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Variability in sensitivity among different host origin-*Macrophomina phaseolina* isolates to azoxystrobin fungicide

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ABSTRACT

The charcoal rot fungus, *Macrophomina phaseolina*, was isolated from chickpea, pigeonpea, groundnut and jute root tissues collected from Bihar and Uttar Pradesh. Variability in isolates was recognized *i.e.* feathery growth for pigeonpea and jute isolates, and restricted growth for chickpea and soybean isolates. The sensitivity of *M. phaseolina* from the four hosts was tested for azoxystrobin, a respiration inhibitor (QoI group) fungicide. The minimum inhibitory concentration was lower (10 ppm) for isolates with restricted growth and higher (between 100 and 150 ppm) for isolates with feathery growth. Concentrations of this fungicide pose significant impact ($P < 0.01$) on time requirement for growth of isolate. We found strong effectiveness of azoxystrobin to inhibit the growth of slow–growing population of *M. phaseolina*. Moreover, this fungicide can also exploit for the fast–growing population of *M. phaseolina* but more time will be required, to act on such isolates, for better result of azoxystrobin. Our results indicate that the response of different isolates varied to concentrations of azoxystrobin; this could be interpreted that the fungicidal application may be performed only after the quantitative estimation of the prevailing population type in the field as because various populations of *M. phaseolina* may be available in an area. Therefore, our results advocate for judicious use of fungicide (azoxystrobin) application, which ultimate restrict the hazardous impact on soil health.

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INTRODUCTION

Charcoal root rot, also known as black root rot, is a disease commonly affecting many agricultural and horticultural crops. The fungus is reported to cause infection in more than 500 plant species (Singh *et al.*,

1990; Su *et al.,* 2001 and Saleh *et al.,* 2010). Charcoal rot is also known by damping-off, dry root-rot, wilt, leaf blight and ashy stem blight due to various nature of the expression of symptom (Singh *et al.,* 1990). The causal agent of this disease is a fungus, *Macrophomina phaseolina* (Tassi.) Goid., which is imperfectly known as *Rhizoctonia bataticola*. This is a pathogen with multiple host range and can infect several plant organs like roots, stems, leaves and pods of different plant species. The common hosts with agricultural importance of this pathogen include sorghum, maize, tall grass prairie, peanut, soybean, sesame, sunflower, chickpea, lentil, cowpea, bean, cotton, chili pepper, tomato and watermelon. *M. phaseolina* is one of the most destructive plant pathogens in the tropics and subtropics (Reuveni *et al.,* 1983 and Kaur *et al.,* 2012). The pathogen is a threat to chickpea, pigeonpea, groundnut, and jute fields in Bihar where dry of subtropical environment provokes the disease. The ideal temperature for the active fungal growth is 28-32ºC under moisture stress condition.

Impact of climate change has accelerated the incidence and severity of this disease in many provinces of India where the geography is supported with low soil moisture availability. The charcoal rot is a disease, being favoured by high temperatures and water stress, has increased with importance in recent years. This disease is currently categorised as acute to emerging disease severity level (Savary *et al.,* 2011). As management option, crop rotation is not considered efficient alternative since the fungus has competitive saprophytic ability (Pearson *et al.,* 1984). The management also includes the use of clean seeds as well as their coating with fungicides before sowing. Theoretically, the most practical and economical way to manage the charcoal rot problem is to employ resistant cultivars; however, not many genotypes are found to be resistant to this disease. Additionally, it has been often seen the ineffectiveness of fungicide application to manage this disease is due to variability among populations of the same pathogen showing different properties. The variation, indeed, aid the pathogen to adapt and survive in diverse environments (Kushwaha *et al.,* 2017).

Both monocots and dicots can be infected by the soil borne pathogen *M. phaseolina*. The heterogeneous host specificity enables this pathogen for non-uniform distribution in the soil (Mayek-Perez *et al.,* 2001 and Su *et al.,* 2001). Thus, better understanding of *M. phaseolina* population diversity among various host origin will assist the strategists in optimization the management to enable different crops secure over broader geographical areas. It is therefore necessary to evaluate the fungicide to improve the management strategy against this pathogen. We evaluated azoxystrobin to different isolates of *M. phaseolina* obtained from various hosts. Azoxystrobin is isolated from *Strobilurus tenacellus*, which is a wood decaying mushroom. It has the property to inhibit respiration in plant pathogenic fungi. The principle action of azoxystrobin is inhibition of mitochondrial respiration by prohibiting the electron transport system in the fungal mitochondria. The process is operated by binding at a specific site on cytochrome b. The mycelial disintegration and inhibition of sporulation are the important effects of azoxystrobin on the plant pathogenic fungi (Harrison and Tedford, 2002). Therefore, emphasising the impact of this fungicide, the objectives of this study were to evaluate the reaction of isolates from diverse hosts to azoxystrobin. The present study is performed to evaluate the efficiency of a second generation fungicide (azoxystrobin, a respiration inhibitor) having trade name Amistar™, Syngenta-India (23% a.i.). The novelty of our investigation lies in the fact that the use of azoxystrobin fungicides has not been evaluated against charcoal rot pathogen of various host origin.

