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RESEARCH PAPER

Tackling Fusarium seed infection in maize: Integrating *in vitro* and *in vivo* approaches

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Abstract Treating maize seed against *Fusarium* spp. is essential, as it forms the first line of defense, ensuring healthy germination and robust early plant growth. This soil-borne fungi not only reduce yield and grain quality but also produce mycotoxins harmful to human and animal health. In the present studies, effectiveness of five fungicides and one native strain of *Trichoderma harzianum* were evaluated against *Fusarium verticillioides* both under *in-vitro* and *in-vivo* conditions. Under *in vitro* conditions, mancozeb 50% + carbendazim 25% WS and carbendazim 50% WP demonstrated complete inhibition of mycelial growth of *F. verticillioides* at 25 ppm, whereas sedaxane 12.16% + azoxystrobin 3.15% + thiamethoxam 22.06% FS and Thiram 75 WS were found to be the least effective against *F. verticillioides*. Dual culture technique showed 68.35 per cent inhibition by *Trichoderma harzianum* against *F. verticillioides*. Under laboratory conditions, roll towel method showed maximum germination (95%) and highest shoot (22.4 cm) and root length (61.55 cm) were observed with carbendazim 50% WP followed by mancozeb 50% + carbendazim 25% WS. Under field conditions, carbendazim 50% WP @ 3g/kg seed and mancozeb 50% + carbendazim 25% WS @ 2g/kg seed were found highly effective, enhancing seed germination by 95 and 94.66 per cent and reducing seedling blight severity by 7.38 and 8.0 per cent and seedling mortality by 3.33 and 4.33 per cent respectively. Seed treated with carbendazim 50% WP at 3g/kg exhibited the highest shoot and root length as well as maximum grain yield, while seeds treated with sedaxane 12.16% + azoxystrobin 3.15% + thiamethoxam 22.06% FS at 3ml/kg displayed the least vigor and minimum grain yield.

Keywords: Fungicides • *Fusarium verticillioides* • Maize • *Trichoderma harzianum* • Seed rot • Seedling blight

Introduction

Seed rot and seedling blight pose a serious threat to maize (*Zea mays* L.) cultivation during *Kharif* and spring seasons particularly under north Indian conditions (Solorzano and Malvick, 2011). *Fusarium* species, among various pathogens, are notably prevalent on maize seeds and contribute to approximately 25 per cent of total production losses (Cramer, 1967). This leads to diminished germination rates, reduced field yields, and also cause seed rotting, seedling blight, stalk rots, ear rots as well as post-harvest losses (Munkvold *et al*., 1997; Styer and Cantliffe, 1984; Wilke *et al.,* 2007). Beyond yield losses, Fusarium-infected maize seeds also produce mycotoxins, compromising grain quality and nutritional value. In India, rotting of ears, roots and stalks cause losses upto 5 per cent while seed rot and seedling blight resulted in 0.1 per cent loss (Parihar *et al*., 2011). This pathogen, *F. verticillioides* systemically colonize maize plants via infected seeds or plant roots in the rhizosphere and directly infects germinating seedlings through natural openings (Yates *et al.,*1999). The severity of this disease is exacerbated in cool, poorly drained, compacted, and wet soils, especially in low-lying areas with temperatures

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below 13°C. Greater fungal interactions with seeds are the result of higher *Fusarium* inoculum concentrations, which ultimately lowers seed germination (Ellis *et al*., 2011). However, under *in vitro* conditions, a temperature range of 25-30°C, high humidity and an acidic pH (3.5-7) promote the growth and sporulation of *Fusarium* spp., thereby enhancing its pathogenicity on maize seeds (Cruz *et al.,* 2019).

Among various management strategies, seed treatment has emerged as a primary approach to mitigate yield loss due to Fusarium-induced diseases. However, limited information exists on the efficacy of new fungicide seed treatments against *F. verticillioides*, particularly in North Indian conditions. This study was conducted to address the dearth of *in vitro* and *in vivo* research on fungicides against maize seed rot and seedling blight under Punjab conditions. Additionally, their impact on maize seed yield and vigor, focusing on seedling emergence and growth in the presence of *F. verticillioides* was also studied under laboratory as well as field conditions.

