



International Journal of Research in Agronomy

E-ISSN: 2618-0618

P-ISSN: 2618-060X

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www.agronomyjournals.com

2024; 7(7): 721-730

Received: 18-04-2024

Accepted: 25-05-2024

CP Manjula

All India Coordinated Research Project on sunflower and Directorate of Research, UAS, GKVK, Bangalore, Karnataka, India

Sangeeta AG

¹⁾ All India Coordinated Research Project on sunflower and Directorate of Research, UAS, GKVK, Bangalore, India

²⁾ Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bangalore, Karnataka, India

Yamanura

All India Coordinated Research Project on Castor and Directorate of Research, UAS, GKVK, Bangalore, Karnataka, India

Divyashree

¹⁾ All India Coordinated Research Project on sunflower and Directorate of Research, UAS, GKVK, Bangalore, India

²⁾ Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bangalore, Karnataka, India

G Punith

¹⁾ All India Coordinated Research Project on sunflower and Directorate of Research, UAS, GKVK, Bangalore, India

²⁾ Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bangalore, Karnataka, India

Sushmita

Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bangalore, Karnataka, India

Harish J

Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bangalore, Karnataka, India

Lakshmeesha R

Department of Plant Biotechnology, University of Agricultural Sciences, Bangalore, Karnataka, India

Corresponding Author:

Divyashree

¹⁾ All India Coordinated Research Project on sunflower and Directorate of Research, UAS, GKVK, Bangalore, India

²⁾ Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bangalore, Karnataka, India

Comprehensive investigation into pathogenicity, cultural characteristics, germplasm resistance and management strategies *Macrophomina phaseolina* causing castor root rot

CP Manjula, Sangeeta AG, Yamanura, Divyashree, G Punith, Sushmita, Harish J and Lakshmeesha R

DOI: <https://doi.org/10.33545/2618060X.2024.v7.i7i.1121>

Abstract

Castor (*Ricinus communis* L.) is an important non-edible oil seed crop in the world. The crop is prone to many diseases, among them root-rot poses serious threat to the crop resulting in reduced crop stand and yield. It affects all parts of the plant viz., root, stem and collar region. The fungus isolated from infected region were characterized through morphological and which was identified and confirmed as *Macrophomina phaseolina* (Tassi) Goid. The fungus showed highest growth in Sabouraud's dextrose agar (88.77 mm) followed by Potato dextrose agar (84.32 mm). The pathogen exhibited good growth with increase in temperature up to 40 °C (87.00 mm) and an optimum pH range of 6.0 to 7.0. Under *in vitro* assessment, fungicides viz., Mancozeb and Propineb (500, 750 and 1000 ppm), Tebuconazole (50, 100 and 150 ppm) and Carbendazim + Mancozeb (100, 250, 500 and 1000 ppm) recorded 100 per cent inhibition of the pathogen. Among the fungal antagonists, *Trichoderma harzianum* GJ 16B (52.84%) and *T. viride* 8 (52.23%) and bacterial antagonists *Bacillus pumilis* (33.10%) exhibited maximum inhibition *in vitro*. Out of 54 germplasm screened, BCG-2 showed absolute resistance while MI-54 exhibited high resistance to root-rot.

Keywords: *M. phaseolina*, castor, germplasm resistance, fungicides

Introduction

Castor is an important non-edible oilseed crop, with wide adaptability. India leads the international castor oil trade by producing highest share of seed and oil. Castor being a hardy crop, is prone to many pathogen infections. *M. phaseolina* is one of important pathogen that causes root rot has wide host range. *M. phaseolina* causes different symptoms on castor viz., seedling blight, dieback, stem blight, collar rot, root rot and twig blight (Moses and Reddy, 1987). The fungus is soil-borne and survives in soil for long periods in the form of sclerotia. Pycnidia which are produced on aerial plant parts help in the secondary spread ^[1].

There is huge difference among cultural practices and also genotypes grown in different regions. It is necessary to understand the severity of the disease noting to so many variation in its cultivation practices and factors influencing the disease development, this will help in devising effective management applications that are feasible, under the prevailing climatic conditions and sowing seasons.

Materials and Methods

Isolation and identification of the pathogen

Plants with root rot symptoms like reduced growth, collar rot, stem blight, and shredded bark were collected from fields at ZARS, GKVK, UAS, Bengaluru. These diseased samples were washed and examined under a microscope for pathogens. The fungus was then isolated and cultured on Potato Dextrose Agar and incubated culture was confirmed for *M. phaseolina* microscopically through morphological structures and conidia ^[2-5].

In culture, the colony color of *M. phaseolina* varies from black to brown or gray, darkening with age. Abundant aerial mycelium with embedded microsclerotia is produced. Hyphae are septate, initially hyaline, turning honey or black. Numerous dark brown to black microsclerotia are visible on the reverse side of the culture plate. The vegetative mycelium forms monilid or barrel-shaped cells with septa near branching points. Branching occurs at acute or right angles to the parent hyphae. Microsclerotia, black in color and ranging from 50-150 µm, are formed from aggregated hyphae with melanin pigment, and their size varies depending on the host and media used [6-7].

Pathogenicity of isolated fungus was tested following *in vivo* sick pot method.

***In vivo* sick pot method**

The mass multiplied *M. phaseolina* on jowar grain was used for the pot experiment. Seeds of susceptible variety GCH-4 were disinfected by immersing them in 2.5% NaOCl for 15 min, rinsed in sterilized water to remove chemical residues and air dried. Castor seeds were sown in pots containing sterilized soil infested with *M. phaseolina* isolate @ 2 gKg⁻¹ soil. Pots without inoculum served as control. The pots were then placed in a net house at 30 ± 20°C. Observations were recorded as the symptoms on germinated seedling.

Cultural and physiological studies of the pathogen

Solid media

Thirteen media of different compositions were used to understand the growth and sporulation of *M. phaseolina* under different nutrient constituents. These agar based sterilized media were poured, into 90 mm diameter sterilized Petri plates @ 20 mL/plate. After solidification, 5 mm diameter culture block of nine days old pure culture of *M. phaseolina* was cut with the help of sterilized cork borer and placed in the centre of the Petri plates. Three replications were kept for recording observations on the colony diameter, sclerotial formation and colony character of the fungus. The Petri plates were incubated at room temperature (28 ± 2 °C) and observations were recorded after seven days of incubation.

