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RESEARCH PAPER

Evaluation of microbial consortia against *Sclerotium rolfsii* Sacc. causing foot rot of wheat

PRIYANKA CHITTU JADAV, GURUDATT M. HEGDE, SHAMARAO JAHAGIRDAR AND VITHAL NAVI

Department of Plant Pathology, College of Agriculture
University of Agricultural Sciences, Dharwad - 580 005 Karnataka, India
E-mail: priyankajadav1998@gmail.com

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Abstract : The microbial consortium (combination of bioagents) is being currently used for crop protection over single bio inoculant in order to overcome the inconsistent performance shown by the introduced biocontrol agent. Foot rot of wheat caused by *Sclerotium rolfsii* Sacc. is a serious soil borne disease in rainfed areas of Karnataka. The biocontrol agents (*Trichoderma harzianum* IOF strain, (MH027645.1), *Pseudomonas fluorescens* IOF strain, (NAIMCC-B-01981), *Bacillus subtilis* IOF strain, (MT383652.1) and *Neofusicoccum parvum* (Endophyte) were evaluated against *S. rolfsii* under *in vitro* condition. The potentiality of bioagents against *S. rolfsii* was higher in the consortium compared to the single bioagent. All four combination of bioagents (*T. harzianum* + *P. fluorescens* + *B. subtilis* + *N. parvum*) had shown superiority over other treatments in arresting the mycelial growth of *S. rolfsii* (80.37%). Among three combination of bioagents, highest inhibition was recorded in *T. harzianum* + *B. subtilis* + *N. parvum* (78.52%) and the least inhibition was recorded in the *T. harzianum* + *P. fluorescens* + *B. subtilis* (74.57%). Among two combinations of bioagents, *B. subtilis* + *N. parvum* has recorded highest inhibition (72.96%) and least inhibition was observed in *P. fluorescens* + *B. subtilis* (45.56%). Among individual antagonists, *N. parvum* has proved maximum inhibition against *S. rolfsii* (47.41%) followed by *T. harzianum* (46.66%) and were statistically on par with each other. Least inhibition was recorded in *P. fluorescens* (14.07%) and *B. subtilis* (17.04%). However, the standard chemical check [(carboxin 37.5% + thiram 37.5%)WP] has shown cent per cent of inhibition of pathogen.

Key words: Bioagents, Microbial consortia, *Sclerotium rolfsii* , Wheat

Introduction

The management of chemical-free plant health has become a new challenge for agriculture as a result of rising awareness of residue-free crops and environmental health. Therefore, biological technologies, including the use of hostile microbes, are becoming important in crop protection for both domestic and international markets, due to the rising cost of chemical pesticides and increased awareness of the harmful effects of their indiscriminate use (Thakkar and Saraf, 2015). Biological control methods invade the rhizosphere, the place that has to be protected, and leave no hazardous leftovers, in contrast to pesticides (Tewari and Mukhopadhyay, 2001).

However, most approaches for biocontrol of plant disease have been using the single biocontrol agents as antagonist to counteract a single pathogen. This may partially account for the inconsistent performance by biocontrol agents (Rajasekhar *et al.*, 2016). The single biocontrol agents are not likely to be active in all soil types against all phytopathogens (Nandakumar *et al.*, 2001). Therefore, the microbial consortium (combination of microorganisms that interact synergistically) are being currently used for crop protection over single bio inoculant. A microbial consortium is two or more microbial groups living symbiotically or it is a "group of species of microorganism that act together as a community". Single strains of diversely capable microbial organisms are substantially less effective than microbial consortia (Sudharani *et al*., 2014).

Wheat (*Triticum aestivum* L.) is the most important cereal crop and cultivated globally in an area of 220 mha with the production of 763 mt and the productivity of 3470 kg/ha (Anon, 2019). In India, the estimated area is around 31.35 mha with a production of 107.86 mt and productivity of 3,440 kg/ha (Anon, 2019). In Karnataka, it is cultivated in an area 1.5 mha of land with an annual production of 1.79 mt and productivity of 1198 kg/ha (Anon, 2019).

