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Evaluation of Bioagents as Seed Treatment on Dominant Seed Mycoflora of Chilli Var. Gvc 111 *In Vitro*

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Abstract

Study to check the efficacy of seed treatment by bioagents on the seed germination and vigour by inhibiting the most dominant seed mycoflora (A. *niger, Colletotrichum* sp. and *Fusarium* sp.) of chilli variety GVC 111 was carried out by paper towel method *in vitro*. Treatment of seeds with T. *harzianum*+ P. *fluorescens* (5+6g/kg seeds) and P. *fluorescens* (6g/kg seeds) found effective in seeds pretreated with A. *niger*, while T. *harzianum*+P. *fluorescens* (5+6g/kg seeds) and P. *fluorescens*+B. *subtilis* (6+6g/kg seeds) found effective in seeds pretreated with *Colletotrichum* sp. and P. *fluorescens*+B. *subtilis* (6+6g/kg seeds) and T. *viride*+P. *fluorescens* (5+6g/kg seeds) found effective in seeds pretreated with *Fusarium* sp. However, overall results indicated that seed treatment with P. *fluorescens*+B. *subtilis* @ 6g + 6g/kg seeds and T. *harzianum*+P. *fluorescens* @ 5g + 6g/kg seeds proved very effective with higher seed germination, seedling length and vigour index in all pretreated seed mycoflora i.e, A. *niger*. *Colletotrichum* sp. and *Fusarium* sp.

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A. *niger*; Bioagents; Chilli; *Colletotrichum* sp; *Fusarium* sp; Paper towel; Seed germination; Seed mycoflora.

Introduction

Capsicum annuum L. (chilli) is an important spice crop of India which is cultivated in an area of 774.9 thousand hectare with productivity of 1.93 tonnes per hectare.[1] The word “capsicum” is derived from Greek word "Kapsimo" which means "to bite”. Chilli is also known as bell pepper, pod pepper, hot pepper, red pepper, cayenne pepper, paparika and capsicum in different parts of the world. It is believed tobe originated in Mexico as a wild crop around 7500 BC and was first introduced to India by Portuguese during 15th century, more particularly in Goa.[2]

Heavy losses in capsicum can occur due to fungi, viruses, nematodes and bacteria many of which survive in seed. Seed borne mycoflora reported 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.,

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Cladosporium sp., *Curvularia* sp., *Drecheslera* sp., *Macrophomina phaseolina, Rhizopus* sp. and *Mucor* sp.[3,4] Many of these pathogens were carried on seed either internally or externally and can result in reducing seed germination and seedling vigour which leads to poor yield.

Association of seed borne mycoflora with chilli seeds results in loss of seed viability and seedling mortality (post and pre emergence), discolouration of fruit and rotting of fruit. Fruit rot is of great concern as it declines the market value of fruit and the low seed quality may cause yield losses of upto 50 per cent.[4] Dead chilli seedling in field serves as a potential source of inoculum for further spread of pathogen in field. To increase the production and productivity of chilli in terms of quality and quantity, farmer requires healthy seeds, with high germination and purity.

Hence, it is of vital importance that seeds should be treated before they are used in the field. This investigation was carried out to check the efficacy of bioagents on seed mycoflora of chilli.

Materials and Methods

Collection of Seed Samples

Seeds of chilli variety GVC-111 was selected for research purpose and were collected from the Regional Horti cultural Research Station (RHRS), Navsari Agricultural University, Navsari. Seeds were packed and preserved in polythene bags at room temperature for further investigation.

Identification of Dominant Seed Mycoflora

A. *flavus*, A. *niger*, *Alternaria* sp., *Colletotrichum* sp., *Penicillium* sp., M. *phaseolina*, *Curvularia* sp., *Fusarium* sp., sterile septate and aseptate fungi were isolated from chilli seed variety GVC 111 by agar plate and standard blotter method during the experiment. Among those isolated fungi, A. *niger*, *Colletotrichum* sp. and *Fusarium* sp. were found to be more common and dominant fungi as it was noticed from almost all the seed samples.

