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In vitro Assessment of Bioagents and Fungicides against *Rhizoctonia solani,* the Causative Agent of Potato Black Scurf Disease

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ABSTRACT

Potato (*Solarium tuberosum* L.) is the most important staple food crop in the world. Its ranking 3rd in terms of total production with over 365 million tonnes/year after rice and wheat. India is the 2nd place after China produced 52588.98 thousand metric tons potato. In India, among all the states Uttar Pradesh stands first with the production of 15812.62 thousand metric tonnes followed by West Bengal with the production of 12782.00 thousand metric tons in the year 2018-19. Potato is infected by a number of soil and tuber borne diseases and pest. Among all, black scurf (*Rhizoctonia solani* Kuhn.) is destructive disease of potato. The most common phase of this disease is formation of sclerotial masses on the tuber resulting in black scurf which considerably reduces market value of edible tubers. In present investigation different bioagents and chemicals was tested for management of disease *in vitro* condition. Among the bioagents, maximum inhibition per cent (67.78%) of pathogen was recorded with *Chaetomium globosum*, followed by *Bacillus subtilis* (63.33%). Among fungicides, the complete mycelial growth inhibition (100%) of *R. solani* was recorded in Boric acid @ 3 % followed by Mencozeb (77.78%) and Monceren (77.03%) @ (0.2%) concentration.

Keywords *In vitro*, Potato, Bioagents, Antagonism, *Rhizoctonia solani,* Fungicides.

INTRODUCTION

Potato occupies largest area under any single vegetable in the world it is also known as king of vegetable. Potato has been introduced into Spain about 1570 AD from Peru (South America) and later to neighboring European countries and in less than 100 years it was being grown extensively in many regions of Europe. Potato was introduced as a garden crop in Ireland by Spanish people. In 18th century many of the ordinary people in Ireland use of potato as their staple food. In 1845 a disease called Potato Blight ruined potato crops all over Ireland. Millions of people died from hunger because they had no other food to eat. This is known as the Great Irish Famine. It lasted for almost 5 years. Many people had to leave Ireland in search of a food for survival of their life.

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Among all the states in India, Uttar Pradesh stands first with the production of 15812.62 thousand metric tonnes followed by West Bengal with the production of 12782.00 thousand metric tonnes in the year 2018-19. In the year 2018-19 India produced 52588.98 thousand metric tones potato (Horticulture Statistics Division, Ministry of Agriculture and Farmers Welfare 2018-19). Over one million people around the world consume potatoes, and a billion people in developing countries consume them. FAO statistics estimates world utilization of current potato production as 45% for human food, 30%for animals, 15% for seed, 2% for starch, and about 8% as waste. Potato tuber contains 80% water, 17% carbohydrates, 0.1% fat, 2% minerals, 0.6% fiber and high in potassium. Potato has low sodium and low vitamins (B, C, and B_2) which play a pivotal role for its nutrition (Walt and Merill 1963).

Potato is infected by a number of soil and tuber borne diseases such as common scab (*Streptomyces scabies*), powdery scab (*Spongospora–subterranean*), brown rot (*Ralstonia solanacearum*), black leg (*Erwinia carotovora* sp.), sclerotium wilt (*Sclerotium rolfsii* Sacc.), *Verticillium* wilt (*Verticillium-albo-atrum,* Reinke & Berth), black scurf (*Rhizoctonia solani* Kuhn), sclerotinia stem rot (*Sclerotinia sclerotlorum* L.) and also insect pest such as Potato tuber moth (*Phthorimea operculella,* Zeller), Aphids (*Myzus persicae*),Whiteflies (*Bemisia tabaci*), Leaf hoppers (*Amrasca biguttula*), White grub (*Holotrichia longipennis*), Mite (*Polyphagotarsone muslatus,* Banks) Root knot Nematode (*Meloidogyne incognita*), Potato cyst nematodes (*Globoderaro stochiensis, G. pallida*), Potato virus *Y* (PVY), Potato virus *X* (PVX) and Potato leaf roll virus (PLRV). Among these, black scarf caused by *Rhizoctonia solani* appears in severe proportions in the state, causing considerable yield losses. The incidence of black scurf has been reported up to 37% from different parts of state.