Wheat suffers from several soil borne pathogens among which foot rot disease caused by *Scelrotium rolfsii* Sacc is serious problem mainly in the rainfed areas of Karnataka, Gujarath and Madhya pradesh (Khapariye and Dinseh, 2007). The pathogen is soil borne and damaging the crop by causing pre and post emergence death from seedling stage to maturity of the crop and also causes 25-50 per cent loss through infection of seedlings (Kalappanavar and Patil, 2000). Wheat plants affected by foot rot disease (*S. rolfsii*) are characterised by yellowing of leaves followed by loss of vigour and premature death. The diseased plants can be easily pulled out from the soil and show poor root growth with white mycelial growth on the crown and root region. The pathogen causes pre and post emergence mortality in seedlings (Nargund, 1981).

Therefore, in recent years, more emphasis is laid on the combined use of biocontrol agents with different mechanisms of actions for improved disease control and also to overcome the inconsistent performance of the introduced single biocontrol agents against phytopathogens (Thakkar and Saraf, 2015). Considering the benefits of microbial consortia, the present investigations was undertaken.

Material and methods

The laboratory study was conducted in the Department of Plant Pathology, College of Agriculture and at Institute of

Organic Farming (IOF), University of Agricultural Sciences, Dharwad.

The potential antagonists *viz.*, *Trichoderma harzianum* Rifai (MH027645.1), *Pseudomonas fluorescens* Migula (NAIMCC-B-01981), *Bacillus subtilis* Cohn (MT383652.1) and Endophyte (*Neofusicoccum parvum*) (Pennycook and Samules) were collected from Institute of Organic Farming, UAS, Dharwad. The foot rot infected root and collar portion of wheat seedlings were collected from experimental plots of wheat in Main Agricultural Research Station (MARS), University of Agricultural Sciences (UAS), Dharwad.

Isolation of pathogen

The pathogen *Sclerotium rolfsii* was isolated from diseased wheat plant by tissue isolation method (Rangaswami and Mahadevan, 1999). Small pieces of tissue of about 3 mm from infected root region along with some healthy tissue were cut with sterile scalpel. Then the pieces were surface sterilized with 0.1 per cent sodium hypochlorite for 30 sec. followed by three washings in sterile distilled water to remove traces of NaOCl on the bits of tissue. These bits were transferred to potato dextrose agar (PDA) plated Petri plates. Plates were incubated at 28 ± 2°C and observed periodically for growth of the fungus.

Dual culture technique

The bio-efficacy of fungal and bacterial biocontrol agents were evaluated under *in vitro* condition against inhibition of mycelial growth of *S. rolfsii* by dual culture technique. (single antagonist + pathogen and compatible bioagents + pathogen) (Dennis and Webster, 1971). Twenty ml of sterilized and cooled potato dextrose agar were poured into sterile Petri plates and allowed to solidify. For evaluation of fungal biocontrol agent, 5 mm mycelial disc of test fungus *S. rolfsii* was inoculated at one end of the Petri plate and antagonistic fungus were placed opposite to it on the other end. To test the efficacy of antagonistic bacterium, it was streaked at one side of plate. On the opposite side to the antagonist 5mm mycelial disc of *S. rolfsii* was placed.

The bio-efficacy of combination of compatible bioagents were tested against *S. rolfsii* by placing the 5mm disc pathogen at the centre of the PDA plates and compatible bioagents on either side of the pathogen at an equal distance. The efficacy of fungicide (Carboxin 37.5 % + Thiram 37.5 %)WP at 0.2% was assayed under *in vitro* using poisoned food technique (Sharvelle, 1961) against *S. rolfsii* as chemical check. The required concentrations of chemical were weighed and incorporated into sterilized, cooled potato dextrose agar. Twenty ml of cooled molten PDA medium was poured into 90 mm sterilized Petri dishes and all the plates was inoculated with actively growing five mm mycelial disc of the pathogen separately.

Three replications were maintained for each treatment with one control maintaining only test fungus. The plates were incubated at 27 ± 1°C for five days. The extent of antagonistic activity (single and consortia) against fungal pathogens (*S. rolfsii*) were recorded by measuring the growth of pathogen in dual culture plates and control plate. The per cent inhibition of pathogens was calculated using the formula suggested by Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Inhibition of mycelial growth (%)

T = Radial growth of pathogen in treatment (centi meter)

C = Radial growth of pathogen in control (centi meter)

Results and discusion

The results of the study revealed that all bioagents have shown significant inhibition against *S. rolfsii* indicated in Table 1, Fig 1 and Plate 1. Antagonistic activity of fungal and bacterial bioagents was higher in consortium than that of individual activity. Among the individual antagonists *N. parvum* (T_4) has

Table 1. *In vitro* evaluation of microbial consortia against *Sclerotium rolfsii* Sacc.

