

*Studies on Management of Black scurf disease of
Potato (*Rhizoctonia solani* Kuhn) through
Biopesticides and Nanoparticles*

काशी हिन्दू
विश्वविद्यालय



BANARAS HINDU
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THIS IS SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF

Masters of Science
in
Plant Biotechnology

Submitted by

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Dear Sir,

I have great pleasure in forwarding the thesis entitled **“Studies on Management of Black scurf disease of Potato (*Rhizoctonia solani* Kuhn) through Biopesticides and Nanoparticles”** submitted by **Mr. Shubham Lad, ID. No. 19430PLB023** in partial fulfillment of the requirements for the degree of **Master of Science in Plant Biotechnology** from Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

I certify that the entire scheme of investigation reported herein, was planned and carried out by the candidate under my guidance to the best of my knowledge and belief; the data presented in the thesis are genuine and original. No part of the work has been submitted for any degree/or distinction.

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Forwarded by

Yours sincerely

Head (Course coordinator)

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**Studies on Management of Black scurf disease of Potato
(*Rhizoctonia solani* Kuhn) through Biopesticides and Nanoparticles**

By

Shubham Lad

Thesis submitted in partial fulfilment of the
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ACKNOWLEDGEMENT

*First and foremost, I would like to thank goddess of knowledge **Maa Sarasvati**, for giving me the potential, knowledge, opportunity and ability to undertake this research and work and to preserve and complete it satisfactorily.*

The success and final outcome of this thesis required a lot of guidance and assistance from many people and I am extremely privileged to have got this all along the completion of my thesis. All that I have done is only due to such supervision and assistance and I would not forget to thank them.

*Indeed, with knee down, I bow my head and offer flower of reverence to **Bharat Ratna Mahamana Pandit Madan Mohan Malviya ji**, the founder of the Banaras Hindi University, for his life time sacrifice and efforts in establishing such a great temple of learning for the cause of millions of students like me.*

*I express my deep sense of gratitude and respect to my supervisor **Dr. Shrvan Kumar**, Assistant Professor, (Department of mycology and Plant Pathology, Institute of Agriculture, Rajiv Gandhi South Campus, BHU) for providing me the opportunity to do my thesis work, for his patience, motivation, enthusiasm, and immense knowledge. Managing all this at the same time was not at all simple and requires lots of difficulties but it was made straightforward due to his inspirations and motivations. His meticulous guidance helped me during all the time of research and writing this thesis. I could not have imagined having a better advisor for my thesis research work. I owe my deep gratitude to him for taking keen interest in my thesis work and guide me all along, till the completion of thesis for his encouragement, insightful comments, ingenious suggestions, constructive criticism during the preparation of the manuscript, hard questions and providing all the necessary information for developing a good system.*

Without his supervision and insistent help this thesis would not have been possible I am also thankful to my co-supervisor, **Dr. Ravindra Prasad**, (Assistant Professor, Department of Genetics and Plant Breeding, Institute of Agriculture, Rajiv Gandhi South Campus, BHU) and **Dr. Abhinav Singh**, (Assistant Professor, Department of Agriculture Statistics, Institute of Agriculture, Rajiv Gandhi South Campus, BHU) for giving me the support and guidance which made my complete the project duly. I am extremely thankful to him for providing such a nice support and guidance. Besides my advisor and co-advisor, I would also like to thank the rest of my thesis advisory committee: **Dr. Vishal srivashtav** (Assistant Professor, Genetics and Plant Breeding, RGSC, BHU), **Dr. Ashok Kumar** (Assistant Professor, Genetics and Plant Breeding, IAS, RGSC, Mirzapur), and my teacher **Dr. Rajesh Kumar** (Assistant Professor, Genetics and Plant Breeding, RGSC, BHU) for their guidance and suggestion during my study and thesis work.

I sincerely express my gratitude to Head of Department, **Dr. B. Sinha**, Professor-In-Charge, **Dr. V. K. Mishra** for providing me with a stimulating atmosphere, external support, guidance and keen interest for the research. In course of paying gratitude, I would like to refer the name of lab staff, **Mr. Madan** and **Mr. Ram Jatan** for their sincere help and co-operation during the course of investigation. I am thankful and fortunate enough to get constant encouragement support and guidance from teacher of plant biotechnology laboratory (department of genetics and plant breeding) and B.Sc (Ag.) laboratory, Rajiv Gandhi South campus B.H.U, Barkaccha, Mirzapur, U.P, which help me successfully completing my thesis. Also, I would like to extend my sincere esteem to all staff in laboratory for their timely support.

I want to thanks **God Shiva** forgiving the patience and always bestowed upon me their kind blessing and showed me path of right virtues. I want to express my appreciation to my wonderful family for their silent sacrifice and their ceaseless blessing. Nobody has been more important to me in the pursuit of this thesis work then the members of my family.

*I express my honest gratitude to my parents they deserve special thanks for giving me the very best that they could. Thanks to my father, **Mr. Dilip Lad**, and my Mother **Mrs Saroj Lad**, for their affection caring and supporting me always and for the constant prayers. They are ultimate role models. Thank you so much for being so good to me and all that I am today is because of you only. I would always remember helping hand and loving attitude of my younger sister **Anushka Lad** for the motivation, encouragement and constant faith in me throughout my study for which I always remain indebted.*

*I would be grateful to thank my friends for their constant support and special thanks to my friend **Anamika, Ashustosh, Rahul and Sohail** for their love, care and everlasting vibrant encouragement, moral support more over for motivation and inspiration to achieve the goal.*

At last but not least, I am thankful to many people whose names were not mentioned here but we are directly or indirectly involved in helping me and I apologize to those whom I forgot to mention in my acknowledgement but they equally deserve. I wish happiness and success to all my friends' fellows and plant biotechnology family.

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ABBREVIATIONS

AgNPs	Silver nanoparticles	MgONPs	Magnesium oxide nanoparticles
°C	Degree celsius	mg	Milligram
CuONPs	Copper oxide nanoparticles	Mm	Millimeter
dia.	Diamtere	ml	Milli-liters
DW	Dry weight	min	Minutes
et al.	Et alii (and others)	×	Multiply
f	Frequency	no.	Number
FW	Fresh weight	%	Percentage
g	Gram	PDA	Potato dextrose agar
i.e.	Id est (that is)	PDB	Potato dextrose broth
M	Molar	ppm	Part per million
μl	Micro-litre	psi	Pound per square inch
μm	Micrometer	syn.	Synonym

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is an indispensable crop among all vegetables and has an essential role in our daily diet. It is an essential food crop of the world both in terms of area and production. The potato belongs to the family Solanaceae and genus Solanum, with a basic set of 12 chromosomes ($2n=2x=24$) and a commonly cultivated potato have ($2n=4x=48$). All cultivated species are 72% diploid ($2n=24$) and nearly 12% tetraploid ($2n=48$). The remaining are triploid ($2n=36$), pentaploid ($2n=60$), and hexaploid ($2n=72$). The extensively cultivated potato belongs to the tetraploid species *Solanum tuberosum* (Watanabe *et al.*, 2015)

Potato is a widely used vegetable crop also used for producing processed foods. Potatoes are also additionally used in various industries for manufacturing starch, processed food, and alcoholic beverages. It is a major source of carbohydrates and starch. Potato tubers contain 77% moisture and 21% dry matter, while about 70-90% starch. Nutritionally, potatoes are flush with vitamins like (B_1 , B_2 , B_3 and B_6), minerals (Phosphorus, potassium and magnesium), riboflavin, and pantothenic acid (Rodriguez *et al.*, 2011). Potatoes are a balanced food in our diet that have less energy but nutritionally higher in quality of protein, minerals, and essential vitamins including trace elements and essential amino acids. Potato is one of the most important crops in the world after wheat, rice, and maize. Next to cereals, potato is the only crop which can supply the needs of the food of the country (Das *et al.*, 2021).

Asia contributes half of the world's potato production, and China and India account for nearly about 38% of that production. India is the second largest producer of potato in the world after china. In 2018-19, our country is cultivated in an area of 21.72 lakh ha area, with an annual production of 50.18 MT and productivity 230.96 Qt/ha (Anonymous, 2020).

Potatoes are grown in almost all the states of India, but the main regions where potatoes grown most are the regions of middle and upper Gangetic plain states.

Mainly potato producing states are West Bengal, Himachal Pradesh, Uttar Pradesh, Madhya Pradesh, Andhra Pradesh, Telangana, Punjab, Bihar, Odisha, Chhattisgarh, Assam, and Haryana. Among the potato-growing states in India, Uttar Pradesh ranks first in production (15.32 MT) followed by West Bengal position (11.00MT) and Bihar (8.15 MT) in 2018-19 (Anonymous, 2020).

With the rising population, the demand of food grains all over the world also were increased, hence a greater efforts are necessary to increase crop yield thus production of the crops. Improvement of potato crop by conventional breeding methods i.e., intra or inter-specific hybridization, helps to improve the quality and quantity of the crops (Guo *et al.*, 2018). There are various genotypes associated with the identification related with several abiotic stresses such as phosphorous efficient traits and iron deficiency chlorosis tolerance which has been developed for increased in growth and yield for many crops. (Dahal *et al.*, 2019)

There are several biotic and abiotic factors that are responsible for low production and productivity of potato crop. Late blight, caused by *Phytophthora infestans*, is recognized to be the most prominent single biotic pressure of potato, although degeneration of vegetative planting tubers, caused by a virus, causes even more prominent yield losses. Arthropod pests likewise cause more serious damage, on a global scale i.e. potato tuber moth (*Phthorimaea operculella*), leaf miner fly (*Liriomyza huidobrenis*). Biotic stress along can cause 15–20% yield loss. Also there are many abiotic stress which causes low production and productivity some of which include heat, drought, salt stress, etc. (Kumar *et al.*, 2021).

Potatoes are infected by several diseases of fungal, viral, and bacterial origin. Some of which are are early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), Pink rot (*Phytophthora erythroseptica*), Charcoal rot (*Macrophomina phaseolina*), and black scurf (*Rhizoctonia solani*) (Rauf *et al.*, 2007; Majeed *et al.*, 2014). Some of the virus includes *Potato virus Y* (PVY), *Potato virus A* (PVA), *Potato virus V* (PVV), *Wild potato mosaic virus* (WPMV), *Potato virus X* (PVX), *Potato leaf roll virus* (PLRV), *Potato latent virus* (PotLV) (Kreuze *et al.*, 2020) and bacterial diseases are bacterial wilt (*Pseudomonas solanacearum*), bacterial soft rot

(*Erwinia carotovora* subsp. *atroseptica*), Pink eye (*Pseudomonas fluorescens*), Common scab (*Streptomyces scabies*).

Among these, one of the major disease of potato is black scurf disease caused by *Rhizoctonia solani* Kuhn. This disease of potato is an important disease of potato which is soil as well as tuber borne disease (Bakali and Martín, 2006). Black scurf usually doesn't have effects on the yield of potato crop but the quality of tuber is gradually affected resulting in the poor market price. Erampalli and Johnston (2001) explained that *R. solani* Kuhn deteriorates the tuber quality as well as quantity. In potato (*Solanum tuberosum*), it is correlated with stem cankers and tubers defects. These diseases can affect both quantitative and qualitative loss to the potato crop. Quantitative losses may occur due to infection of stems, stolons, and roots which influence tuber size and numbers, whereas, qualitative losses occur through the production of deformed tubers and the growth of sclerotia on the tuber surface (known as black scurf). The deterioration of quality caused by black scurf, especially for fresh market potato, economic loss is the most damaging aspect of this disease (Willersinn *et al.*, 2015). In recent years, with an increase in the potato production area and continuous cropping, soil-borne diseases, including potato black scurf caused by *R. solani* have become more serious. According to Woodhall *et al.*, (2008), yield losses caused by black scurf reached up to 40 percent, severely affecting potato production thus results in economic losses. It spread in India in various regions with diverse levels of severity where potato is cultivated year after year in the same field (Arora, 2013). Infected seeds are the principal sources of infection. It attacks roots, stems, tubers. The disease has two phases, *viz.* stem canker and black scurf.

The disease becomes difficult to control when infected tubers are used as planting materials and become sources of inoculum. The *R. solani* persists in the form of black sclerotia which can regrow under wet and cold soil conditions. The potato emerging from such tubers is distinguished by a black and brown sunken lesion on tuber and under-developed tissues (Larkin *et al.*, 2017).

There are certain management strategies to manage black scurf of potato-like chemicals, fungicides, inorganic components, etc. but all these strategies are not Eco

friendly and cause long-term problems like loss in soil productivity, low productions, and quality of the produced crop.

This necessitates its immediate management of *R. solani* for increasing production in the country. Among the different management options, the use of *Trichoderma* formulation (bioagent), botanicals, and nanoparticles are found to be effective and efficient in managing the *R. solani* under *in vitro* conditions and could be applied to field management.

Botanicals are one of the best methods to manage potato black scurf and are proved in controlling the *R. solani*. Botanicals are natural antifungal agents that have the potential to surmount fungal diseases without human health risks (Shuping and Eloff, 2017). On another hand, the plants containing secondary metabolites cause fungal inhibition of pathogens by dysfunction of mitochondria, suppression of cell division, and disrupting cell membrane, cell wall, DNA, RNA, and protein synthesis (Jagar *et al.*, 2014). Poisonous and non-biodegradable synthetic fungicides are noxious to the environment and humans therefore botanicals could be used as natural fungicides (Gwa *et al.*, 2018). Botanicals have been proved more beneficial than pesticides as they are environmentally friendly and cause a notable reduction in fungal growth. The use of botanical for disease management could be a more suitable option for controlling the disease- (Amber *et al.*, 2020).

Milletia pinata is a common tree that is native to India and South-East Asia. All plant parts of the tree were found to be medicinal for treating several diseases and human diseases. *Pongamia pinnata* was investigated for its antifungal properties.

Congress grass, *Parthenium hysterophorus*, family Asteraceae, is an annual weed known for its disreputable role as an environmental, agricultural hazard, and medicals. It is believed that congress grass was introduced into India from North America, this weed has developed as the major problem in India. *Parthenium hysterophorus* were assessed for their fungitoxicity against *R. solani* Kuhn under *in vitro* conditions.

In the occurrence of *R. solani*, biological control leads to either the introduction of organisms that are antagonistic to the pathogen or diminish its effects. *Trichoderma* is often studied to inhibit the growth of *Rhizoctonia* diseases which show inhibitory action against the growth of *R. solani*. (Scarselletti and Faull, 1994), Trichodermin is an antimycotic compound that helps in suppressing the growth of *R. solani* (Bertagnolli *et al.*, 1998). A large number of *Trichoderma* isolates have been recorded to suppress a broad range of *Rhizoctonia solani*, often performing the role of strong biocontrol. *Trichoderma asperellum* is a species of fungus in the family Hypocreaceae. It can be recognized from *T. viride* by molecular and phenotypic traits. The most prominent molecular features are diverging ITS-1 and 18S sequences in DNA and RFLP genes. The main phenotypic characteristics are conidial growth pattern and branching of the conidiophores on the mycelia. The species has been practiced commercially and experimentally as a bioagent for plant disease control management. *Trichoderma asperellum* species check the growth of *R. solani* in many plant diseases and are utilized as a potent bioagent. (Trillas *et al.*, 2006). *Trichoderma asperellum* was used as a potential bio control agent for seed-borne rice diseases. (Watanabe *et al.*, 2006).

The importance of nanomaterials becomes widely influenced by drug delivery, cancer cure, bio medical, agriculture, etc. Nanoparticles are generally involved in the break down cellular structures, start producing reactive oxygen species, also alter the signal transduction mechanism.

A nanoparticle is described as a particle of interest that is somewhat between 1 and 100 nanometres in diameter. AgNPs play a vital role in nanoscience and nanotechnology, especially in nanomedicine. The term is seldom applied for larger particles, up to 400 nm, and tubes that are smaller than 100 nm. Nanoparticles are very powerful materials both for experimental approaches and for practical purposes. Although many metals exist in nature, out of them only a few such as gold, silver, copper, magnesium, platinum, and palladium are extensively amalgamated in the nanostructure.

Silver nanoparticles (AgNPs) have been recognized for their anti-microbial attributes and therefore, potentially useful in controlling fungal plant pathogens. In this research, we have investigated the mycelial growth of *R. solani* in the presence of AgNPs. The outcomes show that AgNPs have the potential to check *R. solani* growth and the consequent spread of symptoms (Oktarina *et al.*, 2021).

A copper nanoparticle is a copper-associated nanoparticle that has 1 to 100 nm in size (Khan *et al.*, 2011). There are many other kinds of nanoparticles, a copper nanoparticle can be synthesized by natural means or through chemical means. (Heiligttag & Niederberger, 2013). These nanoparticles are selective due to their effective application as anti-microbial and biomedical. Copper nanoparticles show unique property including antifungal/antibacterial and catalytic properties. Firstly, copper nanoparticles confirm a very effective catalytic action, a feature that can be linked with their catalytic surface area. With the diminutive great porosity and size, the nanoparticles can accomplish a higher yield within a shorter time when used as reagents in controlling fungal growth (Heiligttag Florian J., 2013).

CuONPs are remarkably small and hold a great surface area ratio that can also act as antibacterial/antifungal agents (Ramyadevi *et al.*, 2011). The antimicrobial action is produced by their tight interaction with microbial cell membranes. As the copper oxidize they start liberating cupric ions that can produce toxic hydroxyl free radicals against the lipid membrane. Then, the free radicals strike lipids in cell membranes through oxidation to deteriorate the membranous structure. As a result, intracellular bodies flow out from the cells; after which the cells are not able to provide fundamental biochemical processes. All these changes inside the cell generate free radicals which eventually lead to cell death (Heiligttag & Niederberger, 2013).

Magnesium nanoparticles are spherical dark black with high surface area particles typically 25-65 nm in size with a particular surface area extending from 30 to 80 m²/g. These nanoparticles are also prepared in ultra-high purity with coated, and scattered forms. Magnesium oxide nanoparticle (MgO) is a metal-based antimicrobial nanoparticle that can be used as an antimicrobe in various plant pathogenic diseases. For the antimicrobial properties of MgO for medical use, it is important to ascertain

the minimal inhibitory, bactericidal, and fungicidal concentrations of MgO against prevalent infectious bacteria, fungi, and yeasts. There are various methods and conditions to unveil and directly examine the efficacy of MgO against pathogenic microorganisms (Cai *et al.*, 2018).

Among these metals mentioned here, AgNPs, MgONPs, and CuONPs have brought ample attention due to their novel physical, chemical, and biological properties. These comprise electrical, optical, thermal, and biological properties related to bulk silver in many applications including agricultural purposes, pharmaceuticals, air filtration, water detoxification, food, textile manufacturers, and oil industries, which catalyze oxidation reactions, medical, health care, consumer, and industrial purposes Nejad *et al.*, (2016).

