Penicillium pinophilum has the potential to reduce damping-off caused by *Rhizoctonia solani* in sugar beet

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Abstract

Rhizoctonia solani is an economically important pathogen of sugar beet (*Beta vulgaris* L.) causing seedling damping-off, and root and crown rot. Cultural practices, partially resistant cultivars, and fungicides are among the methods most used to manage *R. solani*. *Penicillium pinophilum*, a potential bio-control agent for Rhizoctonia damping-off, was isolated from sugar beet. Our objective was to evaluate the bio-control potential of *Penicillium pinophilum* against *R. solani* AG 2-2 under laboratory and greenhouse conditions. *In vitro* co-culture of both fungi showed that *R. solani* growth was inhibited by *P. pinophilum*. A greenhouse inoculation study was done using sclerotia of *R. solani* and a conidia suspension of *P. pinophilum* to evaluate the response of a *Rhizoctonia* susceptible cultivar. Treatments included *R. solani* sclerotia, *P. pinophilum* conidia suspension, a combination of *R. solani* sclerotia with *P. pinophilum* conidia suspension, and a mock inoculation with water (control). One 2-cm deep furrow was made in the middle of peat filled trays into which 10 seeds were planted. Each treatment was applied adjacent to each seed and covered with peat. There were four replicates per treatment arranged in a completely randomized design. The sole sclerotia treatment caused 75% damping-off and severe root rot on surviving plants whereas the combination of sclerotia with *P. pinophilum* conidia suspension reduced damping-off by 7553~~53~~50%. No damping-off incidences were observed with the *P. pinophilum* conidia suspension or the mock-inoculated control. It was concluded that *P. pinophilum* has the potential to reduce damping-off caused by *R. solani* but use of the most appropriate *P. pinophilum* concentration and its mitigation mechanisms need further studies.

Keywords: Biological control, *Beta vulgaris*, Antagonistic, inoculum.

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Introduction

Rhizoctonia solani Kühn (teleomorph: *Thanatephorus cucumeris* [Frank] Donk) is a necrotrophic pathogen that causes damping-off, and Rhizoctonia crown and root rot diseases in sugar beet (*Beta vulgaris,* L.) (O'Brien, 1996). This pathogen is of monocyclic infection and it overwinters in the soil and on crop debris as sclerotia (Sherwood, 1967; Adams and Papavizas, 1970; Papavizas, 1970). Sclerotia germinate to form infective hyphae that penetrate into the root cortex and cause infections to the tissue (Armentrout and Downer, 1987; Armentrout et al., 1987; Flentje et al., 1963). This soil-borne fungus varies in morphogenetic diversity including hyphal fusion or anastomosis, virulence, cultural appearance, and physiology of the biotypes (Carling et al., 2002; O'Brien, 1996). There are 13 anastomosis groups (AGs) of *R. solani* (Carling et al., 2002; Parmeter et al., 1969), while the main AGs detrimental to sugar beet in Minnesota and North Dakota are AG 2-2 IIIB and AG 2-2 IV (Brantner and Windels, 2009; Windels et al., 1997; Windels and Nabben, 1989). Other AGs and sub-groups, including AG 4, AG 1, and AG 5 have also been reported in other US states but at low frequency (Windels et al., 1997). *R. solani* has been reported to reduce sugar beet yield loss by 30% to 50% (Neher and Gallian, 2011).

Severities of damage caused by *R. solani* depend on characteristics of the AG, host, and environment. Integrated pest management (IPM) strategies are considered essential for minimizing disease severity. Cultural strategies such as crop rotation at least every third year with non-host cereal crops such as barely, wheat, and oats are ~~the best treatment~~commonly followed to reduce primary inoculum of *R. solani* (Behn et al., 2012; Boine et al., 2014; Buhre et al., 2009; Buttner et al., 2002; Dircks et al., 2014). Nevertheless, some AGs have a polyphagous nature to surmount this strategy, such as AG 2-2 IIIB which has a wide range of hosts including corn and soybean (Engelkes and Windels, 1996; Ithurrart et al., 2004). It takes many years to develop quantitative

