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Gopal Pawar
Department of Botany, R.G. Bagdia, Science; S.B. Lakhotia, Arts; and R. Bezonji, Science, College, Jalna, Maharashtra, India

Pooja Rajkar
Department of Botany, R.G. Bagdia, Science; S.B. Lakhotia, Arts; and R. Bezonji, Science, College, Jalna, Maharashtra, India

Yogesh Urdukhe
Department of Botany, R.G. Bagdia, Science; S.B. Lakhotia, Arts; and R. Bezonji, Science, College, Jalna, Maharashtra, India

Umesh Mogle
Department of Botany, R.G. Bagdia, Science; S.B. Lakhotia, Arts; and R. Bezonji, Science, College, Jalna, Maharashtra, India

Correspondence
Gopal Pawar
Department of Botany, R.G. Bagdia, Science; S.B. Lakhotia, Arts; and R. Bezonji, Science, College, Jalna, Maharashtra, India

Studies on bioagents and fungicides efficacy on seed mycoflora, germination, and vigour index of selected vegetables

Gopal Pawar, Pooja Rajkar, Yogesh Urdukhe and Umesh Mogle

Abstract
The current investigation focuses on comprehensively analyzing the mycoflora associated with seeds, germination rates, and overall vigor indices of two prominent plant species: Chilli (*Capsicum annuum* L.) and Cabbage (*B. oleracea* var. *oleracea* L.). The study explores the effects of diverse leaf extracts both independently and in combination with *Trichoderma* and fungicides, employing an *in vitro* approach. Notable seedborne fungi such as *Penicillium* sp., *Rhizoctonia solani*, *Aspergillus niger*, *Fusarium oxysporum*, *Aspergillus flavus*, *Fusarium solani*, and *Alternaria* sp. were identified on Chilli and Cabbage seeds. The findings highlight the profound inhibitory impact of *Trichoderma viridae* in synergy with plant extracts against *Aspergillus* sp. *and Fusarium* spp., while the fungicide Dithane M-45 exhibited the greatest inhibition against *Aspergillus* sp., *Fusarium* spp., *Penicillium*, and *Alternaria.* Seeds treated with bioagents demonstrated remarkable enhancements in germination rates, leading to significantly increased root and shoot lengths, ultimately resulting in the cultivation of more robust and vigorous seedlings.

Keywords: Seed germination, seedling vigour index, seed mycoflora

Introduction
The safest, most affordable and effective method to increase germination, vigour index, and manage the majority of seed-borne illnesses is seed priming or seed dressing. A critical stage in the treatment of crop diseases is the diagnosis of seed-borne pathogens by seed health testing.

The chilli is the plant that is most extensively cultivated for vegetables globally (*Capsicum frutescens* L.). It is a solanaceous fruit vegetable that is cultivated largely for the production of dried chilli, a key spice in international trade, and edible green fruits. Plenty of vitamins C, A, and B are present in it. In India, it is an important cash crop that is farmed for both the domestic and global markets. A fungal disease caused by chillies lowers the market value of fruit, and low-quality seeds can cause production losses of up to 50%. The Madras Presidency's Coimbatore chilies are where India's first case of this illness was initially reported. Fusarium oxysporum, a fungus that also causes seed and fruit rot, is known to induce fruit rot in chillies [1].

Cabbage (*B. oleracea* var. *oleracea* L.) has long been grown as a major vegetable crop and source of vitamins, minerals, and fibre, especially during the cold seasons in temperate countries. Cabbage and other cruciferous vegetables (members of the Brassicaceae family) have lately been identified as key sources of chemoprotective phytochemicals in the diet. A variety of diseases typically harm these plants, particularly when resistant types are not planted. Several of the most prevalent issues are addressed here, such as young plant root and stem rots, black rot, downy mildew, and viruses [2].

