Evaluating *Pythium oligandrum* as a foliar biocontrol for *Alternaria solani* and inhibitory effect against co -infections by *Alternaria solani* and *Phytophthora infestans* and against *Rhizoctonia solani*

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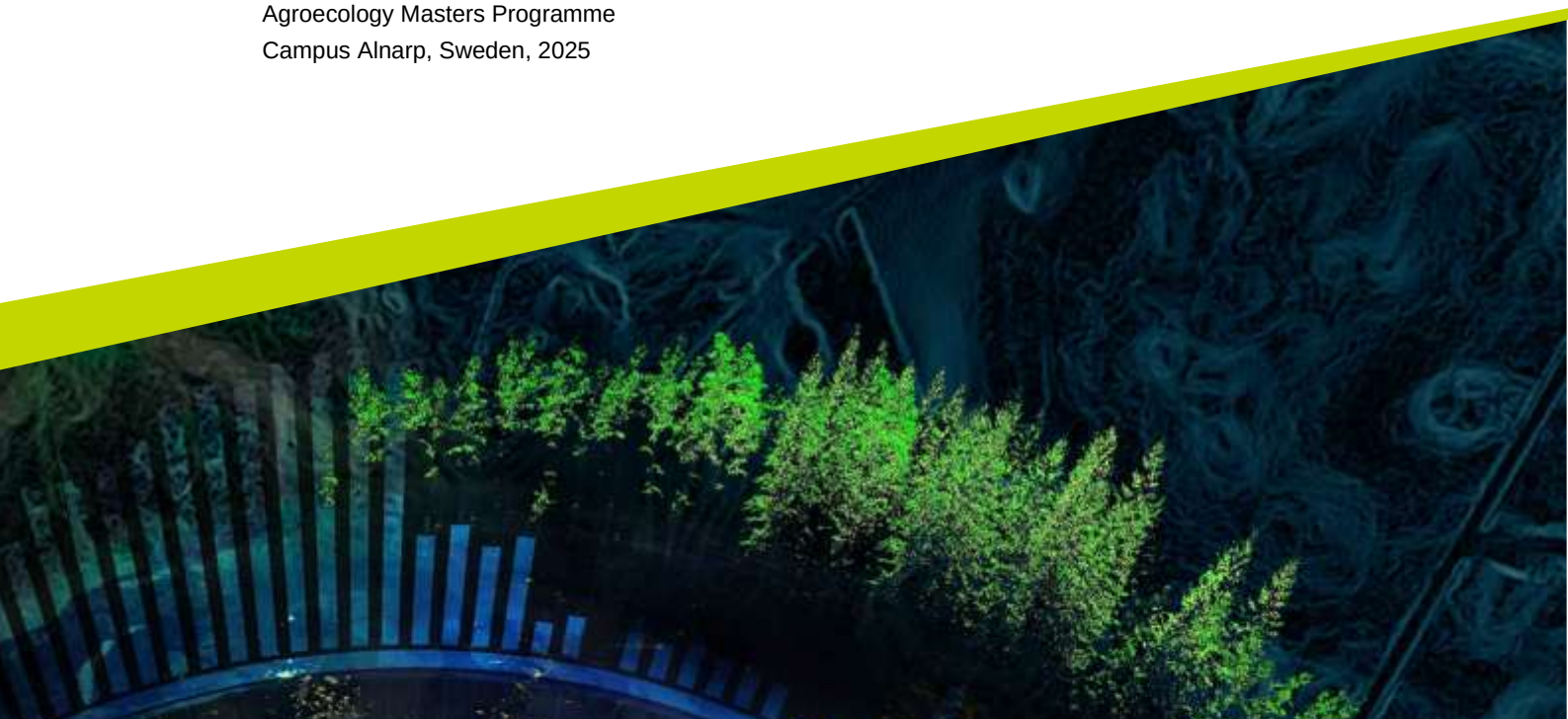

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Abstract

Potato is one of the most important staple crops worldwide. Potato productivity is severely threatened by pathogens such as *Alternaria solani* (early blight), *Phytophthora infestans* (late blight), and *Rhizoctonia solani* (black scurf). Chemical control is the dominant management strategy for these diseases, but it is unsustainable due to the development of fungicide-resistant pathogens, environmental risks, and economic costs. This study evaluated the effectiveness of *Pythium oligandrum* as a biological control agent through *in vitro* and *in planta* experiments. According to the results, *P. oligandrum* significantly inhibited both *A. solani* and *P. infestans* growth when co-inoculated. Dual culture assays of *P. oligandrum* and *R. solani* demonstrated strain-specific susceptibility through strong inhibition of AG3 but limited inhibition of AG5. Mycoparasitic interactions of *P. oligandrum* against *R. solani*, including hyphal coiling observed in microscopy. *In planta* trials, the 'Desiree' cultivar showed that foliar application of *P. oligandrum* reduced early blight lesion development rather than soil application. No significant suppression was observed in 'Kuras' due to its physiological stress by edema. Collectively, these findings indicate the possibility of *P. oligandrum* as a sustainable strategy in Integrated Pest Management (IPM) frameworks. Further research on application amounts and time periods, strain compatibility, and host–pathogen–biocontrol interactions will be essential for resilient and environmentally sound production systems.

Keywords: *Pythium oligandrum*, biological control, potato diseases, *Alternaria solani*, *Phytophthora infestans*, *Rhizoctonia solani*, Integrated Pest Management

Foreword

I worked in the agricultural sector in Sri Lanka and had direct contact with farmers in different cropping systems. Due to changes in fertilizer and pesticide regulations in 2021, the Sri Lankan agricultural sector faced many challenges. This experience highlighted the importance of sustainable solutions for the building resilience in agricultural systems. I was motivated to improve my knowledge of sustainable agriculture by pursuing the Agroecology Master's Programme at the world-leading agricultural university, SLU. I realize that this programme has helped me to enhance my knowledge as well as transform my way of thinking, approaching problems, and engaging with the complex realities of food and farming systems.

I believe that farmers need reliable and practical solutions when transitioning toward sustainable agricultural systems. Through my experience, I have observed that farmers often use large amounts of chemical inputs, even as a precautionary measure. This practice leads to serious environmental and health concerns. This inspired my interest in biocontrol agents as part of Integrated Pest Management strategies that can be implement to sustainable agricultural system.

For my final-year thesis, I studied *Pythium oligandrum* as a potential biocontrol agent against three major potato diseases. This research was both exciting and challenging and it highlighted how complex it can be to introduce one organism to manage another. The project taught me how to formulate research questions, design experiments, and handle practical difficulties in data collection and analysis. It also emphasized the limitations of laboratory experiments and the importance of connecting scientific findings to real field conditions. The study strengthened my patience, adaptability, critical thinking, and reflective mindset. I hope that the results presented in this thesis contribute not only to academic knowledge but also to practical approaches that support farmers and promote sustainable food systems.

Throughout my two years of studying, this programme has helped me to develop as a scientific researcher and to consider the social, ecological, and practical dimensions of agriculture. Although most of our focus was on laboratory research, I learned the importance of social perceptions in successfully implementing sustainable systems. I have strengthened my critical thinking and learned to approach problems with curiosity, humility, and persistence. Finally, it emphasizes that achieving sustainable agriculture requires not only scientific knowledge but also empathy, collaboration, and a deep understanding of the people at farming systems.

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Abbreviations

Abbreviation	Description
BCA	Biological Control Agent
IPM	Integrated Pest Managemet
AG	Anastomosis Group
MS medium	Murashige and Skoog medium
dpi	Days post inoculation
FAO	Food and Agriculture Organization
EU	European Union

1. Introduction

Solanum tuberosum L. (potato) is an important food crop throughout the world and is the most consumed crop among root and tuber crops. It is consumed by more than a billion people and ranks as the fourth-largest staple food crop in the world (Aksoy et al. 2021; Prakash et al. 2020). In Sweden, potato is the highest-yielding food crop and is strongly bound with the food culture (Eriksson et al. 2016).

However, susceptibility to plant pathogenic fungi, oomycetes such as *Alternaria solani* (early blight), *Rhizoctonia solani* (black scurf), and *Phytophthora infestans* (late blight) during cultivation is a significant challenge that affects both quantity and quality of potatoes (Tsror 2023). In an agricultural field, it is possible to have multiple pathogens at the same time. Since these co-infection can strongly influence epidemiological dynamics, co-infected plants should also be taken into account when developing effective disease control programs (Brouwer et al. 2023).

Chemical control remains the primary and most common method of managing these diseases, but its intensive use raises environmental and health risks and also contributes to the development of fungicide-resistant strains, which limit the long-term efficacy of conventional chemical controls (Wharton et al. 2013). As a sustainable alternative, the application of biological control agents (BCA) can be an option to prevent the development of resistance in pathogens and toxicity in the environment (Villavicencio-Vásquez 2025). Among these BCA, oomycete *P. oligandrum* has shown antagonistic activity against a wide range of plant pathogenic oomycetes and fungi through mechanisms such as mycoparasitism, antibiosis, and alteration of the host hyphae (Benhamou et al. 2012; Belonoznikova et al. 2022).

P. oligandrum is commonly used as a seed treatment or rhizosphere inoculant (Brožová & Jana 2002). However, Takenaka et al. (2009) showed that foliar application of its cell wall proteins can rapidly induce defense-related genes and provide targeted resistance at infection sites. Therefore, it is important to identify the most effective method of application for a BCA when it is introduced as an effective control strategy for a sustainable agricultural system.

1.1 Problem statement

Even though *P. oligandrum* shows effectiveness against several pathogens, its performance as a control agent under co-infection scenarios remains understudied (Brouwer et al. 2023). Moreover, the interaction of *P. oligandrum* with *R. solani* is still insufficiently understood, particularly whether inhibition is mediated by direct mycoparasitism or by secondary mechanisms such as volatile compound production. Since few studies have focused on foliar application of *P. oligandrum*, further research is needed to evaluate the effectiveness of foliar use of *P. oligandrum*. Addressing these research gaps is important for understanding the

potential to intergrate *P. oligandrum* into Integrated Pest Management (IPM) frameworks and contribute to agroecological sustainability.

This study focuses on evaluating the effectiveness of *P. oligandrum* as a BCA against three major pathogens related to potato diseases and to examine the efficacy of *P. oligandrum* as a foliar treatment against foliar pathogens.

1.2 Aim of the study

The study aims to evaluate the effectiveness of *P. oligandrum* in managing co-infections of *A. solani* and *P. infestans*, to investigate its inhibitory mechanism against *R. solani*, and to determine the potential of foliar application as a sustainable disease management strategy.

1.3 Objectives

- To measure the growth inhibition of *A. solani* and *P. infestans* when co-cultured with *P. oligandrum*.
- To examine the effectiveness of foliar versus soil application of *P. oligandrum* in reducing *A. solani* infection in potato leaves.
- To assess lesion development in potato leaves under *A. solani* infection following *P. oligandrum* treatment.
- To investigate the inhibitory potential and mode of action of *P. oligandrum* against *R. solani* AG3 and AG5.

1.4 Hypothesis

- *P. oligandrum* inhibits co-infections of *A. solani* and *P. infestans* by reducing pathogen growth.
- Foliar application of *P. oligandrum* will reduce *A. solani* infection severity more effectively than soil application in two different potato cultivars.
- Soil application of *P. oligandrum* will reduce *A. solani* infection severity.
- *P. oligandrum* will exhibit mycoparasitic activity against *R. solani*, through direct inhibition of its growth through physical interaction.

2. Background

2.1 Potato cultivation

The potato (*S. tuberosum*), a member of *Solanaceae* family, is one of the most crucial food crops in worldwide. It originated in the Andes Mountains of South America, has more than 4, 000 native varieties and over 180 wild potato species, which are a valuable reservoir of genetic diversity, including natural resistance to pests, diseases, and environmental stressors (International Potato Center n.d.).

Considering human consumption, potato ranks fourth in global production after wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), and maize (*Zea mays* L.) and it is the third most important food crop after rice and wheat, with over a billion people worldwide. As the result, global production exceeds 300 million metric tons annually (International Potato Center n.d.). Since the 1960s, potato cultivation and production have increased rapidly and become an important crop for food security, specially in regions of South America, Africa, and Asia (Hedberg & Lounsbury 2021).

From the 17th to the 20th century, the potato crop continuously developed from a staple crop to a cash crop throughout Europe (FAO 2009; Belgapom 2015). Within the European Union, Germany, France, the Netherlands, the United Kingdom, and Belgium are the leading potato growing countries (Eurostat 2020). In 2023, the total harvested production across the EU reached approximately 48.3 million tonnes (The EU Potato Sector n.d.).

Potato cultivation has significant cultural and economic importance in Sweden for over two centuries. At the beginning, it was introduced as an emergency food source and a supplement for bread production. Then potatoes gradually turned into a central component of the Swedish diet by the late 19th century (Eriksson et al. 2016). People in Sweden prefer floury potato cultivars (e.g. Desiree, Estima, and King Edward), while other Europeans prefer firmer potatoes. In 2024**,** Sweden produced approximately 477, 700 tonnes of table potatoes and 406, 800 tonnes of starch potatoes. In this period, table-potato growing area has declined to around 14, 480 ha, while starch-potato area has increased to 9, 100 ha **(**Statistics Sweden 2024).

‘Desiree’ and ‘Kuras’ are two of widely cultivated potato varieties in Northern Europe, including Sweden, with distinct end uses and agronomic traits. ‘Desiree’ is a red-skinned, multipurpose dietary potato popular for its moderate starch content, good taste, and diversity in cooking ways (EuroPotato n.d.; Bhat 2015). In contrast, ‘Kuras’ is a starch potato developed in the Netherlands, characterized by its very high tuber yield and starch content when compare with other varieties, usually it is higher than 22% starch by fresh weight, making it suitable for industrial processing (Bhat 2015).

Potato crops are mostly susceptible for both biotic and abiotic stresses due to their shallow root systems and physiological sensitivity during critical developmental stages such as stolonization, tuber initiation, and yield formation. These vulnerabilities are exceeded by the effects of global climate change, especially increased temperatures, precipitation, and extreme weather events. Such changes are not only affecting plant physiology but also increasing the risk and severity of pest and disease outbreaks (Bomers et al. 2024; Morugán-Coronado et al. 2024).

2.2 Overview of diseases and pathogens involved

Potato production is threatened by several diseases, including late blight (P. infestans), early blight (Alternaria spp.), and black scurf (R. solani) (Morugán-Coronado et al 2024; Pawelzik & Möller 2014). Susceptibility to diseases during cultivation poses a significant challenge that affect both yield and quality of potatoes (Birch et al. 2012 ; Tsror 2023). Therefore, it is economically important and require diverse management strategies to reduce yield losses (Secor & Gudmestad 1999).

2.2.1 Early blight

Early blight, also known as *Alternaria* blight, is a significant foliar disease in potatoes that appears in worldwide, specially the regions that are having the suitable environmental conditions (CIP 1996). Early blight is primarily caused by two species from the same genus *Alternaria*: *A. solani* and *A. alternata*. These pathogens thrive in warm climates with alternating periods of dryness and high humidity, especially in sandy, light-textured soils that are low in organic matter (Tsedaley 2014).

Although it says "early" blight, the disease typically appears on mature potato foliage rather than during the early stages of the growing season (Rowe & Powelson 2007). The name ‘early’ blight suggest because it attacks early maturing cultivars more severely than medium or late maturing ones (Smith 2001). It can appear in all potato-growing regions. However, the significant yield losses and quality deterioration are specially observed in the areas that are have warm and wet conditions during the early stages of crop growth. These conditions enhance the rapid disease development. Young and middle-aged potato plants generally have less susceptibility for early blight, but disease incidence increases with crop maturity, particularly from the onset of tuber formation (Rotem 1994). Mature plants are vulnerable due to progressive susceptibility associated with plant aging, nutrient depletion, and stress factors such as injury or environmental extremes (Tsedaley 2014).

The disease mainly damages potato foliage, reducing photosynthetic capacity and causing premature leaf death. Physiological alterations such as increased photosynthesis in unaffected tissues and decreased respiration have been noted, but are difficult to quantify. Therefore, it is generally evaluate the crop losses based on visible disease severity in the damaged plants (Tsedaley 2014).

Early blight symptoms typically first appear on mature, senescing leaves. Initial lesions are small, dark brown to black necrotic spots that range from pinpoint size to about 1.25 cm in diameter (Figure 1). When the lesions enlarge, they mostly develop concentric rings, producing a distinctive "target spot" or "bull's eye" appearance on the leaves. Yellow halos can see surround the lesions, and the affected areas are usually bordered by leaf veins. Severe infections can lead to widespread yellowing and premature defoliation in the plants (Tsedaley 2014).

Figure 1: Early blight lesions are characterized by an alternating series of light and dark concentric rings surrounded by a narrow band of chlorotic tissue (Wharton & Wood 2013).