Commented [LD2]: To be consistent we have corrected to "soilborne" instead of "soil-borne" in the manuscript.

resistant cultivars against *R. solani* and resistant cultivars typically show poorer potential yields than susceptible commercial cultivars (Panella and Ruppel, 1996; Ruppel et al., 1995). There is no commercial cultivar that is immune to *R. solani* that also has resistance to the other major diseases of sugar beet with yields equivalent to the current approved varieties. As such, producers typically use cultivars with partial resistance to *R. solani*, but high yield potential, combined with fungicides to maximize recoverable sucrose. Chemical strategies such as seed treatment (penthiopyrad), and in-furrow application of fungicides such as azoxystrobin at planting provide effective control in greenhouse and field research (Khan et al., 2017; Khan et al., 2010; Liu and Khan, 2016; Liu et al., 2020). Among the quinone outside inhibitor (QoI) fungicides, azoxystrobin, is the most widely used in major sugar beet growing states such as Minnesota, North Dakota, Montana, and Michigan (Harveson et al., 2002; Kirk et al., 2008). Although timely application of fungicides do provide effective control of *R. solani*, fungi often develop resistant biotypes under selection pressure when used repeatedly in commercial fields. QoI resistance has been reported in AG2-2IIIB in turfgrass, and AG 3 in potato (Blazier and Conway, 2004; Djebali et al., 2014; Olaya et al., 2012).

The incorporation of an effective biocontrol agent in a strategy to holistically manage *R. solani* can be environmentally safe and will help in reducing the risk of developing resistant biotypes. Several fungal biocontrol agents including *Stachybotrys elegans* (Benyagoub et al., 1994), *Bacillus amyloliquefaciens, B. subtilis*, and *Trichoderma harzianum* have been reported as providing biocontrol of Rhizoctonia diseases in sugar beet (Abada, 1994; Homma, 1996; Jacobsen et al., 1997; Karimi et al., 2016; Kiewnick et al., 2001). Moreover, the ascomycete *Laetisaria arvalis* and non-pathogenic *Rhizoctonia zeae* have been used with limited success to control Rhizoctonia-damping off in sugar beet (Lewis and Papvizas, 1992; Webb et al., 2015). The

soilborne fungus *Penicillium pinophilum* Hedgcock, *Basionym* (Synonymy *Talaromyces pinophilus* (Hedgcock) Samson, Yilmaz, Frisvad & Seifert, comb. nov. MycoBank) has been demonstrated as a mycoparasitic fungus against *Botrytis cinerea* causing onion scalp and umbel blights (Samson et al., 2011; Abdel-Rahim and Abo-Elyousr, 2018). *Penicillium pinophilum* was also reported to reduce soil-borne pathogens *Pythium* and *Rhizoctonia*-induced damping-off in cucumber in Oman (Kazerooni et al., 2019). We found sugar beet roots from a commercial field in Minnesota, USA with *P. pinophilum*. Our research objective was to evaluate the biological potential of *P. pinophilum* at controlling *R. solani*-induced damping-off on sugar beet.

Materials and Methods Fungal Isolates of *R. solani* and *P. pinophilum*

Clones of *R. solani* AG 2-2 IIIB (Genbank accession: MN128569), isolate #MN569 was maintained on amended clarified V8 (30 g agar, 15 mg pimaricin, 15 mg rifampicin, 375 mg ampicillin, 30 mg rose bengal, and 180 mg PCNB per liter of medium, according to Hansen et al. (1990).~~ACV8)~~ (25 ± 2º C). Sclerotia and mycelia were developed from subcultures on ACV8 and were used for in vitro and in vivo study.

