Studies on seedborne nature of *Colletotrichum* spp. and testing the efficacy of different organics on germination and seedling vigour

ABSTRACT

Background: Chilli is one of the important spice cum vegetable crop cultivated worldwide. It is an important source of vitamins, minerals, oleoresin etc. There are different biotic and abiotic factors responsible for the reduction in the yield of chilli in India.One of the most serious fungal diseases of chilli which causing the major pre and post harvest loss is anthracnose caused by *Colletotrichum* species.Primary infection of the disease is due to the seed borne nature of pathogen.So for the better management of the disease, control measures should be taken by understanding the seed borne nature of pathogen.

Methods: The present investigation was taken up to studying the seed borne nature of disease so that suitable management practices can be recommended to reduce the yield loss. Seeds of the eight infected chilli genotypes were collected and presence of pathogen was detected using agar plate method and standard blotter method. Efficacy of five different organics were tested by using germination test by calculating germination percentage and seedling vigour index of infected seeds of susceptible chilli variety (Byadgi Dabbi)

Result: Among the eight different genotypes tested through agar plate and standard blotter method five genotypes (Bhadra, Shilpa, Ujwala, Arka Tanvi, Green Thunder and Sitara) recorded 100 % however, Arka Tejasvi and WS-2207 showed 0 % seed infection. Among the five organics tested maximum germination percentage and seedling vigour index was recorded when the infected seeds were treated with vermiwash and cow urine.

Key words: Chilli, Anthracnose, *Colletotrichum* spp., Agar plate method, Standard blotter method, Organics

INTRODUCTION

Chilli (*Capsicum annum* L.), or red pepper, is an important member of the Solanaceae family and a versatile vegetable crop grown worldwide. It is a remunerative vegetable, spice cum cash crop of the Indian subcontinent and a principal constituent of many foods, adding flavour, colour and pungency. Chillies are good sources of Vitamin A, B and C, oleoresin, carbohydratesand minerals like calcium, phosphorus, ferrous, sodium copper in trace amounts and the allied pungent principles. Chillies produce alkaloids, capsaicinoids, carotenoids and pigments (Capsorubin and Capsanthin), which make chilli hot and pungent.

The decline in yields of the harvested produce, with the subsequent decline in the total export, has been attributed to many factors, paramount among them being diseases caused by fungi, bacteria, virus and nematodes. Among the different diseases, anthracnose is a severe pre and post-harvest disease of chilli known worldwide caused by Colletotrichum spp, with a wide range of hosts including cereals, legumes, vegetables, perennial crops and fruit trees (Bailey and Jeger, 1992). The diseases are mainly problematic on mature chilli fruits, causing severe losses due to pre and post-harvest fruit decay (Hadden and Black, 1989; Bosland and Votava, 2003). Anthracnose was reported for the first time in Coimbatore of Madras Presidency – India (Sydow, 1919). The losses due to the impact of chilli anthracnose was estimated about 50% in different parts of India (Pakdeevaraporn*et al*., 2005). The average disease incidence level ranges between 66-84 per cent, resulting in yield loss up to 12-50 per cent (Thind and Jhooty, 1985).

This disease is seed and airborne and has a more significant effect on seed germination and vigour (Saxena *et al*., 2016). It damages the crop right from the early stage and continues till harvest. Hot and humid conditions are favourable for transmission of this disease. Small anthracnose lesions on chilli fruits reduce their marketable value due to black spots appearing on fruits (Manandhar *et al*., 1995). Proper identification of these pathogens is essential for mitigating the risk of incursion of new pathogens, which may have devastating consequences for the local industries. In addition, the exact identification of the species is vital for resistance breeding programs and in identifying the host range. This study was carried out to determine the seed borne nature of *Colletotrichum* spp. and to test the efficacy of different organics on germination and the seedling vigour of chilli seeds infected with anthracnose disease.

MATERIALS AND METHODS

The chilli fruits with typical symptoms of anthracnose disease were collected from different locations of Karnataka and one from Kerala. The fungal mycoflora was detected by following the Agar plate method and standard blotter technique (Anon., 1996). Eight varieties of chilli were utilised for this study. Three replication was maintained for each variety.Efficacy of different organics such as cow urine, neem oil, vermiwash, *Trichoderma harzianum* and *Pseudomonas fluorescens* were tested against*Colletotrichum*spp causing anthracnose disease in susceptible variety Byadgi Dabbi.