Different media's viz., Sabouraud's Dextrose Agar (SDA), Carrot Agar (CA), Malt Extract Agar (MEA), Richard's Synthetic Agar (RSA), Corn Meal Agar (CMA), Vegetable Juice Agar Medium (V₈), Rose Bengal Agar (RBA), Czapek's Dox Medium (CDM), Potato Dextrose Agar (PDA), Water Agar (WA), Oat Meal Agar (OMA), Modified Brown and Scott Agar (MB&SA), Standard Nutrient Agar (SNA) are prepared.

Temperature

The growth of *M. phaseolina* was tested at 15, 20, 25, 30, 35 and 40 °C. Sterilized Potato Dextrose Agar was poured into 90 mm diameter sterilized Petri plates. After solidification, 5 mm disc from actively growing culture was cut and inoculated in the solidified Petri plates and incubated for 8 days in the incubator adjusted to required temperature levels. Each treatment was replicated thrice. After the incubation period, radial growth and sclerotial formation on solid media were recorded.

Hydrogen-ion concentration (pH)

The effect of different hydrogen-ion concentration on growth and sporulation of *M. phaseolina* was studied by growing the pathogen at six pH levels viz., 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 in 50 mL Potato Dextrose Broth. The pH levels were adjusted by using 0.1 NH₄Cl or 0.1 N NaOH solutions with the help of pH meter. 50 mL broth of different pH levels were prepared and

then sterilized, inoculated with mycelial disc and were incubated for 7 days at 27 ± 2 °C temperature. Three replications of each pH was maintained. Observation on growth as dry weight of the mycelia until we get a constant weight and sporulation was recorded [7].

Evaluation of castor germplasm lines for root rot resistance

Total of 54 germplasm accessions including GCH-4 as susceptible check and RG- 2787 as resistant check were evaluated against root rot in sick pot culture method under net house conditions. Pots were maintained containing sterilized soil and culture mix under net house conditions. Twenty seedlings were maintained for each accession, ten seeds per pot with two replications and control plants were maintained. The plants were observed for root rot symptoms 15 days after inoculation till 60 days at an interval of 15 days. The percentage of dead plants was recorded. The germplasm were grouped into different categories on the basis of root rot disease reaction [7].

In vitro* evaluation of bio-agents and fungicides against *M. phaseolina

In this study, several isolates of bacterial and fungal bio-control agents, were assessed for their ability to reduce the growth of *M. phaseolina* under laboratory conditions following dual culture technique. There were three replications for each isolate of biocontrol agent with one control each of only the pathogen. They were incubated at 25°C and grown for 6-7 days. The diameter of the colony of both the biocontrol agent and the pathogen was measured in two directions and the average was calculated. Per cent inhibition of growth of the pathogen was calculated. *In-vitro* evaluation was done with three replications. Poisoned food technique was employed for the evaluation of fungicides in the laboratory. 100 mL of Potato Dextrose Agar medium was prepared in the 250 mL conical flask. Required quantity of test fungicides were added to the respective medium separately. These flasks containing poisoned medium were shaken well to have even and uniform distribution of the fungicides and about 20 mL was poured in labelled respective sterilized Petri plates and allowed to solidify. The solidified plates were inoculated with the pure culture of *M. phaseolina*. Three replications were maintained for each treatment. The control plates without fungicide were also inoculated and kept for incubation. The treated plates were incubated at 25 ± 2°C. Observations on the colony diameter were recorded after 5 days. The percentage of inhibition was estimated using the following formula [8].

$$I = \frac{C-T}{C} \times 100$$

Where.,

I = Per cent inhibition of mycelium C= Growth of mycelium in control

T = Growth of mycelium in treatment

Statistical analysis

The data generated for different experiments were analyzed using the WASP (Web Based Agricultural Statistics Software Package), software developed by ICAR- Central Coastal Agricultural Research Institute, Goa and the inferences were made with a probability of one percent for all the laboratory experiments.

Results

Cultural and physiological studies of *Macrophomina phaseolina*

Effect of different culture media on growth of *M. phaseolina*

The growth response of *M. phaseolina* was evaluated across 13 different culture media. Among these, Sabouraud's Dextrose Agar (SDA) demonstrated the highest mycelial growth, reaching 88.77 mm in diameter. This was followed closely by Potato Dextrose Agar (PDA) with a growth of 84.32 mm. Czapek Dox Agar (82.44 mm) and Richard's Synthetic Agar (80.77 mm) also supported substantial mycelial development. The following media exhibited considerable mycelial growth: Modified Brown and Scott Agar: 78.22 mm, Malt Extract Agar: 77.44 mm, Rose Bengal Agar: 77.33 mm, Oat Meal Agar: 76.44 mm, Corn Meal Agar: 73.88 mm, Carrot Agar: 73.77 mm, Standard Nutrient Agar: 71.88 mm. Each of these media effectively supported the growth of *M. phaseolina* mycelia, indicating their suitability for culturing this pathogen. In contrast, the least mycelial growth was observed on Water Agar, which exhibited a growth diameter of 59.88 mm with a notably thin layer of mycelia. Notably, none of the media tested, including those that supported robust mycelial growth, facilitated the production of microsclerotia. This absence of microsclerotia formation across all media suggests specific requirements for this developmental stage that were not met under the conditions provided by these media. This finding is crucial for understanding the growth dynamics of *M. phaseolina* and for selecting appropriate media for further physiological and pathological studies. (Figure 1, Table 1).

Effect of pH on the growth of mycelia and microsclerotia

Based on our observations, the highest dry mycelial weight was recorded at pH levels of 6.0 and 6.5, with values of 1.463 mg/100 mL. The mycelial weights observed at pH levels of 7.0 and 7.5 were slightly lower, at 1.34 mg/100 mL and 1.20 mg/100 mL respectively. Other pH levels, specifically 4.5, 5.0, and 5.5, also demonstrated considerable mycelial growth. From these results, we can conclude that the optimal pH range for the development of *M. phaseolina* lies between 6.0 and 6.5. On average, the mycelial growth did not exceed 1.33 mg/100 mL. Furthermore, moderate formation of microsclerotia was observed after an additional seven days of incubation, following an initial six-day period, at pH levels of 6.0 and 6.5. In contrast, microsclerotia formation was scant at pH levels of 5.5, 7.0, and 7.5, and no microsclerotia formation was observed at pH levels of 4.5 and 5.0 (Figure 2A and Supplementary Table 1).