Early blight is a major challenge to control due to its higher possibility to produce secondary inoculum. Consequently, potato fields are treated with high amounts of fungicides to minimize the yield losses and protect the production (Tsedaley 2014).

However, reliance on fungicide sprays, particularly protectant fungicides applied during warm seasons, has led to inconsistent timing of application and unnecessary chemical use. Rather than chemical management, cultural practices can play a crucial role in controlling early blight. It can be done by eliminating cull piles, removing extra potato plants, and using proper harvesting and storage techniques to reduce pathogen survival, reproduction, and dispersal (Tsedaley 2014).

2.2.2 Alternaria solani

Early blight, caused by the fungus *A. solani*, is a destructive disease of potato that persists in infected leaf and stem tissues either on the soil surface or buried within the soil (van der Waals 2002). The fungus overwinters in plant debris from the previous season and it is well adapted to survival due to the dark pigmentation of its hyphae, which increases resistance to degradation. Spore production occurs on infested debris at the soil surface or on active lesions, under alternating wet and dry conditions across a broad temperature range. These spores, or conidia, are dark to black, asexual, multicellular, and often pear-shaped with both transverse and

longitudinal septa (Figure 2). They form simple conidiophores that detach easily, allowing them to be carried by air currents, wind-blown soil, splashing rain, irrigation water, and insects (Warton & Kirk 2012; Van der Waals 2001).

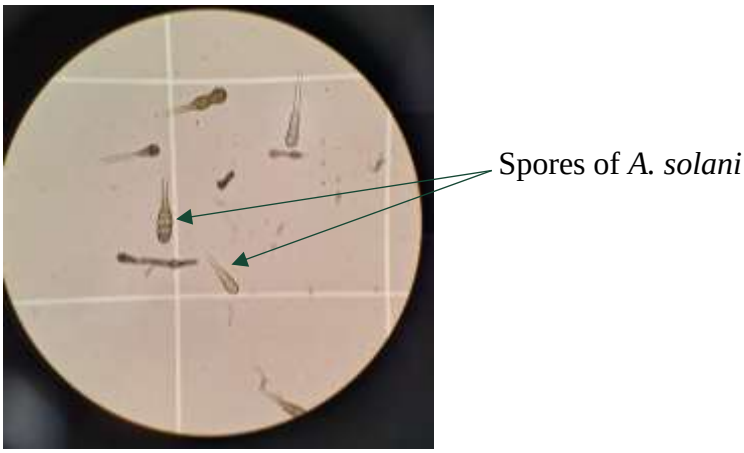

Figure 2: Spores of A. solani

A. solani is a polycyclic pathogen, that has a capability for multiple infection cycles within a single season. Spore germination and infection of susceptible leaf or stem tissues are favored by warm, humid conditions, heavy dew, or rainfall. Favorable moisture from rain, irrigation, fog, or dew and temperatures between 20–30°C are optimal for spore germination and infection, though infection can occur at temperatures as low as 10°C and above 35°C under suitable moisture and inoculum conditions. Infection occurs through direct epidermal penetration, stomata, or wounds caused by mechanical damage, sand abrasion, or insect feeding (Warton & Kirk 2012).

Lesions typically develop within 2–3 days after the infection. Mycelium in these necrotic lesions produces conidia, that leads to secondary cycles of infection. Sporulation is most common in between 5°C and 30°C, with an optimum around 20°C, particularly following periods of heavy rain or dew and during alternating wet and dry conditions (Tsedaley 2014). The conidia remain viable in dry, fallow fields, infected debris, and seed tubers, as a carryover of the disease between seasons a serious concern (Van der Waals 2001). Tuber infection occurs mainly at harvesting time, when tubers are lifted through infested soil (Figure 3). Wounds act as the entry points for the fungus, making immature tubers and those of white or red-skinned varieties especially vulnerable. Infection also can occur through natural openings like lenticels, particularly when soils are wet (Tsedaley 2014).

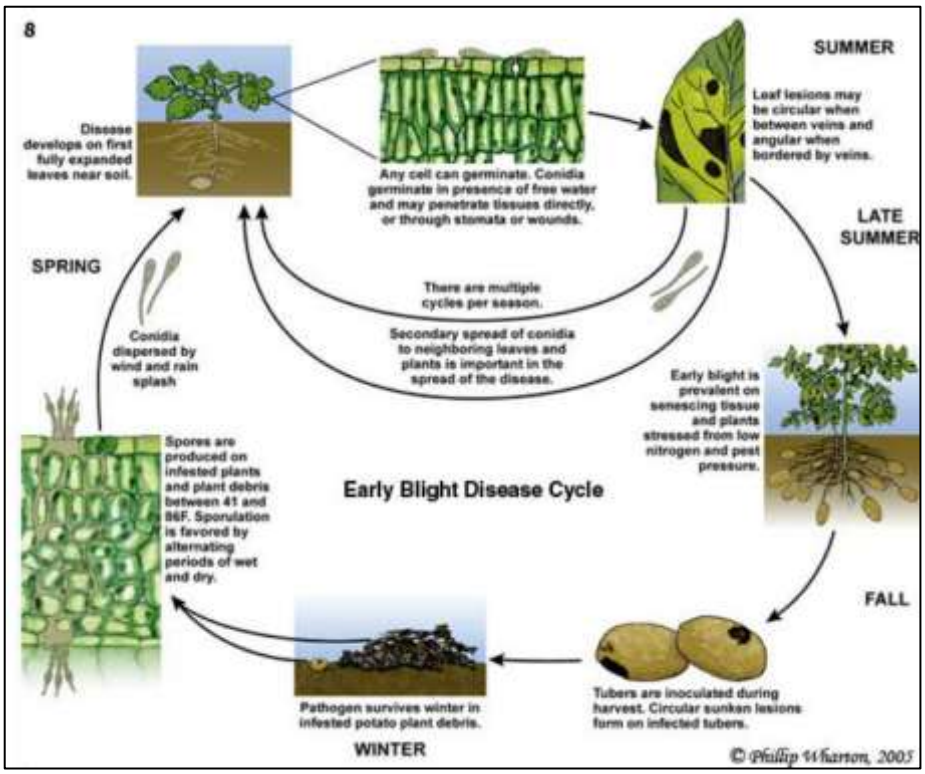

Figure 3: Disease cycle of the early blight pathogen, A. solani (Warton and Kirk 2012).

2.2.3 Late blight

Late blight is another severe disease of potatoes which is caused by the oomycete pathogen *P. infestans*. Although potatoes are the primary host, the disease can also affect other *solanaceous* plants such as tomatoes (Judelson & Blanco 2005). The disease can infect both foliage and tubers at any stage of developing potato plant. Initial symptoms appear as small, irregular, water-soaked lesions with light to dark green in color (Figure 4). These lesions mainly develop near the tips and margins of leaves and then quickly expand into large, necrotic brown to purplish-black areas (Arora n.d.; Kirk 2009). And also, a white, mold-like mildew composed of sporangia and spores can see on the lower surface of infected leaves, especially

around lesion margins,. As the infection progresses, light to dark brown lesions may girdle stems and petioles, leading to tissue collapse (Gevens et al. 2013).

Figure 4: Late blight symptom development in the field (Late Blight in Potato | NDSU Agriculture n.d.)

Under favorable conditions of high humidity and moderate temperatures (10°C – 23°C), it is possible to spread the disease rapidly and destroy entire fields within 5 to 10 days (Yuen 2021). Infected tubers from late blight are contain irregular, reddish-brown to purplish patches that extend into the internal tissues. Tubers are typically infected during the harvesting by contacting with contaminated soil or water.

Economically, late blight has a significant impact. A U.S.survey estimated annual fungicide costs at $77.1 million, with an average of $507 per hectare excluding additional non-chemical control measures (Guenthner et al. 2001).

Although the global management strategy for late blight depends heavily on fungicide applications, the emergence of fungicide-resistant strains poses increasing challenges for control. Understanding the symptoms and disease cycle of this rapidly progressing disease is crucial for timely implementation of integrated management practices (Schumann & D'Arcy 2000).

2.2.4 *Phytophthora infestans*

P. infestans is an oomycete a member of the class Oomycetes, which are taxonomically distinct from true fungi (Shaw & Khaki 1971). The genus *Phytophthora* includes several important plant pathogens. Among those pathogens,

P. infestans is the most studied and most destructive major pathogen in potato crops (Jones 1998).

This pathogen shows both asexual and sexual modes of reproduction. Asexually, the mycelium forms branched sporangiophores that produce lemon-shaped sporangia (Figure 5) at their tips. Characteristic swellings form at the points of sporangia production (Agrios 2005). Sporangia may germinate in two ways. One is by releasing 3 to 8 motile zoospores at lower temperatures (below 12°C –15°C) or by producing a germ tube directly at higher temperatures (above 15°C) (Agrios 2005).

Figure 5: (I) and (II) Sporangia of late blight pathogen P. infestans.

Sexual reproduction in *P. infestans* occurs when both A1 and A2 mating types are present together. When these types grow in close, the female hypha (oogonium) grows through the male antheridium that leading to fertilization and then it forms thick-walled oospores (Tsedaley 2022; Fry et al. 1998). These oospores are resilient, capable of surviving unfavorable conditions, and germinate to initiate new infections either via a sporangium or directly into mycelium.

When continuous wet conditions appered, all aboveground tender parts of susceptible plants may become blighted, rot, and emit a characteristic foul odor (Agrios 2005). Since *P. infestans* has ability to complete multiple reproductive cycles during a single growing season, disease outbreaks can escalate rapidly once the pathogen becomes established.

2.2.5 Black scurf

Black scurf is a significant potato disease caused by the soil-borne fungus *R. solani*. Its most recognizable symptom is the appearance of dark brown to black sclerotia on tuber surfaces (Figure 6), which resemble soil particles. Ground symptoms are more damaging, including stem cankers at the base, yellowing and curling of lower leaves, stunting, purple discoloration of upper leaves, and poor root development—leading to reduced vigor or plant death (Malik et al. 2014; Tsror 2010; Zheng et al. 2014).

Figure 6: Black scurf symptoms in potato tubers (Sagar et al.2014).

The disease prefers cooler, moist, poorly drained soils. It can survive between seasons through sclerotia or mycelium in soil or infected debris. Infected seed tubers are the primary source of inoculum (Carling et al. 2002). Disease management strategies are mainly focus on integrated approaches: using certified seed, practicing crop rotation, improving soil drainage, and applying fungicidal seed treatments to limit infection and spread (Carling et al. 2002).

2.2.6 Rhizoctonia solani

R. solani is also a major soil-borne pathogen for potato.It is causing significant yield losses due to black scurf, root rot, stem canker, and damping-off (Ahmad et al. 1995). *R. solani* can survive in soil and infected tubers as sclerotia or mycelium. It persists in plant debris and weeds, especially in tropical regions, and spreads primarily through infected seed or contaminated soil which is more difficult to control easily (Das & Pattanayak 2022).

R. solani has classified in to several groups based on hyphal anastomosis interactions and also has organized into subgroups known as anastomosis groups (AGs). Anastomosis is the fusion of hyphae when contact with other compatible hyphae. This process allows identify the individuals of the same or closely related groups (Gondal 2019). This AG-based classification is useful for understanding host range, genetic diversity of organism, and disease epidemiology. To date, thirteen AGs have been identified—designated AG-1 through AG-13, along with AGBI (Carling et al. 2002). Among these, AG-3, AG-5, AG-8, AG-4, and AG-2 have been reported as pathogenic for potato. Considering the pathogenicity on potato plant, AG-3 isolates are significantly more virulent than those from other AGs (Figure 7) (Woodhall et al. 2007).

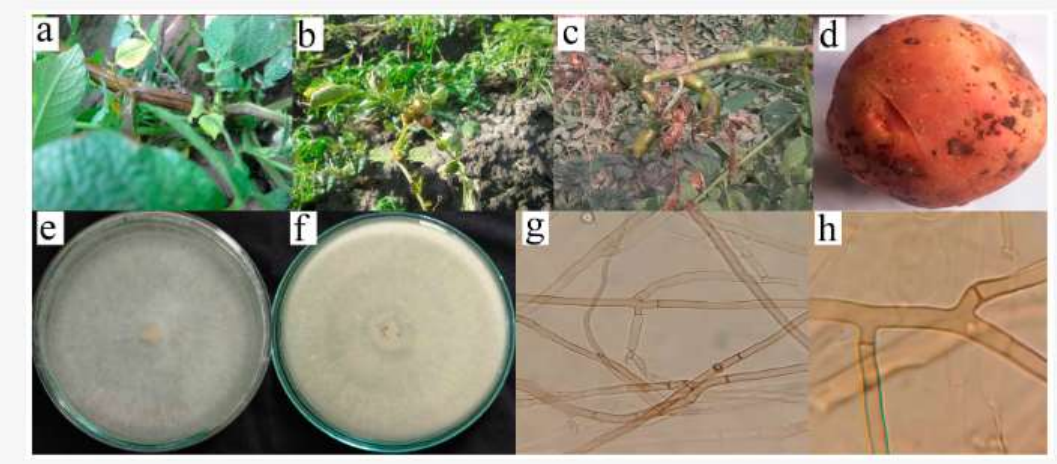

Figure 7: Symptomatology, morphological characteristics, and pathogenicity of the R. solani AG-3 ARS-05 isolate. (a) Whitish mold growth on potato foliage. (b) Rolling of leaves with aerial tubers. (c) Green-colored aerial tubers exhibiting stem canker symptoms. (d) Black sclerotia formation on potato tubers. (e) Growth of R. solani on the surface of a Petri plate. (f) Growth of R. solani on the reverse side of the Petri plate. (g, h) Microscopic images showing characteristic R. solani hyphae with right-angled branching and constriction at the branch origin (Naqvi 2024).

2.2.7 Co-infection and disease complexity

Co-infection by multiple pathogens at the same time can significantly influence disease development and severity in plants. Even including potato, often resulting in complex and unpredictable problems that affect the production. When pathogens concern on similar host, their interactions may be competitive or facilitative, depending on the specific pathosystem involved (Tollenaere et al. 2016). For example, in a previous study of co-inoculation of *Pseudomonas syringae*, a bacterium and *Alternaria brassicicola* on *Arabidopsis thaliana* in the same leaf and it resulted larger lesions than in the control. *A. brassicicola* lesions compared to controls, suggesting a facilitative interaction. However, the observations were different when the pathogens were inoculated on separate leaves alone (Spoel et al. 2007). Belhaj et al. (2017) also demonstrated how one pathogen can change host susceptibility to another by showing that the oomycete *Albugo laibachii* colonizing *A. thaliana* made the non-host plant vulnerable to *P. infestans* infection later on. Both pathogens formed haustoria in the same plant cells. These findings highlight the importance of identifying the pathogen's interaction and preferences to determine disease outcomes and make management strategies.

In potatoes, mixed infections by pathogens from different genera are frequently observed under natural conditions and can affect virulence, transmission, and pathogen evolution (Barrett et al. 2021). Notably, species from the genera *Pectobacterium* and *Dickeya* are major contributors to blackleg disease. Field studies in Finland indicated that single-species infections were more prevalent than mixed infections (Degefu 2021), yet co-inoculation with *Dickeya dianthicola* and *Pectobacterium parmentieri* in the northeastern USA led to greater disease severity than infection by either pathogen alone (Ge et al. 2021), emphasizing the synergistic effects that can occur during co-infection.

In 2017, *A. solani* appeared early, and although *P. infestans* inoculum was present, only minimal late blight symptoms developed. In contrast, early blight severity increse by 20% (Brouwer et al. 2023). These observations describe that *A. solani* may inhibit *P. infestans* growth either through direct antagonistic interactions, production of any inhibitory compounds, or by triggering a plant defense response that limits subsequent infection. Laboratory and field trials on late and early blight in potato further illustrate the complexity of disease dynamics under co-infection scenarios. *A. solani* had a direct inhibitory effect on *P. infestans in vitro* and *A. solani* also had a disruptive effect on sporangia and mycelium of *P. infestans*. In planta infection showed that simultaneous co-inoculation of both pathogens resulted in larger necrotic lesions than single inoculations (Brouwer et al. 2023). Collectively, these findings describe how co-infections can reshape disease development and emphasize the need to consider pathogen interactions for develop disease management strategies.

2.2.8 Limitations of conventional disease management

Even the conventional disease management practices are historically effective to some extent, they exhibit several critical limitations that hinder safety, and long-term efficacy and sustainability in the system. These practices typically heavily depend on chemical inputs and monocultural strategies, which pose risks to both the environment and crop resilience (Benhamou et al. 2012).