However, with a growing world population and a constantly deteriorating environment, food safety has become a primary challenge throughout the world, especially in Asia. Keeping in view the economic importance of potato crop our present research work entitled “**Studies on Management of Black scurf disease of Potato (*Rhizoctonia solani* Kuhn) through Biopesticides and Nanoparticles**” was expressed with the following objectives–

1. To isolate, identify and maintain *Rhizoctonia solani* culture on PDA.
2. To study the efficacy of biopesticides against *R. solani*
3. Antagonistic properties of nanoparticles on *R. solani* under *in vitro* conditions



REVIEW OF LITERATURE

Potato (*Solanum tuberosum*) is the most commonly cultivated tuber crop and fourth-most major food crop next to wheat, rice, and maize. The potato belongs to the family Solanaceae and genus *Solanum*, with a basic set of 12 chromosomes ($2n=2x=24$) and a commonly cultivated potato have ($2n=4x=48$). Potato is a widely used vegetable also used for producing processed foods. Potatoes are also additionally used in various industries for manufacturing starch, alcoholic beverages. The botanical name, *Solanum tuberosum* was given by Linnaeus in his book “Species Plantarum”. The history of potatoes originated from the Andes Mountains of South America about 8000 and 5000 BC near Bolivia and Peru. Wild potatoes from the humid coastal plains of South America were first consumed by people as early as 13,000 years ago. Some wild potato species confer resistance to insects and diseases as they contain compounds like glycoalkaloids (solanine and chaconine), but they impart a bitter taste and are poisonous to humans. All cultivated species are 72% diploid ($2n=24$) and nearly 12% tetraploid ($2n=48$). The remaining are triploid ($2n=36$), pentaploid ($2n=60$), and hexaploid ($2n=72$). The extensively cultivated potato belongs to the tetraploid species *Solanum tuberosum* L. (Watanabe *et al.*, 2015).

Acknowledging the significance of the potato crop and the present topic that has been chosen for the studies. Keeping in mind the above data, a selected review of literature has been made on an area of investigation covered in this thesis work. A concise resume of remarkable feature findings is achieved by many researchers on seed-borne fungal disease related to potato crop has been mentioned in this chapter. An effort was made to cover the research achieved during the past twenty years. Reports on the black scurf of potatoes have been made with the following objectives–

2.1 Isolation, and identification of *R. solani*

Ogoshi (1987) identified tubers with sclerotia were washed in tap water and identified under a dissecting microscope. Hyphal tip growth were placed on potato dextrose agar (PDA) which was then incubated at 22° C for 3 days.

Balali et al. (1995) assessed the causes of *R. solani* infection include seed tubers incorporated with sclerotia and mycelium, and the soil itself, which may include different types, as well as shifting levels of inoculum.

Banville et al. (1996) determine the destruction of potato plants by *R. solani* occurs fundamentally beneath the soil surface, where developing subterranean stems and stolons are particularly susceptible to attack by the pathogen.

Matsumoto et al. (2003) conducted the isolation of *R. solani* by a Water Agar medium at 26°C. A total of 166 isolates were collected from the rice sheath disease of rice; around 44 isolates of the *R. solani*, were collected. Maintenance of these isolates was carried on potato dextrose agar (PDA) media.

Hide et al. (2008) reported black scurf to be the most conspicuous disease of potato, caused by *R. solani*. It emerges late in the growing season and is described by the change of black, irregular sclerotia of variable sizes on the tuber. A modification in the lesion zone, size, and several infected tubers can fall out, and in severe infections, tubers can be deformed and cracked.

Woodhall et al. (2008) perform the isolation and investigation of *R. solani* that were multiplied on potato dextrose agar at 25° C. Several anastomosis groups (AGs) were confirmed using a combination of methods including hyphal fusion assays, PCR, and DNA sequencing.

Singh et al. (2014) isolated different strain of *Rhizoctonia solani* and studied the morphological characteristics of *R. solani* under *in vitro* conditions

Oyetunde et al. (2017) identify the *Rhizoctonia* spp. related with seedling diseases in soybean crop, *Rhizoctonia* isolates were recovered from soybean seedlings with damping off and root rot symptoms.

Kumar et al. (2017) recognized the black scurf disease of potato produces brown-black sclerotia on tubers that limits the maturity of underground tubers and thus causes severe economic loss to the potato crop and production. *Rhizoctonia*

solani dwells symptom less during colonizing phase until infection to be established in form of short swollen marks on tubers.

Alfadhl et al. (2019) conducted a study to isolate and identify two pathogenic fungi namely, *R. solani* and *Fusarium solani* which causes root rot and seedling damping-off disease of cucumber, (*Cucumis sativus*).

Sandoval et al. (2019) isolate Forty-two *Rhizoctonia* isolates from rice, mung bean, and grasses from Laguna, Philippines. Sixteen isolates were binucleate *Rhizoctonia* (BNR), while 26 were multinucleate *Rhizoctonia* (MNR). Twenty MNR isolates produced big (>1.0 mm), light to dark brown sclerotia, three produced salmon-colored masses in the medium. Twenty-three MNR isolates were identified as *R. solani* AG1-IA using specific primers. Deduced Internal Transcribed Spacer (ITS) sequences of BNR isolates D1FL, NVL, and ScNL shared 100, 97, and 100% identity with *R. oryzae-sativae*, respectively,

2.2 In vitro studies on the efficacy of Biopesticides and Nanoparticles on plant pathogenic fungi *R. solani*.

2.2.1 Evaluation of Botanicals

Jortani et al. (1996) detected oleandrin as a lethal cardiac glycoside detected in the poisonous plant, oleander (*N. oleander* L.). As a chief phytochemical property of oleander, oleandrin is associated with the toxicity of oleander sap and has similar to digoxin.

Somani (2009) reported eco-friendly, non-hazardous way of controlling black scurf (*R. solani*) disease of potato, experiments apply the fresh leaf extracts of some natural botanicals in water 15-25% for 30 minutes.

Hadizadeh et al. (2009) reported the anti-mycotic activity of the ethanol derives from Konar Oleander (*Nerium oleander* L.) and Thorn Jujube (*Ziziphus spina-christi* L.) plant parts were studied *in vitro* against four major plant pathogenic fungi viz., *Fusarium oxysporum*, *Fusarium solani*, *Alternaria alternate*, and *R. solani* using agar dilution bioassay. Extracts showed antifungal activity against all the four fungi.

Reddy et al. (2010) investigated the effect of certain plant products for controlling the fungal growth associated with the seed of the crop. They estimate the hazardous and toxin-producing fungi during the storage and transport period. Plant essential oils and plant leaf extract were used as a biological control.

Aye et al. (2011) investigated the results of sixteen naturally ready botanicals and tested *in vitro* for their potential to manage phytopathogens of rice, such as *Rhizoctonia oryzae*, *R. solani*, *Rhizoctonia oryzae-sativae*, and *Sclerotium hydrophilum*. All of the established fungal mycelial were suppressed 100% by using clove botanical. Neem leaf, *Pelargonium*, and rosemary were found to provide the second-best suppression against the examined fungi. Neem leaf extract inhibited the growth of *R. oryzae* by 92.6%, *R. solani* by 87.6%, and *R. oryzae-sativae* by 80%. However, the equivalent extract suppressed *S. hydrophilum* by only 49.1%. Rosemary extract gave an inhibition of 73.89% for *S. hydrophilum*, 67.7% for *R. solani*, and 86.0% for *R. oryzae-sativae*.

Russell et al. (2011) screen the *Calotropis procera* which showed that the leaves contained tannins, cardenolides, terpenoids, glycosides, flavonoids, saponins, and alkaloids. The leaves also contained a bitter compound mudarine and other glycosides, calotoxin calotropin, etc.

Asif et al. (2012) reported *A. indica* (neem) confers therapeutics uses in the health department due to their richness in various components. The most significant active component is Azadirachtin and the others are nimbin, nimbidol, nimbidin, and quercetin. Leaves also contains components such as nimbin, 6-desacetylnimbinene, nimbandiol, 17-hydroxyazadiradione, and 7-desacetyl-7-benzoylgedunin.

Mushir et al. (2016) investigated the effect of a leaf extract from *Calatropis procera*, *Eucalyptus globulus*, *Melia azedarach*, *Datura stamonium*, and *Acalypha indica* on the seed-borne fungi *R. Solani*. Among these botanicals, *E. Globulus* in 20% concentration proved to be the best effective in reducing mycelium growth. Other botanicals were also effective as biological control.

Baka and Rashad (2016) assayed *C. procera* on the mycelial growth of *R. solani*. In the presence of *C. procera*, the variability in the growth inhibition was

observed, as the best growth inhibition was observed in *C. procera* at 3, 5, 10 and 15 percentage concentration.

Abushaala et al. (2017) evaluated the effect of plant leaf extract of a different plant in different concentrations against seed-borne fungi of *R. raetam* extract with 15% and 10% showed to be the most efficient in damping of the seed-borne pathogens, whereas *F. moniliforme* and *F. semitictum* were presented most powerful inhibitory zone.

Rahmatzai et al. (2017) investigated to evaluate various botanical oils on the radial growth of *R. solani* and reduction of black scurf incidence and severity. Oils from various plants such as, Clove (*Syzygium aromaticum*), Jojoba (*Simmondsia chinensis*), Garlic (*Allium sativum*), Sesame (*Sesamum indicum*), Castor (*Ricinus communis*), Eucalyptus (*Eucalyptus globulus*), Cinnamon (*Cinnamon zylanicum*), mustard (*Brassica nigra*), and lemon (*Citrus limon*) were tested at a concentration of 0.1, 1 and 3 percent to determine their inhibitions on the mycelia growth of *R. solani*.

Liton et al. (2019) conducted the study to estimate the effect of different bioagent (*Trichoderma* spp.) and botanicals on potato black scurf caused by *R. solani*. Among the different botanicals, *C. procera* was the most effective in inhibiting the mycelial growth-72.66% of *R. solani* isolate at a 15% concentration followed by *M. pinnata* leaf extract (68.72%). The lowest inhibition percentage (52.78% and 54.277%) also were found by *P. hysterothorus* and *A. indica*, respectively.

Siddhartha et al. (2020) investigated the efficacy of various botanicals on the mycelial growth of *R. solani* under *in vitro* conditions. From the investigation, it could be concluded that all eight examined botanicals evaluated for their efficacy inhibiting *R. solani* at different concentration of 10% and 20% under *in-vitro* conditions.

Nagini et al. (2021) reported bioactive compounds like nimbolide, *Azadirachtin* of neem are examined to have a tremendous ability to regulate many biological processes *in vitro* and *in vivo*.

2.2.2 Evaluation of Biocontrol agents

Elad *et al.* (1981) demonstrated *Trichoderma hamatum* is a commonly occurring rhizosphere-dwelling microorganism of the genus which has industrial importance due to its ability to improve plant biomass and its potential as a natural biological control agent.

Elad (1983) studied the antagonistic actions of *Trichoderma harzianum* and *T. hamatum* with *R. solani* and discovered that *Trichoderma* spp. attached to the pathogen by coiling, nearby hyphae and penetrating and making holes in hyphae and ultimately results in lysed.

Durak (2016) investigated the effect of *Trichoderma* isolates that were isolated from the sample obtained from the different regions on black scurf caused by *Rhizoctonia solani* of the potato cultivation. A total of 80 *Trichoderma* isolates were investigated. All the isolate showed above 30% inhibition on *R. solani*.

Abbas *et al.* (2017) demonstrated somewhat better control of these diseases using *Trichoderma asperellum* may provide effective biocontrol efficacy. *Trichoderma harzianum*, and cattle manure compost implemented in-furrow could reduce black scurf incidence. Incorporation of *T. asperellum* applied to the soil had a relatively antagonistic effect compared to the in-furrow treatment. Treatment of two isolates of nonpathogenic *Rhizoctonia* significantly decreased the incidence of infected tubers.

Chinnaswami *et al.* (2021) were examined *Trichoderma* spp. from native rice rhizosphere soil for their antagonistic efficiency to suppress the two soil-borne rice pathogens, viz., *R. solani* and *S. oryzae*. Morphological, biochemical and molecular characterisation of the isolates led to the identification of species as *T. asperellum*. The isolates of *Trichoderma* spp. were found to be positive to IAA release and phosphate solubilisation and were screened against *R. solani* and *S. oryzae* *in vitro* and *in vivo* in pots under glass house conditions.

Kaur et al. (2021) were reported *Trichoderma* spp., biological control agents to antagonize *R. solani* through coordinated action of several cell wall-degrading enzymes including endochitinase and β -1,3-glucanase. In this study two antifungal genes, encoding endochitinase and β -1,3-glucanase, isolated from *Trichoderma* sp. antagonistic to *R. solani*, were cloned individually in His-tagged expression vectors and mobilized in *Escherichia coli* for protein expression. The purified proteins assayed *in vitro* with *R. solani* impeded pathogen growth independently by causing hyphal distortions revealed through scanning electron microscopy. The combined use of endochitinase and β -1,3-glucanase did not enhance the inhibition. The distortions caused by endochitinase were due to catalytic activity of Glu172 and Asp241 residues on glycosidic linkages in chitin polymers, whereas Glu628, Tyr631, and Asp569 in β -1,3-glucanase targeted glucan polymers.

2.2.3 Evaluation of nanoparticles

Karlsson et al. (2008) reported the Cu and CuO nanoparticles have proved expressly great toxicity in several studies while compared to various other metal and metal oxide nanoparticles. recorded that CuO NPs exhibited the potency in causing DNA damage and cell death when compared to other metal oxides (ZnO, TiO₂, Fe₂O₄, CuZn, Fe₃O₄, Fe₂O₃).

Vanwinkle et al. (2009) anticipated that CuNPs are oxidized to produce Cu ions when associating with the membrane because the cell membrane has a higher O₂ concentration. The metal discharges (H₂O₂) locally at the cell membrane, which cause cell membrane disintegration.

Tejeda et al. (2009) reported the antimicrobial property of (CuNPs) is to a lesser extent compared to that of ZnO or Ag requiring higher ppm to accomplish the same results. However, copper is less expensive than other nano metals and can be used as nanocomposites for accelerative antibiotic efficacy.

Jeyaraman et al. (2012) who observed that copper nanoparticles showed inhibitory action against *Staphylococcus aureus*, *Micrococcus luteus*, *Pseudomonas*

aeruginosa, and *Escherichia coli* and fungi .i.e. *Aspergillus niger*, and *Aspergillus flavus*.

Khare et al. (2014) found that the copper nanoparticles show prodigious results as antimicrobial agents. The most commonly practiced substrates are polymeric beads prepared from divinylbenzene and methacrylic acid including silica beads. These substrates have been impregnated *ex-situ* with metal nanoparticles for the preparation of copper nanoparticles to improve the porosity.

Nezhad et al. (2014) investigated the copper nanoparticles can be simply incorporated with polymers and are comparatively stable in terms of both physical and chemical properties. They may be especially valuable antimicrobial agents because they can be prepared with extremely powerful surface areas.

Khan et al. (2015) demonstrated an assuring and commonly applicable method to make elemental copper nanoparticles using the chemical reduction method. XRD outcomes showed that the starch-stabilized Cu₂O nanoparticles are cubic with mean sizes of 25.16 nm. This whole pathway is particularly suitable for large-scale synthesis of Cu and Cu₂O nanoparticles connected to its easy process and economical cost. It is likely that the development of advanced synthetic methods for Cu and Cu₂O nanocrystals particles.

Abd et al. (2015) investigated that the high efficiency of MgO nanoparticles which shows 100% inhibition ratio at 2 and 3 ppm concentrations concentration in *A. flavus* fungus inhibition in the collected seeds of corn and Marziye (2014) highlighted the MgO nanoparticles efficacy of inhibition on *Fusarium oxysporum* f. sp. *lycopersici* fungus that cause fungus cell decomposition and its growth inhibition also.

Muhannad (2018) investigate the effect of MgO nanoparticles in inhibition of the pathological *R. solani* fungus at 1%, 2%, and 3% compared with the control sample, and the inhibition rates were 96%, 100%, and 100% respectively where the MgO nanoparticles affected exceedingly and reached to 100% in the pathological *R. solani* fungus inhibition on eggplant.

Nejad et al. (2016) investigated the effects of silver nanoparticles on plant pathogenic fungi. Fungal phytopathogens, especially for sclerotium-producing species *Sclerotinia sclerotiorum*, *R. solani*, and *Sclerotinia minor* were chosen due to their significant roles in the survival and disease cycle of the pathogen. Tests for the fungal hyphal growth showed that silver nanoparticles notably repress the hyphal growth.

Aleksandrowicz et al. (2017) described the importance of CuNPs and AgNPs *in vitro* on pathogens causing damping-off, *Fusarium oxysporum*, as well as wood-decay fungi; i.e. *R. solani*, *Phytophthora cactorum*, and *F. redolens*. Outcomes designate selective anti-fungal properties of the nanoparticles, as implemented at concentrations of 5, 15, 25, or 35 ppm.

Cai et al. (2015) demonstrated the MgONPs nanoparticles can be utilized in petrochemical products, electronics, bioscience, biotechnology, coatings, and various other fields. The potential utilization of magnesium oxide nanoparticles are as follows: they are high-temperature dehydrating agents used for the making high-grade ceramic body, silicon sheet, electronic industry, additive, and adhesive in the chemical industry, high valued electrode rod, and electrode sheet; high-frequency magnetic-rod, insulating substance and various transmitters used in the radio industry.

Pariona et al. (2019) conducted the study in *in-vitro* experiments, the antifungal potency against *Fusarium oxysporum*, and *Fusarium solani*. Although the antifungal action varies for each fungal species, it was found that the Cu-NPs provoke powerful morphological variations in the mycelium. Additionally, the destruction of the cell membranes of the pathogens was unveiled by microscopic observations.

Varympopi et al. (2020) show that copper nanoparticles (CuNPs) can be used as an alternative for copper bactericides to repress the growth of bacterial resistance. Physical and chemical approaches have been described to synthesize Copper nanoparticles but their application as bactericides in plants has been understudied. In the study, two separate copper nanoparticles have been used i.e., CuNP1 and CuNP2 (1500 ppm or 300 ppm) for managing the mycelial growth of *R. solani*.

Oktarina et al. (2021) studied the growth of a *Rhizoctonia solani* in the presence of AgNPs. The outcome of AgNPs at two different levels (20 and 50 ppm) on hyphal growth and sclerotium growth was investigated. The results explained that at 20 mg AgNPs suppress *R. solani* hyphal growth and production of sclerotia. The outcomes also indicate that AgNPs have the potential to inhibit *R. solani* mycelia.

Bukhari, et al. (2021) were reported the synthesis of copper oxide nanoparticles (CuONPs) using cell-free culture supernatant of marine *Streptomyces* sp. MHM38. For the optimized production of CuONPs, the influence of some parameters, such as the concentration of copper sulfate (CuSO₄), reaction time, filtrate to substrate ratio, and pH, was studied. 5 mM of CuSO₄ was optimal for nanoparticle (NP) production. UV-visible spectroscopy analysis of CuONPs exhibited a peak at 550 nm, which corresponds to the surface plasmon resonance of CuONPs. Most of the particles were spherical and were 1.72–13.49 nm when measured using a transmission electron microscope. The antimicrobial activity of CuONPs was determined using a well diffusion method against *Enterococcus faecalis* ATCC 29212, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8939, fungi (*Rhizoctonia solani*, *Fusarium solani*, and *Aspergillus niger*), and yeast (*Candida albicans* ATCC 10237). The highest antimicrobial activities were recorded against *Candida albicans* ATCC 10237, whereas *Salmonella typhimurium* ATCC 14028 and *Escherichia coli* ATCC 8939 showed the less activity.



MATERIAL AND METHODS

The present investigation entitled “**Studies on the Management of black scurf disease of Potato through Biopesticides and Nanoparticles**” was performed in the Plant Protection Laboratory, Rajiv Gandhi South Campus, IAS, BHU, Barkachha, Mirzapur, (UP)-231001. This chapter includes all the materials and approaches adopted in the investigation and the procedures are detailed below.

3.1 Materials and requirements

The glassware, plastic ware, chemicals, and other miscellaneous items were procured from Plant Biotechnology Laboratory and Plant Protection Laboratory, RGSC, BHU, Barkachha, Mirzapur.

