Journal of Experimental Agriculture International

Volume 46, Issue 5, Page 18-25, 2024; Article no.JEAI.113312
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Assessment of Different Organics' Efficacy on Seed-Borne Pathogen *Colletotrichum spp.* of Chilli

Anagha G. [a*], Suresha D. Ekabote [b], A. N. Ramesh [c], P. Hemanth Kumar [d], Ravichandra [a] and H. N. Mouna [a]

[a] Department of Plant Pathology, College of Agriculture, KSNUAHS, Shivamogga, Karnataka, 57704, India.
[b] Department of Horticultural Crop Protection, CoH, Hiriyur, KSNUAHS, Shivamogga, Karnataka, 577204, India.
[c] Department of Plant Biotechnology, College of Horticulture, Hiriyur, KSNUAHS, Shivamogga, Karnataka, 577204, India.
[d] Department of Floriculture and Landscaping, CoH, Mudigere, KSNUAHS, Shivamogga, Karnataka, 577204, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2024/v46i52351

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/113312

Original Research Article

Received: 17/12/2023
Accepted: 21/02/2024
Published: 06/03/2024

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.

**Corresponding author: E-mail: anaghag175@gmail.com;*

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)

Results: Among the eight different genotypes tested through agar plate and standard blotter method six genotypes (Bhadra, Shilpa, Ujwala, Arka Tanvi, Green Thunder and Sitara) recorded 100 % however, Arka Tejasvi and WS-2270 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.

Keywords: Chilli; anthracnose; Colletotrichum spp.; agar plate method; standard blotter method; organics.

1. 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, carbohydrates and minerals like calcium, phosphorus, ferrous, sodium and 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 [1]. The diseases are mainly problematic on mature chilli fruits, causing severe losses due to pre and post-harvest fruit decay [2,3]. Anthracnose was reported for the first time in Coimbatore of Madras Presidency – India [4]. The losses due to the impact of chilli anthracnose was estimated about 50% in different parts of India [5]. The average disease incidence level ranges between 66-84 per cent, resulting in yield loss up to 12-50 per cent [6].

This disease is seed and airborne and has a more significant effect on seed germination and vigour [7]. 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 [8]. 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.

2. 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 [9]. 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.

2.1 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 darkness cycles. 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 [10];

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

2.2 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 [10] was used to calculate per cent seed infection.

2.3 Efficacy of Organics on Germination and Seedling Vigour

Twenty-five seeds of Byadgi dabbi 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 [9]. 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 [11];

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

3. 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 1-2 and Figs. 1-2.

