Original Research Article

IN-VITRO AND IN-VIVO MANAGEMENT OF SEED BORNE FUNGAL DISEASES OF MAIZE.

ABSTRACT

The research experiment was conducted during 2021-22 to test the efficacy of seed dressing fungicides and bioagents against seed borne fungal infections *in-vitro*and *in-vivo* conditions. The results of the experiment revealed the lower per cent seedinfectionwas noticed in seed treatment with carboxin 37.5% + thiram 37.5% @ 2 g/ kg of seedsand mancozeb 50% + carbendazim 25% @ 2 g/ kg of seed and they also contributed for the vigorous seedling growth. Among the bioagents tested*Pseudomonas fluorescens* @ 10 g/ kg of seeds recorded highest germination per cent by its various mode of action. In the field the seed treatment with mancozeb 50% + carbendazim 25% WS @ 2 g/kg seeds better performance in reducing the disease severitythere by increasing the yield and shelling percentage.

Key words: bioagents, disease severity, fungicides, maize, seed infection

INTRODUCTION

Maize (*Zea mays* L.) known as the "Queen of Cereals," because of its genetic yield potential. It is the world's third most significant cereal crop after rice and wheat. It is cultivated primarily for grain, but also for fodder and raw material for industrial processes. The major constraints to maize production in the country include both abiotic and biotic stresses, such as drought, nutrient deficiencies, weeds, diseases and insect pests. Among the biotic stresses, one of the most important limiting factors being diseases in maize production (Khokhar et al. 2014). One hundred and twelve diseases are known to appear in maize crops, where more than 70 diseases are seed-borne. The three major groups of pathogens *viz.*, bacteria, fungi and viruses all can be seed transmitted and affect the seed health quality of maize. Sixty fungi are known to be seed-borne or seed transmitted in maize (Rai et al. 1991). Often seeds are majorly used as planting material in maize crop production. Hence, seed is the most crucial and vital component in crop production. The seed material used for sowing has a big impact on crop performance. Quality seed is critical for unlocking the species and variety's full yield potential. But seed quality is deteriorated by many factors, majorly environmental and biological factors, biological factors include pests and pathogens. The fungal invasion may occur during both pre-harvest and post-harvest conditions. In post-harvest storage, seeds may develop discolouration, seed rotting and caking, mycotoxin contamination and loss of viability leading to poor crop stand.Therefore, application of seed treatment to maize seed is an important step that help in ensuring crops establishment and grow to their full potential. Seed treatment has a key role to play in the first four to six weeks after planting, the period when young seedlings are most at risk from external threats such as insect and disease pressure. It is known fact that the choice of chemicals and bioagents for seed treatment exerts a positive effect on the quality of crop stand and crop performance. So that present investigation is taken up to know the efficacy of seed dressing fungicides and bioagents in reducing the seed infection, improving the seed quality parameters under *in- vitro* conditions and reducing the seed fungal disease severity in field condition.

MATERIALS AND METHODS

The present investigation was carried out during 2021-22 in laboratory of Department of Plant Pathology and field experiment was conducted under All India Co-ordinated Research Project, Main Agriculture Research Station, University of Agricultural Sciences, Dharwad.

Rolled towel method

Rolled towel method was employed to know the effect of seed-borne inoculum on seed quality parameters of maize i.e., to carry out germination and vigour tests of apparently healthy and infected seeds of maize and also to see the effect of different seed treatments as listed in Table 1, on seed-borne inoculums as per the International Seed Testing Association Rules (Anon, 1996). Randomly selected 50 seeds in three replications were placed on two layers of moist germination paper, which were placed on a polythene paper and rolled carefully to avoid any excess pressure on seeds which consists of nine treatments including control and each treatment was replicated thrice. These towels were incubated in seed germinator at 20±2ºC for eight days. All morphologically normal seedlings were counted and germination was expressed in percentage. To find out the seedling vigour, ten seedlings were taken from the germination test at random and the root length was measured from the collar region to the tip of the primary root and the mean root length was expressed in cm. The same seedlings were used for the measurement of shoot length. The shoot length was measured from the collar region. The mean shoot length was expressed in cm. Vigour index was calculated by the following formula, given by Abdul Baki and Anderson, 1973.