Materials and methods

In vitro evaluation

Five fungicides viz., Mancozeb 50% + carbendazim 25% WS (Sprint 75WS), Carbendazim 50% WP (Bavistin 50WP), Sedaxane 12.61% + azoxystrobin 3.15% + thiamethoxam 22.06% FS (Vibrance integral), Imidacloprid 18.5% + hexaconazole 1.5% FS (Neonix 20FS) and Thiram 75WS were assessed through *in vitro* assays against *F. verticillioides* on potato dextrose agar (PDA) medium supplemented with 1, 10, 25, 50, 100, 200 and 500 ppm concentrations of each fungicide. Control assays utilized PDA medium lacking fungicide supplementation (Grover and Moore, 1962). In dual culture, consisting of native strain of *Trichoderma harzianum* and *F. verticillioides* was grown on petri plates containing PDA media. Each combination of fungal isolate and fungicide concentration was evaluated in three replicate plates. After 7-day incubation period, colony diameters were measured to determine the percentage of inhibition of fungal growth compared to the colony diameter of control plates. Per cent mycelial inhibition was determined by the formula devised by Vincent (1947) and individual ED_{50} values were calculated using Probit analysis (Finney, 1952).

Laboratory evaluation using roll towel method

Mass culturing and seed inoculation: Fusarium verticillioides culture was multiplied on potato dextrose broth media and a suspension containing 5×10^5 spores/ml was prepared using haemocytometer. Healthy seeds of the maize hybrid PMH 10 were pre-soaked in water for 2 hours, followed by immersion in the spore suspension of *F. verticillioides* for an additional 2 hours. Subsequently, the spore suspension was drained and the seeds were air-dried on filter paper.

Seed treatment with fungicides and biocontrol agent: The air-dried seeds were then treated with five fungicides viz., Mancozeb 50% + carbendazim 25% WS (Sprint 75WS) @ 2g and 3g/kg seed, Carbendazim 50% WP (Bavistin 50WP) @ 3g/kg seed, Sedaxane 12.61% + azoxystrobin 3.15% + thiamethoxam 22.06% FS (Vibrance integral) @ 3ml/kg seed, Imidacloprid 18.5% + hexaconazole 1.5% FS (Neonix 20FS) @ 3ml/kg seed and Thiram 75WS @ 3g/kg seed and one biocontrol agent using talc based formulation of *Trichoderma harzianum* @10g/kg seed. Each chemical was applied as a wet seed treatment by making a slurry with water. Untreated seeds inoculated with *F. verticillioides* served as the control treatment. The seeds of all seven treatments with three replications along with control were placed on germination paper and incubated at 25±2°C for 7 to10 days (ISTA, 1993). Per cent seed germination and seedling growth were recorded after 10 days of incubation.

Field evaluation

Mass culturing and seed inoculation: Healthy seed of maize hybrid PMH 10 was pre-soaked in water for 2 hours, followed by soaking in 5×10^5 spores/ml spore suspension of *F. verticillioides* for an additional 2 hours.

Seed treatment with fungicides and biocontrol agent: Seed were then treated with five fungicides viz., Mancozeb 50% + carbendazim 25% WS (Sprint 75WS) @ 2g and 3g/kg seed, Carbendazim 50% WP (Bavistin 50WP) @ 3g/kg seed, Sedaxane 12.61%+ azoxystrobin 3.15%+ thiamethoxam 22.06% FS (Vibrance integral) @3ml/kg seed, Imidacloprid 18.5% + hexaconazole 1.5% FS (Neonix 20FS) @ 3ml/kg seed and Thiram 75WS @ 3g/kg seed and one biocontrol agent using talc based formulation of *Trichoderma harzianum* @10g/kg seed.

Field layout: The field experiment was conducted at field experimental area, Maize section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana during spring 2023. Each plot consisted of 5 rows of 2m each with row to row and plant to plant spacing of 60cm × 20cm respectively and each treatment with three replications was sown as per standard package of practices for spring season (Anonymous, 2022). Each treatment was replicated thrice.

Observations

Field emergence and seedling mortality: Per cent field emergence in each treatment was recorded. Seedling mortality was observed in each treatment at 30 days after sowing.

