RESEARCH ARTICLE

EVALUATION OF FUNGICIDES AS SEED TREATMENT ON DOMINANT SEED MYCOFLORA OF CHILLI VAR. GVC 101 *IN VITRO*

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Abstract: *In vitro* study was carried out to check the efficacy of seed treatment by fungicides on the seed germination and seedling vigour by controlling the most dominant seed mycoflora of chilli variety GVC 101 was carried out by paper towel method. Seed treatment with carbendazim + mancozeb @ 2.50g/kg seeds found most effective with significantly higher seed germination and seed health parameters for GVC 101 seeds pretreated with *A. niger*. Seed treatment with metalaxyl + mancozeb @ 3.50g/kg seeds found most effective with significantly higher seed germination and seed health parameters for GVC 101 seeds pretreated with *Colletotrichum* sp. Whereas, in seeds pretreated with *Fusarium* sp., significantly higher seed germination and seed health parameters were recorded in seed treatment with metalaxyl + mancozeb @ 3.50g/kg seeds seeds for GVC 101. Captan and metalaxyl + mancozeb were found very effective in controlling or inhibiting the growth of most dominant seed infecting fungi of chilli. It was also noticed that all the seeds treated with tebuconazole @ 1.5g/kg seeds resulted in cent per cent inhibition in seed germination. This indicates toxic effect of tebuconazole @ 1.5g/kg seeds and at higher dose on chilli seeds.

Keywords: *A. niger*, Chilli, *Colletotrichum* sp., *Fusarium* sp., Seed germination, Seed mycoflora

INTRODUCTION

Chilli (*Capsicum annuum* L.) is one of the most valuable commercial crop grown in India. It belongs to the nightshade family Solanaceae and is also called as hot pepper, bell pepper, red pepper, pod pepper, cayenne pepper, paparika and capsicum in different parts of the world (Anonymous, 2009). India is the world's largest producer, consumer and exporter of chilli. Indian share in global production of chilli is 50 to 60 per cent (Anonymous, 2018). In India chilli occupies an area of 774.9 thousand hectare and production of 1492.10 thousand tonnes and the productivity was 1.93 tonnes per hectare (Geetha and Selvarani, 2017). It is known as the universal spice of India because of its diverse utilities for spice, condiment, culinary supplement, medicine and vegetable.

Chilli is propagated by seeds and the role of seed is immense because of its dual role as propagating material and staple food for human (Chamling, 2011). Seed borne diseases can spread easily from one area to another and act as initial source of inoculum in field (Nishikawa *et al*., 2006) Their effect can be adverse on seed health, germination and seedling vigour and also transmit pathogens to the seedlings, accelerates the deterioration in storage, introduce pathogens into new areas and increases the inoculum source in the field (Alves *et al*., 2012). Microorganisms including fungi, bacteria, virus and nematodes can cause seed borne diseases of which fungi are more frequent, and are considered as seed mycoflora.

The association of seed borne mycoflora can reduce the quality, quantity and longevity of seeds and also results in transmission of various diseases. It can adversely affect the nutritive value of the produce and also the market value. Most of these fungi produce mycotoxins as secondary fungal metabolites which are harmful to humans, animals and plants. These mycotoxins has its primarily affect on seed germination, viability, quality, seedling vigour, growth of root and shoot.

Seed borne mycoflora in chilli includes, *Aspergillus niger* Tiegh., *Aspergillus flavus* Link, *F. oxysporum*, *P. aphanidermatum*, *Colletotrichum* sp., *Fusarium moniliforme* J. Sheld., *Alternaria alternata* (Fr.) Keissl., *Penicillium* sp., *Cladosporium* sp., *Curvularia* sp., *Drecheslera* sp., *M. phaseolina*, *Mucor* sp. and *Rhizopus* sp. Among these chilli fruit rot (*Colletotrichum* sp.), damping off (*P. aphanidermatum*) and wilt (*F.oxysporum* f.sp. *capsici*) were reported to cause considerable losses in yield and also observed as seed-borne in nature. It is of vital importance that seeds must be treated before they are sown in the field because the sporulating structures emerging out from the dead chilli seedlings serves as the potential source of inoculum for further spread of pathogen in field.

To increase the production of chilli qualitatively and quantitatively farmer requires healthy and quality seeds, with high percentage of germination and purity. Hence, present investigations were attempted to study the seed treatment with chemical fungicides for preventing seed borne diseases and for producing healthy seeds.