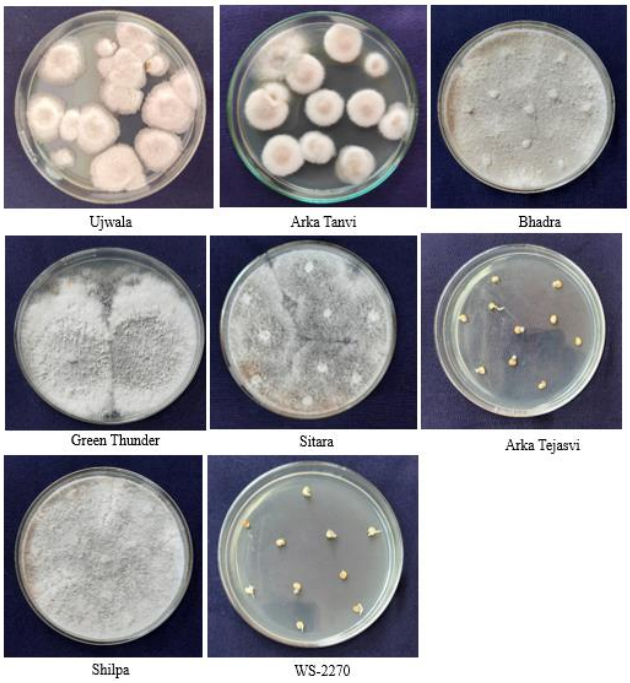

Fig. 1. Percent seed infection in eight genotypes of chilli by agar plate method

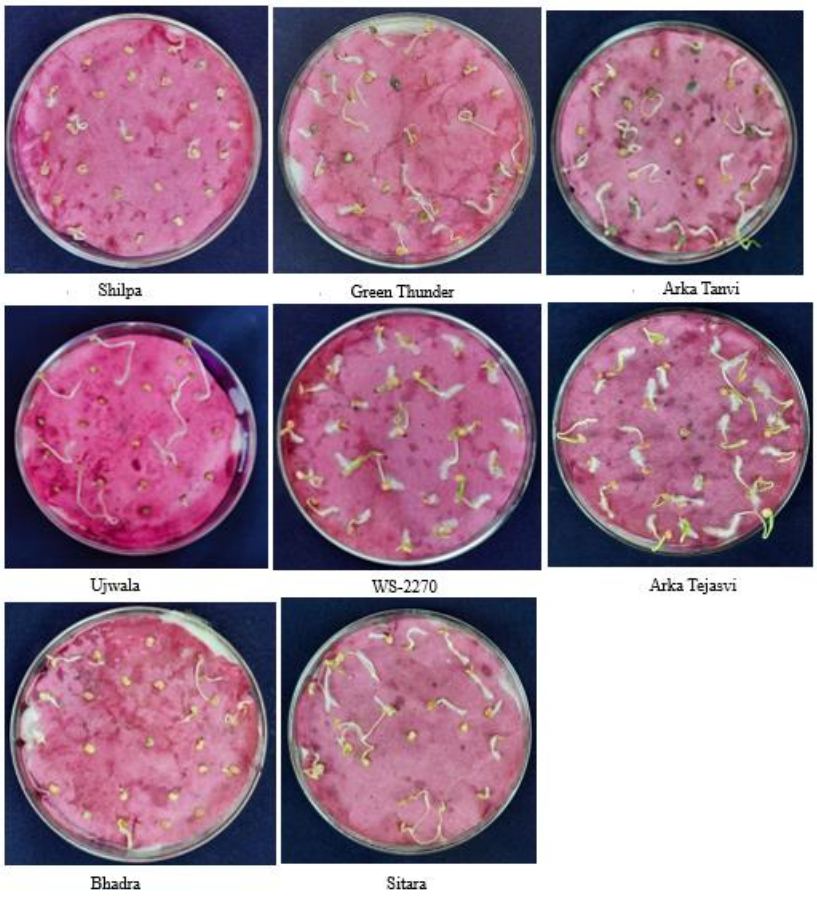

Fig. 2. Percent seed infection in eight genotypes of chilli by standard blotter method

Table 1. Per cent seed infection in agar plate method

Sl. No.	Varieties	Percent seed infection (%) Mean
1)	Arka Tejasvi	0.00 # (1.01) *
2)	Sitara	100 (89.03)
3)	WS-2270	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. Percent seed infection in standard blotter method

Sl.No.	Varieties	Percent seed infection (%) Mean
1)	Arka Tejasvi	0.00 # (1.01) *
2)	Sitara	36.88 (37.42)
3)	WS-2270	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*

3.1 Agar Plate Method

In the agar plate method, among the eight genotypes collected, six genotypes (Bhadra, Shilpa, Ujwala, Arka Tanvi, Green Thunder and Sitara) recorded 100 % however, Arka Tejasvi and WS-2270 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*. [12]. Arka Tejasvi is highly pungent F1 hybrid of chilli developed by Indian Institute of Horticultural Research, Bengluru.WS-2270 is a hybrid of chilli with very high pungency and developed by Welcome crops pvt. Ltd. High pungency in this chilli can be a factor for its resistance against anthracnose.

3.2 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-2270 recorded complete germination of seeds and no seed infection.

Afutu [13] 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.

3.3 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 and seedling 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 byadgi dabbi seeds. Germination test and seedling vigour were calculated following the methods outlined in "Materials and Methods." Results are presented in Table 3-4.

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

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	

 # Mean of three replications

** Figures in the parenthesis are arcsine transformed values*

Table 4. Efficacy of organics on seedling vigour of byadgi dabbi 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*

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 %), while minimum 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), while minimum 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*. [14] where, seed treatment with *P. fluorescens* and *T. polysporum* had no significant enhancement on seed germination infected with *C. capsici. Vermiwash is statistically superior over cow urine. Trichoderma harzianum, Pseudomonas fluorescens and neem oil are on par with each other.*Antimicrobial peptides present in the vermiwash help in controlling the seed infection, whereas the bioavailable minerals, macro and micronutrients, earthworm secretions, hormones and vitamins help in increasing germination percentage and seedling vigour. Cow urine consist of water (95 %), urea (2.5 %), minerals (2.5 %), salts, hormones and enzymes. This promotes growth and germination of seedlings and the anti microbial agents present in cow urine helps to reduce the rate of infection caused by seed borne fungal pathogens.

Welideniya *et al*. [15] 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.

Similarly, Gowtham *et al*. [16] evaluated eight different PGPR isolates for testing the efficacy of PGPR isolates on seedling vigour and germination percentage of chilli seedlings, among these isolates, B. *amyloliquefaciens* offered highest seed germination and seedling vigor of 84.75 % and 1423.8, respectively. It was followed by B. *cepacia* and P. *rettgeri*. Out of these, B. *amyloliquefaciens* offered maximum protection of 71 % against C. *truncatum* infection.