One major limitation is the overdependence on chemical fungicides specially for managing soil-borne pathogens such as *R. solani* and foliar diseases like late blight, caused by *P. infestans*. Fungicides such as Dithane-M, Mancozeb, and Captan are commonly used as pretreatments on seeds to reduce yield losses due to *R. solani* (Ogoshi et al. 1996). Not only that, uses of fungicides remains as the main strategy for manage late blight. This is mainly because of limited adoption of resistant cultivars, which often lack market appeal (Adolf et al. 2020; Liljeroth et al. 2016). Due to this huge dependency on chemicals, potatoes has become one of the most fungicide-dependent crops globally (Yuen 2021). For instance, in Sweden, although potatoes occupy just 0.9% of arable land, they account for 21% of all fungicides used in agriculture (Eriksson 2016), with 2.0 kg fungicides applied per hectare in potato compared to only 0.1 kg/ha in cereals (Vilvert et al. 2022).

The excessive use of fungicides raises environmental pollution, human health risks, and the economic burden on farmers (Wharton et al. 2013). Moreover, continuous chemical applications may lead development of fungicide resistant pathogen strains and further diminishing the long-term effectiveness of these chemicals (Benhamou et al. 2012). Crop rotation of potato with barley, beans, or alfalfa over 3–5 years has been shown that reduce the survival of *R. solani* in soil (Larkin & Honeycutt 2006). Sometimes, such a cultural practices are difficult to implement in intensive farming systems, specially where land availability is limited and crop profitability dictates continuous potato cultivation.

While sustainable alternatives such as composts, biochar, and plant-based treatments are introducing to the field, their adoption remains low due to variable

possibilities under field conditions. Compost, though promising in suppressing pathogens like *R. solani*, may require specific formulations and quality control for consistent results (Hoitink et al. 1997).

As outlined in EU Directive 2009/128/EC, by combining cultural, biological, and chemical methods, Integrated Pest Management (IPM) offers a more balanced approach to managing pest problems in cultivation (Berlin 2018). However, successful IPM implementation needs a combination approch of disease monitoring, predictive tools, and technical knowledge, application at the correct time, which are often lacking in conventional farming systems (Fry 2008; Adolf et al. 2020). Furthermore, although biological control agents and plant resistance inducers (PRIs) are under active development and are projected to grow in importance, there is limited field-level validation of their effectiveness, necessitating more research under real agricultural conditions (Devaux et al. 2020).

2.3 Biological control and *Pythium oligandrum*

BCA can be defined as a living organism (or a product derived from it) that is used to suppress, prevent, or reduce the negative effects of pests, pathogens or weeds (Lal et al. 2016). There are different mechanisms that can be found, including microbial competition, antibiosis, hyperparasitism, and induction of systemic resistance in the host plants (Hoitink et al. 2001). Though it is an urgent need to find alternatives for chemical pesticides and a sustainable solution, the adoption of biological control agents (BCAs) in Europe remains limited. In the present, there are only 14 genera of fungal, oomycete, and bacterial microorganisms such as *Trichoderma* spp, *P. oligandrum*, and *Bacillus* spp have officially registered under European Regulation No. 1107/2009 (Kiptoo et al. 2021). Among these, scientists are mainly concern on *Pseudomonas* spp, *Trichoderma* spp, and *Fusarium oxysporum* for there studies. However, other promising agents specialy like *P. oligandrum* have gained increasing scientific interest over the past decade due to there effectiveness and multiple benefits to the crops (Kiptoo et al. 2021).

2.3.1 P. oligandrum

The first description of the oomycete *P. oligandrum* was in 1930, when it was identified by Drechsler, but it was regarded as a non-pathogenic microorganism for many years (Rey et al. 2008). However, now the studies are highlighting the strong biocontrol potential of *P. oligandrum* against a wide range of plant pathogens (Benhamou et al. 1997; Picard et al. 2000a; Rey et al. 2008). Notably, *P. oligandrum* has been isolated from the rhizosphere of various plant species indicating that this microorganism has a wide range of host plants (Martin & Hancock, 1986; Mulligan & Deacon 1992). *P. oligandrum* also acts as a growth promoter in several important crop species such as suger beet (Veselý 1989), cucumber (Kratka et al. 1994), and rice (Cother & Gilbert 1993). This plant growth

promortion is associated with increasing the yield and overall plant fitness (Bˇelonoˇzníkova ´ et al. 2022).

Its broad-spectrum biocontrol activity has been notified across various crops and pathogens. Including sugar beet infected by *Pythium ultimum* (Martin & Hancock 1986), Verticillium wilt in pepper (Al-Rawahi & Hancock 1998) and bacterial wilt in tomato (*Ralstonia solanacearum*) (Hase et al. 2006). Not only the root pathogens, it also has the efficacy against foliar diseases caused by aerial pathogens such as gray mold (*Botrytis cinerea*) in tomato (Le Floch et al. 2003a, b) and early blight in potato (Stridh et al. 2022).

P. oligandrum exerts its biocontrol activity through multiple mechanisms— which include both direct and indirect ways. Direct mechanisms are shown by mycoparasitism**,** antibiosis**,** and competition for nutrients and space (Benhamou et al. 1998), while helping to induce the plant resistance and promote the plant growth as indirect mechanisms (Rey et al. 1996). Surprisingly, its interaction with plant roots does not cause any damage to the plant system; rather, it penetrates root tissues as rapidly as pathogenic *Pythium* species, but without eliciting disease symptoms as pathogens (Rey et al. 1996). Both field and greenhouse experiments have demonstrated that *P. oligandrum* has the possibility to reduce pathogen attacks significantly, with efficacy ranging from 15% to 100% depending on the host plant, the target pathogen, and the mode of application (Takenaka et al. 2006)

Considering the morphology and life cycle of *P. oligandrum*, it is a homothallic species, which means it has the ability to self-fertilize. It has both sexual and asexual life cycle. In the asexual cycle, it makes diploid zoospore which has ability to move through water. It forms oospores in its sexual cycle. Oogonia fertilizes by antheridia and makes thick walled oospores. Majority of oogonia develop by parthenogenesis. Immature oospores develop into mature oospores which have spikes around it (Figure 8). When the condition are favorable, oospores germinate and form several hyphae. Inflated sporangia can form in these hyphae and these sporangia differentiate to release motile wall-less zoospores which can germinate later (Andersen 2023).

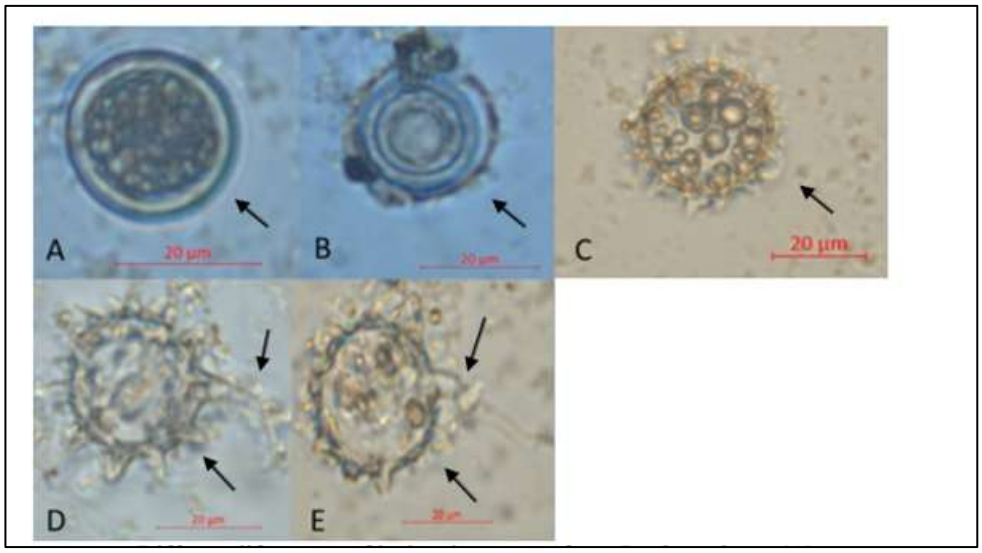

Figure 8: Different life stages of isolated oospores from P. oligandrum. (A) Arrow points towards an unripe oospore. (B) Intermediate ripening stage; the arrow points towards the initial spike formation. (C) Ripe oospore, ungerminated but metabolically active; the spherical circles are possibly lipid formations. (D – E) Ripe oospores that are germinating with one or several hyphae. Picture acquisition performed with a Zeiss Axio Observer inverted microscope at 40X magnification (Andersen 2023).

2.3.2 Application of *P. oligandrum* to plants

Although *P. oligandrum* applies as a soil application or seed treatment, previous studies supported that it is also effective as a foliar application to control leaf diseases. On the above ground surface of the plant, BCAs can compete with the pathogens for nutrients and space and kill the pathogens by direct contact or by direct penetration (mycoparasitism or microbial predation). Also it is possible to produce antibiotics to reduce the germination of pathogen spores. Not only that, BCAs can stimulate plant defense responses against the pathogen (Palmieri et al. 2022). Takenaka and Tamagake (2009) highlight that spraying purified cell wall protein fractions from *P. oligandrum* on sugar beet leaves expressed the defense - related gens in sugar beet and reduced Cercospora leaf spot severity.

2.4 Experimental approaches in previous studies

2.4.1 Coinfection assay

To evaluate the potential of candidate microorganisms as BCAs against phytopathogenic fungi or oomycetes, antagonistic tests are commonly used. These testes normally called as dual culture, plate confrontation, or zone of inhibition assays. Antagonistic tests typically involve co-inoculating the biocontrol agent and the target pathogen on a preferable solid or liquid culture media, and then analyse their interaction through the changes can see in mycelial growth, secreations,

inhibition zones or morphological alterations (IVAMI n.d.; Anith et al. 2021). Among these methods, the solid agar plate confrontation test is commonly used for the preliminary screening of biocontrol agents.It is a useful predictive tool in early-stage biocontrol evaluation because it provides a straightforward way that can exhibit inhibitory activity, particularly with regard to mycelial suppression, and it has demonstrated a high correlation with in planta results (IVAM, n.d.; Anith et al. 2021). However, it is important to note that variations in methodological setup according to microbes such as media type, inoculation timing, or distance between inocula can lead to inconsistent or non-comparable results across studies. Several attempts need to capture a more comprehensive assessment by considering factors like competition for space and nutrients, cell-surface interactions, and the induced or constitutive secretion of volatile or soluble antimicrobial metabolites (IVAMI n.d.).

Confrontation assays have been used in several recent studies to evaluate the dynamics between pathogens or BCAs. One example is, to find out the relationship in between *A. solani* and *P. infestans* on their growth in the same growth media, Brouwer et al. (2023) performed a radial growth experiment. According to the results, *P. infestans* showed a significant growth reduction when cultured in the presence of *A. solani*, indicating a potential antagonistic interaction, while *A. solani* showed unchanged radial growth at the co-culture (Brouwer et al. 2023). It has examined the interaction in between *P. oligandrum* and the necrotrophic oomycete *Pythium myriotylum* by Sheikh et al. (2023). According to the outcomes of their dual culture tests, *P. oligandrum* exhibited definite parasitic activity, which promptly led to the death of *P. myriotylum* cells. Therefore, this confrotation assays demonstrated the suppression to real disease prevention by reducing *P. myriotylum*'s infectious potential in host plants such as ginger in a quantifiable manner and highlight the importance of doing *in vitro* experiment befor apply for field experiment (Sheikh et al. 2023).

2.4.2 *In planta* studies in controlled environmental conditions

For doing a field experiment, it is effective to do an experiment in a controlled environment condition to get an idea, how pathogens and BCA interact each other. Biotron setups provide semi-controlled environments which match with standardized pathogen inoculations, plant growth, and treatment comparisons, while allowing for variation in temperature, light, and humidity that more closely mimics field conditions. Andersen (2023) evaluated *P. oligandrum*'s impact on *A. solani* in potato plants through planta trials conducted in greenhouse settings. The results showed that *P. oligandrum* induce the disease suppression in controlled greenhouse environments, but the field trials were less perform well with high disease pressure. This illustration shows how important to do research in controlled environmental conditions to identify the effect of treatment before it in the field.

3. Materials and Methods

3.1 Preparation of culture media and inoculum production

3.1.1 Rye solid medium

The protocol was followed as described by Caten and Jinks (1968). Sixty grams of rye seeds were thoroughly cleaned to remove weed seeds and other debris. Seeds were surface sterilized using 5% sodium hypochlorite, followed by rinsing with sterile water. The sterile seeds were soaked overnight in a minimal volume of tap water. The soaked rye was ground into 3–4 pieces with the addition of 100 mL of distilled water, then transferred to a beaker. Approximately 600 mL of distilled water (approximately three times the volume of the rye) was added, and the mixture was boiled at 80 °C for 4 hours in a water bath. After boiling, 20 g sucrose and 15 g Bacto agar (Sigma – Aldrich) were added. The final volume was adjusted to 1L before autoclaving.

3.1.2 V8 medium

Two formulations of V8 juice were used for the preparation of V8 media.
Base V8 Juice Formulations:

- Standard V8 Base: 100 mL of vegetable juice (Kung Markattas ekologiska Grönsaksjuice).
- Modified V8 Base: 100 mL prepared by combining 70 mL of tomato juice (Kiviks Naturens Bästa Tomatjuice), 15 mL of carrot juice (Kung Markatta Morotsjuice), and 15 mL of beetroot juice (Kung Markatta Rödbetsjuice).

Preparation of V8 Media: In both cases, 1.5 g $CaCO_3$ was added to the respective V8 juice base, and the volume was adjusted to 1 L with distilled water. The mixture was stirred for 20 minutes. For V8 liquid medium, the pH was adjusted to 5.7 prior to autoclaving. For V8 solid medium, 15 g/L of Bacto agar (Sigma – Aldrich) was added. The protocol followed was based on Andersen et al. (2023).

3.1.3 Pea solid medium

A total of 125 g peas were boiled for 1 h in sufficient water to cover them. The extract was strained through cheesecloth, and 15 g Bacto agar (Sigma – Aldrich) was added. The volume was adjusted to 1 L, and the pH was set to 7.25 before autoclaving at 121 °C, 103.4kPa for 20 min.

3.1.4 Murashige and Skoog (MS) medium

For *in vitro* plant growth, 2.2 g MS media (Duchefa Biochemie) and 20 g sucrose were dissolved in Milli-Q water. The pH was adjusted to 5.8, followed by the addition of 7.2 g Phyto agar (Duchefa Biochemie). The volume was brought to 1 L before autoclaving at 121 °C, 15 psi for 20 min.

3.1.5 Preparation of *P. oligandrum* Oospore suspension

The protocol followed was based on Andersen et al. (2023) with some modifications. *P. oligandrum* was cultured in V8 broth at 22 °C in darkness. The tops of the bottles were covered with aluminium foil instead of using lids. After approximately 14 days, when spiny oogonia were visible, mycelia were macerated in a high-speed blender (30 seconds) and filtered through cheesecloth to collect mature oospores (Figure 9). Oospore concentration was determined using a hemocytometer and adjusted to a final concentration of 1.25×10^4 oospores per mL.

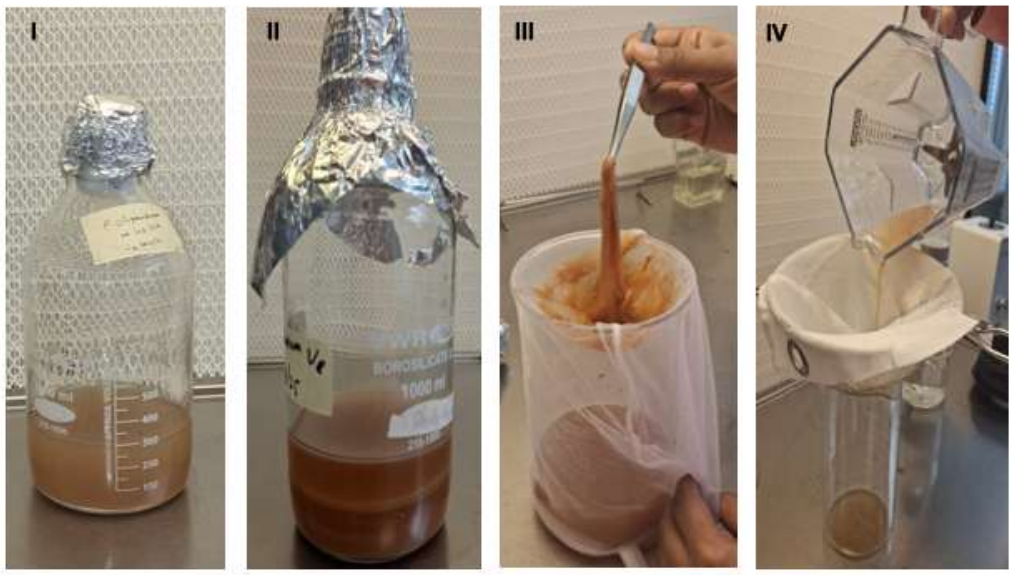

Figure 9: Preparation of P. oligandrum oospore suspension. (I) P. oligandrum was cultured in V8 broth. (II) After approximately 14 days, the mycelium had colonized the entire medium. (III) The V8 broth was filtered to separate the mycelium. (IV) Collected mycelium was macerated in a high-speed blender for 30 seconds and then filtered through cheesecloth to obtain mature oospores.

