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Effect of Culture Filtrates of Dominant Seed Mycoflora of Chilli on Seed Germination and Seedling Vigour

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A B S T R A C T

Effect of culture filtrates of most common and dominant fungi *viz.*, *Aspergillus niger*, *Colletotrichum* sp. and *Fusarium* sp. of chilli seed variety GVC 101 and GVC 111 was studied on seed germination, seedling length, fresh weight, dry weight and vigour index.In GVC 101 variety, the seeds treated with culture filtrate of *A. niger* showed maximum decrease in seed germination (43.35%) followed by *Colletotrichum*sp. (20.23%) and *Fusarium* sp. (9.83%). Average seedling length was also decreased maximum in seeds treated with culture filtrate of *A. niger* (43.70%) followed by *Colletotrichum* sp. (40.22%) and *Fusarium* sp. (23.91%). Seeds treated with culture filtrate of *A. niger* showed maximum reduction of vigour index by 68.09 per cent followed by *Colletotrichum* sp. (52.31%) and *Fusarium* sp. (31.34%). In GVC 111 variety, the seeds treated with culture filtrate of *A. niger* showed maximum decrease in seed germination (35.12%) followed by *Colletotrichum* sp. (17.89%) and *Fusarium* sp. (15.12%). Average seedling length was also decreased maximum in seeds treated with culture filtrate of *A. niger* (61.14%) followed by *Colletotrichum* sp. (36.64%) and *Fusarium* sp. (25.54%). Seeds treated with culture filtrate of *A. niger* showed maximum reduction of vigour index by 74.73 per cent followed by *Colletotrichum*sp. (47.91%) and *Fusarium* sp. (36.73%).

Introduction

Chilli (*Capsicum annuum* L.) is an important spice crop having various commercial and therapeutic values. It is mainly grown for fruits, which are used in green as well as ripe red form for its pungency. Chilli is a fruit of the plant belonging to the nightshade family Solanaceae with chromosome number (2n = 24). It is grown in almost all parts of tropical and subtropical regions of world and is propagated by seed. The role of seed is immense, as it has a dual role of being the propagating material for seed bearing crops and serving as a staple food for the mankind (Chamling, 2011). Plant diseases cause serious losses to crop plants and from the various modes of spread of plant diseases, seeds play a vital role in the transmission of pathogens and development of plant diseases. Chilli suffers from many diseases caused by fungi, bacteria, viruses and also abiotic stresses. Among them, wilt (*Fusarium*

oxysporum f.sp. *capsici* Schlecht.), cercospora leaf spot (*Cercospora capsici* Heald & F.A. Wolf.), fruit rot and die back (*Colletotrichum capsici* Syd. &P.Syd.), damping off **(***Pythium aphanidermatum* (Edson) Fitz.), powdery mildew (*Leveillula taurica* (Lev.) G. Arnaud), bacterial leaf spot (*Xanthomonas campestris* pv. *Vesicatoria* Doidge), Bacterial soft rot (*Erwinia carotovora* Jones) and common viral disease *viz.*, Tobacco Mosaic Virus (TMV), Tomato leaf curl virus (TLCV).

Among these fruit rot (*Colletotrichum* sp.), damping off (*P. aphanidermatum*) and wilt (*F.oxysporum* f.sp. *capsici*) are reported to cause considerable losses in yield and also observed as seed-borne in nature (Anonymous, 2017).

Seed borne diseases can easily spread from one place to another and serve as an initial source of inoculum potential (Nishikawa *et al.,* 2006). They have adverse effect on seed health, reducing germination ability, poor seedling vigour and transmit fungus to the seedlings, accelerating the deterioration in storage, introducing pathogens into new areas and increasing the inoculum source in the field (Alves *et al.,* 2012). Seed borne diseases are caused by microorganisms including fungi, bacteria, virus and nematodes. Among them, the fungi encounter on seeds are more frequent, and are considered as seed mycoflora.

The seed mycoflora reduce the quality, quantity and longevity of seeds and transmit various diseases. The seed borne fungi not only affect the nutritive value of the produce but also adversely affect the market value. Many of these fungi produce secondary fungal metabolites called mycotoxins which are toxic to humans, animals and plants. These metabolites primarily affect the seed germination, quality, viability, seedling vigour, growth of root and coleoptile.