The pathogen is thought to be soil-borne, and sclerotia are commonly found in soil. However, there is little information on its long-term management, and it is typically treated with chemical applications. Overuse of the chemical could lead to environmental, human health, and pest resistance issues. The growing awareness of fungicide-related dangers has emphasised the importance of using biological methods as an appropriate disease control method that is also environmentally friendly (Khare *et al.* 2010). Biological treatment seems to be the best solution for long-term sustainable practices and efficient implementation of soil-borne disease, with the potential to significantly reduce disease incidence (Howell 2003). Bioagents have previously been used to successfully manage *R. solani* on a variety of crops (Meena *et al.* 2003, Atef 2008, Hajieghrari *et al.* 2008). Given the growing importance of sore shin disease and environmental concerns, the current *in vitro* study was conducted to control *R. solani* on potato by *T. viride, T. harzianum,* and *B. subtilis.*

MATERIALS AND METHODS

Field experiments were carried out at the Sardar Vallabhbhai Patel University of Agriculture and Technology's Crop Research Center (CRC), Meerut, and laboratory experiments were carried out at the Center of Excellence for Sanitary and Phytosanitary (SPS), Department of Plant Pathology. During 2019-2020, the College of Agriculture will be located on the main campus of the Sardar Vallabhbhai Patel University of Agriculture and Technology in Meerut, Uttar Pradesh. Meerut district is located at an elevation of 237 meters above mean sea level, between 29° 01'N latitude and 77° 45'E longitude. Meerut is located in the Upper Gangatic plains' northwestern plains subregion.

Collection, isolation, identification, purification, maintenance and pathogenicity of the pathogen

Collection of diseased specimens of Potato plants showing the typical symptoms of stem canker lesion and sclerotia formed on tuber were collected from farmer's field and university campus at 'Crop Research Center' during the cropping season of 2019-20. The specimens were collected on the basis of visual symptom and brought to the laboratory and critically examined and studied the symptoms of disease and isolation of the pathogen.

For the isolation of *R. solani,* sclerotia grown on the skin of infected potato tubers cv Spunta, showing typical black scurf symptoms were first washed free

of excess soil in tap water. A portion of each tissue was transferred to 2 min in 2% sodium hypochlorite solution then rinsed twice in sterile water. The treated tissue pieces with sclerotia were blot dried and then transferred to petri plates containing sterilized potato dextrose agar (PDA) medium with five pieces per plate. All plates were incubated at 25 ± 2°C for 7 days. The pure cultures of isolate of *R. solani* were stored on PDA medium at 4°C in the dark for experimentation. The fungal isolate were identified observing the morphological and microscopic characteristics described by Parmeter and Whitney (1970), Sneh *et al*. (1991) and Tredway and Burpee (2001). Then pathogenicity test of the fungal isolate were carried out to the prove (Koch'spostulate 1876). During the experiment the inoculum was applied in two ways i.e. by mixing the inoculum to pots filled with the sterilized soil before sowing the seeds and the placement of inoculums near plant after sowing the seeds in pots filled with sterilized soil. Sterilized soil was filled in thirty cm diameter earthen pots. Fifteen days old culture grown on PDA medium was mixed thoroughly in the upper soil layer at 1% weight basis. Then apparently healthy, surface washed 2-3 tubers were sown in each pot. The control consisted of a set of pots with sterilized soil inside, 2-3 tubers sown in each pot, and no inoculums. The soil's moisture retention capacity of 25% was maintained throughout the time by adding sterilized water on a weight basis. The plants were hauled out of the ground and properly cleansed with distilled water after 45 days incubation and displayed the characteristic stem canker condition as black brown symptoms. Re-isolations from these artificially infected plants were made, and the resulting cultures were compared to the original cultures.