Agar plate method

Potato dextrose agar medium (PDA) was poured aseptically into the sterilized Petri plates. The seed, before plating, was surface sterilized in 1 per cent sodium hypochlorite, and excess moisture was removed by keeping the seed on sterilized blotting paper. Seeds were placed at the rate of 10 seeds per Petri plate on the solidified PDA plates.These Petri dishes were incubated at 27±1°C for seven days under 12 hours, alternating near UV light and darknesscycles. Three replications of each germplasm and a similar set without surface sterilization were maintained, and seeds were examined for fungi after eight days of incubation. Per cent seed mycoflora associated was calculated based on the formula given by Jha (1995);

Per cent seed infection =Number of seeds infected /Total number of seeds placed

Standard blotter method

Twenty five seeds of eight chilli genotypes were surface sterilized with 1 per cent sodium hypochlorite. Seeds were placed on sterilized Petri plates: 15 in the outer ring, 9 in the inner ring and one in the centre containing three layers of sterilized moistened blotter paper. Plates were incubated at 27+1°C for 12 hours, alternating near UV light and darkness cycles. Three replications of each germplasm and similar set without surface sterilization were maintained, and observations *viz*., per cent mycoflora of individual fungi was recorded after eight days of incubation. Formula given by Jha (1995) was used to calculate per cent seed infection.

Efficacy of organics on Germination and seedling vigour

Twenty-five seeds of Byadgidabbi were soaked in different organics for 3 hours *viz*, neem oil, vermiwash, cow urine, *Pseudomonas fluorescens*, and *Trichoderma harzianum*at 5, 10, 15 per

cent concentration along with control (sterile distilled water). After air drying, soaked seeds were placed on moistened germination paper following the rolled paper towel method (Anonymous, 1996). Data on per cent seed germination, shoot length and root length in all treatments was recorded after 14 days of incubation in the germination chamber. The seedling vigour index was calculated by using the formula suggested by Abdul Baki and Anderson (1973);

Seedling vigour Index = Seed germination (%) x Seedling length [Shoot length (cm) +Root length (cm)]

RESULTS AND DISCUSSION

The agar plate method and standard blotter method can detect fungus from diseases which are seed borne in nature. Chilli anthracnose disease is seed borne in nature. These two methods were carried out in eight different genotypes of chilli infected with anthracnose to check the presence of fungal pathogens in seeds. Results are presented in Table 1-4.

Agar plate method

In the agar plate method, among the eight genotypes collected, five genotypes (Bhadra, Shilpa, Ujwala, Arka Tanvi, Green Thunder and Sitara) recorded 100 % however, Arka Tejasvi and WS-2207 showed 0 % seed infection. Microscopic observations of these fungal mycoflora confirmed the presence of *Colletotrichum* spp. Ujwala and Arka Tanvi recorded the presence of *Alternaria* spp. along with *Colletotrichum* spp. Similar findings of the association of *Alternaria* along with *Colletotrichum* were recorded by Mesta *et al*. (2007).

Standard blotter method

In standard blotter method, among the eight different genotypes, Bhadra showed 99.97 % seed infection followed by Shilpa (72 %), Ujwala (57.33 %), Green Thunder (49.43 %), Sitara (36.88 %) and Arka Tanvi (36.27 %) whereas, Arka Tejasvi and WS-2207 recorded complete germination of seeds and no seed infection.

Afutu (2012) reported that *Colletotrichum dematium* colonised the seed coat and peripheral layers of chilli seed endosperm. Major parts of the seed contained inter and intracellular mycelium,

resulting in the breakdown of parenchymatous layers of the seed coat and the depletion of food material in the endosperm.

Efficacy of organics on germination and seedling vigour

Byadgi Dabbi is highly susceptible variety for anthracnose disease. Seed treatment with organics helps to terminate harmful seed-borne fungi and to protect the seeds against infection. It is done to avoid germination failure andseedling infection, destroy external and internal seed borne fungi and provide a protective zone around the seed in the soil. In this investigation, five organics have been used to soak the byadgidabbi seeds. Germination test and seedling vigour were calculated following the methods outlined in "Materials and Methods." Results are presented in Table 5-6

Among the different organics tested, at 5 % concentration, maximum germination of 67.33 % was recorded in vermiwash, followed by cow urine (62.67 %), neem oil (54.67 %) and *Trichoderma harzianum*(49.33 %). In contrast, the least germination was recorded in *Pseudomonas fluorescens*(48.00 %). At 10 % concentration, maximum germination was recorded in vermiwash (81.33 %), followed by cow urine (64.00 %) and *Trichoderma harzianum*(61.33 %), wherein minimum germination was recorded in *Pseudomonas fluorescens* (57.33 %) and neem oil (57.33 %). At 15 %, maximum germination was recorded in vermiwash (89.33 %), followed by cow urine (65.33 %), Neem oil (60.00 %), *Trichoderma harzianum*(58.67 %), whileminimum germination was recorded in *Pseudomonas fluorescens* (57.33 %). In control, germination recorded was 36 %.

