Original Research Article

Piriformospora indica suppresses natural incidence and severity of black leaf mold of tomato incited by *Pseudocercospora fuligena* over strobilurin and triazole fungicides with enhanced growth and yield

ABSTRACT

Aim: To study the effect of *Piriformospora indica*-root colonisation and spraying of azoxystrobin, pyraclostrobin (strobilurins), propiconazole and tebuconazole (triazoles) fungicides for the management of black leaf mold of tomato.

Study Design: Completely randomized design for lab; Randomized block design for field studies.

Place and Duration of Study: College of Agriculture (Kerala Agricultural University), Vellayani and Coconut Reserarch Station, Balaramapuram, Thiruvananthapuram during 2022-2024.

Methodology: *In vitro* evaluation of strobilurin and trizole fungicides against *Pseudocercospora fuligena* was done by poisoned food technique. Field evaluation of *P. indica*-colonized plants var. Vellayani Vijai was performed to assess the incidence and severity of black leaf mold of tomato over systemic fungicides.

Results: The strobilurin fungicides partially and the trizoles completely inhibited the mycelia growth of the pathogen. *P. fuligena* was completely inhibited at 100 ppm of triazole fungicides, but at least 25 per cent growth was observed in strobilurin fungicides even at 1000 ppm. *P. indica* significantly reduced the natural incidence and severity of black leaf mold disease at 45 DAT and it extended to the entire crop period in the field condition. The disease incidence was 15.00 per cent in the *P. indica*-colonised plants; whereas it ranged from 28.12 to 31.85 per cent in the systemic fungicides sprayed plants; and in control plants, it was significantly high with 47.50 per cent. Similarly, the disease severity was least (PDI: 7.99) in the *P. indica*-colonised plants at 45 DAT. But the disease severity in the fungicides alone sprayed plants were 15.89 to 18.96; whereas in control plants, the severity was

40.83. Moreover, *P. indica* enhanced plant height, early flowering, fruit setting, flowers, fruits and average yield over control and systemic fungicides sprayed plants.

Conclusion: *P. indica* confers tolerance to black leaf mold disease over the strobilurin and triazole fungicides apart from the enhanced crop yield.

KEY WORDS: *Piriformospora indica*, endophytes, tomato, black leaf mold, *Pseudocercospora fuligena*, systemic fungicides

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.), a member of solanaceae family, is the second-most important vegetable crop in the world. It is a good source of minerals and antioxidants including vitamins C and E, lycopene, β-carotene, lutein, and flavonoids. The cultivation of tomatoes is affected by various biotic and abiotic factors. Tomato plants are susceptible to a large number of fungal, bacterial and viral diseases that reduce its yield and quality (El-Abyad *et al*., 1993). Black leaf mold (BLM), caused by *Pseudocercospora fuligena*, is a major plant growth and yield limiting disease for tomato production in the humid tropics (Zahn *et al*., 2011). The disease develops under conditions of warm temperatures (28 to 30°C), high relative humidity (90 to 95%) and long periods of leaf wetness, which are typical for the humid tropics and protected cultivation in tropical and subtropical climates (Hartman *et al*., 1991).

In order to control the diseases caused by fungi, farmers regularly rely on systemic fungicides (Sharma *et al*., 2017). Overtime, a new class of fungicides known as new generation fungicides was developed which are more eco-friendly. New generation fungicides are systemic in nature and needed in less quantity for larger area compared to other fungicides. New generation fungicides also include strobilurins and triazoles fungicides (Adeniyi *et al.,* 2021). The timely application of fungicides can prevent crop loss, though these chemicals have negative impacts on the biome (Pirozzi *et al*., 2016) in addition to the fungicide residues in farm produces if the frequency of harvesting is more.

Many beneficial microorganisms form symbiosis with plants and improve plant health (Smith and Smith 2011). *Piriformospora indica* is an endophytic root colonizing fungus of the order Sebacinales, class Basidiomycota and was isolated from the Thar desert of India (Varma *et al*., 1999). *P. indica* has been experimentally proven to improve water, mineral and nutrient absorption, early

flowering, seed germination and seed production, growth rate, photosynthetic capability, alter the production of secondary metabolites, promote tolerance, adaptation, and resistance to biotic and abiotic stress (Oelmuller *et al.,* 2009; Johnson *et al.,* 2014a; Gill *et al.,* 2016). The study was carried out to study the effect of *P. indica*-root colonisation and spraying of azoxystrobin and pyraclostrobin (strobilurins) fungicides and propiconazole and tebuconazole (triazoles) fungicides for the management of black leaf mold of tomato. Here, we report that *P. indica* drastically reduced the incidence and severity of black leaf mold disease compared to spraying the systemic fungicides in tomato plants.