In-vitro evaluation of different bioagent against *Rhizoctonia solani*

Antifungal activity of the bacterial and fungal bioagents such as *B. subtilis,* and *T. harzianum, Chaetomium globosum* was tested against *R. solani* isolates using dual culture technique (Rabindran and Vidyasekaran 1996). After making the potato dextrose agar (PDA), 100 ml of the media was poured into each 250 ml flask and sterilized. Each petri plate was then filled with 20 cc of molten PDA and left till it solidified. *Rhizoctonia solani* was inoculated on one side of petri plates, and an antagonist was inoculated on the precise opposite side of the identical plate, leaving a gap of approximately 4 cm. This was done to study the effect on the proliferation of the pathogen. For bacterial antagonists, the bacterial culture was streaked at the peripheral of the petri - dish with the use of an inoculation loop. In a petri dish, 5 mm round bits from freshly developing pathogen and inhibitory culture were removed and placed at both peripheral ends. Consequently, developing cultures After 48, 96 and 144 hours of incubation the radial growth of the pathogen was measured. The per cent inhibition of the growth over control was calculated by following the equation given by Vincent (1927). Following Kucuk and Kivanc's method, the *in-vitro* antiproliferative activity of *Trichoderma viride, Trichoderma harzianum, A. niger,* and *Penicillium* spp. versus *Rhizoctonia solani* was investigated using the dual culture method. The approach outlined by (Dennis and Webster 1971) was used to determine the impact of the volatile chemical emitted by *Trichoderma viride* and *Trichoderma harzianum* on the pathogen's ability to grow.

In vitro evaluation of fungicides against *Rhizoctonia solani*

In vitro tests of fungicides against *Rhizoctonia solani* the result of three fungicides from different groups was tested *in vitro* at 0.05%, 0.1%, and 0.2% concentrations for their efficacy to inhibit the pathogen's growth to the greatest extent possible. R's growth is affected. Nene and Thapliyal investigated solani using the poisoned food technique. PDA (potato dextrose agar) was prepared, and 100 ml of the medium was sterilized in 250 ml flasks. The fungicides were added separately to the molten cooled sterile medium and thoroughly mixed to achieve the required concentration levels for each fungicide. Each 90 mm sterilized petri plate received twenty (20) ml of poisoned medium. At 27 ± 2^0C, each plate was cultured with a five mm disc of mycelium in the center and incubated. For each treatment, three replications were kept. Potato dextrose agar medium was used as a control, and plates were incubated at 27 ± 2^0C until the colony reached the periphery in the control. The per cent inhibition of the growth over control was calculated by following the equation given by Vincent (1927).

$$\text{Percent inhibition (PI)} = \frac{C - T}{C} \times 100$$

Where,

PI = Per cent inhibition over control,
C = Diameter of fungal growth in control plate,
T = Diameter of fungal growth in test plate.

Statistical Analysis

The complete randomized design (CRD) was used, and the resulting data were then statistically examined. (Steel *et al.* 1997) used the statistical analysis of variance (ANOVA) method and estimated the critical difference (CD) at the 5% degree of significance for compared with other treatments.

RESULTS AND DISCUSSION

Pathogen

The present investigations were carried out on the "*In vitro* Assessment of Bioagents and Fungicides against *Rhizoctonia solani* the Causal Agent of Black Scurf Disease of Potato" among the different diseases of potato, black scurf of potato caused by *Rhizoctonia solani* is one of the major soil and seed borne disease

