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Pseudomonas based Formulation for Biocontrol of *Sclerotium rolfsii* causing Collar rot of Chickpea

Ritesh Kumar[1*], Priya Bhargava[2] and Diksha Sinha[2]
[1]Department of Plant pathology, MSSSoA,
Centurion University of Technology and Management, (Odisha), India.
[2]Department of Plant Pathology, CSKHPAU, Palampur, (Himachal Pradesh), India.

(Corresponding author: Ritesh Kumar)*
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ABSTRACT: Collar rot disease caused by soil borne fungus, *Sclerotium rolfsii* is an important disease of chickpea that causes significant losses every year. The soil borne nature of pathogen makes it difficult to manage by the use of other management practices except biocontrol agents due to its various mechanism of action. The present investigation is on antagonistic ability of *Pseudomonas fluorescens*, a potent Plant Growth Promoting Rhizobacteria (PGPR), isolated from the root zone *Amaranthus viridis*, against collar rot pathogen. The PGPR reflected good antagonistic activity against five different soil borne plant pathogenic fungi *viz. Sclerotium rolfsii, Rhizoctonia solani, Fusarium solani, F. oxysporum* and *Botrytis cinerea*, and showed maximum inhibition (70.56%) against *S. rolfsii* and minimum against *F. solani* (33.61%). The talcum based formulation of the PGPR, showed highest population of 9.07 Log CFU g^{-1} in the formulation after 1 month while decreased gradually with increase in storage month i.e. 7.05 Log CFU g^{-1} at the end of 6 months. Under pot conditions, disease incidence (DI) due to collar rot was reported minimum in case of chemical spray followed by the application of PGPR in a combination of soil treatment, liquid spray and seed treatment. Thus, the use of PGPR, *P. fluorescens* is an ecofriendly, cost effective and farmers' friendly approach to manage the disease.

Keywords: Disease incidence, PGPR, *Pseudomonas fluorescens*, Soil-borne plant pathogens, Talc.

INTRODUCTION

Sclerotium rolfsii Sacc. causes collar rot, is a non-target plant pathogen, having a broad host range. It is among the most damaging soil-dwelling fungus, having an ability to cause significant (55–95 %) seedling mortality in chickpea under favourable climatic conditions such as 25-30ºC temperature and heavy rainfall (Sharma and Ghosh, 2017). In India, the disease causes losses of 2-5 % every year while under severe conditions, it causes losses up to 60 % (Sravani and Chandra, 2021). Rolfs (1892) first identified the fungus as a source of tomato blight in Florida, but the name *S. rolfsii* was given by Saccardo (1911). According to McClintock, 1917, for the first time, this fungus produced collar rot disease in the United States and as according Butler and Bisby, 1931, it caused disease in India. Collar rot is a widespread disease that causes significant losses in Bolivia, China, Egypt, India, Taiwan, Thailand, and the United States (Bowen *et al*., 1992). In India, collar rot affects all pulse-growing states like Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh etc. where pulses are majorly farmed. Presence of inoculum leads to symptoms such as pre-emergence and post-emergence along with seedling mortality (Agrawal and Kotashane, 1971). The mycelial mat may extend several millimeters above the soil surface on the stem portion with numerous tan to brown, spherical structure known as sclerotia of roughly mustard seed size, which is also observed on infected plant debris (Fig. 1) as well as under *in-vitro* growth of the fungus on Potato Dextrose Agar (PDA) medium (Fig. 2) (Kumar *et al*., 2017).

Fig. 1. Mycelial growth of *Sclerotium rolfsii* on the collar portion of chickpea plant.

Fig. 2. Successive mycelial growth of *Sclerotium rolfsii* and formation of sclerotia.