Five isolates of *P. pinophilum* were obtained from sugar beet tap roots collected in 2018 from a field in Moorhead, MN (46.8738° N, 96.7678° W). The fungal colonies were observed with blue-green velvety and white margins on the root periphery. Conidia were hyaline, globose, and conidiophores were densely penicillated. The morphological characteristics of the fungus were similar to *Talaromyces* species (Yilmaz et al., 2014). A single spore isolation method was used to prepare five independent isolates and genomic DNAs were extracted from those isolates. For the PCR assay, the internal transcribed spacer (ITS) ITS4/ITS5 primers were used to amplify the ITS genomic region. PCR products were cleaned via E.Z.N.A ®Cycle Pure Kit, OMEGA and sent to Sanger sequencing by GenScript (GenScript, Piscataway, NJ). A Blastn analysis of the ITS

sequences of the five isolates showed 100% alignment to *Talaromyces pinophilus* (*Penicillium pinophilum*), accession no. AB455516.1 (596 bp genomic sequence). The amplified genomic sequence (539 bp) was submitted to NCBI (GenBank accession no. MK757839.1). Conidia suspension of *P. pinophilum* were prepared from the clone of MK757839 and were used for *in vitro* and *in vivo* study.

In Vitro Co-culture of Two Forms of _R. solani_ Inocula and Conidia of _P. pinophilum_ on 50% Potato Dextrose Agar (50% PDA)

To understand the potential of *P. pinophilum* as a growth suppressor to *R. solani,* two forms of *Rhizoctonia* inocula – sclerotia and mycelial plug (6 mm^2) were individually co-cultured with conidial suspensions of *P. pinophilum* (1×10^6 conidia/ml) on 50% PDA in petri dishes (100 mm x 15 mm) with four replicates. Each replicate contained 4-sclerotia or 4-mycelial plugs, one in each quarter of the culture plate using sterilized forceps. *P. pinophilum* conidia suspensions (200 µl) were transferred immediately adjacent to each sclerotium/mycelium plug using a dropper. Four replicates of non-conidia suspension (only autoclaved water) were used as mock-inoculations that contained only sclerotia or only mycelial plugs of *R. solani* and were arranged in the plates as described above. All the plates were sealed with parafilm and kept in an incubator at 25 ± 2ºC. This experiment was conducted twice. Additional treatments were included for observations using VWR N. A. 0.30 microscope at 4, 5, and 6 days post treatment initiation.

In Vitro Inoculation of Seeds on 50% PDA Using _R. solani_ and _P. pinophilum_

The efficacy of *P. pinophilum* as a biocontrol agent of *R. solani* on seeds of a *Rhizoctonia* susceptible cultivar (Crystal 101RR, Proprietary material, Crystal Beet Seed, Moorhead, MN 56560) placed on 50% PDA plates was evaluated. Four treatments were included: (1) one mycelial plug of *R. solani* and sugar beet seed; (2) conidiophore plug of *P. pinophilum* with sugar beet seed;

(3) mycelial plug of *R. solani* with seed and a conidiophore plug of *P. pinophilum*; and (4) non-inoculated seeds. Sugar beet seeds (Crystal 101RR) were washed with 70% ethanol for 1 minute and rinsed twice with sterile water. Seeds were then dried on sterile blotter paper under a laminar airflow cabinet. Three seeds were placed with sterile forceps at 1 cm apart on each culture plate followed by each form of inocula being placed close to each seed. Four replicates per treatment were evaluated. All the plates were wrapped with parafilm and kept in a growth chamber at 25 ± 2ºC. This experiment was conducted twice. Germination observations were recorded at 7 days post inoculation (dpi).

Greenhouse Evaluation of Antagonistic Potential of *P. pinophilum* to *Rhizoctonia* Inocula

