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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(8): 11-15

www.thepharmajournal.com
Received: 18-05-2022
Accepted: 02-07-2022

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In vitro evaluation of different plant extracts and bio-agents against dry root rot of greengram caused by *Macrophomina phaseolina*

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Abstract
Greengram (*Vigna radiata*) is a plant species of Fabaceae which is also known as mung bean. It is one of the main pulse crops in India. It is the third most important pulse crop in India, grown in nearly 16% of the total pulse area of the country. Dry root rot (DRR) is an economically devastating disease. The Roving survey was conducted and the results revealed that the maximum disease incidence of 29.75 percent was recorded at Namakkal.

Macrophomina phaseolina isolate NKL1 was isolated from the infected greengram roots showing typical symptoms of dry root rot collected from the crop cafeteria, PGP College of Agricultural Sciences, Namakkal. Six different solid media were tested for growth of pathogens, with maximum radial growth of mycelium (topography) on potato dextrose agar (9 cm). Among the two bio-control agents, *Trichoderma viride* and *Bacillus subtilis*, *Trichoderma viride* shows the maximum percent inhibition of mycelial growth (72.96%) and strong antagonistic activity against *Macrophomina phaseolina* under *in vitro* conditions.

Among the various botanicals (Neem leaf extract, Pungam leaf extract, and garlic extract) used against *Macrophomina phaseolina* NKL1 at different concentrations (5%, 10%, and 15%), garlic extract with 10% and 15% concentration shows 100% inhibition of mycelial growth of *Macrophomina phaseolina* isolate NKL1.

Keywords: Greengram, Dry root rot, *Macrophomina phaseolina*, Bio-agents, Botanicals

1. Introduction

1.1 Greengram

Greengram (*Vigna radiata*) is a plant species of Fabaceae which is also known as mung bean. It is one of the main pulse crops in India. It can be cultivated as a Kharif as well as a summer crop. It has a well-developed root system. It is a warm-season crop and frost-tolerant plant. Greengram is suitable for being planted in tropical and sub-tropical regions. It is primarily grown in East Asia, Southeast Asia, and the Indian subcontinent. It is the third most important pulse crop in India, grown in nearly 16% of the total pulse area of the country. It's a rich source of protein (20–25%) along with fibre and iron, vitamin B and easily digestible carbohydrates, with a calorific value of 62.62 g per 100 g of matured seeds.

India contributes 70% of the world's greengram production. It has contributed 10% to the total pulse population. According to the government of India, greengram production in 2020-2021 was 2.64 million tonnes. The important greengram producing states are Rajasthan, Maharashtra, Andhra Pradesh, Madya Pradesh, and Bihar.

1.2 Dry Root Rot of Greengram

The yield of mung beans is affected by several biotic and abiotic factors. Among biotic factors, fungal diseases are very responsible for yield loss in production, up to 40-60% in Greengram. Biotic factors that cause many diseases, such as powdery mildew, yellow mosaic virus, dry root rot, leaf spots, leaf crinkle virus, rust, etc., are of prime importance in reducing crop yield. Dry root rot (DRR) is an economically devastating disease (Mohit Kumar *et al*., 2019) [10]. Green gram-specific strains of a necrotic fungal phyto-pathogen, *Macrophomina phaseolina*, cause DRR. Micro-sclerotia of this fungus are capable of withstanding harsh environmental conditions, such as primary inoculums. Initial symptoms are scattered necrotic spots in roots, progressing to rotting and withering lateral roots accompanied by pre-maturely dried straw-colored foliage.

The damage which is caused by pathogens not only decreases crop yield but also has an impact on nitrogen fixation that results in increased pathogen density in the soil (Ahmed, 2021) [9].

1.3 Disease Management

Management of dry root rot caused by *Macrophomina phaseolina* is more challenging because of the pathogen's long-term survival and wide host variety. Managing soil-borne fungal pathogens is difficult. These pathogens are not only saprophytes that coexist with other soil species in the soil, but they are also spread by seed. Synthetic fungicides are often used to control phyto-pathogenic fungi, but their use is restricted due to environmental and health concerns because of their negative consequences and the emergence of resistance in crop pathogens. Biological management of soil-borne pathogenic fungi by using bio-agents on the soil is a non-chemical method that is shown to be a cost-effective and efficient way to treat soil disease. Biological monitoring is environmentally safe, leaves no residual toxicity, is cost-effective and can be used effectively in the context of advanced disease prevention. Plant extracts were also evaluated *in vitro* for antifungal activity, and these are more environmentally safe bio-compounds for the treatment of plant diseases using various types of botanicals, and they have been found to be environmentally friendly and effective against the targeted pathogen (Ahmed, 2021) [9]. The aim of this study is to determine the most effective bio-control agents and botanicals to develop an integrated strategy to effectively manage the disease.