Effect of different temperature on growth of *M. phaseolina*

The growth of the isolate exhibited minimal expansion at 15 °C. However, as the temperature increased, there was a notable enhancement in growth. At 20 °C, the growth measured 65.33 mm, which further increased to 66.33 mm at 25 °C. The growth continued to rise significantly, reaching 78.00 mm at 30 °C and 81.00 mm at 35 °C. The maximum growth was observed at 40 °C, with a measurement of 87.00 mm. These observations suggest that higher temperature regimes are conducive to the growth of *M. phaseolina*, which correlates with the increased incidence of the disease in regions with elevated temperatures (Figure 2B and Supplementary Table 2).

Temperature plays an important role in infection and disease development. *M. phaseolina* requires dry and high temperature for growth and survival and also for disease incitation. Maximum mean colony diameter of *M. phaseolina* was recorded at 40 °C and least at 15 °C. In the present study, it was observed

that with the increase in temperature (up to 40 °C), the fungus showed enhanced growth but there was no production of microsclerotia. The results are in accordance with Csondes *et al.* (2007), wherein they recorded that the most favourable temperature range for mycelial growth of *M. phaseolina* was 25 to 40 °C and growth was very low at 10 and 15 °C. It did not form microsclerotia in any of the temperature range.

Evaluation of castor germplasm lines for root rot resistance

The ideal, easy and cheapest method of management is choice of resistant varieties for the control of plant diseases. Screening was undertaken with recognized 54 castor germplasm lines against root rot of castor. The results of screening against root rot incidence were recorded at an interval of 15, 30 and 60 DAS. Per cent disease incidence and reaction of different germplasm lines are given potential yield. Out of 54 germplasm lines screened against *M. phaseolina*, BCG-2 showed absolute resistance (0% disease incidence) and MI-54 (10%) was the only germplasm line with high resistance.

Germplasm lines viz., BCG-17 (14.29%), BCG-19 (20%), BCG-24 (20%), BCG-27 (14.28%), MI-53 (20%), MI-83 (20%), MI-50 (20%), MI-87 (12.57%), ICS-240 (16.66%), ICS-241 (20%) and HCG-104 (20%) were resistant to root rot incidence. About 13 lines showed moderate resistance viz., RG-2787 (10%), BCH-101 (25%), ICS-264 (25%), ICS-266 (28.57%), HCG-107 (28.57%), RG-392 (33.33%), RG-3160-1 (28.57%), BCH-117 (28.57%), BCH-99 (30%), BCG-4 (28.57%), BCG-20 (20%), HCG-91 (28.57%) and BCH-115 (22.22%). Eleven germplasm lines namely BCG-1 (40%), BCG-21 (33.33%), BCG-22 (40%), BCG-25 (33.33%), BCG-29 (33.33%), BCG-105 (33.33%), BCH-97 (40%), MI-71 (33.33%), K. local (40%) and BCH-104 (33.33%) were moderately susceptible. BCG-12 (44.44%), BCG-18 (50%), ICS-242 (50%), ICS-253 (50%), ICS-273 (42.85%), RG-3477 (50%) BCH-122 (50%) and BCG-23 were susceptible to the disease. Ten germplasm lines viz., GCH-4 (66.67%), BCG-3 (62.5%), BCG-13 (100%), BCG-16 (87.5%), BCG-15 (75%), BCG-16 (87.5%), BCG-28 (66.66%), BCH-111 (60%), MI-86 (70%), BCH-33 (55.56%), BCG-11 (100%) exhibited highly susceptible reaction to the disease incidence (Figure 3 and Table 2).

In vitro evaluation of fungal bio-control agents against *M. phaseolina*

Among the ten fungal antagonists tested for their efficacy in inhibiting mycelial growth, *T. harzianum* GJ 16B exhibited the highest mycelial inhibition at 52.84%, which was statistically on par with *T. viride* 8, showing an inhibition of 52.23%. This was followed by *T. harzianum* 4B with an inhibition rate of 48.9%. The least inhibition was observed with *T. harzianum* B2, which recorded an inhibition rate of 24.57%. The inhibition rates of the other antagonists were as follows: *T. harzianum* 10 (45.93%), *T. viride* (42.75%), *T. viride* 1 (35.56%), *T. harzianum* 41 (33.58%), *T. harzianum* 55 (33.41%), *T. harzianum* 56 (31.24%), and *T. harzianum* 14 (26.92%). These results highlight the varying degrees of efficacy among the different *Trichoderma* strains in inhibiting mycelial growth (Figure 4A and Table 3).

In vitro evaluation of bacterial bio-control agents against *M. phaseolina*

Among the eight bacterial bio-agents evaluated, *Bacillus pumilis* exhibited highest mycelial inhibition (33.10%). Next in the order were *B. velezensis* P₄₂ (26.17%), *Azotobacter chroococcum* (18.07%) and *B. megaterium* (18.03%), *Bacillus velezensis* A₆

(17.91%), *Pseudomonas fluorescens* (17.41%), *Azospirillum brasilense* (14.07%) and least mycelial growth was recorded in *Bacillus subtilis* (13.83%) as shown in Figure 4B and Table 4. In the present investigation the *in vitro* evaluation of bio-agents on inhibition of growth of *M. phaseolina* were carried out by dual culture technique. Among all the fungal and bacterial bio-agents used, *T. harzianum* GJ 16B, *T. viride* were found to be most effective in inhibiting the mycelial growth when compared with bacterial antagonists.

In vitro* evaluation of contact fungicides against *M. phaseolina

Among the five contact fungicides assessed, maximum mean per cent inhibition (100%) was observed in two of the fungicides viz., mancozeb and propineb. Next in order was captan with 83.21 per cent inhibition of mycelial growth, and chlorothalonil (70.17%). All the fungicides tested were significantly superior over the control. Copper oxychloride with 63.29 per cent, exhibited least mean per cent inhibition of mycelial.

