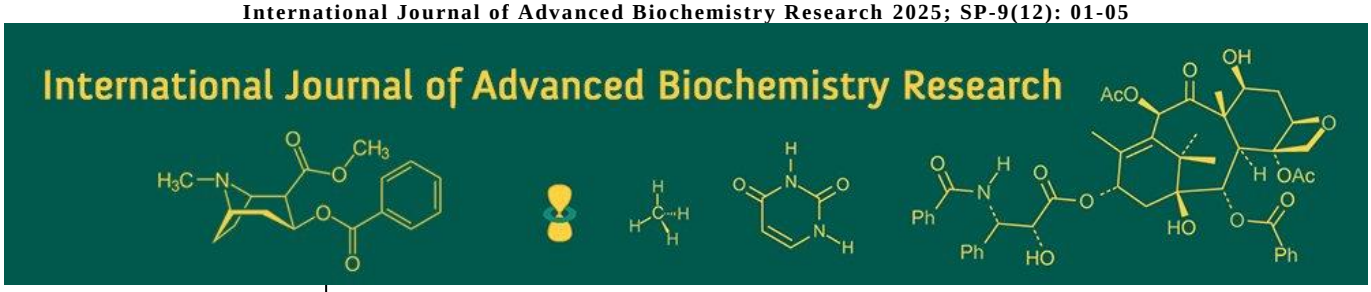

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Potential of fungal and bacterial biocontrol agent under *in vitro* conditions against *Macrophomina phaseolina* causing pigeonpea stem canker

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Abstract
Stem canker caused by *Macrophomina phaseolina* (Tassi) Goid is one of the most important and widespread and destructive diseases of Pigeonpea [*Cajanus cajan* (L.) Mill spaugh], causing responsible quantitative and qualitative losses. Present study was undertaken on the stem canker caused by *M. phaseolina* (Tassi) Goid of pigeonpea during 2023-24. A total of nine bioagents/antagonist i.e. Sevan fungal antagonists *viz., Trichoderma asperallum, T. harzianum, T. lignorum, T. virens, Metarhizium anisopliae, Aspergillus niger* and two Bacterial antagonists *Bacillus subtilis* and *Pseudomonas fluorescens* was evaluated *in vitro* against *M. phaseolina*, applying Dual culture technique. The bio efficacy of fungal and bacterial biocontrol antagonist on *Macrophomina phaseolina* (Tassi) Goid., which incite stem canker of pigeonpea were studied. In dual culture technique all the antagonist/bioagents evaluated, exhibited fungistatic/antifungal activity against test pathogen and significantly inhibited its growth over untreated control. *Trichoderma harzianum* was found most effective with significantly highest inhibition (97.29%) of test pathogen. The second and third inhibitor antagonists found were *Aspergilus flavus* (93.99%) and *A. niger* (92.92%). The bacterial antagonist were *Bacillus subtilis* (87.40%), *Pseudomonas fluorescens* (85.08%). These were followed by *T. asperellum* (84.09%), *T. lingorum* (78.26%), *Metarhizhium anisopliae* (59.87%) and *T. koningii* (34.57%), respectively of the test pathogens inhibition.

Keywords: Bio-control/antagonists, Pigeonpea stem canker, dual culture technique, *Macrophomina phaseolina, Trichoderma sp.*

Introduction
Pigeonpea [*Cajanus cajan* (L.) Mill spaugh] is one of the major legume crops grown in the tropics and sub tropics which accounts for about 5 per cent of world legume production with India being the largest producer. It is hardy, widely adopted and drought tolerant crop with a large temporal variation (90-300 days) for maturity. The crop is able to fix atmospheric nitrogen in the soil thereby increases the soil fertility (Smitha *et al.*, 2015) [22]. However, production of pigeonpea in the Indian subcontinent and other countries in Asia is severely affected by many plants' pathogenic fungi, bacteria, viruses and nematodes which caused the diseases such as *Fusarium* wilt, *Phytophthora* blight, powdery mildew, dry root rot, stem canker and sterility mosaic. Among these *Macrophomina phaseolina* (Tassi) Goid., which causes stem canker is becoming severe in most of the pigeonpea growing regions of India (Bajpal *et al.*, 1999) [2]. *M. phaseolina*, which causes stem canker in a wide range of hosts is one of the most destructive plant pathogens in the tropics and subtropics. Disease development is favoured by high temperature (30-35 °C) followed by moisture stress and a good source of inoculum (Dhingra and Sinclair, 1973a) [4].

