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Integrated approaches for management of anthracnose of chilli (*Capsicum annuum* L)

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Abstract
The experiment was conducted in the field to test the effect of organic amendments, (*viz.* FYM, poultry manure, sheep manure, neem cake and ground nut cake) with *Pseudomonas fluorescens* and carbendazim are used against *Colletotrichum capsici*, anthracnose disease of chili and chilli growth parameters. Eight treatments were under taken with three replications and data was analyzed using RBD. Minimum disease intensity was recorded in T_1 – NPK+ carbendazim (26.13%) with maximum yield (103.83 q/ha) followed by T_5 - FYM + *Pseudomonas fluorescens* (26.40%) with yield (95.79 qa/ha),T_3 - *Pseudomonas fluorescens* seedling treatment + *Pseudomonas fluorescens* (28.56%), with yield (92.52qa/ha), T_2 - Neem cake+ *Pseudomonas fluorescens* (32%), with yield (90.25 qa/ha), T_6 Sheep manure + *Pseudomonas fluorescens* (33.14%) with yield (85.44 qa/ha), T_7-Poultry manure + *Pseudomonas fluorescens* (35.55%) with yield (82.11 qa/ha), T_4-Groundnut cake+ *Pseudomonas fluorescens*-(37.77%) with yield (77.86 qa/ha) maximum disease intensity was recorded in control (49.03) with lowest yield ((36.71 qa/ha)

Keywords: Anthracnose, *Colletotrichum capsici*, Organic amendments, *Pseudomonas fluorescens*.

Introduction
Chilli is affected by several fungal, bacterial and viral diseases, of which, chilli anthracnose causes considera-ble damage, inflicting severe quantitative and qualitative losses. The estimated loss due to this disease ranged from 8 to 60% in different parts of India. The fungus C*olletotrichum capsici* infects both unripe (green) and ripe (red chilli) fruits and survives on seed as acervuli and micro sclerotia (Raj and Christopher 2009) [23].The disease symptoms can occur on leaves, stems, and both pre- and post-harvest fruits. Typical anthracnose symptoms on chilli fruit include sunken necrotic tissues, with concentric rings of acervuli (Agrios 2005) [1]. Infection of *C. capsici* is higher at the mature fruit stage than in the early fruit stage. The fungus pathogen is both seed borne and air borne and affects seed germination and vigour to a greater extent. Several fungicides have been reported to be effective in the management of fruit rot of chilli (Gopinath *et al*., 2006; Shovan *et al*., 2008) [11, 25]. It damage not only in the field but also during storage and is one of the main causes for post-harvest decay of chilli. Ripe fruits turning red are affected by the disease and turn straw coloured from normal red. In advanced cases the seeds are covered by a mat of fungal hyphae, which later turn rusty in colour. The disease has been shown to be caused by more than one *Colletotrichum* species including *C. acutatum*, *C. capsici*, *C. gloeosporioides* and *C. coccodes* (Than *et al*., 2008) [29]. Currently the disease is mostly managed by using chemical fungicides. The continuous and indiscriminate use of fungicides leads to toxic residues on chilli products, development of fungicide resistance and also serves as a cause for environmental pollution (Suji and Raj 2015) [28]. To overcome the undesirable effects of chemical usage, use of organic amendments and bioagents to control the infection came at rescue. Investigations proved that application of organic amendments significantly reduced the fruit rot incidence and enhanced the fruit yield and dry matter production. The different organic amendments *viz.*, fish compost (12.5 t/ha), farm yard manure (FYM) (12.5 t/ha), neem cake (250 kg/ha), mahua cake (250 kg/ha), sheep manure (12.5 t/ha), paddy husk (3 t/ha), press mud (12.5 t/ha), poultry manure (12.5 t/ha), coir pith compost (12.5 t/ha) and groundnut cake (250 kg/ha), efficacy against chilli anthracnose. (Suji and Raj 2015) [28]. The experiment was conducted *in vitro* and micro-plot to test the efficacy of six organic manures *viz.* FYM, poultry manure, water hyacinth compost, spent mushroom compost, goat dung and pigeon excreta among them effect of organic manures on plant growth parameter on, poultry manure treated field was significantly higher in plant height, followed by FYM, pigeon excreta, goat dung, water hyacinth compost and spent mushroom compost. (James and Simon 2015) [23] The use of manure application enhances soil productivity, increases the soil organic carbon content, soil micro-organism, improves soil crumb structure, the nutrient status of the soil and enhance crop yield.