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., *Cladosporium* sp., *Curvularia* sp., *Drecheslera* sp., *M. phaseolina, Mucor* sp. and *Rhizopus* sp. (Kumari, 2011 and Anonymous, 2017). Association of different seed borne pathogens with chilli seeds are responsible for loss in seed viability and seedling mortality (post and pre emergence), discolouration of fruit and fruit rot. Fruit rot is very important as it reduce the market value of fruit and seed quality may cause yield losses of upto 50 per cent (Anonymous, 2017).

Sporulating structures emerging out from the dead chilli seedlings serves as the potential source of inoculum for further spread of pathogen in field.

Hence, it is of vital importance that seeds must be treated before they are sown in the field. With this background, in the present study, effects of culture filtrates produced by most dominant seed mycoflora of chilli on seed germination and seedling vigour was evaluated.

Materials and Methods

Collection of seed samples

Chilli varieties GVC-101and GVC-111 were selected for the experiment and were collected from Regional Horticultural Research Station (RHRS), Navsari Agricultural University, Navsari. The collected seeds were preserved in plastic polythene bags at room temperature for further investigations.

Detection of seed mycoflora

For isolation of seed borne mycoflora of chilli, two different incubation methods i.e, standard blotter method and agar plate method was employed as prescribed by International Seed Testing Association (ISTA, 1999).

After purification, each of the fungi isolated from different variety of chilli seeds was identified on the basis of their cultural and morphological characteristics and also compared with literature.

Identification of seed-borne fungi

The isolated fungi were identified as *A. niger*, *Aspergillus flavus*, *Colletotrichum* sp., *Fusarium* sp. and *Alternaria* sp., *Penicillium* sp., *Curvularia* sp., *Macrophomina phaseolina*, septate and non sporulating fungi. *A. niger*, *Colletotrichum* sp., *Fusarium* sp. were found most commonly associated mycoflora with chilli seeds using standard blotter and agar plate method, hence considered as dominant.

Effect of culture filtrates of dominant seed mycoflora of chilli on seed germination and seedling vigour

Production of toxin in culture filtrate by pathogenic fungi was studied by growing most common and dominant mycoflora isolated from chilli seeds on Potato dextrose (PD) broth. Fifty ml of PD broth medium was poured in 150ml conical flasks, autoclaved and inoculated separately by adding one ml of standard spore suspension of each of the test fungi. The flasks were incubated at room temperature (27 ± 2^0C) for 10 days. The culture filtrate was collected in pre sterilized blotter by filtering the content through Whatman filter paper no. 1.

200 seeds of most susceptible variety (GVC 111 and GVC 101) were surface sterilized with 0.1% mercuric chloride then washed three times with sterilized distilled water. Seeds were suspended in respective culture filtrate of seed mycoflora and is incubated at room temperature (27 ± 2^0C) for 24 hours.

After that seeds were removed from the culture filtrates. Next these seeds were placed equidistantly between two previously wetted germination paper. Control with only sterile distilled water treatment was kept. The paper were rolled without disturbing the position of the seed and labelled properly. The ends were closed with rubber bands, kept in polythene bag and incubated in upright position. After 7 days of incubation period, they were observed for germination and vigour index and following parameters were measured and calculated.

Germination percentage (%)

Germinated and ungerminated seeds were counted from both healthy and infected seeds. Emergence of seedling from the 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 (Khare and Bhale, 2000).

Per cent germination

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

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 in centimeters. Lot showing the maximum seedling length was considered as vigorous.

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

For fresh weight of seedlings, the same samples which were used for seedling length as above was used for recording fresh seedling weight from each repetition separately on electronic weighing balance.

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

To measure seedling dry weight, 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

Effect of mycotoxin (culture filtrate) of common and dominant fungi *i.e*, *A. niger*, *Colletotrichum* sp. and *Fusarium* sp. isolated from chilli seeds were studied on seed germination and seedling health of chilli seeds of varieties GVC 101 and GVC 111.

GVC101

The results on seed germination and seed health parameters of variety GVC 101 (Table 1, Plate 1 and Plate 3) revealed that culture filtrates of all the three pathogens significantly reduced the germination and other seed health parameters to a great extent.