MATERIALS AND METHODS

Collection of seed samples:

Chilli variety GVC-101 was selected for experimental purpose and is collected from Regional

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Horticultural Research Station (RHRS), Navsari Agricultural University, Navsari.The seeds were preserved in polythene bags at room temperature for subsequent investigation.

Identification of dominant seed mycoflora:

Different fungi including *A. niger*, *A. flavus*, *Colletotrichum* sp., *Fusarium* sp., *Alternaria* sp., *Penicillium* sp., *Curvularia* sp., *M. phaseolina*, sterile septate and aseptate fungi were isolated from chilli seed variety GVC 101 by agar plate and standard blotter method. Among them*A. niger*, *Colletotrichum* sp. and *Fusarium* sp. were found to be more common and dominant fungi as it has recorded from almost all the seed samples of chilli.

Evaluation of fungicides as seed treatment on dominant seed mycoflora:

In vitro study to check the efficacy of seed treatment with fungicides on the seed germination and seedling vigour by controlling the most dominant seed mycoflora was carried out by paper towel method. The spore suspension of most dominant mycoflora isolated from chilli seed was prepared separately in sterilized distilled water from 10 days old cultures. The concentration of spores in the suspension was adjusted to 5 X 10^4spores/ml with the help of an improved double neubaur haemocytometer. This suspension was filtered through a sterilized muslin cloth to separate out the mycelium from the spore suspension.

The healthy seeds of GVC-101 were surface sterilized with 0.1% mercuric chloride then washed three times with sterilized distilled water and then soaked in the spore suspension of most dominant fungi for 8 hours separately. Soaked seeds were then drawn out from the solution and spread over the blotter paper for drying. Suspensions of respective fungicides were prepared in sterilized distilled water as per dose given in Table 1. Chilli seeds were then mixed in the above suspension and again kept for 8 hours. Soaked seeds were then drawn out from the solution and spread over the blotter paper for drying. Next these treated seeds were placed equidistantly between two previously wetted germination papers. Seed soaked only in spore suspension of corresponding pathogen were kept as control.

Three repetitions for each of the fungal species in each variety were kept. The paper towels were rolled without disturbing the position of the seed and should be properly labelled. Close the ends with rubber bands, kept in polythene bag and incubate in upright position. After 7 days of incubation period, they were observed and following parameters were measured and calculated.

Germination percentage (%)

Germinated and ungerminated seeds were counted from both healthy and infected seeds. Seedling emergence from seed was considered as successful germination. The germination is expressed as percentage of the ratio of number of normal seedlings to the sum of the normal, abnormal and ungerminated seeds, *i.e*., total planted seeds.

$$\text{Per cent germination} = \frac{\text{Number of normal seedlings}}{\text{Total planted seeds}} \text{ X } 100$$

Seedling length (plumule and radical length) (cm)

After germination, ten normal seedlings from each of the repetition were selected randomly and total seedling length including radical and plumule length was measured. Lot showing the maximum seedling length was considered as vigorous.

Seedling fresh weight (plumule, radical and remaining seed fresh weight) (mg).

The same samples which were used for seedling length as above was used for recording fresh seedling weight from each repetition seperately on electronic weighing balance.

Seedling dry weight (plumule, radical and remaining seed dry weight) (mg)

The same samples which were used for recording fresh seedling weight was utilized. The seedlings were kept in hot air oven at 60^0C for 20 minutes. Oven dry weight of each sample was recorded on electronic weighing balance.

Seedling vigour index.

A combination of standard germination test with seedling length provides broad evaluation of seedling vigour. Seed with high vigour index is considered as vigorous. Vigour index was calculated by using following formulae (Abdul-Baki *et al*., 1973).

Vigour index **(%)** =

Average seedling length on final count (plumule + radical length (cm)) x per cent germination

RESULTS AND DISCUSSION

Results revealed that average per cent seed germination, seedling length, seedling fresh weight, dry weight and seedling vigour index were significantly increased in all the treatments tested over control except in seed treatment with tebuconazole. The results on effect of fungicides as seed treatment on most dominant seed mycoflora (*A. niger*, *Colletotrichum* sp., *Fusarium* sp.)of chilli in var. GVC 101 were presented and discussed here as under.