Pigeonpea stem canker caused by *Macrophomina phaseolina* (Tassi) Goid. is becoming very severe in many parts of pigeonpea growing regions in the country. *Macrophomina* is primarily a soil and seed-borne fungal pathogen that incites the disease by producing microsclerotia/pycnidia (Farr *et al.*, 1995 and Pun *et al.*, 1998) [6, 19]. It has a wide host range of more than 500 species from 75 families with heterogeneous host specificity i.e. the ability to infect monocots as well as dicots and can exhibit non-uniform distribution in the soil (Mayek-Perez *et al.*, 2001 and Su *et al.*, 2001) [16, 23].

In India, the first report of stem canker of pigeonpea occurrence was reported by Kannaiyan *et al.* (1979) [10] in part of Eastern Uttar Pradesh (Varanasi and Mirzapur districts). Recently, the stem canker incited by *M. phaseolina* has emerged as one of the important pathogens of different agricultural crops, including pigeonpea (Kaur *et al.*, 2012b) [12]. *Macrophomina* Stem Canker disease (MSC), caused by *M. phaseolina* is potentially a serious disease in pigeonpea that occurs from flowers to physiological maturity. The disease also occurs in states of Bihar, Madhya Pradesh, Maharashtra, Orissa, Uttar Pradesh, Andhra Pradesh, Tamil Nadu, Gujarat and Rajasthan (Kannaiyan *et al.*, 1981) [11]. The disease is also reported from Australia, Myanmar, Sri Lanka, Vietnam and Zambia (Nene *et al.*, 1996) [17]. The fungus produces necrotic lesions on the stem and girdles the plant at the base leading to premature flower drop, drying of branches that eventually results in total witling and the death of the plant. It causes huge economic losses ranging from 10-100 percent (Smitha *et al.*, 2015) [22]. The decline in pigeonpea production was recorded in Maharashtra and Karnataka during 2023 and 2024 due to high incidences of *Macrophomina* stem canker. None of the cultivated cultivars could withstand the disease overcome. The climate change has been influencing this lesser-known Macrophomina stem canker disease into major epidemic. The dry and warm climate favourable to the disease development are frequently witnessed in the recent three years. In view of the severe outbreak of this disease, new investigation into the management became necessary to curtail the disease and its spread. The current study was conducted to know the efficacy of different antagonist against the pathogen for its effective control under *in vitro*. The results of the study were anticipated to draw the strategies for the lab and field management of the disease.

Materials and Methods

Sevan fungal antagonists *viz., Trichoderma asperallum, T. harzianum, T. lignorum, T. virens, Metarhizium anisopliae, Aspergillus niger* and two Bacterial antagonists *Bacillus subtilis* and *Pseudomonas fluorescens* was evaluated *in vitro* against *M. phaseolina*, applying Dual culture technique (Arora and Upadhay, 1978) [1].

Seven days old cultures of the test bioagents and the test pathogen (*M. phaseolina)* grown on agar media was used for further study. The culture disc (5 mm) of the test pathogen and bioagent were cut out with sterilized cork borer, from a week-old culture. Then two culture discs, one each of the test pathogen and bioagent was placed aseptically at equidistance and exactly opposite with each other on solidified PDA medium in Petri plates and plates were incubated at 28+2 °C. Three replications were maintained. PDA plates inoculated only with culture disc of the test pathogen was maintained as untreated control.