A greenhouse study was done to further evaluate the potential of *P. pinophilum* in preventing or suppressing growth and infection by *R. solani*. Four treatments were applied to a *Rhizoctonia* susceptible cultivar as follows: (1) one *R. solani* sclerotium; (2) *P. pinophilum* conidia suspension (1×10^6 conidia/ml, ?? ml/G potting soil); (3) combination of sclerotium of pathogen with *P. pinophilum* conidia suspension (1×10^6 conidia/ml), and (4) mock-inoculation (autoclaved water) per seed. Plastic pots (27 x 13 x 13 cm, T.O. Plastics, Inc.; Clearwater, MN, USA) were filled with vermiculite and perlite mixer (PRO-MIX FLX) amended with osmocote (N-P-K:15-9-12) fertilizer (Scotts Company; Marysville, OH). Ten surface sterilized Crystal 101RR sugar beet seeds were sowed in each plastic pot in a 2 cm deep furrow at 1 cm apart (Noor and Khan, 2015). Each treatment was applied next to each seed and then covered with the vermiculite and perlite mixer. There were four replicates per treatment and the experiment was set up as a completely randomized design. The greenhouse temperature during the experiment period was 27 ± 2ºC, with 80% relative humidity, and a 12-hour photoperiod. Plants were watered as needed to maintain adequate soil moisture conducive for plant growth and disease development.

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Seedling emergence and damping-off were recorded at 28 days post inoculation (DPI). Percent stand counts and root rot ratings data were collected at 42 dpi. At 42 dpi, surviving plants were removed from pots, and roots were washed and rated for root rot severity using a modified 0-7 rating scale, where 0 = clean roots and no infection, 1 = ≤ 10% of root surface with black/brown symptoms, 2 = ≥10-20% of root surface with black/brown symptoms, 3 = ≥20-30% of root surface with black/brown symptoms; similarly, 4 = ≥30-40% , 5 = ≥40-50%, 6 = ≥50-60% of root surface with black/brown symptoms, and 7 = ≥ 60% dead plant (withered) (Ruppel et al., 1979).

Statistical Analyses

Experiments were conducted twice as a complete randomized design (CRD) with four replicates. Categorical/discrete root rot severity data were transformed to a percent of disease severity index (%DSI) using the following modified formula: $\%DSI = \left[\frac{\{(a\times0)+(b\times1)+(c\times2)+(d\times3)+(e\times4)+(f\times5)+(g\times6)+(h\times7)\}}{\{(a+b+c+d+e+f+g+h)\times i\}}\right] \times 100,$ where *a, b, c, d, e, f, g*, and *h* represent the number of plants with disease scores of 0, 1, 2, 3, 4, 5, 6, and 7, respectively, and *i* represents the highest root rot severity rating (Li et al., 2014). Levene's test of homogeneity of variances was done to determine whether two trials could be combined for analysis. Data were analyzed using R-studio (Version 3.6.1, St. Louis, Missouri, USA). A post hoc test of the Fisher's Protected Least Significant Difference (LSD) was used to separate treatment means using the same R-package (3.6.1). Treatment means were compared and separated by the calculated Fisher's LSD at $p = 0.05$ probability level.

Results

In Vitro Growth Inhibition of *R. solani* Inocula by *P. pinophilum* on 50% PDA

Co-culture of the two fungi showed a consistent growth suppression of *R. solani* inocula (sclerotia and mycelia) by propagules of *P. pinophilum*. Microscopic examination showed that *P. pinophilum* inhibited the hyphal proliferation of *R. solani.* In the plates without *P. pinophilum*, *R. solani* sclerotia and mycelia proliferated vigorously on 50% PDA. Both the independent culture of sclerotia and mycelia initiated sclerotia production at 14 DPI, while no sclerotia were observed with the *P. pinophilum* conidia suspension treatment (Figure 1). Microscopically, the co-cultivation of *R. solani* and *P. pinophilum* on 50% PDA showed growth inhibition of *R. solani* hyphae coinciding with profuse production of conidial mass of *P. pinophilum* (Figure 2).

In Vitro Inoculation of Seed with *P. pinophilum* Reduced *R. solani* Damping-off

Co-cultivation of sugar beet seed and mycelia of *R. solani* demonstrated 100% damping-off at 7 dpi in 50% PDA, while 90% seedling emergence was observed in the combined treatment of mycelia of *R. solani* with conidia suspension of *P. pinophilum*. No damping-off incidences were observed in the non-inoculated controls or the sole conidia treatments (Figure 3~~2~~). The results indicated that *P. pinophilum* conidia suspension suppressed mycelial proliferation, inhibited infections and mitigated damping-off under ambient conditions.