Among the three concentrations tested, 1000 ppm was significantly superior with a mean of 92.44 per cent of inhibition. Next was 750 ppm (87.36%) and 500 ppm concentrations with 70.19 mean per cent inhibition. Mancozeb and propineb at all the three concentrations and captan at 1000 ppm recorded cent per cent mycelial inhibition. The least mycelial inhibition was noticed in copper oxychloride (18.03%). The interaction of fungicides and concentrations varied. These results with respect to inhibition of mycelial growth by five contact fungicides at three concentrations were recorded and tabulated in (Figure 4C and Table 5)

In vitro* evaluation of systemic fungicide against *M. phaseolina

The highest mean per cent of inhibition was observed in Tebuconazole which showed 100 per cent inhibition of mycelial growth, which was on par with Propiconazole (96.55%). All the fungicides tested were significantly superior to control. Least mycelial inhibition was observed in Azoxystrobin (34.47%). Among all the concentrations, the highest concentration (150 ppm) of fungicides was significantly superior over others with maximum mean mycelial growth inhibition (73.24%). Next was 100 and 50 ppm with 70.97 and 67.06 per cent inhibition.

The interaction study revealed that Tebuconazole at 50, 100 and 150 ppm; Propiconazole at 100 and 150 ppm and Hexaconazole at 150 ppm recorded complete inhibition of mycelial growth of *M. phaseolina*, and least inhibition of 27.33 per cent in Azoxystrobin at 100 ppm. The interaction of fungicides and concentrations varied (Figure 4D and Table 6).

In vitro* evaluation of combi-product fungicide against *M. phaseolina

Between the combi-products analysed, mancozeb 63% + carbendazim 12% WP recorded 100 per cent inhibition of growth. Least per cent inhibition was observed in metalaxyl 4% + mancozeb 64% with 61.20 per cent.

Among the tested concentrations highest mean per cent of inhibition was observed in 1000 ppm (93.06%), significantly superior over other concentrations i.e., 500 ppm (88.62%), 250 ppm (83.15%) and 100 ppm (74.74%). Mancozeb + carbendazim at all the three concentrations and Tebuconazole + Trifloxystrobin and Azoxystrobin + Difenconazole at 1000 ppm recorded cent per cent mycelial inhibition.

The least mycelial inhibition was noticed in Copper oxychloride (18.03%). The interaction of fungicides and concentrations

varied. In the present investigation, five non-systemic, 10 systemic and four combi-products were tested at three concentrations each (500, 750 and 1000 ppm for non-systemic and 50, 100 and 150 ppm for systemic fungicides) and four concentration for combi-products (100, 250, 500 and 1000 ppm). The present study reveals that among the contact fungicides, Mancozeb and Propineb were effective in inhibiting the mycelial growth of *M. phaseolina* which may be due to the multisite action of Mancozeb and Propineb i.e., to inactivate the sulfhydryl groups of amino acids, without any risk of resistance. Among the 10 systemic fungicides tested, maximum mycelial inhibition was recorded by Tebuconazole followed by Propiconazole. The effectiveness of the Triazoles like Tebuconazole, Propiconazole and Hexaconazole may be attributed to their interference with the biosynthesis of fungal sterols and inhibition of the ergosterol biosynthesis. It was clear from the study that among the combi-products tested Mancozeb 63% + Carbendazim 12% WP, was found to be the most effective in inhibiting the mycelial growth and the least was Metalaxyl 4% + Mancozeb 64%. This may be due to inclusive effect of systemic and one non-systemic fungicide which might have inhibited the mycelial growth.

Discussion

Studies on castor root rot caused by *M. phaseolina* (Tassi) Goid, was undertaken with respect to collection of sample, characterization of the isolate, proving its pathogenicity for confirmation of the pathogen, cultural and physiological studies to understand the prerequisites of the isolate and management strategies using fungicides and bio-control agents under *in vitro* conditions, screening of castor germplasm lines to know their reaction towards the disease.

The growth response of *M. phaseolina* was evaluated on 13 different culture media. Sabouraud's Dextrose Agar (SDA) showed the highest mycelial growth at 88.77 mm, followed by Potato Dextrose Agar (PDA) at 84.32 mm. Czapek Dox Agar (82.44 mm) and Richard's Synthetic Agar (80.77 mm) also supported substantial growth. Other media, including Modified Brown and Scott Agar, Malt Extract Agar, Rose Bengal Agar, Oat Meal Agar, Corn Meal Agar, Carrot Agar, and Standard Nutrient Agar, supported mycelial growth ranging from 71.88 mm to 78.22 mm. Water Agar exhibited the least growth at 59.88 mm with a very thin mycelial layer. The results are in agreement with Parmar *et al.* (2018), where Potato Dextrose Agar and Richard's Synthetic Agar supported best mycelial growth. Grover and Sakhuja (1981) and Kumar and Chaudhary, 2020, also reported Potato Dextrose Agar as best media, but none of the media reported the growth of pycnidial stage.

The highest dry mycelial weight of *M. phaseolina* (1.463 mg/100 mL) was seen at pH values of 6.0 and 6.5, according to observations. Slightly lower weights were observed at pH levels of 7.0 and 7.5 (1.34 mg/100 mL and 1.20 mg/100 mL, respectively). Notably, pH levels of 4.5, 5.0, and 5.5 also supported significant mycelial growth.

These results indicate that the optimal pH range for *M. phaseolina* development is between 6.0 and 6.5. On average, mycelial growth did not exceed 1.33 mg/100 mL. Additionally, moderate microsclerotia formation was noted after a total of 13 days of incubation at pH 6.0 and 6.5, whereas scant formation occurred at pH 5.5, 7.0, and 7.5. No microsclerotia were observed at pH 4.5 and 5.0.

Edaphic factors such as soil moisture, temperature, salinity and pH critically affect the survival of *M. phaseolina* and influence the incidence of charcoal rot. According to the study conducted by [9-10], the ideal pH for the pathogen was between 4.0 and 6.0,

whereas Dhingra and Sinclair (1978.) found good growth of the pathogen between pH5.0 and 8.0. Thus, it can be concluded that the growth rate of *M. phaseolina* at different pH levels depends on the isolate and its conditions of origin. The results of the present study are in agreement with [11-15].