Aspergillus niger:

Table 2 indicates that the average seed germination was significantly higher in seed treatment with captan (96.67%) as compared to the rest of the treatments and was statistically at par with carbendazim + mancozeb (94.00%). Next best in order of merit was thiram (92.67%) which was at par with carbendazim (90.67%), mancozeb (88.67%) and metalaxyl + mancozeb (87.33%). The lowest seed germination was recorded in the control (31.33%). All seeds treated with Tebuconazole exhibited no germination.

Average seedling length of germinated seed was found significantly higher in seed treatment with captan (3.00cm) as compared to rest of the treatments, followed by carbendazim + mancozeb (2.82cm) and thiram (2.57cm). Next best in order of merit was carbendazim (2.08cm) followed by mancozeb (1.75cm) and metalaxyl + mancozeb (1.04cm). The shortest seedling length was recorded in control (0.85cm). Tebuconazole treated seeds recorded zero seedling length as no germination was recorded.

Average seedling fresh weight of germinated seed was significantly higher in seed treatment with captan (0.83mg) and was statistically at par with carbendazim + mancozeb (0.77mg). Next best in order of merit was thiram (0.61mg) which is at par with carbendazim (0.55mg), which in turn at par with mancozeb (0.50mg) and which in turn again at par with metalaxyl + mancozeb (0.45mg). The least weight was recorded in the control (0.35mg). No fresh weight was recorded as no seeds were germinated in seed treatment with tebuconazole.

Average seedling dry weight of germinated seed was higher in seed treatment with captan (0.22mg) as compared to the rest of the treatments. Next best in order of merit carbendazim + mancozeb (0.18mg) which was statistically at par with thiram (0.17mg) followed by carbendazim (0.13mg). Comparatively lower seedling dry weight was observed in mancozeb (0.10mg) which was at par with metalaxyl + mancozeb (0.09mg). The least seedling dry weight was recorded in the control (0.08mg). No dry weight was recorded in seeds treated with tebuconazole.

Average seedling vigour index of germinated seed was higher in seed treatment with captan (289.53) as compared to rest of the treatments, followed by carbendazim + mancozeb (265.11) and thiram (237.74). Next best in order of merit was carbendazim (188.32) followed by mancozeb (154.91). Comparatively shorter seedling vigour index was noticed in metalaxyl + mancozeb (90.76). The shortest seedling vigour index was recorded in control (26.76). Tebuconazole treated seeds recorded zero vigour index as no seed germination was found in this treatment.

***Colletotrichum* sp. :**

Results presented in Table 3revealed that average seed germination was significantly higher in the seed treatment with metalaxyl + mancozeb (79.33%) as compared to the rest, followed by mancozeb (72.00%) which was statistically at par with carbendazim (69.00%). Next best in order of merit was captan (60.00%), followed by thiram (54.00%). Comparatively lower seed germination was found in carbendazim + mancozeb (50.33%), while least seed germination was found in control (45.67%). All seeds treated with tebuconazole exhibited no germination.

Average seedling length of germinated seed was higher in seed treatment with metalaxyl + mancozeb (1.98cm) and was at par with mancozeb (1.89cm). Next best in order of merit was carbendazim (1.78cm) followed by captan (1.60cm). Comparatively shorter seedling length was noticed in thiram (1.48cm) followed by carbendazim + mancozeb (1.20cm). The shortest seedling length was recorded in control (1.11cm). Tebuconazole treated seeds recorded zero seedling length as no germination was recorded.

Average seedling fresh weight of germinated seed was significantly higher in seed treatment with metalaxyl + mancozeb (0.80mg), followed bymancozeb (0.65mg) which was statistically at par with carbendazim (0.62mg). Next best in order of merit was captan (0.50mg) which was at par with thiram (0.45mg), which in turn at par with carbendazim + mancozeb (0.38mg). The least weight was recorded in the control (0.33mg). No fresh weight was recorded as no seeds were germinated in seed treatment with tebuconazole.

Average seedling dry weight of germinated seed was higher in seed treatment with metalaxyl + mancozeb (0.26mg), which was statistically at par with mancozeb (0.22mg). Next best in order of merit was carbendazim (0.16mg), which was at par with captan (0.15mg). Comparatively lower seedling dry weight was observed in thiram (0.12mg) which was statistically at par with carbendazim + mancozeb (0.11mg). The least weight was recorded in the control (0.05mg). No dry weight was recorded in seeds treated with tebuconazole.