As per this study, among the eight different chilli genotypes evaluated Arka Tejasvi and WS-2270 were showed resistance against the seed borne pathogen *Colletotrichum* spp. in chilli. Pungency of chilli have an impact in providing resistance against the pathogen. Out of the organics used under this study, vermiwash and cow urine were found to be best in enhancing germination and seedling vigour in the anthracnose infected seeds of susceptible variety Byadgi dabbi. Anti microbial peptides present in both the organics helps in controlling the disease development.

4. CONCLUSION

Anthracnose disease in chilli is caused by a seed borne pathogen *ie, Colletotrichum* spp. which makes the disease more problematic. Disease cause yield loss during pre and post harvest season.To treat diseases with primary sources of infection as its seed, seed treatment and use of resistant genotypes are 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 antimicrobial peptides in vermiwash and anti microbial agents present in cow urine helps in preventing anthracnose disease in the infected chilli seeds. Presence of different enzyme, hormones, minerals, urea in vermiwash and cow urine have the efficacy to enhance germination and vigour of Byadgi dabbi seeds infected with anthracnose disease. Agar plate method and standard blotter method studies revealed that among the eight different chilli genotypes studied WS-2270 and Arka Tejasvi are resistant against anthracnose disease in chilli. Genotypes such as Bhadra, Sitara, Arka Tanvi, Green Thunder and Shilpa Ujwala are highly susceptible to anthracnose disease. High pungency in Arka Tejasvi and WS-2270 provides resistance against anthracnose caused by *Colletotrichum* spp.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bailey JA, Jeger MJ. *Colletotrichum*: Biology, pathology and control, wallingford: Commonwealth mycological institute. 1992;388.
2. Hadden JF, Black LL. Anthracnose of pepper caused by *Colletotrichum* spp. proceeding of the international symposium on integrated management practices: Tomato and pepper production in the tropics. Asian Vegetable Research and Development Centre, Taiwan. 1989;189-199.
3. Boseland PW, Votaven EJ. Peppers: Vegetable and spice capsicums. England: CAB International. 2003;233.

4. Sydow H. Aufzahlungeingier in der provizen Kwangtung Und kwangsi (Sub-china) gesammelter prize. Annales, Mycologia. 1919;17:140-143.
5. Pakdeevaraporn JP, Wasee S, Taylor PWJ, Mongkolporn O. Inheritance of resistance to anthracnose caused by *Colletotrichum capsici* in capsicum. Plant Breeding. 2005; 124(2):206-208.
6. Thind TS, Jhooty JS. Relative performance of fungal diseases of chilli fruits in Punjab. Indian J. Mycol. Plant Pathol. 1985;40:543-545.
7. Saxena A, Raghuwanshi R, Gupta VK, Singh HB. Chilli anthracnose: The epidemiology and management. Front. Microbiol. 2016;30(7):1527.
8. Manandhar JB, Hartman GL, Wang TC. Conidial germination and appressorial formation of *Colletotrichum Capsici* and *Colletotrichum gloeosporoides* isolates from pepper. Plant disease. 1995;79(4): 361-366.
9. Anonymous. ISTA; 1996.
10. Jha D. Laboratory manual on seed pathology. First edition Vikas publishing house, New Delhi, India. 1995;7-9.
11. Abdul Baki AA, Anderson JD. Vigour determination in soyabean seed by multiple criteria. Crop Sci. 1973;13:630-633
12. Mesta RK, Kulkarni VR, Rao MSL. Studies on seed borne nature of *Colletotrichum capsici* causing seedling blight and its control through chemicals. Int. J. Agric. Sci. 2007;3(1):35-38.
13. Afutu E. Seed-borne fungi of chilli (*Capsicum frutescens*) and studies on the seed transmission of *Colletotrichum dematium* in the coastal savannah zone of central region of Ghana. M.Sc. (Ag) Thesis. Univ. Cape Coast; 2012.
14. Choudhary CS, Jain SC, Kumar R, Choudhary JS. Efficacy of different fungicides, biocides and botanical extract seed treatment for controlling seed borne *Colletotrichum* sp. in chilli (*Capsicum annuum* L.). The Bioscan. 2013;8(1):123-126.
15. Welideniya WA, Rienzie KDRC, Wickramaarachchi WART, Aruggoda AGB. Characterization of fungal pathogens causing anthracnose in capsicum pepper (*Capsicum annuum* L.) and their seed borne nature. Ceylon J. Sci. 2019;48(3): 261- 269.
16. Gowtham, H. G., Murali, M., Singh, S. B., Lakshmeesha, T. R., Murthy, K. N., Amruthesh, K. N. and Niranjana, S. R., 2018, Plant growth promoting rhizobacteria-Bacillus amyloliquefaciens improves plant growth and induces resistance in chilli against anthracnose disease. Biological Control, 126: 209-217.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/113312