2. Materials and Methods

Experiments related to work on *"In vitro* Evaluation of Different Plant Extracts and Bio-Agents against Dry Root Rot of Greengram Caused by *Macrophomina phaseolina*" have been carried out in the Plant Pathology laboratory, Department of Crop Protection, PGP College of Agricultural Sciences, Namakkal, which is situated at 11.229545° latitude and 78.200957° longitude and at an elevation of 218m above MSL. The details of materials used and the methods adopted in the present research are described in brief hereunder.

2.1 Survey for the intensity of the disease

A roving survey was conducted to assess the incidence of *Macrophomina phaseolina* in greengram in Namakkal district in 2022. Dry root rot infected samples were collected from different areas of Namakkal district. *viz*., Mohanur, Nammakal, Sendhamangalam, Rasipuram.

The percent disease incidence was calculated by the following formula (Baria *et al*., 2020) [2].

Disease Incidence as a Percentage = (Number of Infected Plants/Total Plants) × 100

2.2 Pathogen Isolation and Identification

2.2.1 Pathogen isolation

Greengram crops showing typical symptoms of dry root rot were collected from crop fields around Namakkal in May 2022. A standard tissue isolation procedure was followed to isolate the pathogen. The infected tissues were cut and surface sterilised for 30 seconds with a 0.1 percent mercuric chloride solution, and such bits were transferred three times to Petri dishes containing sterile water, drained the water, and the hits were placed on sterile tissue paper and transferred into Petri dishes containing 15 ml of potato dextrose agar medium and incubated at 28 °C for seven days (Mallikarjuna *et al.*, 2015) [8]. A pure culture of the fungus was obtained by the hyphal tip method.

2.2.2 The Hyphal tip method

Infected tissues are placed in the PDA. After the 3rd day, a 9mm disc of well sprouted or mycelium growth culture at the hyphal tip end was taken with the help of a cork borer and then transferred into the medium. Such cultures stored at 4 °C in a refrigerator were used for further studies.

2.3 Pathogen Identification and Characterization

The cultures were identified based on morphological characteristics seen in the petri dishes. Cultural characters are mycelial growth, topography, colour of mycelium, and colour of pigmentation, and morphological characters like sclerotiak, pycnidia-size, colour, and shape are noticed.

2.4 Culture Maintenance and Preservation

The slant culture of *Macrophomina phaseolina* was maintained at 4 °C in a refrigerator and sub-cultured periodically at an interval of 30 days during the course of the investigation. (Ashwathi *et al.*, 2017) [1].

2.5 Morphological investigations

2.5.1 Spore size and shape

Spores of *Macrophomina phaseolina* were taken from 7-day old culture plates and mounted on a clean glass slide. Spores were mixed with lacto phenol thoroughly in order to obtain a uniform spread, over which a cover slip was placed.

2.6 Cultural studies in different media

The growth characteristics of *Macrophomina phaseolina* were studied on six solid media, *viz*., potato dextrose agar, Czapek's agar medium, carrot juice agar medium, Sabourd's Agar Medium, and water agar, Richard's Agar medium. All the media were sterilised at 1.1 kg/cm^2 pressure for 15 minutes in the autoclave. To carry out the study, 15 ml of each of the mediums was poured into each Petri dish separately. Such Petri dishes were aseptically inoculated with 9 mm disc cut-outs from the periphery of an actively growing culture and incubated at 28 °C for a period of seven days. Each treatment was replicated thrice. Observations were taken with respect to colony size on the 5th day after inoculation. The mycelial colour and substrate colour were recorded at 5 days after inoculation. The records on radial growth were examined statistically.

The composition and preparation of different media were followed as given in Tuite, 1969 [15] (Potato dextrose agar), Cantrell and Dowler, 1971 [3] (Czapek's agar medium), Olsen and Bakken, 1987 [11] (Water Agar), Thawar*et al.* (2016) [14] (Richards Agar), Guerin *et al.*, 1994 [6] (Sabouraud's Agar Medium and Carrot Juice Agar Medium).