Tr. No.	Treatments details	Per cent inhibition
T_1	*Trichoderma harzianum* IOF strain, Dharwad	46.66(43.06)*
T_2	*Pseudomonas fluorescens* IOF strain, Dharwad	14.07(22.01)
T_3	*Bacillus subtilis* IOF strain, Dharwad	17.04(24.33)
T_4	*Neofusicoccum parvum* IOF, AC, Dharwad	47.41(43.49)
T_5	*T. harzianum* + *P. Fluorescens*	54.44(47.53)
T_6	*T. harzianum* + *B. subtilis*	57.04(49.02)
T_7	*T. harzianum* + *N. parvum*	68.52(55.87)
T_8	*P. fluorescens* + *B. subtilis*	45.56(42.42)
T_9	*P. fluorescens* + *N. parvum*	56.67(48.81)
T_{10}	*B. subtilis* + *N. parvum*	72.96(58.65)
T_{11}	*T. harzianum* + *P. fluorescens* + *B. subtilis*	74.57(59.69)
T_{12}	*T. harzianum* + *P. fluorescens* + *N. parvum*	77.28(61.51)
T_{13}	*T. harzianum* + *B. subtilis* + *N. parvum*	78.52(62.37)
T_{14}	*P. fluorescens* + *B. subtilis* + *N. parvum*	77.04(61.35)
T_{15}	*T. harzianum* + *P. fluorescens* + *B. subtilis* + *N. parvum*	80.37(63.67)
T_{16}	(Carboxin 37.5%+ Thiram 37.5%) WP (chemical check)	100.00(90.00)
	S.Em.±	0.75
	C.D. (0.01)	2.17

*Angular transformation or Arc sine values

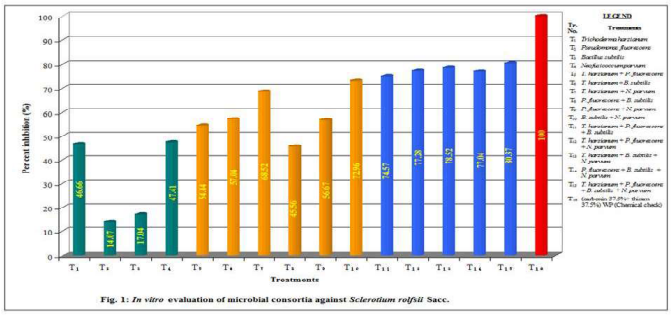

Fig. 1. *In vitro* evaluation of microbial consortia against *Sclerotium rolfsii* Sacc

Plate 1: *In vitro* evaluation of microbial consortia against *Sclerotium rolfsii* Sacc.

shown maximum inhibition against *S. rolfsii* (47.41%) followed by *T. harzianum* (T_1) (46.66%) and were statically on par with each other. Least inhibition was recorded in *P. fluorescens* (T_2) (14.07%) and *B. subtilis* (T_3) (17.04%). The results of the efficacy of combination of bioagents against *S. rolfsii* found that among the two combinations of bioagents, *B. subtilis* + *N. parvum* (T_{10}) has recorded highest inhibition (72.96%) followed by *T. harzianum* + *N. parvum* (T_7) (68.52%). Next best was *T. harzianum* + *B. subtilis* (T_6) (57.04%) followed by *P. fluorescens* + *N. parvum* (T_9) (56.67%) followed by *T. harzianum* + *P. fluorescens* (T_5) (54.44%). The treatments T_6, T_9 and T_5 were statically on par with each other. Least inhibition was observed in *P. fluorescens* + *B. subtilis* (T_8) (45.56%).