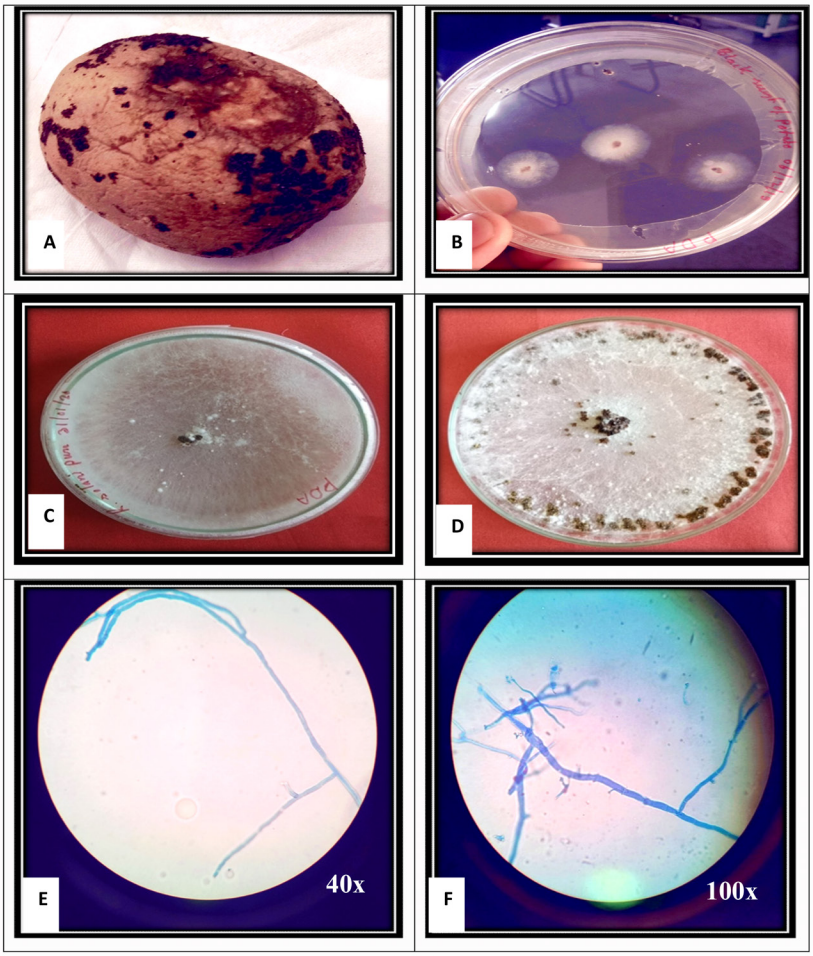

Plate No. 1. A) Infected tuber with Sclerotia, B) Single colony of *R. solani*, C) Pure mycelial growth of *R. solani,* D) Sclarotial formation of *R. solani* on PDA and E and F) Microscopy observation that see right at 90^0 angle mycelia.

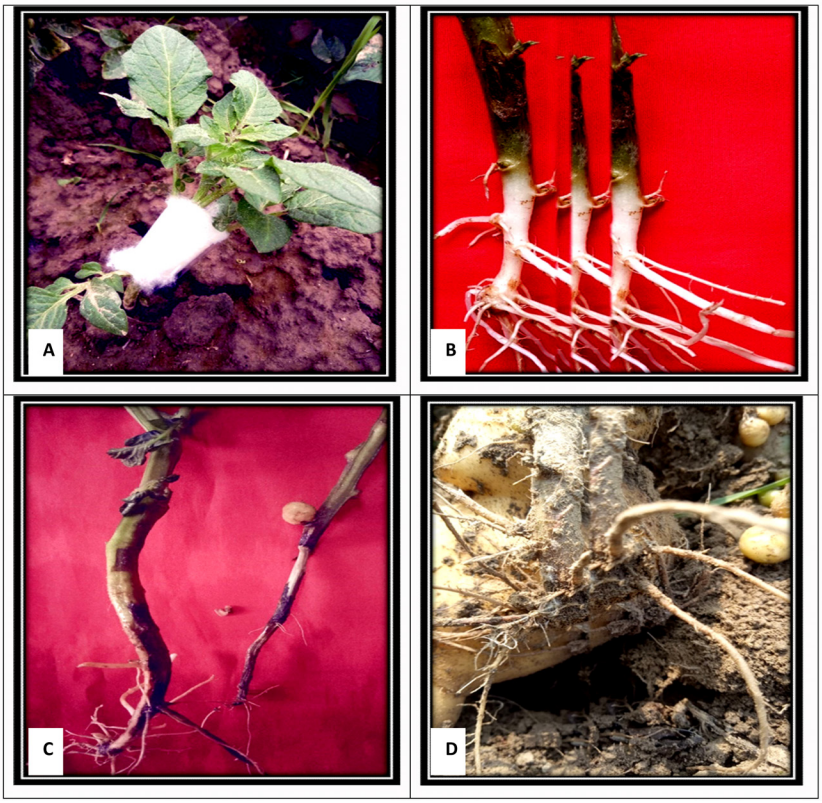

Plate No. 2 A) Inoculation of pathogen in healthy plant B) Caused on stem and initially appearance of symptom C) Severe symptom of *R. solani* on stem D) Sclerotia formation on tuber.