2. MATERIALS AND METHODS

2.1 Maintenance and culturing of fungal root endophyte *P. indica* in potting mixture

Beneficial fungal root endophyte *P. indica* (No. INBA3202001787) was maintained in potato dextrose agar (PDA) medium. Potting mixture was made by mixing dried fine farm yard manure and coco peat in the ratio 1:1 amended with 2 per cent gram flour (w/w) (Jojy *et al*., 2020) and moisture content was maintained between 35 to 40 per cent. Sterilized potting mixture was then filled in sterilized plastic trays. Fungal mat was uniformly mixed with the potting mixture at 1% w/w. Trays were covered using cling film and kept for complete fungal growth which took about a week.

2.2 Co-cultivation of *P. indica* with tomato var. Vellayani Vijai and its root colonization

Potting mixture was transferred to sterilized protrays. Seeds of tomato variety Vellayani Vijai were surface-sterilized with 0.1 per cent mercuric chloride for 10 seconds. Then the seeds were sown in the protrays and kept in dark for two days. Protrays were maintained in temperature and humidity controlled conditions for uniform growth. Roots of tomato plants were examined for root colonization by *P. indica* at 7 and 15 days interval after co-cultivation. Roots were cut into pieces of 1 cm length. Cut pieces were then carefully transferred to a test tube containing freshly prepared 5 ml of 10 per cent KOH. Test tube was then heated in water bath at 65°C for 5 min. Root bits were washed with water again and transferred to a test tube containing 1 per cent HCl for 5 min. Root bits were washed with water and kept in lactophenol trypan blue for 2 min to stain the fungus and were observed under microscope (Leica-ICC50HD, USA) to examine the colonization of the fungus with the presence of hyphae and chalmydospores.

2.3 Isolation and maintenance of black leaf mold pathogen, *P. fuligena*

Leaf samples with the specific symptoms of black leaf mold of tomato were collected from the tomato field of Coconut Research Station, Balaramapuram, Thiruvananthapuram. A small portion of leaf bit (3 mm) was cut out which included a freshly infected portion of 1 mm length and a healthy tissue of 1 mm length. Surface-sterilization of the leaf bit was done with 0.1 per cent mercuric chloride followed by two subsequent washing in sterile water. Leaf bits were then placed in sterilized PDA medium and allowed the fungus to grow which was subsequently sub-cultured. Healthy and mature tomato leaves were collected and surface-sterilized using 70 per cent alcohol. A small moistened cotton piece was kept at the cut end of petiole to prevent desiccation of leaf. Micro injuries were made on the leaf using a needle and an actively growing fungal bit was placed above it. Fungal disc was covered using a small piece of moistened cotton to retain the moisture and was maintained in humid chamber.

2.4 *In vitro* evaluation of strobilurin and triazole fungicides against *P. fuligena*

New generation systemic fungicides such as azoxystrobin, pyraclostrobin, propiconazole and tebuconazole at different concentrations viz., 50, 100, 250, 500 and 1000 ppm were evaluated against *P. fuligena* in PDA medium by poisoned food technique. Fifty ml of autoclaved distilled water and 50 ml of autoclaved double strength PDA medium were prepared separately. Double the required concentration of the fungicide was mixed with 50 ml distilled water which was subsequently mixed with molten double-strength PDA to make concentrations 50, 100, 250, 500 and 1000 ppm. Then the medium was plated in sterilized petri plate and kept for solidification. At the centre of each petri plate, actively growing mycelial disc (5 mm) of *P. fuligena* was placed. Plates with non-poisoned medium were maintained as control. Radial growth of the mycelium was measured periodically. Percentage inhibition of growth was calculated using the formula.

$$I = (C-T)/C \times 100$$

Where, I = Percentage of inhibition

C= Growth of *P. indica* in control PDA medium

T= Growth of *P. indica* in the fungicide poisoned PDA medium

2.5 Evaluation of *P. indica* and systemic fungicides for the management of black mold of tomato under field conditions

Field studies were conducted at Coconut Research Station, Balaramapuram, Thiruvananthapuram during the period of March 2023 to June 2023 in Randomised Block Design (RBD). Each treatment was replicated four times. Separate blocks were maintained to differentiate the following treatments.

