

Evaluation of Bio-Agents, Botanicals and Fungicides under *in vitro* conditions against *Macrophomina phaseolina* (Tassi) Goid Causing Charcoal Rot of Cowpea

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ABSTRACT: In recent years, it has been observed that charcoal rot of cowpea caused by *Macrophomina phaseolina* is becoming major problem in arid regions of India especially Rajasthan. The main aim of this investigation was to find out the suitable controlling measure for *Macrophomina phaseolina* which will benefit farmers in increasing the production of cowpea. The *in vitro* study was taken to evaluate the efficacy of bio-agents, botanicals and fungicides against *M. phaseolina*. The antagonistic action of two fungal bio-agents viz., *Trichoderma harzianum* and *T. viride* and two bacterial bio-agents viz., *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated. Similarly, seven botanicals viz., *Azadirachta indica*, NSKE, *Datura stramonium*, *Tinospora cordifolia*, *Leptadenia pyrotechnica*, *Allium sativum* and *Ocimum sanctum* and five fungicides viz., tebuconazole 50% + trifloxystrobin 25% WG, carbendazim 12% + mancozeb 63% WP, captan 70%WP, hexaconazole 5% + captan 70%WP and carboxin 37.5% + thiram 37.5% WS were evaluated against *M. phaseolina*. Among the bio-agents maximum growth inhibition was recorded in *T. harzianum* (73.33%) as compared to other bio-agents. Among botanicals the maximum mycelial growth inhibition was recorded by *Allium sativum* (53.70%, 79.26%, 87.04% and 98.15%) followed by *Azadirachta indica* (39.63%, 43.33%, 52.59% and 57.41%) at 5, 10, 15 and 20% concentrations respectively. Least growth inhibition was found in *Ocimum sanctum* (6.67%, 8.15%, 12.96% and 18.89%). Among fungicides, mycelial growth inhibition at all the tested concentrations viz., 0.05, 0.1 and 0.2 % was found maximum in tebuconazole 50% + trifloxystrobin 25% WG which showed 100% growth inhibition at all concentrations while captan 70% WP was found least effective (70.70%, 83.59% and 100% respectively).

Keywords: Bio-agents, botanicals, fungicides, *in vitro*, *Macrophomina phaseolina*.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) has become a crucial legume crop in Indian sub-continent owing to its versatility. It is found in tropical, sub-tropical, arid and semi-arid regions all over the world with differing morphology and ecology (Ng, 1990, Quinn, 1999 and PainoD'urzo *et al.*, 1990). It has origin from Africa and belongs to the family Fabaceae (Cobley, 1956) It can be used as pulse, vegetable, fodder and green manure crop and generally grown as an intercrop with millets such as sorghum (Asiwe, 2007). Cowpea is also known as 'vegetable meat' due to its high content of protein (26.61%). It is also a rich source of carbohydrates (56.24%), lipids (3.99%) and gross energy (1.51%) (Owolabi *et al.*, 2012). The crop is usually preferred by farmers because it enriches the soil with nitrogen through biological nitrogen fixation and produce highly nutritious cattlefeed (Blade *et al.*, 1997).

The crop is affected by many fungal, bacterial, viral, phytoplasmal and nematodal diseases (Emechebe and Lagoke 2002). The major diseases are root rot, charcoal rot, rust, wilt, leaf spots, cercospora leaf blight, powdery mildew, yellow mosaics etc. Among all the fungal diseases of cowpea, the most destructive is charcoal rot of cowpea caused by *Macrophomina phaseolina* causing potential yield losses (Lodha and Singh 1984). The pathogen *Macrophomina phaseolina* is reported to be soil, seed and stubble borne (Smith, 1969). A charcoal like appearance can be observed at the collar region of affected host plants, hence the disease is named charcoal rot. *Macrophomina phaseolina* affects the cowpea crop at all the developing stages from germination to flowering to adult. In general, they gain entry into host by natural openings (Bressano *et al.*, 2010). The severe incidence is usually observed at post-flowering stage. In advance stage

scattered sclerotial bodies may be seen on the affected tissues (Singh and Srivastava 1988). The disease is most severe at a temperature range of 28-32°C. Owing to its seed and soil borne nature, the disease can be managed in an integrated manner through the use of bio-agents (El-Barougy *et al.*, 2009), botanicals (Dubey *et al.*, 2009) and chemical fungicides (El- Baz, 2007). Considering the importance of the disease and the heavy losses incurred by it, the investigation was undertaken.

