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SHORT COMMUNICATION

Protection against *Fusarium* wilt disease in bell pepper through abiotic resistance inducers

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Introduction

Bell pepper (*Capsicum annuum* L. var. *grossum* Sendt.) is an important high nutritive value vegetable crop. In India, it is grown in an area of 38,000 ha with a production of 556,000 MT (Agriculture and Farmers Welfare, 2022). It is an important off-season vegetable of the western Himalayas and offers the potential to boost the economy of farmers of hilly regions. Fusarium wilt, caused by *Fusarium oxysporum* (Schlect.) Emend. Synd. and Hans. f. sp. *capsici* Riv, is one of the most destructive and important diseases of bell peppers, which reduces the overall yield of a crop. This disease has become a serious menace to farmers who are growing bell peppers as a commercial crop year after year. The inoculum level has increased in such fields, which causes huge economic losses. For the management of disease, extensive application of fungicides is required (Attri et al., 2019). Moreover, the continuous use of fungicides raises concerns regarding the development of fungicide-resistant strains of the pathogen. There is a need to search for alternatives to fungicides for the management of Fusarium wilt of bell pepper. Hence the present investigations were carried out to manage Fusarium wilt disease of bell peppers through abiotic inducers of resistance.

The pathogen was isolated from infected plants by following the standard isolation method on potato dextrose agar (PDA) medium and was identified as *Fusarium oxysporum* (Schlect.) Emend. Synd. and Hans. f. sp. *capsici* Riv. on the basis of morphological characters viz., colony growth, color, conidia shape and size, and sick pots with *F. oxysporum* f. sp. *capsici* (Foc) were prepared (Attri et al., 2018). Different abiotic resistance inducers were evaluated to induce resistance in bell peppers against Fusarium wilt disease in these sick pots. Roots of 40-days old seedlings of bell pepper cultivar 'Solan Bharpur' were dipped for 45 minutes in solutions of different abiotic resistance inducers at specific concentrations *viz.* β-aminobutyric acid (1 mM), acibenzolar-S-methyl (0.05 mM), potassium oxalate (50 mM), potassium dihydrogen phosphate (200 mM), potassium chloride (300 mM), salicylic acid (1 mM), sodium salicylate (10 mM) and oxalic acid (1 mM). Treated seedlings were transplanted in sick pots with the growth of Foc. The experiment was conducted in a Completely Randomized Design (CRD) and each treatment was replicated thrice. The pots were incubated in plant growth chamber at 25 ± 2°C temperature, maintaining 70 to 80% relative humidity. The data on the number of wilted plants, plant height, plant weight and number of healthy leaves were recorded after 10 days of transplanting. Disease incidence was calculated in each treatment by the formula: Disease incidence (%) = (Number of infected plants/total number of plants observed) x 100. The efficacy of various abiotic resistance inducers was further studied under field conditions at the experimental farm of the Department during crop season 2016 where disease incidence was recorded as high in previous years. The experiment was conducted by using a susceptible cv. 'Solan Bharpur'. The experiment was laid out in a randomized block design. Each treatment was repeated thrice. The plot size was 2.0 × 2.0 m, having four rows 45 cm apart. Five plants were transplanted in each row and a total of 20 plants per plot were transplanted. Seedlings (40 days old) were treated by dipping the roots in a solution of abiotic resistance inducers for 45 minutes before transplanting. Foliar spray (FS) of abiotic resistance inducers was also applied on grown-up plants after 25 days of transplanting and repeated once after 15 days of the first spray. Numbers of wilted plants were recorded and disease incidence in each treatment was calculated. The data on green fruit yield were also recorded on each harvesting periodically. The inoculum load of Foc was estimated in each treatment of the field trial. At the last harvest and uprooting of plants of a field trial, the soil was collected from each replication of each treatment. All replicates of each treatment were mixed and homogenized. A portion, i.e., 1-g of the soil from each treatment, was directly used to determine the colony forming unit (CFU) through the serial dilution plate method.

