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INHIBITORY EFFECTS OF MANCOZEB ON GROWTH AND STIMULATION OF RESISTANCE AGAINST *FUSARIUM* WILT OF BRINJAL

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ABSTRACT

Mancozeb was tested in four concentrations (0.05%, 0.10%, 0.15% and 0.20%) against *Fusarium oxysporum* f.sp. *melongenae* which causes destructive yield losses in brinjal. The results indicated that 0.20% showed maximum inhibition of 62.2% followed by 0.15% which recorded 57.7% inhibition. All the treatments were tested for seed germination and vigour studies at 3 h and 6 h in which 0.20% recorded maximum germination of 62% and vigour of 427.6 at 3 h whereas at 6 h it showed 58.6% germination and 387.6 seedling vigour. In control, poor germination of 44%, seedling vigour of 287.4 and 40.6% seed germination, vigour of 220.8 was observed at 3 h and 6 h respectively. All the treatments were analysed for vegetative growth, HR and disease protection studies and results stated that, the same 0.20% concentration was effective in increasing Plant height (8.4 cm), SFW (1.8 g), SDW (1g), RFW (1 g), RDW (0.6 g), HR (62%) and offered 51% protection in comparison to control and other treatments tested. Therefore, from the study it was concluded that, Mancozeb at 0.20% concentration was effective in inhibiting the pathogen growth, increased plant growth, HR and offered considerable protection in *in vitro* and green house studies.

INTRODUCTION

The crop brinjal is widely cultivated and consumed as vegetable by people across the world due to its unique taste, nutritional and medicinal importance. *Fusarium* Wilt of brinjal caused by *Fusarium oxysporum* f.sp. *melongenae* is a destructive disease of brinjal. It's a soil borne fungus which causes severe wilting and death of the upper parts of the plants by invading through vascular bundles thereby blocking the xylem transport system (Altinok, 2005). The disease is observed all over the India and in other Asian countries and is more prevalent especially in south Karnataka. Disease symptoms were observed from late spring through the end of summer with temperatures above 25ºC. The pathogen survives for long time as chlamydospores in plant and soil debris even after rotation of non-susceptible crops (Gordon and Martyn, 1997). Mancozeb is a dithiocarbamate non-systemic agricultural fungicide with multi-site, protective action on contact. It is a combination of two other dithiocarbamates: maneb and zineb (Morgan, 1982). The mixture have got potency in protecting plants from various fungal diseases in a wide range of field crops, fruits, nuts, vegetables and ornamentals. It is marketed as Penncozeb, Trimanoc, Vondozeb, Dithane, Manzeb, Nemispot and Manzane. Many of the research scientists reported the use of Mancozeb in stimulation of plant growth and resistance through seed treatment in crop plants like cotton, potatoes, corn, safflower, sorghum, peanuts, tomatoes, flax and cereal grains (Berg, 1988; Hayes and Laws, 1991; Meister, 1992). Similarly, Choulwar *et al*., in 1989 reported that Mancozeb was active in inhibiting the colony growth of *Alternaria solani* at 0.2% concentration and improved resistance in controlling early blight disease of tomato caused by *A. solani* (Singh *et al*., 2001). Biotic agents like rhizobacteria, non-pathogenic fungi, plant extracts and inducers like acibenzolar-S-methyl, salicylic acid were used as alternatives to chemical fungicides in control of *Fusarium oxysporum* f.sp. *melongenae* to minimize the hazardous effects of fungicides on environment (Yildiz *et al*., 2012; Altinok and Dikilitas, 2014; Bashar *et al*., 2015, Naziya and Sharada, 2017). Besides this, farmers prefer fungicides for quick, easy and effective management of fungal diseases. Fungicides are given more preference as they are effectively used in control of many diseases such as coriander root rot, stem rot of Indian mustard and leaf spot of chilli (Bhaliya and Jadeja, 2014; Bharti *et al*., 2015; Muthukumar *et al*., 2016).

