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In vitro investigations of different bio-agents efficacy against *Sclerotinia sclerotiorum* (Lib.) De Bary cause sclerotinia stem rot of pea (*Pisum sativum* L.)

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Abstract
Pea is third most important pulses crop at global level, after dry bean and chick pea and third most important Rabi pulse of India after chick pea and lentil. Present investigation was done to evaluate the efficacy of different fungal and bacterial bio-agents *viz., Trichoderma hamatum Trichoderma viride, Chaetomium spp. Bacillus subtilis and Pseudomonas fluorescens* against *Sclerotinia sclerotiorum* (Lib.) de Bary cause sclerotinia stem rot of pea (*Pisum sativum L.*). Indiscriminately use of chemical fungicides for protection of crops from diseases is not merely injurious to human health but also unsafe for our environment. The results were obtained from the present investigation showed that the minimum mycelial growth and maximum mycelial inhibition growth of tested fungus were observed in *Trichoderma viride* followed by *Trichoderma harzianum* and *Chaetomium* sp., while maximum mycelial growth and minimum inhibition zone was observed in case of *Bacillus subtilis* as compared to control. Therefore we say that on basis of present investigation the more study are needed for field application of these Bio-agents to manage the disease incidence and severity of sclerotinia stem rot of pea.

Keywords: Bio-agents, fungal, bacterial, efficacy, sclerotinia, inhibition per cent

Introduction
Pea (*Pisum sativum* L.) is an important legume belonging to family Fabaceae (formerly Leguminosae (Mikić *et al*., 2006) [11]. Pea is third most important pulses crop at global level, after dry bean and chick pea and third most important rabi pulse of india after chick pea and lentil. It can be divided into two categories (Field pea and Gardan pea). In general, pea seeds contain 20-25% protein, 40-50% starch and 10-20% fiber (Dahl, *et al*., 2012) [5] and (Tulbek *et al.* 2016) [16]. Pea protein is a relatively new type of plant proteins and it becomes more and more popular in global food industry due to its availability, low cost, nutritional values and health benefits (Boye, *et al*., 2010) [3] and (Roy; Lam *et al.* 2018) [10]. Indian production contributes around 7% to the total world's production with production figures of 7.8 lakh tonnes. Uttar Pradesh is a major field pea-producing state in India producing about 60% of the country's produce. The yield and seed quality of field pea is affected by fungal as well as bacterial diseases such as Ascochyta blight (*Ascochyta pisi.*), Powdery mildew (*Erysiphe pisi*), Fusarium wilt (*Fusarium oxysporum*), Downy mildew (*Peronospora pisi*), pre-emergence damping off (*Pythium* spp.), Grey mold (*Botrytis cinerea*), common root rot (*Fusarium* spp.) and Sclerotinia *stem rot* (*Sclerotinia sclerotiorum*) are the important fungal diseases. Among these fungal diseases, Sclerotinia stem rot is a serious disease of pea caused by *Sclerotinia sclerotiorum* (Lib) de Bary, which causes 5 to 40% yield loss. (Hartman *et al*., 1999) [7]. White cottony mycelium of the pathogen grows on the surfaces of infected aerial tissues of the pea plant. The hyphae produce enzymes and oxalic acid, creating water-soaked lesions, frequently with a distinct margin. Secondary symptoms such as wilting, bleaching, and shredding also can be observed on above-ground tissues including stems, leaves, petioles and reproductive organs. At later stages of the disease, the cottony hyphae of the pathogen aggregate into (typically) pea-sized clumps of mycelium. These clumps eventually mature into hard black sclerotia which are most commonly found on the outer surface of the diseased tissue, but sometimes inside of soft host tissues or cavities such as floral receptacles, fruits and the pith of stems. The optimum temperature for disease development ranges from 15 to 21 °C. If favourable temperature and moisture conditions prevail during the growing season, the incidence of the disease can be high and its development can be extensive.