Average seedling vigour index of germinated seed was higher in seed treatment with metalaxyl + mancozeb (156.84) followed by mancozeb (136.29). Next best in order of merit was carbendazim (122.55) followed by captan (96.07). Comparatively shorter seedling vigour index was noticed in thiram (80.08), followed by carbendazim + mancozeb (60.62). The shortest seedling vigour index was recorded in control (50.79). Tebuconazole treated seeds recorded zero vigour index.

***Fusarium* sp.:**

Results presented in Table 4 revealed that average seed germination was significantly higher in the seed treatment with metalaxyl + mancozeb (92.67%) as compared to the rest of the treatments, followed by mancozeb (87.33%). Next best in order of merit was captan (69.33%) which was at par with carbendazim (64.33%). Comparatively lower seed germination was found in thiram (57.67%) which was at par with carbendazim + mancozeb (52.00%), while least seed germination was found in control (50.67%). All seeds treated with tebuconazole exhibited no germination.

Average seedling length of germinated seed was significantly higher in seed treatment with metalaxyl + mancozeb (2.90cm) and at par with mancozeb (2.81cm). Next best in order of merit was captan (2.10 cm) followed by carbendazim (1.77cm). Comparatively shorter seedling length was noticed in thiram (1.62cm) wh-ich was at par with carbendazim + mancozeb (1.60cm). The shortest seedling length was recorded in control (1.36cm). Tebuconazole treated seeds recorded zero seedling length as no germination was recorded.

Average seedling fresh weight of germinated seed was maximum in seed treatment with metalaxyl + mancozeb (1.03mg) and was statistically at par with mancozeb (0.98mg). Next best in order of merit was captan (0.78mg) which was at par with carbendazim (0.75mg). Comparatively lower average seedling fresh weight was found in thiram (0.60mg) followed by carbendazim + mancozeb (0.40mg). The least weight was recorded in the control (0.37mg). No fresh weight was recorded as no seeds were germinated in seed treatment with tebuconazole.

Average seedling dry weight of germinated seed was higher in seed treatment with metalaxyl + mancozeb (0.24mg) as compared to the rest of the treatments. Next best in order of merit was mancozeb (0.21mg) which was statistically at par with captan (0.18mg). Comparatively lower seedling dry weight was found in carbendazim (0.16mg) followed by thiram (0.12mg) and carbendazim + mancozeb (0.10mg). The least weight was recorded in the control (0.05mg). No dry weight was recorded in seeds treated with tebuconazole.

Average seedling vigour index of germinated seed was higher in seed treatment with metalaxyl + mancozeb (268.91) and was followed by mancozeb (245.01). Next best in order of merit was captan (145.67) followed by carbendazim (113.66). Comparatively shorter seedling vigour index was noticed in thiram (93.40) which was statistically at par with carbendazim + mancozeb (83.24). The shortest seedling vigour index was recorded in control (68.97). Tebuconazole treated seeds recorded zero vigour index.

Table 1. List of fungicides and their dose used in the experiment

Tr. No.	Technical name of fungicides	Dosage (g/kg seeds)
T_1	Carbendazim 50% WP	2.00
T_2	Tebuconazole 250 EC (25.9% w/w)	1.50
T_3	Thiram 75% WP	3.50
T_4	Captan 50% WP	3.50
T_5	Mancozeb 75% WP	3.00
T_6	Carbendazim 12% + Mancozeb 63% WP	2.50
T_7	Metalaxyl 4 % + Mancozeb 64 % WP	3.50
T_8	Control (treated with respective pathogen only)	-

Table 2. Effect of seed treatment with fungicides on chilli seeds of var. GVC 101 pretreated with *A. niger*

Tr. No.	Treatment	Dosage(g/kg seeds)	Av. seed germination (%)	Av. seedling length(cm)	Av. fresh weight of seedling (mg)	Av.dry weight of seedling (mg)	Av. seedling vigour index
T_1	Carbendazim	2	72.26* (90.67)	8.28 (2.08)	4.25 (0.55)	2.06 (0.13)	13.72** (188.32)
T_2	Tebuconazole	1.5	0.57 (0.00)	0.57 (0.00)	0.57 (0.00)	0.57 (0.00)	0.71 (0.00)
T_3	Thiram	3.5	74.50 (92.67)	9.21 (2.57)	4.48 (0.61)	2.36 (0.17)	15.42 (237.74)
T_4	Captan	3.5	81.59 (96.67)	9.97 (3.00)	5.24 (0.83)	2.70 (0.22)	17.02 (289.53)
T_5	Mancozeb	3	70.41 (88.67)	7.59 (1.75)	4.05 (0.50)	1.78 (0.10)	12.44 (154.91)
T_6	Carbendazim +Mancozeb	2.5	75.92 (94.00)	9.66 (2.82)	5.03 (0.77)	2.40 (0.18)	16.28 (265.11)
T_7	Metalaxyl +Mancozeb	3.5	69.21 (87.33)	5.85 (1.04)	3.84 (0.45)	1.75 (0.09)	9.53 (90.76)
T_8	Control (treated with *A. niger* only)	-	34.01 (31.33)	5.30 (0.85)	3.38 (0.35)	1.65 (0.08)	5.17 (26.76)
		S.Em.±	2.14	0.08	0.09	0.06	0.13
		C.D. at 5%	6.40	0.24	0.28	0.19	0.39
		C.V. %	6.19	1.96	4.27	5.72	1.98