T_1: *P. indica*-root colonisation alone

T_2: Foliar spraying of azoxystrobin (125 g a.i. ha^{-1})

T_3: Foliar spraying of pyraclostrobin(100 g a.i.ha^{-1})

T_4: Foliar spraying of propiconazole(125 g a.i. ha^{-1})

T_5: Foliar spraying of tebuconazole (125 g a.i. ha^{-1})

T_6: Absolute control

P. indica-colonized and control seedlings were raised in separate portrays. One month old tomato seedlings (var. Vellayani Vijai) were transplanted to the main field at a spacing of 60 cm× 60 cm and irrigated daily. Manures, fertilizers and intercultural operations were done at periodic intervals as per the Package of Practices Recommendation of Kerala Agricultural University 2016. Systemic fungicides such as azoxystrobin, propiconazole and tebuconazole were sprayed at 125 g a.i. ha^{-1} and pyraclostrobin was sprayed at 100 g a.i. ha^{-1}. Foliar spray of the systemic fungicides were given at vegetative stage *i e.,* 40 and fruit setting stage, 70 days after sowing as the disease occurs during these stages. Black leaf mold disease incidence and severity were taken after 15 days of sprayings. Percentage disease incidence was calculated using the formula:

$$PDI = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100$$

Percentage disease severity of black leaf mold of tomato was calculated using the modified score chart by Reang *et al*. (2018). The grades with descriptions are detailed in Table 1.

$$\text{Percentage disease severity} = \frac{\text{Sum of all disease ratings}}{\text{Total number of leaves observed} \times \text{Maximum disease grade}} \times 100$$

Table 1. Score chart for assessing severity of black leaf mold of tomato caused by *P. fuligena*

Grade	Description
0	No infection
1	<1 per cent leaf area infected
3	1 to 10 per cent leaf area infected
5	11 to 25 per cent leaf area infected
7	26 to 50 per cent leaf area infected
9	More than 50 per cent leaf area infected

Biometric observations such as plant height, number of leaves, number of days taken for flowering, number of days taken for 50 per cent flowering, number of days taken to initiate fruit set, number of days taken for 50 per cent fruiting, number of fruits per plant, average weight of fruit per treatment were taken.

2,6 Statistical analysis

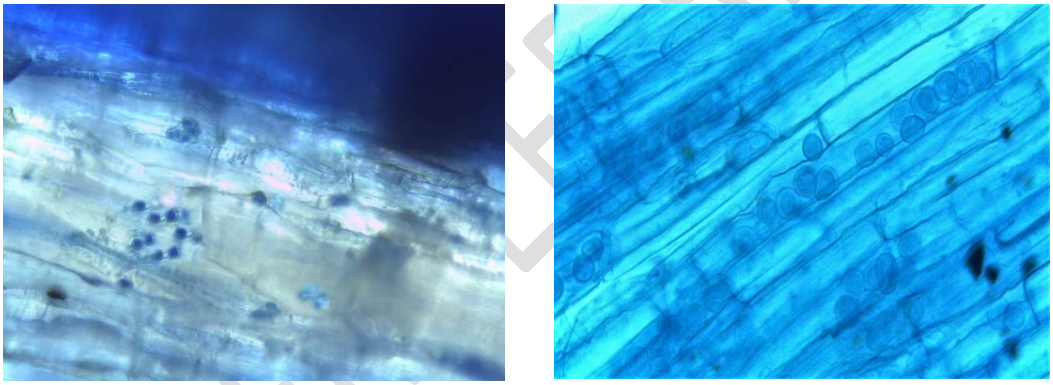
All the *in vitro* experiments followed CRD and the data was analysed using one way analysis of variance (ANOVA). Statistical significance between the treatments was compared by least significant difference (LSD) test at $p < 0.05$ probability level. Field experiments were laid out as per randomised block design. All the statistical analyses were performed using the statistical software of Kerala Agricultural University, GRAPES 1.0.0, developed by Gopinath *et al.* (2021).