Chemical fungicides are near to ineffective against these soil-borne pathogens like *S. rolfsii*, and the disease persistence remains to be an issue (Huang *et al*., 2012). Beside this, these chemicals are routinely employed and their widespread use may have an environmental impact (De-Oliveira *et al*., 2010). As a result of increased public concern about chemical residues in food and environment, there is a growing demand for developing alternative approaches, such as biocontrol (Spadaro and Gullino, 2005). The microorganisms, like *Pseudomonas fluorescens* present in the rhizospheric region are of great importance for their contribution to plant nutrition, hormonal control, disease suppression etc. (Bastida *et al*., 2009; Kai *et al*., 2016), which are known to be Plant Growth Promoting Rhizobacteria (PGPR). The colonization level of Pseudomonads represents 0.1 to 1% of the culturable aerobic rhizobacterial population under natural condition (Chatterton *et al*., 2004). Being Biocontrol Agents (BCAs), they stimulate plant growth by supply of nutrients from the soil and suppression of phytopathogens, which is mediated by different mechanisms, like competition for iron, secondary metabolites with antibiotic production and systemic resistance (Defago and Has, 1990). The effectiveness of *P. fluorescens* has been also reported by several workers against chickpea collar rot (Maurya *et al.*, 2008; Gaurkhede *et al.*, 2015; Wavare *et al.*, 2017) but few studies have been done on the shelf life of its formulations. In this context, the present study is focused on the *in-vitro* antagonistic effect of *P. fluorescens*, development of its talc based formulations and evaluation of its shelf life.

MATERIALS AND METHODS

The rhizospheric *P. fluorescens* was isolated from the root tissues of *Amaranthus viridis* and was confirmed on the basis of morphological characteristics and phylogenetic analysis of the 16S rRNA gene sequences. The cross-streak method (Toumatia *et al*., 2014) was used to evaluate the antagonistic activities of *Pseudomonas* against 5 different soil borne plant pathogenic fungi *viz*. *Sclerotium rolfsii*, *Rhizoctonia solani*, *Fusarium solani*, *F. oxysporum* and *Botrytis cinerea*. Spore suspension of *Pseudomonas fluorescens* was mixed with 100 g of autoclaved talc powder, 1.5 g of calcium carbonate and 10 g of carboxymethyl cellulose for the formation of talc based formulation of the microbe. After an interval of 1 month, the formulation was checked for its viability and microbial count. To eliminate the effect of any other soil borne microorganism, the garden soil was autoclaved twice in a polypropylene bag containing 1.5 Kg of soil. Before filling the earthen pots, they were properly cleansed with tap water, rinsed with 1% formalin, and dried in the sun for one day. Sclerotia were multiplied in large numbers and the sterilised soil was combined with the sclerotia at a rate of 100 sclerotia per 100 g of soil in the pots (Yaqub and Shahjad, 2005) to study the effect of *P. fluorescens* on the collar rot using different treatments like T1- soil treatment, T2- liquid Spray, T3- seed treatment, T4-combination of soil treatment + liquid Spray + seed treatment along with a T5- Chemical spray with Hexaconazole 5% and T6- Control.

RESULTS AND DISCUSSION

P. fluorescens showed positive antagonistic activities against all targeted fungi and the percentages of inhibition were greater than 33% against all the pathogens. The strongest antagonistic activity with a percentage of inhibition 70.56% was observed against *S. rolfsii*, followed by *R. solani* (54.72%), *B cineria* (50.28%), *F. oxysporum* (34.44%) and *F. solani* (33.61%) (Table 1). Several Pseudomonads have been reported as potential bioagents and plant growth promoter against various soil-borne phytopathogenic fungi (De-Oliveira *et al*., 2010).

Table 1: Antifungal activity of *P. fluorescens* against phytopathogenic fungi.

Sr. No.	Target Pathogen	Inhibition Percentage (%)
1.	*Sclerotium rolfsii*	70.56(±1.43)[a]
2.	*Rhizoctonia solani*	54.72(±1.06)[b]
3.	*Fusarium solani*	33.61(±1.40)[d]
4.	*Fusarium oxysporum*	34.44(±1.81)[d]
5.	*Botrytis cineria*	50.28(±1.90)[c]

The data shown are the mean of four independent replicates ± standard deviation.
Similar letters depict non significantly difference ($p \geq 0.05$).
The differences were determined using Duncan's multiple range test at 5% error of transformed values of Inhibition percentage.