Vigour Index = Seed germination (%) × Seedling length (Shoot +Root length (cm)

Field experiment

A field experiment was conducted during *rabi* 2021-2022. Maize genotype G-25 was used for the study. All the package of practice were followed and kept common to all the treatments. The experiment was laid out in Completely Randomized Block Design (RBD) with three replications. The treatments were randomly allotted to the plots.

Observations were recorded at silk drying stage on Per cent Disease Index (PDI) of turcicum leaf blight on 0 to 9 scale prescribed in Annual Progress Report, 2016, Indian Institute of Maize Research, Ludhiyana. The incidence of stalk rot was recorded at physiological maturity stage by splitting up of stem recording for presence of disease symptoms. characterized by a pinkish or reddish discoloration with rotting symptoms inside the stalk when split open was recorded as the fusarium stalk rot and the presence of many minutes black round structures inside the stalk when split open that can give it a grey to black appearance was recorded as the charcoal rot incidence.

Further, the per cent disease incidence was assed by formula

$$\text{Per cent disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants examined}} \times 100$$

Table1: Treatment details

Sl. No.	Treatment detail	Trade name	Dosage (g/kg seeds)
1.	*Trichoderma harzianum*	-	10
2.	*Trichoderma harzianum*	-	5
3.	*Pseudomonas fluorescens*	-	10
4.	*Pseudomonas fluorescens*	-	5
5.	*Bacillus subtilis*	-	10
6.	*Bacillus subtilis*	-	5
7.	Carbendazim 50WP	Bavistin	2
8.	Mancozeb 50% + Carbendazim 25% WS	Sprint	2
9.	Carboxin 37.5% + Thiram3705% WP	Vitavax Power	2
10.	Thiram 75% WP	Thiram	3
11.	Control (Untreated check)		

RESULTS

In- vitro evaluation

Efficacy of four seed dressing fungicides and three bioagents in two different dosages were tested against seed-borne fungal infections of maize (Genotype: G-25) using rolled towel method, as explained in ‘Material and Methods’ revealed that, seed treatment with carboxin 37.5% + thiram 37.5% WP @ 2 g/kg seeds showed least seed infection (4.66%), the seed infection was decreased by 92.85 per cent over the untreated control followed by mancozeb 50% + carbendazim 25% WS @ 2 g/kg seeds (6.99%) where the infection was decreased by 89.79 per cent over untreated control. However, both treatments were statistically on par with each other.

The highest seed germination was recorded in seed treatment with *Pseudomonas fluorescens* @ 10 g/kg seeds (96.66%) which was at statistically on par with all the chemicals evaluated along with the seed treatment with *Bacillus subtilis* @ 10 g/kg seeds. Whereas seedling vigour was recorded highest in seed treatment with carboxin 37.5% + thiram 37.5% WP @ 2 g/kg of 3882 i.e., 32.44 increase in seedling vigour over untreated control. Whereas the seed treatment with mancozeb 50% + carbendazim 25% WS @ 2 g/kg seeds and carbendazim 50 WP @ 2 g/kg seedswere on par with the highest one with vigour index of 3731 and 3648 respectively (Table 2).

Table 2: Efficacy of seed dressing fungicides and bioagents against seed borne fungalinfection and other seed quality parameters of maize by rolled towel