causing heavy potato crop losses. Hence, the present investigations for *In vitro* evaluation of bioagents and fungicides were carried out at Department of Plant Pathology, College of Agriculture, Sardar Vallabhbhai Patel University of Agriculture and Technology Meerut. The results of different experiments pertaining to the study are presented in this appropriate tables and illustrations.

Collection, isolation and purification of *Rhizoctonia solani*

Infected tubers of potato were collected from potato field at Chirori farm of the University at Meerut and farmers field in the vicinity of the University where disease was prevalent. The laboratory received samples for isolation and additional research. Under aseptic circumstances, the fungus was grown on PDA from a potato tuber that was infected. On PDA, it was discovered that the fungus growing from the tuber parts had abundant aerial mycelium that afterwards turned greyish brown to black. The hyphal tip approach was used to purify the culture.

Identification of the pathogen

Based on cultural traits and morphological characteristics of sclerotia, which could be seen with the unaided eye and under a microscope, the isolated fungus was identified. The observed characters are listed below, characteristics of the growth habit using potato dextrose agar medium, pale to dark grey white colo-

nies with puffy mycelium were formed. The fungus developed copious amounts of sclerotial-body-containing surface mycelium. The sclerotia developed on the extracellular matrix and were greater than the sclerotia dispersed around the culture's surface. Mycelium on an agar medium, mycelium ranged in breadth from 4.5 to 7.3 µm and in length from 3.5 to 7.2 µm. Initially greyish white in color, it eventually turned dark brown with age. The older portion of the mycelium had more septa than the younger one. Branches frequently develop at a straight angle with restriction at their origin point, and a septum was almost always present nearby. Characteristics of the colony, Colonies were fluffy, quickly expanding, dark brown with plenty of sclerotia formation, margin smooth, and covered the entire plate in 3–4 days. On the back of the petri plate, the colony had a black appearance. Sclerotia were spherical to oval or irregularly spherical in shape, and darkish brown in colour. Microscopic observation, there was always a septum present close to the point of formation of the mycelial branches, which frequently emerge at acute angle (90^0) with constriction at their origin. On both the potato and tuber dextrose agar media, sclerotia were abundantly produced. They were spherical to round or irregular in shape, and darkly brown in color. (Plate no.1) A) Infected tuber with Sclerotia, B) Single colony of *R. solani,* C) Pure mycelial growth of *R. solani*, D) Sclarotial formation of *R. solani* on PDA and E and F) Microscopy observation that see right at 90^o angle mycelia.

Pathogenicity

When seed and soil were artificially inoculated to potato plant under artificial inoculation conditions, the (*Rhizoctonia solani* Kuhn.) isolated from infected tuber of potato was found pathogenic. After 30 days, the common signs of stem canker rot appeared. The most obvious symptom was the prevalent cause of potato seedlings. The first stem begins to discolor and dry, and even the root rot follows, eventually killing the entire plant. The diseased plant's stem had brown to greyish lesions. *Rhizoctonia solani* was isolated from an artificially inoculated plant in (Plate no. 2) A) Inoculation of pathogen in healthy plant, B) Caused on stem and initially appearance of symptom, C) Severe symptom of *R. solani* on stem,