The microbial purity of the formulated products was verified. The spore suspensions obtained from the talcum powder based formulation of *P. fluorescens* showed pure cultures of the strain and no microbial contamination was noted. Kinetics of viable spore counts evaluated at 1-month intervals for 6 months are described in Fig. 3, showed the presence of 9.07 log CFU g^{-1} in the formulation after 1 month and decreased

simultaneously i.e. 8.45, 8.15, 7.57, 7.22 and 7.05 log CFU g^{-1} in the next consecutive 6 months respectively. The data on collar rot incidence is given in Fig. 4.

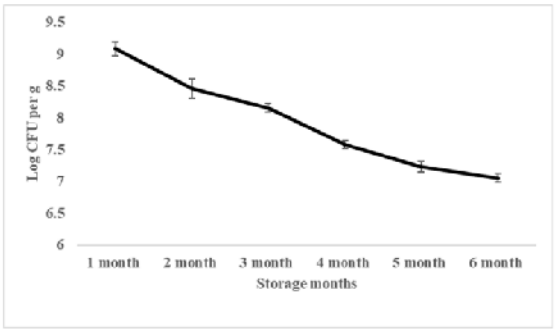
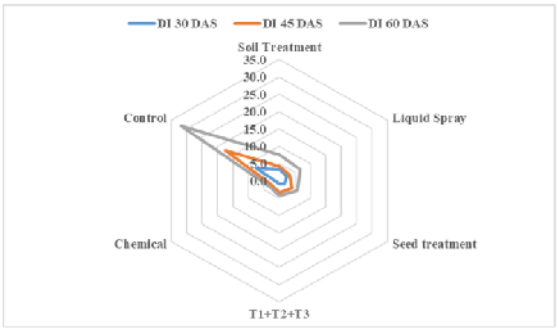


Fig. 3. Population of *P. fluorescens* in talc based formulation at monthly interval.



CD(0.05%) 30 DAS: 2.9, 45 DAS: 2.6 and 60 DAS: 3.6

Fig. 4. Collar rot Disease incidence of Chickpea after different time intervals of sowing.

For collar rot, the lowest DI (%) i.e. 0.8% was recorded 30 DAS in combined application of soil treatment, liquid spray and seed treatment (T4) of *P. fluorescens* formulation and in chemical spray (T5) also the DI (%) was recorded same (0.8%), which was followed by T3- seed treatment (1.7%), T2- Liquid spray (2.5%) and T1- soil treatment (3.3%) while the highest DI (7.5%) was reported in T6- control, where no treatment was applied. After 45 days of sowing, lowest DI (%) of 2.5% was recorded in T5- chemical spray, followed by T4- combined application of *P. fluorescens* as soil treatment, liquid spray and seed treatment (3.33%) and T2- liquid spray (3.33%). T1 and T3 recorded DI (%) of 4.2% each followed by 17.5%, which was reported in T6- control. A minimum DI (%) of 3.3% was observed in T5- chemical treatment at 60 DAS, followed by T4- combination of soil treatment, liquid spray and seed treatment (4.2%), T3- seed treatment (5.8%), T2- Liquid spray (6.7%) and T1- soil treatment (7.5%). However, maximum DI of 31.7% was reported in T6- no treatment. Singh *et al.,* (2012) also reported antibiotic potential of *Pseudomonas sp.* against *S. rolfsii*. Gaurkhede *et al.,* (2015) also reported the effectiveness of combined application of *Pseudomonas sp.* as seed and soil treatment against collar rot. Sahni and Prasad (2020) also reported that integration of *Pseudomonas* strain in management practices can effectively manages the collar rot pathogen by inducing defence enzymes production. Thus, *P. fluorescens* can be used as combined application and alone for sustainable management of the disease.

The present preliminary investigation has laid an initiative and widened future scope with biochemical and molecular aspects that clearly defines about different paths of the mechanisms involved *viz.* auxins, siderophores, and phosphate solubilization. Furthermore, methodologies to integrate the various subjective areas in order to develop a predictive function for the effectiveness and implementation of PGPR mechanisms in alternative crop species can also be recommended.

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Conflict of Interest. None.

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