Tr. No.	Treatment	Dosage (g/kg seeds)	Per cent seed germination	Per cent germination increase over control	Per cent seed infection	Per cent seed infection decrease over control	Vigour index	Per cent increase in vigour over control
1	*Trichoderma harzianum*	5	90.00 (71.59)*	16.66	18.66 (25.54)	71.42	3192	8.91
2	*Trichoderma harzianum*	10	88.66 (70.41)	12.96	35.33 (36.45)	45.91	2931	-1.12
3	*Bacillus subtilis*	5	92.00 (73.62)	25.95	28.00 (31.92)	56.12	3263	15.97
4	*Bacillus subtilis*	10	93.33 (75.77)	22.22	24.00 (28.33)	75.50	3270	15.03
5	*Pseudomonas fluorescens*	5	90.00 (71.59)	16.66	28.66 (32.35)	57.14	3399	11.32
6	*Pseudomonas fluorescens*	10	96.66 (79.56)	35.18	16.00 (23.54)	63.26	3371	11.57
7	Carbendazim 50WP	2	96.00 (78.68)	33.33	8.66 (17.09)	86.73	3648	24.46
8	Mancozeb 50% + carbendazim 25% WS	2	94.00 (75.82)	23.80	6.99 (14.92)	89.79	3731	27.28
9	Carboxin 37.5% + thiram 37.5% WP	2	95.33 (77.55)	31.48	4.66 (12.41)	92.85	3882	32.44
10	Thiram 75% WP	3	93.33 (75.77)	25.92	13.33 (21.39)	79.59	3140	7.13
11	Control (untreated check)		84.00 (66.42)	-	56.33 (48.63)	-	2898	-
	S.Em. ±		1.56		0.77		95.97	
	CD(p=0.01)		4.61		2.27		283.29	
	CV (%)		3.66		4.78		4.85	

Field experiment

Turcicum blight produces small, yellowish, round or oval spots on the leaves initially later these spots extend along the leaf and coalesce into longitudinal bands, these bands form lesions that are elliptical and tan in colour, developing distinct dark areas as they mature. The seed treatment with mancozeb 50% + carbendazim 25% WS @ 2 g/kg seeds recorded a low per cent disease index at silk drying stage with per cent disease index of 29.63. It has decreased the disease by 24.98 per cent over the untreated control. The seed treatment with carboxin 37.5% + thiram 37.5% WP @ 2 g/kg seeds was found on par with mancozeb 50% + carbendazim 25% WS @ 2 g/kg seeds with per cent disease index of 30.86 and 21.86 per cent decrease in disease over the untreated control. Among biocontrol agents seed treatment with *Trichoderma harzianum* @ 10 g/kg seeds was found effective in reducing the disease recording the per cent disease index of 32.72 (Table:3).

Fusarium spp. is the most commonly reported fungus infecting maize. Infections can be endophytic (asymptomatic) or pathogenic resulting in disease symptoms, namely seedling blights, root rots, stalk rots, and ear rots. Fusarium stalk rot *is* characterized by a pinkish or reddish discoloration with rotting symptoms inside the stalk when split open. The reduction in disease assessed by recording the per cent disease incidence at physiological maturity stage. The low per cent disease index was observed in seed treatment with mancozeb 50% + carbendazim 25% WS @ 2 g/kg seeds (4.32%) by reducing the disease incidence by 51.80 per cent over the untreated control, followed by seed treatment with carboxin 37.5% + thiram 37.5% WP @ 2 g/kg seeds (4.46%) by reducing the disease incidence of 50.22 per cent over the untreated control. (Table:3)

The charcoal stalk rot disease is characterized by the presence of many minutes black round structures *i.e.,*microsclerotial bodies inside the stalk when split open that can gives it a grey to black appearance. The incidence was observed based on the characteristic symptom at silk drying stage and low incidence was observed in seed treatment with mancozeb 50% + carbendazim 25% WS @ 2 g/kg seeds (7.80 %) which decreased the disease incidence by 48. 45 per cent over untreated control, followed by carboxin 37.5% + thiram 37.5% WP @ 2 g/kg (8.32 %) which decreased the disease incidence by 45.00 per cent compared to untreated control. Along with these two-treatment seed treatment with carbendazim 50 WP @ 2 g/kg seeds was found statistically on par with each other. However, all treatments differed significantly over control in minimizing charcoal stalk rot incidence (Table:3).

The seedlings mortality was observed at 30 DAS and lowest mortality observed in treatment T_9*i.e.* seed treatment with carboxin 37.5% + thiram 37.5% WP @ 2 g/kg (25.33 %) followed by mancozeb 50% + carbendazim 25% WS @ 2 g/kg (27.12 %). Shelling per cent and hundred seed weight was found highest in seed treatment with mancozeb 50% + carbendazim 25% WS @ 2 g/kg recording 84.21 per cent and 28.33 grams respectively. The highest yield of 45.76 q/ha was found in seed treatment with mancozeb 50% + carbendazim 25% WS @ 2 g/kg followed by45.39(Table:3).