In today's scenario, management of fungal diseases is one of the major issue in plant disease management because fungi have gained resistance to almost all fungicides which pose big challenge to researchers and urgent need to develop effective management strategy in order to control fungal diseases therefore the present study was aimed to test the effects of Mancozeb in plant growth promotion through seed germination and vigour analysis, vegetative growth parameters and stimulation of resistance by means of pathogen inhibition, HR and disease protection under *in vitro* and greenhouse conditions.

MATERIAL AND METHODS

Preparation of Mancozeb (MAN)

Different concentrations of 0.05%, 0.10%, 0.15% and 0.20% MAN was prepared by weighing 0.05 g, 0.10 g, 0.15 g and 0.20 g MAN and the final volume of the solution was make up to 100 mL using sterile distilled water (SDW). The solution was mixed well to ensure complete solubilisation (Thippeswamy *et al*., 2006).

In vitro inhibitory potential of Mancozeb against _Fusarium_ wilt pathogen by poisoned food technique

Different concentrations of Mancozeb were tested for its *in vitro* inhibitory activity against *Fusarium oxysporum* f.sp. *melongenae* using poisoned food technique of Grover and Moore, 1962. In this, 15 mL of Potato dextrose agar media (PDA) was poured into the petri plates and to this 2 mL of different concentrations of MAN (mentioned above) were added, mixed well and allowed for solidification. Next, 5 mm mycelial discs of *Fusarium oxysporum* f.sp. *melongenae* (seven-day-old) were taken and aseptically inoculated at the centre of the PDA plates. Simple PDA media plates devoid of MAN served as control. All the Plates were incubated at 25 ± 2° C for seven days and mycelial growth of the pathogen was recorded and % inhibition of mycelia growth was calculated using the formula of Vincent, 1947.

$$\%\text{ inhibition of mycelia growth} = \frac{\text{growth of mycelium in control(mm) - growth of mycelium in treatments(mm)}}{\text{growth of mycelium in control(mm)}} \times 100$$

In vitro evaluation of seed priming with Mancozeb on brinjal seed germination and vigour using paper towel method

As per standard ISTA rules of 2005, 400 brinjal seeds cv. Dhenu were taken, primed with each concentrations of MAN (0.05%, 0.10%, 0.15% and 0.20%) for 3 h and 6 h separately. Seeds primed with Sterile distilled water (SDW) served as control. The primed seeds were placed on moist paper towels in an equal distance, rolled and incubated at room temperature for fourteen days. The experiment was repeated thrice containing four replicates of 100 seeds per treatment. Mean shoot length, Mean root length was recorded, % germination and vigour index (VI) was calculated using the formula of Abdul Baki and Anderson, 1973).

$$PG = \frac{\text{Total number of seed germinated}}{\text{Total number of seed plated}}$$

Vigour Index (VI) = (Mean Root Length + Mean Shoot Length) x Per cent Germination (PG)

Evaluation of Mancozeb on vegetative growth parameters of brinjal under greenhouse conditions

MAN and SDW treated seeds of 3 h duration were sown in 30 cm diameter plastic pots which contain 5 kg of sterilized soil, sand and farmyard manure (FYM) in 3:1:1 ratio. The pots were watered in regular intervals and maintained at 25 ± 2° C for up to 60 days. The experiment was carried out in triplicates. Vegetative growth parameters like plant height, shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW) and root dry weight (RDW) was recorded after 60 days of sowing according to the methods described by Agarwal, 1994.