3. RESULTS AND DISCUSSION

3.1 *P. indica* and its root colonization in tomato var. Vellayani Vijai

P. indica completely grow on the PDA plate in 14 days after sub-culturing and the growth as fungal mat in potato dextrose broth after 18 days of inoculation. *P. indica* grows better on potato dextrose agar medium followed by kidney bean agar, alkyl ester agar, oat meal agar and Luria Bertani agar media. Spore production was early on oats meal agar followed by kidney bean agar and potato dextrose agar medium (Hu *et al*., 2024). The endophyte is grown in both synthetic and natural media rich in carbohydrates and proteins.

The *P. indica* colonized tomato root bits were observed under microscope at 7 and 15 days interval after co-cultivation of the fungus (Plate 1). The presence of chlamydospores of the fungus in the roots of tomato seedlings was observed from 5th day onwards. *P. indica*-colonization was confirmed by observing hyphae and chlamydospores of the fungus at the cortical region of roots from fifth day onwards. The size and number of chlamydospores were increased in subsequent days of observation. The results were in accordance with Sam (2021) and Aruna *et al.* (2023).



a **b**

Plate 1. *In vivo* colonization of *P. indica* in roots of tomato variety Vellayani Vijai a) 7DAC and b) 15 DAC (DAC - Days after colonization)

3.2 Isolation of *P. fuligena* and its pathogenicity

P. fuligena isolated as pure culture on potato dextrose agar medium showed radial mycelium growth of 0.77 cm on the seventh day, 2.30 cm on the 20th day and 4.50 cm on the 45th day. Growth rate of the fungus was observed as 1.1 mm/day (Table 2). Colour of the mycelia of *P. fuligena* was white on the upper side on seventh day after inoculation and grey on the 20th day and finally to brown on 45th day; the colour of the mycelia was black on the rear side of the petri plate throughout the growth of the fungus in PDA. Nature of mycelial growth was sparse with concentric zonations and

regular margin (Table 2). The pathogen was also isolated in carrot leaf decotion agar, biomalt agar, carrot leaf oats meal agar, malt extract agar, water agar, potato carrot agar and V_8 juice gar medium in the decreasing order of growth rate (Mersha and Hu, 2008). The slow growth of the pathogen may be due to the absence or inadequacy of essential nutrients needed for the growth of *P. fuligena* in the media that is available in plant system.

Table 2. Nature of mycelial growth of *Pseudocercopora fuligena* causing black leaf mold of tomato in PDA medium

Sl. No.	Growth parameters of *P. fuilgena* in PDA medium		Fungal growth in PDA medium		
			7^{th} day	20^{th} day	45^{th} day
1	Radial mycelial growth (cm)		0.77	2.30	4.50
2	Colour of the mycelia	Upper surface	White	Grey	Brown
		Lower surface	Black	Black	Black
3	Nature of the mycelial growth		Sparse mycelial growth with concentric rings	Sparse mycelial growth with concentric rings	Sparse mycelial growth with concentric rings
4	Margin of mycelial growth		Regular	Regular	Regular
5	Growth rate (mm / day)		1.10	1.15	1.00

Pathogenicity of *P. fuligena* was proved by performing detached leaf assay. The symptoms start appearing from the 5^{th} day onwards after inoculation of *P. fuligena*. The lesion size produced by the pathogenic fungus was 0.30 cm on the 5^{th} day, 1.50 cm on the 10^{th} day and 2.00 cm on the 15^{th} day after inoculation. Nature and type of symptoms were distinct for black leaf mold of tomato. The symptoms were initially chlorotic spot which later transform into chlorotic lesion without definite margin on the upper surface and with sooty mold growth of the fungus on the corresponding lower surface (Plate 2).

a b c

Plate 2. Inoculation of *P. fuligena* causing black leaf mold in tomato leaves var. Vellayani Vijai to prove Koch's postulates a) Healthy leaf; b) Inoculation with the fungal mycelium; c) Appearance of symptom