All the treatments of abiotic inducers resulted in disease reduction as compared to control in sick pots (Table 1). However, seedling dip treatment of acibenzolar-S-methyl (0.05 mM = 0.002%) was very effective and no disease incidence of Fusarium wilt was recorded. Seedling dip treatment of ß-aminobutyric acid and salicylic acid at 1 mM concentration was also found effective and resulted in 13.33 and 33.33% disease incidence, respectively. Seedling dip treatment of potassium chloride (2.0%) also reduced disease incidence to 50.07%. In contrast, potassium oxalate 50 mM (0.1%) and potassium dihydrogen phosphate 200 mM (2.7%) were less effective and resulted in 73.33 and 66.66% disease incidence, followed by sodium salicylate (10 mM) with 53.33% disease incidence, respectively. Oxalic acid 1 mM (0.01%) was the least effective, with only a 7.15% reduction in disease incidence. The application of abiotic resistance inducers showed a positive effect on plant growth and health. The plants treated with abiotic inducers of resistance had more healthy leaves compared to the pathogen-inoculated control (Table 1). All the leaves of plants treated with acibenzolar-S-methyl were healthy. It was followed by 60.37 and 44.27% healthy leaves in plants treated with ß- aminobutyric acid and salicylic acid, respectively (Table 1). On the contrary, oxalic acid and potassium oxalate resulted in the lowest percentage of healthy leaves, i.e., 6.25 and 10.30%, respectively, compared to 4.90% of healthy leaves in pathogen-inoculated control. Further, data also revealed that all the treatments resulted in an increase in fresh plant weight significantly as compared to the control treatment (Table 1). However, acibenzolar-S-methyl treatment resulted in the highest increase in plant weight (1.13 g) followed by ß- aminobutyric acid (0.89 g)

Table 1: Effect of abiotic inducers resistance against Fusarium wilt of bell pepper in sick pots

Abiotic resistance inducer	*Conc. (mM)*	*Dose (%)*	*Disease incidence (%)*	*Disease Reduction (%)*	*Healthy leaves (%)*	*Plant weight (g)*	*Plant height (cm)*
ß-aminobutyric acid	1	0.01	13.33 (17.71)^{g}	85.72	60.37 (50.98)^{b}	0.89^{ab}	9.1^{ab}
Acibenzolar-S-methyl	0.05	0.002	0.00 (0.00)^{h}	100.00	100.00 (90.00)^{a}	1.13^{a}	10.3^{a}
Potassium oxalate	50	0.1	73.33 (59.19)^{bc}	21.43	10.30 (18.35)^{fh}	0.44^{ch}	5.3^{cg}
Potassium dihydrogen phosphate	200	2.7	66.66 (55.35)^{cd}	28.58	16.26 (22.71)^{fg}	0.61^{cf}	7.0^{bf}
Potassium chloride	300	2.0	46.60 (43.07)^{ef}	50.07	31.44 (34.07)^{de}	0.73^{bd}	7.3^{bd}
Salicylic acid	1	0.015	33.33 (34.99)^{fg}	64.29	44.27 (41.70)^{c}	0.86^{ac}	8.8^{ac}
Sodium salicylate	10	0.15	53.33 (46.91)^{de}	42.86	30.68 (33.59)^{dd}	0.70^{be}	7.1^{be}
Oxalic acid	1	0.01	86.66 (72.28)^{ab}	7.15	6.25 (11.73)^{fi}	0.52^{cg}	5.0^{ch}
Control	-	-	93.33 (81.14)^{a}	-	4.90 (7.52)^{fj}	0.34^{d}	4.2^{ci}
$CD_{(0.05)}$			(19.086)		(11.923)	0.324	2.741

Figures in the parenthesis are arc sine transformed value; Figures denoted by same letter do not differ significantly