Evaluation Of Bioagents As Seed Treatment On Dominant Seed Mycoflora

The efficacy of bioagent seed treatment on inhibition of seed borne mycoflora through seed germination and seedling vigour was studied by paper towel method *in vitro*. Spore suspension of most dominant seed mycoflora (A. *niger*, *Colletotrichum* sp. and *Fusarium* sp.) recorded from seeds of chilli variety GVC 111 were prepared separately in sterilized distilled water from 10 days old cultures. By using an improved Double Neubaur Haemocyto meter, concentration of spores was maintained to 5 X 104 spores/ml in the suspension and it was then filtered with muslin cloth to separate the mycelium from spore suspension.[5]

Table 1: Various bioagents treatment evaluated against the most dominant seed mycoflora of chilli *in vitro*

Treatment No.	Name of bioagents	Dose (g/kg seeds)
T_1	*Trichoderma viride* (Navsari isolate)	5
T_2	*Trichoderma harzianum* (Navsari isolate)	5
T_3	*Pseudomonas fluorescens* (Navsari isolates)	6
T_4	Bacillus subtilis (Navsari isolates)	6
T_5	*Trichoderma harzianum*+ *Pseudomonas fluorescens*	5+6
T_6	*Trichoderma viride*+ *Pseudomonas fluorescens*	5+6
T_7	*Pseudomonas fluorescens* + Bacillus subtilis	6+6
T_8	Control (treated with respective pathogen only)	-

(Fungal antagonists @ 10^6cfu/g and bacterial antagonists @ 10^8 cfu/g)

Healthy seeds were surface sterilized with mercuric chloride (0.1%) and then washed three times with sterilized distilled water. Those seeds were soaked in the spore suspension of most dominated fungi for 8 hours separately. The soaked seeds then taken out from the solution and kept on blotter paper for drying. After that suspension of talc based formulation of respective bioagents @ 5g/kg for fungal bioagents (10^6cfu/g) and @ 6g/kg for bacterial bioagents (10^8cfu/g) was prepared (Table 1). Then mix the seeds in suspension and again wait for 8 hours. Soaked seeds were taken out from the suspension and dried in blotter paper. Treated seeds were placed equidistantly between two previously wetted germination papers. Seed soaked only in spore suspension of corresponding pathogen was 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 labelled properly. The ends of paper towels were closed with rubber bands, kept in polythene bag and incubated in upright position. After 7 days of incubation period, they were observed and following parameters were measured and calculated.

Germination Percentage (%)

Count of both germinated and ungerminated seeds was taken from healthy and infected seeds. Seedling emergence from seed was considered as successful germination. The germination percentage was calculated as the ratio of number of normal seedlings to the total planted seeds i.e., sum of the abnormal, normal and ungerminated seeds.[6]

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

Seedling Length (Plumule and Radical Length)

Ten normal seedlings from all repetitions were randomly selected and total seedling length including plumule and radical length was measured. Lot with maximum seedling length was taken as vigorous.

Seedling Fresh Weight (Plumule, Radical And Remaining Seed Fresh Weight) (Mg).

The samples which used for seedling length were also used for recording fresh seedling weight from each repetition separately on electronic weighing balance.

Seedling Dry Weight

The samples used for recording seedling fresh weight were kept in hot air oven at 60^0C for 20 minutes. Dry weight of each sample was taken with electronic weighing balance.

Seedling Vigour Index.

It is the combination of standard germination test with seedling length. Vigorous seed may be those who give high vigour index. Vigour index was calculated by using formula of Abdul-Baki.[7]

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

Results and Discussion

The average per cent seed germination, seedling length, fresh weight, dry weight and vigour index were found to be increased in all treatments tested over control. The effect of bioagents as seed treatment on most dominant seed mycoflora (A. *niger*, *Colletotrichum* sp., *Fusarium* sp.) of chilli in var. GVC 111 are presented and discussed as under.

Aspergillus niger

Results presented in Table 2 revealed that average seed germination was significantly higher in seed treatment with T. *harzianum* + P. *fluorescens* (93.33%) as compared to other treatments and was statistically at par with P. *fluorescens* (90.00%). Next best significant group was B. *subtilis* (86.00%), T. *harzianum* (84.67%) and T. *viride* + P. *fluorescens* (82.00%), where all of them were at par with each other. Relatively lower seed germination was seen in P. *fluorescens*+ B. *subtilis* (80.00%), followed by T. *viride* (64.67%). While lowest seed germination was found in in control (59.33%). Average seedling length was more in the seed treated with T. *harzianum*+ P. *fluorescens* (3.02cm) and was found significantly superior over the rest of treatments. Next best in order was seed treatment with P. *fluorescens* (2.57cm), which was at par with B. *subtilis* (2.42cm) and T. *harzianum* (2.39cm). The length of germinated seeds

was comparatively shorter in T. *viride*+ P. *fluorescens* (2.32cm), followed by P. *fluorescens*+B. *subtilis* (2.01cm) and T. *viride* (1.62cm). The lowest seedling length was recorded in control (1.35cm).