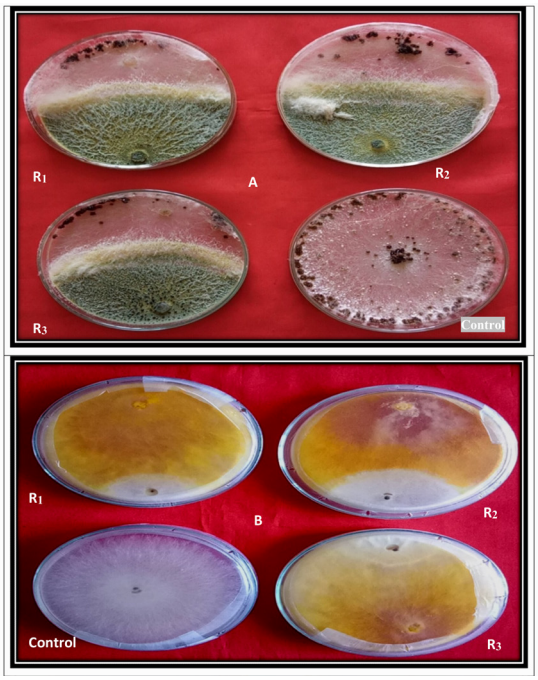

Plate No. 3. *In-vitro* assessment of several biological against *Rhizoctonia solani*; A) *Trichoderma harzianum* B) *Chaetomium globosum.*

D) Sclerotia formation on tuber.

Assessment of several biological agents and fungicides against pathogens *in-vitro* assessment of several biological against *Rhizoctonia solani*

Antagonistic activities of three antagonists' viz., *Trichoderma harzianum, Chaetomium globosum,* and *Bacillus subtilis* was evaluated against black scurf fungus *Rhizoctonia solani* under *in vitro* conditions. The data for results is indicated in (Table 1, Plate no. 3) the results from the table indicate that, significant difference observed in per cent inhibition of mycelial growth of *Rhizoctonia solani* by all the tested bioagents. Among the maximum inhibition per cent (67.78%) of *Rhizoctonia solani* was recorded with *Chaetomium globosum* after 144 hours, which is significantly superior from all the tested isolates followed by *Bacillus subtilis* (63.33%) and While least mycelial growth inhibition was recorded with *Trichoderma harzianum* (60.37%). The findings of (Seema and Devaki 2012) who found that in a dual culture assay, the percentages of growth inhibition

Table 1. Assessment of bio-agents against *Rhizoctonia solani in vitro.*

Sl. No.	Treatments	Radial growth was measured after 144 hr in (mm)	Growth inhibition (%) data were calculated after 144 hr
1	*Trichoderma harzianum*	26.66	60.37
2	*Chaetomium globosum*	20.00	67.78
3	*Bacillus subtilis*	24.16	63.33
4	Check	90.00	00.00
CD at 5%		**3.90**	
SE (m)		**1.18**	
CV		**5.08**	

of *T. viride, T. harzianum, A. niger, B. subtilis,* and *Penicillium* spp. on *R. solani* were 70%, 67%, 57%, 50%, and 44%, respectively. The results of the present investigation support these findings. This resulted in the reduction of the mycelial growth of the *R. solani.* Through the use of a dual culture approach, (Prasad *et al.* 2015) evaluated the effectiveness of twenty-four fungal biocontrol agents and twelve bacterial biological control against the phytopathogenic fungus *Rhizoctonia solani*. Under *in vitro* circumstances, *Rhizoctonia solani's* mycelium was reported to be effectively inhibited by *Trichoderma harzianum*-1 and *Pseudomonas fluorescence*-2 (62.53%, 62.20%) (Walther and Gindrat 1988). Discovered that *Chaetomium globosum* on *R. solani* underwent hyphal coiling in response to *C. globosum* on agar, which was one of the antagonistic actions seen *in vitro.*

Table 2. *In vitro* assessment of fungicides against *Rhizoctonia solani.*

Sl. No.	Fungicides	Radial growth (mm) after 144 hrs	Growth inhibition (%)
T1	Monceren @ 0.05%	26.67	60.37
T2	Mancozeb @ 0.05%	25.83	61.30
T3	Boric acid @1%	16.67	71.48
T4	Monceren @0.1%	23.33	64.08
T5	Mancozeb @0.1%	20.01	67.77
T6	Boric acid @2%	13.33	75.19
T7	Monceren @0.2%	11.67	77.03
T8	Mancozeb @0.2%	11.00	77.78
T9	Boric acid @3%	0.00	100.00
T10	Control (Check)	90.00	-
CD at 5%		**=2.90**	
SE±m		**=0.97**	
CV		**=7.10**	

In vitro assessment of fungicides against *Rhizoctonia solani*

The Poisoned Food Technique was used to examine the effectiveness of non-systemic fungicides at concentrations of 0.05%, 0.1%, and 0.2%. The results, which were shown in is indicated in (Table 2, Plate no.