Details of experiment

Design: Completely Randomized design (CRD)
Replications: Three
Treatments: Ten

Fungal antagonists

Tr. No.	Treatments
T_1	*Trichoderma asperellum*
T_2	*T. harzianum*
T_3	*T. lignorum*
T_4	*Metarhizium anisopliae*
T_5	*T. koningii*
T_6	*Aspergillus niger*
T_7	*Aspergillus flavus*

Bacterial antagonists

Tr. No.	Treatments
T_8	*Bacillus subtilis*
T_9	*Pseudomonas fluorescens*
T_{10}	Control

Observations on linear colony growth/diameter (mm) of the test pathogen and the test bioagent was recorded at an interval of 24 hrs of incubation and continued up to seven days or till the untreated control plates were fully covered with mycelial growth of the test pathogen. Based on cumulative data, per cent mycelial growth inhibition of the test pathogen with the test bioagents, over untreated control was calculated by applying following formula (Arora and Upadhay, 1978) [1].

$$\text{Per cent Growth Inhibition} = \frac{\text{Colony growth in control plate-Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

Results and Discussion

The results obtained on mycelial growth and inhibition of *M. phaseolina* with seven fungal and two bacterial antagonists were represented in Table 1.

Table 1: *in vitro* bioefficacy of the bioagents against *M. phaseolina*, causing stem canker of pigeonpea

Tr. No.	Treatments	Colony Diam. of test pathogen * (mm)	% Inhibition
T_1	*Trichoderma asperellum*	14.32	84.09 (66.49)
T_2	*T. harzianum*	02.44	97.29 (81.13)
T_3	*T. lingorum*	19.57	78.26 (62.21)
T_4	*T. koningii*	58.89	34.57 (36.01)
T_5	*Metarhizhium anisopliae*	36.12	59.87 (50.69)
T_6	*Aspergilus niger*	06.37	92.92 (74.57)
T_7	*Aspergilus flavus*	05.41	93.99 (75.81)
T_8	*Pseudomonas fluorescens*	13.43	85.08 (67.28)
T_9	*Bacillus subtilis*	11.34	87.40 (69.21)
T_{10}	Control (untreated)	90.00	00.00 (00.00)
S.E. ±		0.51	0.94
C.D. (P = 0.01)		1.52	2.80

*Mean of three replications. Figure in parenthesis are arc sine transformed values.
Diam.: Diameter.

Results (PLATE1, Table 1 and Fig. 1) revealed that all of the bioagents evaluated exhibited fungistatic action against *M. phaseolina* cuasing stem canker of pigeonpea and significantly inhibited its growth, over untreated control. However, *Trichoderma harzianum* was found most effective with significantly least colony growth of the pathogen (02.44 mm) and its significantly highest inhibition (97.29%) which was followed by *Aspergilus flavus* (05.41 mm and 93.99%), *A. niger* (06.37 mm and 92.92%), *Bacillus subtilis* (11.34 mm and 87.40%), *Pseudomonas fluorescens* (13.43 mm and 85.08%), *T. asperellum* (14.32 mm and 84.09%), *T. lingorum* (19.57 mm and 78.26%), *Metarhizhium anisopliae* (36.12 mm and 59.87%) and *T. koningii* (58.89 mm and 34.57%), respectively of the pathogens colony growth and its inhibition.

Thus, the bioagents *viz*., *Trichoderma harzianum*, *Aspergilus flavus*, *A. niger*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *T. asperellum* were found most potential antagonists against *M. phaseolina* causing stem canker of pigeonpea. Thus, all of the nine bioagents evaluated by using dual culture technique were found fungistatic against *M. phaseolina* and significantly inhibited its mycelial growth, over untreated control. However, the bioagents found most effective in the order of merit were *Trichoderma harzianum*, *Aspergilus flavus*, *A. niger*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *T. asperellum*, *T. lingorum*, *Metarhizhium anisopliae* and *T. koningii*, respectively of the pathogen's colony growth and its inhibition.