Evaluation of Mancozeb in offering disease protection in brinjal against _Fusarium_ wilt under greenhouse conditions

60-day-old-plants raised from different concentrations of MAN, non-treated (SDW) of 3 h duration in 30 cm diameter plastic pots containing 5 kg of sterilized soil, sand and farmyard manure (FYM) in 3:1:1 ratio were sprayed with *F. oxysporum* f.sp. *melongenae* inoculum at 1x10^6 conidia mL^{-1} (Altinok, 2005). These plants were monitored regularly for the typical appearance of *Fusarium* wilt symptoms like chlorosis, leaf curling, yellowing and wilting of leaves. Total treatments consisted of 15 pots with 5 seedlings per pot and the experiment was repeated thrice. Disease protection was calculated after 30 days of pathogen inoculation using the formula described below,

$$\text{Disease Protection (\%)} = \frac{\text{\% Fusarium wilt disease in control plants - \% Fusarium wilt disease in treated plants}}{\text{\% Fusarium wilt disease in control plants}}$$

Hypersensitive reaction (HR)

HR was carried out by modifying the procedure of Kumudini *et al*., 2001. Fourteen day old, MAN and SDW treated seedlings of 3 h duration were challenge inoculated with *F. oxysporum* f.sp. *melongenae* (1x10^6 conidia mL^{-1}) for 24 h duration. The experiment consists of 25 seedlings per treatment and repeated four times. The total number of seedlings showing necrotic spots were counted and the % HR was calculated using the formula mentioned below,

$$\%HR = \frac{\text{Number of seedlings with necrotic spots}}{\text{Total number of seedlings taken}} \times 100$$

RESULTS AND DISCUSSION

In vitro antagonist effects of Mancozeb on inhibition of _Fusarium_ wilt pathogen

The ability of Mancozeb was assessed against *Fusarium oxysporum* f.sp. *melongenae* under *in vitro* conditions using poisoned food technique. It was noticed that Mancozeb substantially inhibited the growth of the pathogen in all the concentrations tested. The maximum per cent inhibition of 62.2 was observed at 0.20% concentration followed by 0.15% which showed 57.7% inhibition. In the other two concentrations *i.e.*, 0.05%, 0.10% of MAN, an inhibition of 53.3% and 54.4% was noticed. On the other hand, in control PDA plate's full growth of the pathogen was observed (Table 1, Fig. 1). The present finding of testing's of various concentrations of Mancozeb in inhibiting the growth of *Fusarium oxysporum* f.sp. *melongenae* are in line with the earlier findings wherein antagonistic activity of twelve fungicides, six bioagents and ten botanicals were tested against *Alternaria solani* causing early blight of tomato in which results showed that contact fungicide Mancozeb at 0.2%, systemic fungicide, Hexaconazole at 0.1 % and the combi fungicide Hexazconazole 4% + Zineb 68% at 0.2% recorded the inhibition of 87.21%, 88.88% and 88.88% (Roopa *et al*., 2014). Similar group of study in which different fungicides along with plant extracts were tested in brinjal in which maximum of 100% inhibition was recorded in Carbendazim (50%) and Propiconazole (25%) against *Fusarium solani* and *Fusarium oxysporum*. However Dithane M-45 (80% Mancozeb) at 400 ppm recorded 76.93 and 71.35% inhibition respectively (Bashar *et al*., 2015). Likewise, copper fungicides generated the highest 100% mycelial growth inhibition followed by the acid citric and borax fungicides which recorded 10.82% and 12.93% against *Citrus* Gummosis Caused by *Phytophthora, Phytopythium* and

Table 1: *In vitro* evaluations of Mancozeb (MAN) treatments in inhibition of *Fusarium oxysporum* f.sp. *melongenae*

Sl.No	Treatments	Mean colony diameter (mm)	% growth inhibition
1	Control	9.0 ± 0.00[a]	0.00 ± 0.00[a]
2	0.05% MAN	4.2 ± 0.10[b]	53.3 ± 1.10[b]
3	0.10% MAN	4.1 ± 0.1[b,c]	54.4 ± 1.80[b,c]
4	0.15% MAN	3.8 ± 0.10[c]	57.7 ± 1.50[c]
5	0.20% MAN	3.4 ± 0.03[d]	62.2 ± 0.04[d]

Values are means of three replicates (n = 3) ± indicate Standard errors. Values with same letter(s) within the same column are not significantly ($p \leq 0.05$) different from each other according to Duncan multiple range test.