Organic manure is also very cheap and effective as good as source of nitrogen for sustainable crop production (Lata and Veenapani 2011) [16].

Pseudomonas fluorescens suppress the pathogens by various modes of actions namely competition for nutrients and space, antibiosis by production various antibiotics, siderophores and lytic enzymes. Other mechanisms include of production of hydrogen cyanide (Defago *et al*., 1990) [8] and degeneration of toxins (Borowitz *et al*., 1992) [4]. Moreover, *pseudomonas fluorescens* produce plant growth promoting substances such as auxins and gibberellins and enhance plant growth and yield. Hence, they are collectively called as plant growth-promoting rhizobacteria (PGPR) (Dubeikovasky *et al*., 1993) [9]. In addition to direct antagonism against pathogen and plant growth promotion, inducing systemic resistance (ISR) by enhancement of plant defense capacity. *Pseudomonas fluorescens* is a new strategy for plant disease management (Ramamoorthy *et al*, 2001) [24]. The potential for biological control of *Colletotrichum* species had been suggested as early as in 1976 by Lenné and Parbery (1976). Jeger and Jeffries (1988) [17, 15] also stressed the possibilities of biological control of post-harvest fruit diseases by using *Pseudomonas fluorescens*. Application of *pseudomonas fluorescens* as seed treatment alone or as seed treatment plus soil application has induced systemic resistance in cucumber plants against *Colletotrichum orbiculare* and chilli against *Colletotrichum capsici* inciting anthracnose disease and resulted in reduction of disease incidence under field conditions (Ramamoorthy and Samiyappan 2001) [24]. *Pseudomonas fluorescens* along with 2 sprays of 1 % *Pseudomonas fluorescens* against *Colletotrichum capsici* is effective (Ekbote 2003) [10].

Isolation and multiplication of *Colletotrichum capsici* from chilli

Isolation of pathogen was done following the procedure by Linu *et al*., 2017 [18]. Infected fruit specimens were collected from central research field SHUATS Allahabad. The fruit specimens were washed with tap water, the discolored parts cut into small pieces (5 mm), sterilized with 0.1% NaOCl for two min and rinsed in sterilized water for three times and dried between folds of sterilized filter paper. The sterilized fruit pieces were transferred on sterilized oat meal agar plates and PDA plates and incubated at room temperature for 5 days. Mycelial bits were transferred to sterile petridishes containing oat meal agar medium; later it was purified by hyphal tip method and transferred to Potato dextrose agar (PDA) slants and pure cultures of the pathogens were maintained for further studies. Multiplication of pathogen was done by using potato broth and oat meal agar media.

Nursery preparation

Nursery bed is prepared by adding 1:1:1 of sand, soil and fym in the Department of Plant Protection of SHUATS. After one week chilli seeds of variety G-4 is sown on the nursery bed and covered with paddy straw. Seedlings are ready within 45 days for transplantation.

Amendment of organic manures Well decomposed organic manure viz., FYM, sheep dung, poultry manure, neem cake, groundnut cake are applied in the selected treatment @ 12.5 t/ha, 12.5 t/ha, 12.5 t/ha, 250 kg/ha, 250 kg/ha respectively, and ploughed again following the method given by (Suji and Raj 2015) [28]. NPK basal application is done one week before transplantation.