3.3 Inhibition of *P. fuligena* by strobilurin and triazole fungicides

Both strobilurin and triazole fungicides significantly inhibited the growth of *P. fuligena.* Triazole fungicides are more effective than strobilurin fungicides in controlling the pathogen. Growth of *P. fuligena* was completely inhibited at 100 ppm of triazole fungicides, but at least 25 per cent growth was observed in strobilurin fungicides even at 1000 ppm (Table 3 and Table 4). There was more than 55 per cent growth inhibition upto 250 ppm; whereas about 60 and 74 per cent growth inhibition was recorded at 500 and 1000 ppm of strobilurin fungicides (Table 3). Similar results were obtained by Lee *et al.* (2015) where the triazole fungicide, metconazole suppressed growth of the pathogen up to 59 per cent and 97 per cent at concentrations 0.5 µg/ml and 4 µg/ml respectively whereas difenconazole inhibited growth of the pathogen up to 90 per cent and 98 per cent at concentrations 1.25 and 2.5 µg/ml respectively. Kumar *et al*. (2024) evaluated the efficiency of triazole and strobilurin fungicides against *Cercospora arachidicola* causing early leaf spot in ground nut in both lab and field conditions. The results showed that tebuconazole was the most effective in inhibiting the mycelial growth of the pathogen followed by combination of trifloxystrobin+ tebuconazole. In field study also tebuconazole recorded maximum disease control that is 70.73 per cent with maximum pod yield; followed by combination of trifloxystrobin +tebuconazole.

Table 3: Effect of strobilurin fungicides on the radial mycelial growth of *P. fuligena* by poisoned food technique at 30 DAI

Concentrations of fungicide (ppm)	Radial growth of mycelium (cm) *		Percentage inhibition over control	
	Azoxystrobin	Pyraclostrobin	Azoxystrobin	Pyraclostrobin
50	3.87 ± 0.02[a]	3.80 ± 0.07[a]	29.54	29.54
100	2.87 ± 0.02[b]	3.00 ± 0.08[b]	47.73	47.73
250	2.50 ± 0.08[c]	2.50 ± 0.05[c]	54.54	54.54
500	2.15 ± 0.13[d]	2.10 ± 0.21[d]	60.91	60.91
1000	1.42 ± 0.02[e]	1.80 ± 0.12[e]	74.09	74.09
Control	5.50 ± 0.00	5.50 ± 0.00	-	
SE (m) ±	0.04	0.04	-	
CD (0.05)	0.13	0.12	-	

* Values are mean of 5 replications ± standard deviation; Superscripts with same alphabets indicate on par values and those in different alphabets indicate significant difference at 5 per cent level of significance

At 50 ppm and 100 ppm of propiconazole, the mycelial growth of the pathogen was 1.37 cm and 1.20 cm corresponding to a growth inhibition of 75.00 per cent and 78.18 per cent over control (Table 4). However, at 250 ppm of propiconazole onwards, the mycelial growth of the pathogen was completely inhibited in the fungicide poisoned PDA medium. Except 50 ppm, all higher concentrations of tebuconazole could completely inhibit the mycelial growth of the pathogen. At 50 ppm, *P. fuligena* has grown up to 1.21 cm with a growth inhibition of 79.01 per cent over control (Table 4). Hence, both triazole fungicides, propiconazole and tebuconazole, are more effective in inhibiting the mycelial growth of *P. fuligena* than strobilurin fungicides at different concentrations tested. However, comparing both triazole fungicides, tebuconazole fungicide is more effective than propiconazole fungicide. Kumar *et al*. (2024) found that tebuconazole is the most effective systemic fungicide in inhibiting the mycelial growth of *C. arachidicola*.

Table 4: Effect of triazole fungicides on the radial mycelial growth of *P. fuligena* by poisoned food technique at 30 DAI

Concentrations of	Radial growth of mycelium (cm) *	Percentage inhibition over control

fungicide (ppm)	Propiconazole	Tebuconazole	Propiconazole	Tebuconazole
50	1.37 ± 0.01[a]	1.21 ± 0.06[a]	75.1	78.0
100	1.20 ± 0.08[b]	0.00 ± 0.00	78.2	100
250	0.00 ± 0.00	0.00 ± 0.00	100	100
500	0.00 ± 0.00	0.00 ± 0.00	100	100
1000	0.00 ± 0.00	0.00 ± 0.00	100	100
Control	5.50 ± 0.00	5.50 ± 0.00	-	
SE (m) ±	0.02	0.01	-	
CD (0.05)	0.08	0.04	-	

* Values are mean of 5 replications ± standard deviation; Superscripts with same alphabets indicate on par values and those in different alphabets indicate significant difference at 5 per cent level of significance

