

Original Research Article

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Field Evaluation of Native Pink Pigmented Facultative Methylophs for Growth Promotion and Anthracnose Management in Chilli

Savitha Santosh^{1,2*} and M. N. Sreenivasa²

¹ICAR – Central Institute for Cotton Research (CICR), Nagpur, Maharashtra - 440010, India

²Department of Agricultural Microbiology, University of Agricultural Sciences (UAS),
Dharwad, Karnataka - 580005, India

*Corresponding author

ABSTRACT

Field studies were conducted to study the role of native pink pigmented facultative methylophs (PPFMs) in plant growth promotion and anthracnose management in chilli. The efficient PPFM isolates were identified for field evaluation upon *in-vitro* functional characterization. Three selected isolates (PPFM6, PPFM170 and PPFM35) along with their consortium, the reference strain of PPFM (*Methylobacterium extorquens* AM1), chemical control (carbendazim) were included in field experiment. Increased plant height, dry matter and chlorophyll content were recorded with inoculation of PPFM isolates at different crop stages. Reduction in anthracnose incidence and higher yield levels along with improved capsaicin content of chilli was observed with inoculation of PPFM over uninoculated control. Chemical control recorded least disease incidence and yielded more chilli compared to PPFM isolates. The study clearly establishes PPFM's as plant growth promoters of chilli having remarkable biocontrol efficiency against *C. capsici*.

Keywords

PPFM, Chilli,
Anthracnose,
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Introduction

Chilli (*Capsicum annum L.*) is cultivated throughout the world for its pungency, colour, flavour and aroma and is a key component of our Indian cuisine, without which food is considered unfinished. Among many production constraints of chilli, diseases caused by fungi, bacteria, viruses, nematodes

inflict major crop losses worldwide, limiting its productivity. Chilli anthracnose caused by *Colletotrichum* spp. alone accounts for 10-54% yield losses in India (Lakshmesha *et al.*, 2005; Ramachandran and Rathnamma, 2006). Management of anthracnose is imperative to increase productivity of chilli as it affects the economic part. Though, large numbers of agrochemicals are commercialized for plant

growth promotion and effective anthracnose management in chilli, but environmentally and economically, they are not benign. In addition, they enhance resistance development in pathogens and reduce beneficial microflora population. Therefore, biological approaches using rhizosphere and phyllosphere microorganisms are considered as viable alternative for sustainable chilli production.

The biocontrol agents against *Colletotrichum* sp. which play significant role in anthracnose management include strains of *Trichoderma*, *Pseudomonas* and *Bacillus* etc. Considerable number of bacterial species, mostly associated with the plant rhizosphere have been evaluated and found to be beneficial for plant growth, yield enhancement and quality improvement (Pyrlak and Kose, 2009). Studies to explore phyllosphere bioagents as plant growth promoters and biocontrol agents are very sparse. Among phyllosphere microorganisms, members of pink pigmented facultative methylophilic (PPFM) potentially dominate the phyllosphere population. Diverse association of PPFM with plants are reported from free-living to epiphytic, endophytic and symbiotic forms (Sy *et al.*, 2001; Jackson *et al.*, 2006). Therefore, methylophilic have received a great attention in the recent times due to their abundance in the biosphere and their potential commercial applications.

PPFMs are known to improve plant growth (Radha *et al.*, 2009, Kim *et al.*, 2010; Yim *et al.*, 2012., Savitha *et al.*, 2013, 2019) with remarkable biocontrol activity against plant pathogens (Madhaiyan *et al.*, 2004, 2006; Indiragandhi *et al.*, 2008., Janahiraman *et al.*, 2016). However, the native isolated strains are seldom evaluated for growth promotion and yield enhancement. Hitherto, to the best of our knowledge, there are no reports on field evaluation of native PPFM for

management of chilli anthracnose. To address this knowledge gap, a study was planned to understand the role of native PPFMs in plant growth promotion and management of chilli anthracnose.

Materials and Methods

A field experiment was conducted with randomised block design comprising seven treatments and three replications to assess the effect of three selected isolates of PPFM (PPFM6, PPFM170 and PPFM35), their consortia (PPFM6+PPFM170+PPFM35; here after referred as PPFM consortia) and reference strain (*Methylobacterium extorquens* AM 1) on growth, yield and anthracnose incidence in chilli. Chemical control (carbendazim) and uninoculated control served as checks. The chilli (variety Byadagi dabbi) seedlings were raised in the nursery bed of size 1m × 1m. The recommended packages of practises were followed to raise the healthy seedlings. The 45 days old chilli seedlings were uprooted from nursery and transplanted in field.