Table 2: Evaluation of abiotic resistance inducers against Fusarium wilt of bell pepper under field conditions

Resistance inducers	*Conc. (mM)*	*Dose (%)*	*Application type*	*Disease incidence (%)*	*Disease reduction (%)*	*Yield (kg/plot)*
ß-amino-butyric acid	1	0.01	SD	18.33 (25.30)[f]	73.81	14.50[a]
Acibenzolar-S-methyl	0.05	0.002	SD	13.33 (21.33)[g]	80.95	15.50[a]
Potassium oxalate	50	0.1	SD+FS	35.00 (36.23)[d]	50.00	12.80[c]
Potassium oxalate	50	0.1	FS	51.66 (45.97)[b]	26.20	11.00[e]
Potassium dihydrogen phosphate	200	2.7	SD+FS	28.33 (32.13)[d]	59.52	13.00[c]
Potassium dihydrogen phosphate	200	1.35	FS	46.60 (43.07)[b]	33.42	11.50[d]
Potassium chloride	100	2.0	SD+FS	25.00 (29.79)[e]	64.28	13.50[b]
Salicylic acid	1	0.02	SD+FS	23.33 (28.85)[e]	66.67	14.00[b]
Sodium salicylate	10	0.15	FS	45.00 (42.08)[c]	35.71	12.60[c]
Oxalic acid	1	0.01	FS	53.83 (46.94)[b]	23.10	10.50[e]
Control	-	-	-	70.00 (56.82)[a]	-	8.50[f]
$CD_{(0.05)}$				(7.778)	-	1.402

SD-Seedling Dip, FS-Foliar Spray; Figures in the parenthesis are arc sine transformed values;
Figures denoted by same letter do not differ significantly

and salicylic acid (0.86 g) compared to control (0.34 g). Analogically, all the treatments significantly resulted in higher plant height as compared to control (Fig. 1; Table 1). Plant height was found to be maximum in acibenzolar-S-methyl treatment (10.3 cm) followed by ß- aminobutyric acid (9.1 cm) and salicylic acid (8.8 cm). Oxalic acid and potassium oxalate showed minimum plant height of 5.0 and 5.3 cm, respectively, compared to 4.2 cm in control.

The efficacy of abiotic resistance inducers to induce resistance in bell pepper plants against Fusarium wilt under field conditions was evaluated and it is clearly reflected from the data in Table 2 that significantly low disease incidence was observed in all the treatments compared to high disease incidence of 70% in untreated control. Least disease incidence of 13.33 and 18.33% were observed in seedling dip treatment of bell pepper with acibenzolar-S-methyl 0.05 mM and ß-amino-butyric acid 1 mM, respectively. Next in order was statistically similar 23.33 and 25.00% disease incidence in seedling dip + foliar spray with salicylic acid and potassium chloride, respectively. Since acibenzolar-S-methyl and ß-amino-butyric acid are relatively expensive chemicals, hence only one application, as seedling treatment, was evaluated for induction of resistance in bell pepper against Fusarium wilt to keep down the cost of disease management chemicals.

It is further evident from the data in Table 2 that all the treatments were effective in reducing the disease incidence as compared to control under field conditions. However, seedling dip of acibenzolar-S-methyl 0.05 mM was found most effective and reduced the disease to 80.95% over control, which was followed by ß-amino-butyric acid (1 mM) and salicylic acid (1 mM) with 73.81 and 66.67% disease reduction, respectively. Foliar sprays alone of potassium oxalate at 50 mM and oxalic acid at 1 mM were least effective against the disease with 26.20 and 23.10% reduction in disease incidence over control, respectively. However, a combination of foliar sprays with seedling dip treatment increased disease reduction. Seedling dip + foliar spray with potassium dihydrogen phosphate provided a 59.52% reduction in Fusarium wilt, whereas foliar spray alone with the same inducer provided only 33.42% disease reduction compared to the control. Similarly, seedling dip + foliar spray with potassium oxalate at 50 mM provided 50.00% disease reduction, whereas foliar spray alone with the same inducer provided only 26.20% disease reduction compared to control. All the treatments significantly increased the overall yield of harvested green fruits as compared to the control (Table 2). Maximum yield of green fruits, i.e., 15.50 kg/plot was obtained from the acibenzolar-S-methyl treated plants, followed by ß-amino-butyric acid 14.50 kg/plot, which were statistically at par with each other, followed by salicylic acid and potassium chloride with 14.0 and 13.5 kg/plot while least fruit yield was recorded in oxalic acid.