The management of the disease can be done through cultural, biological, chemical methods and use of resistant varieties. In the absence of resistance/tolerant variety it is difficult to manage the disease caused by soil borne pathogens because of complex soil environment of physical, chemical and biological origin. Some studies reported that *Sclerotinia sclerotiorum* can be control by using biological and chemical methods. Biological methods may include the application of specific fungal antagonists such as *Conlothyrium minitans, Sporidesmium sclerotivorum, Trichoderma hamatum* (Adams and Ayers 1982) [1] and (Mischke, 1998) [12] *T. harzianum, T viridae* (Shaat and Argawy, 2011) [13] *Trichoderma harzianum* has been reported to parasitize on the mycelium and sclerotia of *S. sclerotiorum* and destroyed the sclerotia within 15 days in white mould of pea incited by *Sclerotinia sclerotiorum*. The *Trichoderma harzianum* inhibited the carpogenic and ascospore germination of *Sclerotinia sclerotiorum* (Sumida *et al.*, 2014) [15].

2. Materials and Methods

2.1 Experimental site

The present investigation was conducted in the Laboratory, Department of Plant Pathology, Sardar Vallabhbhai Pate University or Agriculture and Technology, Modipuram, Meerut 250110 (Uttar Pradesh). This university is situated on the western side of the Delhi-Dehradun highway at a distance of 10.0 km in the north of Meerut City. The Meerut district is situated between 29^0 01'N latitude and 77^0 45'E longitude at an altitude of 237 meters above the mean sea level.

2.2 Establishment of pure cultures

The culture of tested pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary and bio-agents *viz., Trichoderma hamatum Trichoderma viride, Chaetomium* spp. *Bacillus subtilis* and *Pseudomonas fluorescens* were isolated from sclerotia of tested pathogen and rhizosphere of pea plant. The cultures of tested pathogens *Sclerotinia sclerotiorum* (Lib.) de Bary and bio-agents were multiply in sterilized Petri plate containing Potato Dextrose Agar (PDA) for further with the help of single hyphal tip culture technique and these cultures were incubated at 21±2 °C in BOD incubator.

2.3 Efficacy of bio-agents against pathogen

For the present investigation to evaluate the efficacy of different bio-agents against *Sclerotinia sclerotiorum* (Lib.) de Bary cause sclerotinia stem rot of pea (*Pisum sativum* L.) five fungal and bacterial bio-agents *viz., Trichoderma hamatum Trichoderma viride, Chaetomium* spp. *Bacillus subtilis* and *Pseudomonas fluorescens* were tested *in vitro* condition using dual culture technique as described by (Dhingra and Sinclaire, 1995) [6]. For this purpose 20 ml of sterilized PDA was aseptically poured in sterilized Petri dishes and allowed to solidify. Five mm disc of 7^{th} to 10^{th} old culture of tested pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary and Bio-agents, each cut with the help of sterilized cock borer were inoculated on solidify PDA in such manners so that they may be placed on either of the side of Petri dish 60 mm apart. The Petri-plates were inoculated without bio-agents served as control. These inoculated Petri-plates were incubated at 21 ± 2 °C. After the 48, 96 and 144 Hrs. inoculation, the colony diameter of the pathogen was measured and the per cent inhibition of *Sclerotinia sclerotiorum* (Lib.) de Bary was calculated by adopting the following formula.

$$\text{Per cent inhibition} = \frac{\text{Radial growth in control (C)} - \text{Radial growth in treatment (T)}}{\text{Radial growth in control (C)}} \times 100$$

2.4 Statistical analysis

The Complete Randomized Design (CRD) was applied and the data thus obtained were analyzed statistically. Analysis of variance (ANOVA) technique and critical difference (CD) was calculated at five percent level of significance for comparison with other treatment (Kumar *et al*., 2020) [9].

3. Results and Discussion

The results are presented in tabulated form exhibited that the mycelial growth of tested pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary were reduced to great extent by extensive growth of the antagonists in the dual culture. The inhibition zone of mycelial growth of tested pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary by *Trichoderma viride, Trichoderma hamatum, Chaetomium sp., Pseudomonas* and *fluorescens Bacillus subtilis* were observed visually by clearcut demarcating zones between their respective mycelial growth of the tested pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary and Bio-agents. *Trichoderma viride* formed maximum inhibition per cent (76.13 mm) and minimum mycelia growth (21.46) followed by *Trichoderma harzianum* (72.00 mm and 25.20 mm) and *Chaetomium* sp. (68.51 mm and 28.31 mm) while minimum (58.46 mm and 37.35) inhibition zone was observed in case of *Bacillus subtilis* as compared to control (00.00 and 89.93) respectively. However, the sclerotia production in the culture Petri-plats with bio-agents was much less as compared to the control. Rotting of the sclerotia of the pathogen was frequently observed by *Coniothyrium minitans*. Thus, growth of the pathogen and formation of sclerotia were greatly reduced by parasitizing habit and antibiosis of bio-agents. The results are almost in accordance with the findings of (Singh and Singh, 2019) [14] reported (14 mm) inhibition zone. The present investigation is in conformity of finding reported by Ahmed and Tribe (1977) [2] who noticed *Coniothyrium minitans* as mycoparasites of *Sclerotinia sclerotiorum*. Similar findings were also observed by Budge *et al.* (1995) [4].