PPFM inoculum and its inoculation

The isolated strains of PPFM from disease free chilli plants in anthracnose infected fields were functionally characterised for plant growth promotional ability viz., IAA, GA and cytokinin production, biocontrol efficiency against major pathogens of chilli under *in vitro* conditions (Savitha *et al.*, 2013, 2015 and 2019). Based on *in-vitro* studies, three isolates were selected for field evaluation. The 72 hours old, log phase PPFM culture in ammonium mineral salts (AMS) broth was used for further mass multiplication of bioinoculant. The flasks were kept in a temperature controlled shaker at $28 \pm 2^{\circ}\text{C}$ for 5 days to get a population of 10^9 cfu/ml of liquid culture. Preparation of carrier based bioinoculant was achieved by mixing broth

culture with sterilized lignite powder at 1:3 ratio using which seed treatment was carried out. One kg carrier based inoculant was mixed with 15 litre of sterilized distilled water was used for seedling dip. The seedlings were dipped in the slurry for 30 min before transplanting. The spraying inoculum was diluted at 1:1 ratio with sterilized distilled water and sprayed with a hand sprayer at the rate of 25 ml/plant on the leaves in the morning hours to achieve uniform wetting (Holland and Polacco, 1994) at 30, 45 and 60 days after transplanting (DAT).

Plant parameters, microbial population and disease intensity

The plant growth indicators like height of the plant and chlorophyll content were recorded at 30, 60, 90 and 130 DAT. Estimation of capsaicin content was done by colorimetric method (Mahadevan and Sridhar, 1986). The population of PPFM on rhizosphere and phyllosphere at different intervals was enumerated by serial dilution and plating technique. The colonies appearing on plates after 72 hours of incubation were counted and expressed as number of cfu/g sample. The total dry matter was measured at the time of harvesting (130 DAT) by oven drying samples at 70°C till constant weight is achieved. The anthracnose incidence was recorded by scoring five plants in each replication using 0-9 scale (Mayee and Datar, 1986). Per cent disease index (PDI) was calculated with the disease scales as per Wheeler (1969) while, per cent fruit rot infection (FRI) was calculated by dividing number of infected fruits by total number of fruits observed.

Statistical analysis

The data pertaining to plant growth promotional ability and biocontrol activity of different PPFM isolates were subjected for

statistical analysis by following the appropriate statistical procedures (Panse and Sukhatme, 1985). The values of $P < 0.05$ were considered as statistically significant. Percentage values of FRI, PDI and capsaicin content were arc-sin transformed before statistical analysis (Gomez and Gomez, 1984). PPFM population in rhizosphere and phyllosphere values were log-transformed before subjecting to ANOVA.

Results and Discussion

Influence of PPFM isolates on plant growth parameters

Inoculation of different PPFM isolates showed an increase in plant growth compared to uninoculated control. Plant height in different treatments was recorded at 30, 60, 90 and 130 DAT (Table 1). The maximum plant height was recorded with inoculation of PPFM6 (26.32cm) followed by PPFM consortia (26.00 cm). Chemical control (22.35cm) and uninoculated control (22.00 cm) showed significantly lesser plant height. Similar trend was observed for plant height by different treatments at 60DAT, 90DAT and 130DAT. Plant biomass (total dry matter content) recorded at 130DAT showed significant increase with treatments PPFM consortia (90.25g) and PPFM6(90.00g). The least biomass was recorded in uninoculated control (72.00 g). Chlorophyll content, a vital photosynthetic element of plants at 30 DAT was found to be high in treatments with PPFM consortia (26.50 SPAD) followed by PPFM6 (26.00 SPAD) (Table 2) while, the chemical control (22.00 SPAD value) and uninoculated control (22.00 SPAD value) recorded least chlorophyll content. Similar trend of chlorophyll content was observed with different treatments at 60DAT, 90DAT and 130DAT. The inoculation of PPFM to chilli plants significantly increased plant height, total biomass and chlorophyll content

as compared to uninoculated plants. These findings were validated in other crops as well including cotton (Madhaiyan *et al.*, 2005), rice (Senthilkumar, 2003), groundnut (Reddy, 2002), tomato (Thangamani and Sundaram, 2005). Reduction in chlorophyll content of plants was observed in anthracnose infected plants and it may be attributed to toxic effect of the pathogen (Muthuchelian *et al.*, 1990).

Pathogen is known to initiate leaf chlorosis by producing chlorophyllase enzyme and eventually necrosis condition in the phylloplane, thus reducing the number and size of the chloroplasts. PPFM may be involved in suppressing chlorophyllase activity or they produce the needed organic acids for the host growth and development.