The pathogen of Fusarium wilt of bell pepper, i.e., Foc was quantified in each treatment of field trial and CFU was estimated. It was found that a substantial amount of inoculum ranging from 1.95×10^3 to 2.79×10^3 CFU was present in the soil in all treatments of a field trial. Information pertaining to the efficacy of abiotic resistance inducers against Fusarium wilt of bell pepper is scarce in literature; hence, literature available in other crops was mentioned for comparison. Application of Benzo-(1,2,3)-thiadiazole-7-carbothioic acid S- methyl ester (BTH) and DL-3-aminobutyric acid (DL-3-ABA) were effective against wilt of tomato and resulted in symptoms suppression and reduced disease severity as compared to control (Chamsai et al., 2004). Application of salicylic acid-induced plant defense mechanisms and provided resistance against *F. oxysporum* f. sp. *asparagi* in asparagus and against *F. oxysporum* f. sp. *lycopersici* in tomato (He & Wolyn, 2005; Mandal et al., 2009). The effectiveness of various systemic acquired resistance inducers, including acibenzolar-S-methyl and potassium dihydrogen phosphate on pot and field conditions by seed soaking and foliar application was reported against root rot and vine decline of melon (Aleandri et al., 2010). Phosphate and sodium salts provide antifungal activity against fungal pathogens by inducing resistance in plants (Doliopoulos et al., 2010). The abiotic resistance inducers are effective against w wide range of plant pathogens, including fungi, oomycetes and bacteria. Two foliar sprays of acibenzolar-S-methyl and ß-amino-butyric acid were effective in reducing the prevalence of Phytophthora leaf blight and fruit rot of bell pepper caused by soil-borne oomycetous phytopathogen *Phytophthora capsici* (Sharma et al., 2016). Foliar application of potassium phosphite induced resistance in potatoes against late blight caused by *Phytophthora infestans* (Feldman et al., 2020). The application of abiotic inducers of resistance brings structural and biochemical changes in pathogen-challenged plants (Sood et al., 2023). Potassium is crucial for the healthy growth of plants and is rapidly absorbed by the plant tissues and is extremely mobile within tissues. Calcium ions play an important role in the production of salicylic acid and chitinase, both closely linked with induced resistance. Salicylic acid is well known for its endogenous signal molecule playing an important role in the development of systemic resistance in plants. Induced resistance has the potential to provide long-term resistance against a number of diseases.

A novel approach to managing Fusarium wilt of bell pepper through seedling treatment and foliar spray of abiotic resistance inducers *viz.,* potassium oxalate, potassium chloride, acibenzolar-S-methyl (ASM), oxalic acid, ß-aminobutyric acid (BABA), potassium dihydrogen phosphate, salicylic acid (SA) and sodium salicylate is presented in the present study. In sick pots, seedling dip treatment of ASM (0.05 mM = 0.002%) provided complete control of Fusarium wilt with no disease incidence. BABA and SA at 1 mM concentration resulted in 13.33 and 33.33% disease incidence, respectively. All abiotic inducers of resistance also increased the plant height plant weight and resulted in higher healthy leaves percentage compared to the control. Further, an evaluation of abiotic resistance inducers under field conditions showed that ASM, BABA, SA and potassium chloride were effective against the disease and provided protection against the pathogen. These treatments also resulted in a significant increase in yield over the untreated control.

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