2.7 Isolation of bio-control agents

The bio-control agents were purchased from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, and stored at 4 °C until used. *Trichoderma viride* and *Bacillus subtilis* cultures were sub cultured using potato dextrose agar (PDA) and nutrient agar (NA) medium by the fungal disc method and later pure cultures were obtained. The

purified isolates were preserved at 4 °C and used during the course of the study.

2.8 Biocontrol agent *in vitro* assay against *Macrophomina phaseolina* (dual plate technique)

Macrophomina phaseolina was tested against antagonists such as *Trichoderma viride* (Sreedevi *et al.,* 2011) [13] and *Bacillus subtilis* (Hashem *et al.,* 2017). The bio-control agent and test fungus were cultured on potato dextrose agar in order to get fresh and active growth of fungus. On the potato dextrose agar medium, 9 mm of the fungal disc of the antagonist along with the test fungus were kept in the opposite direction. The plates were incubated for a week at 28 °C. The mycelial growth of antagonistic fungus and the growth of pathogens were also recorded separately. The observation of the interaction zone or inhibition zone was recorded. After the period of incubation, the growth of the mycelia of *Macrophomina phaseolina* was recorded and the percent inhibition of the mycelia over control was calculated.

2.9 *In vitro* assay of various botanicals

The relative efficacy of three plant extracts, *viz*., *Azadirachta indica*, *Pongamia pinnata*, and *Allium sativum, was* tested against the pathogens in the laboratory by the Poison Food Technique (Gobika *et al.,* 2021) [5]. The extracts are mixed with the medium in different concentrations (5%, 10%, and 15%) and make up the medium. Pour different concentrations of medium into petri dishes (9 cm). Allow them to incubate at 28 ºC for 5 days. The observation of the interaction zone or inhibition zone was recorded. After the period of incubation, the growth of the mycelia of *Macrophomina* was recorded and the percent inhibition of the mycelial growth over control was calculated.

3. Experimental Results and Discussion

The dry root rot disease of greengram caused by *Macrophomina phaseolina* has been severe during the vegetative stage, affecting growth and yield. The present investigations into this disease have been as per the objectives mentioned earlier, and the results of the experiments have been conducted on various aspects with reference to a survey on dry root rot disease, symptomology, cultural studies on the growth of *Macrophomina phaseolina, in-vitro* evaluation of botanicals and bio-control agents against *Macrophomina phaseolina*. The results of the experiments are presented hereunder.

3.1 Survey on Dry root rot disease

3.1.1 Disease intensity during 2022

A survey was conducted to assess the incidence of *Macrophomina phaseolina* in greengram in Namakkal district in 2022. Dry root rot infected samples were collected from different areas of Namakkal district, *viz.* Mohanur, Nammakal, Sendhamangalam, and Rasipuram. The survey conducted revealed that the maximum disease incidence of 29.75 percent was recorded at Namakkal.

3.2 Isolation and characterization of *Macrophomina phaseolina*

3.2.1 Isolation purification of fungus

Macrophomina phaseolina culture was isolated from greengram plants infected with dry root rot. The mycelial colour of *Macrophomina phaseolina* is white in the initial stage, later changing to dark black in colour on PDA medium (Plate 2). The mycelial topography was raised by aerial mycelium.

3.2.2 Morphology factors of *Macrophomina phaseolina*

Macrophomina phaseolina produces asexual fruiting body pycnidia but is unable to be observed in a microscope as clearly. Sclerotia of *Macrophomina phaseolina* isolate were dark black and irregular in shape and were able to be observed in microscopic detail (Plate 3). Similar results were reported by Malllikarjuna *et al.,* 2015 [8].

3.3 Growth Behavior of *Macrophomina phaseolina* on Different Solid Media

The cultural characters of *Macrophomina phaseolina* were studied on different solid media as described in material and methods. Observations on radial growth, mycelial colour, topography, and substrate colour on different media were recorded at 5 days after inoculation. The radial growth of *Macrophomina phaseolina* was maximum (9 cm) on Potato dextrose agar and Carrot juice agar, followed by Sabouraud's Agar Medium (8.77 cm). The lowest growth rate of mycelium was observed on Richard's agar medium (2.77 cm) and there was no mycelial growth in water agar or Czapek's agar.