Transplanting of seedling

45 days old seedlings of cultivar G4 were transplanted in the prepared organic amended field according to the treatment. Transplantation of seedling was done in the evening after the sunset with the spacing of 60 x 45 cm (row X row and plant X plant) Gopakkali and Sharanappa (2014) [12]. *Pseudomonas fluorescens* root dipping is done for one treatment before transplanting.

Preparation and application of conidial spray

The mycelia growth of *Colletotrichum capsici* from the culture test tube was scraped with the help of sterilized glass rod after pouring 10 ml of distilled water. The solution is filtered through double layered muslin cloth to remove mycelia and cultural debris and obtained the conidial spore. The conidial concentration was adjusted to 1×10^5 ml-1 with adding of sterile distilled water and observed with haemocytometer. The suspension was sprayed after 15 days after transplanting. Sprays are done with the help of hand micro sprayer Rahman et al., (2011) [22].

Spraying of *Pesudomonas fluorescens* 2% and carbendazim@ 0.1% as foliar application.

Spraying of *P. fluorescens* @ 2% per cent and carbendazim @ 0.1% immediately after the first appearance of disease symptoms followed by two sprays at 15 days interval by using hand sprayer according to treatments. (Ahiladev I., 2013, Ngullie *et al*, 2010, Chauhan *et al.*, 2014) [2, 21, 6]

Disease intensity

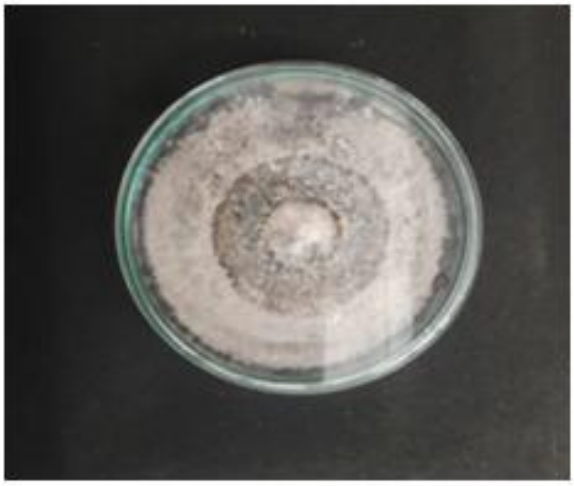
Standard disease rating scale (0-9 scale) for accessing PDI of anthracnose of chilli given by (Mayee and Datar, 1986) [19].

Rating Scale	Disease intensity
0	No symptoms on the leaf or branch or fruit.
1	Small, irregular brown spots covering 1 percent or less area of the leaf or branch or fruit.
3	Brown, dirty, pin headed spots covering 1-10 per cent area on the leaf or branch or fruit.
5	Dark brown, dirty black spots with blackish margin covering 1 per cent of the area of leaf or branch or fruit.
7	Dark brown, circular or irregular spots with blackish covering 26-50 per cent area of leaf or branch or fruit.
9	Dark brown, circular or irregular spots with blackish covering 51 per Cent and above area of leaf or branch or fruit

The per cent disease index (Chester, 1959 and Wheeler, 1969) [7, 30] was calculated as

$$\text{Per cent disease index PD} = \frac{\text{Sum of all individual disease rating}}{\text{Total No. of plants assessed maximum rating}} \times 100$$

Leaf, Fruit and Stem symptoms of *Colletotrichum capsici*



Growth of *C. capsici* in PDA

Setae and conidia of *C. capsici*

Untreated control plant

P. fluorescens treated plant

FYM+*P. fluorescens* treated plant

Results and Discussion

Table 1: Effect of organic amendments and *Pseudomonas fluorescens* on disease intensity of *Colletotrichum capsici* on chilli plant at 45, 60, 75 DAT (days after transplanting).