Seedling blight severity: Each seedling was assessed at 10, 20 and 30 days after planting and seedling blight severity was recorded following 0-5 scale (Zhang *et al.,* 2012) given below-

Rating scale	Description
0	Healthy plant
1	Yellowing of lower leaves (<25% chlorotic/necrotic leaves)
2	Yellowing of higher leaves (25–50% chlorotic/necrotic leaves)
3	Browning and discoloration (50–75% chlorotic/necrotic leaves)
4	Rotting at base (>75% chlorotic/necrotic leaves)
5	Death of plant

Seedling vigour: Shoot length and root length of ten uniform seedlings per replication were measured at three intervals- 10, 20 and 30 days after sowing using *Biovis* root scanner software. The seedling vigor index of uprooted seedlings was calculated at 10 days after sowing using the standard formula given by International Seed Testing Association (ISTA 1985):

Vigour index = Per cent germination × (Root length + Shoot length)

Grain yield: Maize cobs were harvested as soon as the husk cover turned brown and the silk became completely dry. Grain yield per plot was recorded in each treatment.

Statistical analysis: The ANOVA model was performed for experiment carried out under controlled as well field conditions and organized according to completely randomized design (CRD) and randomized block design (RBD). Data were subjected to a two-way analysis of variance and the mean comparisons were done by calculating CD and SE at the 5% level of significance. Graphics data representation and statistical analyses were carried out using MS Excel spreadsheet software and OPSTAT statistical software.

Results

In vitro evaluation

All the test fungicides significantly inhibited the mycelial growth of *F. verticillioides* over untreated control. The perusal of data in Table 1 clearly indicated that among five fungicides, mancozeb 50% + carbendazim 25% (Sprint 75WS) and carbendazim 50% WP (Bavistin 50WP) proved highly effective inhibiting the colony growth by 74.08 and 62.31 per cent respectively at 10 ppm concentration, however both these chemicals completely inhibited colony growth at 25 ppm (Figure 1). Thiram 75 WS and sedaxane 12.61% + azoxystrobin 3.15% + thiamethoxam 22.06% (Vibrance integral) were found least effective giving 84.03 per cent and 78.22 per cent inhibition, respectively at 500 ppm. Among five fungicides, ED_{50} value for mancozeb 50% + carbendazim 25% (Sprint 75WS) and carbendazim 50% (Bavistin 50 WP) were 1.17 and 3.99 µg/ml respectively. Sedaxane 12.61% + azoxystrobin 3.15%+ thiamethoxam 22.06% FS (Vibrance integral) with ED_{50} value of 40.19 µg/ml was found least effective. However, in dual culture technique, *Trichoderma harzianum* showed 68.35 per cent inhibition of mycelial growth of *F. verticillioides* after four days of incubation (Figure 2).

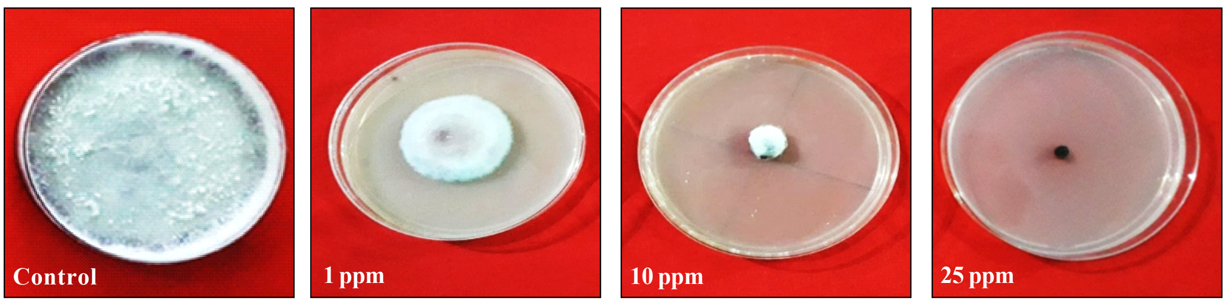
Laboratory evaluation using roll towel method

Seed germination: Data in Table 2 showed that, the maximum germination (95%) was observed in seed treated with carbendazim 50% WP (Bavistin 50 WP) @ 3g/kg seed and the least germination (82%) was observed in seed treated with sedaxane 12.61% + azoxystrobin 3.15% + thiamethoxam 22.06% FS (Vibrance integral) @ 3ml/kg seed. Seed treated with Thiram 75WS and talc-based formulation of *Trichoderma harzianum* showed 86 and 87 per cent germination respectively which was significantly superior over control (71%).