3.1.6 Preparation of *A. solani* conidial suspension

The preparation of an *A. solani* suspension, as described in the study by Brouwer et al. (2023), involves the following steps. *A. solani* strain AS112 was cultured on V8 solid medium at 22 °C in darkness for 5 days, then transferred to an 18 °C incubator equipped with UV-C light bulbs (OSRAM HNS15G13, λ = 254 nm) providing 8 h UV-C exposure per day for 9 days to induce sporulation. Conidia were harvested by flooding plates with 10 mL sterile tap water with 0.01% Tween 20 and gently rubbing the colony surface with a sterile L-shaped spatula. Conidial concentration was determined using a haemocytometer and adjusted to 2.5×10^4 conidia per mL (Figure 10).

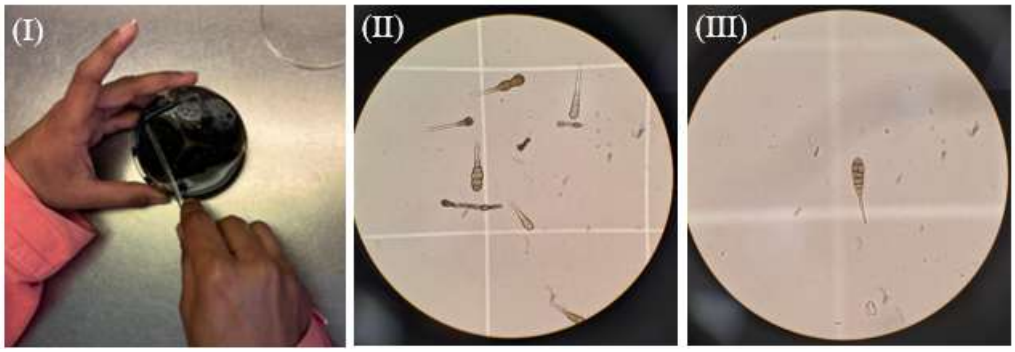

Figure 10: Preparation of A. solani conidial suspension. (I) Harvesting conidia by flooding the plates with tap water containing 0.01% Tween 20 and then gently rubbing using a sterile L-shaped spatula. (II and III) A. solani conidia observed in haemocytometer.

3.2 Plant material maintenance

In vitro plantlets of potato cultivars 'Desiree' and 'Kuras' were maintained on MS medium (Figure 11.I). Two-week-old plantlets were transplanted into 3.5 L pots containing a 50:50 (v/v) sand (S:T ERIKS) : compost (SW Horto) mixture (Figure 11.I). Plants were grown in a controlled environment chamber (biotron) at 20 °C, 65% RH, under a 14 h photoperiod with a light intensity of 160 μmol m^{-2} s^{-1} (Brouwer et al. 2023).

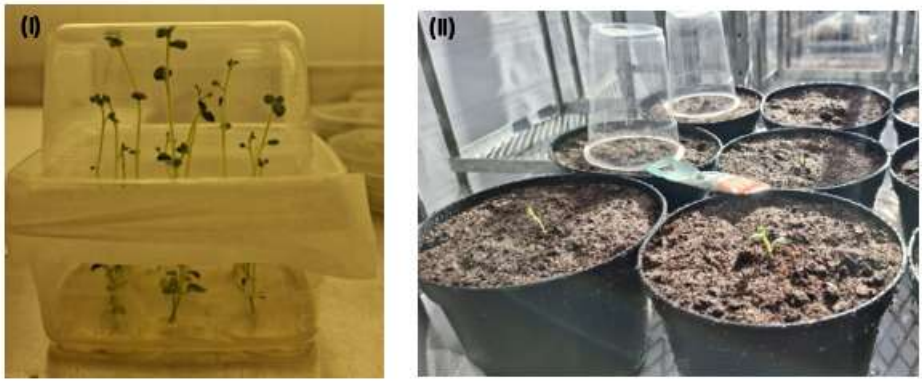

Figure 11: Maintenance of the plant materials. (I) The potato plantlets that are maintained on MS medium. (II) Transplanted two-week-old plantlets in 3.5 L pots containing a 50:50 (v/v) sand–compost mixture and then covered them with transparent plastic cups for two weeks to maintain a suitable microenvironment. Plants were grown in a controlled environment chamber.

3.3 Experiment 1 - Co-inoculation of *A. solani*, *P. infestans*, and *P. oligandrum*

P. infestans strain 88069 was grown on rye solid medium at 20 °C. *A. solani* AS112 was cultured on V8 medium at room temperature in darkness, and *P. oligandrum* was grown on V8 medium at 20 °C. Fourteen to twenty days old cultures were used for co-inoculation on both V8 and rye solid media in 9 cm diameter Petri plates. Agar plugs (7 mm diameter) containing mycelium were placed 4.8 cm apart at the same time (Figure 12). Plates were incubated at 20 °C for 5 days. The following confrontations were prepared between *P. infestans*, *P. oligandrum* and *A. solani* (three replicates per combination):

Three *P. infestans* plugs
Three *A. solani* plugs
Three *P. oligandrum* plugs
Two *A. solani* plugs + one *P. oligandrum* plug
One *A. solani* plug + two *P. oligandrum* plugs
Two *P. infestans* plugs + one *P. oligandrum* plug
One *P. infestans* plug + two *P. oligandrum* plugs
One *A. solani* + one *P. infestans* + one *P. oligandrum* plug
One plug of *A. solani*
One plug of *P. infestans*
One plug of *P. oligandrum*

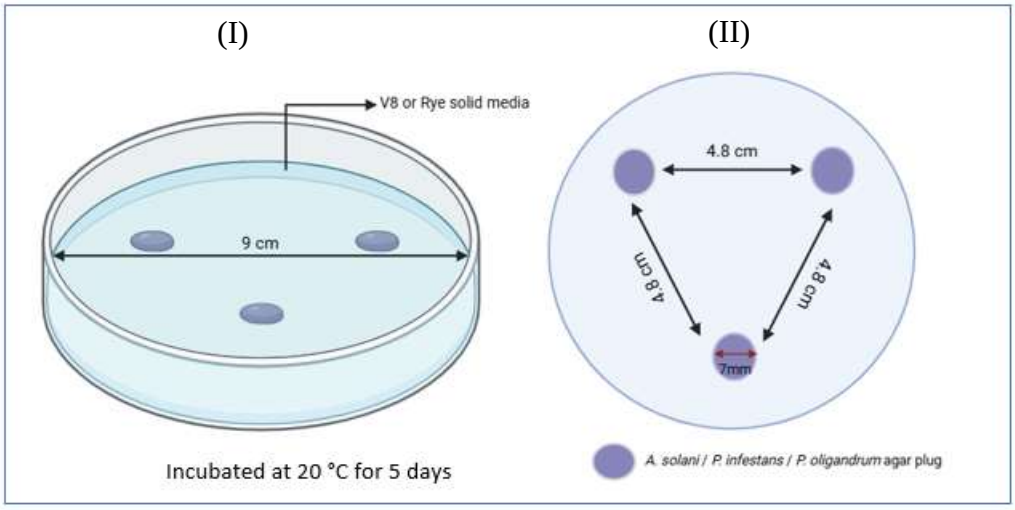

Figure 12: Schematic representation of triple culture confrontation assays. (I): Petry dish overview with three plugs of microbes. (II): Top view of the setup.Microbes were placed 4.8 cm apart.

3.3.1 Observations and data collection

Radial growth of individual cultures was measured on days 2, 3, 4, and 5 after inoculation. Growth inhibition was calculated by using the following equation.

Growth inhibition % = $\frac{C-T}{C} \times 100$

(C=radial growth of control, T= radial growth of treatment)

3.4 Experiment 2 - *In planta* experiment

The experiment was followed a two-factor factorial design with repeated measures, with two potato cultivars (‘Desiree’ and ‘Kuras’) and six treatment combinations. Six-week-old potato plants were enclosed in polythene covers to create a high-humidity microenvironment. A total of 30 plants per cultivar were used, allocated as follows:

Control (no inoculation) – 4 plants
P. oligandrum foliar inoculation – 4 plants
P. oligandrum soil inoculation – 4 plants
A. solani inoculation – 4 plants
A. solani + *P. oligandrum* soil inoculation – 7 plants
A. solani + *P. oligandrum* foliar inoculation – 7 plants

A. solani inoculation was done by using conidial suspension. Three fully expanded top leaflets were drop-inoculated with four 10 µL(concentration was 2.5×10^4 in

1mL) droplets of conidial suspension per leaflet. Two leaflets per plant were inoculated (maximum number of drops per plant is eight). For *P. oligandrum* inoculation, an oospore suspension was utilized. 10 mL from this suspension at a concentration of 1.25×10^4 in 1mL per plant was used. The application administered either as a foliar spray using a high-pressure hand sprayer or as a soil drench applied to the base of the plant (Figure 13). Two applications of *P. oligandrum* were carried out: the first was applied two days prior to pathogen inoculation, and the second was applied two days after pathogen inoculation. Number of lesions were counted at days 3, 5, and 7 after the second *P. oligandrum* application.

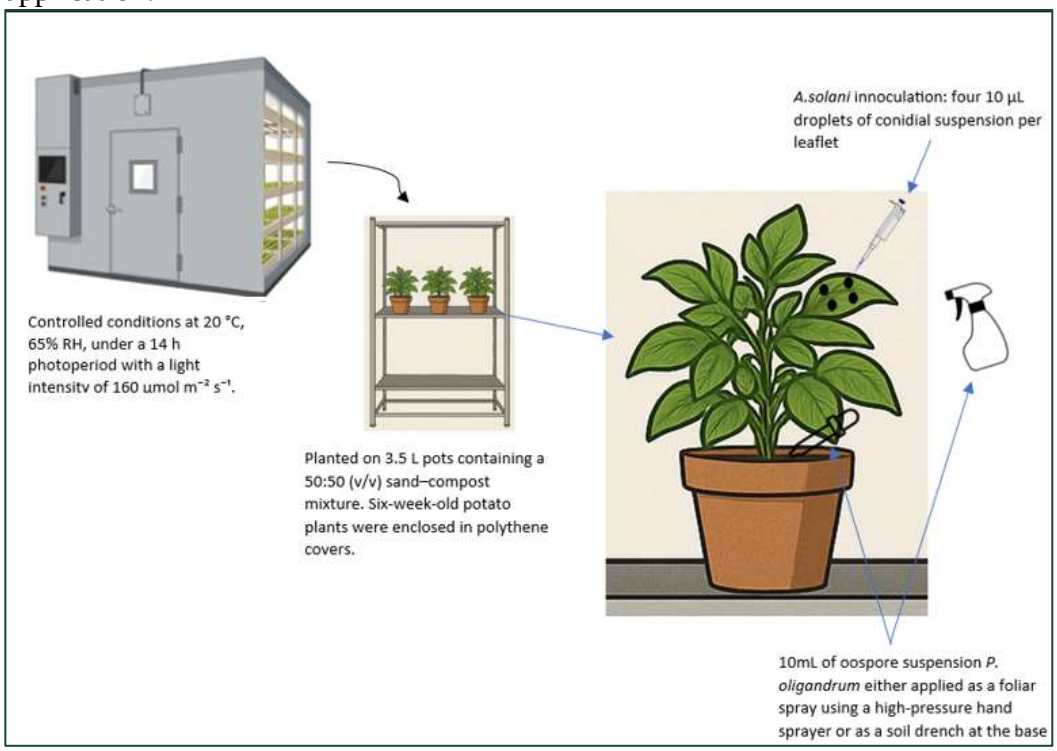

Figure 13: Schematic representation of in vitro plant experiment. Thirty plants per cultivar were used. A. solani was applied as a conidial suspension (leaf drop inoculation), while P. oligandrum was applied as an oospore suspension (foliar spray or soil drench).

3.5 Experiment 3 - Dual culture of *R. solani* strains with *P. oligandrum*

R. solani strains 22880 and 22897 were maintained on pea solid medium at 20 °C, while *P. oligandrum* was maintained on V8 medium at 20 °C. 14 days old cultures were used for dual culture assays on pea solid medium in 9 cm diameter Petri plates. Agar plugs (7 mm diameter) were placed 4 cm apart at the same time (Figur 14). The following combinations were prepared (three plates per combination):

Two *R. solani* 22880 plugs
Two *R. solani* 22897 plugs
Two *P. oligandrum* plugs
One *R. solani* 22880 plug + one *P. oligandrum* plug
One *R. solani* 22897 plug + one *P. oligandrum* plug
One *R. solani* 22880 plug + one *R. solani* 22897 plug
One plug of *R. solani* 22880
One plug of *R. solani* 22897
One plug of *P. oligandrum*

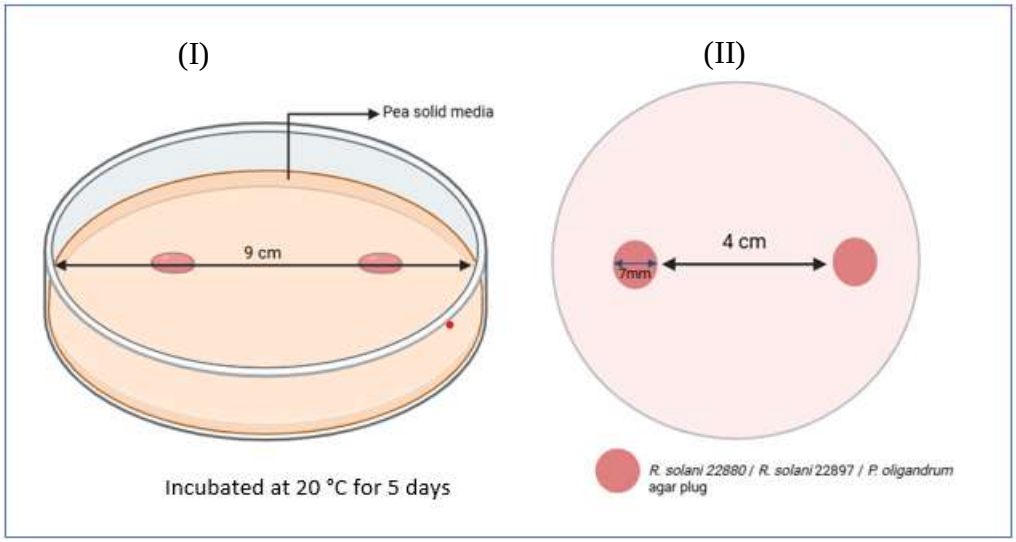

Figure 14: Schematic representation of dual culture confrontation assays. (I): Petry dish overview with three plugs of microbes. (II): Top view of the setup.Microbes were placed 4 cm apart.

Plates were incubated at 20 °C for 7 days. Radial growth of individual cultures was recorded on days 2, 3, 4, and 7. Growth inhibition was calculated by using the following equation.

Growth inhibition % = $\frac{C-T}{C} \times 100$

(C=radial growth of control, T= radial growth of treatment)

3.6 Statistical analyses

All datasets were statistically analyzed using RStudio (version 4.5.0). Analysis of Variance (ANOVA) was used to determine significant differences among treatments, and across time points. Post-hoc Tukey's HSD test was employed to evaluate pairwise differences at a significance level of $p < 0.05$. Plots, boxplots, and bar charts were generated using the ggplot2 package in RStudio.

4. Results

4.1 Co-inoculation of *A. solani* and *P. infestans* with *P. oligandrum*

We conducted preliminary tests with various media to identify the most suitable for this experiment.. *P. oligandrum* and *A. solani* exhibited more extensive growth on V8 medium, whereas *P. infestans* grew faster on rye medium. When comparing the two media, V8 was clear and allowed easier observation of microbial growth, while rye medium contained small rye particles hindering the clear observation of mycelial development. Despite these visual challenges, we ultimately selected both rye solid medium and V8 medium for the confrontation analyses to assess potential differential interactions and growth patterns of the microbes on substrates with varying nutritional and physical properties.

A. solani, colonies showed a greenish appearance on rye medium, while on V8 medium developed a black and yellow coloration. Apart from these pigmentation differences, the radial growth pattern was similar across both media. *P. infestans* demonstrated slower growth on V8 medium compared to rye, white colony appearance was similar. Mycelial growth was often difficult to observe from the under side of the plate. *P. oligandrum* displayed a fluffy mycelial morphology on V8 medium, but produced denser, internally growing hyphae on rye (Figure 15.III and VII). *P. oligandrum* growth was faster when in proximity to other microbes, with complete plate coverage within four days (Figure 15).