*Figures outside the parentheses indicate arc sine transformation values **Figures outside the parentheses indicate SQR (X+0.5) transformation values

Figures in parentheses indicate original values

Table 3. Effect of seed treatment with fungicides on chilli seeds of var. GVC 101 pretreated with *Colletotrichum* sp.

Tr. No.	Treatment	Dosage (g/kg seeds)	Av. seed germination (%)	Av. seedling length (cm)	Av. fresh weight of seedling (mg)	Av. dry weight of seedling (mg)	Av. seedling vigour index
T_1	Carbendazim	2	56.16* (69.00)	7.65 (1.78)	4.50 (0.62)	2.27 (0.16)	11.07** (122.55)
T_2	Tebuconazole	1.5	0.57 (0.00)	0.57 (0.00)	0.57 (0.00)	0.57 (0.00)	0.71 (0.00)
T_3	Thiram	3.5	47.27 (54.00)	6.99 (1.48)	3.84 (0.45)	2.00 (0.12)	8.95 (80.08)
T_4	Captan	3.5	50.76 (60.00)	7.26 (1.60)	4.04 (0.50)	2.24 (0.15)	9.80 (96.07)
T_5	Mancozeb	3	58.04 (72.00)	7.90 (1.89)	4.62 (0.65)	2.70 (0.22)	11.67 (136.29)
T_6	Carbendazim +Mancozeb	2.5	45.17 (50.33)	6.29 (1.20)	3.54 (0.38)	1.87 (0.11)	7.77 (60.62)
T_7	Metalaxyl + Mancozeb	3.5	62.94 (79.33)	8.07 (1.98)	5.13 (0.80)	2.90 (0.26)	12.52 (156.84)
T_8	Control (treated with *Colletotrichum* sp. only)		42.49 (45.67)	6.04 (1.11)	3.31 (0.33)	1.32 (0.05)	7.12 (50.79)
		S.Em.±	1.13	0.06	0.10	0.07	0.19
		C.D. at 5%	3.34	0.19	0.30	0.20	0.56
		C.V. %	4.24	1.69	4.74	5.69	3.70

*Figures outside the parentheses indicate arc sine transformation values **Figures outside the parentheses indicate SQR(X+0.5) transformation values
Figures in parentheses indicate original values

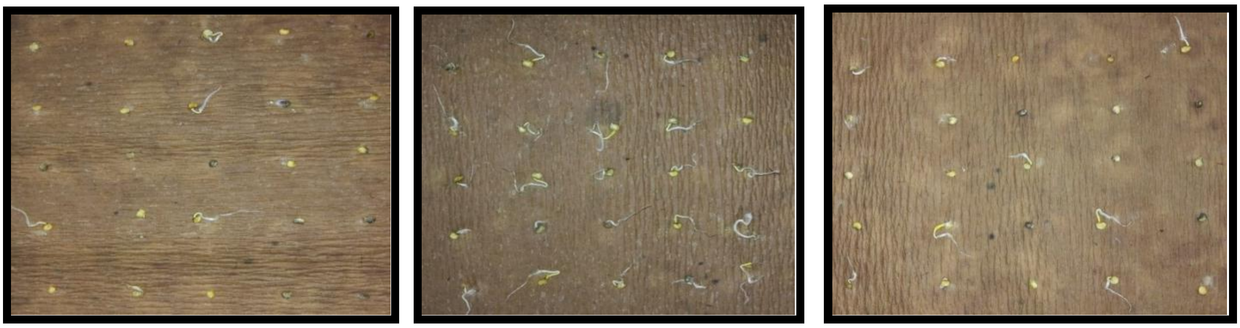
Table 4. Effect of seed treatment with fungicides on chilli seeds var. GVC 101 pretreated with *Fusarium* sp.