1. **Autoclave-** For sterilization of glassware and decontamination of equipment, they were autoclaved at 121.6°C at 15 psi for 15 minutes.
2. **UV Chamber-** For quick sterilization of tools every time before using, they were wiped off with absolute alcohol and kept in UV Chamber with both wavelengths of UV light running for 15 min.
3. **BOD incubator-** For growth and multiplication of fungi BOD was set at 25± 1°C with humidity maintained accordingly.
4. **Spectrophotometer-** For detection of nanoparticles used in experiment, they were confirmed by their spectral absorbance 420 nm.
5. **Zoom stereo microscope with camera-** To take closer look at fungal colonies on seeds and for their identification Zoom stereo -microscope was used.
6. **Binocular Microscope with camera-** For taking high quality pictures and studying conidia and spores structure this Microscope was used with a 5 megapixel camera. (4x, 10x, 40x, 100x).

7. **Microwave oven-** For frequent boiling and heating of Medium and Potato used in experimentation.
8. **Hot Air Oven-** For storing sterilized glassware for further use.
9. **Laminar Air Flow-** For isolation, purification & amplification; inoculation of pathogens.
10. Electronic Digital Balance for weighing.
11. Forceps, needles, blades, knives & scalpel.
12. Spirit lamp for sterilization of inoculation needle tip.
13. Rotary shaker for agitation.
14. Micro pipette (20 μ l, 200 μ l, 1000 μ l) for different measurements.

3.2 Sterilization

Glassware used for the present investigation was kept in sterilization tins or wrapped in silver foil paper and then they were sterilized in a hot air oven at 160 °C for 90 minutes. With the help of cotton gauze which is dipped in spirits (pure) the surface of Laminar Air Flow (LAF) was disinfected through wiping. Inoculation needle, cork-borer and scalpel were sterilized by dipping in alcohol and heating to red hot. Culture media and distilled water were disinfected in an autoclave at 15 psi for 15 minutes. The working surface was wiped off clean with alcohol-dipped cotton to ensure an aseptic environment was required for our experiments.

3.3 Collection of samples

Potato, tuber were collected from various regions of the local market of Balehara, Mirzapur (Uttar Pradesh). These samples were shade dried before introducing them in the lab and then stored in paper bags at room temperature. Potato surfaces were then inspected under Zoom stereo-microscope to find out the infestation of *R. solani*. The following method was used as described below–

3.4 Culture media used

The following media was used for the isolation, purification, and maintenance of *R. solani* in the laboratory.

Potato Dextrose Agar (PDA) medium

PDA medium was prepared from the commercially available D-Glucose (SRL Company) and Agar-Agar powder. The recommended value contains 20.0g dextrose, 20.0g agar, and 200.0 g potatoes infusion form. The final pH should be about 5.6 \pm 0.2. It was then constantly mixed to dissolve while boiling. It was then autoclaved at 15 psi (121.6° C) for 15 min. The medium should not be re-melted and use more than once.

Preparation of media

Potato Dextrose Agar (PDA) was used for the isolation and maintenance of the *R. solani* in pure cultures. Its ingredients are as follows-

Following Composition of PDA was used for this experiment.

Ingredients	gm/L
Dextrose	20 g
Potato	200 g
Agar-Agar	20 g
Water	1000 ml
pH	5.6 \pm 0.5, temp- 25°C

Fresh and clean potatoes were peeled, sliced, and boiled in 500 ml of water till the potato pieces were softened. Potato extract was then filtered through a clean muslin cloth to separate it from the potato. Agar was separately boiled in 500 ml of

distilled water by constant stirring and dextrose was added to the filtered and melted agar. The volume of potato extract was made up to 1000 ml. The medium was poured into the conical flasks and plugged with non-absorbent cotton plug and sterilized in an autoclave for 15 minutes at 121°C at 15 psi.

Potato dextrose broth (PDB)

Potato dextrose broth is used for the multiplication of fungi in the laboratory setting. Suspended the 200-250 gm potatoes, dextrose 20g in 1 liter of distilled water. Heat the media until it completely dissolves. After that media is filtered by muslin cloth and then finally filtered media is sterilized by autoclave at 15 psi (121.6° C) for 15 minutes.

Plating on PDA

The sterilized PDA media was melted and poured into Petri plates (9 cm diameter) at the rate of 20 ml per plate aseptically under the laminar airflow chamber and allowed to solidify. The plates containing the PDA media were used for culturing and maintenance of *R. solani* in the laboratory.

3.5 Isolation and Identification of *R. solani*

In this study, the isolation of the *R. solani* was made from the infected tuber of potato (Black scurf). The identification and isolation of *R. solani* fungus was examined under lab conditions by blotter plate method, using Binocular Microscope with camera. The fungus was named *Rhizoctonia* which was recognized by Julius Kuhn a fungus on diseased potato tubers and called it as *Rhizoctonia solani*. Characters like colony colour colony, texture, radial growth, hyphal diameter, hyphal structure. Sclerotia characteristics, distribution pattern, relative abundance, sclerotial surface were identified by Singh *et al.* (2014). The culture was purified by the Hyphal Tip Technique and blotter paper method. Based on cultural and morphological characteristics, the fungus was identified as *R. solani* (Desvani *et al.*, 2014). *R. solani* detected on potato samples by blotter paper methods (**Plate 3.1**) tentatively were identified but for the confirmation they were isolated on PDA

medium. As soon as the colonies became visible, they were transferred to the Petri plate carefully with the help of an inoculation needle. The inoculate tubes were incubated at $25 \pm 1^\circ\text{C}$.

3.5.1 Slide preparation and Identification of *R. solani*

In the present study, the microscopic examination of *R. solani* was done by slide mounting, a part of the colony in a drop of distilled water on a clean slide. Isolated fungi were identified on the basis of colony, colour, sclerotia, etc. using Binocular microscope and was confirmed with the help of literature- (Singh *et al.*, 2014; Kareem, and Hassan, 2015).

3.5.2 Morphological characteristics of *R. solani*

The morphological features of the *R. solani* were studied under stereoscope microscope which was kept under petri plates. Potato dextrose agar medium is used in the laboratory to maintain the culture of the *R. solani*.

Mass Multiplication

Mass multiplication of *R. solani* fungi was done by inoculating Petri plates filled with 20 ml of PDA medium with the help of inoculation needle and further incubated in BOD for 3 days at $25 \pm 1^\circ\text{C}$. These pure cultures were used for the investigation of physiological characteristics of the colony, spore structure, and mycelium production. Later they were stored in the refrigerator for further practice at 4°C . Temperature is one of the crucial factors for the growth and sclerotia production of an organism which additionally affects the occurrence and spread of disease and most of the organisms grow between 0 to 42°C (Wolf and Wolf, 1947). During the present investigation, maximum mycelial growth and sclerotia production of the fungus was observed at 30°C after 3 days of incubation. Optimum temperature. i.e. 25°C to 35°C is maintained for mycelial growth and sclerotia production for *R. solani* has also been witnessed by Ray and Kumar (2009).

3.6 Methods of preparation of biopesticides and nanoparticles

3.6.1 Botanicals

Following biopesticides and Nanoparticles prepared at Plant Protection Laboratory, Rajiv Gandhi South Campus, Banaras Hindu University, Mirzapur, UP, were used for the control and management of *R. solani* associated with potato black scurf disease.

The plant chosen for the study were **Table 3.1** *Azadirachta indica* (Neem), *Calotropis procera* (Madar), *Milletia pinnata* (Karanj), *Nerium oleander* (Kaner), *Ocimum sanctum* (Tulsi) and *Parthenium hysterophorus* (Congress grass). All the botanicals used in this present study was well known for their medicinal values and importance. These botanicals were well formed by extracting their leaves extract which was then used as a treatment at different concentrations.

Table 3.1: List of botanicals evaluated against *Rizoctonia solani*

S.No.	Botanical name	Common Name	Family	Plant Part Used	Active Substances
1	<i>Azadirachta indica</i>	Neem	Meliaceae	Leaves	Azadirachtin/ Nimbolinin Bhavani <i>et al.</i> ,(2019)
2	<i>Calotropis procera</i>	Madar	Apocynaceae	Leaves	Calotropin, Digoxin Kumar <i>et al.</i> ,(2021)
3	<i>Milletia pinnata</i>	Karanj	Fabaceae	Leaves	Flavonoids
4	<i>Nerium oleander</i>	Kaner	Apocynaceae	Leaves	Oleandrin Al-Snafi (2020)
5	<i>Ocimum sanctum</i>	Tulsi	Lamiaceae	Leaves	3,4-dimethoxycinnamic acid, Eugenol, Permethrin Leila Mousavi(2018)
6	<i>Parthenium hysterophorus</i>	Congress grass	Asteraceae	Leaves	Sesquiterpene lactone, parthenin Khalid <i>et al.</i> (2020)

Preparation of plant extracts

Four different concentration has been prepared for the application of botanicals on potato broth media. The following concentration were demonstrated in the table given below:

Fresh leaves of *A. indica*, *C. procera*, *M. pinnata*, *N. oleander*, *O. sanctum*, and *P. hysterophorus* were cut and washed with water to remove all contaminants; they were then crushed with help of grinder to extract their chemical properties. The paste was then dissolved in methanol with equal proportion. They were all left to steep in covered containers for 24 hrs. The extract was squeezed out with a double-layered sterilized cheesecloth and filtered whatman filter No.1. Extra methanol were evaporated till half quantity of solution in a hot air oven at 50°C. The extracts thus collected were counted as of 100 % concentration. The effect of all six botanicals was examined at four different concentrations (3, 5, 10 & 15 percent) following the method Singh and Majumdar (2001) with slight modifications.

Required quantity of both plant extract i.e. stock solution was incorporated thoroughly in potato broth, to get coveted concentration, just before pouring in sterilized Petri dishes. Individually plate was inoculated with a 10 mm disc of a mycelial bit taken with the help of a sterilized cork borer and the disc was taken from the periphery of a week-old culture of *R. solani* growing on PDA. Three replications were set up and the entire treatment was placed in a BOD chamber with a temperature set at 25±1°C for 3 days. *R. solani* cultured on potato broth plates with no plant extracts but with only sterilized distilled water serves as a control plate. A control was also prepared where the medium was not supplied with any plant extract. The investigation was accomplished in Completely Randomized Design (CRD). Colony fresh and dry weight was recorded on the 3rd day of incubation. Percent growth inhibition was measured by the Vincent's (1947) formula as follows:

$$\text{Inhibition \%} = \frac{C-T}{C} \times 100$$

Where,

C = Fungus colony weight (in gm) in control plate

T = Fungus colony weight (in gm) in treated plate

Average of three replications of each test is taken for calculations.

Table 3.2: Calculation on Botanicals for application on potato broth media

Percentage concentration (in %)	Botanicals (in ml)	Broth media (in ml)	Net volume (in ml)
3	0.6	19.4	20
5	1	19	20
10	2	16	20
15	3	17	20

3.6.2 Bio control agents

Potentially, biocontrol agents, was used in various studies to control many plant pathogenic fungi. In the present investigations, the antagonistic action of these two species of *Trichoderma* (*T. asperellum* and *T. hamatum*) was tested against *R. solani* fungi. An effort was made in the present study to manage dominant fungi i.e. *R. solani* through the application of biocontrol agents (*T. asperellum* and *T. hamatum*). Biocontrol agents including *Trichoderma asperellum*, and *Trichoderma hamatum* were tested *in vitro* for their antagonistic activity against *Rhizoctonia soloni*. For studying the effect of biocontrol agents on *R. solani* fungi, a dual culture technique was employed.

For the dual culture technique, an agar disc of 10 mm in diameter has been taken from 7 days old culture of *R. solani* which was then placed away from the

periphery of a Petri dish. Both the culture of *Trichoderma* spp. were then placed 1cm away from the periphery on the opposite side of *Rhizoctonia* in two different Petri dish. After in inoculation both the plates were incubated at $25\pm 1^{\circ}$ C for 3 days in incubator. Mycelial growth inhibition was calculated after 3 days. Observation were recorded on the basis of development of the inhibition zone. The diameter (in mm) of the mycelial growth was recorded and compared with the control plates (Huang and Hoes, 1976).

Percent inhibition of mycelial growth was calculated by the following formula-

Vincent's (1947) formula as follows:

$$\text{Inhibition \%} = \frac{C-T}{C} \times 100$$

Where,

C = Diameter of fungus colony (mm) in control plate

T = Diameter of fungus colony (mm) in treated plate

Average of three replications of each test is taken for calculations.

3.4.3 Nanoparticles

In the present research work three nanoparticle has been taken to detect their antagonistic properties against *R. solani* under *in vitro* conditions. The following concentration were used in current research which has been tabulated below:

Recently the nanotechnology has also emerged as a lucrative option for management of several plant disease. Nano based particles like AgNPs, MgONPs, and CuONPs can be used for estimate the efficacy of these three nanoparticles against *R. solani* under *in vitro* conditions.

Biosynthesis of Silver Nanoparticles (AgNPs)

Usually, the synthesis of nanoparticles has been synthesized by three distinct approaches, including chemical, physical, and biological approaches. In physical approach, nanoparticles are synthesized by evaporation and condensation in a tube at atmospheric pressure.

Chemical methods utilize organic solvents or water to make silver nanoparticles. This process commonly has three main components, such as reducing agents, metal precursors, and capping agents (Dhand *et al.*, 2015).

In this experiment, 10 mg of silver foil was reacted with 40 % HNO₃ and incubate for 1 hour the reaction as follows



Biological method for bio synthesis of the silver nanoparticles (AgNPs) the culture of the *Trichoderma asperellum* was encouraged according to the (Casagrande *et al.*, 2019) in potato broth supplemented with 0.01 mM AgNO₃, at ambient condition and temperature, Culture of *Trichoderma asperellum* was grown in Petri dish for 72 hours under shaking condition (200 rpm) at 27°C. After incubation, the mycelial biomass was then insulated from the broth by centrifugation (500 rpm) and rinsed thrice with sterilized double-distilled water. The recovered nanoparticles were physically characterized by UV-VIS Spectrophotometry and monochromatic laser light.

Here, AgNO₃ was utilized as the initial reactant for Silver nanoparticle synthesis and 1% Trisodium citrate (TSC) and Ascorbic acid as reducing agents and stabilizers, respectively. About 80 ml of laboratory prepared AgNO₃ was added to 20 ml of 1 % of TSC and put on ice and added Ascorbic acid step by step up to solution become colorless and constant stirring. When the solution becomes colorless, it is stored at room temperature and was characterized by a UV-Vis Spectrophotometer. Characterization of nanoparticles by biological and chemical method was carried out by two approaches the reduction of AgNO₃ was monitored by visual inspection, growth in *Trichoderma* spp. and in second approach the periodical measurement of Absorbance of the solution at the 300 to 700 nm using spectrophotometer.

Synthesis of Magnesium Oxide nanoparticles (MgONPs)

Preparation of the precursor

Synthesis of MgONPs requires 5 grams of Magnesium sulphate (MgSO₄) which was liquified in 100 ml of sterilized distilled water and 5 grams of sodium hydroxide (NaOH) was dissolved in 100 ml of sterilized distilled water separately. Then after 50 ml of Magnesium sulfate solution and 50 ml of Sodium hydroxide solution were mixed **Plate 3.4**. The solution was continuously stirred with a magnetic stirrer for about 2 hrs after which Magnesium hydroxide was precipitated. The solution obtained after the precipitation was heated at 100°C for 2 hours in a hot air oven. The precursor, Magnesium hydroxide (MgONPs) synthesized.

Preparation of MgONPs

The precursor was then put in a muffle furnace at 300oC for 3 hrs after which MgONPs were produced.

UV-Visible spectroscopy

UV-vis spectrophotometer is used to describe UV-visible spectra of the prepared MgONPs in the form of absorbance, which represent the wavelength range between 200–900 nm which assure the degree of absorbance of MgONPs. The observation were recorded the absorption bands of MgO from 200-750 nm. The spectrum of MgO contains several absorption peaks in between 260-330 nm (Rastogi, 2015) another method by which MgONP were confirmed was laser diffraction method.

Synthesis of Copper nanoparticles (CuNPs)

Materials

All the chemicals utilized in the experiment were comprised of scientific grade. Copper sulfate CuSO₄ (0.1 M), Ascorbic acid C₆H₈O₆ (0.2 M), Starch

(C₆H₁₀O₅)_n (1.2 %), and Sodium hydroxide NaOH (1M) were used from SRL company. Sterilized distilled water were used for the experiment.

Method

The CuONPs obtained were synthesized by chemical reduction means using copper sulfate (CuSO₄) as precursor and starch was used as a capping agent. The preparation procedure begins with the addition of 0.1 M copper sulfate solution into 120 mL of starch solution (1.2 %) by using magnetic stirrer for 30 minutes. In the next step, 50 mL of 0.2 M ascorbic acid solution was added to the synthesis solution which was also mixed for next 30 minutes by using magnetic stirrer. Subsequently, 30 mL of 1 M Sodium hydroxide solution was gradually added to the prepared solution by continuous stirring and heating at 80°C for 2 hrs. The color intensity of the solution turned from yellow to ocher. After the end of the reaction, the solution was obtained from the heat and left to settle overnight and the supernatant solution was then discarded carefully. The precipitates were isolated from the solution by filtration and rinsed with deionized water and ethanol three times to get out the unnecessary starch dressed with the nanoparticles. Ocher color precipitates (**Plate -3.4**) obtained are dried at room temperature. After drying, nanoparticles were collected and stored in a glass vessel for further interpretation.

The UV–vis absorption spectra

Synthesized CuONPs were characterized using UV–vis spectra, reading was recorded between the wavelengths of 190–800 nm in a spectrometer. The UV–vis absorption spectra of CuONPS were shows a peak at 210 nm which is associated with the formation of cuprous oxide nanoparticles (Sivaraj, 2014).

Treatment of nanoparticles on the growth of *R. solani in vitro*

Nanoparticles forms of Copper oxide were evaluated *in vitro* against *R. solani* at five concentrations; 25, 50, 75, and 100 ppm. The examined nanoparticles were added individually to Petri plates containing sterilized PDA medium to obtain the correct concentrations then mixed gently before solidification, the treated medium

with (CuONPs) was poured into 3 sterilized Petri plates (9 cm diameter) per each treatment. After media solidification, PDA plates were individually inoculated in the center with even mycelial bit (10 mm) and incubated at $25\pm 1^\circ\text{C}$ for 3 days. The inoculated PDA plates with only mycelial bit served as a control. The diameter of grown colonies was measured when fungus filled the control plate. The percentage of inhibition on the mycelial colony diameter was calculated by using formula suggested by Sirirat *et al.* (2009) as follows-

$$\text{Reduction \%} = (\text{de} - \text{di}) / \text{de} \times 100$$

Where, de is the growth of mycelium in control plates,

di is the growth of mycelium in treated plates.

The understanding of MgO nanoparticles inhibition of the pathogenic fungus *R. solani* is associated with the high surface area of the MgO nanoparticle and to the sharp arrangement of the nanoparticle surface sites and the nanoparticles surface area acts as media for solutions reception and this makes it inappropriate for fungus growth and eventually results in inhibition of the fungus. The sharp structure of the nanoparticle surface acts on the host cell wall that shows disintegration and makes out all cell contents which after will inhibit fungus growth.

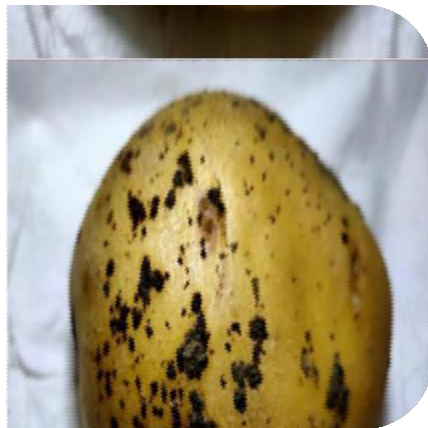
Light scattering tests have been proved one of the most assuring and beneficial techniques for *in situ* analyses of size, size distribution, and shape of low dimensional nanoparticles. The scattering angle of elements of the scattering matrix (nanoparticles, aerosols, hydrosols, etc.) can be detected in the laboratory by using monochromatic laser light scattering experiments (Travis *et al.*, 2002).

