ANTIFUNGAL ACTIVITY OF PSEUDOMONAS FLUORESCENS AND ITS BIOPESTICIDE EFFECT ON PLANT PATHOGENS

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Abstract

Antifungal activity of different strains of *Pseudomonas fluorescens* were tested against some plant pathogens such as *Alternaria cajani, Curvularia lunata, Fusarium sp., Bipolaris sp.* and *Helminthosporium sp.* in invitro. Different concentrations (1000, 2000, 3000, 4000 and 5000 µg/ml) of *Pseudomonas fluorescens* were used and maximum spore germination of fungus was inhibited at 4000 and 5000 µg/ml. The result indicated that all the strains of *Pseudomonas fluorescens* presented a most significant value against *Alternaria cajani* and *Curvularia lunata.* Out of the five strains studied, the best result was shown by Strain 1, which showed almost complete inhibition against pathogenic fungi such as *Curvularia lunata* and *Fusarium sp.* at 4000 and 5000 µg/ml while Strain - 5 was resistant against Fusarium sp. and *Helminthosporium sp.* at 5000 µg/ml. Among the fungus tested, bacterial strains Strain - 2 and Strain - 5 were found to be more sensitive to *Fusarium sp.* and *Helminthosporium sp.*

Keywords: Plant pathogen, Antifungal activity, Strain, Bio pesticides

I. INTRODUCTION

Biopesticides (also known as biological pesticides) are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. For example, garlic, mint, and baking soda all have pesticidal applications and are considered biopesticides. Microbial pesticides contain a microorganism (bacterium, fungus, virus, protozoan or alga) as the active ingredient. The most widely known microbial pesticides are varieties of the bacterium *Bacillus thuringiensis,* or Bt, which can control certain insects in cabbage, potatoes, and other crops. Biopesticides produce a protein that is harmful to specific insect pests. Certain other microbial pesticides act by out-competing pest organisms. Microbial pesticides need to be continuously monitored to ensure that they do not become capable of harming non-target organisms, including humans (Kinkel, 2001).

Fungal plant diseases are one of the major concerns to agricultural production. It has been estimated that total losses as a consequence of plant diseases reach 25% of the yield in western countries and almost 50% in developing countries (Blakemanand,1987). Conventional practice to overcome this problem has been the use of chemical fungicides which have adverse environmental effects causing health hazards to humans and other non-target organisms, including beneficial life forms. Hence there is increasing concern towards the toxicity and biomagnification potential of these chemicals in agriculture(Frammell,2003). Currently practices based on molecular biology techniques involve development of transgenic plants which are resistant to plant pathogens, are being used.(Shickiler, 1997).Microbial insecticides are another kind of biopesticide. They come from naturally-occurring or genetically altered bacteria, fungi, algae, viruses or protozoans. They suppress pest by producing a toxin specific to the pest; causing a disease; preventing establishment of other microorganisms through competition; or other modes of action (Fokkema, 1993)

A. Pseudomonas fluorescens

The genus *Pseudomonas* sp is gram-negative, strictly aerobic, polarly flagellated rods. They are aggressive colonizers of the rhizosphere of various crop plants, and have a broad spectrum anatagonistic activity plant pathogens, such as antibiosis (the production of inhibitory compounds) (Cartwright et al., 2007), siderophores production (iron-sequestering compounds) and nutrition or site competition. Some species of Pseudomonas can also produce levels of HCN that are toxic to certain pathogenic fungi (David and O'Gara, 2004). These characteristics make *Pseudomonas* species good candidates for used as seed inoculant and root dips for biological control of soil-borne plant pathogen.