3.4 *In vitro* screening of *Trichoderma viride and Bacillus subtilis* isolates against *Macrophomina phaseolina*

The bio-control agents were purchased from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore. The antagonist effect of *Trichoderma viride* and *Bacilus subtilis* isolate was studied against *Macrophomina phaseolina* in dual culture under *in-vitro* conditions. The results revealed that *Trichoderma viride* isolates recorded 72.96% mycelial inhibition, followed by *Bacillus subtilis* (51.11%) (Table 2; Plate 5 & 6). Similar results were reported by Sowmya *et al.,* 2015 [12].

3.5 *In vitro* screening of Botanicals against *Macrophomina phaseolina*

The antagonist effects of different botanicals were studied against *Macrophomina phaseolina* food poisoning technique under *in vitro* conditions. The results show that garlic extract at 10% and 15% concentrations completely inhibits *Macrophomina phaseolina* mycelial growth. Garlic extract with 5% concentration recorded a significant result with 98.51% inhibition of mycelial growth, followed by Neem leaf extract with 15% concentration, which recorded 75.18% (Table 1; Plate 4).

The *in vitro* evaluation of both botanicals and plant extracts was found to be considerably successful in preventing the percentage mycelia growth of *Macrophomina phaseolin*a as compared to an untreated control. (Dhingani *et al.,* 2013) [4].

Table 1: Effect of Various Plant Extracts on Mycelial Growth of *Macrophmina phaseolina*

Treatments	Mycelial Growth* (mm)			Per Cent Inhibition of Mycelial Growth*		
	5%	10%	15%	5%	10%	15%
Neem	4.8	4.5	2.23	46.66	49.99	75.18
Pungam	7.63	6.9	5.9	15.18	23.33	34.44
Garlic	0.13	0	0	98.51	100	100
Control	9	9	9	0	0	0
CD (P=0.05)	0.5262			5.8469		

*Average of Three replications

Table 2: Effect of Various Bio-control Agents on Mycelial Growth of *Macrophmina phaseolina*

Treatment	Mycelial Growth* cm)	Per Cent Inhibition of Mycelial Growth*
Trichoderma viride	2.43	72.96
Bacillus subtilis	4.40	51.11
Control	9.00	0.00
CD (P=0.05)	0.6248	7.0299

*Average of Three replications

Plate 1: Greengram Dry Root Rot Symptoms

Plate 2: *Machrophomina phaseolina* Culture

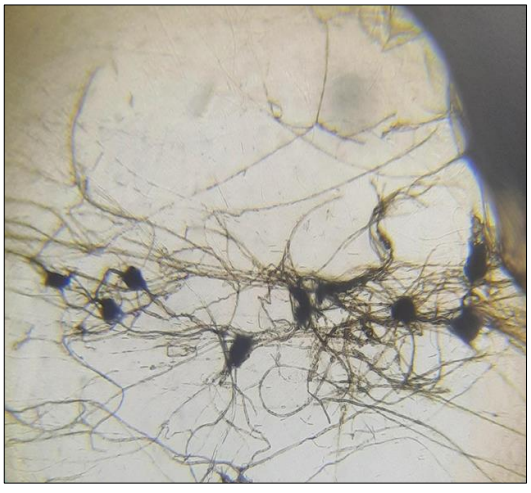

Plate 3: Microscopic View of Sclerotia

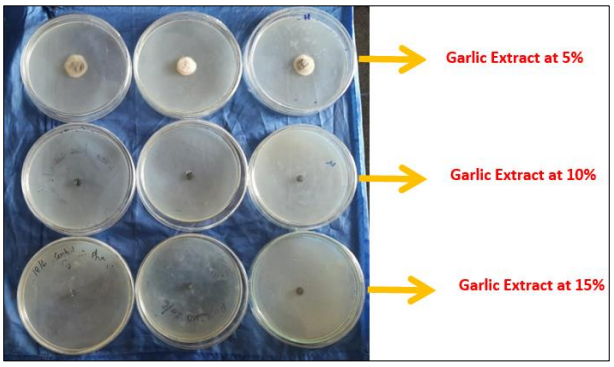

Plate 4: Garlic extract treated plates against *Macrophomina phaseolina*

Plate 5: Effect of *Trichoderma viride* on the growth of *Macrophomina phaseolina*

Plate 6: Effect of *Bacillus subtilis* on the growth of *Macrophomina phaseolina*

4. Conclusion

The significant results of this study show that garlic extract at a 5% concentration is the most effective bio-agent against *Macrophomina phaseolina*, followed by *Trichoderma viride*. So, in the future, the use of bio-agents and botanicals can be increased to save our earth from environmental hazards caused by dangerous chemicals.

5. Acknowledgment

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