Statistical Analysis

All the calculated parameter were analyzed in three replications. The data obtained by different treatments and concentrations were determine by the completely randomized design (CRD) for studying the significance of various data.

Table 3.3: Calculation on Nanoparticles for application on PDA Medium

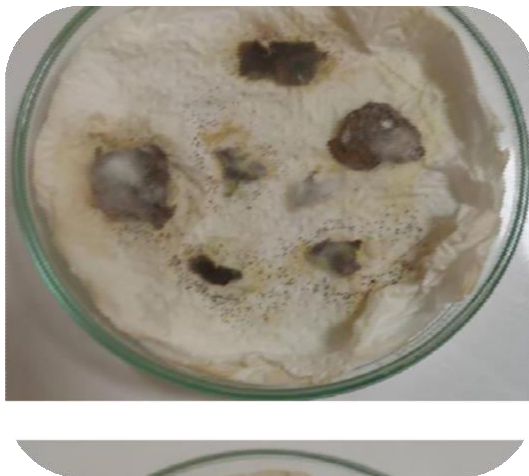
Nanoparticles Concentration (in ppm)	Nanoparticles Solution (in ml)	PDA Medium (in ml)	Net volume (in ml)
25	1.5	58.5	60
50	3.0	57.0	60
75	4.5	55.5	60
100	6.0	54.0	60



Infected potato tuber



Sclerotia on potato tuber



Mycelial growth



Culture on PDA



**Hyphal branching at
Right angle**



**Pitted sclerotium with
honeydew**



Mature mycelium

Plate 3.1. Isolation, identification and culture maintain of *R. solani* on PDA



Ocimum sanctum (Tulsi)



Calotropis procera
(Madar)



Nerium oleander (Kaner)



*Parthenium
hysterophorus* (C. grass)



Milletia pinnata (Karanj)

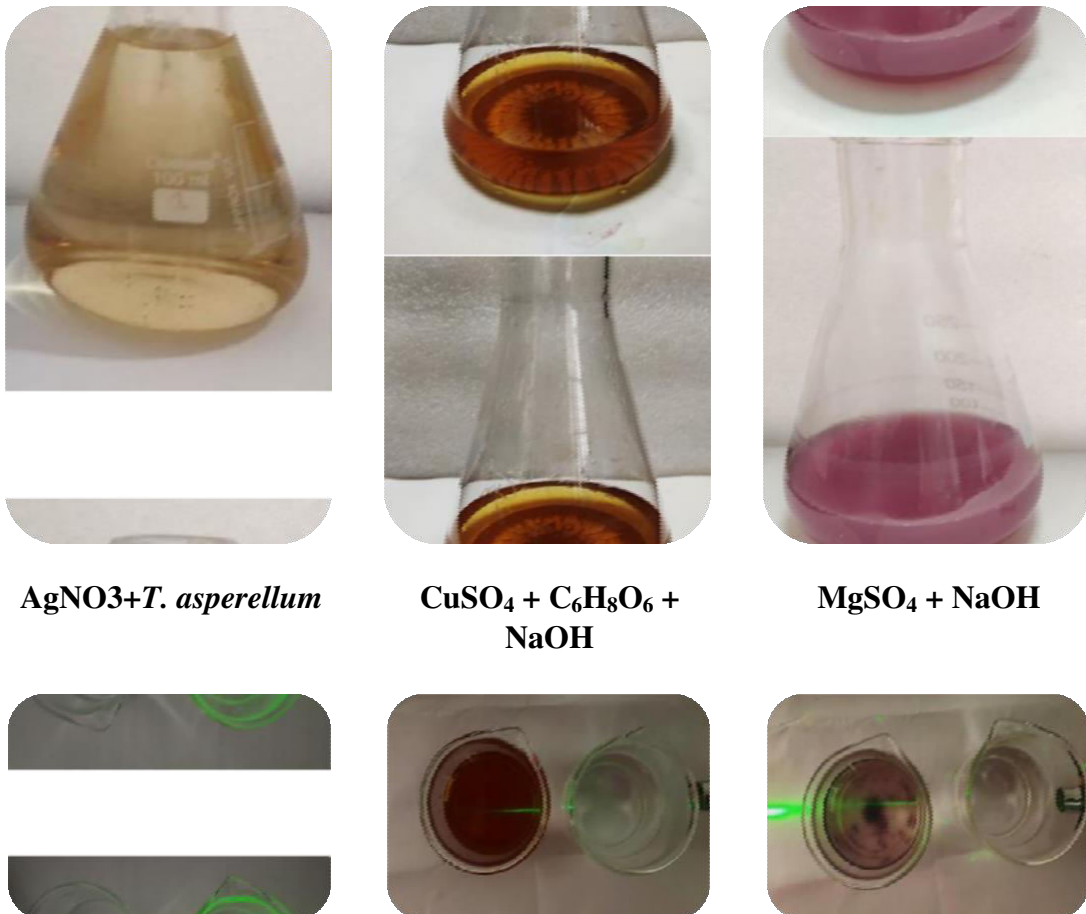


Azadirachta indica
(Neem)

Plate 3.2. Names of plants and parts used for Plant extracts



Plate 3.3. Extraction process of Botanicals



$\text{AgNO}_3 + T. asperellum$

$\text{CuSO}_4 + \text{C}_6\text{H}_8\text{O}_6 + \text{NaOH}$

$\text{MgSO}_4 + \text{NaOH}$

Plate 3.4: Nanoparticles synthesis and detection through laser light scattering



RESULTS AND DISCUSSION

The present investigation entitled “Studies on Management of Black Scurf of Potato through Biopesticides and Nanoparticles” was conducted in a Completely randomised (CRD) at the Plant Biotechnology Laboratory and Plant Pathology Laboratory, RGSC, BHU, Barkachha, Mirzapur. To identify the *R. solani* on potato tuber, blotter plate method was used. Mycelial growth inhibition of *R. solani* through Botanicals, Bio control agents and Nanoparticles was assessed. The experimental results obtained from the present investigation with important objectives are presented as follows:

4.1 Collection of potato samples

An extensive study for the existence of *R. solani* on potato were performed throughout the research period. Diseased potato tuber was collected from Balehara, Barkachha, Mirzapur (U.P), and brought to the laboratory. The pieces (5.0*5.0 mm) of infected potato tuber were kept within moist blotter plate chamber for 3 days. The fungus was isolated from infected tuber of potato under aseptic conditions on PDA (Plate 3.1). The culture was purified by the hyphal tip technique.

4.2 Identification of *Rhizoctonia solani* Kuhn

The identification and pathogenicity of isolated and purified fungus was examined under lab conditions by blotter plate method, using Binocular Microscope with camera. The fungus was named *Rhizoctonia* which was recognized by Julius Kuhn a fungus on diseased potato tubers and called it as *Rhizoctonia solani*. Mycelial disc (10 mm diameter) from the actively growing edge of 3-day old pure culture of each isolate was transferred at the center of petri plate containing PDA and incubated for 15 days at 25±1° C. Morphological characters like radial growth, angle of hyphal branching, constriction at the point of origin and width of hyphae were examined without staining. Three replications of each isolate were maintained (Singh *et al.*, 2014).

Cultural characters like colony colour is pale brown, colony texture- sparse, flappy, radial growth is fast and have hyphal diameter of $9.35\pm 0.25\mu\text{m}$ that have right angle hyphal structure. Sclerotia characteristics, initiation period of sclerotia- production is within 6 days, distribution pattern- peripheral and sub-central, relative abundance- moderate to high, sclerotial surface- pitted with honeydew formation, and sclerotial width- 2.48 ± 0.32 mm in diameter. The observed characteristics were in accordance with finding of Singh *et al.*, (2014).

4.3 Management of *R. solani* under *in vitro* condition

4.3.1 Efficacy of different botanicals

In recent years, countless botanicals are being utilized as fungitoxicants for management of numerous plant diseases. The present investigation was performed by using following six natural botanicals i.e. *Azadirachta indica* (Neem), *Calotropis procera* (Madar), *Milletia pinnata* (Karanj), *Nerium oleander* (Kanner), *Ocimum sanctum* (Tulsi) and *Parthenium hysterophorus* (Congress grass) to understand their antimycotic behavior on the mycelial growth of *R. solani* following the Poisoned Food Technique (Nene and Thapliyal, 1993).

The data on colony fresh, dry weight (in gm) and percent growth inhibition were studied in term of mycelial growth of *R. solani* presented in **Table-4.1 and Plate-4.1-4.6**. Antifungal activity of *A. indica*, *C. procera*, *M. pinnata*, *N. oleander*, *O. sanctum* and *P. hysterophorus* were tested with the increasing concentration order. The highest mycelial growth inhibition was at 15 percent by *Calotropis procera* i.e. 72.66 percent and the lowest mycelial growth inhibition was by *Parthenium hysterophorus* i.e. 52.79 percent. At 15% concentration all six botanicals were found to be effective against *R. solani* that resulted in 52.79 to 72.66 percentage inhibition of mycelial growth. However, at 10% concentration *A. indica* were found to be less effective among all six plant extract i.e. 40.04 percent and maximum inhibition by *M. pinnata* i.e. 63.37 percent mycelial growth inhibition. The maximum mycelial fresh and dry weight (in gm) of *R. solani* were found in *C. procera* (2.08 and 1.50) followed by *A. indica* (1.90 and 1.43) at 3 % concentration. However, minimum mycelial fresh and dry

weight (in gm) of *R. solani* were found in *P. hysterophorus* (0.69 and 0.28) followed by *C. procera* (0.87 and 0.27) at 15% concentration. Percent mycelial growth inhibition of *R. solani* was observed based on dry weight between 25.25-72.66 maximum in *C. procera* and 41.70-68.72 in *M. pinnata* among the all tested concentrations. However, all the treatments exhibited significant mycelial growth inhibition over control.

Hassanein *et al.*, (2008) demonstrated the efficacy of leaf extracts of neem (*Azadirachta indica*) against grass carp, *Ctenopharyngodon idella*. By slightly modification the *in vitro* activity of neem leaf extract were compared against *R. solani* pathogenic fungi. Leaf extract of different concentrations (3%, 5%, 10%, and 15 %) were prepared by neem and methanol in equal proportion and added to growth media before inoculation. Inhibition values recorded a distinguishing variation by increasing concentration of botanicals. In the case of neem, inhibition percentages were 20.21%, 30.53%, 40.04 %, and 54.28% for *R. solani* in the four used concentrations. Methanol extracts of neem leaves assayed at a concentration of 15%, fully suppressed the growth of *R. solani*. All used concentrations of neem extract effectively suppressed the mycelial growth of *R. solani* pathogenic fungi in increasing order with respect to different concentration and this effect gradually increased with increasing concentration. Our tested botanicals namely *Azadirachta indica*, *C. procera*, *M. pinnata* and, *N. oleander* were tested at 3%, 5%, 10%, and 15% percent concentration that inhibited mycelial mass growth and sclerotia production of *R. solani* *in vitro*. *C. procera* extract gave maximum inhibition of mycelial growth and sclerotia formation at higher concentrations followed by *N. oleander* leaf extract. The present result are also in accordance with the finding of Sehajpal *et al.*, (2009).

Rao *et al.*, (2012) screened different concentrations of leaf extracts of *M. pinnata* against *R. solani* under invitro conditions. screened different percentages of concentrations used were 3%, 5%, 10% and 15% which showed 41.70, 48.18, 63.37, and 68.72 percentage mycelial growth inhibition, respectively.

Hadizadeh, *et al.*, (2009) reported the anti-mycotic property of the ethanol extracts from Kaner (*N. oleander* L.) floral parts against *R. solani* using agar dilution bio assay. Extracts of *N. oleander*. percent concentration (0.6%, 1%, 2%, and 3 %)

showed antifungal active inhibition of 33.73%, 37.50, 54.35%, and 59.03 percent respectively against *R. solani*. *Nerium oleander* was the effective extract by decreasing the growth of *R. solani*. These effects showed that extracts could be viewed as a suitable alternative to chemical additives for the limitation of fungal diseases in plants.

Rao *et al.*, (2012) were tested Congress grass, *Parthenium hysterophorus*, of the family Asteraceae, known for its disreputable role as medical, environmental, and agricultural hazards. When evaluated against the black scurf of potato shows significantly effective inhibition with the increasing concentration of the leaf extract showed following percentage mycelial growth inhibition 21.24, 37.73, 45.04 and 52.79 percent with a concentration of 3, 5, 10, and 15 percent, respectively.

Antifungal property of aqueous leaf extract of Neem (*Azadirachta indica* L.), Madar (*Calotropis procera*), Karanj (*Milletia pinnata*), Kaner (*Nerium oleander* L.), Tulsi (*Ocimum sanctum*), and Congress grass (*Parthenium hysterophorus*), was investigated against *R. solani*. At 3, 5, 10, 15 percent concentration, the maximum antifungal potential was observed with the extracts of *M. pinnata*, which showed 68.72% percent inhibitory activity against *R. solani*, followed by leaf extract of *O. sanctum*, and *C. procera*. The application of botanical extracts for disease management could be advisable option for controlling disease. Six plant extracts namely *A. indica*, *C. procera*, *M. pinnata*, *N. oleander*, *O. sanctum*, and *P. hysterophorus* were tested at 3, 5, 10, and 15 percent which shows significant inhibition on mycelial growth and sclerotia production of *R. solani in vitro*. *Calotropis procera* extract gave maximum inhibition of mycelial growth and sclerotia formation at higher concentrations followed by *N. oleander* leaf extract. Similar results had been recorded by our experimental work while working with *R. solani in vitro*.

Our finding on six plant extracts (**Table 4.1**) were tested *in vitro* at four concentrations viz., 3%, 5%, 10%, and 15% percentage against *R. solani* within potato broth. Among six, botanicals *A. indica*, *C. procera*, *M. pinnata*, *N. oleander*, *O. sanctum*, and *P. hysterophorus* was observed effective in inhibiting mycelial growth (20 to 72 %) of *R. solani* at 3, 5, 10 and 15 percentage. Both the concentrations 10,

and 15 percent of all the tested botanicals were found significantly superior in comparison to control against *R. solani* pathogen. *Calatropis procera* extract gave maximum inhibition of 72.66% on mycelial growth and sclerotia formation at higher concentrations followed by *N. oleander* leaf extract. Similar results have been recorded by Sehajpal *et al.*, (2009) while working with *R. solani* *in vitro*.

Table 4.1: Mycelial growth inhibition of *Rhizoctonia solani* by different concentration of botanicals in potato broth culture

Name of Botanicals	Concentration (in %)	Control average (in g)	Fresh weight (in g)	Inhibition average (in %)	Control average (in g)	Dry weight (in g)	Inhibition average (in %)
<i>Azadirachta indica</i>	3	2.00	1.90	10.41	1.80	1.43	20.21
	5	2.12	1.73	18.99	1.88	1.30	30.53
	10	2.15	1.43	25.97	1.60	0.95	40.04
	15	1.93	0.86	35.45	1.08	0.50	54.28
<i>Calotropis procera</i>	3	1.33	2.08	13.09	2.00	1.50	25.25
	5	2.40	1.25	36.67	1.46	0.81	42.42
	10	1.97	1.19	36.19	1.20	0.54	54.70
	15	1.86	0.87	47.69	1.01	0.27	72.66
<i>Milletia pinnata</i>	3	1.69	1.21	38.07	1.29	0.72	41.70
	5	1.95	0.87	43.41	0.91	0.47	48.18
	10	1.53	0.60	42.40	1.82	0.66	63.37
	15	1.08	0.36	53.62	1.69	0.53	68.72
<i>Nerium oleander</i>	3	0.79	1.63	27.27	1.81	1.19	33.73
	5	2.27	1.47	30.24	1.67	1.04	37.50
	10	2.10	1.02	40.76	1.19	0.54	54.35
	15	1.72	0.56	46.80	0.92	0.38	59.03
<i>Ocimum sanctum</i>	3	1.05	1.77	25.10	1.90	1.07	43.84
	5	2.37	1.53	29.46	1.61	0.79	50.52
	10	2.17	0.83	46.00	0.97	0.45	53.72
	15	1.53	0.79	35.16	1.03	0.43	58.31
<i>Parthenium hysterophorus</i>	3	1.22	1.75	22.46	1.27	0.96	21.24
	5	2.26	1.35	35.94	0.90	0.55	37.73
	10	2.12	0.95	49.45	0.92	0.51	45.04
	15	1.89	0.69	55.79	0.59	0.28	52.79
C.D. (at 5 %)		0.41	0.30	18.97	0.30	0.20	15.97
SE±(m)		0.144	0.105	6.65	0.11	0.07	5.60

Notes: *Average of three replications and Mycelial growth inhibition was recorded after 3 days of inoculation.

4.3.2 Efficacy of bio control agents

The following bio agents were used for study viz., *Trichoderma hamatum* and *T. asperellum*. The fungus *Trichoderma* is particularly efficient in the controlling *R. solani* pathogen.

The bioagents viz., *T. asperellum*, *T. hamatum* were evaluated for their antifungal activity against *R. solani* under in vitro condition by dual culture technique. Inhibition zone (in mm) was measured and the percent inhibition was calculated and results are presented in **Table– 4.2 and Plate–4.7**. The bioagents significantly inhibited the growth of *R. solani* either by over-growing or by exhibiting inhibition zone. The *Trichoderma* spp. showed considerable antifungal activity against *R. solani*. The inhibition of radial growth of *T. asperellum* (57.60%) was significantly higher as compared to *T. hamatum* (49.90%).

Wang *et. al.* (2019) investigated the dual culture assay to identify the growth inhibition actions of the *Trichoderma* spp. against *R. solani*. They investigated *Trichoderma* isolates that distinctly inhibited the growth of *R. solani* in which *T. pyramidale*, and *T. simmonsii* and showed the most eminent inhibition rate (76%), followed by *T. simmonsii* (82%). *Trichoderma paratroviride* and *T. atrobrunneum* had quite low inhibitions (<60%). Hyphal interaction and sporulation of *Trichoderma* spp. in dual cultures were also reported. The antifungal analysis was done to investigate the potential of *Trichoderma* isolates to control the growth of *R. solani*. Among the 25 selected isolates tested, 10 were found effective against the growth of *R. solani* (>10%) with seven of them showing strong inhibition of (>30%).

The mycelial growth from the interaction between *Trichoderma* spp. and *R. solani* on dual culture has also been tested. The diameter of hyphae of *Trichoderma* spp. was 1.5-3.5 µm and the diameter of *R. solani* was 5-7 µm, hence they could be easily distinguished between *Trichoderma harzianum* and *Trichoderma hamatum*. Results also

concluded that the hyphae of *T. hamatum* were very dense than those of *T. asperellum*. In the present study effects of *Trichoderma asperellum* on the growth of *R. solani* was screened (**Plate 4.7**) and it showed antagonistic activity (55.83%) against *R. solani* in the dual culture. While, *T. hamatum* exhibited comparatively lower growth inhibition (49.90%) against *R. solani* (**Table 4.2**).

These observations are in agreement with the findings of Kumar *et al.*, (2017). They reported that *T. hamatum* followed by *T. asperellum* was found significantly superior in reducing the mycelial growth of *R. solani*. The multiple combinations of biological control agents (including *T. harzianum* and *T. hamatum*) and organic supplements that were advised to control soil borne plant pathogens. They asserted that such combinations could significantly reduce the inhibition of disease caused by *R. solani*.