Symbol	Name of the Treatment	45 days	60 days	75 days
T_0	Control	26.65	30.40	49.03
T_1	NPK + carbendazim	12.05	15.46	26.13
T_2	Neem cake + *P. fluorescens*	15.97	21.06	32.00
T_3	*P. fluorescens* root dip + *P. fluorescens*	14.64	19.73	28.56
T_4	Ground nut cake + *P. fluorescens*	22.86	28.80	43.77
T_5	Farm yard manure + *P. fluorescens*	14.29	18.40	26.40
T_6	Sheep manure + *P. fluorescens*	16.88	23.00	34.47
T_7	Poultry manure + *P. fluorescens*	19.99	24.80	37.77
C.D.(0.05)		2.201	2.156	4.159
SE. d		1.026	1.173	1.938

Table 2: Effect of organic amendments and *Pseudomonas fluorescens* on plant growth parameters and yield of chilli

Symbol	Name of the Treatment	Plant height mean @			Length of Fruit mean	Average yield g/treatment	Average yield q/ha
		45 DAT	60 DAT	75 DAT			
T_0	Control	14.49	30.64	35.54	6.65	929	36.71
T_1	NPK + carbendazim	23.70	42.10	54.08	10.64	2527	103.83
T_2	Neem cake + *P. fluorescens* foliar application	19.92	37.85	49.02	8.85	2190	90.25
T_3	*P. fluorescens* root dip + *P. fluorescens* foliar application	22.05	40.22	51.10	9.22	2248	92.52
T4	Ground nut cake + *P. fluorescens* foliar application	18.08	36.31	46.28	7.22	1889	77.86
T5	Farm yard manure + *P. fluorescens* foliar application	19.61	37.67	49.23	9.74	2330	95.79
T6	Sheep manure + *P. fluorescens* foliar application	19.53	37.09	47.67	8.46	2075	85.44
T7	Poultry manure + *P. fluorescens* foliar application	20.8	38.28	50.15	8.07	1995	82.11
C.D.(0.05)		1.180	1.375	2.548	0.375	94.25	3.927
S.Ed		0.559	0.642	1.185	0.171	43.94	1.831

Results

Result based on field experiment, the effectiveness of the soil amendments and biocontrol agent (NPK, farm yard manure, poultry manure, sheep manure, neem cake and groundnut cake and *Pseudomonas fluorescens* on the plant growth parameters increases significantly as compared to control. The maximum plant height was observed in the treatment T_1 (NPK + carbendazim -54.66) followed by T_2 (*Pseudomonas fluorescens* seedling treatment with *P. fluorescens* foliar application - 51.10 cm), T_7 (Poultry manure + *Pseudomonas fluorescens* - 50.15 cm), T_5 (FYM + *Pseudomonas fluorescens* - 49.23 cm), T_2 (Neem cake + *Pseudomonas fluorescens* - 49.02 cm), T_6 (Sheep manure + *Pseudomonas fluorescens* - 47.67 cm) and T_4 (Groundnut cake+ *Pseudomonas fluorescens*- 46.28 cm). while minimum of plant height was observed in untreated control (35.54 cm).

In case of length of fruit the result reveals that T_1 (NPK + carbendazim (10.64) followed by T_2 (FYM + *P. fluorescens* - 9.74), T_3 (*Pseudomonas fluorescens* seedling treatment + *Pseudomonas fluorescens* - 9.22 cm), T_2 (Neem cake+ *Pseudomonas fluorescens* - 8.85 cm), T_6 (Sheep manure + *Pseudomonas fluorescens* - 8.46 cm), T_7 (Poultry manure + *Pseudomonas fluorescens* - 8.07 cm) and T_4 (Groundnut cake+ *Pseudomonas fluorescens* - 7.22 cm).While minimum length of fruit was observed in untreated control (6.65).

In case of yield of chilli maximum yield was observed inT_1(NPK + carbendazim -103.83 qa/ha) followed by T_5 (FYM + *P. fluorescens* -95.79 qa/ha), T_3 (*Pseudomonas fluorescens* seedling treatment + *Pseudomonas fluorescens*-92.52qa/ha), T_2 (Neem cake+ *Pseudomonas fluorescens* – 90.25 qa/ha), T_6 (Sheep manure + *Pseudomonas fluorescens*-85.44 qa/ha), T_7 (Poultry manure + *Pseudomonas fluorescens*-82.11 qa/ha) and T_4 (Groundnut cake+ *Pseudomonas fluorescens*- 77.86 qa/ha). while minimum yield was observed in untreated control (36.71 qa/ha).