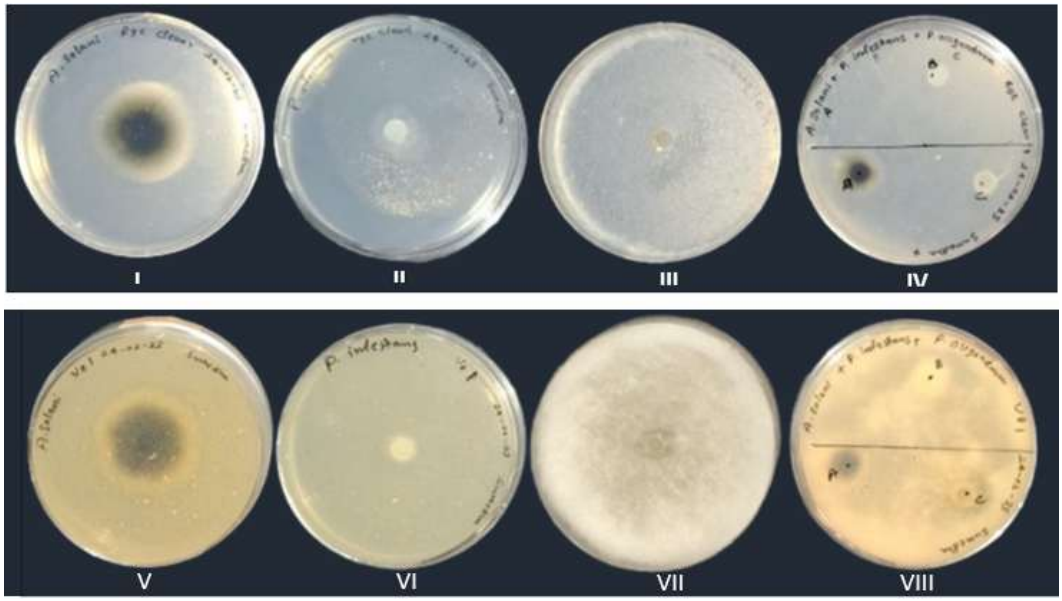

Figure 15: Culture growth on media after 5 dpi. (I) A. solani on rye, (II) P. infestans on rye, (III) P. oligandrum on rye, (IV) co-inoculation of A. solani (A), P. infestans (B) and P. oligandrum (C) on rye, (V) A. solani on V8, (VI) P. infestans on V8, (VII) P. oligandrum on V8, and (VIII) co-inoculation of A. solani (A), P. infestans (B), and P. oligandrum (C) on V8.

The radial growth of *A. solani* mycelia was higher in the control and self-pair confrontations on both rye and V8 media, but was significantly reduced when co-cultured with *P. oligandrum* (p-value 2×10^{-16} on rye; p-value 6.89×10^{-08} on V8) with the strongest inhibition observed in the triple combination (Figure 16). During the initial two days, *A. solani* grew at a comparable rate but its growth was subsequently reduced in *P. oligandrum* presence (Figure 16). *A. solani* growth inhibition was higher with two plugs of *A. solani* and one of *P. oligandrum* (Figure 17). When examining the growth inhibition percentage of *A. solani*, higher inhibition was observed in *P. oligandrum* presence. Conversely, the self-culture of A. solani showed no evidence of self-inhibition withing the observation period.

Regarding *P. infestans* culture growth, it exhibited slower growth compared to *A. solani* and *P. oligandrum* across all control and confrontation plates. Among these, growth was significantly different between control cultures and confrontations when co-cultured with *A. solani* and *P. oligandrum*, particularly on rye medium (p-value 7.89×10^{-08}). As growth was generally reduced on V8 medium, significant differences between control and treatments were less pronounced. Also it showed more inhibition when it was with two *P. infestans* plugs rather than one *P. infestans* plug (Figure 17). The lowest growth occurred in the combination with three microbes (Figure 16). For *P. infestans*, higher growth inhibition was observed when cultures contained two plugs of *P. infestans* and one plug of *P. oligandrum*. Moreover, the inhibition was greater on V8 medium compared to rye medium (Figure 16).

For *P. oligandrum* growth pattern, the presence of two pathogen plugs enhanced growth more than a single plug. *P. oligandrum* also showed significant differences between its control and confrontation plates in both rye medium (p-value 6.386×10^{-14}) and V8 media (p-value 35×10^{-10}).

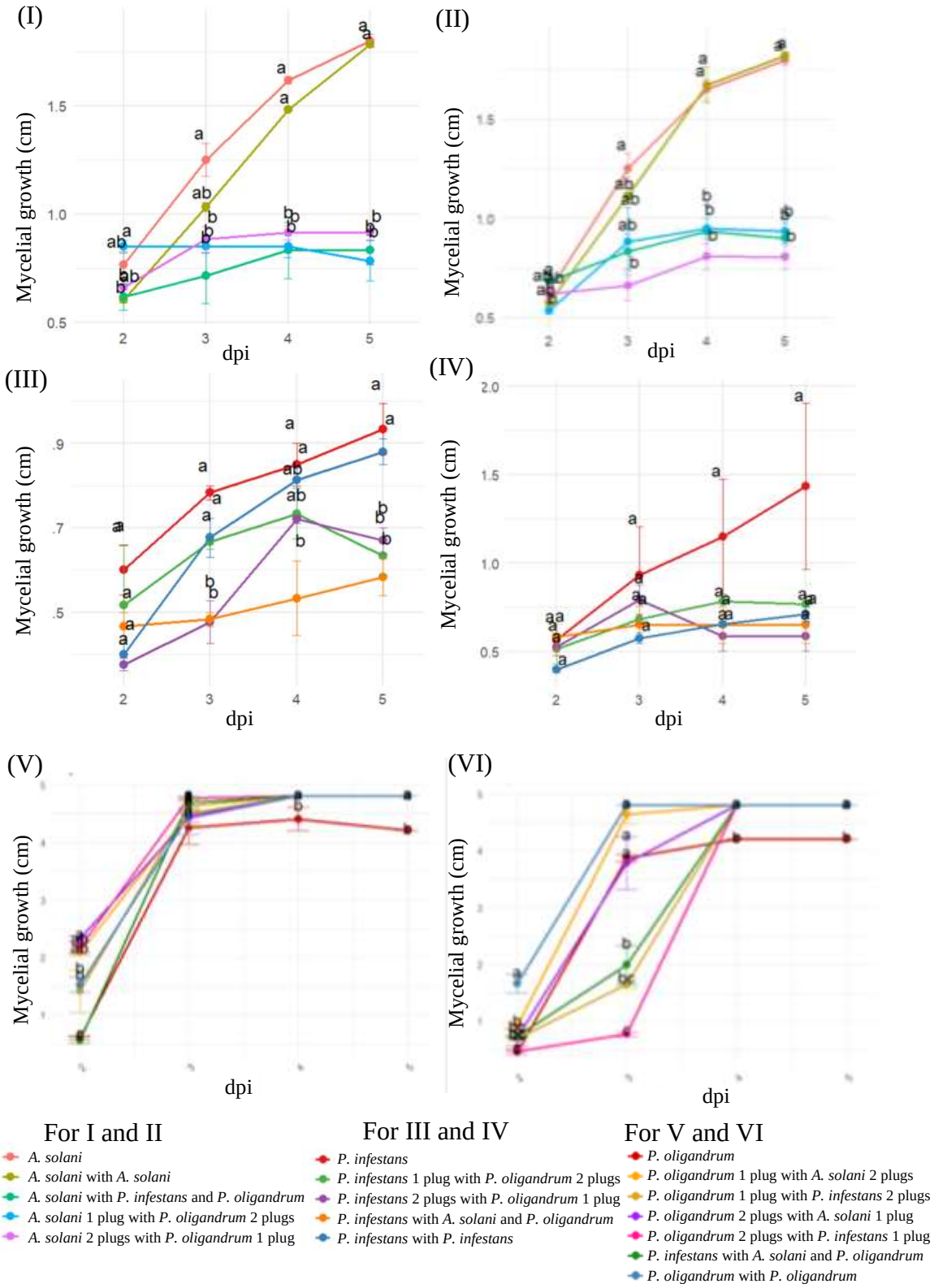

Figure 16: Mycelial growth means of A. solani, P. oligandrum and P. infestans over four time points with significant differences state with letters after 2, 3, 4, 5 dpi on rye and V8 media. (I) A. solani growth on rye media. (II) A. solani growth on V8 media. (III) P. infestans growth on rye media. (IV) P. infestans growth on V8 media. (V) P. oligandrumi growth on rye media. (VI) P. oligandrum growth on V8 media.

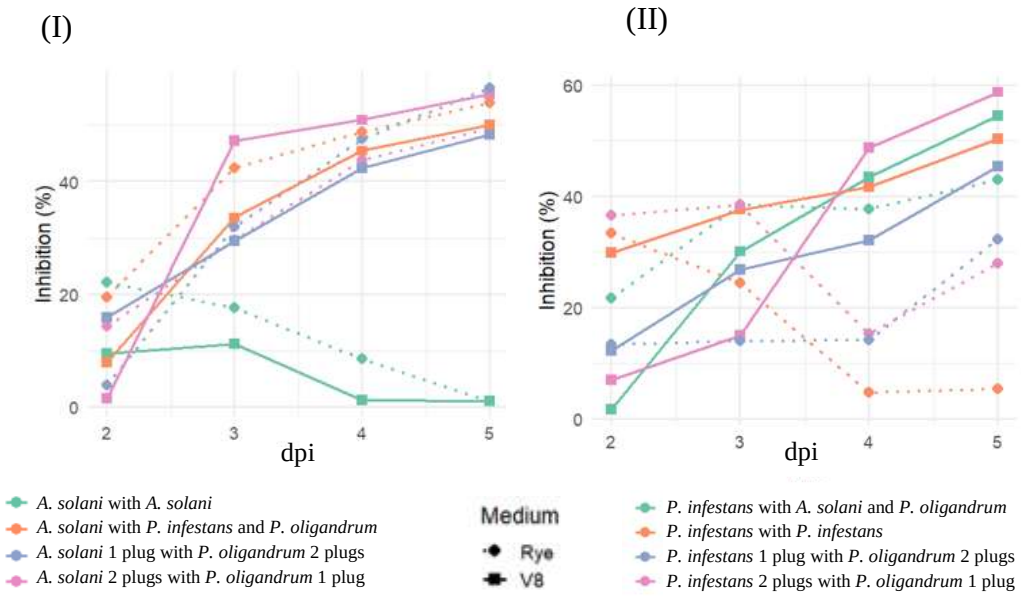

Figure 17: Growth inhibition after 2, 3, 4, and 5 dpi. (I) Inhibition of A. solani growth by confrontation on rye and V8 solid media. (II) Inhibition of P. infestans growth by confrontation on rye and V8 solid media

4.2 *In planta* experiment

Two-week-old 'Desiree' and 'Kuras' potato cultivars were planted in the biotron under controlled environmental conditions (20℃, humidity 65%, and light 160 µmol m^{-2} s^{-1}). Plants were watered when the soil began to dry. After four weeks of growth in the biotron, inoculation was carried out.

Lesions appeared three days after pathogen inoculation, with necrotic lesions developing at the *A. solani* inoculation sites. Furthermore, yellowish discoloration also observed on some leaves at the same time period. The necrotic lesions were clearly visible in 'Desiree' plants without edema, whereas in 'Kuras' plants, where edema was severe, fewer lesions were observed, and their measurement was more challenging due to leaf shrinkage and extensive edema spotting and browning. From 3 to 7 dpi, lesion diameters increased slowly, reaching approximately 3-4 mm. In both cultivars, some leaves exhibit yellowing and a shriveled appearance (Figure 18 and Figure 19).

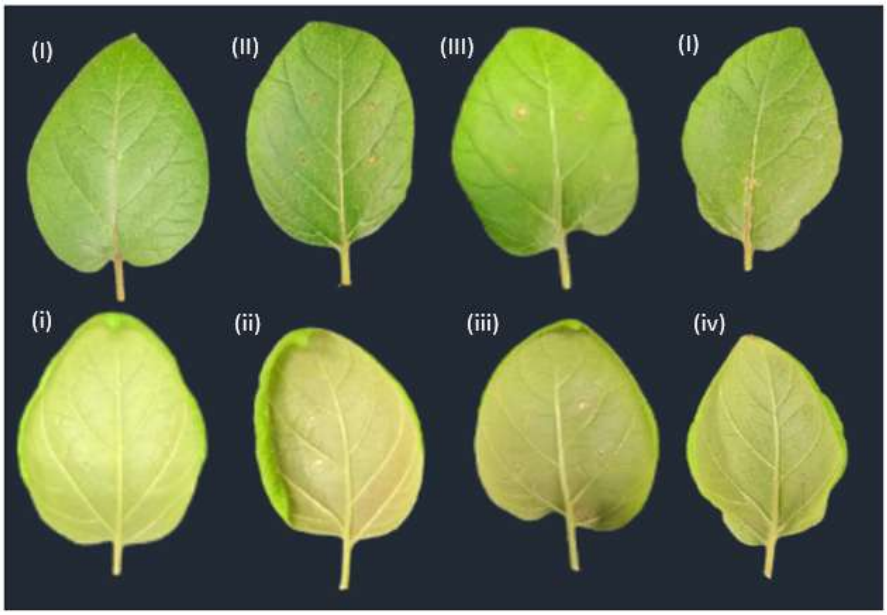

Figure 18: Symptom development on 'Desiree' potato leaves at 7 days post-A. solani inoculation (dpi) (I, i) Uninoculated control. (II, ii). Inoculated with A. solani. (III, iii). Leaf inoculated with A. solani and P. oligandrum via soil. (IV, iv). Leaf inoculated with A. solani and P. oligandrum via foliar application.

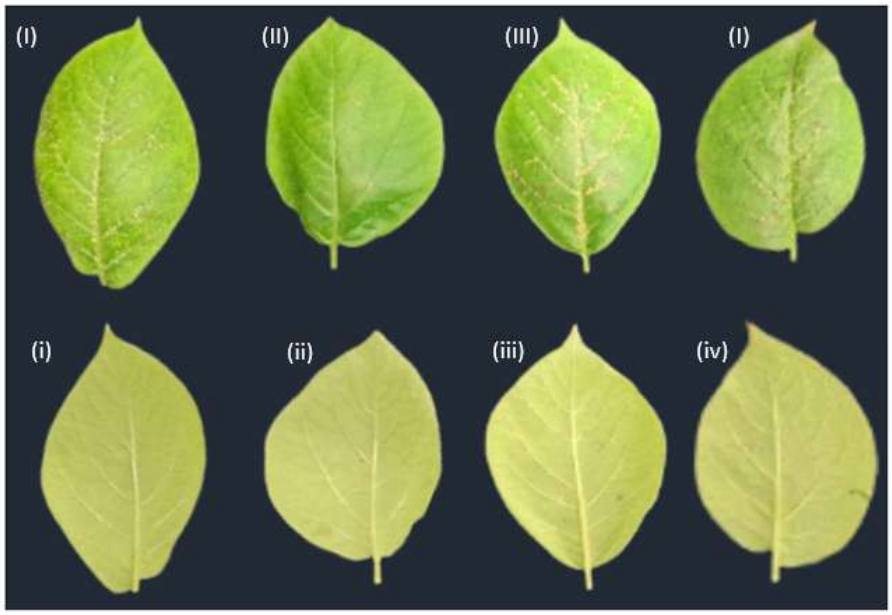

Figure 19: Symptom development on 'Kuras' potato leaves at 7 days post-A. solani inoculation. (I,i) Uninoculated control. (II, ii). Inoculated with A. solani. (III, iii). Inoculated with A. solani and P. oligandrum via soil. (IV, iv). Inoculated with A. solani and P. oligandrum via foliar application.

Despite maintaining theoretical favorable growth conditions withing the biotron, edema symptoms developed in the plants and progressively worsened, particularly in the 'Kuras' cultivar. In 'Desiree' , edema was observed in only three plants,

whereas all ‘Kuras’ exhibited edema symptoms by the end of the experiment (Figure 20).

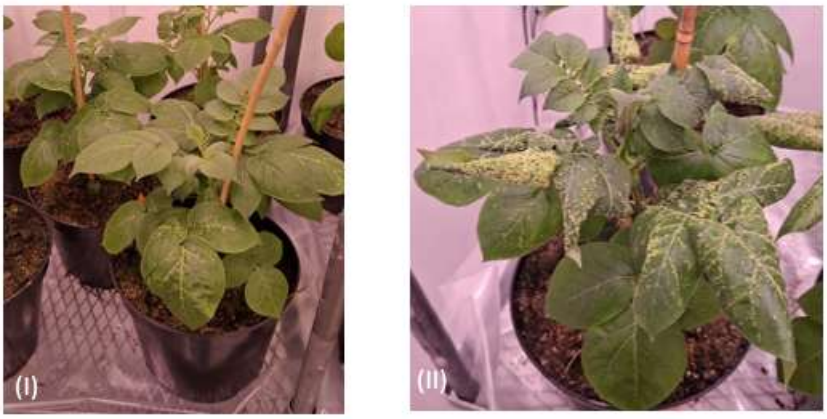

Figure 20: Edema in the ‘Kuras’ potato cultivar. (I) in 4 weeks old and (II) 6 weeks old plant

Following foliar application of *P. oligandrum*, white patches originating from substences comming with the inoculum were observed on the leaves and these patches were carefully removed 24 hours after inoculation. In plants that received soil application of *P. oligandrum*, white mycelial growth was observed on the soil surface.