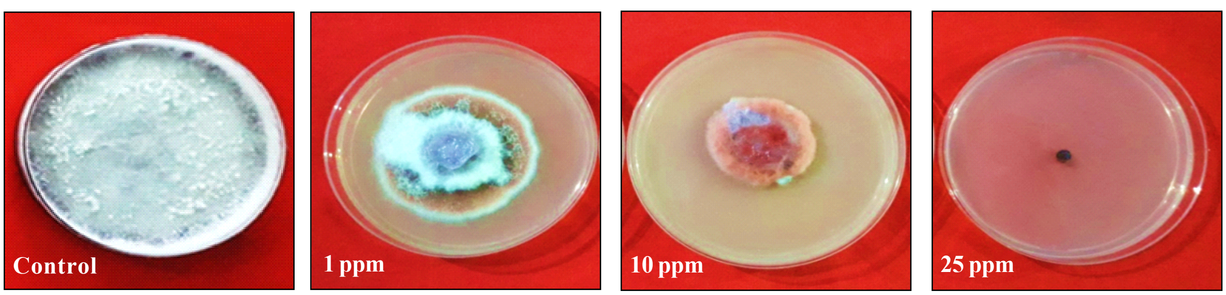
Table 1. Evaluation of different chemicals against *Fusarium verticillioides* causing seed rot and seedling blight of maize under *in vitro* conditions

Chemical	Per cent inhibition of mycelial growth at different concentration (ppm)							ED_{50}**
	1	10	25	50	100	200	500	
Mancozeb 50% + carbendazim 25%WS (Sprint 75 WS)	49.03 (44.44)*	74.08 (59.39)	100.00 (89.68)	100.00 (89.68)	100.00 (89.68)	100.00 (89.68)	100.00 (89.68)	1.17
Carbendazim 50%WP (Bavistin 50WP)	32.17 (34.55)	62.31 (52.12)	100.00 (89.68)	100.00 (89.68)	100.00 (89.68)	100.00 (89.68)	100.00 (89.68)	3.99
Imidacloprid 18.5% + hexaconazole 1.5% FS (Neonix 20 FS)	22.16 (28.07)	46.07 (42.74)	64.67 (53.53)	66.98 (54.93)	70.12 (56.87)	84.32 (66.70)	89.29 (70.90)	11.02
Thiram 75WS	19.73 (26.36)	36.47 (37.15)	51.44 (45.82)	54.68 (47.68)	70.24 (56.94)	75.89 (60.59)	84.03 (66.46)	22.67
Sedaxane 12.61% + azoxystrobin 3.15% + thiamethoxam 22.06% FS(Vibrance integral)	18.03 (25.12)	27.66 (31.73)	39.63 (39.01)	52.53 (46.45)	60.66 (51.15)	70.67 (57.21)	78.22 (62.18)	40.19
CD (p=0.05)	Fungicide = 0.40, Concentration =0.47 Fungicide × Concentration =1.05							

*Figures in parentheses represent growth inhibition in arc sine transformed value
**Values determined after plotting per cent growth inhibition against fungicide concentrations



(a) Mancozeb 50% + carbendazim 25%WS



(b) Carbendazim 50%WP

Figure 1. *In vitro* evaluation of (a) Mancozeb 50% + carbendazim 25% WS and (b) Carbendazim 50%WP against *Fusarium verticillioides* using poisoned food technique

Figure 2. Antagonistic effect of *Trichoderma harzianum* on *Fusarium verticillioides* using dual culture technique

Seedling vigour: Maximum shoot length (22.4 cm) and root length of seedlings (61.55 cm) was observed in seed treated with carbendazim 50%WP (Bavistin 50 WP) as compared to 4.67 cm and 6.99 cm respectively in inoculated control (Table 2). Treatment of mancozeb 50% + carbendazim 25% WS (Sprint 75 WS) @ 2g/kg seed and 3g/kg seed showed the shoot length of 20.75 and 17.18cm and root length of 55.7 and 46.51 cm respectively. Minimum shoot length (10.43 cm) and root length (33.33 cm) was observed in seed treated with

Table 2. Efficacy of seed treatment of fungicides and biocontrol agent against *Fusarium verticillioides* causing seed rot and seedling blight of maize using roll towel method under laboratory conditions

