with the help of Haemocytometer. The concentration of *Fusarium solani* was adjusted to 1×10^4 conidia ml^{-1} and two concentrations of *Paecilomyces sp.* (1×10^4 conidia ml^{-1} and 1×10^6 conidia ml^{-1}) were prepared. Plants were treated by dipping method. Plants were dipped in conidial concentration of *Paecilomyces sp*. for 10 minutes followed by *Fusarium solani* with a 10 minutes break in between the two treatments. Then the plants were sown in pots having sterilized soil in the screen house. For comparison, untreated plants used were considered as control.

Treatment combinations used in the screen house experiment

T1=P0B0, T2 = P0B1, T3= P0B2, T4= P1B0, T5 = P1B1, T6= P1B2

Where, P0 =No *Fusarium solani* P1 = $1x10^4$ conidia $ml^{-1}$of *Fusarium solani*

B0 = No *Paecilomyces sp.* B1= $1x10^4$ conidia $ml^{-1}$of *Paecilomyces sp.*

B2= $1x10^6$ conidia $ml^{-1}$of *Paecilomyces sp.*

Ten days after inoculation, data was taken for disease symptoms at two days intervals for six weeks. The disease severity was assessed through visual observation. Following scale reported by De Cal *et al*. [7] was used.

Scale	Percent disease severity	Remarks
1	0- 24%	Healthy plant, all leaves green
2	25-49%	Lower leaves yellow
3	50-74%	Lower leaves dead and upper leaves yellow
4	75-99%	Upper leaves wilted, lower leaves dead
5	100%	Dead plant

Data on the following agronomic parameters were also recorded for investigating the plant growth promoting effect of the biocontrol agent.

- Fresh shoot and root weight
- Dry shoot and root weight
- Plant height
- Fruit length
- Fruits $plant^{-1}$

Chilli plants were uprooted 60 days after planting and their roots were washed with tap water. The plant heights were measured and the fresh and dry weight of roots and shoots were weighed. Dry root and shoot weight was also recorded.

Data analysis

Two factor factorial CR design was used in this research. Data analysis was done through statistics software (Statistix 8.1) with various parameters as dependent variable and analysis of variance (ANOVA) with treatments as independent variables.

3. Results

Isolation of rhizosphere fungi

In Present experiment many fungi were isolated (Table 1) from the rhizosphere of two medicinal plants i.e. *Aloe vera* (Ghee Kuwar) and *Punica granatum* (Pomegranate). Identification was done by observing the structure of spores, and colony morphology.

Effect of *Paecilomyces sp.* on the growth of different fungal pathogens in dual culture experiment

Paecilomyces sp. made inhibition zones against different fungal pathogens in dual culture experiment (Table 2) which varied with the pathogens. The highest zone of inhibition (9 mm) was formed against *Fusarium solani* and the lowest zone (2.7 mm) was formed against *Helminthosporium*. This showed the antifungal potential of *Paecilomyces sp* against plant pathogens in dual culture.

Table 1: List of fungi isolated from the rhizosphere of medicinal plants *Aloe vera* and *Punica granatum* L.

S. No.	Fungal genus	*Aloe vera*	*Punica granatum*
1	*Penicillium sp.*	+	-
2	*Paecilomyces sp.*	+	+
3	*Fusarium oxysporum*	+	_
4	*Alternaria*	+	+
5	*Rhizopus sp.*	+	+
6	*Aspergillus flavus*	+	+
7	*Aspergillus niger*	+	+
8	*Unknown fungi	+	+

*Not identified.
+ = presence of fungus
- = absence of fungus

Table 2: Effect of *Paecilomyces sp.* on the growth of different pathogenic fungi in dual culture experiment

S. No	Pathogen	Inhibition zone (mm)	Over growth
1	*Aspergillus flavus*	*4±0.58	-
2	*Aspergillus niger*	-	+
3	*Rhizopus sp.*	5.3±0.88	-
4	*Curvularia sp.*	3.3±0.67	-
5	*Helminthosporium sp.*	2.7 ±0.33	-
6	*Alternaria*	6.66±0.33	-
7	*Fusarium oxysporum*	3±0.58	-
8	*Fusarium solani*	9±0.58	-
9	*Rhizoctonia solani*	6.66±0.88	-

*Mean±SE
+ = presence of inhibition zone or overgrowth
- = absence of inhibition zone or overgrowth

Effect of root treatment with *Paecilomyces sp.* on the severity of Fusarium wilt of chilli in screen house experiment

Root treated with different conidial concentrations of *Paecilomyces sp.* resulted in significant disease reduction of Fusarium wilt of chilli crop. The percent disease reduction (Table 3) was different at different inoculum concentrations of *Paecilomyces sp.* used in the experiment. No disease symptoms were observed in P0B1 treatment while in case of P0B2 the disease severity was 13.6%. The highest disease severity (76.4%) was recorded in P1B0 treated plants whereas in P1B1 treated plants disease severity was 9.9%. In case of P0B0 25.2% disease was recorded. The reason might be the spread of pathogen with water drops during watering the pots and soil particles carried by wind.