For ‘Desiree’ cultivar, statistical analysis showed a significant overall effect of treatment on lesion development when averaged across all days (p-value 3.2×10^{-03} ; Figure 21). However, differences in lesion number between 3, 5, and 7 days were not statistically significant, and there was no significant interaction between treatment and day, indicating that the relative ranking of treatments remained consistent over time. There was a significant difference between *A. solani* inoculation alone and *A. solani* with foliar-applied *P. oligandrum* (p-value 2.7×10^{-03}). The difference between *A. solani* alone and *A. solani* with soil-applied *P. oligandrum* was not significant, and the results between foliar and soil application of *P. oligandrum* also not significantly difference.

However, for ‘Kuras’ cultivar no significant differences were detected on number of necrotic lesions between treatments at any time point. Although there was a slight trend toward changes across days, the effect was not statistically significant after correcting for sphericity.

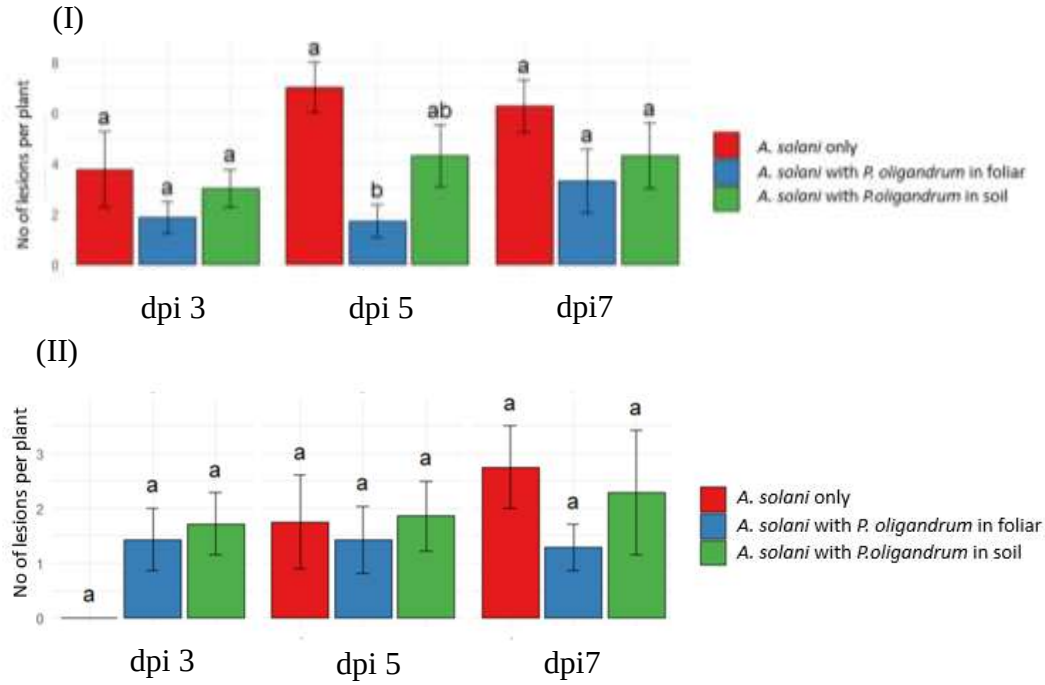

Figure 21: Number of necrotic lesions in dpi 3, 5, and 7 days after inoculation in (I) 'Desiree' (II) and 'Kuras'.

Observations after 7 dpi, showed further symptom development (Figure 22). At this stage, lesions had extended beyond the inoculation sites, indicating systemic spread of *A. solani* within the leaf. However, these observations were excluded from the statistical analysis.

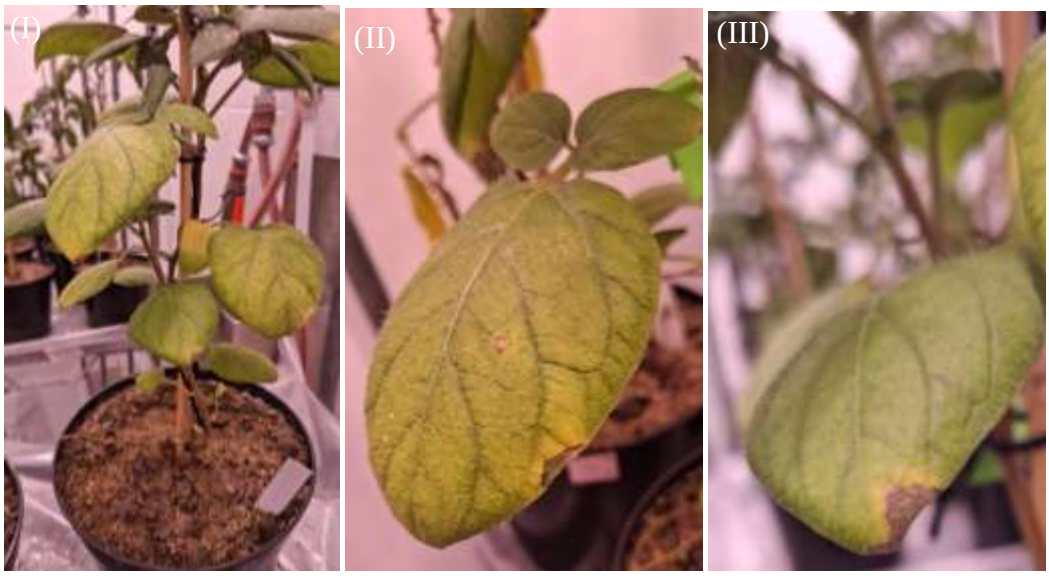

Figure 22 : A. solani infected symptoms. (I) A. solani innoculated 'Desiree' plant. (II and III) infected leaves after 14 days

4.3 Interaction of *R. solani* strains with *P. oligandrum*

The *R. solani* 22897 (AG3) strain was clearly inhibited by *P. oligandrum*, which grew rapidly toward AG3 and suppressed its expansion (Figure 23.A). In contrast, the *R. solani* 22880 (AG5) strain grew similarly to the control plate, unaffected by the presence of *P. oligandrum*, showing no visible inhibition and even overgrowing the *P. oligandrum* mycelium (Figure 23.B; Table 1).When AG5 and AG3 were confronted, the AG5 strain grew toward AG3 and restricted its development, demonstrating a stronger competitive ability. In self-pairings, AG5 formed a clear inhibition line upon contact with its own mycelium, while AG3 mycelia merged without inhibition (Figure 23.C).

Confrontation assays were performed on pea solid medium in 9 cm Petri plates. The transparent nature of the medium facilitated clear observation of mycelial development. Both AG5 and AG3 initially produced white mycelium, which developed brownish pigmentation around day 5 post-inoculation (Figure 23.A and B). Strain AG3 produced more intense pigmentation than AG5. Additionally, AG5 exhibited characteristic concentric ring patterns during growth, whereas both strains displayed a fluffy mycelial morphology. *P. oligandrum* grew extensively on pea medium, in a manner comparable to its growth on V8 medium, and also showed a fluffy morphology.

Detailed observation of microbial interactions in the dual culture confrontation assay further confirmed these trends. In the combination of *P. oligandrum* and *R. solani* AG3, overgrowth of AG3 by *P. oligandrum* was already evident by day 2, and by day 7 *P. oligandrum* had completely overgrown (>50%) the AG3 colony. AG3, however, did not has the ability to grow over *P. oligandrum* at any time point. In contrast, when *P. oligandrum* was paired with *R. solani* AG5, both organisms overlapped during early growth stages, but by day 7 AG5 had overgrown *P. oligandrum* by more than 50%. In the confrontation between AG3 and AG5, neither strain showed signs of overgrowth up to day 5; thereafter, AG5 gradually expanded over AG3, demonstrating its stronger competitive nature (Table 1).

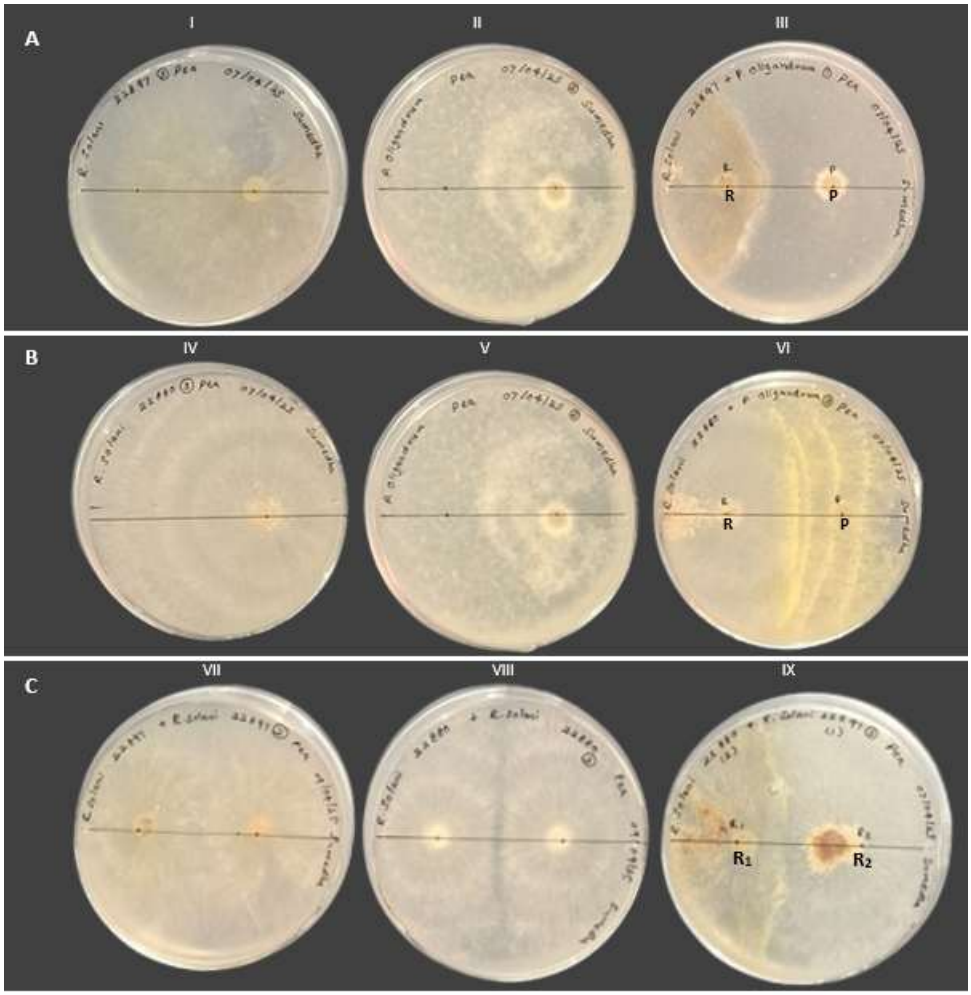

Figure 23 : (A); AG3 culture growth on pea media with P. oligandrum at 7dpi.(I) R. solani AG3 strain, (II) P. oligandrum and (III) co-inoculation of R. solani AG3 strain (R)and P. oligandrum (P)

(B); AG5 culture growth on pea media with P. oligandrum at 7dpi(IV) R. solani AG5 strain, (V) P. oligandrum and (VI) co-inoculation of R. solani AG5 strain (R)and P. oligandrum (P)

(C); Culture growth of R. solani AG3 with AG5 on pea media at 7dpi (VII) R. solani AG3 strain with it self, (VIII) R. solani AG strain with it self and (IX) co-inoculation of R. solani AG3 strain (R1)and R. solani AG5 strain (R2).

Table 1: Microbial interaction in dual culture confrontation assay between P. oligandrum and R. solani strains observed on days 2, 3, 4, and 7. Overgrowth of one organism over the other was visually recorded and categorized as no overgrowth (yellow), partial overgrowth <50% (light green), and complete overgrowth >50% (dark green). Considering microb has been highlighted with (*).

Day	2	3	4	7
P. oligandrum * against *R. solani* AG3				
R. solani AG3 * against *P. oligandrum*				
P. oligandrum * against *R. solani* AG5				
R. solani AG5 * against *P. oligandrum*				
R. solani AG5 * against *R. solani* AG3				
R. solani AG3 * against *R. solani* AG5				

	No over grow
	Partialy overgrow (less than 50%)
	Completely over grow (more than 50%)

For the Growth comparisons indicated that the AG5 strain achieved the highest radial growth under control conditions and also when paired with *P. oligandrum*, followed by growth with AG3. Its lowest growth occurred in self-pairing. Conversely, AG3 displayed the weakest growth when challenged by *P. oligandrum* and moderate growth when paired with AG5 or with itself. *P. oligandrum* grew vigorously alone and in combination with AG3, but its growth was reduced when paired with AG5, suggesting that AG5 was capable of outcompeting it.

R. solani AG3 strain had a significant main effect of date (p-value $3.5\times 10^{-}3$), indicating growth changed over time. The effect of confrontation combination was highly significant (p-value 1.08×10^{-9}), and the confrontated microorganism per date interaction was also significant (p-value 7.6×10^{-13}), suggesting that differences among treatments varied over time (Figure 24. I).

Confrontation combinations of AG5 and *P. oligandrum* had a significant effect(p-value 2×10^{-16}), and differences across dates were also significant (p-value 2.79×10^{-7}), which warranted post-hoc comparisons per date. For P. oligandrum, both the main effect of treatment (p-value 2×10^{-16}) and the treatment per date interaction (p-value 4.89×10^{-11}) were highly significant. Post-hoc analyses confirmed that *P. oligandrum* growth was notably reduced in the presence of *R. solani* (Figure 24. II and III).

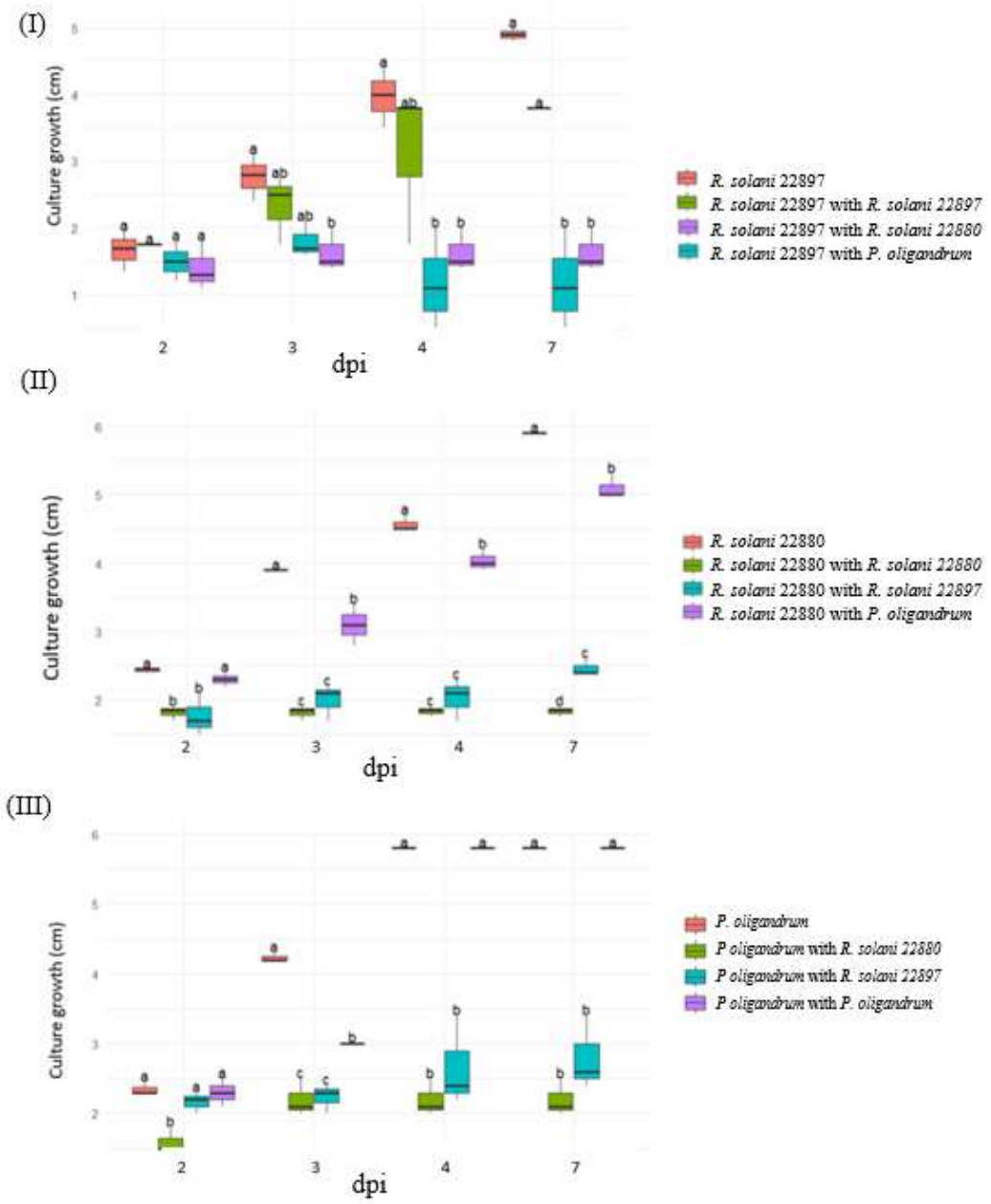

Figure 24 : Mycelial growth means of R. solani strain after 2, 3, 4, and 7 dpi. (I) R. solani AG3 strain (II) R. solani AG5 strain and (III) P. oligandrum over time in pea solid media.