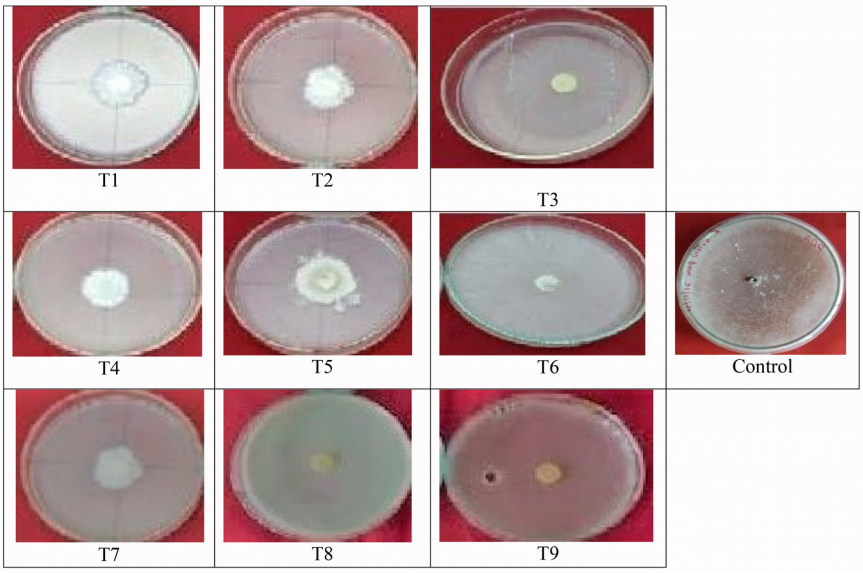

Plate No. 4. *In vitro* assessment of fungicides against *Rhizoctonia solani.*

4) showed a considerable difference in the fungicides' ability to block *Rhizoctonia solani's* mycelial growth to a certain extent. The complete mycelial growth inhibition (100%) of *Rhizoctonia solani* was recorded with 3% of Boric acid followed by Mencozeb 77.78% and Monceren 77.03% @ 0.2% concentration in both. Whereas, minimum mycelial growth inhibition 60.37 % was recorded in Monceron @ 0.05% concentration followed by 61.30% was observed in Mancozeb @ 0.05% compared to control. The result obtained in the present investigation are in accordance with the findings of (Thind *et al.* 2002) conducted an experiment to determine the efficacy of Pencycuron with other fungicide against black scurf of potato and observed that fungicide Pencycuron completely inhibited the mycelial growth of *Rhizoctonia solani* at 25 ug/ml *in vitro* condition. It was discovered to be particularly helpful in reducing potato black scurf. Muhammad *et al.* (2015) conducted an *in vitro* evaluation of three fungicides, namely Monceren (pencycuron), Curon (pencycuron), and Topsin M (thiophenate methyl), against *R. solani.* While Topsin M was effective at 2500 ppm with 74% inhibition, Moncerene and Curon worked best at 700 ppm with 96% and 87% suppression of the pathogen's mycelial growth, respectively.

CONCLUSION

In vitro study among all the tested fungicides Boric acid @3% was found most effective followed by Mencozeb 75% WP @ 0.2 % and Pencycuron (250 SC) @ 0.2% concentrations for inhabiting the growth of pathogen. While, among tested bio-agents, *Chaetomium globosum* was highly effective followed by *Trichoderma harzianum* and *Bacillus subtilis* for mycelial growth inhibition of the pat. While alternative of chemical farmers may also be use bioagent *Bacillus subtilis* for the management of black scurf disease.

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