Sl. No.	Treatment	Per cent Charcoal stalk rot incidence	Charcoal stalk rot incidence per cent decrease over untreated control	Turcicum leaf blight (PDI)	Turcicum leaf blight disease decreases over untreated control	Per cent fusarium stalk rot incidence	Fusarium stalk rot incidence Per cent decreases over untreated control	Mortality (%)	Shelling (%)	Yeild(q/ha)
1	Seed treatment (ST) with *Trichoderma harzianum* @ 5 g/kg seeds	11.75 (20.04)*	22.31	34.57 (36.01)	10.92	6.20 (14.41)	30.79	35.25 (36.42)*	76.30 (60.86)	42.43
2	ST with *Trichoderma harzianum* @ 10 g/kg seeds	10.14 (18.56)	33.00	32.72 (34.88)	17.17	5.26 (13.26)	41.26	33.58 (35.41)	77.66 (59.92)	43.90
3	ST with *Bacillus subtilis* @ 5 g/kg seeds	12.58 (20.77)	16.83	35.19 (36.38)	10.92	7.83 (16.25)	12.59	35.47 (36.55)	74.89 (61.45)	41.13
4	ST with *Bacillus subtilis* @ 10 g/kg seeds	10.42 (18.83)	31.14	34.57 (36.01)	15.61	6.98 (15.31)	22.11	34.09 (35.72)	77.17 (59.92)	42.32
5	ST with *Pseudomonas fluorescens* @ 5 g/kg seeds	11.25 (19.59)	25.65	33.95 (35.63)	14.04	6.88 (15.20)	23.20	31.54 (34.16)	75.18 (60.11)	41.61
6	ST with *Pseudomonas fluorescens* @ 10 g/kg seeds	8.84 (17.29)	41.60	33.33 (35.26)	15.61	5.73 (13.84)	36.09	30.94 (33.79)	78.14 (62.82)	42.35
7	ST with carbendazim 50WP @ 2 g/kg seeds	8.67 (17.12)	42.69	32.10 (34.51)	18.73	4.79 (12.64)	46.53	29.77 (33.06)	80.27 (63.62)	44.90
8	ST with mancozeb 50%+ carbendazim 25% WS @ 2 g/kg seeds	7.80 (16.21)	48.45	29.63 (32.97)	24.98	4.32 (11.99)	51.80	27.12 (31.38)	84.51 (66.82)	45.76
9	ST with carboxin 37.5%+ thiram 37.5% WP @ 2 g/kg seeds	8.32 (16.76)	45.01	30.86 (33.74)	21.86	4.46 (12.19)	50.22	25.33 (30.15)	82.92 (65.58)	45.39
10	ST with thiram 75% WP 3g/kg seeds	9.05 (17.50)	40.18	31.48 (34.13)	20.30	4.98 (12.89)	48.91	30.68 (33.63)	79.14 (62.82)	44.07
11	Control (untreated check)	15.13 (22.89)	-	39.51 (38.94)	-	8.96 (17.42)	-	43.18 (43.95)	64.36 (53.34)	40.24
	S.Em. ±	0.41		1.27		0.25		1.73	3.34	1.04
	CD @ 5%	1.26		3.72		0.75		5.07	9.80	3.05
	CV%	8.36		9.69		7.39		9.10	7.48	6.30

Table 3: Field evaluation of seed dressing fungicides and bio agents for the management of seed-borne fungal diseases and yield parameters of maize