MATERIAL AND METHODS

Sample collection of fungal isolates :

Isolates of *Macrophomina phaseolina* were collected from five districts comprising Bihar and Uttar Pradesh (Table A). A total of 23 isolates were sampled from four hosts *viz.*, chickpea, pigeonpea, groundnut and jute. Characteristics symptom expressing plants, bearing fungal mycelium and exhibiting charcoal powder, were chosen for sample collection. Diseased samples were packed in paper bags and then in 10 × 15 cm polyethylene bags, labeled, brought to the laboratory and stored at 4°C until processed for identification.

Isolation, purification and storage of *M. phaseolina*:

The fungus was isolated from the collar and root regions of the plant tissue from the mentioned crops. The diseased samples were processed within a day after appearing the laboratory. The infected samples were cut into 0.5–1.0 cm-pieces and surface sterilized with 1 per

Table A: Isolates of *Macrophomina phaseolina* from different hosts

Sr.No.	Isolate	Host of origin	Location of collection	Year of collection
1.	MpCP4	Chickpea	Mokameh	2013
2.	MpCP6	Chickpea	Mokameh	2013
3.	MpCP7	Chickpea	Mokameh	2013
4.	MpCP8	Chickpea	Mokameh	2013
5.	MpCP9	Chickpea	Mokameh	2013
6.	MpCP11	Chickpea	Sabour	2013
7.	MpCP16	Chickpea	Sabour	2013
8.	MpCP23	Chickpea	Sabour	2014
9.	MpCP28	Chickpea	Sabour	2014
10.	MpCP31	Chickpea	Sabour	2014
11.	MpCP32	Chickpea	Sabour	2014
12.	MpCP36	Chickpea	Sabour	2015
13.	MpCP37	Chickpea	Sabour	2015
14.	MpCP41	Chickpea	Sabour	2015
15.	MpPP12	Pigeonpea	Varanasi	2012
16.	MpPP7	Pigeonpea	Sabour	2014
17.	MpPP10	Pigeonpea	Sabour	2015
18.	MpGN1	Groundnut	Jalalgarh	2014
19.	MpGN2	Groundnut	Jalalgarh	2014
20.	MpJ3	Jute	Katihar	2014
21.	MpJ6	Jute	Katihar	2014
22.	MpJ7	Jute	Katihar	2015
23.	MpJ8	Jute	Katihar	2015

cent sodium hypochlorite for 1 minute and thereafter rinsed thrice in sterilized distilled water for 20 seconds at each time. The pieces were placed on potato dextrose agar (PDA) medium in Petri dishes and incubated under dark condition at 27±2°C for 7–8 days. The actively growing mycelium at the margin of the colony of *M. phaseolina* was taken, and inoculated onto fresh Petri dishes containing glucose agar medium (glucose, 20 g; agar, 20 g and water, 1 lit). These plates were also incubated under dark condition at 27±2°C for 7–8 days. For an isolate, the mycelium was looped and transferred into a PDA slant and incubated as described above. The mycelium in the slants allowed to grow until the surface of PDA was covered with a dense layer of the fungal colony. Three sets of slants for each isolate were developed.

Identification of *M. phaseolina*:

Two types of growth pattern of mycelium were recognized *viz.*, feathery and restricted (Table 1). Colony colour was also observed and ranged between white and grayish-black. These patterns of fungal colonies confirmed with our previous attempts (*not communicated*), which made apparent our experience in order to obtain *M. phaseolina* isolates. None of the isolates found with sclerotia producing ability. Therefore, a pathogenicity test performed to be confirming the *M. phaseolina* isolate.