Bacteria of the genus *Pseudomonas* comprise a large group of the active biocontrol strains as a result of their general ability to produce a diverse array of potent antifungal metabolites. These include simple metabolites such as 2,4-diacetylphloroglucinol, phenazine-1-carboxylic acid and pyrrolnitrin [3-chloro-4-(2'-nitro-3'-

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Strains of Pseudomonas fluorescence shows known biological control activity against certain soil-borne phytopathogenic fungi and has the potential to produce known secondary metabolites such as siderophore, HCN and protease that showed antagonistic activity against *Macrophomina phaseolina, Rhizoctonia solani, Phytophthora nicotianae var. parasitica, Pythium sp. and Fusarium sp.* (Ahmadzadeh et al., 2006). Pseudomonas fluorescens is being researched as a biological control organism

Pseudomonas spp. are ubiquitous Gamma subclass of Proteobacteria that are inhabitants of a wide range of soil, water and plant surfaces. Many pseudomonas live in a commensally relationship with plants, utilizing nutrients

S.NO	Fungus	Host	Strains	Concentration (µg/ml) Spores inhibition (%)					
				Control	1000	2000	3000	4000	5000
1	Alternaria cajani	Cajanus cajan	Strain 1	33.33	50	78.57	86.21	87.5	92.86
			Strain 2	31.25	75.53	80.72	85.00	90.48	94.00
			Strain 3	23.81	57.78	82.14	85.25	86.67	94.29
			Strain 4	28.57	35.29	73.68	76.81	79.31	81.82
			Strain 5	33.93	64.76	65.33	73.91	87.84	90.28
2	Curvularia lunata	Sorghum vulgare	Strain 1	47.31	53.13	76.92	91.67	100	100
			Strain 2	27.27	73.68	80.88	91.21	91.46	94.64
			Strain 3	31.75	67.65	70.00	83.78	84.21	91.11
			Strain 4	37.14	48.28	53.33	63.16	75.00	84.62
			Strain 5	40.91	75.00	75.00	83.64	87.67	89.71
3	Fusarium sp.	Cajanus cajan	Strain 1	23.30	73.18	88.89	88.14	89.47	97.94
			Strain 2	8.89	17.07	18.37	21.57	22.54	26.67
			Strain 3	23.26	65.38	66.67	70.00	81.82	90.70
			Strain 4	50.61	66.67	86.89	88.14	89.47	97.94
			Strain 5	14.89	15.79	17.54	18.18	20.69	22.73
4	Bipolaris sp.	Oryza sativa	Strain 1	49.57	71.79	85.19	87.50	95.92	96.43
			Strain 2	18.73	35.48	55.17	58.33	62.82	73.27
			Strain 3	10.64	25.71	30.77	35.14	54.29	59.26
			Strain 4	6.59	10.53	12.07	23.53	26.67	27.27
			Strain 5	34.21	61.11	81.25	86.84	94.59	95.65
5	Helminthos porium sp.	Saccharum officinarum	Strain 1	10.27	35.71	69.64	71.43	72.22	73.68
			Strain 2	17.86	20.00	23.40	23.68	28.30	33.33
			Strain 3	12.12	25.37	28.89	39.39	41.38	50.00
			Strain 4	77.78	79.41	88.14	91.43	94.92	100
			Strain 5	17.02	25.00	27.12	27.12	28.07	31.58

tolarvae as well as pupae of vector mosquitoes (Hamdan, 2005).

III. RESULTS AND DISCUSSION

Five strains of *Pseudomonas* fluorescens were screened for their antimicrobial activity against different fungi viz. *Alternaria cajani, Curvularia lunata, Fusarium sp., Bipolaris sp.* and *Helminthosporium sp.* in vitro. The Pseudomonas strains showed antifungal activity against all tested strains. Below table presents the different concentrations obtained for each strain tested.

