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Use of Non-pathogenic Microorganism (*Pseudomonas fluorescens)* as a Bio control Agent against Stemphylium Blight of Lentil under *Invitro* Condition

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A B S T R A C T

Microbial intervention of plant diseases is the promising approach where no hazardous to human body as well as environment. Therefore the present experiment was conducted on biological control of stemphylium blight of lentil by few isolates of *Pseudomonas fluorescens* under *in vitro* condition. The isolate Pf-4 exhibited maximum inhibition (52.83%) followed by Pf-2 (49.59 %) and very lowest was found in Pf-3 where inhibition was 39.35% over control. This indicated that there was moderate antagonistic effect of *Pseudomonas fluorescens* (Pf-4) against this pathogen. Therefore there is need of further isolation of effective strain of *Pseudomonas fluorescens* which has highly antagonistic potentiality against this pathogen.

Introduction

Lentil (*Lens culinaris* Medik) is the second most important pulse crop in terms of both area and production (Anon., 2014). The crop is vulnerable to many diseases. Among the diseases, *Stemphylium* blight is a major one in our country. Generally, it appears at flowering stage of the crop. Relatively high temperature (around 21°C) and high humidity (90%) enhance the disease development. The disease is caused by the pathogen *Stemphylium botryosum* Wallr (Pleosporales, P!eosporaceae) (teleomorph: Pleospora herbarum (Fr) Rab:).This disease is reported on lentil from Bangladesh (Bakr 1993), Canada(Morrall 2003), Egypt, Syria (Bayaa and Erskine 1998) and the USA (Wilson and Brandsberg 1965). The disease has the potential to cause yield losses up to 62% under conducive conditions (Bakr 1993).

Effective control of stemphylium blight disease of lentil involves the use of one or a combination of the following: resistant cultivars, cultural control and chemical control.

Intensive use of chemical fungicides has resulted in accumulation of toxic compounds potentially hazardous to humans and environment and also in build-up of resistance of pathogens. In this view, investigation and application of biological control agents seems to be one of the promising approaches (Cook, 1985).

Bio control agents involve the use of naturally occurring non pathogenic microorganisms that are able to reduce the activity of the pathogens and thereby suppress the disease outbreaks. Generally bio control agents compete with other pathogens for nutrients, inhibit multiplication of pathogens by secreting antibiotics or toxins or reduce pathogen population through hyperparasitisms.

Pseudomonas fluorescens is the non-pathogenic rhizobacteria which suppress the soil-borne pathogens through rhizosphere colonization, antibiosis, iron chelating by siderophore production and ISR. It is mainly used for biological control of soil-borne and foliar plant pathogens. In the past three decades, numerous strains of *fluorescent pseudomonas* have been isolated from the rhizosphere soil and plant roots by several workers and their bio-control activity against soil-borne and foliar pathogens were reported.

But there is no report available in the control of stemphylium blight of lentil by *Pseudomonas* sp. at the present. Therefore in the present investigation, attempt was made to test the antagonistic potentiality of *Pseudomonas fluorescens* against *Stemphylium botryosurm*.

Materials and Methods

Pathogen

The disease samples were used for the isolation of the pathogen collected from Murshidabadh during survey 2016. The isolated pathogen causing stemphylium blight of lentil is cultured on PDA at 28°c. The working culture of *Stemphylium botryosum* is employed for antagonistic activity incubated for seven days in Petri dish. The conidia of isolated *Stemphylium botryosurm* are oblong, olive to brown, ovoid to subdoliiform, occasionally constricted at 1-3 transverse septa and at the 1-3 longitudinal septa.

Antagonists

The isolates of antagonistic microorganism *Pseudomonas fluorescens* were employed for the experiment were obtained from rhizosphere soil of lentil collected from Murshidabad (Pf-1), Bardhaman (Pf-4), Hooghly (Pf-3) and Nadia (Pf-1). The antagonistic microorganism has been isolated by using serial dilution method on King's B medium and maintained at 32°c on same medium for growth and subculture for the subsequent experiments. In king's B medium *Pseudomonas fluorescens* produce greenish yellow pigmentation which is important characteristics for identification.

Compatibility test of *Pseudomonas fluorescens* isolates with *Rhizobium* sp. isolated from nodule of lentil

This experiment simply was carried out on Potato Dextrose Agar medium (PDA) on Petri dishes. Smear of *Rhizobium* sp. was prepared first on PDA on each Petri dish with the help of spreader. Then two days old culture of four isolates of *Pseudomonas fluorescens* were spotted on four places on PDA where already smeared with Rhizobium in each Petri dish

with the help of inoculating needle. These inoculated dishes are incubated at 28°c for 2 to 3 days for observation of their growth characteristics. It was found that whole plate was covered by Rhizobium with limited growth of these four isolates of *P. fluorescens.*

It indicates that there is mild harmful effect of these isolates on growth and multiplication of Rhizobium. From this point of view it may be suggested that these isolates can be used as a bio control agent against those organisms causing harmful diseases of lentil.