Among the different organics tested at 5 % concentration, maximum seedling vigour (549.67) was recorded in vermiwash, followed by cow urine (454.69), neem oil (342.15) and *Trichoderma harzianum*(324.80 %), whereas, minimum vigour was recorded in *Pseudomonas fluorescens* (282.19). At 10 % concentration, maximum vigour was recorded in vermiwash (669.87), followed by cow urine (577.51), neem oil (462.23) and *Trichoderma harzianum*(366.87), wherein minimum vigour was recorded in *Pseudomonas fluorescens* (340.07). At 15 %, maximum vigour was recorded in vermiwash (841.67), followed by cow urine (783.57), Neem oil(543.13) and *Trichoderma harzianum* (490.20), whileminimum vigour was recorded in *Pseudomonas fluorescens* (350.77). In control, the seedling vigour recorded was 278. 28.This is in accordance with the findings of Choudhary *et al*. (2013) where, seed treatment with *P. fluorescens*and *T. polysporum* had no significant enhancement on seed germination infected with *C. capsici*. Antimicrobial peptides

present in the vermiwash help in controlling the seed infection, whereas bioavailable minerals, macro and micronutrients, earthworm secretions, hormones and vitamins help in increasing germination percentage and seedling vigour.

Welideniya*et al*. (2019) discovered that both *Colletotrichum capsici* and *C. gloeosporioides* could damage major components of the seeds internally and externally, resulting in higher losses during germination, both before and after emergence. The severity of pod infection has a direct impact on seed germination.

CONCLUSION

To treat diseases with primary sources of infection as its seed, seed treatment and use of resistant genotypesare the best methods to be followed to prevent infection. Chemical seed treatments have many ill effects on the beneficiary micro organism and are causes of environmental pollution so we need to replace it with organics.From this study, it was revealed that vermiwash and cow urine have the efficacy to enhance germination and vigour of Byadgidabbi seeds infected with anthracnose disease. Agar plate method and standard blotter method studies revealed that among the eight different chilli genotypes studied WS-2207 and Arka Tejaswi are resistant against anthracnose disease in chilli.

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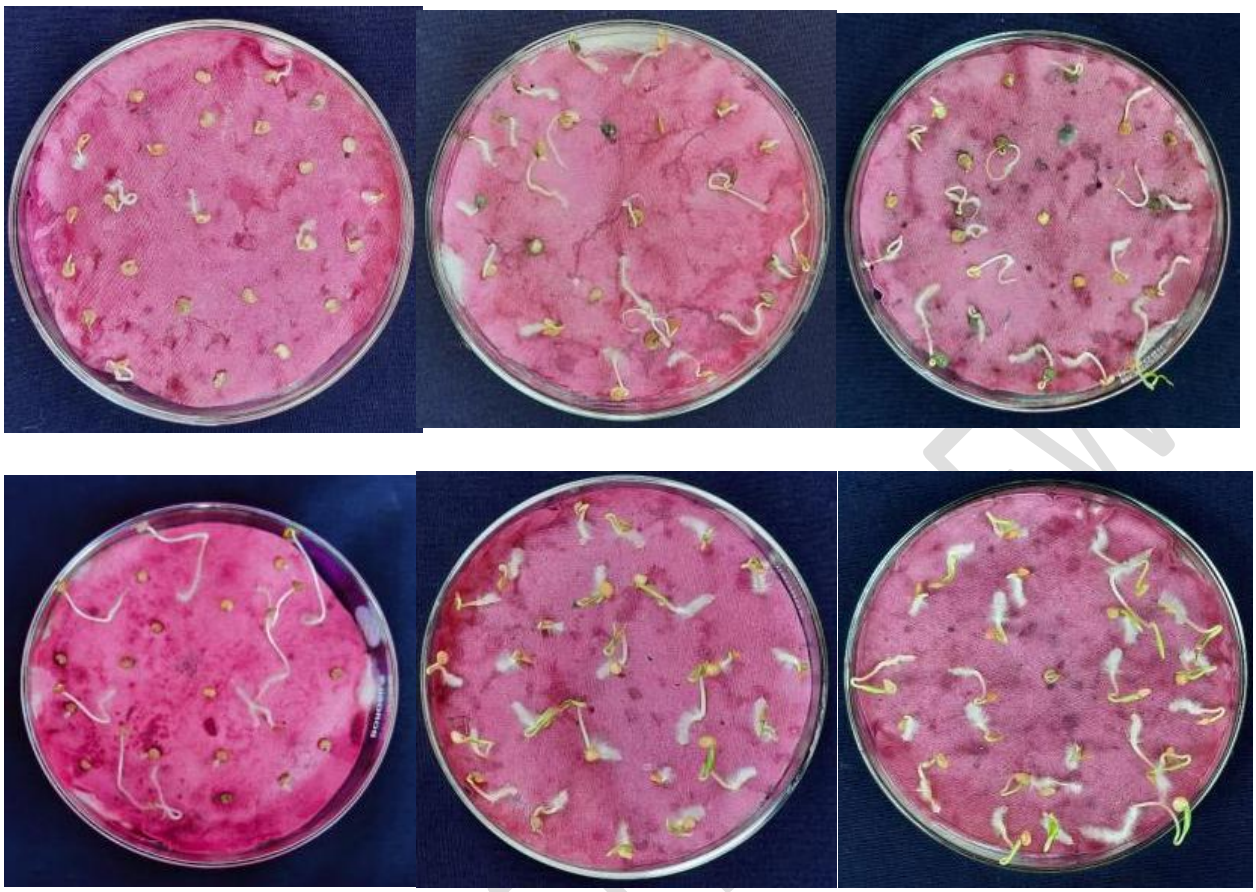