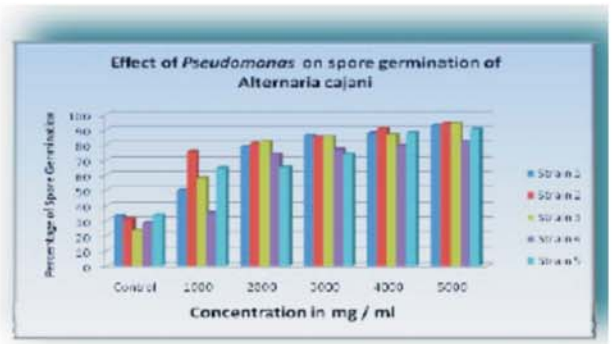

Fig.1. Effect of Pseudomonas on Spore Germination of Alternaria Cajani

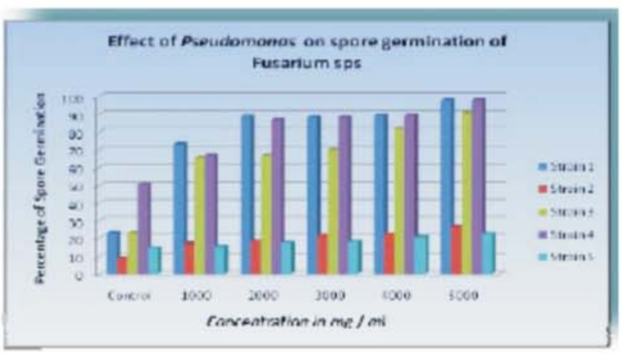

Fig. 2. Effect of Pseudomonas on Spore Germination of Fusarium Sp

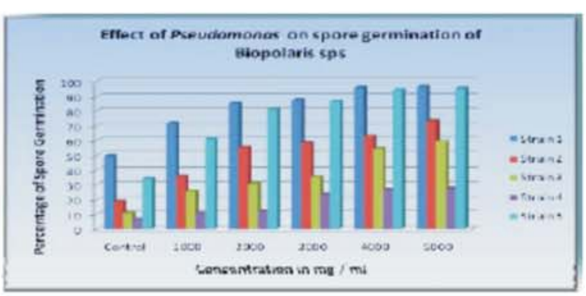

Fig. 3. Effect of Pseudomonas on Spore Germination of Bipolaris Sp

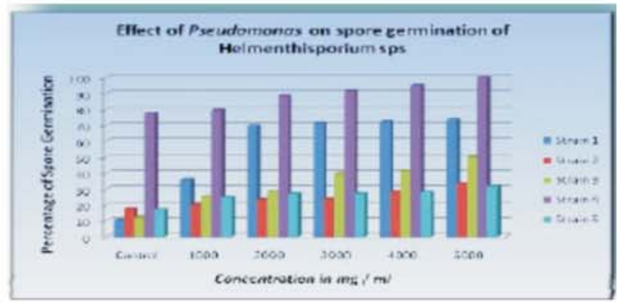

Fig. 4. Effect of Pseudomonas on Spore Germination of Helminthosporium Sp

All the five strains showed varied levels of antifungal activity and the highest concentration of Pseudomonas strains were capable of inhibiting the growth of the pathogenic microorganism. At 5000 µg/ml, all the Pseudomonas strains (Strain 1, 2, 3, 4 and 5) were highly resistant and showed highest inhibition percentage (81% to 100%) against fungus Alternaria cajani and Curvularia lunata. Out of the five strains studied, the best result was shown by Strain 1, which showed almost complete inhibition and maximum activity against Curvularia lunata and Fusarium sp. at 4000 and 5000 µg/ml while strain Strain 4 was resistant against Fusarium sp. and Helminthosporium sp. at 5000 µg/ml. The moderate activity was shown by the strains Strain 2 and Strain 3 against Bipolaris Strain 1 and Strain 3 against Helminthosporium. The most sensitive strains were Strain 2 and Strain 5 against pathogenic fungi Fusarium sp. and Helminthosporium sp. while strain Strain 4 against Bipolaris sp. at 5000 µg/ml. These data revealed that Pseudomonas strains exhibited significant antifungal activity.

IV. CONCLUSION

The data obtained exhibit the antifungal activity of Pseudomonas strains and indicate the possibility of using Pseudomonas fluorescens as a biological control agent of some plant pathogenic fungi. However, this requires further screening of a large number of Pseudomonas strains from different regions of India. The antimicrobial activity of Pseudomonas may be attributed to the various phytochemical constituents have even more potency with respect to the inhibition of microbes.

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