DISCUSSION

In-vitro evaluation of seed dressing fungicides and bioagents by rolled towel method revealed the highest seed germination was recorded in seed treatment with *Pseudomonas fluorescens* @ 10 g/kg seeds with 35.18 per cent increase in seed germination over untreated control. As that of Bharathi et al. (2013)where study was carried out to evaluate the response of biopeticides and biofertilizers on seed mycoflora and seed quality parameters of sesame (*Sesamum indicum L.*), germination percentage was maximum in the treatment *Trichoderma* + *Pseudomonas* formulation recording 96 per cent. Rao *et al*. (2009) reported the efficacy of *Pseudomonas fluorescens* by bio-priming the sunflower seeds for the effective management of alternaria blight of sunflower and the best results were obtained in treatment with *Pseudomonas fluorescens* (0.8%) in jelly. *Pseudomonas fluorescens* is known as one of the important plant biocontrol agents as it also acts as plant growth promoting rhizobacteria, it plays major role in plant growth promotion by production of wide spectrum of bioactive metabolites and induce systematic resistance. Collectively all these qualities may have contributed to better germination of seeds. However, all the fungicides used were on par with the *Pseudomonas fluorescens* @ 10 g/kg seeds as the chemicals are effective in reduction of infection by pathogens there by promoting the germination (Fig 1a).

The least per cent seed infection was observed in seed treatment with carboxin 37.5% + thiram 37.5% WP @ 2 g/kg seeds followed by seed treatment with mancozeb 50% + carbendazim 25% WS @ 2 g/kg seeds. Kumar *et al*. (2021) also reported that, the least per cent seed infection of 7.33 per cent was noticed in seed treatment with carboxin 37.5% + thiram 37.5% @ 2 g/ kg of seed with per cent germination of 91.67 and seedling vigour index of 785.11 which is found to be on par with seed treatment with mancozeb 50% + carbendazim 25% @ 2 g/ kg of seed with infection of 10 per cent, germination of 89.33 per cent and seedling vigour index of 708.04. In study conducted by Sheelavant (2021) to assess the efficacy of seed treatment with fungicide on seed-borne inoculum of *Colletotrichum gloeosporioides*and seed quality parameters by rolled towel method, the seed treatment with mancozeb + carbendazim @ 0.2% recorded the highest per cent seed germination and seedling vigour, low per cent seed infection (94%, 2906.17 and 7.33% respectively) and was on par with seed treatment with carboxin + thiram @ 0.2%.Carboxin 37.5% + thiram 37.5% WP is a broad spectrum, dual action (systemic and contact) fungicide which controls seed and soil borne diseases and also acts as plant growth stimulant (Fig 1b).

The seedling vigour in seed treatment with *Trichoderma harzianum* @ 10 g/kg seeds was decreases by 1.12 per cent over untreated control because the bioagent was itself colonised the seeds there hindering the germination and vigour (Fig:1c).

The lowest per cent disease incidence of charcoal stalk rot and fusarium stalk rot was observed in seed treatment with mancozeb 50% + carbendazim 25% WS @ 2 g/kg. Gogoi *et al*. (2022) evaluated the new chemical formulations against the post-flowering stalk rot in maize, which among them carbendazim 20 which is carbendazim based product was found to reduce the disease to maximum extent. Mancozeb 50% + carbendazim 25% WS has a combination of both curative and preventive activity. It has broad-spectrum contact and systemic fungicide specially formulated for the control of seed and early soil borne diseases and this may be the reason for effective control of stalk rot pathogens which are known to transmit through seed or infect during early crop stages and exhibit symptoms in later post flowering stages.

Biological seed treatments can be highly effective, it must be recognized that they differ from chemical seed treatments by their utilization of living microorganisms. Storage and application conditions are more critical than with chemical seed protectants and differential reaction to hosts and environmental conditions may cause biological seed treatments to have a narrower spectrum of use than chemicals. Among the biocontrol agents *Trichoderma harzianum* @ 10 g/kg seeds have known to reduce the charcoal rot and fusarium stalk rot incidence by 33 per cent and 41.26 per cent over the control respectively.It is known that the *Trichoderma*-based biocontrol mechanisms mainly rely on mycoparasitism, production of antibiotic and/or hydrolytic enzymes, competition for nutrients, as well as known to induced plant resistance and numerous secondary metabolites produced by *Trichoderma* species could directly inhibit the growth of several plant pathogens. These mechanisms may act directly or indirectly against the plant pathogen there by reducing the severity of diseases. As that of results obtained by Khan and Khan (2015) where they conducted the field experiment to evaluate the seed treatment with bio-wilt X (*Trichoderma harzianum*), bio-derma (*T. viride*) and Abtec Pseudo (*Pseudomonas fluorescens*). The greatest increase in growth parameters (30–36%) and yield (28–40%) was recorded with bio-wilt X (*T. harzianum*) this was because soil population of *M. phaseolina* was significantly reduced in presence of bio-control agents (Fig 2b and 2c).