Results presented in Table 1 showed that maximum reduction in average seed germination was found in culture filtrates of *A. niger* (49.00%) followed by culture filtrates of *Colletotrichum* sp. (69.00%) and *Fusarium* sp. (78.00%). Highest seed germination was recorded in control (86.50%). Seed treatment with culture filtrate of *A. niger* reduced seed germination by 43.35 per cent. It was followed by *Colletotrichum* sp. (20.23%) and *Fusarium* sp. (9.83%).

Average seedling length was found significantly lowest in seed treated culture filtrates of *A. niger* (1.20cm) followed by *Colletotrichum* sp. (1.27cm) and *Fusarium* sp. (1.62cm). Highest average seedling length was recorded in control (2.12cm). Seed treatment with culture filtrate of *A. niger* reduced average seedling length by 43.70 per cent followed by *Colletotrichum* sp. (40.22%) and *Fusarium* sp. (23.91%).

Average seedling fresh weight was found significantly lowest in culture filtrates of *A. niger* (0.44mg) followed by *Colletotrichum* sp. (0.54mg). Whereas, the seed treated with culture filtrate of *Fusarium* sp. (0.72mg) showed minimum reduction in seedling fresh weight.

The highest seedling fresh weight was obtained in control (0.92mg). Average seedling fresh weight was reduced in seed treatment with culture filtrate of *A. niger* by 51.70 per cent followed by *Colletotrichum* sp. (41.50%) and *Fusarium* sp. (21.77%).

Average seedling dry weight was found significantly lower in seed treated with culture filtrate of *A. niger* (0.09mg) followed by *Colletotrichum* sp. (0.12mg) and *Fusarium* sp. (0.17mg). The highest seedling dry weight was obtained in control (0.22mg). Average seedling dry weight was reduced in culture filtrate of *A. niger*by57.56 per cent followed by *Colletotrichum* sp. (44.77%) and *Fusarium* sp. (19.77%).

Average seedling vigour index was found significantly lowest in culture filtrates of *A. niger*(58.57). Comparatively higher seedling vigour index was observed in culture filtrates of *Colletotrichum* sp. (87.55) followed by *Fusarium* sp. (126.03). Highest vigour index was recorded in control (183.57). Seed treatment with culture filtrate of *A. niger* reduced average seedling vigour index by 68.09 per cent followed by *Colletotrichum* sp. (52.31%) and *Fusarium* sp. (31.34%).

GVC 111

The results on seed germination and seed health parameters of variety GVC 111 (Table 2, Plate 2 and Plate 4) revealed that culture filtrates of all the three pathogen significantly reduced the germination and other seed health parameters to a great extent.

Table.1 Effect of culture filtrates of most dominant seed mycoflora on seed germination and seedling growth of chilli variety GVC 101

Sr. No.	Treatment	Av. seed germination		Av. seedling length		Av. fresh weight of seedling		Av. dry weight of seedling		Av. seedling vigour index	
		(%)	Decrease percentage	(cm)	Decrease percentage	(mg)	Decrease percentage	(mg)	Decrease percentage	-	Decrease percentage
1	*Colletotrichumsp.*	56.17* (69.00)	20.23	6.46 (1.27)	40.22	4.19 (0.54)	41.50	1.97 (0.12)	44.77	87.55	52.31
2	*Fusarium sp.*	62.11 (78.00)	9.83	7.29 (1.62)	23.91	4.85 (0.72)	21.77	2.37 (0.17)	19.77	126.03	31.34
3	*A. niger*	44.41 (49.00)	43.35	6.27 (1.20)	43.70	3.80 (0.44)	51.70	1.72 (0.09)	57.56	58.57	68.09
4	*Control*	68.59 (86.50)	-	8.37 (2.12)	-	5.49 (0.92)	-	2.65 (0.22)	-	183.57	-
S.Em.±		0.97	-	0.04	-	0.09	-	0.05	-	2.68	-
C.D. at 5%		2.81	-	0.11	-	0.27	-	0.15	-	7.77	-
C.V. %		4.76	-	1.57	-	5.84	-	6.89	-	6.66	

***Figures outside the parentheses indicate arc sine transformation values Figures in parentheses indicate original values**