Table.1 Influence of PPFM isolates on plant height and total dry weight of chilli under field condition

Treatment details	Plant Height (cm)				Total dry matter (g/plant) at 130 DAT
	DAT				
	30	60	90	130	
PPFM 6	26.32	43.95	60.15	71.15	90.00
PPFM 170	24.60	42.00	58.50	69.50	87.28
PPFM 35	23.50	41.10	58.00	69.00	86.15
PPFM Consortia	26.00	44.15	60.50	71.70	90.25
Reference strain of PPFM (<i>M. extorquens</i> AM 1)	23.50	40.00	56.50	67.60	85.80
Chemical control (carbendazim)	22.35	37.70	55.50	67.50	82.00
Uninoculated control	22.00	36.00	51.00	57.00	72.00
S. Em ±	0.33	0.34	0.46	0.53	0.35
CD (0.05)	1.03	1.06	1.42	1.62	1.09

Note: DAT- Days after transplanting

Table.2 Influence of PPFM isolates on chlorophyll content of chilli under field condition

Treatment details	Chlorophyll content (SPAD value)			
	DAT			
	30	60	90	130
PPFM 6	26.00	40.00	33.00	26.80
PPFM 170	24.50	37.50	31.28	24.00
PPFM 35	24.00	36.00	30.00	22.50
PPFM Consortia	26.50	40.20	33.25	27.00
Reference strain of PPFM (<i>M. extorquens</i> AM 1)	24.00	35.80	30.00	22.50
Chemical control (carbendazim)	22.00	34.00	30.00	22.50
Uninoculated control	22.00	32.00	26.00	18.00
S.Em ±	0.37	0.45	0.39	0.44
CD (0.05)	1.14	1.40	1.20	1.35

Note: DAT- Days after transplanting

Table.3 Population dynamics of PPFMs at different stages of chilli growth under field condition

Treatment details	Population of PPFM in rhizosphere (10 ⁴ cfu/g soil) DAT				Population of PPFM in phyllosphere (10 ⁴ cfu/g leaf) DAT			
	30	60	90	130	30	60	90	130
PPFM 6	13.13 (5.12)	14.15 (5.15)	14.75 (5.17)	8.22 (4.91)	34.12 (5.53)	42.32 (5.63)	45.27 (5.66)	37.85 (5.58)
PPFM 170	12.17 (5.08)	12.50 (5.09)	13.13 (5.12)	7.00 (4.84)	33.31 (5.52)	41.25 (5.62)	44.30 (5.65)	36.28 (5.56)
PPFM 35	10.32 (5.01)	11.50 (5.06)	12.53 (5.10)	6.98 (4.84)	32.18 (5.51)	40.21 (5.60)	42.30 (5.63)	35.92 (5.56)
PPFM Consortia	13.00 (5.11)	14.52 (5.16)	15.15 (5.18)	8.93 (4.95)	35.42 (5.55)	43.13 (5.63)	46.18 (5.66)	38.48 (5.59)
Reference strain of PPFM (<i>M. extorquens</i> AM 1)	11.33 (5.05)	12.32 (5.09)	13.15 (5.12)	7.43 (4.87)	32.18 (5.51)	39.48 (5.60)	40.56 (5.61)	34.56 (5.54)
Chemical control (carbendazim)	5.60 (4.75)	5.92 (4.77)	6.40 (4.81)	5.85 (4.77)	16.10 (5.21)	21.40 (5.33)	21.80 (5.34)	14.80 (5.17)
Uninoculated control	5.60 (4.75)	5.80 (4.76)	6.10 (4.79)	5.25 (4.72)	16.00 (5.20)	18.20 (5.26)	13.25 (5.12)	6.20 (4.79)

Note: Figures in the parentheses are log transformed values: - : No population of PPFM, Initial population of PPFM in soil was: 5.50 x 10⁴ cfu/ g soil; DAT- Days after transplanting

Table.4 Influence of PPFM on disease severity and yield of chilli under field conditions