Table 2 : *In vitro* evaluation of Mancozeb (MAN) treatments for 3h and 6h on brinjal seed germination and seedling vigour

Treatments	3 h		6 h	
	PG	*VI*	*PG*	*VI*
Control	44.0 ± 8.1[a]	287.4 ± 44.9[a]	40.6 ± 9.3[a]	220.8 ± 58.8[a]
0.05% MAN	51.3 ± 5.8[b]	329.5 ± 58.2[b]	45.3 ± 11.5[b]	251.3 ± 64.7[b]
0.10% MAN	58.0 ± 10.9[bc]	367.3 ± 43.8[bc]	47.3 ± 5.3[bc]	281.5 ± 31.4[bc]
0.15% MAN	59.3 ± 4.6[c]	382.0 ± 30.9[c]	48.0 ± 13.0[c]	294.5 ± 108.4[c]
0.20% MAN	62.0 ± 3.2[d]	427.6 ± 18.9[d]	58.6 ± 3.6[d]	387.6 ± 75.7[d]

Values are the means of four replicates (n = 4) ± indicate Standard errors. Values with same letter(s) within the same column are not significantly ($p \leq 0.05$) different from each other according to Duncan multiple range test.

Table 3 : Evaluation of Mancozeb (MAN) treatments on vegetative growth parameters of brinjal at 3 h duration after 60 days of sowing under greenhouse conditions.

Treatments	Shoot Fresh weight (g)	Shoot Dry weight (g)	Root Fresh weight (g)	Root Dry weight (g)	Plant height (cm)
Control	0.6 ± 0.2[a]	0.4 ±0.05[a]	0.5 ± 0.03[a]	0.2± 0.06[a]	5.1 ± 0.3[a]
0.05% MAN	0.9 ± 0.1[a]	0.5 ± 0.1[a]	0.6 ± 0.05[a]	0.3 ± 0.08[a]	5.4 ± 0.4[a]
0.10% MAN	1.2 ± 0.1[b]	0.7 ± 0.1[b]	0.7 ± 0.10[a]	0.4 ± 0.00[a]	5.7 ± 0.3[b]
0.15% MAN	1.6 ± 0.1[c]	0.8 ± 0.1[bc]	0.8 ± 0.05[ab]	0.5 ± 0.05[ab]	6.6 ± 0.1[c]
0.20% MAN	1.8 ± 0.1[cd]	1.0 ±0.1[d]	1.0 ± 0.10[ab]	0.6 ±0.03[bcd]	8.4 ± 0.6[d]

Values are means of three replicates (n = 3) ± indicate Standard errors. Values with same letter(s) within the same column are not significantly ($p \leq 0.05$) different from each other according to Duncan multiple range test.

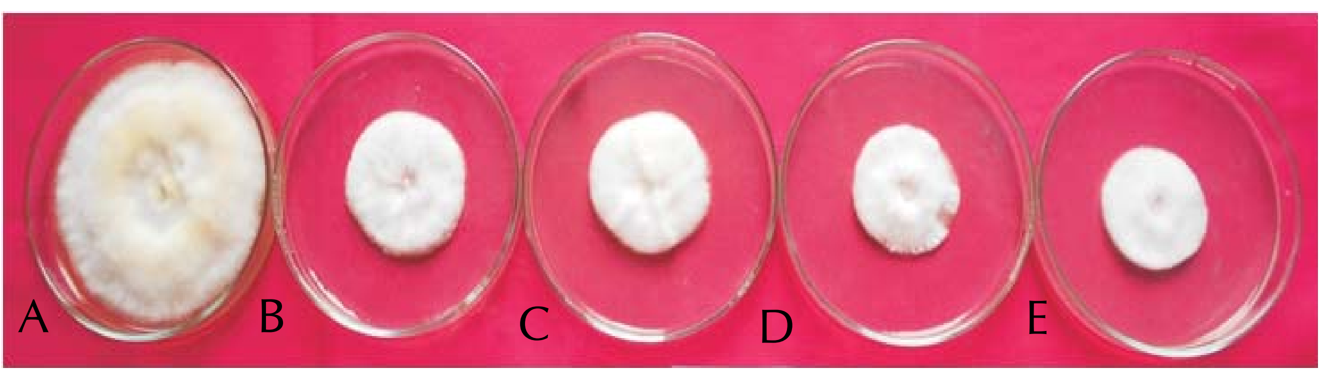
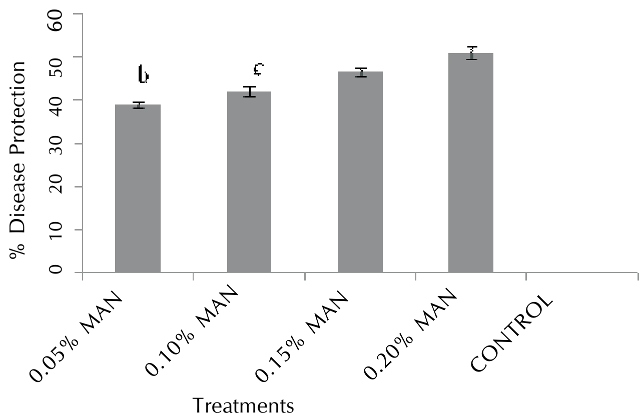