The growth of the *M. phaseolina* isolate was minimal at 15 °C but increased significantly with higher temperatures: 65.33 mm at 20 °C, 66.33 mm at 25 °C, 78.00 mm at 30 °C, 81.00 mm at 35 °C, and reaching a maximum of 87.00 mm at 40 °C. This indicates that higher temperatures favor the growth of *M. phaseolina*, explaining the higher disease incidence in warmer regions. Temperature plays an important role in infection and disease development. *M. phaseolina* requires dry and high temperature for growth and survival and also for disease incitation. Maximum mean colony diameter of *M. phaseolina* was recorded at 40 °C and least at 15 °C. In the present study, it was observed that with the increase in temperature (up to 40 °C), the fungus showed enhanced growth but there was no production of microsclerotia. The results are in accordance with [16-19], wherein they recorded that the most favourable temperature range for mycelial growth of *M. phaseolina* was 25 to 40 °C and growth was very low at 10 and 15 °C. It did not form microsclerotia in any of the temperature range.

The screening of 54 castor germplasm lines for root rot resistance identified BCG-2 as completely resistant (0% disease incidence) and MI-54 as highly resistant (10%). Twelve lines were resistant, thirteen showed moderate resistance, eleven were moderately susceptible, and eight were susceptible. Ten lines exhibited high susceptibility, highlighting the significant variability in resistance among the germplasm lines and emphasizing the importance of selecting resistant varieties for effective and economical disease management. The results are in similar with reports of [20-23].

The varied reaction of castor lines is attributed to the genetic constitution of each line and is suggestive of the resistance available in the germplasm lines screened. Those lines which exhibited absolute resistance are useful for the breeders in developing the varieties/hybrids which are free from the disease which can be tested over locations/sections in the endemic areas for recommending to hybrid development and commercial cultivation. Among the ten fungal antagonists tested, *T. harzianum* GJ 16B showed the highest mycelial inhibition at 52.84%, comparable to *T. viride* 8 with 52.23%. *T. harzianum* 4B followed with 48.9% inhibition. The lowest inhibition was observed with *T. harzianum* B2 at 24.57%. Other strains showed varying inhibition rates: *T. harzianum* 10 (45.93%), *T. viride* (42.75%), *T. viride* 1 (35.56%), *T. harzianum* 41 (33.58%), *T. harzianum* 55 (33.41%), *T. harzianum* 56 (31.24%), and *T. harzianum* 14 (26.92%). These findings demonstrate the

differential efficacy of *Trichoderma* strains in inhibiting mycelial growth

Among the eight bacterial bio-agents evaluated, *B. pumilis* showed the highest mycelial inhibition at 33.10%, followed by *B. velezensis* P42 (26.17%), *A. chroococcum* (18.07%), *B. megaterium* (18.03%), *B. velezensis* A6 (17.91%), *P. fluorescens* (17.41%), *A. brasilense* (14.07%), and *B. subtilis* (13.83%). In the *in vitro* dual culture evaluation, *T. harzianum* GJ 16B and *T. viride* were the most effective in inhibiting the mycelial growth of *M. phaseolina* compared to the bacterial antagonists.

Most fungi have chitin and β (1-3) glucan as essential constituent in their cell wall. Mechanism for bio-control by *Trichoderma* sp. are antibiosis, lysis, competition and mycoparasitism, their ability to suppress pathogens is mainly due to coiling and disintegration of hyphae of the pathogen.

These results are in accordance with [24], who conducted an experiment to check the antagonistic effect of fungal and bacterial bio-control agents on *M. phaseolina*. Among the tested antagonists, *T. harzianum* (Th-R) was found to be more effective with maximum inhibition per cent (41.86%) followed by *T. viride* (39.07%) [25-26], tested the efficacy of different bio-control agents such as *T. viride*, *T. harzianum* and *P. fluorescens* and reported that *T. harzianum* was superior with 73.21 per cent inhibition.

The fungicide Tebuconazole showed the highest mean mycelial inhibition at 100%, followed closely by Propiconazole at 96.55%. All fungicides tested were significantly more effective than the control, with Azoxystrobin showing the least inhibition at 34.47%. Among concentrations, the highest (150 ppm) was most effective with 73.24% inhibition, followed by 100 ppm (70.97%) and 50 ppm (67.06%). The highest mean mycelial inhibition was observed at 1000 ppm (93.06%), significantly outperforming 500 ppm (88.62%), 250 ppm (83.15%), and 100 ppm (74.74%). Mancozeb + carbendazim at all concentrations, as well as Tebuconazole + Trifloxystrobin and Azoxystrobin + Difenconazole at 1000 ppm, achieved 100% mycelial inhibition. These results are in line with the findings of [27], the evaluation of systemic, non-systemic and combi-products against who revealed that Mancozeb 75% WP (500, 750, 1000 ppm), Tebuconazole (50, 100, 150 ppm) and Propiconazole (100, 150 ppm) and Mancozeb 63% + Carbendazim 12% WP (100, 250, 500, 1000 ppm) inhibited cent per cent growth of the pathogen *M. phaseolina*. [28] tested the efficacy of fungicides against *M. phaseolina*, root rot pathogen in cotton under *in vitro* and reported that Mancozeb, Propiconazole and Mancozeb 63% + Carbendazim 12% WP showed cent per cent inhibition of mycelial growth of the fungus [29-34].

Table 1: Effect of different media on cultural characteristics of *M. phaseolina* infecting castor

Tr. No.	Media	Radial mycelial growth (mm)**	Color	Colony texture	Margin
T ₁	Carrot Agar (CA)	73.77 (59.20)	Black with brownish substratum	Loose and fluffy	Regular
T ₂	Corn Meal Agar (CMA)	73.88 (59.28)	White with greyish substratum	Loose and fluffy	Regular
T ₃	Potato Dextrose Agar (PDA)	84.32 (66.68)	Dirty white	Compact	Regular
T ₄	Malt Extract Agar (MEA)	77.44 (61.66)	Dark brown	Moderately compact	Regular
T ₅	Rose Bengal Agar (RBA)	77.33 (61.59)	White with greyish substratum	Moderately compact	Irregular
T ₆	Sabouraud's Dextrose agar (SDA)	88.77 (70.46)	White	Compact	Regular
T ₇	Richard's Synthetic Agar (RSA)	80.77 (64.16)	Black with greyish substratum	Loose and fluffy	Irregular
T ₈	Water Agar (WA)	59.88 (50.71)	Dirty white	Thin mycelia	Irregular
T ₉	Modified Brown and Scott Agar (MB&SA)	78.22 (62.19)	Greyish white	Moderately compact	Irregular
T ₁₀	Standard Nutrient Agar (SNA)	71.88 (58.02)	Grey	Loose and fluffy	Irregular
T ₁₁	Oat Meal Agar (OMA)	76.44 (60.97)	Brown with black substratum	Moderately compact	Regular
T ₁₂	Vegatable Juice Agar (V8)	66.66 (54.74)	Brown	Fluffy	Irregular
T ₁₃	Czapek Dox Agar (CDZ)	82.44 (65.23)	Greyish with brown substratum	Compact	Regular
	S. E. (m) \pm	1.08			
	C. D. at 1%	4.23			