MATERIALS AND METHODS

An experiment was conducted in *Kharif* 2018 for testing the efficacy of bio-agents, botanicals and fungicides against *M. phaseolina* in laboratory conditions.

A. Bio-agents

Two fungal antagonists *viz.*, *Trichoderma harzianum* and *T. viride* and bacterial antagonists *Bacillus subtilis* and *Pseudomonas fluorescens* were evaluated *in vitro* against *M. phaseolina* using dual culture method and paper disc method (Loo *et al.*, 1945) respectively. Seven days old culture of the test fungus (grown on PDA) and bio-agents (fungal bio-agents grown on PDA and *P. fluorescens* and *B. subtilis* grown on PAF and NA media respectively) were used. For testing fungal bio-agents, one mycelial disc each of the test fungus and bio-agent were placed at periphery opposite to each other on solidified PDA media. Three replications were maintained. For testing bacterial antagonists, four sterilized filter paper discs of 5mm diameter were placed in bacterial suspension and were placed equidistantly at periphery with test fungal disc at the centre on solidified PDA media. Three replications were maintained. PDA plates inoculated only with culture disc of test fungus were considered as control. The plates were placed in BOD incubator at 28±2°C. The observations were recorded till maximum mycelial growth occurred in control plates.

B. Botanicals

Seven botanicals *viz.*, *Azadirachta indica*, NSKE, *Datura stramonium*, *Tinospora cordifolia*, *Leptadenia pyrotechnica*, *Allium sativum* and *Ocimum sanctum* were evaluated against *M. phaseolina* under *in-vitro* condition using poisoned food technique (Nene and Thapliyal, 1973). One hundred gram of fresh, healthy, thoroughly washed and air dried plant parts (leaves/stem/cloves/kernals) were macerated in 100ml distilled water (w/v) in mortar and pestle. The extract was strained through muslin cloth and further filtered through Whatmann No. 1 filter paper and centrifuged at 10000 rpm for 5-10 minutes. It was marked as stock solution. The stock solutions of these phytoextracts (5, 10, 15 and 20 %) were mixed with 95, 90, 85 and 80 ml of sterilized and cooled PDA media respectively. This gives 5, 10, 15 and 20 % concentrations of botanical

extract. Twenty ml of such medium was poured in sterilized Petri plates under aseptic conditions, was cooled and solidified. Mycelial discs of *M. phaseolina* (5mm) was cut from the periphery of culture using sterilized cork borer and placed at the centre of each plates. Three replications were maintained. PDA plates inoculated only with culture disc of test fungus were considered as control. The plates were placed in BOD incubator at 28±2°C. The observations were recorded till control plates were fully covered with mycelial growth of test fungus.

C. Fungicides

Five fungicides *viz.*, tebuconazole 50% + trifloxystrobin 25% WG, carbendazim 12% + mancozeb 63% WP, captan 70% WP, hexaconazole 5% + captan 70% WP and carboxin 37.5% + thiram 37.5% WS were tested at a concentration of 0.05%, 0.1% and 0.2% against *M. phaseolina* using poisoned food technique (Nene and Thapliyal, 1973). 100 ml PDA medium was autoclaved at 121.6°C for 15 minutes. Required quantity of fungicides were calculated and mixed thoroughly with the sterilized medium separately. About 20 ml of poisoned media was poured in sterilized Petri plates and inoculated with 5 mm mycelial disc of *M. phaseolina*. Three replications were maintained for each treatment. The control plates without fungicides were inoculated with test pathogen and kept at 28±2°C in BOD incubator.