Sharma *et. al.*, (2014) reported cloudless visible inhibitory zone was observed in the zone of junction between *T. asperellum* and *R. solani*. In the experiment, the reaction type was found to belong to the 1 and, 2 scales i.e. *T. asperellum* and *T. hamatum* overgrew at least two-thirds on the PDA media. One report by Kumar *et. al.*, (2011) stated that *Trichoderma* significantly suppressed the growth of *R. solani* as observed in this study. Evidence shows the antagonistic/mycoparasitic behavior of many species of *Trichoderma*. Kale *et al.*, (2016) reported that the culture of *T. viride* suppressed the mycelial growth and sclerotium of *R. solani*.

Percent inhibition was then scaled which showed the reaction type

- 1: *T. asperellum* and *T. hamatum* entirely overgrew the pathogen and occupied the entire medium surface.
- 2: *T. asperellum* and *T. hamatum* overgrew at least two-thirds of the medium surface.

- 4: The pathogen established small surface area but failed to suppress the *T. asperellum* and *T. hamatum*.

The observation was observed on the radial growth of the *Trichoderma* spp. and test pathogen when the fungus in the control plate reached full of its growth in the plate. The percent growth inhibition of the test pathogen in presence of the antagonist was calculated over control.

Table 4.2: Mycelial growth inhibition of *Rhizoctonia solani* by different concentration of bioagents in potato dextrose agar media

Bioagent treatment	Mycelial growth Average (in %)	Mycelial growth inhibition (in %)
Control	65.50	-
<i>Trichoderma asperellum</i>	28.83	57.60
<i>Trichoderma hamatum</i>	32.73	49.90
C.D. (at 5 %)	2.01	2.74
SE±(m)	2.85	2.93

Notes: *Average of three replications (nine readings) and Mycelial growth inhibition was recorded after 3 days of inoculation.

4.3.3 Efficacy of nanoparticles

Ag nanoparticles

Silver nanoparticles (AgNPs) are known to have anti-microbial properties and therefore have the potency to control fungal plant pathogens including *R. solani*. In this study, we investigated the growth of *R. solani* in the presence of AgNPs. The effect of AgNPs at different ppm concentration (25%, 50%, 75% and 100%) on mycelial growth in *R. solani* was studied. The results showed that at 25, 50, 75 and 100 ppm of AgNPs have suppressed *R. solani* mycelial growth i.e. 38.89, 43.33, 51.74 & 64.86 percent respectively along with the production of sclerotia. In addition, greater than 60% (64.86%) inhibition was observed against *R. solani* when treated with a 100 ppm AgNPs on PDA medium. The lowest level of inhibition was observed against *R. solani* when treated with a 25 ppm concentration i.e. 38.89 percent concentration of AgNPs on PDA (**Table 4.3**). The results symbolize that AgNPs have the potential to manage *R. solani* growth and the development of plant disease symptoms. The presence of AgNPs in growth media limited the growth rate of *R. solani*.

Oktarina *et al.*, (2020) shows that AgNPs at 75 and 100 ppm reduced the mycelial area of *R. solani* significantly when compared to controls (i.e. no AgNPs). A previous study had stated that very low AgNPs concentration (2-6 mg) suppress hyphal growth of *R. solani*. Similarly, the study uses six different strains of *R. solani* that are sensitive to low levels of AgNPs. These findings imply that sensitivity of *R. solani* by silver is likely varied between different fungal strains of the same species but this should be examined further using comparable growth conditions.

Kim *et al.*,(2009) were reported that silver nanoparticles (AgNPs) range from 1nm and 100 nm in size and are widely engaged in research studies due to their antimicrobial properties. Common applications involve the use of AgNPs for antimicrobial coats on particle silica to control bacteria and other microbes they may

adhere to the cell surface, penetrate the cells, change cell membrane characteristics and ultimately result in DNA destruction due to the dissolution of Ag ions. AgNPs also produce reactive oxygen species (ROS), especially hydroxyl radical, and superoxide radical, that result in damage to the cell. The precise anti-microbial mechanism of AgNPs is however not completely understood. The potential of AgNPs to suppress *R. solani* had been studied the effects of silver nanoparticles on sclerotium-forming *R. solani* fungi.

The current study validated that AgNPs have the potency to suppress the mycelial growth of *R. solani*. A study had described that low AgNP concentration (25-100 µl/L) inhibited mycelial growth of *R. solani* due to causing an irregular shape of the hyphal walls which then failed to multiply. These findings insinuate that the sensitivity of silver is likely to inhibit the growth of *R. solani*.

CuO nanoparticles

Copper oxide nanoparticles (CuO NPs) were evaluated *in vitro* against *R. solani* at four different concentrations i.e. 25, 50, 75, and 100 ppm concentration. Results in **Table 4.3** indicate that all evaluated concentrations of nanoparticles suppressed the mycelial growth of *R. solani* in comparison to control. However, copper oxide nanoparticles displayed the highest inhibition mycelial growth of *R. solani* (46.95%) at concentration 100 ppm whereas, lowest inhibition of mycelial growth (21.83%) at 25 ppm concentration. Furthermore, CuONPs exhibited a clear percent reduction in fungal mycelial growth range 21.83-46.95 at all concentration. The acquired results coincided with those examined by Kim *et al.*, (2011) who asserted that the mycelial growth rate of *R. solani* was reduced typically by more than 90% at a 60 ppm the concentration of Nano sized copper and silica hybrid silver complex (NSS). Similar results were also studied by Moreover, Kanhed *et al.*, (2014) investigated CuNPs extraordinary inhibitory action against *A. alternata*,

F. oxysporum, and *C. lunata*. The increased antifungal activity of CuNPs was due to their larger surface area to volume ratio.

Data in **Table 4.3** indicate that all examined nanoparticle at all concentrations subdued infection percent of black scurf disease under *in-vitro* with control. Also, all tested nanoparticles at a concentration of 100 ppm had reduced black scurf disease significantly. Hence treatment with CuONPs nanoparticles at 25, 50, 75, 100 ppm concentration revealed a great reduction of mycelial growth with efficacy of 21.83, 34.50, 39.84 and 46.95 percent, respectively under *in-vitro* conditions.

MgO nanoparticles

This investigation was carried out to test the efficiency of magnesium oxide nanoparticles (MgONPs) to control the plant pathological fungus *R. solani* in the culture media i.e. PDA. The result of table 4.3 showed an effect of MgONPs in inhibition of the *R. solani* fungus at 25, 50, 75, and 100 ppm compared with the control sample, and the inhibition rates were 48.15, 56.09, 57.81, and 61.82 percent, respectively where the MgO nanoparticles affected greatly and reached to 61.82 percent in the *R. solani* mycelial inhibition at 100 ppm. These results coincide with the results of multiple studies which referred to the high activity of nanoparticles and their oxides in inhibition many of the plant pathogenic fungi (Clement *et al.*, 1994; Gonzales- Melendi *et al.*, 2008; Rico *et al.*, 2011; Mahendra *et al.*, 2012). Kim *et al.*, (2012) also reported high effectiveness of AgONPs and MgONP at 100 ppm against plantpathogenic fungi on PDA media.

Botanicals, bio-agents, and fungicides have been used much for the control of *R. solani* under lab conditions. In the present study, botanicals, bio-agents and nanoparticles (AgNPs, CuO NPs, MgO NPs) were used against the black scurf of potatoes *in vitro*. All the botanicals, bio agents, and nanoparticles tested were found effective in controlling *R. Solani* causing black scurf disease. Among botanicals, *C.*

procera (72.66%) extract was most effective followed by *M. pinnata* (68.72%) while in the case of bioagents, *Trichoderma asperellum* (55.83%) followed by *T. hamatum* (49.90%) was observed highly efficacious against *Rhizoctonia solani*. These observations are similar with those listed by Mallesh *et al.*, (2009). They have suggested the effectiveness of many botanicals, bio-agents, and fungicides in managing *R. solani* in the field as well as in the laboratory.

Silver leads to influence a broad range of biological processes in different microorganisms. Silver nanoparticles (AgNPs) can be used for slow oxidative dissolution of the cell membranes of microbes, by production of silver ions and eventually results in death of the cells. A broad spectrum of silver activity assures its use against a number of diverse pathogens including *R. solani*. Silver leads to influence a broad range of biological processes in microorganisms.

Besides, the application of copper and magnesium as micronutrients can increase the resistance against black scurf (*R. solani*). An integrated strategy of fungicides, organic amendment, crop rotation, and cultivation methods were also used. It was interesting in the development of plant protection by nanotechnology method such as application of different nanoparticles at 100 ppm have been reported to show approximately 70% growth inhibition of *R. solani*. Other nanoparticles that have been applied to crop protection are nano- forms of copper (Cu), iron (Fe), silica (Si), silver (Ag), and carbon (C).

Table 4.3: Mycelial growth inhibition of *Rhizoctonia solani* by different concentration of nanoparticles in potato dextrose agar media

Nanoparticles	Concentration (in PPM)	Control Average (in mm)	Mycelial growth average (in mm)	Average of Mycelial growth inhibition (in %)
Silver nanoparticles (Ag NPs)	25	67.40	41.17	38.89
	50	58.44	33.13	43.33
	75	60.80	29.30	51.74
	100	67.53	23.73	64.86
Magnesium oxide (MgO NPs)	25	47.67	24.50	48.15
	50	47.50	20.83	56.09
	75	46.30	19.50	57.81
	100	48.50	18.50	61.82
Copper oxide nanoparticles (CuO NPs)	25	35.40	27.50	21.83
	50	40.50	26.50	34.50
	75	43.20	25.80	39.84
	100	59.40	31.50	46.95
C.D. (at 5 %)		3.25	4.76	13.08
SE±(m)		1.11	1.62	4.46

Notes: *Average of three replications and Mycelial growth inhibition was recorded after 3 days of inoculation.