Tr. No.	Treatment	Dosage (g/kg seeds)	Av. seed germination (%)	Av. seedling length (cm)	Av. fresh weight of seedling (mg)	Av. dry weight of seedling (mg)	Av. seedling vigour index
T_1	Carbendazim	2	53.31* (64.33)	7.63 (1.77)	4.96 (0.75)	2.29 (0.16)	10.66** (113.66)
T_2	Tebuconazole	1.5	0.57 (0.00)	0.57 (0.00)	0.57 (0.00)	0.57 (0.00)	0.71 (0.00)
T_3	Thiram	3.5	49.39 (57.67)	7.30 (1.62)	4.44 (0.60)	2.00 (0.12)	9.66 (93.40)
T_4	Captan	3.5	56.36 (69.33)	8.33 (2.10)	5.07 (0.78)	2.40 (0.18)	12.07 (145.67)
T_5	Mancozeb	3	69.21 (87.33)	9.64 (2.81)	5.69 (0.98)	2.60 (0.21)	15.65 (245.01)
T_6	Carbendazim +Mancozeb	2.5	46.13 (52.00)	7.26 (1.60)	3.62 (0.40)	1.78 (0.10)	9.11 (83.24)
T_7	Metalaxyl +Mancozeb	3.5	74.50 (92.67)	9.81 (2.90)	5.83 (1.03)	2.81 (0.24)	16.40 (268.91)
T_8	Control (treated with *Fusarium* sp. only)		45.36 (50.67)	6.69 (1.36)	3.46 (0.37)	1.32 (0.05)	8.30 (68.97)
		S.Em.±	1.41	0.08	0.13	0.07	0.23
		C.D. at 5%	4.22	0.23	0.38	0.20	0.68
		C.V. %	4.94	1.85	5.18	5.95	3.81

*Figures outside the parentheses indicate arc sine transformation values **Figures outside the parentheses indicate SQR(X+0.5) transformation values
Figures in parentheses indicate original values

Captantreated seeds **Control** **Metalaxyl + Mancozeb treated seeds**

A. niger



Control **Metalaxyl + Mancozeb treated seeds** **Control**

***Colletotrichum*sp.** ***Fusarium* sp.**

Plate 1: Effect of seed treatment with fungicides on chilli seeds var. GVC 101 pretreated with dominant fungi

CONCLUSION

The data of variety GVC 101 pretreated with *A. niger*, *Colletotrichum* sp. and *Fusarium* sp. indicated that seed germination and seed health parameters were significantly superior in all the treatments except Tebuconazole over control. Seed treatment with carbendazim + mancozeb@ 2.50g/kg seeds found most effective with significantly higher seed germination and seed health parameters for GVC 101 seeds pretreated with *A. niger*(Plate 1). Seed treatment with metalaxyl + mancozeb @ 3.50g/kg seeds found most effective with significantly higher seed germination and seed health parameters for GVC 101 seeds pretreated with *Colletotrichum* sp. Whereas, in seeds pretreated with *Fusarium* sp., significantly higher seed germination and seed health parameters were recorded in seed treatment with metalaxyl + mancozeb @ 3.50g/kg seeds seeds for GVC 101. From over all study, it can be revealed that captan and metalaxyl + mancozeb were found very effective in controlling or inhibiting the growth of most dominant seed infecting fungi of chilli.Machenahalli *et al*. (2014) reported that captan 75 WP showed least infection (12.28%) with highest vigour index (754.64) in non-systemic fungicides tested and among combination fungicides, carboxin 37.5% + thiram 37.5% showed least infection (7.25%) with highest vigour index (932.02) followed by metalaxyl 4% + mancozeb 64% (10.09% seed infection, 871.70 vigour index).

From the present study, it was also noticed that all the seeds treated with tebuconazole @ 1.5g/kg seeds resulted in cent per cent inhibition in seed germination. This showed toxic effect of tebuconazole fungicide on chilli seeds at a concentration of 1.5g/kg seeds or more. Considering the toxicity of tebuconazole on seed germination, it is suggested that for seed treatment in chilli lower dose of tebuconazole or any other safer fungicide like captan @ 3.5g/kg seeds or metalaxyl + mancozeb @ 3.5g/kg seeds can be used for getting higher seed germination, seedling vigour and for controlling seed borne fungi.

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