The lowest PDI of turcicum leaf blight was recorded in seed treatment with mancozeb 50% + carbendazim 25% WS @ 2 g/kg. This result was in agreement with Malik *et al.* (2018) conducted the field experiment to evaluate the new fungicide molecules against turcicum leaf blight of maize, result revealed that during *kharif* 2014 minimum per cent disease index (44.66%) was recorded in treatment with carbendazim 12 WP + mancozeb 63 WP at 0.125 per cent and among the five fungicides, propiconazole 25 EC at 0.1% and carbendazim 12% WP + mancozeb 63% WP at 0.125% gave best control of maydis leaf blight and significantly increased the grain yield (Fig: 2a).

REFERENCE

Abdul Baki AA and Anderson JP, 1973, Seedling vigour determination in soyabean seeds by multiple criteria. *Crop Sci.*,**13**: 630-633.

Anonymous, 1996, International rules for seed testing. Seed science and Technology, 24:1-335.

Anonymous, 2016, Annual Progress Report, All India Co-ordinated Maize Improvement Project, Indian Council of Agricultural Research, Indian Institute of Maize Research, Ludhiyana, 1-236.

Bharathi V, Sudhakar R, Parimala K and Reddy VA, 2013, Evaluation of bioagents and biofertilizers for the managament of seed and seedling diseases of *Sesamum indicum* (Sesame). *Int. J.Phytopathol*.,**2(**3): 179-186.

Gogoi R., Sipani NS and Rai S, 2022, Chemical control of post-flowering stalk rot of maize with new fungicide formulations.*Indian Phytopathol*.,**3**:1-9.

Khan U and Khan MR, 2015, Seed treatment with bio-fungicides for management of dry root rot of chick pea caused by *Macrophominaphaseolina. Ann. Plant Prot. Sci.*, **23**(2):302-307.

Khokhar MK, Hooda KS, Sharma SS and Singh V, 2014, Post flowering stalk rot complex of maize-present status and future prospects. *Maydica*, **59**(3):226-242.

Kumar DSD, Rao MSL and Manjunatha SE, 2021, *In vitro* evaluation of seed dressing fungicides, bioagent and priming agents against pod blight complex of soybean (*Glycine max* (L.) Merrill) by rolled towel method. *J.Pharmacogn.Phytochem.*, **10**(1):1552-1555.

Malik VK, Singh M, Hooda KS, Yadav NK and Chauhan PK, 2018, Efficacy of newer molecules, bioagents and botanicals against maydis leaf blight and banded leaf and sheath blight of maize. *Plant Pathol.*, **34** (2):121.

Rai A, Rai BK and Rai A, 1991, Seed mycoflora of *Zea mays* in tribal areas. Indian Phytopathol 44(4):526.

Rao MSL, Kulkarni S, Lingaraju S and Nadaf HL, 2009, Bio-priming of seeds: a potential tool in the integrated management of alternaria blight of sunflower. *Helia* **32**(50):107-114.

Sheelavant S, 2021, Studies on anthracnose of cowpea caused by *Colletotrichum gloeosporioides*(Penz.) Penz. and Sacc., *M.Sc. (Agri) Thesis.* University of Agricultural Science, Dharwad, India.

1a

1b

1c

Fig 1: Efficacy of seed dressing fungicides and bioagents against seed borne fungal infection and other seed quality parameters of maize by rolled towel method

a) Carboxin 37.5% + Thiram 37.5% WP @ 2 g/kg seeds
b) *Trichoderma harzianum*@ 10 g/kg seeds
c) Control (untreated check)

2a

2b

2c

Fig 2. Symports of major seed borne fungal diseases in maize

a) Turcicum leaf blight
b) Charcoal stalk rot
c) Fusarium stalk rot