These results are confirmed with earlier workers Lokesha and Benagi (2007) [14] reported that *T. harzianum*, *T. viride* and *P. fluorescens* significantly inhibited the mycelial growth of *M. phaseolina* causing dry root rot of pigeonpea (74-76%) by using Dual culture technique. Swamy *et al.*, (2018) among the bio-agents tested *T. harzianum* (Th-R) was found more effective as compared to other bio-control agents and inhibited maximum fungal growth (41.86%) of *M. phaseolina* causing stem canker of pigeonpea.

The results are similar with Tandel (2004) [25] reported *in vitro* interaction study of known antagonist by dual culture method and studied strong antagonistic effect on *M. phaseolina* causing leaf blight of green gram with *T. viride*, *T. harzianum*, *B. subtilis*, *T. longibrachyatum*, *T. koningii* and *Chaetomium globosum*. Kumar and Kelaiya (2021) [13], Gajera *et al.* (2012) [8] who studied in the laboratory experiment effect of *Trichoderma* spp. against *M. phaseolina*. The highest growth inhibition of test fungus was recorded by bioagents *T. koningi* (74.3%) followed by *T. harzianum* (61.4%). The results of this study are in harmony with Biswas and Sen, (2000) [3] studied *Trichoderma* spp. was to be a potential antagonist against *M. phaseolina* through colony interaction. Sreedevi *et al.* (2011) stated that all five *Trichoderma* spp. were antagonistic against *M. phaseolina* by using dual culture technique. Elham *et al.* (2016) [5] reported that all three isolates of *T. harzianum* significantly inhibited the growth of *M. phaseolina*. These earlier reports lend support to the present findings. A multiplicity of mechanisms involving mycoparasitism, antibiosis, lysis and hyphal interference could be attributed to the reduction in the mycelial growth of *M. phaseolina*. Gireesha *et al.*, (2025) [9] studied in *in vivo* experiments, it was found that, out of the bioagents tested, *T. harzianum* was the most effective in inhibiting maximum mycelial growth of *M. phaseolina* followed by *T. viride* (67.72%) and *P. fluorescens* (61.41%).

The specific activities of cell wall degrading enzymes such as chitinase, β-1, 3 glucanase, protease and cellulase were also recorded (Silva *et al.*, 2004) [21]. Rathore *et al.*, (2020) [20]. Parmar and Patel (2020) [18] confirmed *T. asperellum*, *T. harzianum*, *T. viride*, *T. longibacter*, *T. koningii* bioagents inhibit the growth of *M. phaseolina in vitro*. Lakhran and Ahir (2022) [15] also reported the effectiveness of *T. viride*, *B. subtilis* and *P. fluorescence* on radial growth of *M. phaseolina*. Gadde *et al.*, (2023a) [7] Bio-controls tested *Trichoderma asperellum* showed maximum mycelial inhibition (81.06%) while *Pseudomonas fluorescence* and *Bacillus subtilis* showed zero percent inhibition against *M. phaseolina* causing stem blight in pigeonpea. Indra *et al.*, (2024) [10] conformity with four fungal antagonists viz., *T. asperellum*, *T. harzianum*, *T. virens* and *T. koningiopsis*, three bacterial antagonists viz., *Bacillus licheniformis*, *B. amyloliquefaciens* and *B. subtilis* were effective against *M. phaseolina* causing root rot in mulberry.

Mycelial growth inhibition of *M. phaseolina* by *Trichoderma* spp. may be attributed to the secretion of extracellular cell wall degrading enzymes such as chitinase β-1, 3-glucanase, cellulose and lectin etc., production of secondary metabolites such as glioviridin, viridian and gliotoxin, also various mechanisms such as competition, lysis, antibiosis, production of volatile/non-volatile substances.