Table.2 Effect of culture filtrates of most dominant seed mycoflora on seed germination and seedling growth of chilli variety GVC 111

Sr. No.	Treatment	Av. seed germination		Av. seedling length		Av. fresh weight of seedling		Av.dry weight of seedling		Av. seedling vigour index	
		(%)	Decrease percentage	(cm)	Decrease percentage	(mg)	Decrease percentage	(mg)	Decrease percentage	-	Decrease percentage
1	*Colletotrichumsp.*	52.62* (63.13)	17.89	7.43 (1.68)	36.64	4.12 (0.52)	44.46	2.08 (0.13)	35.37	105.95	47.91
2	*Fusarium sp.*	53.89 (65.25)	15.12	8.06 (1.97)	25.54	4.33 (0.57)	38.72	2.24 (0.15)	25.61	128.69	36.73
3	*A. niger*	44.90 (49.88)	35.12	5.81 (1.03)	61.14	3.92 (0.47)	49.40	1.83 (0.10)	50.00	51.39	74.73
4	*Control*	61.31 (76.88)	-	9.36 (2.65)	-	5.54 (0.94)	-	2.59 (0.21)	-	203.40	-
S.Em.±		0.97	-	0.04	-	0.10	-	0.05	-	3.24	-
C.D. at 5%		2.81	-	0.13	-	0.29	-	0.13	-	9.39	-
C.V. %		5.16	-	1.70	-	6.50	-	6.01	-	7.49	-

***Figures outside the parentheses indicate arc sine transformation values Figures in parentheses indicate original values**

Plate.1 & 2

Plate.1 Effect of culture filtrates of dominant seed mycoflora on seed germination growth of chilli variety GVC 101

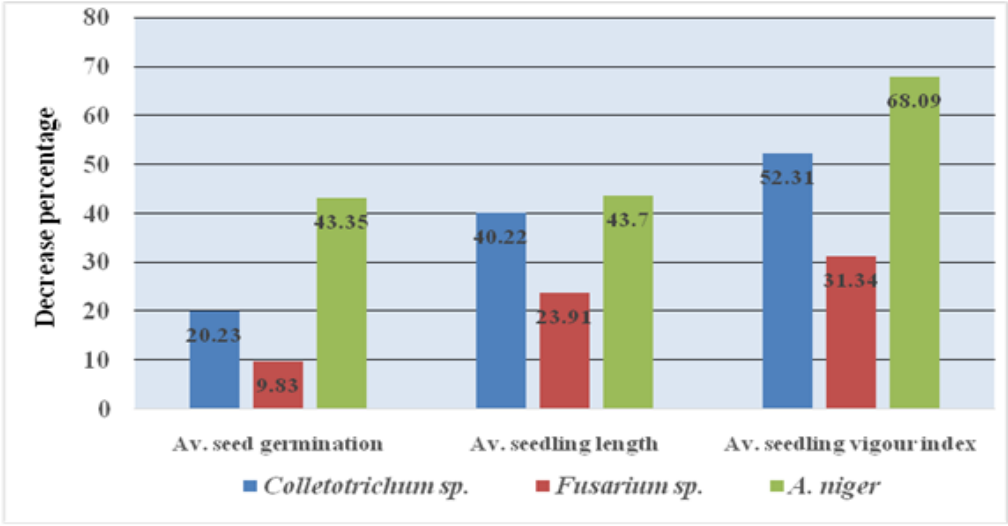


Plate.2 Effect of culture filtrates of dominant seed mycoflora on seed germination and seedling growth of chilli variety GVC 111