Fungicide sensitivity assay :

The experiment was carried out following poisoned food technique to evaluate sensitivity of *M. phaseolina* isolates of four host origin *viz.*, chickpea, pigeonpea, groundnut and jute. The isolates were tested for four concentrations of the fungicide azoxystrobin. At first, a stock solution of Amistar™, Syngenta-India (23% a.i.) was prepared by dissolving an appropriate amount in sterile distilled water. Thereafter, the evaluating concentrations of 10, 50, 100 and 150 ppm were obtained by adding the proper quantity of stock suspension of the

Table 1 : Characteristics of *Macrophomina phaseolina* isolates

Sr. No.	Isolate	Host of origin	Colony colour	Growth pattern	
				Feathery	Restricted
1.	MpCP4	Chickpea	Whitish-gray	–	+
2.	MpCP6	Chickpea	Gray	–	+
3.	MpCP7	Chickpea	Gray	–	+
4.	MpCP8	Chickpea	White	–	+
5.	MpCP9	Chickpea	Whitish-gray	–	+
6.	MpCP11	Chickpea	Gray	–	+
7.	MpCP16	Chickpea	Gray	–	+
8.	MpCP23	Chickpea	Gray	–	+
9.	MpCP28	Chickpea	Gray	–	+
10.	MpCP31	Chickpea	Gray	–	+
11.	MpCP32	Chickpea	White	–	+
12.	MpCP36	Chickpea	Whitish-gray	–	+
13.	MpCP37	Chickpea	Whitish-gray	–	+
14.	MpCP41	Chickpea	Whitish-gray	–	+
15.	MpPP12	Pigeonpea	Grayish-black	+	–
16.	MpPP7	Pigeonpea	Grayish-black	+	–
17.	MpPP10	Pigeonpea	Grayish-black	+	–
18.	MpGN1	Groundnut	Gray	–	+
19.	MpGN2	Groundnut	Gray	–	+
20.	MpJ3	Jute	Grayish-black	+	–
21.	MpJ6	Jute	Grayish-black	+	–
22.	MpJ7	Jute	Grayish-black	+	–
23.	MpJ8	Jute	Grayish-black	+	–

fungicide to sterilised PDA medium at about 45°C. Fungicide was not added to control treatments where the isolates were incubated under same conditions of other fungicide amended treatments on PDA. Fungicide-amended and non-amended PDA for each isolate was poured into 8 cm diameter plates under aseptic conditions. Mycelium-grown of agar plugs (5 mm) were separated from the edge of colony of the original non-amended plates and placed on fresh plates amended with azoxystrobin for 10, 50, 100 and 150 ppm medium as treatments. Fungal growth was measured at 24 hours interval when at least one isolate touched the periphery of the non-amended plate. The fungal growth in fungicide amended plates was compared with non-amended plates. The inoculated plates were incubated at 28±2°C under dark. Additionally, the inhibition per cent was calculated as suggested by Topps and Wain (1957). Different growth pattern was also observed for different isolates obtained from different host of *M. Phaseolina* during research carried out under *in vitro* conditions.

Statistical design and data analysis :

The plates were subjected to factorial arrangement according to completely randomised design (Gomez and Gomez, 1984). Analysis of variance (ANOVA) was performed to identify the effect of isolate response to the concentration of fungicide azoxystrobin measured

Table 2 : Analysis of variance for the effect of different host-derived isolates of *Macrophomina phaseolina* on the concentration of azoxystrobin and the progress of mycelium over time

Sources of variation	df	Host of origin for isolate[a]							
		Chickpea		Groundnut		Pigeonpea		Jute	
Concentration (C)	4	369.1	**	188.1	**	21.8	*	127.1	**
Incubation time (T)	3	53.9	*	36.7	*	429.2	**	912.7	**
C × T	12	31.4	*	19.2	*	2.4	*	19.1	*
CV		31.7		40.4		12.5		7.8	

[a] Digits are F-value

* and ** indicate significance of values at P<0.05 and 0.01, respectively

over period of time (Table 2). The experiment was performed with three replications for each treatment. Radial growth of the fungus for each isolate was measured using a transparent ruler bisecting the colony. Average value was therefore calculated from the two bisecting assessments and used for analysis. The analysis was performed with a statistical software SPSS version 16.

RESULTS AND DISCUSSION

Fungicide azoxystrobin (Amistar™, Syngenta-India) belonging Q_OI group, known for respiration inhibition, was tested to determine its effect on various isolates of *Macrophomina phaseolina* sampled from different hosts. Our previous attempt provides a new vista that the type of population of *M. phaseolina* should be evaluated to avoid non-judicious application of fungicide to the soil (Gupta *et al*., 2014).