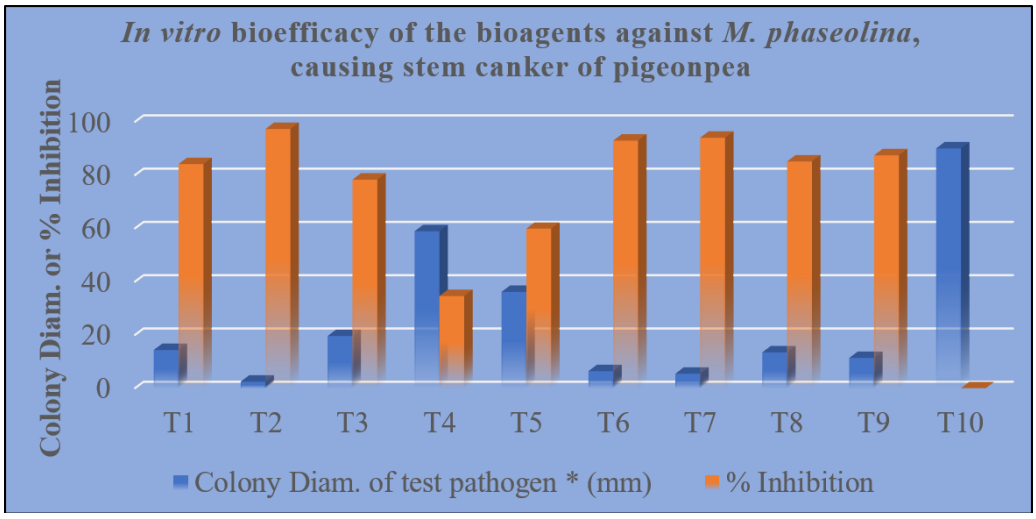

Fig 1: *In vitro* bioefficacy of the bioagents against *M. phaseolina*, causing stem canker of pigeonpea

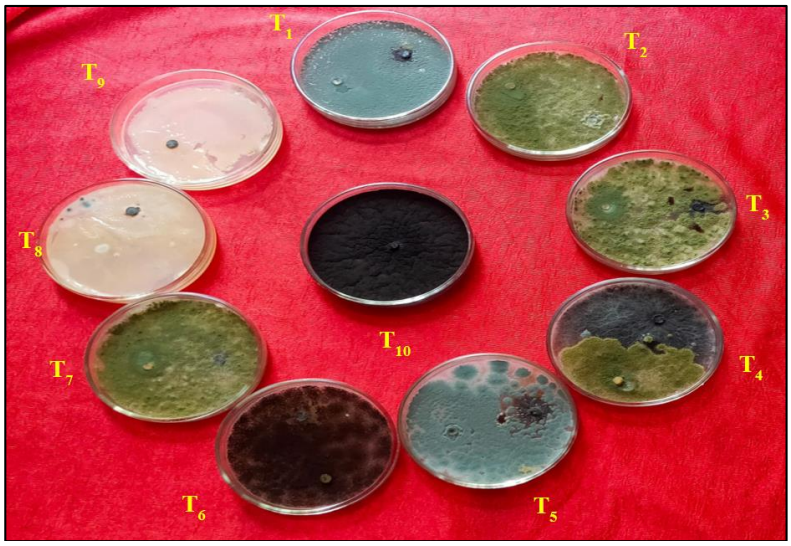

Plate 1: *In vitro* bio efficacy of the bioagents against *M. phaseolina*, causing pigeonpea stem canker

Conclusions

All the seven fungal and two bacterial antagonists tested, exhibited significant mycelial growth inhibition of *M. phaseolina*. However, *Trichoderma harzianum* was found most effective with significantly least colony growth of the pathogen and its significantly highest inhibition which was followed by *Aspergilus flavus*, *A. niger*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *T. asperellum*, *T. lingorum*, *Metarhizhium anisopliae* and *T. koningii* respectively of the pathogen's colony growth and its inhibition.

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Authors' Contributions

KAB developed the protocol for systematic designed and conducted *in vitro* experiment and prepared the original draft, DGH conceptualised, validated the experiment and reviewed the original draft, SNB helped in methodology, formal analysis and manuscript preparation, VMG and ASK helped in data curation. All authors read and approved the final manuscript.

Compliance with Ethical Standards Conflict of Interest: Authors do not have any conflict of interests to declare.

Ethical Issues: None

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