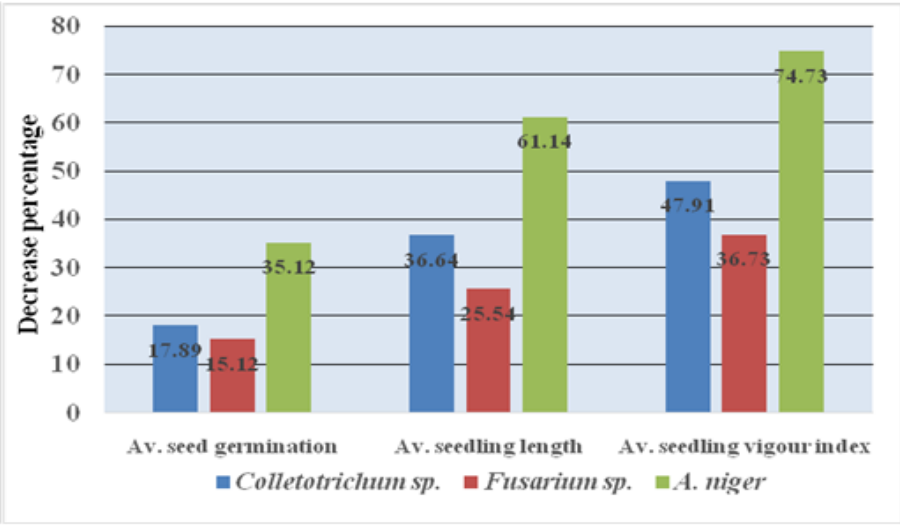


Plate.3 Effect of culture filtrates of dominant mycoflora on chilliseeds (var. GVC 101)

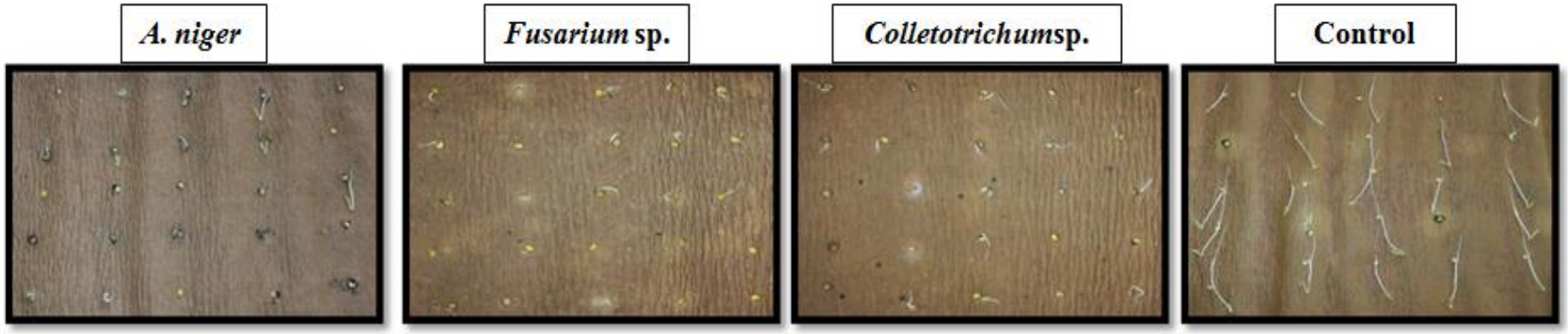
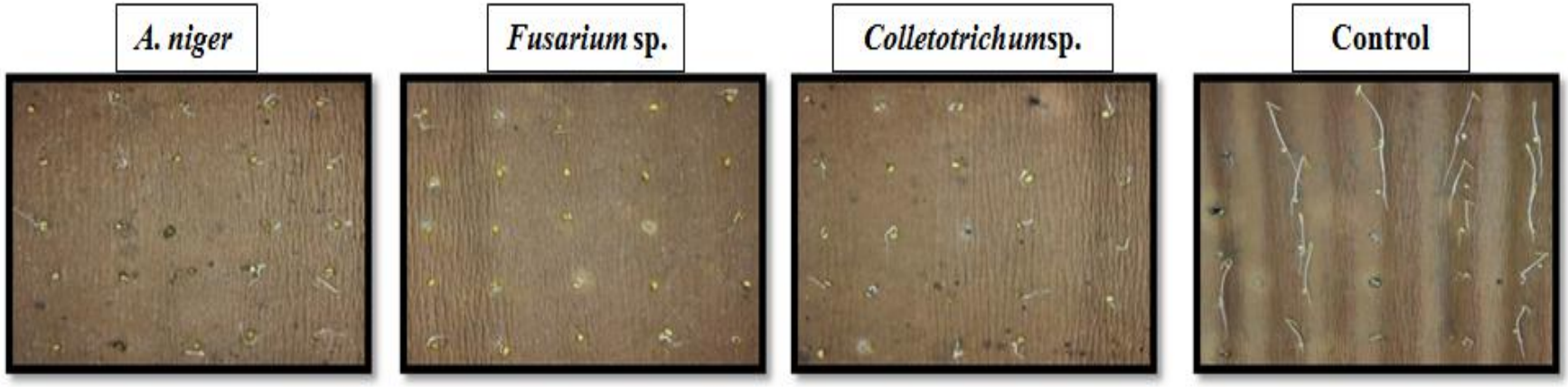