Seed treatment	Dose per kg seed	Percent germination*	Seedling growth parameters (10 days after incubation)		
			Shoot length (cm)	Root length (cm)	Seedling vigour index
Mancozeb 50% + carbendazim 25%WS (Sprint 75 WS)	2g	93 (74.67)	20.75	55.7	7,115.28
	3g	91(72.53)	17.18	46.51	5,802.04
Sedaxane 12.61% + azoxystrobin 3.15% + Thiamethoxam 22.06% FS (Vibrance Integral)	3ml	82(64.90)	10.43	33.33	3,583.24
Imidacloprid 18.5% + hexaconazole 1.5% FS (Neonix 20 FS)	3ml	93(74.98)	14.35	44.88	5,508.93
Carbendazim 50%WP (Bavistin 50WP)	3g	95(77.12)	22.40	61.55	7,973.75
Thiram 75WS	3g	86 (68.05)	16.40	35.18	4,441.40
Talc based formulation of *Trichoderma harzianum*	10g	87(68.96)	17.96	41.46	5,166.48
Inoculated control	-	71(57.55)	9.75	15.82	1,817.33
CD (p=0.05)	-	8.04	4.67	6.99	1,116.40
SE(m)±	-	2.43	1.41	2.11	337.10

*Mean of 50 seed per replication with three replications per treatment

sedaxane 12.61% + azoxystrobin 3.15% + thiamethoxam 22.06% FS (Vibrance integral). There was non-significant difference in seedling vigour of seed treated with carbendazim 50%WP (7973.75) and 2g of mancozeb 50% + carbendazim 25% WS (7115.28), but were statistically significant from inoculated control (1817.33). Seed treatment with talc-based formulation *Trichoderma harzianum* @ 10g/kg seed significantly enhanced seedling growth, resulting in a root length of 41.16cm, shoot length of 17.96 cm and a seedling vigour index of 5166.48 (Table 2).

Field evaluation

Seed emergence: Data in Table 3 revealed that field emergence was maximum (95%) in seed treated with carbendazim 50% WP @ 3g/kg seed closely followed by mancozeb 50% + carbendazim 25% WS @ 2g/kg seed (94.66%) and imidacloprid 18.5% + hexaconazole 1.5% FS (94.33%) @ 3ml/kg seed. The least increase in germination (4.35%) was observed in seed treated with sedaxane 12.61% + azoxystrobin 3.15% + thiamethoxam 22.06% FS @ 3 ml/kg seed as compared to the inoculated control.

Seedling blight severity: At 30 DAS, seedling blight severity (7.38%) and seedling mortality (3.33%) was recorded lowest in seed treated with carbendazim 50% WP as compared to 20.89 and 14.66 per cent respectively in inoculated control (Table 3). Mancozeb 50% + carbendazim 25% WS, when used @ 3g/kg seed showed 5.33 per cent seedling mortality as compared to 4.33 per cent at 2g/kg seed. The un-inoculated control showed 7.66 per cent seedling mortality which was statistically lower than inoculated plots (14.66%). Treatment of seeds with fungicides and biocontrol agents significantly reduced seedling mortality and disease severity compared to the control. The maximum reduction in disease severity (64.67%) and seedling mortality (77.26%) was observed in seeds treated with carbendazim 50% WP @ 3 g/kg seed, followed by Sprint 75 WS@ 2 g/kg seed (Figure 3). The minimum reduction in disease severity (38.54%) and seedling mortality (40.88%) was observed with sedaxane 12.61% + azoxystrobin 3.15% + thiamethoxam 22.06% FS. Whereas, 61.34 per cent decrease in seedling mortality and 54.33 per cent decrease in disease severity were observed in seeds treated with *T. harzianum*.

Grain yield: The data presented in Table 3 revealed that seed treatment with fungicides had significant effect on maize grain yield. Among the treatments, carbendazim 50WP gave highest grain yield of 32.51 q/acre with 31.09 per cent increase in yield over inoculated control. However, lowest yields of 26.28 q/acre and 26.67 q/acre were recorded in seed plots treated with sedaxane 12.61% + azoxystrobin 3.15% + thiamethoxam 22.06% FS and Thiram 75 WS respectively. The seed plots treated with talc based formulation of *T. harzianum* gave 30.85 q/