Table 2: Summary of screening germplasm lines against root rot

Disease Scale	Per cent infection	Disease response	No. of genotypes	Genotype name
0	0.00	Absolute resistant	1	BCG-2
1	0.01-10.00	Highly resistant	1	MI-54
2	10.01-20.00	Resistant	11	BCG-17, BCG-19, BCG-24, MI-53, MI-83, MI-50, MI- 87, ICS-241, HCG-104, ICS-240, BCG-27
3	20.01-30.00	Moderately resistant	13	RG-2787, BCH-101, ICS- 264, ICS-266, HCG-107, RG-392, RG-3160-1, BCH- 117, BCH-99, BCG-4, BCG-20, HCG-91, BCH-115
4	30.01-40.00	Moderately susceptible	10	BCG-1, BCG-21, BCG-22, BCG-25, BCG-29, BCG- 105, BCH-97, MI-71, K. local, BCH-104
5	40.01-50.00	Susceptible	7	BCG-12, BCG-18, ICS-242, ICS-253, ICS-273, RG- 3477, BCH-122, BCG-23
6	>50	Highly susceptible	10	GCH-4, BCG-3, BCG-13, BCG-15, BCG-16, BCG-28, BCH-111, MI-86, BCH-33, BCG-11

Table 3: *In vitro* evaluation of fungal bio-control agents against *M. phaseolina* infecting castor

Tr. No.	Fungal bio agent	Average colony diameter (mm)	Per cent inhibition over control**
T ₁	<i>T. harzianum</i> 14	65.77 (54.23)	26.92 (31.25)
T ₂	<i>T. harzianum</i> 55	59.55 (50.51)	33.41 (35.31)
T ₃	<i>T. harzianum</i> 56	61.88 (51.88)	31.24 (33.98)
T ₄	<i>T. harzianum</i> 41	59.77 (50.64)	33.58 (35.41)
T ₅	<i>T. harzianum</i> B ₂	67.88 (55.55)	24.57 (29.71)
T ₆	<i>T. viridae</i>	51.22 (45.70)	42.75 (40.83)
T ₇	<i>T. viridae</i> 1	57.99 (49.60)	35.56 (36.61)
T ₈	<i>T. harzianum</i> 4B	45.99 (42.70)	48.90 (44.37)
T ₉	<i>T. viridae</i> 8	42.99 (40.97)	52.23 (46.28)
T ₁₀	<i>T. harzianum</i> 10	48.66 (44.23)	45.93 (42.57)
T ₁₁	<i>T. harzianum</i> GJ 16B	42.44 (40.64)	52.84 (46.63)
	Control	90.00 (71.57)	
	S. E. (m) ±	1.27	
	C. D. at 1%	5.05	

Table 4: *In vitro* evaluation of bacterial bio-control agents against *M. phaseolina* infecting castor

Tr. No.	Bacterial bio agent	Average colony diameter (mm)	Per cent inhibition over control**
T ₁	<i>Bacillus megaterium</i>	73.78 (59.20)	18.03 (25.13)
T ₂	<i>Azotobacter chroococcum</i>	73.77 (59.22)	18.07 (25.16)
T ₃	<i>Azospirillum brasilense</i>	77.33 (61.57)	14.07 (22.03)
T ₄	<i>B. velezensis</i> A ₆	73.89 (59.28)	17.91 (25.04)
T ₅	<i>B. subtilis</i>	77.55 (61.75)	13.83 (21.83)
T ₆	<i>B. pumilis</i>	60.22 (50.91)	33.10 (35.12)
T ₇	<i>Pseudomonas fluorescens</i>	74.33 (59.56)	17.41 (24.66)
T ₈	<i>B. velezensis</i> P ₄₂	67.00 (54.94)	26.17 (30.77)
	Control	90.00 (71.57)	
	S. E. (m) ±	0.787	
	C. D. at 1%	3.249	

Table 5: *In vitro* efficacy of contact fungicides against *M. phaseolina* infecting castor

Tr. No.	Fungicide	Per cent inhibition over control (mm)			Mean
		Concentration (ppm)			
		500	750	1000	
T ₁	Chlorothalonil	67.90 (55.49)	69.02 (56.18)	73.58 (59.07)	70.17 (56.90)
T ₂	Mancozeb	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T ₃	Propineb	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T ₄	Captan	65.06 (53.76)	84.57 (66.87)	100.00 (90.00)	83.21 (69.17)
T ₅	Copper oxychloride	18.03 (25.13)	83.21 (65.81)	88.64 (70.30)	63.29 (52.71)
	Mean	70.19 (56.71)	87.36 (69.17)	92.44 (74.04)	83.33 (65.90)
	Source	S. E. (m) ±			C. D. at 1%
	Fungicide (F)	0.69			2.00
	Concentration (C)	0.54			1.55
	F×C	1.20			3.47

Table 6: *In vitro* efficacy of systemic fungicides against *M. phaseolina* infecting castor

Tr. No.	Fungicide	Per cent inhibition over control (mm)			Mean
		Concentration (ppm)			
		50	100	150	
T ₁	Penflufen	61.73 (51.78)	69.75 (56.63)	71.36 (57.64)	67.61 (55.31)
T ₂	Pyrachlostrobin	50.01 (45.01)	50.62 (45.36)	51.00 (45.57)	50.54 (45.31)
T ₃	Carbendazim	86.92 (68.80)	87.28 (69.11)	88.03 (69.76)	87.41 (69.22)
T ₄	Azoxystrobin	40.08 (39.28)	27.33 (31.52)	40.08 (39.28)	34.47 (35.95)
T ₅	Difenconazole	32.60 (34.82)	62.47 (52.22)	75.54 (60.36)	56.87 (48.95)
T ₆	Hexaconaozle	89.88 (71.45)	91.11 (72.65)	100.00 (90.00)	93.66 (75.42)
T ₇	Kresoxim methyl	31.24 (33.98)	39.92 (39.18)	43.46 (41.24)	38.21 (38.18)
T ₈	Tebuconazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T ₉	Propiconazole	89.65 (71.23)	100.00 (90.00)	100.00 (90.00)	96.55 (79.30)
T ₁₀	Thiophonate methyl	77.65 (61.79)	77.91 (61.97)	81.24 (64.33)	78.93 (62.68)
	Mean	67.06 (54.98)	70.97 (57.40)	73.24 (58.85)	70.42 (57.05)
	Source	S. E. (m) ±			C. D. at 1%
	Fungicide (F)	0.40			1.52
	Concentration (C)	0.20			0.76
	F×C	0.70			2.64