Treatment details	Per cent fruit rot infection	Per cent disease index	Yield (q/ha)	Capsaicin content (%)
PPFM 6	44.00 (41.55)	55.00 (47.87)	2.55	0.16 (2.29)
PFM 170	44.50 (41.84)	61.00 (51.35)	2.52	0.15 (2.22)
PPFM 35	47.00 (43.28)	65.00 (53.73)	2.20	0.14 (2.14)
PPFM Consortia	43.50 (41.27)	55.50 (48.16)	2.56	0.16 (2.29)
Reference strain of PPFM (<i>M. extorquens</i> AM 1)	47.00 (43.28)	65.00 (53.73)	2.19	0.14 (2.14)
Chemical control (Carbendazim)	27.50 (31.63)	41.00 (39.82)	4.10	0.23 (2.75)
Uninoculated control	50.00 (45.00)	69.00 (56.17)	1.86	0.13 (2.07)
S. Em ±	0.58	0.44	0.006	0.001
CD (0.05)	1.30	1.35	0.01	0.003

Note: Values in the parentheses are arc sine transformed values

Dynamics of PPFM population

Variation in PPFM population was observed in rhizosphere and phyllosphere regions at different stages of crop growth. Higher population of PPFMs was recorded on phyllosphere as compared to rhizosphere across treatments and stages. Lesser population of PPFM was recorded in different treatments (5.60 to 13.13×10^4 cfu/g soil) at initial stage of crop growth (30DAT), which increased later on at 60DAT (5.80 to 14.52×10^4 cfu/g soil) and 90DAT (6.1 to 15.15×10^4 cfu/g soil) and an drastic decline (5.25 to 8.93×10^4 cfu/g soil) was observed at 130DAT in all treatments tested (Table 3). PPFMs population dynamics at phyllosphere followed the trend of rhizosphere except in uninoculated control at 90 DAT, the population of PPFMs found to decrease as compared to 60 DAT. Dynamics of PPFM population in rhizosphere and phyllosphere recorded at different plant growth stages showed highest population load on phyllosphere compared to rhizosphere. This could be directly related to the emission of methanol through stomatal openings during leaf expansion by pectin demethylation (Nemecek-marshall *et al.*, 1995), which may be utilized by PPFMs to survive on the surface of leaves.

Anthracoze incidence and yield

Among different treatments imposed, spraying crop with carbendazim recorded least disease incidence (27.50% FRI, 41.00 PDI) compared to other treatments (Table 4). In treatments involving PPFM isolates PPFM6 (44.00% FRI, 55.00 PDI), PPFM170 (44.50% FRI) and PPFM consortia (43.50% FRI, 55.50 PDI) recorded least disease incidence compared to uninoculated control (50.00% FRI, 69.00 PDI). PPFM consortia (2.56 q/ha) and PPFM6 (2.55 q/ha) provided higher yields compared to uninoculated control (1.86 q/ha) (Table 4). Carbendazim

sprayed treatment plots recorded significantly higher yield (4.10 q/ha) compared to other treatments in the study. The pungency of chilli which is measured in terms of capsaicin content in different treatments varied from 0.13 to 0.23 per cent. The highest capsaicin content was recorded with carbendazim (0.23 %) treated plot. PPFM consortia (0.16%) showed improved capsaicin content compared to uninoculated control (0.13 %). PPFM inoculated plants revealed lesser incidence of anthracnose disease compared to uninoculated control, nevertheless chemical control with carbendazim showed least disease incidence. Yield parameters like chilli yield and capsaicin content were directly proportional to anthracnose incidence in all the treatments studied. Though, carbendazim provided effective disease control, the environmental contamination and serious health risks in human beings outweigh its merits of disease control. Many studies have highlighted the potential risk to humans and undesirable impact on the environment by these agrochemicals (Jeyaratnam, 1981; Igbedioh, 1991; Forget, 1993) and even the risk of fungicide resistance at the standard dose of agrochemicals (Staub, 1991) is also reported which debits their potential disease control ability. On contrary, microorganisms as bioagents are ecofriendly and sustainable tools for effective disease management. Bioagents also helps to circumvent the contentious issue of chemical residues in export quality chilli.

In conclusion, performance of a plant growth promoting rhizobacteria (PGPR) under field conditions is a fundamental selection criterion for its identification as commercial bio-fertilizer. Selection based on their biocontrol efficiency as well as plant growth promotional ability will enable improved crop productivity. As bioagents are highly vulnerable to changing environment, expected results are often difficult to achieve with unpredictable weather (Bashan, 1998; Lucy *et*

al., 2004). This is more so with the phyllosphere bioagents as they are subjected to highly varying weather vagaries compared to rhizosphere bioagents. The present study revealed that PPFMs are very good plant growth promoters with remarkable biocontrol efficiency against *C. capsici* under field conditions. PPFMs can be a valuable component of integrated disease management and sustainable chilli production, for which more efficient strains need to be identified and their compatibility with the different commercially available bioagents/chemicals needs to be worked.

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