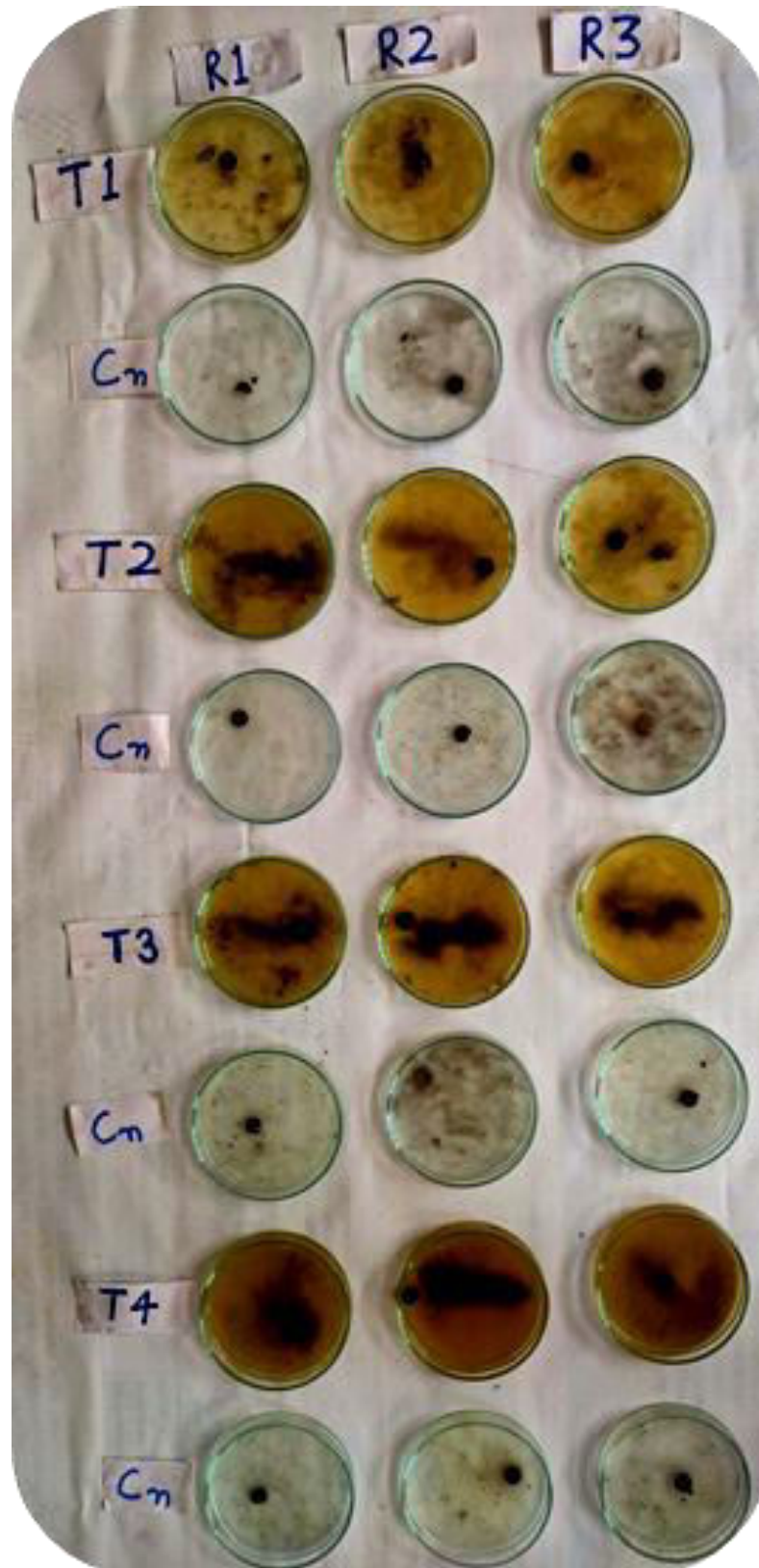


Plate 4.1: Efficacy of *Azadirachta indica* (Neem) against *R. solani*

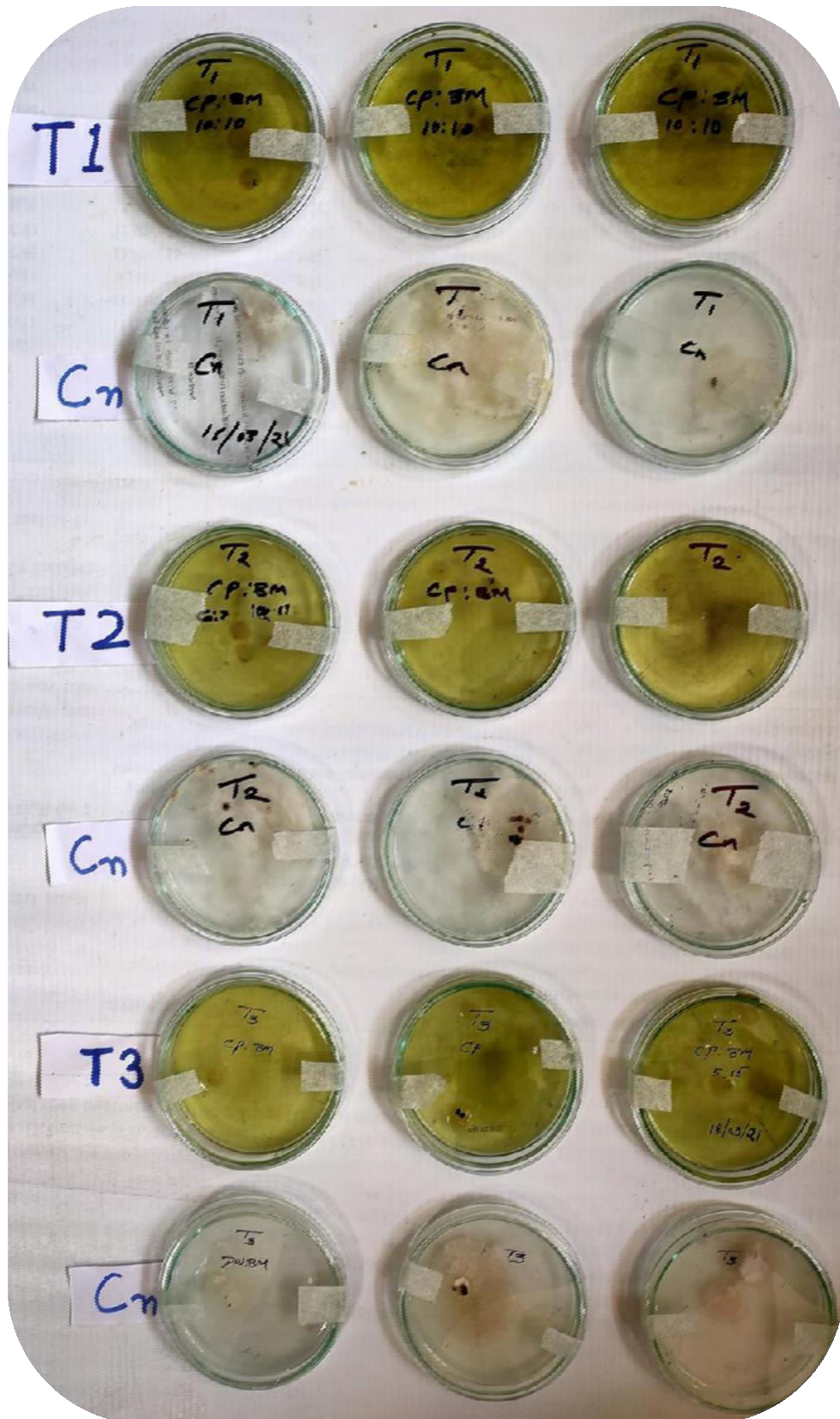


Plate 4.2: Efficacy of *Calatropis procera* (Madar) against *R. solani*

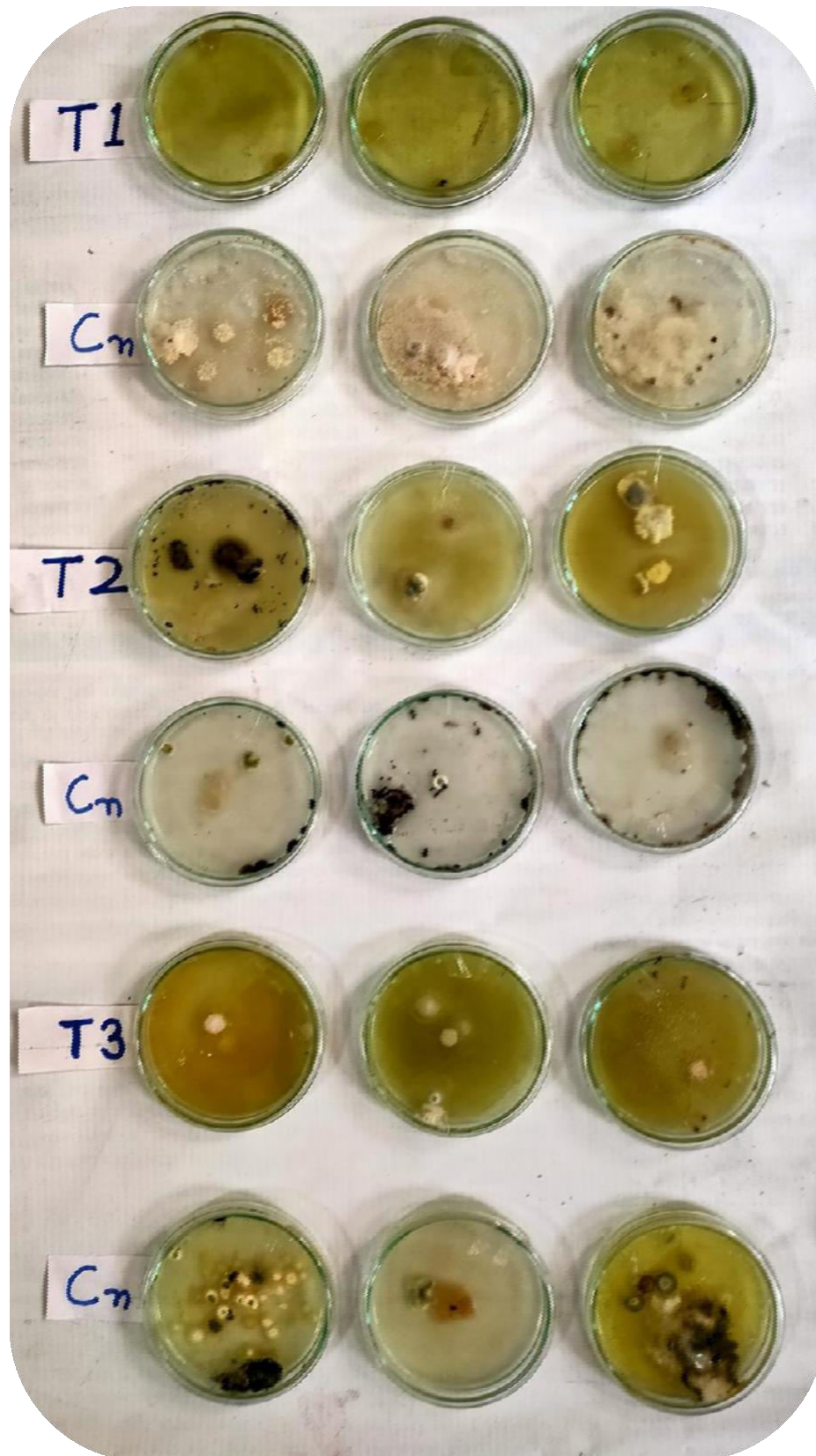


Plate 4.3: Efficacy of *Mellettia pinnata* (Karanj) against *R. solani*

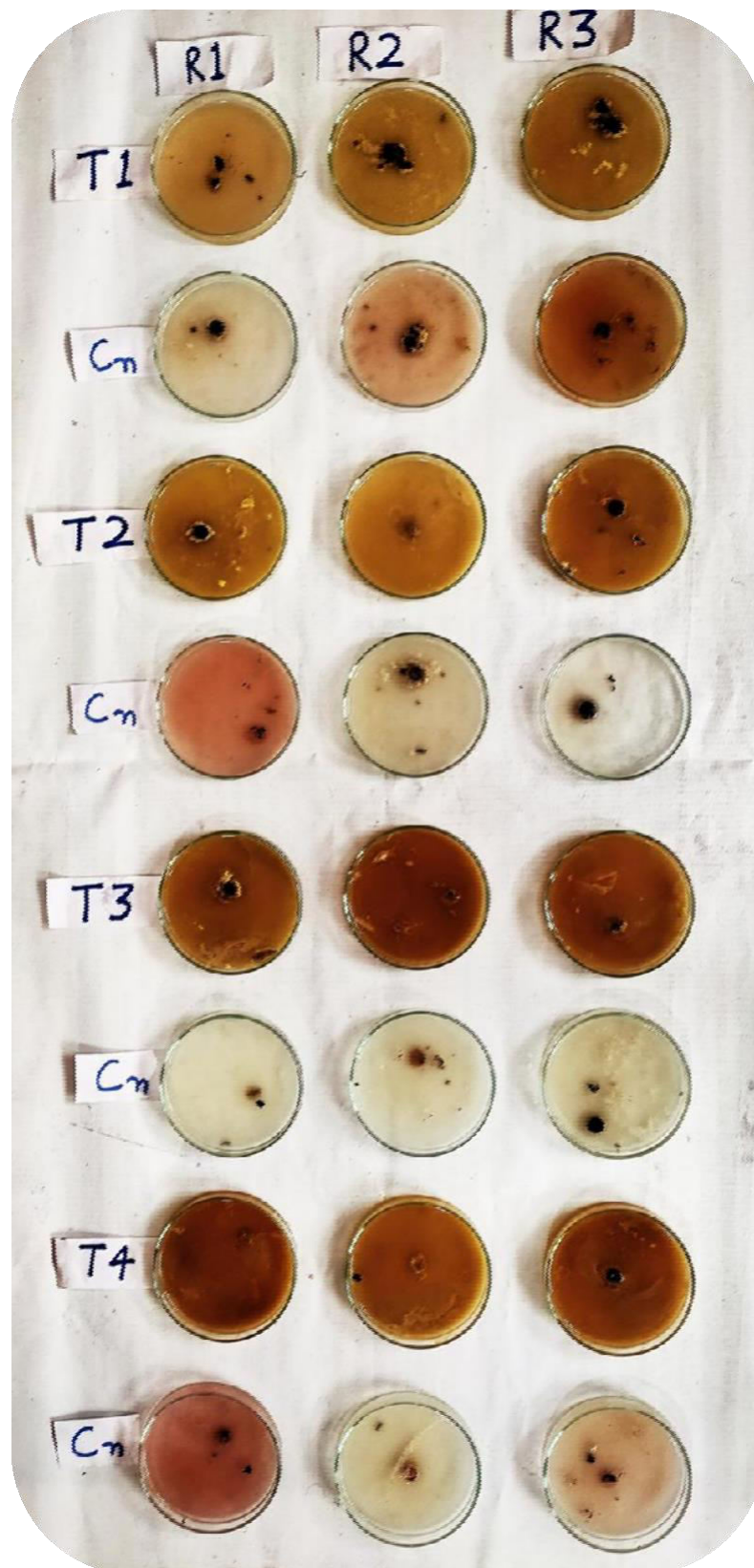


Plate 4.4: Efficacy of *Nerium oleander* (Kaner) against *R. solani*

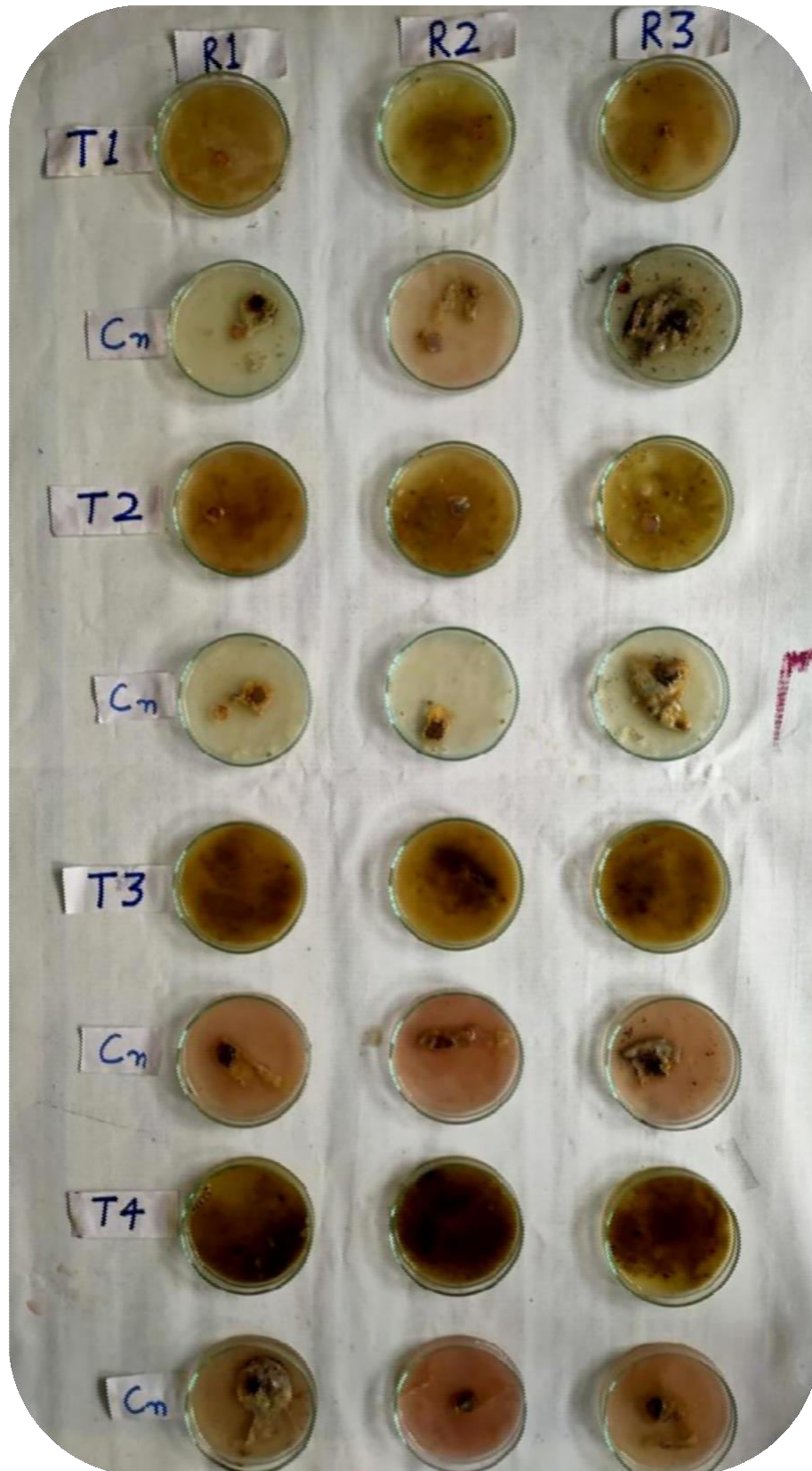


Plate 4.5: Efficacy of *Ocimum sanctum* (Tulsi) against *R. solani*

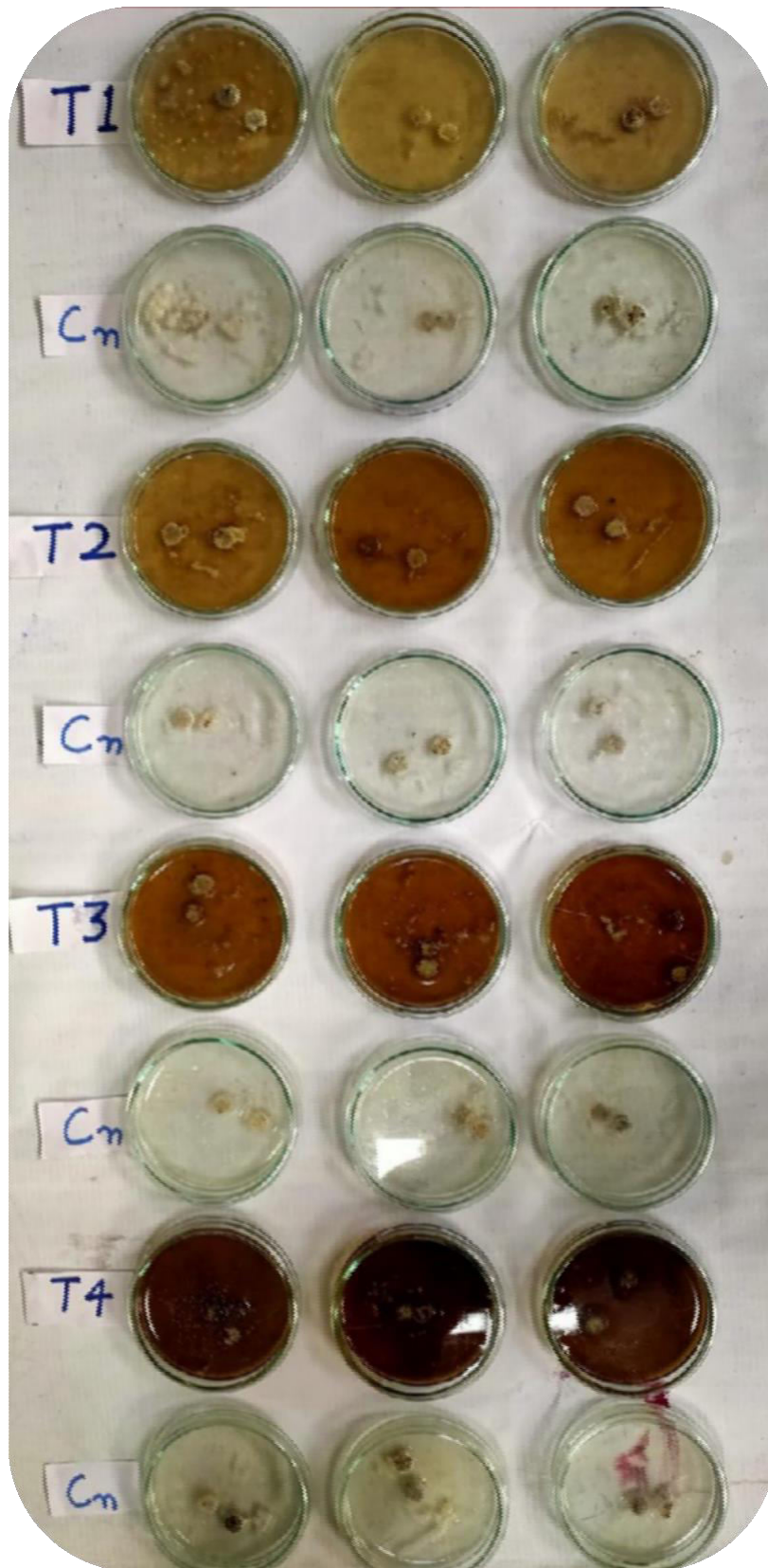


Plate 4.6: Efficacy of *Parthenium hysterophorus* (C. grass) against *R. solani*

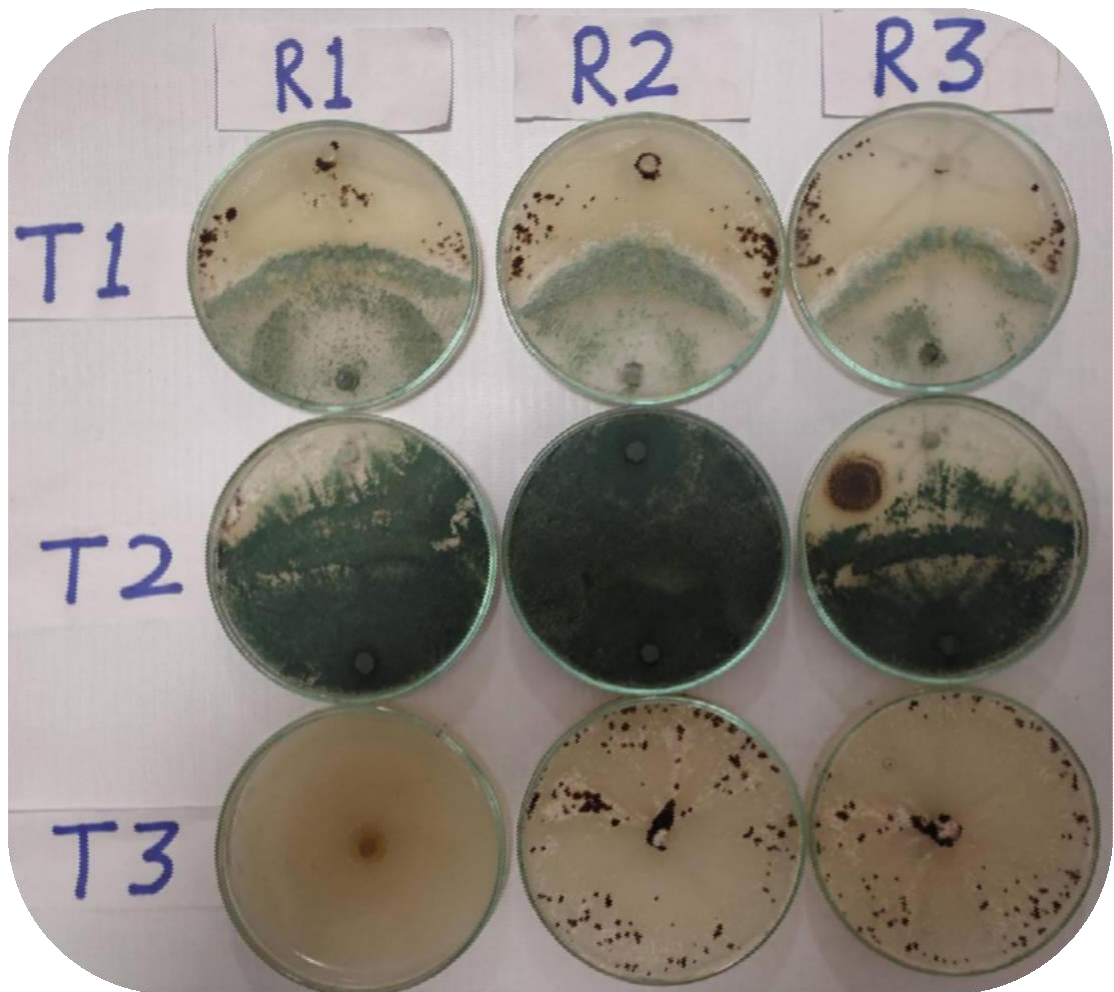


Plate 4.7: Efficacy of *T. asperellum* and *T. hamatum* against *R. solani*

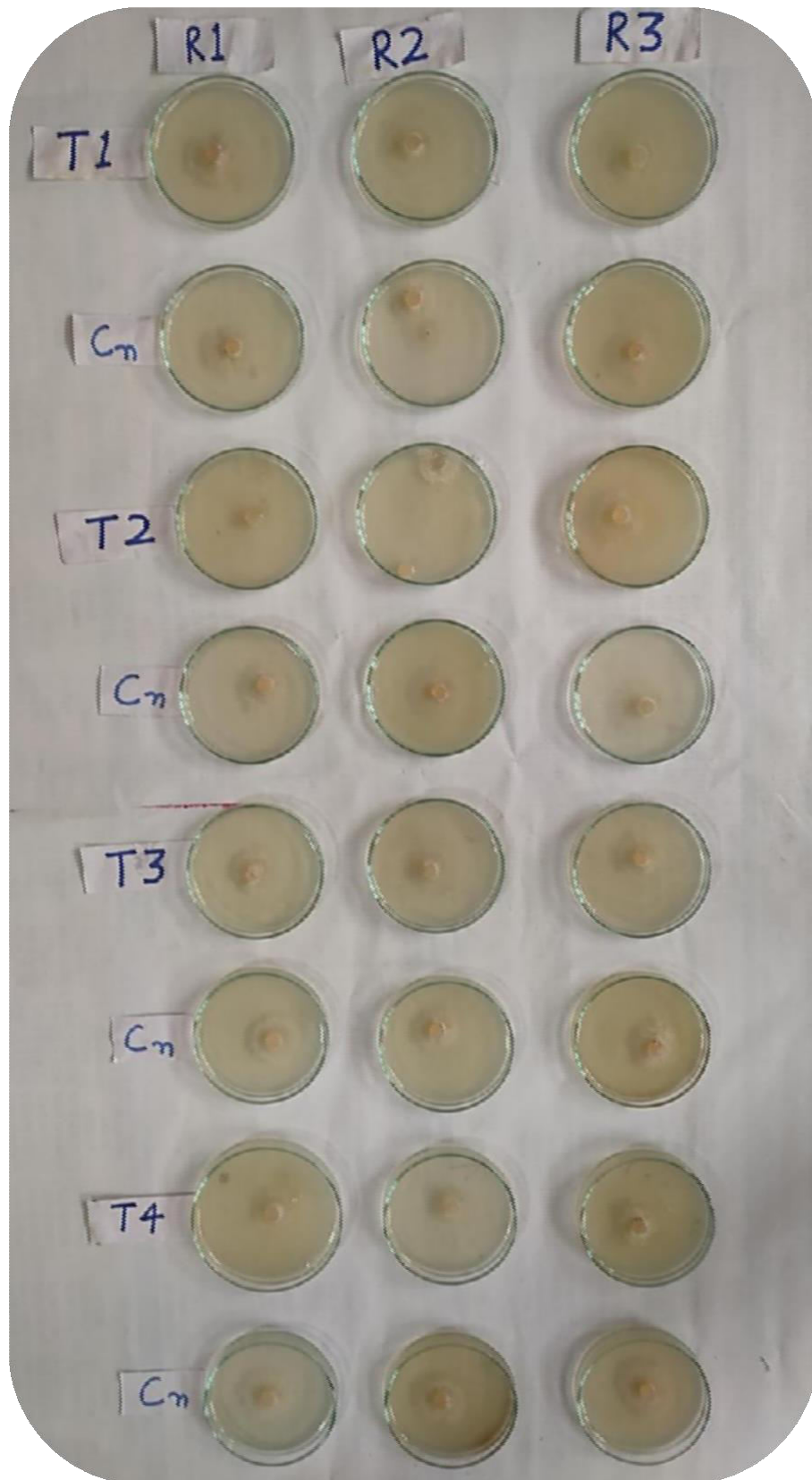


Plate 4.8: Efficacy of silver nanoparticles (Ag NPs) against *R. solani*

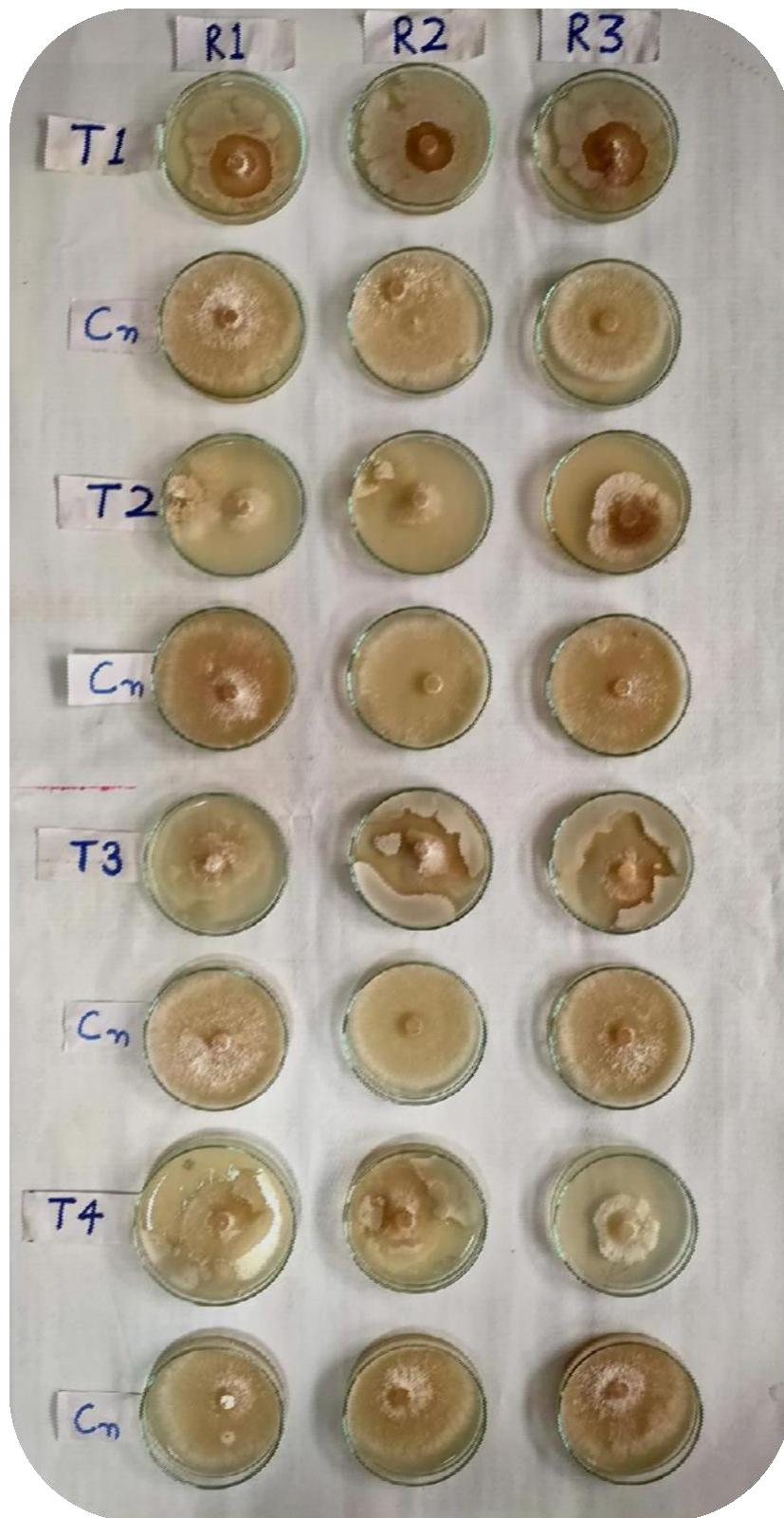


Plate 4.9: Efficacy of magnesium oxide nanoparticles (MgO NPs) against *R. solani*

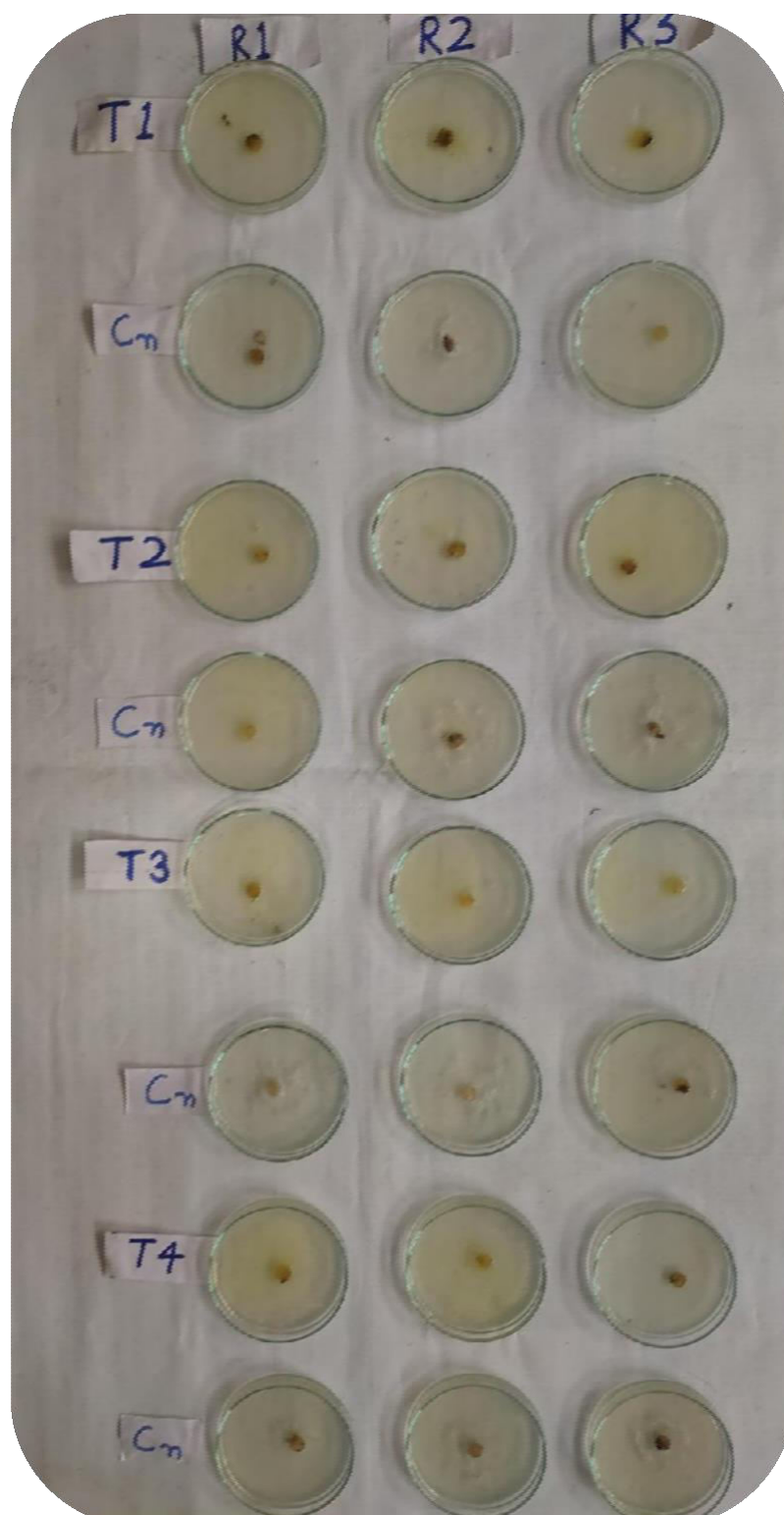


Plate 4.10: Efficacy of copper oxide nanoparticles (CuO NPs) against *R. solani*



SUMMARY AND CONCLUSION

In this dissertation work, keeping the objectives in mind we have studied about isolation and identification of *R. solani* and efficacy of biopesticides and nanoparticles against *R. solani* under *in vitro* conditions. The salient findings of the present investigation include effective and ecofriendly management of black scurf disease of potato caused by *R. solani* are summarized below:

The potato (*Solanum tuberosum*) is an important vegetable crop cultivated in India as well as throughout the world. This crop is attacked by different bacterial, fungal, and viral diseases resulting in total yield as well as a reduction in quality and quantity of the produce. Among the numerous fungal diseases of potatoes, soil-borne black scurf disease had resulted to cause substantial losses every year because it is produced under a wide host range and climatic conditions. In recent years, black scurf disease caused by *Rhizoctonia solani*, has appeared as a serious problem in most of the potato growing areas of India with up to 60-70 percent disease incidence yield losses up to 50 percent. The salient findings of the present investigation on Eco-friendly and effective management of *Rhizoctonia solani* causing black scurf of potato are summarized below:

Among different botanicals used (Table 3.3) Neem (*A. indica*), Madar (*C. procera*), Karanj (*M. pinnata*), Kaner (*N. oleander.*), Tulsi (*O. sanctum*), and Congress grass (*P. hysterophorus*) all the botanicals were found to be effective against *R. solani*. A maximum inhibition percentage was recorded in Madar (*C. procera*) i.e. (72.66%) mycelial growth, followed by Karanj (*M. pinnata*) i.e. (68.72%) and the minimum inhibition percentage were recorded in Neem (*A. indica*) i.e (54.28%) followed by Congress grass (*P. hysterophorus*) i.e. (52.79%).

In PDA media, inhibitory properties of biocontrol agents i.e. (*T. asperellum* and *T. hamatum*) was investigated. Among the Two bioagents, *Trichoderma asperellum* revealed better results which inhibited 57.60% mycelial growth, followed by *T. viride* (49.90%).

Further the efficacy of promising bio fungicides, and nanoparticles was evaluated under lab conditions for the management of black scurf disease of potato. Among all the treatments, minimum mycelial growth inhibition was found with copper oxide nanoparticles (CuONPs) i.e. 46.95% whereas, highest mycelial growth inhibition was found in *C. procera* i.e. 72.66% than the rest of the treatments. Among different botanicals *P. hysterophorus* showed lowest inhibition (52.79%) over the control and the highest inhibition was showed by *C. procera* (72.66%) at 15% botanical concentration. In PDA, *R. solani* treated with two bioagents i.e. *T. asperellum* and *T. hamatum* mycelial inhibition was 57.60% and 49.90% , respectively. *R. solani* treated with different nanoparticles silver nanoparticles (AgNPs) showed highest inhibition of *R. solani* i.e. 64.86% and lowest inhibition was found in copper oxide nanoparticles (46.95%). All the data recorded were collected after 3 days of inoculation and statistically defined by their C.D. values at 5% concentration.

All the bio fungicides and nanoparticles incorporated in the present study were found to be effective in inhibiting the mycelial growth of the *R. solani* under *in vitro* conditions. It can be summarized from the present study that botanicals, bio control agents and nanoparticles are most effective *in vitro* and can be incorporated as a management practice against black scurf disease of potato.

CONCLUSION

It can be concluded from the present study that it is very important to ensure the quality and health of the planting tubers for sowing and further production. The morphological identification and characterization of the *R. solani* is very crucial in disease management. Biopesticides and nanoparticles showed most effective inhibitory action against *R. solani* under *in vitro* conditions. They can be incorporated as the management strategies against this soil-born pathogen in potato. In the present study the application of botanicals are found to be the most effective against *R. solani* mycelial growth at different concentrations. *Trichoderma* spp. (*T. asperellum* and *T. hamatum*) are also found effective against *R. solani* growth on the potato broth media at different concentrations. Also, nanoparticles (AgNPs, CuONPs and MgONPs) are found antagonistic against the mycelial growth of *R. solani* on PDA medium. All these biofungicides and nanoparticles can be useful in understanding the biochemical, and physical resistance against the *R. solani* and these all strategies are an approach for innovative strategies for the management of potato black scurf of potato disease.



REFERENCES

- Abbas, Aqleem & D, Jiang & Y, Fu. (2017). *Trichoderma* Spp. as Antagonist of *Rhizoctonia solani*. *Journal of Plant Pathology & Microbiology*, **08**, 10.4172/2157-7471.1000402.
- Abushaala, F. & Ramadan, A. & Fahej, Mohamed. (2017). In vitro Antifungal Activity of Some Plant Extracts against Seed-borne Pathogens. *IOSR Journal of Agriculture and Veterinary Science*. **10**. 49-57. 10.9790/2380-1004014957.
- Ajayi-Oyetunde, O., & Bradley, C. (2017). Identification and Characterization of *Rhizoctonia* Species Associated with Soybean Seedling Disease. *Plant Disease*, **101**(4), 520-533. doi: 10.1094/pdis-06-16-0810-re