Table 1. Percent seed infection in agar plate method

Sl.No.	Varieties	Per cent seed infection (%) Mean
1)	Arka Tejasvi	0.00 # (1.01) *
2)	Sitara	100 (89.03)
3)	WS-2207	0.00 (1.01)
4)	Bhadra	100 (89.03)
5)	Shilpa	100 (89.03)
6)	Ujwala	100 (89.03)
7)	Arka Tanvi	100 (89.03)
8)	Green Thunder	100 (89.03)
	S.Em.±	1.19
	CD@1%	3.67

Mean of three replications

* Figures in the parenthesis are arcsine transformed values

Table 2. Per cent seed infection in standard blotter method

	Varieties	Per cent seed infection (%) Mean
1)	Arka Tejasvi	0.00 # (1.01) *
2)	Sitara	36.88 (37.42)
3)	WS-2207	0.00 (1.01)
4)	Bhadra	99.97 (89.44)
5)	Shilpa	72.00 (58.12)
6)	Ujwala	57.33 (49.25)
7)	Arka Tanvi	36.27 (37.05)
8)	Green Thunder	49.43 (44.70)
	S.Em.±	0.75
	CD@1%	2.30

Mean of three replications

* Figures in the parenthesis are arcsine transformed values

Table 3. Efficacy of organics on germination of Byadgi Dabbi seeds

Mean of three replications

* Figures in the parenthesis are arcsine transformed values

Sl. No.	Organics	Germination (%)			Mean
		5 %	10 %	15 %	
1)	Cow urine	62.67 # (52.36) *	64.00 (53.16)	65.33 (53.96)	64.00 (53.16)
2)	Neem oil	54.67 (47.70)	57.33 (49.24)	60.00 (50.79)	57.33 (49.24)
3)	*Trichoderma harzianum*	49.33 (44.64)	61.33 (51.58)	58.67 (50.02)	56.44 (48.73)
4)	*Pseudomonas fluorescens*	48.00 (43.88)	57.33 (49.24)	57.33 (49.24)	54.22 (47.45)
5)	Vermiwash	67.33 (55.17)	81.33 (64.43)	89.33 (70.97)	79.33 (62.99)
6)	Control				36.00 (36.89)
		Organics (O)	Concentrations (C)	Interactions (O×C)	
	S. Em.±	0.77	0.60	1.34	
	CD @ 1%	2.24	1.74	3.88	

Table 4. Efficacy of organics on seedling vigourof byadgidabbi seeds

Sl. No.	Organics	Seedling vigour			Mean
		5 %	10 %	15 %	
1)	Cow urine	454.69 #	577.51	783.57	605.26
2)	Neem oil	342.15	462.23	543.13	443.39
3)	*Trichoderma harzianum*	324.80	366.87	490.20	399.74
4)	*Pseudomonas fluorescens*	282.19	340.07	350.77	324.34
5)	Vermiwash	549.67	669.87	841.67	687.07
6)	Control				278.28
		Organics (O)	Concentrations (C)	Interactions (O×C)	
	S. Em.±	6.23	4.83	10.79	
	CD @ 1%	18.00	13.94	31.18	

Mean of three replications

* Figures in the parenthesis are arcsine transformed values