Table 2: Effect of bioagents on seedling growth parameters of chilli seeds pretreated with A. *niger*

Tr. No.	Treatment	Dose (g/kg seeds)	Seed germination (%)	Length of seedling (cm)	Fresh weight (mg)	Dry weight (mg)	Seedling vigour index
T_1	*T. viride*	5	53.51* (64.67)	7.30 (1.62)	3.62 (0.40)	1.44 (0.06)	104.53
T_2	T. *harzianum*	5	67.03 (84.67)	8.88 (2.39)	4.74 (0.68)	1.92 (0.11)	201.72
T_3	P. *fluorescens*	6	71.59 (90.00)	9.21 (2.57)	5.39 (0.88)	2.26 (0.16)	231.10
T_4	B. subtilis	6	68.03 (86.00)	8.94 (2.42)	5.29 (0.85)	2.21 (0.15)	207.73
T_5	T. *harzianum*+ P. *fluorescens*	5+6	75.25	10.01 (93.33	5.69 (3.02)	2.60 (0.98)	282.29 (0.21)
T_6	T. *viride*+ *P. fluorescens*	5+6	64.89 (82.00)	8.75 (2.32)	4.68 (0.67)	1.74 (0.09)	189.87
T_7	*P. fluorescens* + *B. subtilis*	6+6	63.48 (80.00)	8.15 (2.01)	4.19 (0.53)	1.65 (0.08)	161.08
T_8	Control (*treated* with *A. niger only*)	-	50.36 (59.33)	6.66 (1.35)	3.38 (0.35)	1.23 (0.05)	80.20
		S.Em.±	1.39	0.14	0.10	0.04	6.09
		C.D. at 5%	4.16	0.42	0.29	0.12	18.26
		C.V. %	3.74	2.83	3.64	3.67	5.79

*Figures outside the parentheses indicate arc sine transformation value Figures in parentheses indicate original values
(Fungal antagonists @ 10^6cfu/g and bacterial antagonists @ 10^8cfu/g)

Average seedling fresh weight was found maximum in the seeds treated with T. *harzianum* + P. *fluorescens* (0.98mg) and was significantly higher over rest of the treatments. Next best wasP. *fluorescens* (0.88mg), but was at par withB. *subtilis* (0.85mg) T. *harzianum* (0.68mg) and T. *viride*+ P. *fluorescens* (0.67mg). The least weight was observed in the control (0.35mg). Higher average seedling dry weight was found in seeds treated with T. *harzianum*+ P. *fluorescens* (0.21mg), followed by seed treatment with P. *fluorescens* (0.16mg), but both were at par with B. *subtilis* (0.15mg). Next best significant group was T. *harzianum* (0.11mg) followed by T. *viride*+ P. *fluorescens* (0.09mg) and P. *fluorescens*+ B. *subtilis* (0.08mg). The least weight was observed in control (0.05mg).

Table 3: Effect of bioagents on seedling growth parameters of chilli seeds pretreated with *Colletotrichum* sp.

Tr. No	Treatment	Dose (g/kg seeds)	Seed germination (%)	Length of seedling (cm)	Fresh weight (mg)	Dry weight (mg)	Seedling vigour index
T_1	*T. viride*	5	43.83* (48.00)	6.18 (1.16)	3.31 (0.33)	1.36 (0.06)	55.63
T_2	*T. harzianum*	5	45.74 (51.33)	7.18 (1.56)	3.49 (0.37)	1.78 (0.10)	80.10
T_3	*P. fluorescens*	6	58.90 (73.33)	7.44 (1.68)	3.55 (0.38)	2.14 (0.14)	122.85
T_4	*B. subtilis*	6	60.22 (75.33)	7.54 (1.72)	3.84 (0.45)	2.24 (0.15)	129.77
T_5	*T. harzianum*+ *P. fluorescens*	5+6	69.74 (88.00)	8.65 (2.27)	4.59 (0.64)	3.01 (0.28)	199.37
T_6	*T. viride*+ *P. fluorescens*	5+6	63.93 (80.67)	8.08 (1.98)	4.16 (0.53)	2.29 (0.16)	159.73
T_7	*P. fluorescens*+ *B. subtilis*	6+6	64.40 (81.33)	8.19 (2.03)	4.40 (0.59)	2.81 (0.24)	165.47
T_8	Control (treated with *Colletotrichum* sp. only)	-	39.97 (41.33)	4.44 (0.60)	2.70 (0.22)	1.19 (0.04)	24.97
	S.Em.±		1.16	0.10	0.08	0.06	3.31
	C.D. at 5%		3.47	0.29	0.25	0.17	9.91
	C.V. %		3.59	2.30	3.77	4.71	4.88