It was observed that lower conidial concentration of *Paecilomyces sp*. was more effective. Plants treated with only *Fusarium solani* showed severe disease symptoms and those treated with only *Paecilomyces sp*. showed no disease symptoms.

Fresh shoot weight

Fresh shoot weight of chilli plants was also observed to analyse the effect of *Paecilomyces sp*. on growth promotion. Data presented in Table 4 shows that the highest shoot weight (32.0 g) was recorded from P0B1 treated plants while the lowest fresh shoot weight (22.1 g) was recorded in P1B0 treatment. In the combination of *Fusarium solani* with *Paecilomyces sp.* higher shoot weight (29.9 g) was recorded in P0B1treated plants whereas lower shoot weight (23.4 g) was recorded from those plants treated with P0B2. The data showed that lower concentration of *Paecilomyces sp*. was more effective against *Fusarium solani* that promoted good growth of the crop.

Table 3: Effect of treatment of *Paecilomyces sp.* and *Fusarium solani* on disease severity of *Capsicum annum* L.

Fusarium solani	*Paecilomyces sp.*			Mean
	BO (0)	**B1**($1x10^4$)	**B2** ($1x10^6$)	
P0 (0)	25.2b	0.0d	13.6c	12.9b
P1 ($1x10^4$)	76.4a	9.9c	21.3b	35.9a
Mean	50.8a	4.9c	17.5b	

* Column bearing same letter (s) are not significantly different at (P<0.05).

LSD value for *Paecilomyces sp.*	3.6
LSD value for *Fusarium solani*	2.97
LSD value for *Paecilomyces sp.* x *Fusarium solani*	5.1

Table 4: Effect of treatment of *Paecilomyces sp.* and *Fusarium solani* on fresh shoot weight of *Capsicum annum* L.

Fusarium solani	*Paecilomyces sp.*			Mean
	BO (0)	**B1($1x10^4$)**	**B2($1x10^6$)**	
P0 (0)	30.7a	32.0a	30.2a	31.0a
P1 ($1x10^4$)	22.1b	29.4a	23.4b	24.9b
Mean	26.4b	30.7a	26.8b	

* Column bearing same letter (s) are not significantly different at (P<0.05).
LSD value for *Paecilomyces sp* 3.3670
LSD value for *Fusarium solani* 2.7491
LSD value for *Paecilomyces sp*. x *Fusarium solani*4.7616

Dry shoot weight

Different treatment combination effects of *Fusarium solani* and *Paecilomyces sp*. on dry shoot weight of chilli crop is given in Table 5. The highest dry shoot weight (15.0 g) was observed in case of plants with P0B1treatment whereas the lowest dry shoot weight (10.8 g) was recorded in case of plants treated with P1B0. Dry shoot weight of plants in the treatment with P1B1 was higher (13.7 g) as compared to the plants treated with P1B2 that was (11.6 g). In case of P0B0 dry shoot weight was 14.5 g.

It means that the lower conidial concentration of *Paecilomyces sp*. was effective in increasing the dry shoot weight of chilli plants.

Fresh root weight

The measurements of fresh root weight of chilli crop (Table 6) reveal that the highest weight (6.2 g) was taken from P0B1 treated plants while the lowest fresh root weight (3.2 g) was observed in case of P1B0 treatment. In the treatment P1B1 the higher fresh root weight was 5.4 g, while with P1B2 the fresh root weight was 4.1g.

This showed that the lower conidial concentration of *Paecilomyces sp.* was effective in increasing the fresh root weight.

Table 5: Effect of treatment of *Paecilomyces sp.* and *Fusarium solani* on dry shoot weight of *Capsicum annum* L.

Fusarium solani	*Paecilomyces sp.*			Mean
	BO (0)	**B1($1x10^4$)**	**B2 ($1x10^6$)**	
P0 (0)	14.5a	15.0a	14.8a	14.8a
P1 ($1x10^4$)	10.8b	13.7ab	11.6ab	12.1b
Mean	12.7a	14.3a	13.2a	

* Column bearing same letter (s) are not significantly different at (P<0.05).
LSD value for *Paecilomyces sp*. 2.4661
LSD value for *Fusarium solani* 2.0135
LSD value for *Fusarium solani* x *Fusarium solani* 3.4875

Table 6: Effect of treatment of *Paecilomyces sp.* and *Fusarium solani* on fresh root weight of *Capsicum annum* L.