Minimum disease intensity was recorded in T_1 (NPK+carbendazim -26.13%) followed by T_5 (FYM + *Pseudomonas fluorescens*-26.40%),T_3 (*Pseudomonas fluorescens* seedling treatment + *Pseudomonas fluorescens*-28.56%), T_2 (Neem cake+ *Pseudomonas fluorescens* - 32%),T_6 (Sheep manure + *Pseudomonas fluorescens*-33.14%),T_7(Poultry manure + *Pseudomonas fluorescens*-35.55%),T_4 (Groundnut cake+ *Pseudomonas fluorescens*-37.77%) as compare to control (49.03).

Application of organic amendments significantly reduced the fruit rot incidence and enhanced the fruit yield in field conditions. FYM + *Pseudomonas fluorescens* foliar application (26.40%) followed by *Pseudomonas fluorescens* seedling treatment + *Pseudomonas fluorescens* foliar application (28.56%), Neem cake+ *Pseudomonas fluorescens* foliar application (32%), Sheep manure + *Pseudomonas fluorescens* foliar application (33.14%), Poultry manure + *Pseudomonas fluorescens* foliar application (35.55%), Groundnut cake+ *Pseudomonas fluorescens* foliar application (37.77%). The results are in agreement with the findings of Suji and Raj (2015) [28] FYM at 12.5 tons/ha which recorded a 51.3% disease incidence of 357.0 g/plant fruit yield followed by neem cake, 250 kg/ha recorded 54.5% disease incidence, 348.7 g/plant fruit yield. Stone (2003) reported that paper mill residue compost amended to sandy field soils consistently was suppressive to anthracnose and brown spot disease in snap bean.

Pseudomonas fluorescens seedling dip and foliar application performed better than the other treatments through improved plant characters viz., plant height (51.10 cm), length of fruit (9.22) and yield (92.5 q/ha for 3 pickings) and also with reduced disease intensity (28.5) compared to control (49.03).The results are in agreement with the findings of Ngullie *et al.*, (2010) [21] with disease intensity of 20.25 and yield 205 q/ha. Seedling dip of chilli plant for 30 minutes in *Pseudomonas fluorescens* along with 2 sprays of 1 %

Pseudomonas fluorescens against *Colletotrichum capsici* is effective Ekbote (2003) [10].

The efficacy of Bavistin against the fruit rot pathogen was reported by several workers (Mishra 1988; Raju and Rao 1989; Azad. 1992) [20, 27, 3]. Bavistin (0.1%) was significantly superior to the organic amendments and biocontrol agents. This is in agreement with the report of earlier studies of Ngullie *et al*., 2010; Sujatha Bai 1992; Jeyalakshmi and sitharaman 1999). Chaudhary *et al*.,(2013) [21, 14, 5] observed that Bavistin (carbendazim) was most effective that three sprays of carbendazim @ 0.05% at 15 days interval commencing with the onset of the disease not only reduces the disease intensity of anthracnose of chilli but also results in higher healthy fruit yield and benefit ratio.

Conclusion

The present studies clearly indicates that the NPK+ carbendazim shows minimum disease intensity (26.13%) with highest yield (103.83 q/ha) followed by FYM + *Pseudomonas fluorescens (*26.40%) with yield (95.79 qa/ha). So, using organic amendments and bio control agents can be economical, long lasting and free from residual side effects. FYM + *Pseudomonas fluorescens* also gave good result in controlling disease and also increases yield of chilli. The results of present experiment were limited to one season under Allahabad agro-climatic conditions as such more trials should be carried out in future to validate the findings.

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