Figure 1: *In vitro* evaluation of Mancozeb (MAN) treatments against *Fusarium oxysporum* f.sp. *melongenae* (A- Control, B- 0.05% MAN, C- 0.10% MAN, D- 0.15% MAN, E- 0.20% MAN).

Pythium respectively (Benfradj *et al.*, 2016). Also, there are many other research workers who reported the efficiency of different chemical fungicides in inhibition of various pathogens such as *F. solani*, *F. oxysporum* f.sp. *pisi*, *F. moniliforme*, *Sclerotinia sclerotiorum* and *Cercospora capsici* under *in vitro* conditions (Bhaliya and Jadeja, 2014; Sadia *et al.*, 2014; Begum *et al.*, 2015; Bharti *et al.*, 2015; Muthukumar *et al.*, 2016).

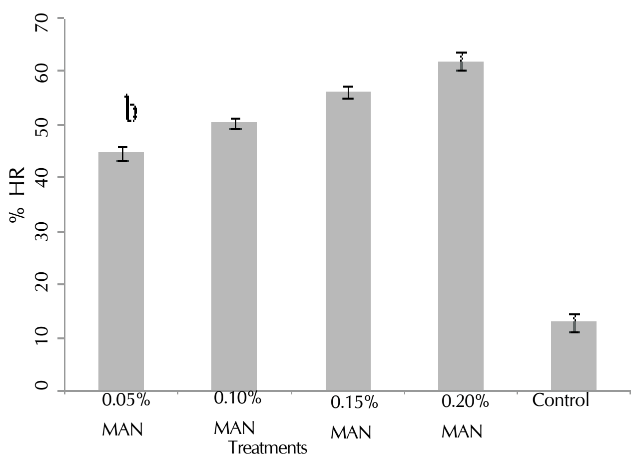
In vitro evaluation of seed priming with Mancozeb on seed germination and seedling vigour of brinjal

Mancozeb tested in four different conditions was considerably effective in enhancing the per cent germination and vigour under *in vitro* conditions in 3 h compared to 6 h by recording highest per cent germination of 62 and seedling vigour of 427.6 at 0.20% concentration followed by 0.15% which showed 59.3% seed germination and vigour index of 382. The 0.05% MAN and 0.10% MAN showed per cent germination of 51.3 and 58 with seedling vigour of 329.5 and 367.3 respectively. However control seeds maintained only with SDW recorded least germination of 44% and 287.4 seedling vigour (Table 2). The present study is in accordance with the research findings of other workers wherein Mancozeb along with other fungicides have showed better potential in enhancement of seed germination and vigour in various crop plants. In case of brinjal, three chemical fungicides namely Captan, Dithane M-45(Mancozeb) and Bavistin at 2g Kg^{-1} recorded seed germination and seedling vigour of 71.15%, 782.84, 69.67%, 769.52 and 70.21%, 776.69 respectively (Srinivas *et al.*, 2005). In addition Mancozeb, Carbendazin and Captan at 0.20% concentration increased seed



Each value is the mean of three replicates (n = 3) and bars sharing different letters are significantly different (p ≤0.05) according to Duncan multiple range test. The vertical bar designates the standard error.