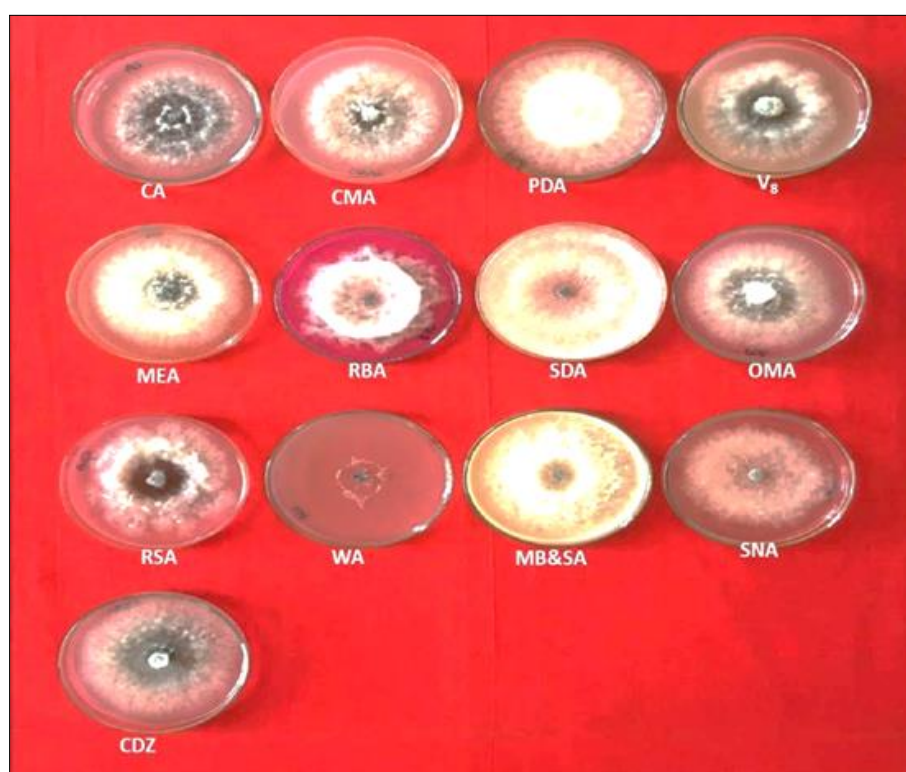
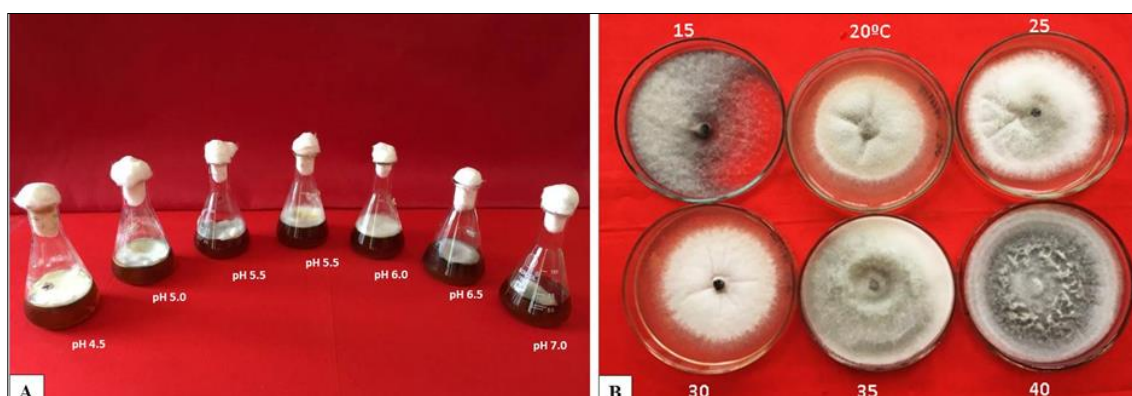
**Fig 1:** Effect of different media on cultural characteristics of *M. phaseolina***Fig 2:** 2A. Effect of hydrogen ion concentration (pH) on growth of *M. phaseolina*; 2B. Effect of temperature on growth of *M. phaseolina*



Fig 3: Screening of different castor germplasm lines against *M. phaseolina* causing root rot in castor. 3A. Castor germplasm lines screening, 3B. Resistant lines, 3C. Response of screening germplasm lines against root rot