Per cent inhibition for bio-agents, botanicals and fungicides was calculated using the following formula (Vincent, 1947):

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where,

C = Radial growth of *M. phaseolina* in control (mm)

T = Radial growth of *M. phaseolina* in presence of antagonist (mm)

RESULTS AND DISCUSSION

A. In vitro bio-efficacy of bio-agents

Results (Table 1, Fig. 1 & Plate 1) indicates that all the bio-agents exhibited antagonistic activity against *M. phaseolina* and significantly inhibited mycelial growth of test fungus over control. The mycelial growth was least in the presence of *T. harzianum* (24 mm) followed by *T. viride* (30.97 mm), *P. fluorescens* (45.03 mm) and *B. subtilis* (54.93 mm). Maximum growth inhibition was recorded in *T. harzianum* (73.33%) which was significantly superior to the rest of the treatments *viz.*, *T. viride* (65.59%), *P. fluorescens* (49.96 %) and *B. subtilis* (38.96%). Babu *et al.*, (2002), Doley and Jite (2012), Bimla and Gaur (2016), Khaledi and Taheri (2016) and Meena and Gangopadhyay (2016) found *Trichoderma* spp., antagonistic to *M. phaseolina* in the research conducted by them.

Table 1: Efficacy of bio-agents on mycelial growth of *M. phaseolina* in vitro

Treatment	Mycelial growth (mm)	Growth inhibition (%)
T ₁ <i>T.harzianum</i>	24.00 (29.31)*	73.33
T ₂ <i>T.viride</i>	30.97 (33.76)	65.59
T ₃ <i>P.fluorescens</i>	45.03 (42.13)	49.96
T ₄ <i>B.subtilis</i>	54.93 (47.82)	38.96
T ₅ Control	90.00 (71.54)	-
S.Em±	1.79	
CD (P = 0.05)	5.71	
CV (%)	6.33	

* Figures in parenthesis are angular transformed values

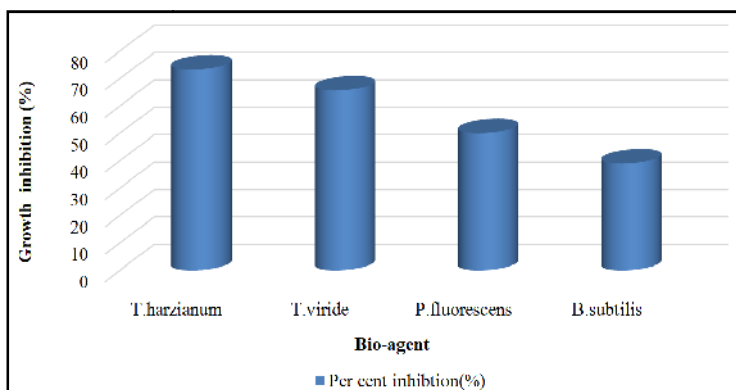


Fig. 1. Efficacy of different bio-agents on mycelial growth of *M. phaseolina* in vitro.



Plate 1. Efficacy of different bio-agents on mycelial growth of *M. phaseolina* in vitro.

B. Testing the botanicals against M. phaseolina in vitro
The results (Table 2, Fig. 2 & Plate 2) revealed that all the botanicals/plant leaf extracts tested *in vitro* were found significantly effective in reducing the percentage mycelial growth of *M. phaseolina* over untreated control.

At 5% concentration least mycelial growth of *Macrophomina phaseolina* was recorded in *Allium sativum* (41.67 mm) followed by *Azadirachta indica* (54.33 mm) and *Datura stramonium* (65.67 mm) thus showing maximum mycelium growth inhibition of

53.70%, 39.63% and 27.04% respectively. Maximum mycelium growth was observed in *Ocimum sanctum* (84 mm) thus exhibiting least mycelial growth inhibition (6.67%).

At 10% concentration of plant extract, least mycelial growth of *Macrophomina phaseolina* was recorded in *Allium sativum* (18.67 mm) followed by *Azadirachta indica* (51mm) and *Datura stramonium* (52.33 mm). *Ocimum sanctum* (82.67 mm) showed maximum mycelium growth. All the tested botanicals significantly reduced the mycelial growth over the control.

Maximum growth inhibition of 79.26% was recorded in *Allium sativum* followed by *Azadirachta indica* (43.33%) and *Datura stramonium* (41.85%). It is evident from the data that 10% concentration was more effective than the 5% concentration from all the tested botanicals and resulted more growth inhibition. *Ocimum sanctum* (8.15%) was least effective.