Figure 2: Evaluations of seed treatments with Mancozeb (MAN) for 3 h on *Fusarium* wilt disease protection in brinjal under greenhouse conditions.



Each value is the mean of three replicates (n = 3) and bars sharing different letters are significantly different (p ≤ 0.05) according to Duncan multiple range test. The vertical bar designates the standard error

Figure 3: Seed treatments with Mancozeb (MAN) induced hypersensitive reaction (HR) in brinjal seedlings on challenge inoculation with *Fusarium* wilt pathogen at 24 h.a.i

germination to 95% in brinjal (Thippeswamy *et al.*, 2006). In maize, BAU-Biofungicide treated seeds recorded the highest germination of 87% (Debnath *et al.*, 2012) and Metalaxil and Mancozeb treated seeds recorded the highest germination rate of 100% on the 7th day (Taye *et al.*, 2013). In chilli, Bavistin was most effective with 93% seed germination and increased seedling vigour index of 506.85. This fungicidal treatment was significant over control in which 68% seed germination and vigour index of 306 was observed (Choudhary *et al.*, 2013). Synthetic fungicide (M-45) at 50% concentration reported to increase seed germination up to 90% and seedling vigour to 1406.7 in Tomato (Vikhe and Reddy, 2015).

Effect of Mancozeb treatments on vegetative growth parameters under greenhouse conditions

Mancozeb treated seeds soaked for 3 h duration was selected for vegetative growth studies as they recorded maximum per cent seed germination and vigour compared to 6 h treatments and control. The seeds were sown in sterilized soil, sand containing FYM and allowed to grow for 60 days. After 60 days, vegetative growth parameters were recorded and the results indicate that when compared to all other concentrations tested, 0.20% MAN treatment at 3 h substantially increased shoot fresh weight to 1.8 g, shoot dry weight to 1 g, root fresh weight to 1 g, root dry weight to 0.6 g and plant height to 8.4 cm followed by 0.15% which recorded shoot fresh weight (1.6 g), shoot dry weight (0.8 g), root fresh weight (0.8 g) root dry weight (0.5 g) and plant height (6.6 cm). In control, shoot fresh weight (0.6 g), shoot dry weight (0.4 g), root fresh weight (0.5 g), root dry weight (0.2 g) and plant height (5.1 cm) was recorded at 60th day (Table 3). The present study is in agreement with earlier studies wherein many fungicides including Mancozeb reported to increase vegetative growth parameters in different plants. The fungicide Difenoconazole treatments reported to increase fresh weight dry weight and root growth in *Mentha piperita* when compared to control (Kavina *et al.*, 2011). Mancozeb in addition with other two fungicides (Metalaxyl and Ridomil) at 2.5 g per Kg of the seed have reported to improve vegetative growth characters in maize but the study showed that Metalaxil treatment was stood best in increasing seedling height (12.84 cm), leaves number (4.28), fresh root weight (9.62 g), fresh shoot weight (16.61 g) and dry weight (2.79 g) followed by Mancozeb which showed seedling height (12.42 cm), leaves number (4.25), fresh root weight (8.17 g), fresh shoot weight (16.08 g) and dry weight (2.56 g) whereas the Ridomil treatment reported least vegetative growth parameters over metalaxil and Mancozeb treatments (Taye *et al.*, 2013). Likewise, Siddartha *et al.*, 2017 reported chemical fungicide named Thiram at 2g per Kg of the seed increased plant height to 18.29, 18.25 in two different packaging materials but proved to be better when used in combination with polymer wherein it increased plant height to 16.07 and 16.14 respectively after 2 months of treatment in tomato.