Greenhouse Evaluation of *R. solani* Mediated Damping-off via Conidia of *P. pinophilum*

Effects of treatments were significant ($p<0.05$) (Table 1). At 28 dpi, the highest mean damping-off was 75% in the sclerotia treatment, whereas the mean damping-off was 25% in the combined treatment of sclerotia and propagules of *P. pinophilum*. No damping-off incidences were observed in the mock-inoculated control and the treatment with only conidia of *P. pinophilum*. Overall, the treatments were significant for stand counts and root rot rating at 42 dpi ($p<0.001$).

192 The highest mean stand count was observed in the mock-inoculated control (95%), followed by
193 the treatment with the sole conidia suspension (94%). The lowest mean stand count was 25% in
194 the treatment with sclerotia. The combined treatment of sclerotia and conidia showed 75% stand
195 count. Among the four treatments, the most severe mean root rot was observed in the treatment
196 with *R. solani* sclerotia, while there was no root rot with the combined sclerotia and conidia of *P.*
197 *pinophilum* treatment. Likewise, the mock-inoculated control and exclusive conidia suspension of
198 *P. pinophilum* treatment did not show any root rot.

199 **Discussion**

200 This study provided in vitro evidence of the inhibitory activity by *P. pinophilum* of *R.*
201 *solani*, and its use for successful biocontrol of Rhizoctonia disease of sugar beet in the
202 greenhouse. At the microscopic level, we have demonstrated that the mycelia growth of *R. solani*
203 was inhibited by spore propagules of *P. pinophilum*. Significantly, the production of *R. solani*
204 sclerotia was also inhibited in the combined co-culture of the two organisms in the plate
205 bioassays, whereas monoculture of *R. solani* sclerotia and mycelia initiated new sclerotia
206 production at 14 days post treatment initiation. Furthermore, *in vitro* co-cultivation of sugar beet
207 seeds (i.e. from a susceptible cultivar), *R. solani* inocula (mycelia), and propagules of *P.*
208 *pinophilum* showed that this combined treatment at 7 dpi reduced damping-off by 80% compared
209 to levels with co-cultivation of sugar beet seeds and mycelia of *R. solani* alone.

210 *R. solani* survives in soil as sclerotia or as melanized mycelia, forms which are the primary
211 source of infection during the seed germination stage in the field (Boland et al., 2004; Lee and
212 Rush, 1983). We, therefore, preferred to use sclerotia in the greenhouse evaluations. We observed
213 that sole sclerotia inoculation was aggressive and capable of causing the highest damping-off in
214 28 days. Others have also observed that sclerotia cause severe damping-off in sugar beet (Gaskill,

1968; Naito and Makino, 1995). In this study, the combined treatment of sclerotia and conidia of *P. pinophilum* suppressed seedling damping-off by ~~75~~~~53~~50% when compared with the treatment using only sclerotia. Among the four treatments, the highest root rot was exclusively observed in the treatment of *R. solani* sclerotia. As expected, the mock-inoculated check, and inoculation with conidial suspension of *P. pinophilum* did not show any root rot. The combined treatment (*R. solani* sclerotia + *P. pinophilum* conidia) on the Rhizoctonia susceptible cultivar did not show any root rot, either. These results provide evidence that the novel *P. pinophilum* isolate significantly inhibited the damping-off potential of *R. solani* and inhibited the growth of *R. solani* sclerotia. Depending on the stages of plant development, *R. solani* causes pre- and post-emergence damping-off of seedlings, crown rot and root rot (Liu et al., 2019). Traditionally, the commercial *Rhizoctonia* resistance cultivars were developed for the latter two symptoms, thus the seedling stage remains most vulnerable to Rhizoctonia. Sugar beet farmers commonly use chemically treated seeds to ward of the damping-off phase. However, with increasing costs and concern of chemical pesticides on the environment, and development of fungicide resistance, biological control of Rhizoctonia damping-off remained a viable option. In this context, control of the fungus both on the seeds and the immediate milieu of the emerging seedlings with *P. pinophilum* as seed treatment or in-furrow application during seed sowing remains to be explored in subsequent experiments.