At 15% concentration of plant extract, least mycelial growth of *Macrophomina phaseolina* was also recorded in *Allium sativum* (11.67 mm) followed by *Azadirachta indica* (42.67 mm) and *Datura stramonium* (48.33 mm). *Ocimum sanctum* (78.33mm) showed maximum mycelium growth. The trends of growth inhibition were similar as reported in 5% & 10% concentrations. Among these botanicals, maximum growth inhibition was recorded in *Allium sativum* (87.04%) followed by *Azadirachta indica* (52.59%) and *Datura stramonium* (46.30%). The least effective botanical was *Ocimum sanctum* (12.96%). Data revealed that 15% concentration is further more effective in growth inhibition as compared to 5% and 10% concentration.

At 20% concentration of botanical extract was found most effective in inhibiting the growth of *Macrophomina phaseolina*. The similar pattern was followed as mentioned in the mycelium growth and growth inhibition by 5%, 10% and 15%. Least mycelial growth of *Macrophomina phaseolina* was recorded in *Allium sativum* (1.67 mm) followed by *Azadirachta indica* (38.33 mm) and *Datura stramonium* (42.33 mm). *Ocimum sanctum* (73mm) showed maximum mycelium growth. Maximum growth inhibition was recorded in *Allium sativum* (98.15%) followed by *Azadirachta indica* (57.41%) and *Datura stramonium* (52.96%). Among all the tested botanical extract the least effective was *Ocimum sanctum* showing a growth inhibition of 18.89%.

The results of present investigation corroborated with the findings of Dhingani *et al.*, (2013); Meena *et al.*, (2014) found that *Allium sativum* significantly inhibited the mycelial growth of *M. phaseolina*.

Table 2: Efficacy of different botanicals on mycelial growth of *M. phaseolinain vitro*.

Treatment	Mycelial growth (mm) at conc.				Growth inhibition (%) at conc.			
	5 %	10 %	15 %	20 %	5 %	10 %	15 %	20 %
T ₁ <i>Azadirachta indica</i> (Neem leaf extract)	54.33 (47.47)*	51.00 (45.56)	42.67 (40.76)	38.33 (38.23)	39.63	43.33	52.59	57.41
T ₂ <i>Azadirachta indica</i> (NSKE)	73.67 (59.11)	63.67 (52.90)	58.33 (49.70)	50.33 (45.17)	18.15	29.26	35.19	44.07
T ₃ <i>Datura stramonium</i> (Datura leaf extract)	65.67 (54.13)	52.33 (46.32)	48.33 (44.03)	42.33 (40.05)	27.04	41.85	46.30	52.96
T ₄ <i>Tinospora cordifolia</i> (Giloe vine extract)	78.00 (62.02)	76.67 (61.11)	74.33 (59.55)	60.67 (51.14)	13.33	14.81	17.41	32.59
T ₅ <i>Leptadenia pyrotechnia</i> (Kheep stem extract)	83.67 (66.29)	81.63 (64.61)	75.33 (60.25)	61.00 (51.33)	7.04	9.30	16.30	32.22
T ₆ <i>Allium sativum</i> (Garlic clove extract)	41.67 (40.19)	18.67 (25.59)	11.67 (19.86)	1.67 (7.39)	53.70	79.26	87.04	98.15
T ₇ <i>Ocimum sanctum</i> (Tulsi leaf extract)	84.00 (66.42)	82.67 (65.38)	78.33 (62.26)	73.00 (58.67)	6.67	8.15	12.96	18.89
T ₈ Control	90.00 (71.54)	90.00 (71.54)	90.00 (71.54)	90.00 (71.54)	-	-	-	-
S.Em±	1.65	0.95	1.51	0.66				
CD (P = 0.05)	5.00	2.87	4.55	1.99				
CV (%)	4.01	2.54	4.35	2.19				

*Figure in parenthesis are angular transformed values

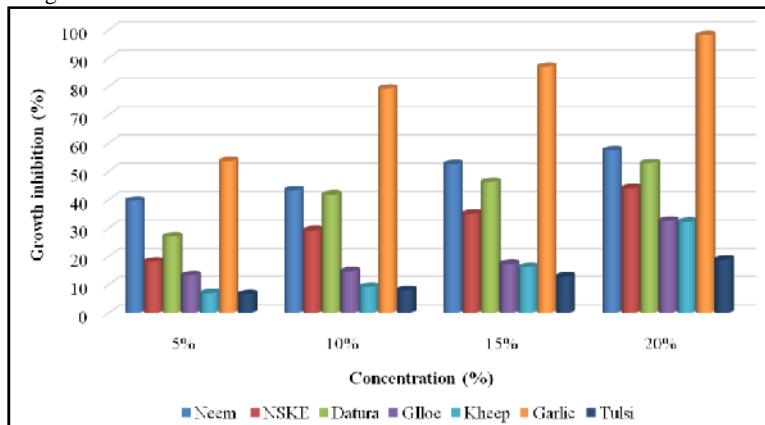


Fig. 2. Efficacy of different botanicals on mycelial growth of *M. phaseolinain vitro*.