Strobilurin fungicides are known as quinone outside inhibitors and they reversibly bind to quinol oxidation site or ubiquinol site of cytochrome b of cytochrome bc1 complex located in the inner mitochondria and stops electron transfer between cytochrome b and cytochrome c1; which subsequently results in reduced NADH oxidation and ATP synthesis that further leads to death of the fungus (Bartlett *et al*., 2002). Ergosterol is an integral part of fungal cell wall. Triazole fungicide act on an enzyme-cytochrome P450 lanosterol 14 alpha demethylase which is involved in the biosynthesis of ergosterol. As a result, fungal cell wall is disrupted which leads to the death of the fungus (Pan *et al*., 2018).

3.4 *P. indica* colonization drastically reduced the natural incidence and severity of black leaf mold disease under field conditions

The root endophyte significantly reduced the natural incidence and severity of black leaf mold disease at 45 DAT (Table 5) and it extended to the entire crop period in the field condition. At 45 days after transplanting, the disease incidence was 15.00 per cent in the *P. indica*-colonised plants; whereas it ranged from 28.12 to 31.85 per cent in the systemic fungicides sprayed plants and in control plants, the disease incidence was significantly high with 47.50 per cent (Table 5). Similarly, the disease severity was least (PDI: 7.99) in the *P. indica*-colonised plants at 45 DAT. But the disease

severity in the fungicides alone sprayed plants were 15.89 to 18.96; whereas in control plants, the severity was 40.83. The above data explicitly show that *P. indica*-colonisation alone is sufficient to significantly reduce the severity of black leaf mold in tomato even without the use of systemic fungicides.

Table 5. Effect of *P. indica*-colonisation and the systemic fungicides sprayings on incidence and severity of black leaf mold caused by *P. fuilgena* in tomato plants var. Vellayani Vijai at 45 days after transplanting

Treatments	Disease incidence (%)	Disease severity - PDI
P. indica	15.00 (22.75)[a]	7.99[a]
Azoxystrobin @ 125 g a.i. ha^{-1}	28.12 (31.97)[b]	15.69[b]
Pyraclostrobin @ 100 g a.i. ha^{-1}	31.87 (34.37)[c]	16.53[b]
Propiconazole @ 125 g a.i. ha^{-1}	31.25 (33.98)[bc]	16.57[b]
Tebuconazole @ 125 g a.i. ha^{-1}	30.13 (32.97)[bc]	18.96[c]
Control	66.25 (54.43)[d]	40.83[d]
SE (m) ±	1.03	0.98
CD (0.05)	2.86	2.58

* Values are mean of 5 replications **±** standard deviation; Values in parentheses are arc-sine transformed values; Superscripts with same alphabets indicate on par values and those in different alphabets indicate significant difference at 5 per cent level of significance

The ability of *P. indica* in reducing the foliar fungal disease incidence and severity was also well established in barley-*Blumeria graminis* f. sp. *hordei*, *Arabidopsis thaliana-Alternaria brassicae* (Johnson *et al*., 2014b), tomato-*Alternaria solani* (Panda *et al.,* 2019), gerbera-*Phytophthora cryptogea* (Wu *et al*., 2022). *P. indica*-priming activated induced systemic resistance in plants and as a result, the disease incidence and severity were drastically reduced (Johnson *et al.,* 2014a; Gill *et al.,* 2016).

3.6 *P. indica* colonization enhances the growth and yield parameters under field conditions

In addition to the disease management, *P. indica* also enhanced different growth parameters of tomato plants such as plant height, number of fruits per plant and average weight of fruit (Table 6). Colonization of *P. indica* significantly enhanced the plant height compared to the control plants. The systemic fungicides had no visible effect on the height of tomato plants in both the colonised and control plants; though the effect was more pronounced in the colonised plants. At 45 DAT, there was less than 7 per cent growth enhancement due to the systemic fungicides; whereas the *P. indica* significantly enhanced the plant height to more than 28 per cent over control plants. *P. indica*-colonisation alone could increase the plant height to 109.07 cm at 45 DAT compared to 84.73 cm in the control plants. The enhanced plant height due to *P. indica*-colonisation accounts for 28.72 per cent increase in plant height compared to the control plants (Table 6).