Plate.4 Effect of culture filtrates of dominant mycoflora on chilliseeds (var. GVC 111)



Results presented in Table 2 showed that maximum reduction in average seed germination was found in culture filtrates of *A. niger* (49.88%) followed by culture filtrates of *Colletotrichum* sp. (63.13%) and *Fusarium* sp. (65.25%).

Highest seed germination was recorded in control (76.88%). Seed treatment with culture filtrate of *A. niger* reduced average seed germination by 35.12 per cent. It was followed by *Colletotrichum* sp. (17.89%) and *Fusarium* sp. (15.12%).

Average seedling length was found significantly lowest in seed treated with culture filtrates of *A. niger* (1.03cm) followed by *Colletotrichum* sp. (1.68cm) and *Fusarium* sp. (1.97cm). Highest average seedling length was recorded in control (2.65cm). Seed treatment with culture filtrate of *A. niger* reduced average seedling length by 61.14 per cent followed by *Colletotrichum* sp. (36.64%) and *Fusarium* sp. (25.54%).

Average seedling fresh weight was found significantly lowest in culture filtrates of *A. niger* (0.47mg) followed by *Colletotrichum* sp. (0.52mg). Whereas, the seed treated with culture filtrate of *Fusarium* sp. (0.57mg) showed minimum reduction in seedling fresh weight. The highest seedling fresh weight was obtained in control (0.94mg). Average seedling fresh weight was reduced in seed treatment with culture filtrate of *A. niger* by 49.40 per cent followed by *Colletotrichum*sp. (44.46%) and *Fusarium* sp. (38.72%).

Average seedling dry weight was found significantly lower in seed treated with culture filtrate of *A. niger* (0.10mg) followed by *Colletotrichum*sp. (0.13mg) and *Fusarium* sp. (0.15mg). The highest seedling dry weight was obtained in control (0.21mg). Average seedling dry weight was reduced in culture filtrate of *A. niger*by50.00 per cent followed by *Colletotrichum* sp. (35.37%) and *Fusarium* sp. (25.61%).

Average seedling vigour index was found significantly lowest in culture filtrates of *A. niger*(51.39). Comparatively higher seedling vigour index was observed in culture filtrates of *Colletotrichum* sp. (105.95) followed by *Fusarium* sp. (128.69). Highest vigour index was recorded in control (203.40). Seed treatment with culturefiltrate of *A. niger* reduced average seedling vigour index by 74.73 per cent followed by *Colletotrichum* sp. (47.91%) and *Fusarium* sp. (36.73%).

The effect of culture filtrate of dominant fungi on chilli seeds resulted in three category of seedling *viz.*, rotted seed/no germination, infected seedling and healthy seedling. Seeds infected with culture filtrate of *A. niger* and *Colletotrichum* sp. resulted in complete inhibition of seed germination and seed rotting. Whereas, infected seedlings were observed in culture filtrates of *Fusarium* sp.

Culture filtrate of *A. niger* reduced maximum per cent seed germination, seedling length and vigour index followed by *Colletotrichum* sp.and *Fusarium* sp., in both varieties GVC 101 and GVC 111. Telang (2010) reported that the maximum inhibition of seed germination was found in culture filtrate of *A. flavus* (17% seed germination) followed by *A. alternata* (18%), *F. moniliforme* (38%) and *C. lunata* (50%). Rajamanickam and Sethuraman (2017) reported that culture filtrate *Colletotrichum* sp treated chilli seeds recorded the lowest seed germination of 26.67 per cent and highest reduction in shoot length (76.92%).Thus, the present findings are more or less in confirmation with above workers. The difference in results may be due to the difference in periods of seed soaking and susceptibility of chilli varieties. Ismael (2010) found that the influence of fungal exudates were less at 6 hours of soaking while the effects increased by raising the periods to 24 hours of soaking.

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