Antagonistic potentiality of *Pseudomonas fluorescens* isolates against *Stemphylium botryosum* causing stemphylium blight of lentil

The assay for antagonism was carried out on PDA on Petri dishes by pyramidal culture technique. To test the antagonistic potentiality of bacteria, a 5mm of mycelia agar disc from pathogen culture was placed at the centre of Petri dish containing PDA medium and a loopful of bacteria (*Pseudomonas fluorescens)* was streaked in pyramidal shape by centralising that mycelium disc placed at centre on the same Petri dish. The paired cultures were incubated at 28°c. Dishes containing only mycelia disc of test pathogen were served as controls. Percent growth inhibition ($^{PGI)}$ was calculated by using following formula.

$$PGI(\%) = \frac{C - D}{C} * 100$$

Where C represent the distance between the point of inoculation and the margin of colony (test pathogen) on control dishes and D represent the distance from the point of inoculation of test pathogens to the colony margin on the treated dishes in the direction of antagonist (Korsten *et al*, 1995).

Results and Discussion

From the Table-1 it was found that among the four isolates of *Pseudomonas fluorescens*, Pf-4 was effective in inhibition of mycelial growth of *Stemphylium botryosum* compare to other isolates. Pf-2 exhibit 49.59 % inhibition over control where Pf-1 inhibit 44.74 % and very lowest in Pf-3 where inhibition was (39.35%). Therefore it indicates that Pf-4 isolate can be used for the control of the disease effectively.

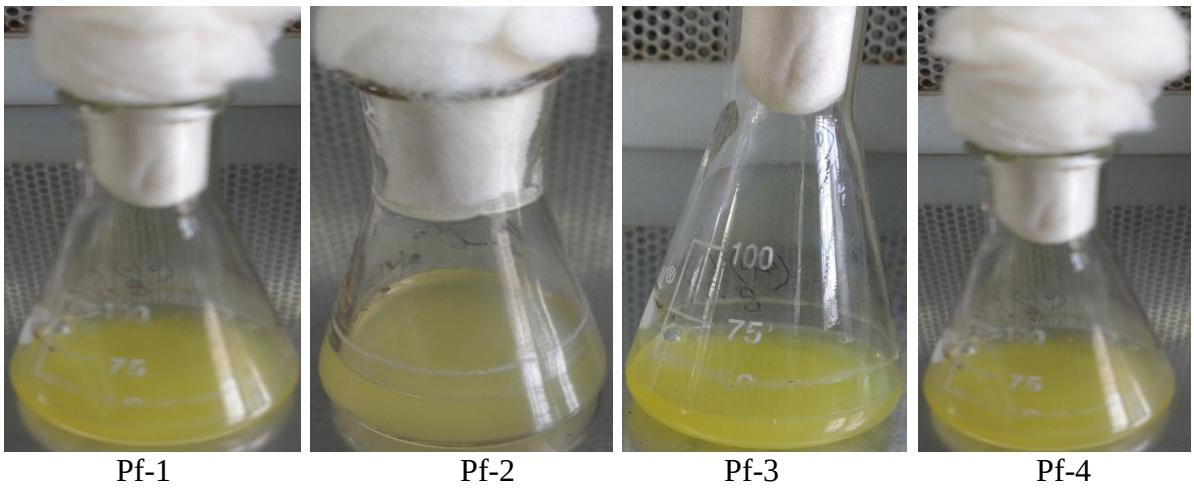
Table.1 Antagonistic potentiality of different isolates of *Pseudomonas fluorescens* against *Stemphylium botryosum* under *in vitro* condition

Treatment / Isolates	Average colony diameter of mycelia growth (cm)	Per cent growth inhibition over control (%)
Pf-1	4.10	44.74
Pf-2	3.74	49.59
Pf-3	4.5	39.35
Pf-4	3.5	52.83
Control	7.42	
S.Em (+-)	0.05	
CD at 5%	0.17	

Fig.1 Compatibility test of *Pseudomonas fluorescens* with Rhizobium

Fig.2 Assay for antagonistic potentiality of different isolates of *Pseudomonas fluorescens* against *Stemphylium botryosum*

Fig.3 Broth culture of different isolates of *Pseudomonas fluorescens* in king's B medium



This result was similar with Hussein *et al* (2007) who found that the highest inhibition of mycelial growth of *Stemphylium vesicarium* was achieved by *Pseudomonas fluorescens, Bacillus subtilis* and *Trichoderma harzianum.*

The findings from the investigation revealed that *Pseudomonas fluorescens* was able in inhibition of the mycelial growth of *Stemphylium botryosum* to a few extents resulting it has mild antagonistic effect on the pathogen causing stemphylium blight.

Therefore there is need of further isolation of *Pseudomonas fluorescens* which have strong antagonistic potentiality against the pathogen.

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