- Aleksandrowicz-Trzcńska, Marta & Szaniawski, Adam & Olchowik, Jacek & Drozdowski, Stanisław (2018). Effects of copper and silver nanoparticles on growth of selected species of pathogenic and wood-decay fungi in vitro. *The Forestry Chronicle*, **94**, 109-116.
- Alfadhl, Fadhl & Al-Abedy, Aqeel & Alkhafije, Duaa. (2019). Isolation and molecular identification of *Rhizoctonia solani* and *Fusarium solani* isolated from cucumber (*Cucumis sativus* L.) and their control feasibility by *Pseudomonas fluorescens* and *Bacillus subtilis*. *Egyptian Journal of Biological Pest Control*, **29**. 10.1186/s41938-019-0145-5.
- Al-Snafi, Ali. (2020). Bioactive ingredients and pharmacological effects of *Nerium oleander*. *IOSR Journal of Pharmacy (IOSRPHR)*, **10**, 19-32.
- Amber, Rahila & Adnan, Muhammad & Tariq, Akash & Niaz, Khan & Mussarat, Sakina & Hashem, Abeer & Al-Huail, Asma & Al-Arjani, Al-Bandari. (2017). Antibacterial activity of selected medicinal plants of northwest Pakistan traditionally used against Mastitis in livestock. *Saudi Journal of Biological Sciences*, **25**, 10.1016/j.sjbs.2017.02.008.
- Amrate, Pawan & Sharma, J.R. & Singh, C. (2013). In vitro evaluation of fungicides, plant extracts and oils against *Alternaria alternata* (Fr.) keissler causing leaf spot of *Aloe barbadensis* (Miller). *Asian Journal of Microbiology, Biotechnology and Environmental Sciences*, **15**, 609-613.
- Arora, Ravinder (2013). Comparative efficacy of boric acid and pencycuron for management of black scurf of potato. *Potato Journal*, **40**.
- Asif, Mohammad (2012). Antimicrobial Potential Of *Azadirachta Indica* Against Pathogenic Bacteria and Fungi. *Journal of Pharmacognosy and Phytochemistry*, **1**, 78-83.

- Baka, Z., & Rashad, Y. (2016). Alternative control of early blight of tomato using plant extracts from *Acacia nilotica*, *Achillea fragrantissima* and *Calotropis procera*. *Phytopathologia Mediterranea*, **55**(1), 121-129. https://doi.org/10.14601/Phytopathol_Mediterr-17161
- Bakali, Abdessamad & Martín, María. (2006). Black scurf of potato. *Mycologist*, **20**, 130-132, 10.1016/j.mycol.2006.03.006.
- Balali, G. R., Neate, S. M., Scott, E. S., Whisson, D. L. & Wicks, T. J. (1995). Anastomosis group and pathogenicity of isolates of *Rhizoctonia solani* from potato crops in South Australia, **44**(6), 1050–1057.
- Banville, G.J., Carling, D. E., & Otrysko, B. E. (1996). *Rhizoctonia* Disease on Potato BT - *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control (B. Sneh, S. Jabaji-Hare, S. Neate, & G. Dijst (eds.)). pp. 321–330). Springer Netherlands.
- Bertagnolli, B. & Daly, S. & Sinclair, J. (2008). Antimycotic Compounds from the Plant Pathogen *Rhizoctonia solani* and its Antagonist *Trichoderma harzianum*. *Journal of Phytopathology*, **146**, 131-135.
- Bhavani, D & Sundari, Nalina & Patel, Chirag (2019). Comparative studies on efficacy of azadirachtin, a plant derived pesticide and cypermethrin, a synthetic pesticide on the control of *Pericallia ricini* [Fabricius 1775]. *Ssrn Electronic Journal*, **6**, 2069-2074.
- Bukhari, S. I., Hamed, M. M., Al-Agamy, M. H., Gazwi, H. S., Radwan, H. H., & Youssif, A. M. (2021). Biosynthesis of copper oxide nanoparticles using *Streptomyces* MHM38 and its biological applications. *Journal of Nanomaterials*, Article ID, 6693302.
- Cai, L., Chen, J., Liu, Z., Wang, H., Yang, H., & Ding, W. (2018). Magnesium Oxide Nanoparticles: Effective Agricultural Antibacterial Agent Against *Ralstonia solanacearum*. In *Frontiers in Microbiology* (Vol. **9**, p. 790).
- Chinnaswami, K., Mishra, D., Miriyala, A., Vellaichamy, P., Kurubar, B., Gompa, J., .. & Raman, M. S. (2021). Native isolates of *Trichoderma* as bio-suppressants against sheath blight and stem rot pathogens of rice. *Egyptian Journal of Biological Pest Control*, **31**(1), 1-10.
- Dahal, K., Li, X.-Q., Tai, H., Creelman, A., & Bizimungu, B. (2019). Improving Potato Stress Tolerance and Tuber Yield Under a Climate Change Scenario – A Current Overview. In *Frontiers in Plant Science* (Vol. **10**, p. 563).
- Das, S., Mitra, B., Luthra, S.K., Saha, A., Hassan, M.M. and Hossain, A. (2021) Study on Morphological, Physiological Characteristics and Yields of Twenty-One

-
- Potato (*Solanum tuberosum* L.) Cultivars Grown in Eastern Sub-Himalayan Plains of India. *Agronomy*, **11**, 335.
- Desvani, S., Lestari, I., Wibowo, H., Supyani, Poromarto, S., & Hadiwiyono. (2018). Morphological characteristics and virulence of *Rhizoctonia solani* isolates collected from some rice production areas in some districts of Central Java. doi: 10.1063/1.5054472
- Dhand, V., Soumya, L., Bharadwaj, S., Chakra, S., Bhatt, D., & Sreedhar, B. (2015). Green synthesis of silver nanoparticles using *Coffea arabica* seed extract and its antibacterial activity. *Materials science & engineering. C, Materials for Biological Applications*, **58**, 36–43.
- Durak, Emre. (2016). Biological control of *Rhizoctonia solani* on potato by using indigenous *Trichoderma* spp. , 1726.
- Elad, Yigal & Chet, Ilan & Y.A., Henis. (1981). A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. *Phytoparasitica*, **9**, 59-67.
- Elad, Yigal. (1983). Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii* -- Scanning Electron Microscopy and Fluorescence Microscopy. *Phytopathology*. **73**. 10.1094/Phyto-73-85.
- Errampalli, Deena & Johnston, H.. (2010). Control of tuber-borne black scurf [*Rhizoctonia solani*] and common scab [*Streptomyces scabies*] of potatoes with a combination of sodium hypochlorite and thiophanate-methyl preplanting seed tuber treatment. *Canadian Journal of Plant Pathology*. March 2001. 68-77. 10.1080/07060660109506911. Esteban-Tejeda, L., Malpartida, F., Esteban-Cubillo, A., Pecharromán, C., & Moya, J. S. (2009). Antibacterial and antifungal activity of a soda-lime glass containing copper nanoparticles. *Nanotechnology*, **20**(50), 505701.
- Guilger-Casagrande, M., Germano-Costa, T., Pasquoto-Stigliani, T. et al. Biosynthesis of silver nanoparticles employing *Trichoderma harzianum* with enzymatic stimulation for the control of *Sclerotinia sclerotiorum*. *Science Reports*, **9**, 14351 (2019).
- Guo, F., Bunn, S. E., Brett, M. T., Fry, B., Hager, H., Ouyang, X., & Kainz, M. J. (2018). Feeding strategies for the acquisition of high-quality food sources in stream macroinvertebrates: Collecting, integrating, and mixed feeding. *Limnology and Oceanography*, **63**(5), 1964–1978.
- Gwa, V.I., A.O., N., and Ekefan, E.J. (2018). Antifungal Effect of Five Aqueous Plant Extracts on Mycelial Growth of *Penicillium expansum* Isolated from Rotted Yam Tubers in Storage. *Acta Scientific Agriculture*, **2**(6), 65–70.