Fusarium solani	*Paecilomyces sp.*			Mean
	BO (0)	**B1($1x10^4$)**	**B2 ($1x10^6$)**	
P0 (0)	4.2c	6.2a	4.6bc	5.0a
P1 ($1x10^4$)	3.2d	5.4ab	4.1cd	4.2b
Mean	3.7b	5.8a	4.3b	

* Column bearing same letter (s) are not significantly different at (P<0.05).
LSD value for *Paecilomyces sp*. 0.6488
LSD value for *Fusarium solani* 0.5297
LSD value for *Fusarium solani* x *Fusarium solani* 0.9175

Dry root weight

To see the effect of *Paecilomyces sp*. on dry root weight was also recorded (Table 7). The highest dry root weight (4.1 g) was recorded in case of P0B1 and the lowest dry root weight (2.3 g) was observed in the treatment of P1B0.While in the interaction of the *Fusarium solani* and the *Paecilomyces sp,* the higher dry root weight (2.7 g) was recorded from the plants treated P1B1 and lower dry root weight (2.4 g) was recorded from the plants treated P1B2.

Plant height

To see the plant growth promoting effect of *Paecilomyces sp,* plant height of chilli crop was also measured (Table 8). The highest plant height (34.2 cm) was observed in case of P0B1 treated plants and the lowest (21.2 cm) was in the case of P1B0 treated plants. While in the interaction of the *Fusarium solani* and *Paecilomyces sp.* the higher plant height (32.7 cm) was recorded from the plants treated with P1B1 and the lowest plant height (24.8 cm) was observed from the plants treated with P1B2.

Table 7: Effect of treatment of *Paecilomyces sp.* and *Fusarium solani* on dry root weight of *Capsicum annum* L.

Fusarium solani	*Paecilomyces sp.*			Mean
	BO (0)	**B1 ($1x10^4$)**	**B2 ($1x10^6$)**	
P0 (0)	2.4b	4.1a	2.7b	3.0a
P1 ($1x10^4$)	2.3b	2.7b	2.4b	2.5b
Mean	2.4b	3.4a	2.5b	

* Column bearing same letter (s) are not significantly different at (P<0.05).
LSD value for *Paecilomyces sp.* 0.5751
LSD value for *Fusarium solani* 0.4696
LSD value for *Fusarium solani* x *Fusarium solani* 0.8133

Table 8: Effect of treatment of *Paecilomyces sp.* and *Fusarium solani* on plant height of *Capsicum annum* L.

Fusarium solani	*Paecilomyces sp.*			Mean
	BO (0)	**B1($1x10^4$)**	**B2 ($1x10^6$)**	
P0 (0)	30.4a	34.2a	30.5a	31.7a
P1 ($1x10^4$)	21.2b	32.7a	24.8b	26.2b
Mean	25.8b	33.5a	27.7b	

* Column bearing same letter (s) are not significantly different at (P<0.05).
LSD value for *Paecilomyces sp.* 3.4942
LSD value for *Fusarium solani* 2.8530
LSD value for *Fusarium solani* x *Fusarium solani* 4.9415

Yield

At the end of field experiment, yield i.e. total number of fruits plant^{-1} of chilli crop was also determined (Table 9). The highest yield (22.7 fruits plant^{-1}) was observed from the plants which were treated with P0B1 and the lower yield (7.6 fruits plant^{-1}) was recorded in case of plants which were treated with P1B0. In the interaction of the *Fusarium solani* with *Paecilomyces sp.* the higher yield (12.3 fruits plant^{-1}) was recorded from the plants treated with P1B1 and the lower yield (11.4 fruits plant^{-1}) was recorded from the plants treated with P1B2.

It was obvious that the lower conidial concentration of *Paecilomyces sp.* affected the fruit setting in chilli crop positively.

Fruit length

Data of fruit length (Table 10) of chilli crop showed that the highest fruit length (7.4 cm) was observed in the treatment of P0B1 and the lowest fruit length (3.7 cm) was in the case of P1B0. Whereas in the combination of *Fusarium solani* and *Paecilomyces sp.* the higher fruit length (6.4 cm) was recorded from the plants treated with P1B1 and lower fruit length (4.6 cm) was noticed from the plants treated with P1B2.