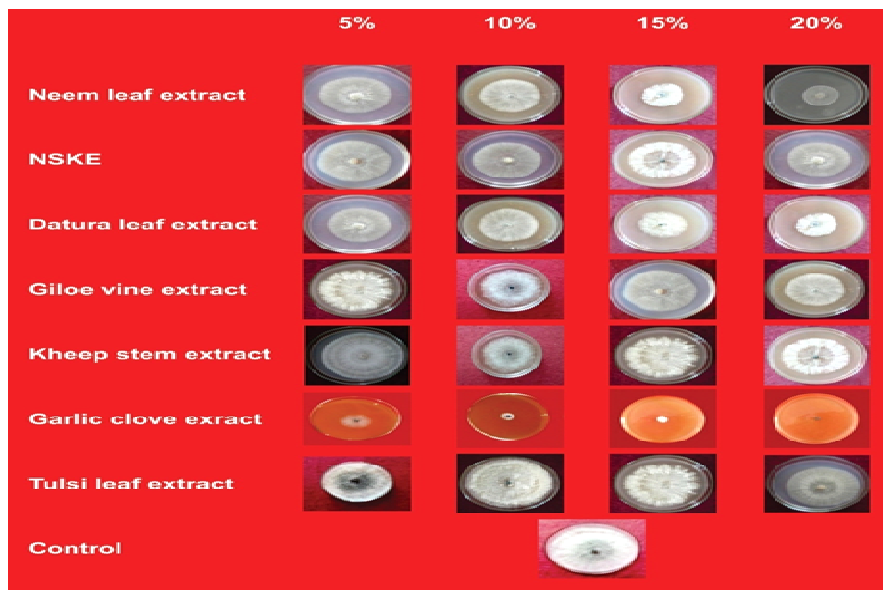


Plate 2: Efficacy of different botanicals on mycelial growth of *M. phaseolinai* in vitro.

C. Efficacy of fungicides

Five fungicides viz., tebuconazole 50% + trifloxystrobin 25% WG, carbendazim 12% + mancozeb 63% WP, captan 70% WP, hexaconazole 5% + captan 70% WP and carboxin 37.5% + thiram 37.5% WS were tested at a concentration of 0.05%, 0.1% and 0.2% against *M. phaseolina* using poisoned food technique (Nene and Thapliyal 1973) in vitro. The table 3 & plate 3 shows the effectiveness of all the tested fungicides.

Among the tested fungicides at 0.05%, least mycelial growth of *Macrophomina phaseolina* was recorded in tebuconazole 50% + trifloxystrobin 25% WG (0 mm) followed by carbendazim 12% + mancozeb 63% WP (6.57 mm) and carboxin 37.5% + thiram 37.5% WS (11.47 mm). Captan 70% WP (26.37 mm) showed the highest mycelia growth. The most effective fungicide regarding growth inhibition was tebuconazole 50% + trifloxystrobin 25% WG (100%) followed by

carbendazim 12% + mancozeb 63% WP (92.30%) and carboxin 37.5% + thiram 37.5% WS (87.26%). Captan 70% WP (70.70%) was found to be least effective.

At 0.1% concentration, no mycelium growth was observed in tebuconazole 50% + trifloxystrobin 25% WG, carbendazim 12% + mancozeb 63% WP and carboxin 37.5% + thiram 37.5% WS. Maximum mycelium growth was recorded in Captan 70% WP (12.23 mm). Cent percent growth inhibition was observed with tebuconazole 50% + trifloxystrobin 25% WG, carbendazim 12% + mancozeb 63% WP and carboxin 37.5% + thiram 37.5% WS. Captan 70% WP (83.59%) was found to be least effective.