Table 6. Effect of *P. indica*-colonisation and the systemic fungicides sprayings on growth and yield parameters of tomato plants var. Vellayani Vijai at 60 days after transplanting

Treatments	Plant height (cm)	Number of fruits per plant	Average fruit weight (g)
P. indica	109.07 ± 4.82^{a}	20.80 ± 1.75^{a}	41.23 ± 4.99^{a}
Azoxystrobin @ 125 g a.i. ha^{-1}	89.55 ± 3.69^{b}	15.68 ± 1.75^{b}	34.87 ± 1.30^{b}
Pyraclostrobin @ 100 g a.i. ha^{-1}	88.78 ± 3.78^{b}	16.08 ± 1.27^{b}	34.57 ± 1.31^{b}
Propiconazole @ 125 g a.i. ha^{-1}	88.68 ± 3.25^{b}	15.18 ± 1.32^{b}	34.12 ± 1.36^{b}
Tebuconazole @ 125 g a.i. ha^{-1}	89.95 ± 2.77^{b}	14.53 ± 1.52^{b}	35.02 ± 1.12^{b}
Control	84.73 ± 3.11^{c}	15.18 ± 1.45^{b}	34.75 ± 1.27^{b}
SE (m) ±	0.71	0.28	0.66
CD (0.05)	1.59	0.78	1.52

* Values are mean of 5 replications **±** standard deviation; Superscripts with same alphabets indicate on par values and those in different alphabets indicate significant difference at 5 per cent level of significance

P. indica-colonization significantly reduced the days taken for flowering and also for 50 per cent flowering compared to the control plants irrespective of the fungicides used. The days taken for

flowering were reduced to 20.93 in the *P. indica*-colonised plants compared to 34.57 in the control plants (Table 7). Similarly, the days taken for 50% flowering was also significantly reduced to 41.75 against 63.00 days in the control plants. The days taken for the flowering and 50% flowering were more (34.57 and 63.00 days) in the control plants (Table 7). The systemic fungicides sprayings also reduced the days taken for flowering and 50% flowering compared to the control plants. The growth promotion, early flowering and enhanced crop yield due to *P. indica*-colonisation had been reported in many crop plants which were reviewed by many authors (Johnson *et al.,* 2014a; Gill *et al.,* 2016; Li *et al.,* 2023).

Table 7. Effect of *P. indica*-root colonization on flowering of tomato var. Vellayani Vijai under field condition

Treatments	Days taken for flowering* (after transplanting)	Days taken for 50% flowering* (after transplanting)
P. indica	20.93 ± 2.26^{b}	41.75 ± 3.50^{b}
Azoxystrobin @ 125 g a.i. ha^{-1}	29.33 ± 2.22^{c}	52.00 ± 2.58^{c}
Pyraclostrobin @ 100 g a.i. ha^{-1}	30.13 ± 1.81^{d}	51.50 ± 1.00^{c}
Propiconazole @ 125 g a.i. ha^{-1}	29.58 ± 2.73^{c}	54.25 ± 0.96^{c}
Tebuconazole @ 125 g a.i. ha^{-1}	30.43 ± 2.24^{c}	51.25 ± 1.5^{c}
Control	34.57 ± 3.84^{d}	63.00 ± 2.16^{d}
SE (m) ±	0.34	1.22
CD (0.05)	1.12	3.58

* Total number of plants observed in each treatment - 40; Values are means of 40 replications ± standard deviation; Superscripts with same alphabets indicate on par values and those in different alphabets indicate significant difference at 5 per cent level of significance

Colonization with the root endophyte also significantly increased the number of fruits per plant and its average weight compared to control plants at 60 DAT (Table 6). The average fruit number was 20.80 and fruit weight was 41.23 g in the *P. indica*-colonised plants as against 15.18 and 34.75 g in the control plants. Strobilurin and triazole fungicides were having little effect on the number of fruits

per plant and its average weight (Table 6). Enhanced number of fruits and its size were reported to be due to the increased mobilisation of nutrients from soil and also the enhanced photosynthetic efficiency.

CONCLUSION

P. indica-colonization in tomato var. Vellayani Vijai could effectively control the black mold disease caused by *P. fuligena* with enhanced growth parameters such as plant height, number of flowers per plant, number of fruits per plant and average yield per plant over the control and systemic fungicides under field conditions.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies and text-to-image generators have been used during writing or editing of this manuscript.

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