Table 1: Effect of different bio-agents on the mycelia growth of *Sclerotinia sclerotiorum*

T. No.	Bio-agents	48 Hrs.		96 Hrs.		144 Hrs.	
		Average growth	Inhibition %	Average growth	Inhibition %	Average growth	Inhibition %
T_1	*Trichoderma harzianum*	10.33	70.01	20.00	67.87	25.20	72.00
T_2	*Trichoderma viride*	16.00	53.55	18.35	70.52	21.46	76.13
T_3	*Chaetomium* sp.	17.00	50.65	21.90	64.81	28.31	68.51
T_4	*Bacillus subtilis*	20.33	40.98	29.35	52.85	37.35	58.46
T_5	*Pseudomonas fluorescens*	21.66	37.12	25.45	59.11	30.66	65.92
T_6	Control	34.45	00.00	62.25	00.00	89.93	00.00
	SE(m)	0.93		0.96		1.33	
	C.D. at 5%	2.89		2.97		4.15	
	C.V. (%)	8.06		5.60		5.94	

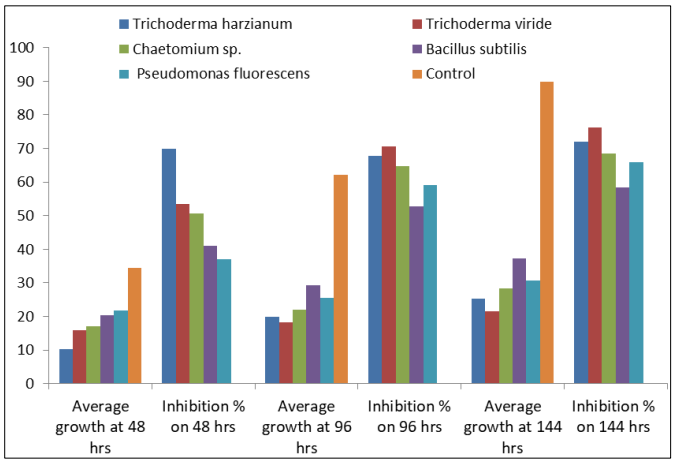

Fig 1: Effect of different bio-agents on the mycelia growth of *Sclerotinia sclerotiorum*

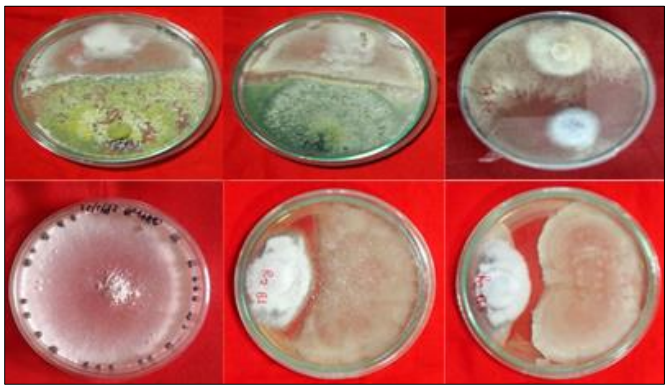

Plate 1: Effect of different bio-agents on the mycelia growth of *Sclerotinia sclerotiorum*

4. Conclusion

In present investigations the minimum mycelial growth and maximum mycelia inhibition growth of tested fungus were observed in *Trichoderma viride* followed by *Trichoderma harzianum* and *Chaetomium* sp., while maximum mycelial growth and minimum inhibition zone was observed in case of *Bacillus subtilis* as compared to control. Therefore we say that on basis of present investigation the more study are needed for field application of these Bio-agents to manage the disease incidence and severity of sclerotinia stem rot of pea.

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