Phenotypic assessments :

At first, we observed the morphological pattern of *M. phaseolina* isolates in the *in-vitro* tests (Table 1). The collected isolates exhibited white to grayish-black colonies in plates. Two types of growth patterns have also been explored *viz.*, feathery and restricted. Feathery isolates are in general darker in appearance and faster to grow under artificial environment (Fig. 1A). However, the isolates with restricted colonies are lighter in colour and relatively poor to grow in similar condition. Isolates with feathery growth reached the periphery of 8 cm diameter plate after 3 days of inoculation, whereas the restricted isolates took over 7 days to cover the plates (Fig. 2). A distinct type of colony was observed by Wang *et al*. (2011) they found some colonies with aerial mycelium in China. A work of Iqbal and Mukhtar (2014) exhibits variation in radial growth of *M. phaseolina* isolates collated from several districts. They found the growth of the fast growing colonies ranged between 32.00 to 87.17 mm observed after 7 days of incubation. They concluded the isolates have gone over 80 mm were considered fast growing colonies and the isolates grown below 61 mm were categorised as slow growing colonies. Our fast growing isolates represents luxurious growth over the findings of Iqbal and Mukhtar (2014).

Efficacy of azoxystrobin on *M. phaseolina*:

Different host-originated isolates of *M. phaseolina* was tested to recognise the efficacy effect of azoxystrobin on such isolates (Fig. 1B). The fungicide was examined for five concentrations *i.e*. 10, 50, 100, and 150 ppm along with 0 ppm which was treated as control. The feathery isolates (collected from jute) had not affected by this fungicide even upto 150 ppm; however, the isolates of pigeonpea shown a meagre

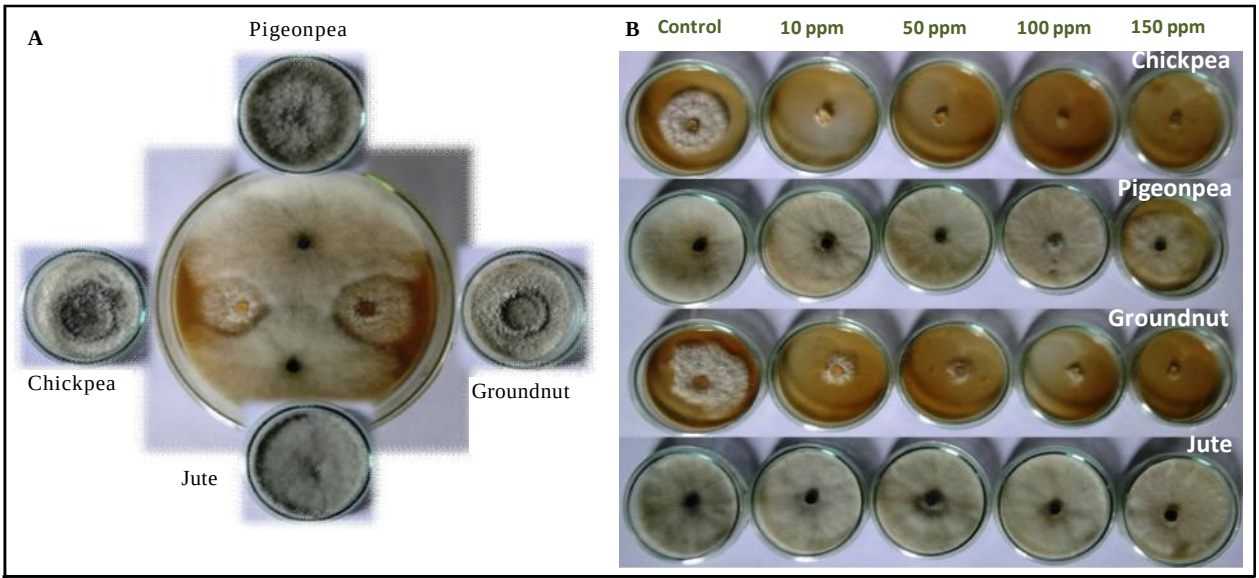

Fig. 1(A): **Two types of growth patterns are visible: feathery growth for pigeonpea and jute isolates and restricted growth for chickpea and groundnut isolates (B) Effect of azoxystrobin fungicide on growth of *Macrophomina phaseolina* isolates originated from different hosts**