The *R. solani* AG5 strain exhibited the greatest self-inhibition in dual culture, followed by inhibition when paired with AG3 strain. The weakest inhibitory effect was observed in co-culture with *P. oligandrum*. In contrast, the AG3 strain displayed a markedly different response pattern: the highest growth inhibition was induced by *P. oligandrum*, followed by AG5 strain, whereas no inhibition was observed in self interactions with AG3 (Figure 25).

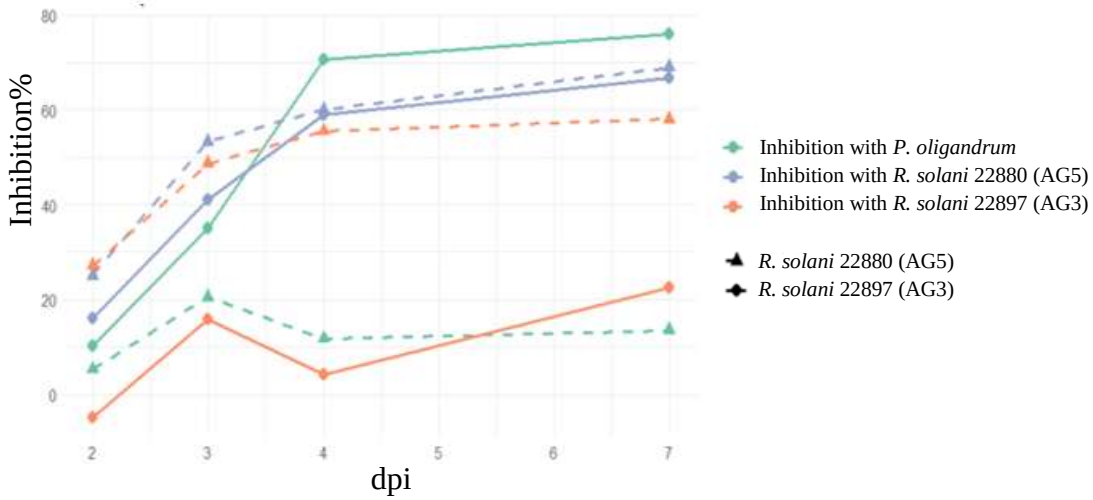

Figure 25 : Growth inhibition of R. solani AG5 strain and R. solani AG3 strain after 2, 3, 4, and 7 dpi at different combinations.

After observing differences in the confrontation analyses between AG3 and AG5 against *P. oligandrum*, we examined the interaction under the light microscope. In the AG3 and *P. oligandrum* interaction, clear coiling of *P. oligandrum* hyphae around AG3 hyphae, accompanied by swelling and disintegration of the AG3 hyphae (Figure 26. I and II). In contrast, during the AG5-*P. oligandrum* interaction, *P. oligandrum* hyphae were observed coiling around AG5 hyphae (Figure 26. III and IV). Additionally, apparent intracellular particles were observed within the hyphae of both AG3 and AG5 (Figure 26. II and IV).

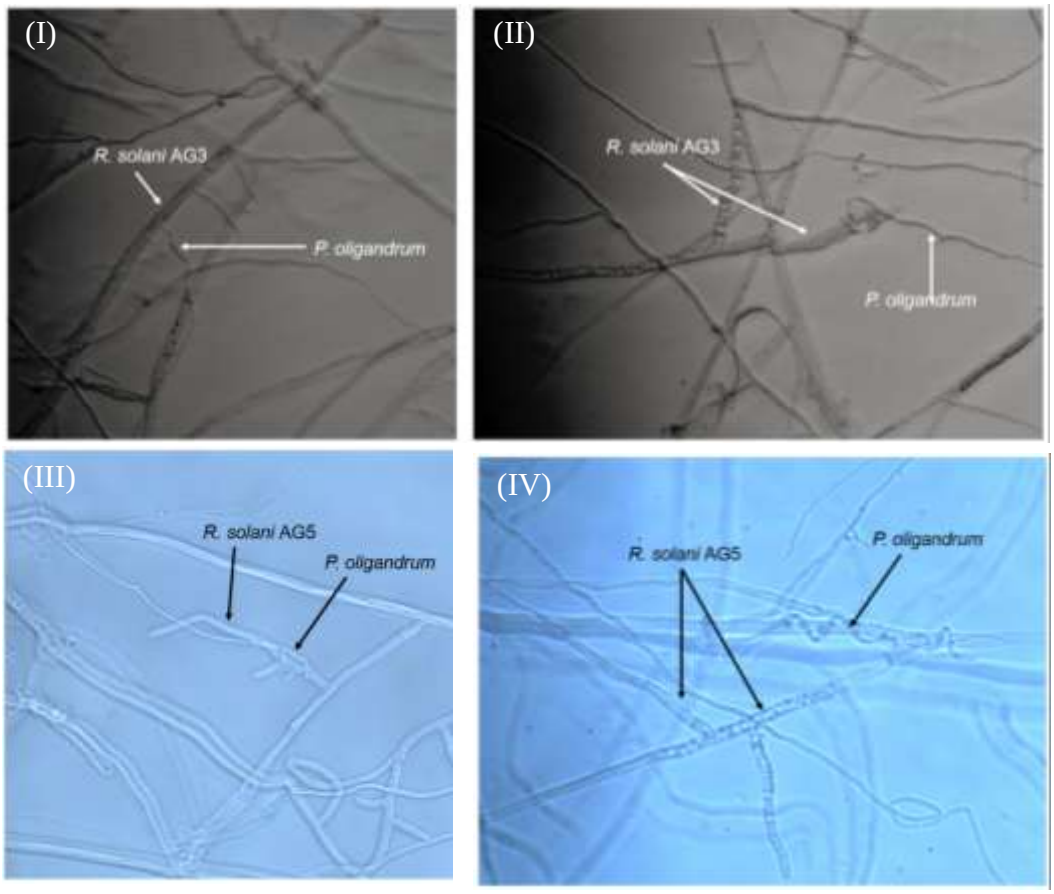

Figure 26 : Microscopic observation of P. oligandrum when interacting with R. solani AG3 and AG5. (I) Start the coiling of P. oligandrum around the hypha of R. solani AG3 strain. (II) The hyphae of R. solani AG3 were enveloped by P. oligandrum hyphae and subsequently exhibited signs of degradation. (III) and (IV) Coiling of P. oligandrum around the hypha of R. solani AG5 strain. Pictures are taken with a Zeiss Axio observer inverted microscope at 40X magnification.

5. Discussion

Microorganisms in the rhizosphere play an important role in plant defense system and promising sources of BCA (Fan et al. 2017). Understanding antagonistic mechanisms of BCA is crucial for select effective organisms and to optimize the conditions to achieve their highest biocontrol potential (Nega 2014). This project focused on biocontrol potential of *P. oligandrum* against three major potato pathogens, *A. solani*, *P. infestans*, and *R. solani* (AG3 and AG5 strains) through *in vitro* confrontation and *in planta* experiments. The results provide valuable new insights into interactions between pathogen-biocontrol organisms and effective strategies for applying *P. oligandrum* to control diseases.

5.1 P. oligandrum interaction with *A. solani* and *P. infestans*

The first experiment focused on the effectiveness of *P. oligandrum*, when *A. solani* and *P. infestans* were present at the same time. The study is relevant because early and late blight can sometimes occur concurrently in potato fields (Brouwer et al. 2023). In Brouwer et al. (2023) study, they investigated the interaction between *A. solani* and *P. infestans* when appears simultaneously, under *in vitro* conditions and also *in planta*. Their results demonstrated that *A. solani* was capable of inhibiting *P. infestans*, reducing its growth *in vitro* as well as its infection success in both laboratory and field environments (Brouwer et al. 2023). With respect to biological control of these two diseases, previous research has shown that *P. oligandrum* is effective against individually for both: it reduces the severity of early blight caused by *A. solani* (Stridh et al. 2022) and also suppresses late blight caused by *P. infestans* through rapid parasitism (Johansson 2025). However, it is essential to know whether *P. oligandrum* can maintain its biocontrol efficacy even with the two diseases appear simultaneously. We hypothesized that *P. oligandrum* inhibits co-infections of *A. solani* and *P. infestans* by reducing pathogen growth. The study results confirmed that *P. oligandrum* effectively controlled both pathogens growth under *in vitro* conditions, providing valuable preliminary data for its possibility to use in IPM systems.

Interestingly, the presence of both pathogens together appeared to enhance the growth of *P. oligandrum*. Furthermore, the plates inoculated with two pathogen plugs showed faster inhibition by *P. oligandrum* compared to those with a single pathogen plug. Even under higher density levels, the pathogens were unable to escape inhibition by *P. oligandrum*. This finding is consistent with earlier reports describing the broad-spectrum antagonistic potential of *P. oligandrum*. Benhamou et al. (1999) studied *P. oligandrum* interactions with several plant pathogens, including *P. ultimum, F. oxysporum, R. solani*, and *Phytophthora megasperma*, and noted that structural alterations in the host pathogens occurred soon after contact with the antagonist, indicating active mycoparasitic interactions. They demonstrated that *P. oligandrum* can directly parasitize pathogenic fungi through hyphal contact and enzymatic degradation. *P. oligandrum* produces hydrolytic

enzymes, including glucanases, cellulases, and chitinases, which enable degradation of both fungal and oomycete cell walls (Benhamou et al. 1999). Additionally, Picard et al. (2000) reported *P. oligandrum* has ability to compete for nutrients and secrete antimicrobial metabolites. The co-occurrence of both pathogens or higher pathogen density may therefore provide more opportunities for *P. oligandrum* to exploit these mechanisms, resulting in enhanced growth and activity compared to when each pathogen is present alone or at lower density. In the experiment, it was difficult to determine the exact inhibition mechanism of *P. oligandrum*, as physical contact with the pathogens occurred only after three days.

Two different media types were used in this study to see how these two pathogens and biocontrol growth change in different nutrient composition. V8 agar was selected because it supports higher growth of *A. solani* due to its nutrient-rich composition supports the growth and sporulation (Ambarish 2013) and the medium typically contains V8 vegetable juice, calcium carbonate, β-sitosterol, and agar, providing essential nutrients and buffering capacity for *P. oligandrum* as well (Benhamou et al. 1999), while rye agar is the standard medium for *P. infestans* due to its ability to consistently support growth, sporulation, and oospore formation growth compared to vegetable based media such as carrot, soybean, or V8 agar (Medina et al. 2002; Raza 2022). The results also supported this, since *P. infestans* showed less growth in V8 than rye even it was alone. And *P. oligandrum* showed fluffy mycelial morphology on V8 medium, but produced internally growing hyphae in rye.

Temperature is important factor for microbes hyphal growth, spore germination and pathogenicity and mycoparasitic activity. Benhamou et al. (1999) highlight that *P. oligandrum* parasitizes effectively between 18–22 °C. This temperature range ensures that *P. oligandrum* remains metabolically active, facilitating the formation of intimate hyphal contact and enzymatic degradation of host pathogens, which are critical for its biocontrol efficacy (Benhamou et al. 1999). *In vitro* studies of *P. infestans* on chick-pea-sucrose agar showed optimum growth at 20° C with a range from 4° C to less than 30° C (Zan 1962). However, Bais et al. (2019) reported that maximum colony growth of *A. solani* occurred at 25 °C, followed by 30 °C and excellent sporulation was observed at 20 °C and 25 °C. This indicates that 25 °C supports both robust growth and effective sporulation of *A. solani* (Bais et al. 2019). Therefore 20 °C that we used in this study can affect for the growth of *A. solani* when compare with other two microbes.

5.2 Foliar vs. soil application of *P. oligandrum in planta*

Foliar application of *P. oligandrum* against *A. solani* is important because it enables the biocontrol agent to directly target foliar pathogens at their infection sites, providing faster and more efficient suppression compared to soil application. Foliar delivery allows immediate interaction with the pathogen and also it can trigger local and systemic plant defense system (Bělonožníková et al. 2022; Benhamou et al. 2012). Benhamou et al. (2012) showed that *P. oligandrum* activate systemic defense pathways such as PR proteins, defense enzymes after foliar application (Benhamou et al. 2012). Since *A. solani* spread rapidly through foliar tissues,

spraying biocontrols on leaves helps to reduce spore density at early. This can slow the disease progression compared to soil application. Bělonožníková et al. (2022) emphasized that foliar application of *P. oligandrum* reduced sporangial density of *P. infestans*. In practical applications, foliar delivery of biocontrol agents such as *P. oligandrum* can be advantageous over soil treatments because it generally requires a smaller application volume to achieve effective disease suppression (Walters et al. 2013). By restricting the application to soil and use foliar treatments may reduce potential disturbances to beneficial soil microbiota while still inducing both local and systemic resistance responses in plants (Benhamou et al. 2012; Bělonožníková et al. 2022).

In this study, we hypothesized that foliar application of *P. oligandrum* would reduce *A. solani* infection severity more effectively than soil application in two different potato cultivars. We further hypothesized that soil application would also reduce infection severity, though to a lesser extent than foliar application. The results partially supported these hypotheses. It showed that foliar application of *P. oligandrum* has reduced the number of lesions develop by *A. solani* in 'Desiree' cultivar. Though the soil application reduce the leasion development, it did not produce significant suppression within the observation period. This difference highlights the efficiency different in between application method. Since *A. solani* initiates its infection on the leaf surface, foliar application ensures immediate contact with pathogen and activate both antagonistic interactions and rapid defense. Previous studies showed that foliar sprays of *P. oligandrum* cell wall protein fractions can induce defense-related gene expression within hours of treatment, providing protection against aerial pathogens (Bělonožníková et al. 2022). A general review on induced systemic resistance highlights that root-colonizing biocontrol agents typically elicit resistance signaling through jasmonic acid and ethylene pathways. But that takes some time to prime defense responses, thus making it a slower mechanism compared to foliar induction (Shoresh et al. 2010). These findings reinforce that foliar application is more efficient by directly target the pathogen and timely strategy against early blight.

In contrast, no significant suppression was observed in the cultivar 'Kuras'. This can be attributed to severe edema symptoms that developed under the controlled growth conditions, which hided lesion development and altered host physiology. Edema is associated with excessive soil moisture, high humidity, poor air circulation, and mineral imbalance, specially low calcium and potassium. This disrupt normal transpiration and make the tissues swelling (Edema | USU n.d.). Eventhough the experiment plants were grown in a biotron, they might suffered stress from lack of air circulation, high moisture or nutrient imbalance.

Edema like abiotic streses can enhance plant defense pathways, improving the speed or intensity of responses to pathogen attack (Bruce et al. 2007; Mauch-Mani et al. 2017; Ding et al. 2013), suppressing in the case of ´Kuras´ lesion development caused by *A. solani*, irrespective of *P. oligandrum* treatment. However, genetic resistance, physiological responses can varying susceptibility level for biotic and abiotic stresses, that can be the reason for less edema appeared in 'Desiree'. This

cultivar-specific outcome highlights the importance of tailoring disease management strategies to both the cultivar and its growing environment.

In this study, *A. solani* was applied only to the adaxial (upper) leaf surface and the symptoms were not very wide. Natural infections do not exclusively occur in one side of the leaf, as the abaxial (lower) surface can be equally or even more susceptible depending on microclimatic conditions and defense chemistry. For example, Caseys et al. (2024) demonstrated that *Botrytis cinerea* displayed differential infection outcomes on adaxial versus abaxial surfaces across crop species. The same situation may had with limiting of inoculation to the adaxial surface in this study can be not enough when compare with natural infections. Overall, the findings of this experiment emphasize the effectiveness of *P. oligandrum* as a biocontrol agent for suppressing early blight in potato, particularly it showed the possibility of appliyng directly to foliar tissues.