Determinations of disease protection against *Fusarium* wilt of brinjal under greenhouse conditions

The efficiency of Mancozeb treatments in offering disease protection against *Fusarium* wilt of brinjal was evaluated under greenhouse conditions. The results designate that after spray inoculation with pathogen to the 60 day old Mancozeb treated (3 h) raised brinjal plants, a substantial disease protection was achieved against *Fusarium oxysporum* f.sp. *melongenae* and the highest disease protection of 51% was observed at 0.20% concentration followed by 0.15% which showed 46.6% protection. In 0.05% and 0.10% MAN, 39 and 42% of disease protection was noticed. Therefore, all MAN treatments were effective in comparison to control wherein no protection was observed (Fig. 2). The findings of the present study corroborated with studies of Sadia *et al.* (2014) and Begum *et al.* (2015) who reported fungicide treatments lower disease incidence in pea (31% in Topsin-M treatment) upon inoculation with *Fusarium oxysporum* f.sp. *pisi* and cabbage (26% in carbendazim treatment) against *Fursarium moniliforme*. In case of brinjal, fungicidal treatment alone *i.e* Metaxyl at 6 g per Kg seed recorded an average reduction of 37.49% and in combination with Captan (1.5 g + 3 g per Kg seed) recorded reduction up to 29.72% against damping off disease caused by *P. ultimum* but higher reduction was observed when fungicides were used in combination with Garlic and biocontrol agent (*T. viride*) up to 18.33% respectively (Gholve *et al*., 2014).

Hypersensitive reaction (HR)

HR serves to inhibit the growth of the invading pathogen by killing infected and uninfected cells, producing a physical barrier composed of dead cells. During HR, the dying plant cells strengthen their cell walls and accumulate certain toxic compounds like phenols and phytoalexins thereby enhancing resistance (Dangl *et al*., 1996). Keeping this in view, in the present study, HR was tested using Mancozeb as a part of defense response in brinjal against the pathogen invaded. All the Mancozeb treatments of 3 h duration were further assessed for hypersensitive reaction in fourteen day old treated inoculated and non-treated inoculated brinjal seedlings at 24 h of pathogen inoculation and the results indicate that, Mancozeb treatments considerably increased HR over control and the maximum of 62% HR was noticed at 0.20% concentration in the form of brown necrotic spots in treated inoculated seedlings followed by 0.15% which showed 56% HR, 0.10% recorded 50.3% and minimum of 44.6% HR was noticed at 0.05% concentration (Fig. 3). The findings of the present study correlated with the previous studies of Kumudini *et al*., 2001 who reported the leaf treatment of *Datura metel* at 2% increases HR up to 90% at 24 h of pathogen inoculation in susceptible pearl millet seedlings challenge inoculated with *Scleorospora graminicola*. In addition, number of proteins, cerebrosides, fungal oligosaccharides are reported to stimulate distal hypersensitive response (HR) against fungal phytopathogens (Shibuya and Minami, 2001; Meng *et al*., 2010; Zheng *et al*., 2011). Chemicals like chitosan and BTH reported to induce hypersensitive response in barley against *Blumeria graminis* f.sp. *hordei* (Bgh) in the form of brown necrotic streaks (Faoro and Iriti, 2005) and in salicylic acid treated inoculated seedlings induced better hypersensitive response of 72.3% at 20 mM concentration in comparison to the Mancozeb tested in the present study against *Fusarium* wilt pathogen after 24 hours of inoculation (Naziya and Sharada, 2017). The above results frame support to the present findings.

From the results, it was concluded that out of four different concentrations of Mancozeb tested, 0.20% concentration was good in inhibiting the pathogen growth and exhibited better performance in enhancement of brinjal seed germination and seedling vigour under *in vitro* conditions. The same concentration considerably increased vegetative growth parameters and offered maximum protection under greenhouse conditions by inducing moderate hypersensitive response against *Fusarium* wilt pathogen. This source of information or data will be helpful further in preparation of schedule for effective plant disease management strategy.

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