*Figures outside the parentheses indicate arc sine transformation values Figures in parentheses indicate original values (Fungal antagonists @ 10^6cfu/g and bacterial antagonists @ 10^8cfu/g)

Average seedling vigour index was found significantly higher in seeds treated with T. *harzianum* + P. *fluorescens* (282.29) as compared to other treatments. Next best was seed treatment with P. *fluorescens* (231.10) followed by B. *subtilis* (207.73), which in turn were at par with

T. *harzianum* (201.72) and T. *viride*+ P. *fluorescens* (189.87). Relatively lower vigour index was observed in seed treatment with P. *fluorescens* + B. *subtilis* (161.08) and T. *viride* (104.53). The lowest vigour index was found in the control (80.20).

Colletotrichum *sp*

Results presented in Table 3 revealed that average seed germination was significantly higher in seed treatment with T. *harzianum*+ P. *fluorescens* (88.00%) when compared to all other treatments. Next best was P. *fluorescens*+ B. *subtilis* (81.33%)and was statistically at par with T. *viride* + P. *fluorescens* (80.67%) followed by B. *subtilis* (75.33%). Comparatively lower seed germination was found in T. *harzianum* (51.33%), which was found statistically at par with T. *viride* (48.00%). While lowest seed germination was found in control (41.33%). Average seedling length was significantly the highest in seed treatment with combination of T. *harzianum*+ P. *fluorescens* (2.27cm). However, the next best treatment wasP. *fluorescens*+ B. *subtilis* (2.03cm), which was at par with T. *viride* + P. *fluorescens* (1.98cm). Significantly the lowest seedling length was recorded in control (0.60cm).

Average seedling fresh weight was found significantly higher in seed treatment with combination of T. *harzianum*+ P. *fluorescens* (0.64mg), which was statistically at par withP. *fluorescens* + B. subtilis (0.59mg). Next best significant group includes T. *viride*+ P. *fluorescens* (0.53mg) followed by B. *subtilis* (0.45mg). Least weight was observed in the control (0.22mg). Average seedling dry weight was significantly higher in seed treatment with T. *harzianum*+ P. *fluorescens* (0.28mg), followed by P. *fluorescens*+ B. *subtilis* (0.24mg). Next best significant group includes T. *viride*+ P. *fluorescens* (0.16mg), B. *subtilis* (0.15mg) and P. *fluorescens* (0.14mg). Least weight was observed in the control (0.04mg).

Average vigour index was found significantly higher in seeds treated with T. *harzianum*+ P. *fluorescens* (199.37) as compared to others. Next best in order of merit was P. *fluorescens*+ B. *subtilis* (165.47), which was at par with T. *viride*+ P. *fluorescens* (159.73). Lower vigour index was observed in T. *harzianum* (80.10) followed by T. *viride*(55.63). The lowest vigour index was found in the control (24.97).

Fusarium Sp

Results presented in Table 4 revealed that average seed germination was significantly higher in seeds treated with P. *fluorescens*+ B. *subtilis* (94.00%) and was at par with T. *viride*+ P. *fluorescens* (93.33%). Next best significant group was T. *harzianum*+ P. *fluorescens* (89.33%) with P. *fluorescens* (86.00%). The lowest seed germination was found in control (50.96%).Average seedling length was maximum in seeds treated with P. *fluorescens*+ B. *subtilis* (2.93cm) and was at par withT. *viride*+ P. *fluorescens* (2.76cm), followed T. *harzianum*+ P. *fluorescens* (2.34cm). Next best significant group was P. *fluorescens* (1.97cm) with T. *viride* (1.86cm). The lowest seedling length was recorded in control (1.15cm).

Average seedling fresh weight was maximum in seed treatment with P. *fluorescens*+ B. *subtilis* (1.17mg), followed by T. *viride*+ P. *fluorescens* (0.97mg) and T. *harzianum*+ P. *fluorescens* (0.70mg). Next best significant group possessed P. *fluorescens* (0.50mg), T. *viride* (0.41mg) andB. *subtilis* (0.31mg). Relatively lower vigour index was noticed in T. *harzianum* (0.18mg). The least was observed in the control (0.17mg). Average seedling dry weight was higher in seed treatment with P. *fluorescens*+ B. *subtilis* (0.32mg), followed by T. *viride*+P. *fluorescens* (0.24mg) and T. *harzianum*+P. *fluorescens* (0.21mg). Least weight was found in control (0.04mg).