The fungicidal concentration of 0.2% is most effective since no mycelial growth and 100% growth inhibition was observed in all tested fungicides. Thus the concentration of 0.2% is highly effective in inhibiting the mycelial growth of *M. phaseolina*.

Table 3: Efficacy of different fungicides against *M. phaseolina* in vitro.

Treatment	Mycelial growth (mm) at conc.			Growth inhibition (%) at conc.		
	0.05 %	0.1 %	0.2 %	0.05 %	0.1 %	0.2 %
T ₁ tebuconazole 50% + trifloxystrobin 25% WG	0.00 (0.00)*	0.00 (0.00)	0.00 (0.00)	100.00	100.00	100.00
T ₂ carbendazim 12% + mancozeb 63% WP	6.57 (14.71)	0.00 (0.00)	0.00 (0.00)	92.30	100.00	100.00
T ₃ Captan 70% WP	26.37 (30.86)	12.23 (20.42)	0.00 (0.00)	70.70	83.59	100.00
T ₄ hexaconazole 5% + captan 70% WP	14.54 (22.26)	4.73 (12.35)	0.00 (0.00)	83.11	94.74	100.00
T ₅ carboxin 37.5% + thiram 37.5% WS	11.47 (19.76)	0.00 (0.00)	0.00 (0.00)	87.26	100.00	100.00
T ₆ Control	90.00 (71.54)	90.00 (71.54)	90.00 (71.54)	-	-	-
S.Em±	1.15	0.69	-			
CD (P= 0.05)	3.58	2.13	-			
CV (%)	8.03	6.66	-			

*Figure in parenthesis are angular transformed values

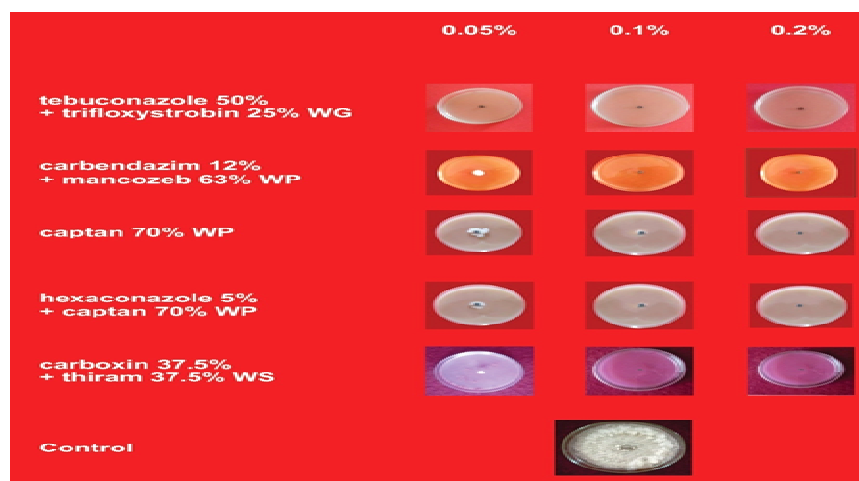


Plate 3: Efficacy of different fungicides against *M. phaseolina* *in vitro*.

The results resembled with the work done by Sangappa and Mallesh (2016) whose findings revealed that carbendazim fungicide gave maximum inhibition of mycelial growth at concentrations of 0.05, 0.1 and 0.2%. Ramadoss and Sivapraskasam (1994) reported that carbendazim completely inhibited the sclerotia development in the pathogen. Nativo resulted in minimum colony diameter of pathogen because it disrupts its metabolism and inhibits its growth and development. It forms covalent bond with sclerotia of pathogen (El-Fiki *et al.*, 2004). Fungicides, carbendazim, carbendazim, + mancozeb and captan were reported inhibitory to *M. phaseolina* (Bainade *et al.*, 2007; Suryawanshi *et al.*, 2008, Kar and Sahu, 2009; Chaudhari and Chaudhari 2012).

SUMMARY AND CONCLUSION

In the following study, in case of bioagents, *Trichoderma harzianum*, *Allium sativum* among phytoextracts and tebuconazole 50% + trifloxystrobin 25% WG among fungicides were found highly effective in inhibiting the mycelial growth of *M. phaseolina* under *in vitro* conditions. Thus promising results can be achieved if they can be taken into consideration at field levels for efficient management of this disastrous pathogen.

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Conflict of Interest. None.

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