The dynamics of *R. solani* epidemics on sugar beet may follow a polycyclic epidemic (Otten et al., 2003), driven by two sources of inoculum: primary resident or incoming inoculum and secondary inoculum produced by infected roots (Gilligan and Kleczkowski, 1997). Overwintering sclerotia or mycelia of *Rhizoctonia* serves as the primary inocula for damping-off, where as in-season buildup of inoculum on sugar beet roots and debris in soil surface, followed

by its spread to crown region by rain splash or intercultural operation are mainly responsible for the secondary infection. The losses and damages caused by *Rhizoctonia* can be reduced by using measures that minimize disease infection sources or suppress spread this disease. This in general can be achieved through elimination of the initial pathogen inoculum and by reducing the production of secondary inoculum through resistant cultivars to slow down the incidence and rate of disease development, as well as by minimizing the time of exposure of the most susceptible stages of the crop to the pathogen. It will be interesting to know if a seed application of the *P. pinophilum* for *Rhizoctonia* biocontrol affects both the primary and the secondary inocula (Gilligan and Kleczkowski, 1997), and also affect one or both disease incidence (i.e., primary infection plus allo-infection) and conditional disease severity (Motisi et al., 2009).

Microbial agents are known to biocontrol through five mechanisms – antibiosis, parasitism, competition for nutrients, production of lytic enzymes and other chemical signals, and induced systemic resistance (Lamichhane et al., 2017). The *P. pinophilum* has been shown to produce 3-O-methylfunicone as a toxin in cultural extract against *R. solani* AG2-1 (Nicoletti et al., 2004). It will also be interesting to investigate if the mechanism of biocontrol of *P. pinophilum* on *R. solani* involves more than just the toxin production, as Abdel-Rahim et al (2018) have shown that the *P. pinophilum* releases cell wall degrading enzymes to attack *Botrytis cinerea*. In this vein, it has been suggested that a single biocontrol agent (BCA) with two biocontrol mechanisms usually results in better control than the use of individual or combinations of two BCAs with single mechanisms of action (Xu et al., 2011). It was then that the conditions under which the experiments were conducted in the greenhouse were close to the optimum for *P. pinophilum*. Efficiencies of *P. pinophilum* need to be studied and validated under conditions more relevant to field conditions such as cool temperatures in early crop establishment. Further detailed investigations on

inhibition of *R. solani* hyphal growth and sclerotia formation by *P. pinophilum* with light and electron microscopy as well as gene expression and enzymatic analyses would shed light on the nature of antagonism of the BCA. Moreover, searches for the optimal concentration of *P. pinophilum*, potential beneficial effects of *P. pinophilum* against other sugar beet damping-off pathogens, and any synergistic interactions with composts, other BCAs and biorationals should be explored for integrated management of sugar beet damping-off diseases (Lamichhane et al., 2017, Roberts et al., 2016).

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Table 1. Percentage of damping-off at 28 days post inoculation (dpi), and plant stand counts and root rot severity (% DSI) at 42 dpi in a Rhizoctonia susceptible cultivar - Crystal 101RR under greenhouse conditions. Means followed by the same letters are not significantly different at $p = 0.05$.

	28 dpi	42 dpi	
Treatment/Inocula	**Damping-off**	**Stand count (%)**	**% DSI[a]**
Mock-inoculated Check	0.0c	95a	0.00b
Sclerotia of *R. solani*	75.0a	25c	80a
Sclerotia of *R. solani* + Conidia of *P. pinophilum*	25.0b	75b	0.00b
Conidia of *P. pinophilum*	0.0c	94a	0.00b

[a]$\%\text{DSI} = \left[\frac{\{(a\times0)+(b\times1)+(c\times2)+(d\times3)+(e\times4)+(f\times5)+(g\times6)+(h\times7)\}}{\{(a+b+c+d+e+f+g+h)\times i\}}\right]\times 100,$ where *a, b, c, d, e, f, g*, and *h* represent the number of plants with disease scores of 0, 1, 2, 3, 4, 5, 6, and 7, respectively, and *i* represents the highest root rot severity rating.