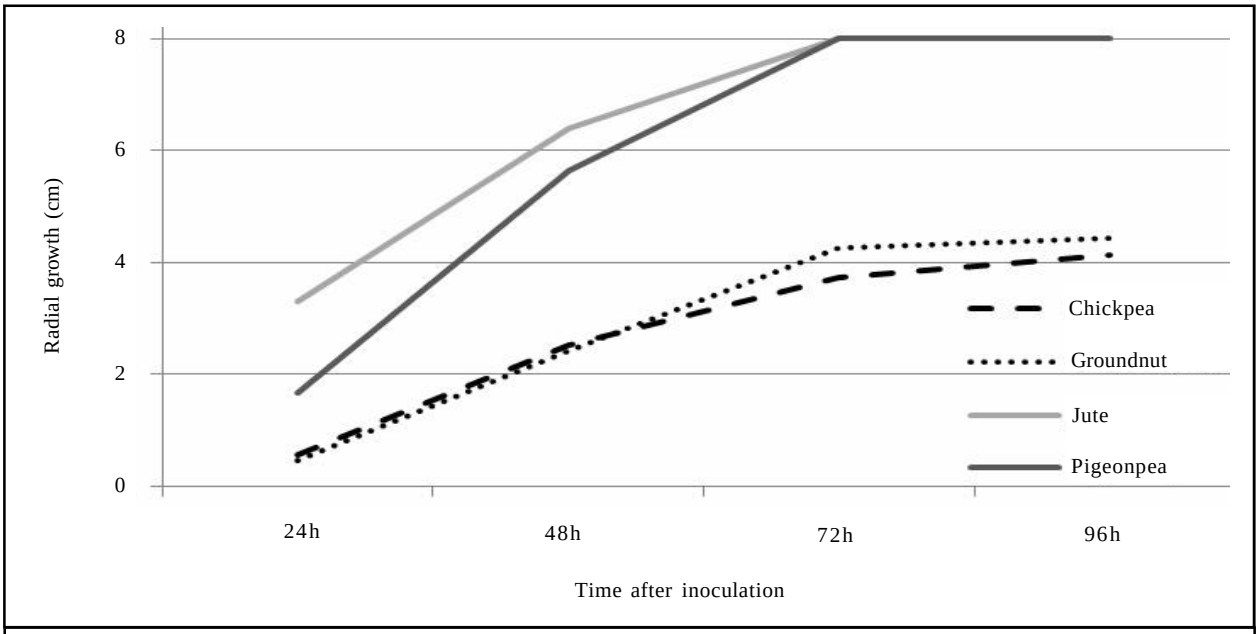

Fig. 2: Progress of radial growth for various isolates of *Macrophomina phaseolina*. This figure is generated with the representative isolate from each host

effect of azoxystrobin at 150 ppm. Significant effect of azoxystrobin was encountered for the isolates with restricted growth (Fig. 1B). Even, the 10 ppm concentration found highly effective for these isolates sampled from chickpea and groundnut. Supporting our findings, Hsiang *et al.* (2004) shown that uredospores of *Puccinia hemerocallidis* were completely suppressed in a treatment of azoxystrobin at a dose of 1.0–100 µg a.i./ml. We found significantly ($P < 0.01$) high effect of concentration of azoxystrobin on the fungal growth of isolates sampled from various hosts (Table 2). However, pigeonpea isolates were less affected with concentration