- Hadizadeh, I., Peivastegan, B., & Kolahi, M. (2009). Antifungal activity of nettle (*Urtica dioica* L.), colocynth (*Citrullus colocynthis* L. Schrad), oleander (*Nerium oleander* L.) and konar (*Ziziphus spina-christi* L.) extracts on plants pathogenic fungi. *Pakistan Journal of Biological Sciences : PJBS*, **12**(1), 58–63.
- Hassanein, Hamdy & Okail, Hanan. (2008). Toxicity determination and hypoglycaemic effect of neem biopesticide on the grass carp “*Ctenopharyngodon idella*. *Egyptian Academic Journal of Biological Sciences. A, Entomology*, **1**, 37-49.
- Heiligtag, F. J., & Niederberger, M. (2013). The fascinating world of nanoparticle research. *Materials Today*, **16**(7), 262–271.
- Hide, G. & Hirst, J. & Stedman, O. (2008). Effects of black scurf (*Rhizoctonia solani*) potatoes. *Annals of Applied Biology*, **74**, 139 - 148.
- Hormozi-Nezhad, M.Reza & Abbasi-Moayed, Samira (2014). A sensitive and selective colorimetric method for detection of copper ions based on anti-aggregation of unmodified gold nanoparticles. *Talanta*, **129**, 227–232.
- Huang, H. & Hoes, J. (2011). Penetration and infection of *Sclerotinia sclerotiorum* by *Coniothyrium minitans*. *Canadian Journal of Botany*, **54**, 406-410. 10.1139/b76-039.
- Jager, Sara. (2014). Correlation between Plant Secondary Metabolites and Their Antifungal Mechanisms—A Review. *Medicinal & Aromatic Plants*, **03**, 10.4172/2167-0412.1000154.
- Jeyaraman, Jeyaraman & Jeyasubramanian, Kadarkaraithangam & Marikani, Arumugam & Rajakumar, Govindasamy & Rahuman, Abdul (2012). Synthesis and antimicrobial activity of copper nanoparticles. *Materials Letters*, **71**, 10.1016/j.matlet.2011.12.055.
- Jortani, S. A., Helm, R. A., & Valdes, R. (1996). Inhibition of Na,K-ATPase by oleandrin and oleandrogenin, and their detection by digoxin immunoassays. *Clinical Chemistry*, **42**(10), 1654–1658.
- K., R., Barnett, H., & Hunter, B. (1972). Illustrated Genera of Imperfect Fungi. *Mycologia*, **64**(4), 930. doi: 10.2307/3757954
- Kale, Sonam Kale & Pradeeprao, Kadu & Dnyanobarao, Gadambe & Agrawal, Toshiy & Kotasthane, Anil. (2016). Efficiency of Different Trichoderma Isolates on Plant Growth Promoting Activity in Rice (*Oryza sativa* L.). *International Journal of Bio-resource and Stress Management*, **7**, 489-500.

- Kanhed, Prachi & Birla, Sonal & Gaikwad, Swapnil & Gade, Aniket & Seabra, Amedea & Rubilar, Olga & Duran, Nelson & Rai, Mahendra. (2014). In vitro antifungal efficacy of copper nanoparticles against selected crop pathogenic fungi. *Materials Letters*, **115**, 13-17. 10.1016/j.matlet.2013.10.011.
- Kareem, Tareq & Hassan, M. (2015). Comparison of *Rhizoctonia solani* Isolated from Soil in Baghdad – Iraq Genetically with World Isolates. *Donnish Journal of Agricultural Research*, **2**, 29-35.
- Karlsson, H. L., Cronholm, P., Gustafsson, J., & Möller, L. (2008). Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes. *Chemical Research in Toxicology*, **21**(9), 1726–1732.
- Kaur, R., Kalia, A., Lore, J. S., Kaur, A., Yadav, I., Sharma, P., & Sandhu, J. S. (2021). Trichoderma sp. endochitinase and β -1, 3-glucanase impede *Rhizoctonia solani* growth independently, and their combined use does not enhance impediment. *Plant Pathology*, **70**(6): 1388-1396.
- Khalid, Shah & Shumail, Hoor & Haq, Syed Inziam Ul. (2021). Antimicrobial activity of Parthenium hysterophorus against five bacterial strains. **10**, 1404-1410. 10.19045/bspab.2021.100146.
- Khare, Prateek & Sharma IITK, Ashutosh & Verma, Nishith. (2014). Synthesis of phenolic precursor-based porous carbon beads in situ dispersed with copper–silver bimetal nanoparticles for antibacterial applications. *Journal of Colloid and Interface Science*, **418**, 216–224.
- Kim, S., Choi, J. E., Choi, J., Chung, K. H., Park, K., Yi, J., & Ryu, D. Y. (2009). Oxidative stress-dependent toxicity of silver nanoparticles in human hepatoma cells. *Toxicology in vitro : An International Journal Published in Association with BIBRA*, **23**(6), 1076–1084.
- Kreuze, J. F., Souza-Dias, J. A. C., Jeevalatha, A., Figueira, A. R., Valkonen, J. P. T., & Jones, R. A. C. (2020). Viral Diseases in Potato. In H. Campos, & O. Ortiz (Eds.), *The Potato Crop: Its Agricultural, Nutritional and Social Contribution to Humankind* (pp. 389-430). [11] Springer. https://doi.org/10.1007/978-3-030-28683-5_11
- Kumar, Gagan & Maharshi, Anupam & Patel, Jai & Mukherjee, Arpan & Singh, Harikesh & Sarma, Birinchi. (2017). Trichoderma : A Potential Fungal Antagonist to Control Plant Diseases. SATSA Mukhapatra - Annual Technical Issue. 206-218.
- Kumar, Gaurav & Loganathan, Karthik & Rao, Bhaskar. (2011). A review on pharmacological and phytochemical profile of *Calotropis gigantea* Linn. *Pharmacologyonline*, **1**, 1-8.

- Kumar, M., Singh, J. K., Kumar, S., & Kumar, A. (2017). A Comprehensive Overview on Black Scurf of Potato. *International Journal of Current Microbiology and Applied Sciences*, **6**(10), 4981–4994.
- Kumar, P., Kumar, P., Sharma, D., Verma, S. K., Halterman, D., & Kumar, A. (2021). Genome-wide identification and expression profiling of basic leucine zipper transcription factors following abiotic stresses in potato (*Solanum tuberosum* L.). *PLOS ONE*, **16**(3), e0247864.
- Kumar, Subhash & Kumar, Devendra. (2020). Abiotic stresses in potato crop.
- Larkin, R. P., Honeycutt, C. W., Griffin, T. S., Olanya, O. M., He, Z., & Halloran, J. M. (2017). Cumulative and residual effects of different potato cropping system management strategies on soilborne diseases and soil microbial communities over time. *Plant Pathology*, **66**(3), 437–449.
- Liton, Md & Bhuiyan, Khurshed & Jannat, Rayhanur & Ahmed, J. & Rahman, Md & Rubayet, Md. Tanbir. (2019). Efficacy of Trichoderma-fortified compost in controlling soil-borne diseases of bush bean (*Phaseolus vulgaris* L.) and sustainable crop production. *7*, 123-136.
- Majeed, Abdul & Chaudhry, Zubeda & Muhammad, Zahir. (2014). Variation in the Aggressiveness of Phytophthora infestans Pathotypes Collected from Different Potato Fields of Khyber Pakhtunkhwa (Pakistan). *International Journal of Agriculture and Biology*, **16**, 1560-8530.
- Malles, S.B. & Narendrappa, T. & Ramanujame, B. (2009). Evaluation of microbial antagonists and organic amendments on root rot of sage (*Salvia officinalis*) by *Fusarium solani* and *Rhizoctonia solani*. *Journal of Agricultural Sciences*, **21**, 301-302.
- Matsumoto, M. (2003). A qualitative baiting technique for selective isolation and DNA diagnosis of *Rhizoctonia* spp., causal agents of rice sheath diseases, from soil. *Journal- Faculty of Agriculture Kyushu University*, **48**, 13-20.
- Mousavi, Leila & Mohd Salleh, Rabeta & Murugaiyah, Vikneswaran. (2018). Phytochemical and bioactive compounds identification of *Ocimum tenuiflorum* leaves of methanol extract and its fraction with an anti-diabetic potential. *International Journal of Food Properties*, **21**, 2390-2399. 10.1080/10942912.2018.1508161.
- Mushir, Ansari & Jahan, Nasreen & MAKBUL, SHAIKH. (2016). A review on Phytochemical and biological properties of *Calotropis gigantea* (Linn) R.Br. *Discovery Phytomedicine*, **3**, 15-21.

- Nagini, S., Nivetha, R., Palrasu, M., & Mishra, R. (2021). Nimbolide, a Neem Limonoid, Is a Promising Candidate for the Anticancer Drug Arsenal. *Journal of Medicinal Chemistry*, **64**(7), 3560–3577.
- Nene YL, Thapliyal RN (1993). Evaluation of fungicides for plant disease control. 3rd edition , IBH Pub. Co. New Dehli. p. 33.
- Ogoshi, A (1987). Ecology and Pathogenicity of Anastomosis and Intraspecific Groups of *Rhizoctonia solani* Kuhn. *Annual Review of Phytopathology*, **25**(1), 125–143.
- Oktarina, Hartati & Singleton, Ian. (2020). Soil Fungal Community Responses to the Silver Nanoparticles Contamination as Assessed by Illumina Next Generation Sequencing (NGS). *Jurnal Rekayasa Kimia & Lingkungan*, **15**, 99-103. 10.23955/rkl.v15i2.16025.
- Oktarina, Hartati & Woodhall, James & Singleton, Ian. (2021). The potential of silver nanoparticles to control *Rhizoctonia solani* (AG3-PT) growth in vitro. *Journal Natural*, **21**, 10.24815/jn.v21i1.17555.
- Pariona, N., Mtz-Enriquez, A. I., Sánchez-Rangel, D., Carrión, G., Paraguay-Delgado, F., & Rosas-Saito, G. (2019). Green-synthesized copper nanoparticles as a potential antifungal against plant pathogens. *RSC Advances*, **9**(33), 18835–18843.
- Rahmatzai, N., Zaitoun, A. A., Madkour, M. H., Ahmady, A., Hazim, Z., & Mousa, M. A. A. (2017). In vitro and in vivo antifungal activity of botanical oils against *Alternaria solani* causing early blight of tomato. *International Journal of Biosciences (IJB)*, **10**(1), 91–99.
- Rajeswara Rao, B.R. & Kv, Syamasundar. (2012). *Millettia pinnata* (L.) Panigrahi: Overview and Biological Properties of Fixed Oil.
- Ramyadevi, J., Jeyasubramanian, K., Marikani, A., Rajakumar, G., Rahuman, A. A., Santhoshkumar, T., Kirthi, A. V., Jayaseelan, C., & Marimuthu, S. (2011). Copper nanoparticles synthesized by polyol process used to control hematophagous parasites. *Parasitology Research*, **109**(5), 1403–1415.
- Rauf, C. & Ashraf, Masri & Ahmad, Iftikhar. (2007). Management of black scurf disease of potato. *Pakistan Journal of Botany*, **39**, 1353-1357.
- Ray, Anjana & P, Kumar. (2009). Influence of different media, pH and temperature on growth and sclerotial production of *Rhizoctonia solani* Kuhn, causing aerial blight of soybean. *Pantnagar Journal of Research*, **7**, 50-53.
- Reddy. (2010). 126-133 To Help Discussion in Plant Derived Feeds.Pdf (p. 9).

- Rodriguez, Roberto. (2011). The Potato and Its Benefits to Human Health.
- Russell, Dennis & Al-Sayah, Mohammad & Munir, M.F. (2011). Volatile compounds produced by *Calotropis procera* (Family: Asclepiadaceae] leaves that aid in the repulsion of grazers. *Journal of Biodiversity and Ecological Sciences*, **1**, 191-196.
- Scarselletti, R., & Faull, J. (1994). In vitro activity of 6-pentyl- α -pyrone, a metabolite of *Trichoderma harzianum*, in the inhibition of *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. lycopersici. *Fungal Biology*, **98**, 1207-1209.
- Sehajpal, A., Arora, S., & Kaur, P. (2009). Evaluation of plant extracts against *Rhizoctonia solani* causing sheath blight of rice. *The Journal of Plant Protection Sciences*, **1**(1), 25–30.
- Sharma, Pratibha & Sharma, M & Manokaran, Raja & Shanmugam, V. (2014). *Status of Trichoderma research in India: A review*, **67**, 1-19.
- Shuping, Daniel & Eloff, Jacobus. (2017). The use of plants to protect plants and food against fungal pathogens: a review. *African Journal of Traditional, Complementary and Alternative Medicines*, **14**, 120-127. doi: 10.21010/ajcam.v14i4.14.
- Siddhartha, Verma, A., Bashyal, B., Gogoi, R., & Kumar, R. (2020). New nano-fungicides for the management of sheath blight disease (*Rhizoctonia solani*) in rice. *International Journal Of Pest Management*, 1-10. doi: 10.1080/09670874.2020.1818870
- Singh, V., Kumar, S., Lal, M., & Hooda, K. S. (2014). Cultural and morphological variability among *Rhizoctonia solani* isolates from trans-gangetic plains of India. *Research on Crops*, **15**(3), 644-650.
- Sivaraj, R., Rahman, P. K., Rajiv, P., Narendhran, S., & Venckatesh, R. (2014). Biosynthesis and characterization of *Acalypha indica* mediated copper oxide nanoparticles and evaluation of its antimicrobial and anticancer activity. *Spectrochimica acta. Part A, Molecular and Biomolecular Spectroscopy*, **129**, 255–258.
- Soltani Nejad, Meysam, Shahidi Bonjar, Gholam Hosein, Khatami, Mehrdad & Amini, Abbas & Aghighi, Sonia. (2016). In vitro and in vivo antifungal properties of silver nanoparticles against *Rhizoctonia solani*, a common agent of rice sheath blight disease. *IET Nanobiotechnology*, **11**, 10.1049/iet-nbt.2015.0121.
- Somani, A. K. (2009). Management of black scurf (*Rhizoctomia solani*) of potato through seed treatment with botanicals. *Potato Journal*, **36**(3–4), 155–159.

- Sundaram, Rajagopal, Ramanathan, Muthiah, Rajesh, R., Satheesh, B. & Saravanan, Dhandayutham. (2012). LC-MS Quantification of rosmarinic acid and ursolic acid in the *Ocimum sanctum* Linn. leaf extract (Holy Basil, Tulsi). *Journal of Liquid Chromatography & Related Technologies - J Liq Chromatogr Relat Techno*, **35**, 634-650.
- Travis, L. & Lacis, Andrew. (2002). Scattering, Absorption, and Emission of Light by Small Particles.
- Trillas, M.I. & Casanova, E. & Cotxarrera, Lurdes & Ordovás, J. & Borrero, Celia & Aviles, Manuel. (2006). Composts from agricultural waste and the *Trichoderma asperellum* strain T-34 suppress *Rhizoctonia solani* in cucumber seedlings. *Biological Control*, **39**, 32-38. 10.1016/j.biocontrol.2006.05.007.
- Vanwinkle, B. A., de Mesy Bentley, K. L., Malecki, J. M., Gunter, K. K., Evans, I. M., Elder, A., Finkelstein, J. N., Oberdörster, G., & Gunter, T. E. (2009). Nanoparticle (NP) uptake by type I alveolar epithelial cells and their oxidant stress response. *Nanotoxicology*, **3**(4), 307–318.
- Varympopi, A., Dimopoulou, A., Theologidis, I., Karamanidou, T., Kaldeli Kerou, A., Vlachou, A., Karfaridis, D., Papafotis, D., Hatzinikolaou, D. G., Tsouknidas, A., & Skandalis, N. (2020). Bactericides Based on Copper Nanoparticles Restrain Growth of Important Plant Pathogens. *Pathogens (Basel, Switzerland)*, **9**(12), 1024.
- Wani, Ab. Hamid & Shah, Mohammad. (2012). A unique and profound effect of MgO and ZnO nanoparticles on some plant pathogenic fungi. *Journal of Applied Pharmaceutical Science*, **2**, 40-44. 10.7324/JAPS.2012.2307.
- Watanabe K. (2015). Potato genetics, genomics, and applications. *Breeding Science*, **65**(1), 53–68.
- Watanabe, Satoshi & Kato, Hideki & Kumakura, Kazuo & Ishibashi, Eiichi & Nagayama, Kozo. (2006). Properties and biological control activities of aerial and submerged spores in *Trichoderma asperellum* SKT-1. *Journal of Pesticide Science*, **31**, 375-379. 10.1584/jpestics.G06-09.
- Willersinn, Christian & Mack, Gabriele & Mouron, Patrik & Keiser, Andreas & Siegrist, Michael. (2015). Quantity and quality of food losses along the Swiss potato supply chain: Stepwise investigation and the influence of quality standards on losses. *Waste Management (New York, N.Y.)*, **46**, 10.1016/j.wasman.2015.08.033.
- Woodhall, James & Lees, Alison & Edwards, Simon & Jenkinson, P. (2008). Infection of potato by *Rhizoctonia solani*: Effect of anastomosis group. *Plant Pathology*, **57**, 897 - 905.

- Yadav, Rajesh & Ghasolia, R.P. & Yadav, Roshan. (2020). Management of *Rhizoctonia solani* of okra (*Abelmoschus esculentus* L. Moench) through plant extracts and fungicides in vitro and field conditions. *International Journal of Chemical Studies*, 8, 2982-2987.
- Yadav, Rajesh & Ghasolia, R.P. & Yadav, Roshan. (2020). Management of *Rhizoctonia solani* of okra (*Abelmoschus esculentus* L. Moench) through plant extracts and fungicides in vitro and field conditions. *International Journal of Chemical Studies*, 8, 2982-2987.