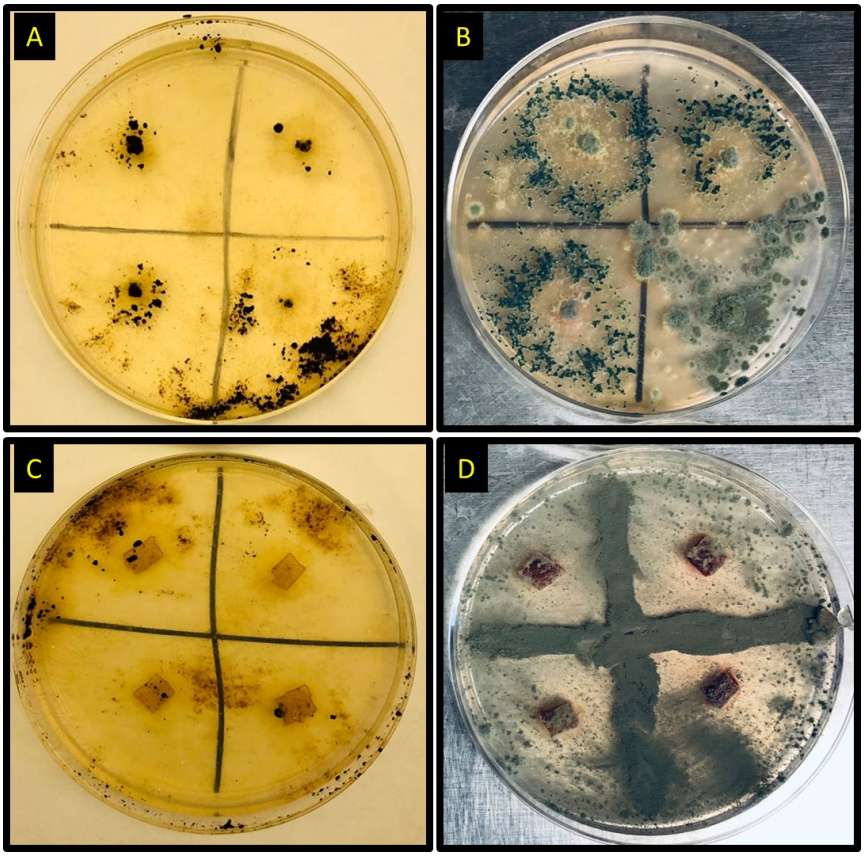

Fig. 1. Contrasting view of growth inhibition of *R. solani* inocula (sclerotia or mycelial plugs) via propagules of *P. pinophilum* on 50% PDA**-** A. exclusively sclerotia which generated sclerotia production, B. co-cultivation of sclerotia of *R. solani* and conidia of *P. pinophilum* in which *P. pinophilum* propagules inhibited the germination of *R. solani* sclerotia, C. exclusively mycelia plug of *R. solani* which generated sclerotia, D. co-cultivation of mycelia plug of *R. solani* and propagules of *P. pinophilum*- resulted in growth inhibition of *R. solani.*