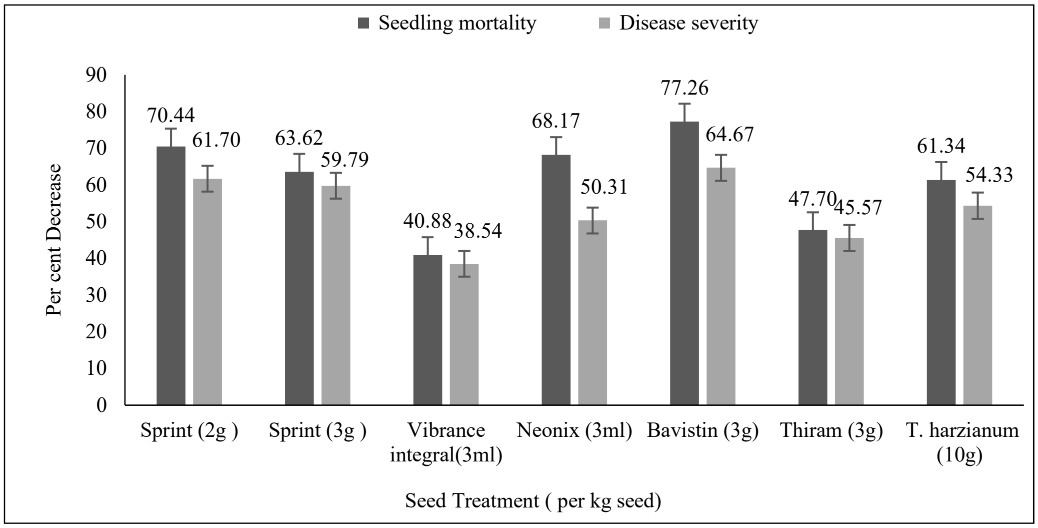


Figure 3. Effect of seed treatment with different fungicides and biocontrol agent on per cent decrease in seedling mortality and disease severity of seed rot and seedling blight of maize under field conditions

Table 3. Efficacy of fungicides and bioagent on seed germination, severity of seed rot and seedling blight and grain yield of maize under field conditions

Seed treatment	Dose per kg seed	Field emergence (%)	Per cent increase in field emergence	Seedling blight severity (%)*			Seedling mortality at 30DAS (%)	Grain yield (q/acre)*	Per cent increase in yield
				10DAS	20DAS	30DAS			
Mancozeb 50% + carbendazim 25%WS (Sprint 75WS)	2g	94.66 (75.86)	14.79	4.88 (12.76)	6.53 (14.79)	8.00 (16.42)	4.33 (11.99)	31.57	29.04
	3g	93.33 (75.11)	13.57	5.64 (13.74)	6.95 (15.29)	8.40 (16.81)	5.33 (13.33)	30.97	27.14
Sedaxane 12.61% + azoxystrobin 3.15% + Thiamethoxam 22.06%FS (Vibrance Integral)	3ml	84.33 (66.84)	4.35	7.52 (19.55)	8.21 (22.33)	12.84 (20.98)	8.66 (17.10)	26.28	14.76
Imidacloprid 18.5% + hexaconazole 1.5%FS (Neonix FS)	3ml	94.33 (76.41)	14.49	6.85 (15.16)	8.05 (16.49)	10.38 (18.78)	4.66 (12.45)	30.68	26.98
Carbendazim 50%WP (Bavistin 50WP)	3g	95.00 (77.22)	15.09	4.32 (11.98)	6.28 (14.50)	7.38 (15.76)	3.33 (10.49)	32.51	31.09
Thiram 75WS	3g	84.66 (66.93)	4.72	7.56 (15.95)	8.56 (17.00)	11.37 (19.68)	6.33 (14.56)	26.67	16.01
Talc based formulation of *Trichoderma harzianum*	10g	91.33 (73.43)	11.68	6.59 (14.86)	7.41 (15.78)	9.54 (17.98)	5.66 (13.75)	30.85	27.39
Uninoculated control	-	89.33 (70.92)	9.70	8.21 (16.64)	9.51 (17.94)	12.86 (21.00)	7.66 (16.06)	29.05	22.89
Inoculated control	-	80.66 (63.90)	-	11.33 (15.92)	14.45 (16.64)	20.89 (27.19)	14.66 (22.45)	22.40	-
CD(p=0.05)	-	5.66	-	Treatment=0.85, Days after sowing=0.49 Treatment × Days after sowing=1.47			2.17	2.87	-
SE(m)±	-	1.87	-	Treatment=0.29, Days after sowing=0.17 Treatment× Days after sowing=0.51			0.72	0.95	-