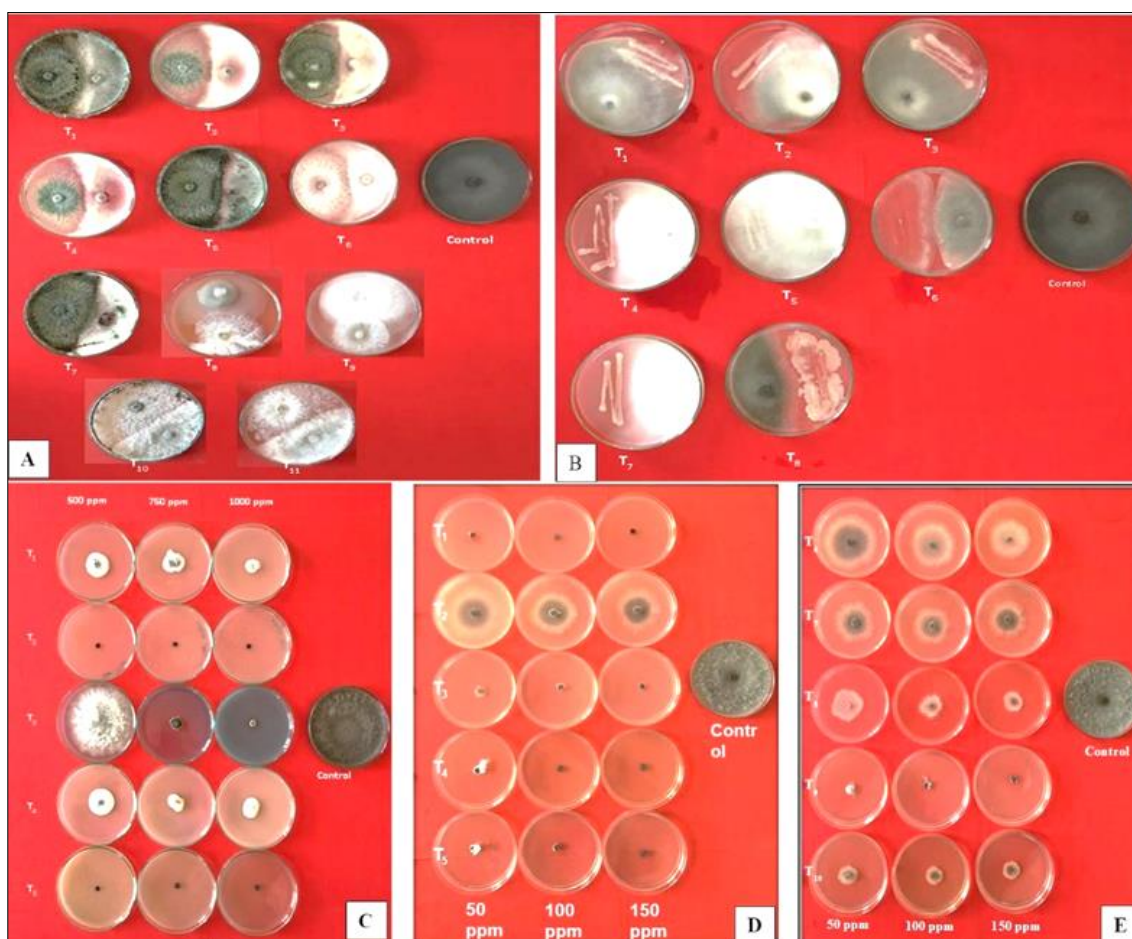


Fig 4: 4A. *In vitro* evaluation of fungal bio-control agents against *M. phaseolina* 4B. *In vitro* evaluation of bacterial bio-control agents against *M. phaseolina* 4C. *In vitro* efficacy of contact fungicides against *M. phaseolina* 4D. *In vitro* efficacy of systemic fungicides against *M. phaseolina* 4E. *In vitro* efficacy of combination product fungicides against *M. phaseolina*

Conclusion

The study demonstrated that Sabouraud's dextrose agar (SDA) is the most conducive medium for the mycelial growth of *M. phaseolina*, achieving a maximum growth of 88.77 mm. Optimal growth conditions for the pathogen were identified as a pH range of 6.0 to 6.5 and temperatures up to 40°C, with the highest recorded growth being 87.00 mm at 40°C. Screening of 54 castor germplasm lines revealed that BCG-2 exhibited absolute resistance to *M. phaseolina*, with 0% disease incidence, while MI-54 showed high resistance with a 10% incidence rate. Among the bio-agents tested, *T. harzianum* GJ 16B and *B. pumilis* were the most effective, inhibiting mycelial growth by 52.84% and 33.10%, respectively. The fungicide evaluations indicated that Tebuconazole, Mancozeb + Carbendazim and Propiconazole (at 100 and 150 ppm) were highly effective, achieving near-complete inhibition of mycelial growth. Conversely, Azoxystrobin was the least effective fungicide tested. Additionally, the combination of Tebuconazole + Trifloxystrobin at 1000 ppm also proved to be highly effective in inhibiting mycelial growth. These findings underscore the importance of selecting resistant germplasm lines and effective fungicides, along with utilizing bio-agents, as part of an integrated management strategy for controlling *M. phaseolina*.

Acknowledgments

We thankful to K. B. Palanna, CoA, UAS, GKVK, Bengaluru-65, for providing facilities to conduct the experiments of the student's thesis work as well as for the research project.

Funding

All India Coordinated Research Project on sunflower and Directorate of Research, UAS, GKVK, Bangalore -560065.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have approved to influence the work reported in this paper.

Ethical Statement

All the experimental procedures involving only on plant species were conducted following the University of Agricultural Science, Bangalore institutional guidelines. There are no human and animal subjects/trials conducted in this article and informed consent is not applicable.

Disclosure Statement

The authors declare that there are no financial/commercial conflicts of interest.

Author Contributions

C. P. Manjula (Conceptualization [supporting], Data curation [lead], Formal analysis [lead], Investigation [lead], Visualization [lead], Writing –original draft [lead]), Sangeeta A G (Supervision [supporting], Validation [equal], Writing –review & editing [equal]), Yamanura. (Supervision [supporting], Validation [supporting]), Divyashree (conceptualization [supporting], Supervision [lead], Formal analysis [lead], Visualization [lead]) G. Punith (Conceptualization, Data curation, Writing –review & editing- supporting) and Sushmita (Writing–original draft [supporting], Data curation [supporting]).

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Supplementary Tables

Supplementary Table 1: Effect of hydrogen ion concentration (pH) on *M. phaseolina* growth infecting castor

Tr. No.	pH	Dry weight of mycelial growth (mg/100 mL) **	Sporulation
T ₁	4.5	1.29 (6.53)	-
T ₂	5.0	1.24 (6.40)	-
T ₃	5.5	1.24 (6.40)	+
T ₄	6.0	1.46 (6.94)	++
T ₅	6.5	1.46 (6.95)	++
T ₆	7.0	1.34 (6.66)	+
T ₇	7.5	1.32 (6.61)	+
	S. E. (m) ±	0.10	
	C. D. at 1%	0.40	

Note: *Means of three replication; -: absent; +: 1-4 scanty; ++: 5-8 moderate; +++: 9-15 good; ++++: >15 abundant

Supplementary Table 2: Effect of temperature on *M. phaseolina* infecting castor

Tr. No.	Temperature	Radial mycelial growth (mm)**
T ₁	15	59.67 (50.59)
T ₂	20	65.33 (53.94)
T ₃	25	66.33 (54.53)
T ₄	30	78.00 (62.04)
T ₅	35	81.00 (64.19)
T ₆	40	87.00 (68.88)
	S. E. (m) ±	0.96
	C. D. at 1%	4.13