5.3 Interaction of *P. oligandrum* with *R. solani*

Rey et al. (1996) showed that *P. oligandrum* antagonistic activity happen through hyphal coiling, penetration, and subsequent disintegration of *R. solani* hyphae. Following this evidence, we also conducted dual culture assays of *P. oligandrum* with *R. solani* AG3 (strain 22897) and AG5 (strain 22880) separately. We hypothesized that, *P. oligandrum* will exhibit mycoparasitic activity against *R. solani*, through direct inhibition of its growth through physical interaction and the results were supported this. However, in this study, antagonism depended on the strain with AG3 exhibiting strong growth inhibition in the presence of *P. oligandrum*, while AG5 was largely unaffected and even outcompeted the biocontrol agent. These observations highlight differences in the susceptibility of *R. solani* anastomosis groups to antagonists and underline the need to tailor biocontrol strategies to specific pathogenic groups.

In terms of growth, AG5 consistently outcompeted *P. oligandrum in vitro*, even overgrowing its mycelium on *P. oligandrum*. AG5 also demonstrated stronger growth rates across the combinations with *P. oligandrum* and AG3, except in self-pairing where it exhibited self-inhibition. This self inhibition is happen due to vegetative incompatibility / somatic incompatibility that can observe in some *R. solani* microbes (Cubeta & Vilgalys 1997). The results aligns with earlier studies reporting variability in growth rates, competitive ability, and ecological adaptation among *R. solani* anastomosis groups (Hendel et al. 2022). The strong competitive ability of AG5 can be the reason for less inhibition by *P. oligandrum* compared to AG3. It may lie in secondary metabolite production by pathogen. *R. solani* is known to produce antifungal metabolites that can inhibit antagonists, and it can possible that AG5 produces these compounds at higher levels than AG3. Such chemical defenses can provide AG5 with a competitive advantage in direct interactions with biocontrol agents.

Our microscopic observations confirmed mycoparasitic interactions by hyphal coiling, swelling, and disintegration of AG3 hyphae. Such direct mycoparasitism is consistent with earlier studies showing that *P. oligandrum* produces hydrolytic

enzymes that degrade host cell walls (Rey et al. 1996). By contrast, AG5 hyphae showed coiling by *P. oligandrum* but no damage was noticed, suggesting that AG5 may possess structural or biochemical features that confer resistance.

However, AG3 is more aggressive pathogen on potato than AG5, being the primary causal agent of black scurf and stem canker (Carling et al. 2002). Even with the high pathogenicity, this selective inhibition of AG3 by *P. oligandrum* highly relevant for disease management. Although AG5 is also pathogenic and causes consistent stem canker (Ogoshi 1996), its lower aggressiveness relative to potato make it a less critical target for biocontrol in potato production. The differential susceptibility observed here therefore suggests that *P. oligandrum* could be particularly useful as a biocontrol agent against AG3, though its limited activity against AG5 raises concerns about its broader applicability across *R. solani* populations.

5.4 Implications in future for sustainable disease management

In vitro results of this study indicate that *P. oligandrum* has a strong potential for act as effective biocontrol agent against major potato pathogens under *in vitro* conditions. However, these observations were obtained under *in vitro* systems that do not fully capture the complexity of plant–soil–microbe interactions. Extending the assay period by using larger Petri plates could allow more reliable assessment of the temporal dynamics of antagonism. And also conducting a experiment with different positioning distances, such as close and far, can provide a clearer understanding of how *P. oligandrum* inhibits the pathogens. In this study focused on one temperature, but testing at wider range of temperatures can provide more reliable results. To apply the laboratory findings to agricultural field. further experiments in greenhouse and field trials are needed for better understanding of the effectiveness of *P. oligandrum* performance across diverse agroecological contexts.

Considering the foliar application of *P. oligandrum*, this study involved two applications of *P. oligandrum* inoculum. However future research should evaluate the effectiveness with number of foliar applications sould done per plant , and also the effective time for application. Such as before pathogen symptom appear or after infection. Optimizing concentration with volume that better to innoculate could help to determine the most effective strategy. It remains difficult to determine whether applying mature or immature spores for better effectiveness. Therefore, conducting a study on the type of inoculation comparing mature versus immature oospores would be highly valuable.

It would also be valuable to assessing responses across different potato cultivars, since genetic variation may affect both susceptibility to *A. solani* and efficacy of induced resistance by *P. oligandrum*. Moreover, extending the observation period beyond seven days is critical, as more *A. solani* disease symptoms appeared later, and short-term assessments may underestimate treatment effects. Chamber

experiments should be refined to reduce abiotic plant stress and mimic field-like conditions. Future molecular analyses (e.g. defense gene expression) could confirm whether biocontrol effects are mediated through induced resistance pathways. Ultimately, field-based trials under natural environmental variability and pathogen pressure will be essential to validate foliar application as a robust, sustainable component of integrated potato disease management. Not only that, conducting qualitative research is also important. Surveys with farmers and other participants in the agricultural sector, is essential to understand the social perceptions of farmers and consumers. Social perception and awareness are crucial for successfully introducing an effective control strategy.

5.5 Relevance to agroecology

The use of *P. oligandrum* as a biological control agent supports for more sustainable agricultural practices. It can use as a IPM stratergy by reducing dependence on chemical disease control. As a soil-born mycoparasitic oomycete, *P. oligandrum* can directly affect on key soil-borne pathogens without damaging the wider root microflora, thereby maintaining overall biodiversity in the rhizosphere (Gerbore 2014). Not only that, by inducing plant defense pathways, it supports plants for broad-spectrum disease resistance while also stimulating growth (Hashemi 2013). This dual function - pathogen suppression plus growth promotion is highly consistent with core agroecological principles, which emphasize ecological regulation and multifunctionality.

As climate change intensifies, it increased pathogen virulence and range expansion including more prevalent soil-borne oomycete pathogens under warmer conditions (Singh 2023). Biocontrol agents like *P. oligandrum* become increasingly invaluable to control these diseases. By offering adaptable, low-input pathogen management, they align perfectly with agroecology's focus on sustainable, locally adapted interventions. Rather than that, *P. oligandrum* offers potent benefits for smallholder and organic farmers, by reducing dependency on costly synthetic fungicides and supporting compliance with organic standards (Klimek-Kopyra 2023). Its application thus can supports affordable, eco-smart crop protection, enhances market alignment with eco-conscious consumers.

In summary, *P. oligandrum* exemplifies an agroecologically robust biocontrol agent, capable of integrating with cultural practices such as crop rotation, promoting multi-trophic beneficial interactions, and advancing both ecological resilience and socio-economic sustainability in modern agroecosystems. However, it is necessary to enhance the awareness of farmers and potato consumers before implementing to the agricultural system, as their perceptions are also an important factor in ensuring the resilience and success of the strategy.

6. References

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7. Popular science summary

The potato is one of the world's most important staple foods, feeding more than a billion people worldwide. However, they are constantly threatened by plant diseases such as early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), and black scurf (*Rhizoctonia solani*). To combat these threats, farmers have traditionally relied on chemical fungicides, a practice that raises significant concerns for our agricultural future. These concerns include environmental harm, high financial costs for farmers, and the worrying rise of fungicide-resistant pathogens.

Our study investigated a more sustainable approach: using the naturally occurring microbe, *Pythium oligandrum*, as a biological control agent. This remarkable organism, found in soil, acts as a "biological control agent". It posses a dual-action ability: it inhibits harmful pathogens and also stimulate plant defenses, offering promising alternative to chemical fungicides in potato farming systems.

The experiments were conducted in both laboratory dishes and in planta under controlled conditions. In laboratory studies, *P. oligandrum* significantly reduced the growth of both early blight and late blight pathogens. Against black scurf pathogen, *R. solani*, *P. oligandrum* showed strain-specific effects, strongly inhibiting one of the strains that we tested and not the other strain. In planta trials, the application method of *P. oligandrum* was observed to understand the effective application method. It was compared foliar sprays (directly on potato leaves) with soil application and the results showed that spraying on leaves was more effective than soil application. The potato variety 'Desiree' responded well to this treatment, but 'Kuras' did not show any difference, likely due to a plant stress disorder called edema. Microscopic studies revealed that *P. oligandrum* act on pathogens by wrapping around the pathogen's filaments and breaking them down. This direct attack, combined with its ability to boost plant immunity, makes *P. oligandrum* an effective candidate for sustainable crop protection in agricultural systems.

Overall, the findings highlight that *P. oligandrum* is important as a strategy in Integrated Pest Management (IPM) for potato production. By offering a natural way to suppress diseases, it helps to reduce the reliance on chemicals and and offers a more resilient and environmentally friendly agricultural system. Further research is needed to optimize its use in real-world farming conditions.

Fighting Potato Diseases with a Friendly Microbe

Evaluating ***Pythium oligandrum*** as a foliar biocontrol for *Alternaria solani* and inhibitory effect against co-infections by *Alternaria solani* and *Phytophthora infestans* and against *Rhizoctonia solani*

AIM OF THE STUDY

Evaluate the effectiveness of *P. oligandrum* in managing co-infections of *Alternaria solani* and *Phytophthora infestans*, to investigate its inhibitory mechanism against *R. solani*, and to determine the potential of foliar application as a sustainable disease management strategy.

INTRODUCTION

Potato is the fourth-largest staple food crop in the world and is consumed by more than a billion people (Aksoy et al. 2021; Prakash et al. 2020). Susceptibility to plant pathogens during cultivation is a significant challenge that affects both the quantity and quality of potatoes (Tsror 2023). Chemical control remains the most common method of managing diseases, but it is not a viable long-term controlling method. It raises environmental and health risks and also contributes to the development of fungicide-resistant strains. As a sustainable alternative, the application of biological control agents (BCA) can be effective stratergy.

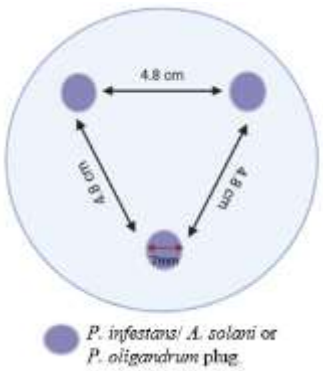
Among BCAs, *P. oligandrum* has shown antagonistic activity against a wide range of plant pathogens through different mechanisms (Benhamou et al. 2012; Belonoznikova et al. 2022). However, its performance as a control agent under co-infection scenarios remains understudied (Brouwer et al. 2023). Moreover, the inhibitory mechanisms of *P. oligandrum* to control *Rhizoctonia solani* are still insufficiently understood.

P. oligandrum is commonly used as a seed treatment or rhizosphere inoculant (Brožová 2002). However, it can be effective as a foliar application as well, specially against foliar diseases. Since few studies have focused on foliar application of *P. oligandrum*, further research is needed to evaluate the effectiveness of foliar use of *P. oligandrum*. Identifying the most effective method of application for a BCA is important when it is introduced as an effective control strategy for a sustainable agricultural system.

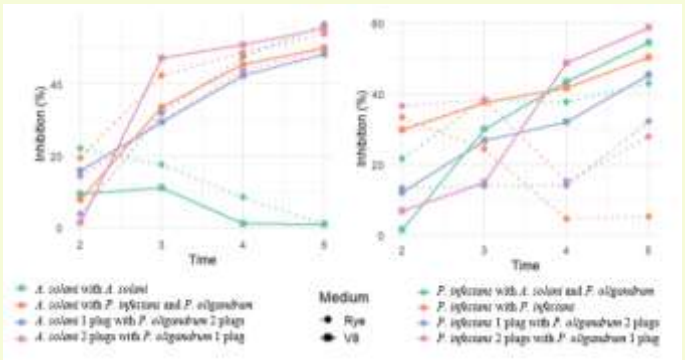
METHODOLOGY AND RESULTS

Experiment 1: Co-inoculation of *A. solani*, *P. infestans*, and *P. oligandrum*

14 - 20 days old cultures from *P. infestans*, *A. solani*, and *P. oligandrum* were used for co-inoculation on both V8 and rye solid media in Petri plates. Agar plugs (7mm) containing mycelium were placed 4.8 cm apart at the same time. Plates were incubated at 20 °C in dark for 5 days. Radial growth was measured on days 2, 3, 4, and 5 after inoculation.

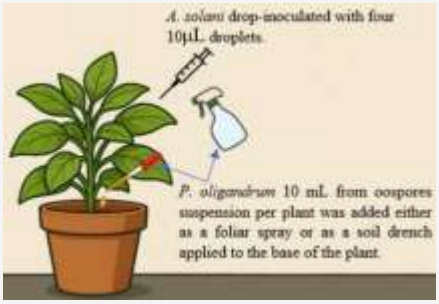


Experiment 1: Results



- *A. solani* mycelia growth was significantly reduced in both media when co-cultured with *P. oligandrum*.
- *P. infestans* culture growth was significantly different between control cultures and confrontations when co-cultured with *A. solani* and *P. oligandrum*, particularly on rye medium.

Experiment 2: *In planta* experiment



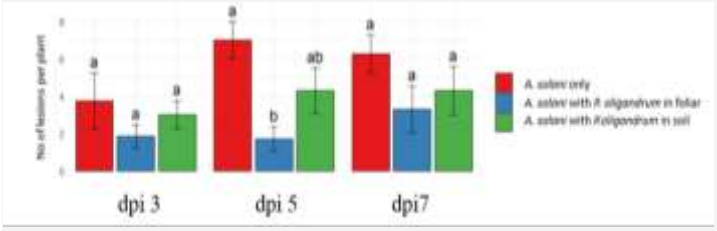
Six-week-old potato plants from two cultivars ('Desiree' and 'Kuras') were used. *A. solani* inoculation was done by using conidial suspension.

Three fully expanded top leaflets were drop-inoculated with four 10 µL (2.5×10^4/ mL) droplets of *A. solani* per leaflet. For *P. oligandrum* inoculation, an oospore suspension was utilized. 10 mL from this suspension (1.25×10^4/ mL) per plant was used. The application was done, either as a foliar spray or as a soil drench applied to the base of the plant. Two applications of *P. oligandrum* were carried out: the first was applied two days prior to pathogen inoculation, and the second was applied two days after pathogen inoculation. Number of lesions were counted at days 3, 5, and 7 after the second *P. oligandrum* application.

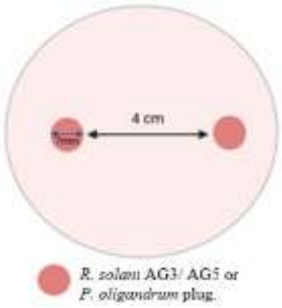
Experiment 2: Results

- 'Desiree' cultivar showed statistically significant overall effect of treatment on lesion development.
- There was a significant difference between *A. solani* inoculation alone and *A. solani* with foliar-applied *P. oligandrum*.
- The difference between *A. solani* alone and *A. solani* with soil-applied *P. oligandrum* was not significant.
- 'Kuras' cultivar did not show significant differences between treatments at any time point.

'Desiree' cultivar



Experiment 3: Dual culture of *R. solani* strains with *P. oligandrum*



14 days old *R. solani* AG3 and AG5 and *P. oligandrum* cultures were used on pea solid medium in Petri plates. Agar plugs (7 mm) were placed 4 cm apart at the same time. Plates were incubated at 20 °C for 7 days. Radial growth of individual cultures was recorded on days 2, 3, 4, and 7.

Experiment 3: Results

- R. solani AG3 growth was significantly inhibited by *P. oligandrum.*
- AG5 and *P. oligandrum* interactions showed highly significant treatment and time effects, and *P. oligandrum* growth was consistently reduced when paired with *R. solani*.
- Microscopic observations revealed that *P. oligandrum* coiled around both AG3 and AG5 hyphae, with AG3 showing pronounced swelling, disintegration, and intracellular particle formation indicative of strong microparasitic activity.

KEY TAKEAWAYS AND FUTURE DIRECTIONS

- *P. oligandrum* shows strong antagonistic activity against major potato pathogens (*A. solani*, *P. infestans*, and *R. solani* AG3), supporting its potential as a broad-spectrum biocontrol agent.
- Foliar application provides faster and more effective suppression of early blight than soil drench, especially in the cultivar 'Desiree'.
- Microscopy confirmed mycoparasitism (hyphal coiling, swelling, and disintegration), particularly against *R. solani* AG3, indicating direct pathogen degradation.
- Strain-specific effects were observed: AG3 was highly inhibited, while AG5 showed resistance, highlighting the need for targeted strategies.
- Environmental factors such as temperature, medium composition, and plant stress (e.g., edema) can influence biocontrol efficacy and cultivar response.
- Future research should focus on field trials, optimizing foliar application timing and dosage, and comparing responses across potato cultivars to validate practical use.

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2025

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