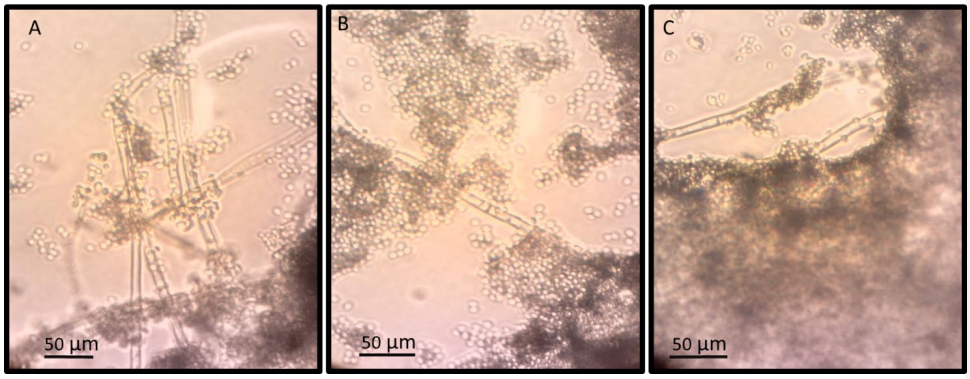

Fig. 2. Co-cultivation of *R. solani* and *P. pinophilum* showed growth inhibition of *R. solani* hyphae by conidial mass of *P. pinophilum* on 50% PDA at three time points-A. 4-days after co-culture, B. 5-days after co-culture, C. 6-days after co-culture. Magnifications was 10x.

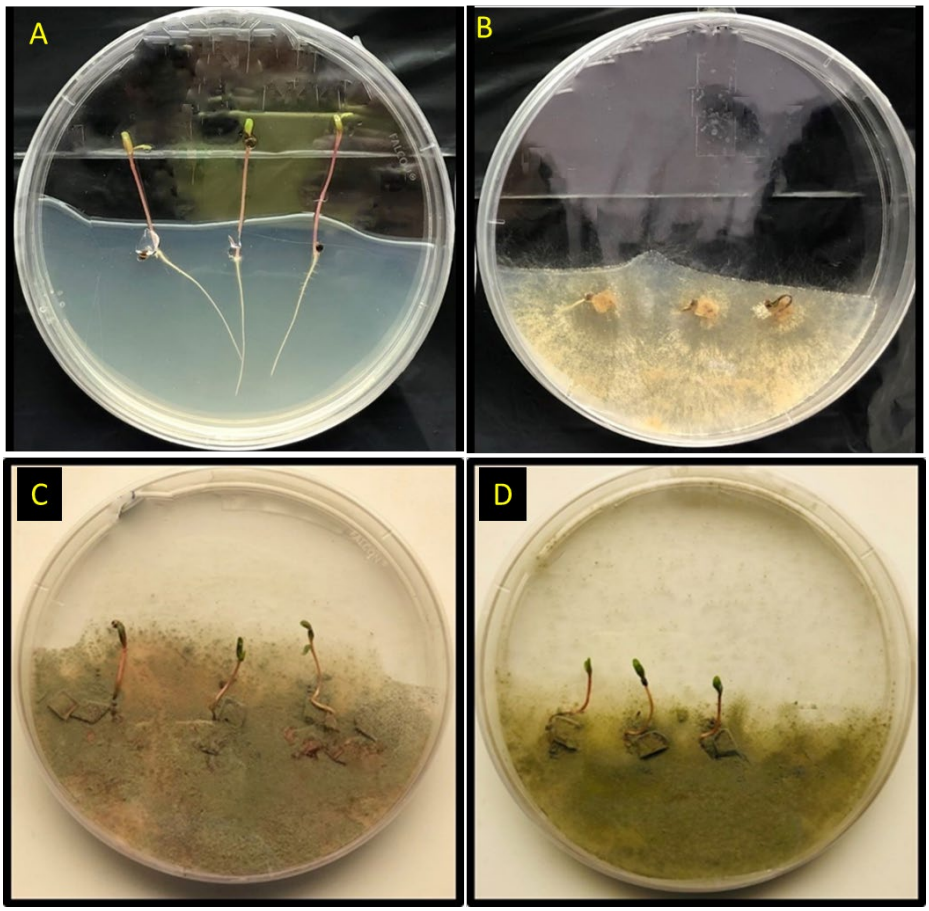

Fig. 3. In vitro inoculation of sugar beet seed on 50% PDA media using three groups of inocula and a control at 7 dpi- A. plate contained only seed (non-inoculated check)- shows 0% damping-off, B. plate contained seed and mycelia plug of *R. solani*- shows 100% damping-off, C. plate contained combined treatment mycelia plug of *R. solani* + propagules of *P. pinophilum*-shows 0% damping-off, D. plate contained seed and propagules of *P. pinophilum*-shows 0% damping-off.