Among the three combination of bioagents, highest inhibition was recorded *T. harzianum* + *B. subtilis* + *N. parvum* (T_{13}) (78.52%) followed by *T. harzianum* + *P. fluorescens* + *N. parvum* (T_{12}) (77.28%) followed by *P. fluorescens* + *B. subtilis* + *N. parvum* (T_{14}) (77.04%) while least inhibition was noticed in the *T. harzianum* + *P. fluorescens* + *B. subtilis* (T_{11}) (74.57%). The treatments T_{12}, T_{13} and T_{14} were statically on par with each other, and T_{11} was statically on par with treatments T_{12} and T_{14}. In case of combination of four bioagents, *T. harzianum* + *P. fluorescens* + *B. subtilis* + *N. parvum* (T_{15}) has shown superiority over all other treatments in arresting the mycelial growth of *S. rolfsii* (80.37 %). The study indicated that the use of consortium of biocontrol agents against *S. rolfsii* has maximum inhibition than the sole of biocontrol agents. Thus, there is lot of potentiality in applying biocontrol agents in combination for effective management of disease. However, the standard chemical check (carboxin 37.5% + thiram 37.5%) (T_{16}) has shown maximum inhibition (100%) of *S. rolfsii.*

Use of potential antagonists for inhibition of phytopathogens could replace the chemical usage in plant disease management. But bioagents are affected by many biotic and abiotic factors which leads to failure in suppression of pathogens by them. In order to overcome these inconsistency performance shown by the individual antagonist, microbial consortium is used for the management of phytopathogens especially for the soil borne pathogens like *S. rolfsii*. Hence, the present study was conducted for the control of *S. rolfsii* by using biocontrol agents as sole and compatible combination of two, three and four by dual culture plate method under *in vitro.*

The results of the experiment revealed that all the biocontrol agents used either sole or in combination have shown significant inhibition of *S. rolfsii.* Among sole bioagents, an endophyte *N. parvum* (T_4) has shown maximum inhibition (47.41%) and least inhibition was recorded in *P. fluorescens* (T_2) (14.07%). Similar findings were obtained by Vastrad (2018) who reported the superiority of endophytes against soil borne pathogens (*Fusarium solani* and *Rhizoctonia solani*). Similarly Brunda (2019) reported the siginificant superiority of *N. parvum* and other endophytes against *Sclerotiim rolfsii, Rhizoctonia bataticola* and *Fusarium oxysporum*. The results of the efficacy of consortium of two combination of bioagents against *S. rolfsii* proved that *B. subtilis* + *N. parvum* (T_{10}) has recorded highest inhibition (72.96%) and least inhibition was noticed in *P. fluorescens* + *B. subtilis* (T_8) (45.56%). Among the three combination of bioagents, *T. harzianum* + *B. subtilis* + *N. parvum* (T_{13}) has shown highest inhibition (78.52%) and least inhibition (74.57%) was recorded in the *T. harzianum* + *P. fluorescens* + *B. subtilis* (T_{11}). While, in combination of four bioagents, *T. harzianum* + *P. fluorescens* + *B. subtilis* + *N. parvum* (T_{15}) has shown maximum superiority over all other treatments in arresting the mycelial growth of *S. rolfsii* (80.37 %). However, the standard chemical check vitavax power (carboxin 37.5% + thiram 37.5%) (T_{16}) has shown maximum inhibition (100%) of *S. rolfsii.* Similar studies was conducted by Archana (2018) wherein, among the consortium of *T. harzianum, P. fluorescens* and *B. subtilis* in two and three combination against *S. rolfsii* under *in vitro*, the consortium of *T. harzianum* + *P. fluorescens* + *B. subtilis* recorded highest inhibition of pathogen (72.22%) and least inhibition was observed in the consortium of + *P. fluorescens* + *B. subtilis* (52.22%).

The possible role of growth inhibition of phytopathogens by bioagents is attributed by different mechanism of actions such as production of antibiotics, hydrogen cyanide, siderophore, secretion of lytic enzymes, competition for the space and nutrients (Weller, 1988; Intana *et al.*, 2008). The combi fungicide carboxin 37.5% + thiram 37.5% is both contact as well as systemic in nature and thus able to suppress the pathogen by inhibiting the growth and development (El-Deeb *et al.*, 2002).

Conclusions

Fungal and bacterial bioagents alone and in combination were found effective against *S. rolfsii* under *in vitro* condition. The microbial consortia of *T. harzianum* + *P. fluorescens* + *B. subtilis* + *N. parvum* has been found to be the most promising in inhibition of the pathogen. Thus, microbial consortia can be explored in successful management of the soil borne plant pathogens.

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