*Mean of 3 replications, Figures in parentheses represent disease severity in arc sine transformed value
#DAS- Days after sowing

Table 4. Seedling vigour and growth parameters of maize seedlings affected by priming of inoculated seed with different fungicides and biocontrol agent under field conditions

Seed treatment	Doses per kg seed	Mean shoot length (cm)* 10DAS	Mean shoot length (cm)* 20DAS	Mean shoot length (cm)* 30DAS	Mean root length (cm)* 10DAS	Mean root length (cm)* 20DAS	Mean root length (cm)* 30DAS	Seedling vigour index (10 DAS)
Mancozeb 50% + carbendazim	2g	23.67	26.53	30.69	37.89	52.44	65.12	5,914.60
25%WS (Sprint 75WS)	3g	24.59	26.08	29.56	37.08	51.22	60.33	5,664.81
Sedaxane 12.61% + azoxystrobin 3.15% + thiamethoxam 22.06% FS (Vibrance Integral)	3ml	19.16	22.24	25.96	25.71	38.52	52.39	3,754.87
Imidacloprid 18.5% + hexaconazole 1.5%FS (Neonix FS)	3ml	20.42	24.00	27.53	33.24	45.10	56.17	5,056.92
Carbendazim 50%WP (Bavistin 50WP)	3g	22.17	25.21	34.18	41.22	59.16	67.77	6,042.67
Thiram 75WS	3g	17.69	21.63	28.85	31.80	42.02	56.72	4,190.00
Talc based formulation of *Trichoderma harzianum*	10g	20.46	22.83	32.65	34.59	47.17	59.15	5,034.52
Uninoculated control	-	19.18	22.62	26.59	26.43	39.49	51.9	4,076.23
Inoculated control	-	16.72	18.59	22.52	21.75	30.59	42.08	3,107.45
CD (p=0.05)	-	Treatment=1.45, Days after sowing=0.83; Treatment × Days after sowing=2.51			Treatment=1.74, Days after sowing=1.00; Treatment × Days after sowing=3.01			621.31
SE(m)±	-	Treatment=0.51, Days after sowing=0.29; Treatment × Days after sowing=0.88			Treatment=0.61, Days after sowing=0.35; Treatment × Days after sowing=1.06			205.47

*Mean of 3 replications; #DAS- Days after sowing

acre grain yield with 27.39 per cent increase in yield as compared to 22.40 q/acre obtained in inoculated control.

Seedling growth parameters: Data in Table 4 revealed that maximum shoot length (34.18 cm) and root length (67.77 cm) of 30 days old seedlings were observed in seed plots treated with carbendazim 50% WP, as compared to 22.52cm and 42.08cm, in inoculated plots. However, among treated seed plots, minimum shoot (25.96 cm) and root lengths (52.39 cm) were observed in seeds treated with sedaxane 12.61% + azoxystrobin 3.15% + thiamethoxam 22.06% FS (Table 4). The effect of seed treatment on seedling vigor index varied significantly among all fungicide treatments. Seeds treated with carbendazim 50% WP exhibited the highest seedling vigor (6042.67), followed by mancozeb 50% + carbendazim 25% WS @ 2g/kg seed (5914.60) and 3g/kg seed (5664.81). Notably, the un-inoculated control demonstrated comparatively higher seedling vigor (4076.23) than the inoculated control (3107.45).