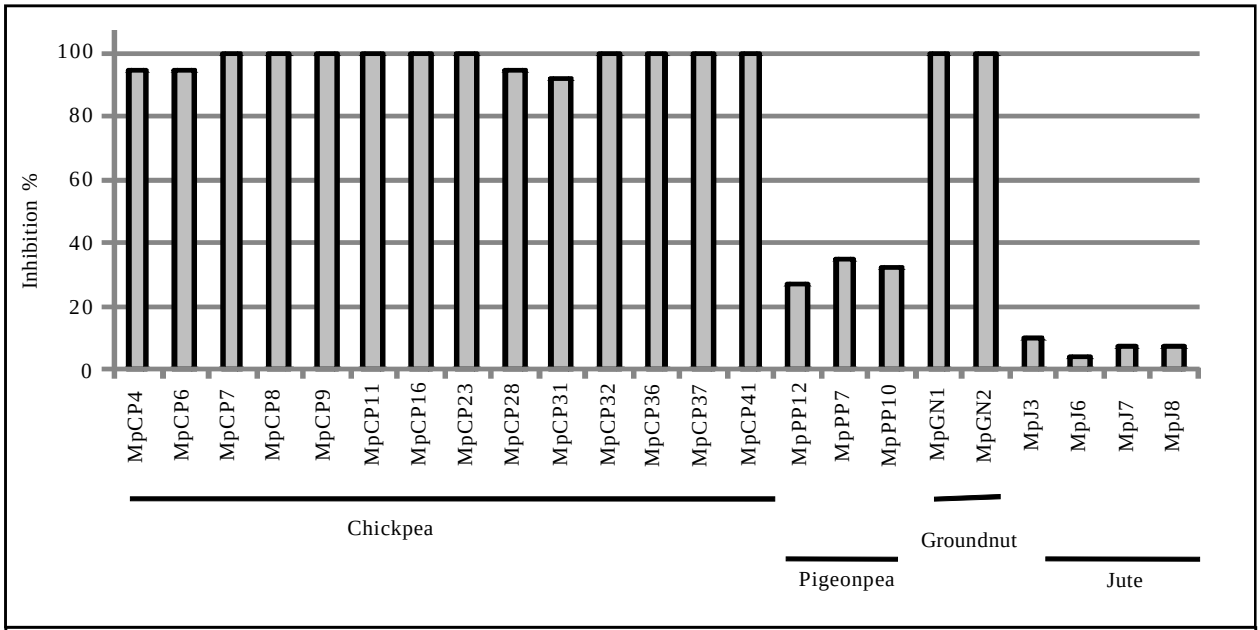

Fig. 3 : Inhibition per cent of *Macrophomina phaseolina* isolates from various hosts to azoxystrobin

of azoxystrobin compared to other isolates. Incubation time has also affected the progress of mycelial growth of *M. phaseolina* isolates. But the feathery isolates (fast growing) had not affected by incubation time ($P < 0.01$) because such isolates were not inhibited by the application of azoxystrobin. The interaction of concentration with incubation time had significant effect on isolates from different hosts ($P < 0.05$). This indicates that even the lower concentration of azoxystrobin with effective time for its action in the field may be useful to manage charcoal rot disease in various crops.

Inhibitory effect of azoxystrobin on *M. phaseolina*:

Inhibition per cent was determined for various isolates on 4th day after inoculation (Fig. 3). This experiment was done at 150 ppm of azoxystrobin. The slow growing isolates from groundnut were 100 per cent inhibited by azoxystrobin but the other group of slow growing isolates from chickpea was also severely inhibited and ranged between 92.5 and 100 per cent. Small number of groundnut isolates studied in this investigation. Probably, a large number of groundnut isolates may justify a better conclusion. Mycelium growth was found to be inhibited by 100 per cent in *Colletotrichum gloeosporioides* causing mango anthracnose upon application of azoxystrobin (Sundravadana *et al.,* 2007). Among the fast growing group of isolates, significantly greater inhibition found in pigeonpea isolates compared to jute isolates. Our result is therefore indicates that the least affected isolates to azoxystrobin are of jute origin. The jute originated isolates of *M. phaseolina* were inhibited 4.2 to 10.0 per cent by azoxystrobin at 150 ppm. At the same concentration, the inhibitory per cent ranged between 27.1 and 35.0 per cent for the isolates of pigeonpea origin. In other phytopathosystems, the growth and sporulation of *Alternaria alternata* (Reuveni and Sheglov, 2002) and *Phomopsis* sp. (Everett *et al.,* 2005) were found to be inhibited by azoxystrobin treatment.

Conclusion :

Azoxystrobin is currently being used for higher members of plant pathogenic fungi. But scarcity of literature in *Macrophomina*–pathosystem is enabling this study imperative. The results from this investigation advocating the identification of *Macrophomina phaseolina* from distinct host–origin is truly important in order to apply a fungicide. We found azoxystrobin is very much effective to control slow–growing population of *M. phaseolina*. However, this fungicide can be used for the fast–growing population if proper time is allowed to get the fungicide activated for its action. A further study could be attained to determine the quantity of azoxystrobin, particularly for fast–growing population, to effectively manage charcoal rot disease. The initiatives and additional studies of azoxystrobin fungicide for soil-borne pathogens would be applicable for field application, and benefit the farmers' welfare, particularly to resolve the complex issue of wilt problem.

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