Table 9: Effect of treatment of *Paecilomyces sp.* and *Fusarium solani* on fruit plant^{-1} of *Capsicum annum* L.

Fusarium solani	*Paecilomyces sp.*			Mean
	BO (0)	B1(1x10^4)	B2 (1x10^6)	
P0 (0)	15.0b	22.7a	16.6b	18.1a
P1 (1x10^4)	7.6d	12.3b	11.4c	10.4b
Mean	11.3 c	17.5a	14.0b	

* Column bearing same letter (s) are not significantly different at (P<0.05).

LSD value for *Paecilomyces sp*. 1.2185
LSD value for *Fusarium solani* 0.9949
LSD value for *Fusarium solani* x *Fusarium solani* 1.7232

Table 10: Effect of treatment of *Paecilomyces sp.* and *Fusarium solani* on fruit length (cm) of *Capsicum annum* L.

Fusarium solani	*Paecilomyces sp.*			Mean
	BO (0)	B1(1x10^4)	B2 (1x10^6)	
P0 (0)	4.5d	7.4a	5.4c	5.8a
P1 (1x10^4)	3.7c	6.4b	4.6d	4.9b
Mean	4.1c	6.9a	5.0b	

* Column bearing same letter (s) are not significantly different at (P<0.05).

LSD value for *Paecilomyces sp*. 0.3234
LSD value for *Fusarium solani* 0.2641
LSD value for *Fusarium solani* x *Fusarium solani* 0.4574

4. Discussion

The unscrupulous use of chemical pesticides in modern agriculture has diverted the attention of biologists to search for alternative ways and means of using biological agents that may help in controlling plant diseases and increasing productivity of agricultural crops. Many pathogenic soil borne fungi are suppressed in some soil types e.g. *Gaeumannomyces graminis*, *Fusarium oxysporum*, *Phytophthora* and *Pythium* species. However, it is not necessary if one soil type suppresses a pathogen may suppress other pathogens. Therefore, biologists are investigating biological control agents that can be used in controlling plant diseases [21].

Due to more colonization and nitrogen fixation by microorganisms in the rhizosphere of medicinal plants as compared to non-medicinal plants, two medicinal plants i.e. *Aloe vera* and *Punica granatum* were selected to investigate their rhizosphere for the presence of potential biocontrol fungi. Many fungi were isolated from the rhizosphere of these two medicinal plants. *Paecilomyces sp*. was selected as potential biocontrol agent due to its presence in the rhizosphere of both medicinal plants. *Paecilomyces sp*. produced different length of inhibition zones against different pathogens but the highest inhibition zone (9 mm) was formed against *Fusarium solani*.

Although the phenomenon of biocontrol activity in dual culture is due to the colonizing and competitive ability of pathogen and biocontrol agent for food [8]. In the present study it was observed that there was no antagonism for space. Thus it is possible that *Paecilomyces sp*. produced some antifungal compounds, the nature of which are not completely known [1]. Due to lack of resources and limited time, present study was carried only on *Paecilomyces sp*. The investigation of antifungal activities of the remaining fungi in future research can reveal more useful results.

Paecilomyces lilacinus and *Pseudomonas aeruginosa* individually or in combination significantly (P<0.05) reduced disease severity of *Fusarium solani* in guar, *Macrophomina phaseolina* and *Fusarium oxysporum* in pumpkin and root knot nematode in guar [15]. The biocontrol agent, *Paecilomyces sp*. used in screen house experiment also showed its efficacy to control Fusarium wilt of *Capsicum annum* L. It was observed that the lower as compared to higher conidial concentration of *Paecilomyces sp.* was more effective in controlling chilli wilt disease. In case of root colonizing fungi treatment of lower conidial concentration has been reported to be more effective as compared to higher concentration [1].

Biocontrol agents help in plant growth promotion due to their ability of nitrogen fixation, nutrients solubilization, balancing ethylene within roots, production of phytohormones while lowering heavy metals [18]. In this experiment application of *Paecilomyces sp*. also promoted the biomass e.g. fresh and dry shoot/root weight of chilli plants.

5. Conclusion and Recommendation

Application of 1×10^4 conidia ml^{-1} concentration of *Paecilomyces sp.* effectively reduced the severity of Fusarium wilt of chilli up to 90.1% under screen house conditions. Application of *Paecilomyces sp.* in the beginning of growing chilli seedlings before transplantation to the screen house or field will help in early establishing *Paecilomyces sp.* in the roots, thus minimizing the losses from the future onset of *Fusarium solani*.

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