Discussion

Fusarium spp., widely distributed plant pathogenic fungi worldwide, comprise a large and complex group of filamentous fungi. Among these, *F verticillioides* poses a major threat to maize cultivation, primarily through diseases such as seed rot and seedling blight, stalk rots and ear rots. In India, losses upto 5 per cent resulted from the rotting of stalks, roots and ears, while, 0.1 per cent loss was contributed by seed rot and seedling blight. Major challenges include resilient pathogen survival, wide host range, scarce resistant varieties and limited genetic diversity in maize germplasm. Keeping this in view, the present study was conducted to manage seed rot and seedling blight of maize using different chemicals and biocontrol agent under both *in vitro* and *in vivo* conditions. Our present study revealed that mancozeb 50% + carbendazim 25% WS and carbendazim 50% WP were found highly effective in inhibiting the mycelial growth of *F. verticillioides*. These results were supported by research work done by Khokhar *et al.* (2014), who observed complete inhibition of mycelial growth of *F. verticillioides* by carbendazim followed by Thiram. Similar studies were carried out by Bohra *et al.* (2001); Trivedi *et al.* (2002); Thori *et al.* (2012) and Chandra *et al.* (2008), demonstrating the efficacy of carbendazim in inhibiting mycelial growth of *F. verticillioides*. Similarly, the efficacy of *Trichoderma* spp. in suppressing the population of *Fusarium* spp. has been reported by Jambhulkar *et al.* (2022); Kaur and Mohan (2013) and Chandra *et al*. (2010). They identified potential native strains of *T. harzianum* against *F. verticillioides*, showing the maximum inhibition zone and restricting its growth upon contact. Conversely, Khokhar *et al*. (2014) and Anita (2016) observed that *T. harzianum* was slightly less effective than *T. viride* in inhibiting the mycelial growth of *F. verticillioides*.

Under laboratory conditions, seed treated with carbendazim 50% WP (Bavistin 50 WP) and mancozeb 50% + carbendazim 25% WS (Sprint 75 WS), yielded higher germination and seedling growth parameters in roll towel method. Carbendazim fungicide disrupts *F. verticillioides* by blocking DNA production and microtubule formation, while Sprint fungicide stops mycelial growth, appressoria formation, and germ tube development. Similar findings were reported by Yadav *et al.* (2021) and Dey *et al*. (1989). The efficacy of treatments observed under field conditions was significantly correlated with the *in vitro* results. Field data revealed that treating seeds with 3g of carbendazim 50% WP and 2g of mancozeb 50% + carbendazim 25% WS (Sprint 75 WS) per kg seed can significantly enhance germination, seedling growth parameters and ultimately, maize yield when grown from seeds infected with *F. verticillioides*. These fungicides gave good protection against seed rot and seedling blight severity compared to other treatments. Similar findings were reported by Yadav *et al.* (2021), who observed that mancozeb 50% + carbendazim 25% WS and carbendazim 50% WP significantly increased seed germination, as well as improved shoot length, root length and seedling vigour of maize seedlings compared to the control. Likewise, Dey *et al.* (1989) observed that seed treatment with Bavistin @ 0.3% against *F. moniliforme* resulted in enhanced germination and seedling survival under both in vitro and in vivo conditions. Several other researchers (Kumar, 1999; Khokhar *et al.,* 2014; Jamil and Ashraf, 2020; Thori, 2011) also supported that seed germination was hastened by Bavistin when treated @ 0.2%. However, Gam (2018) observed maximum germination in seeds treated with mancozeb 63% + carbendazim 12% @ 0.2% and *T. viride* @ 10g/kg seed, along with significant reduction in disease index. Many workers (Anitha *et al.,* 2013; Bana *et al.,* 2017) have proved that seed treatments maintain integrity of seed by covering minor cracks and irregularities in the seed coat, creating barriers against fungal invasion. Moreover, they function as physical barriers, reducing the leaching of inhibitors from the seed covering and limiting oxygen movement, ultimately lowering embryo respiration, slowing down seed aging and enhancing the seed germination.

Conclusion

Seed rot and seedling blight diseases significantly reduce maize productivity and diminish the yields of high-yielding varieties. Treating seeds helps control these diseases effectively while also enhancing grain production, making it a cost-effective and practical approach to improving plant stands and overall productivity in maize cultivation. The present study revealed that treatment with carbendazim 50% WP (Bavistin 50 WP) @ 3g/kg seed or mancozeb 50% + carbendazim 25% WS (Sprint 75 WS) @ 2g/kg seed significantly improved maize seed health and vigor resulting in higher grain yields than other treatments. The incorporation of biocontrol agents such as *Trichoderma harzianum* further enhances disease suppression and offers a sustainable alternative for integrated disease management in maize cultivation. Future research could focus on exploring the synergistic effects of combining chemical fungicides with biocontrol agents under diverse agroclimatic conditions.

Declarations

Conflict of interest: The authors declare that they have no conflicts of interest

Ethical statement: This article does not contain any studies involving animals/human participants performed by any of the authors.

Data availability: All data generated or analyzed during this study are included in this published article

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