Average vigour index was found significantly higher in seeds treated with P. *fluorescens*+ B. *subtilis* (275.77) and was at par with T. *viride*+ P. *fluorescens* (258.05). Next best significant group in order of merit was T. *harzianum*+ P. *fluorescens* (209.16), P. *fluorescens* (169.43) and T. *viride*(149.62). Comparatively lower seedling vigour index was noticed in B. *subtilis* (106.14) followed by T. *harzianum* (84.61). The lowest vigour index was found in the control (69.65).

On an overall the treatments were found significantly superior over control in increasing seed health parameter. Seeds treated with T. *harzianum*+ P. *fluorescens* (5+6g/kg seeds) and P. *fluorescens* (6g/kg seeds) found effective when seeds were pretreated with A. *niger*, while T. *harzianum*+ P. *fluorescens* (5+6g/kg seeds). Similarly P. *fluorescens*+ B. *subtilis* (6+6g/kg seeds) found

effective in seeds pretreated with *Colletotrichum* sp. and P. *fluorescens*+ B. *subtilis* (6+6g/kg seeds) and T. *viride*+ P. *fluorescens* (5+6g/kg seeds) found effective in seeds pretreated with *Fusarium* sp.

Table 4: Effect of bioagents on seedling growth parameters of chilli seeds pretreated with *Fusarium sp.*

Tr. No.	Treatment	Dose (g/kg seeds)	Seed germination (%)	Length of seedling (cm)	Fresh weight (mg)	Dry weight (mg)	Seedling vigour index
T_1	*T. viride*	5	63.93* (80.67)	7.83 (1.86)	3.67 (0.41)	1.71 (0.09)	149.62
T_2	*T. harzianum*	5	58.47 (72.67)	6.19 (1.17)	2.33 (0.18)	1.32 (0.05)	84.61
T_3	*P. fluorescens*	6	68.03 (86.00)	8.06 (1.97)	4.05 (0.50)	2.16 (0.14)	169.43
T_4	*B. subtilis*	6	60.27 (75.33)	6.81 (1.41)	3.19 (0.31)	1.48 (0.07)	106.14
T_5	*T. harzianum*+ *P. fluorescens*	5+6	71.02 (89.33)	8.79 (2.34)	4.78 (0.70)	2.60 (0.21)	209.16
T_6	*T. viride*+ *P. fluorescens*	5+6	75.12 (93.33)	9.56 (2.76)	5.64 (0.97)	2.82 (0.24)	258.05
T_7	*P. fluorescens* + *B. subtilis*	6+6	75.92 (94.00)	9.86 (2.93)	6.20 (1.17)	3.24 (0.32)	275.77
T_8	Control (treated with *Fusarium sp.* only)	-	50.96 (60.33)	6.16 (1.15)	2.45 (0.17)	1.19 (0.04)	69.65
	S.Em.±		1.47	0.12	0.11	0.06	6.05
	C.D. at 5%		4.40	0.36	0.34	0.19	18.13
	C.V. %		3.88	2.64	4.85	5.19	6.33

*Figures outside the parentheses indicate arc sine transformation values (Fungal antagonists @ 10^6cfu/g and bacterial antagonists @ 10^8cfu/g) Figures in parentheses indicate original values

Machenahalli[8] reported that seeds treated with T. *harzianum*+ P. *fluorescens* @5+5g/kg seeds showed lower infection (14.89 %) with higher vigour index (930.74) which was followed by P. *fluorescens* (10.0g/kg) with 14.94 per cent infection and vigour index of 915.27. Reddy[9] showed that seeds dressed with T. *viride* (10g/kg seed) and T. *viride* (5g/kg seed)+ P. *fluorescens* (5g/kg seed)

decreased the seed mycoflora infection in chilli by 81.8 per cent compared to untreated seed. The results were more or less in agreement with earlier works of seeds treated with bioagents on germination of seed and other health parameters of seed. The difference in results can be due to difference in dose of bioagents and varieties used for investigation.

From the present study it can be concluded that P. *fluorescens* + B. *subtilis* @ 6+6g/kg seeds and T. *harzianum*+ P. *fluorescens* @ 6+6g/kg seeds were effective in controlling the growth of seed infecting fungi in chilli variety GVC 111.

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Conflict of Interest
The authors do not have any conflict of interest

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