Although being the most prolific vegetable crop in the nation, the chilli and cabbage, yields are still low because of weak plant stands, insect and disease incidence, poor seed quality, variable soil moisture, and seed mycoflora. These factors have always affected seed viability and germination, which in turn affects crop productivity. Together with the seed-borne mycoflora, several saprophytic organisms degrade seed quality, impair viability, and hinder germination of seeds, producing aberrant seedlings as a result [3, 4, 5]. The objective of the present study was to show the effects of different leaf extracts on seed mycoflora, germination, and vigour index, both alone and in combination with *Trichoderma* and fungicides.

Materials and Methods

The current study was carried out in the Departments of Botany, R.G. Bagdia Arts, S.B. Lakhotia Commerce & R. Bezonji Science College, Jalna. Seeds of Chilli (*Capsicum annuum* L.) and Cabbage (*B. oleracea var. oleracea* L.) were procured from local farmers from Jalna, District Jalna.

Treatment with fungicides

Fungicides *viz*., Bavistin (Benzimidazole), M45 (Mancozeb 75% WP) and SAAF Powder (Mancozeb 63% + Carbendazim 12% WP) were prepared @ 0.3, 0.7, 1.0 % depending on the seeds' weight. 20 g of seeds were placed in a 250 ml conical flask, and 50 ml of each fungicide were added one at a time. The flasks were given a thorough shake before being left for 30 minutes. After that, blotting paper was placed over the treated seeds, and they were left to soak. These seeds were subsequently exposed to fungicides in order to measure seed germination, seedling length, and seedling vigour index.

Plant extract preparation and treatment

Fresh leaves of *Xanthium strumarium*, *Solanum nigrum*, and *Alternanthera sessilis* were used as plant material. These leaves were properly cleaned in tap water. Before being passed through two layers of muslin fabric, each plant material was carefully crushed in a mortar with 50 grammes of distilled water (1:1) [6]. The extracts were centrifuged for 20 minutes at 3000 rpm before being placed in a refrigerator at 4 °C to be utilised later. By soaking the seeds for 30 minutes in the prepared plant extracts, each seed received a treatment. To assess seed germination and mycoflora, the treated seeds were soaked and then spread out on wet blotting paper.

Detection of fungi and seed germination percentage on blotter test method

Blotter testing, as advised by the International Seed Testing Association (ISTA-1966) with some modifications, was used to detect seed mycoflora and seed germination [7, 8]. Three layers of blotting paper were stacked in each Petri dish after being soaked in sterile distilled water. As per ISTA regulations, 100 seeds were selected at random. 25 seeds were inserted per petridish out of a total of 100 seeds. Following that, the petridishes were incubated for seven days at 28 2 °C under dim lighting. The formula below was used to compare treated and control seeds for percentage of seed germination. Germination (%) is equal to (Number of seeds germination/Number of seeds utilised) 100. The plated seeds were inspected using a stereo binocular microscope to look for fungus after seven days of incubation. The developing fungus on the blotter were subcultures on PDA plates for identification using the keys provided by Alexopoulos & Mims (1979) and Gilman (1957) [9, 10].

Seed quality testing

The treated seeds underwent a germination test using the traditional blotter method after being blotted dry (ISTA, 1993). To examine how bioagents and fungicides affect seed quality, 25 treated and untreated seeds of Chilli (*Capsicum annuum* L.) and Cabbage (*B. oleracea var. oleracea* L.) were randomly selected and allowed to sprout on moistened blotter sheets on petri plates at 26±2 °C for 8 days. The number of germinations, the number of infected seeds, the length of the roots, and the length of the shoots were all counted after incubation. It was noted how many seeds germinated, how long the seedlings grew, and how vigorously they grew.

Vigour Index: Vigour index was calculated by using the formula of Baki and Anderson (1973) [11] as shown below: Vigour index (VI) = (Mean shoot length + mean root length) x Germination (%)

Results

Table 1: Effect of some leaf extract and fungicides on seed germination, vigour index of Chilli.

Treatment	Seed Germination %	Mean Root Length (mm)	Mean Shoot Length (mm)	Vigour Index
Control	78	13.3	10.7	1872
A) Leaf extract				
Xs	89	14.3	9.7	2136
Sn	92	18.8	11.1	2750.8
As	80	17.1	14.6	2536
B) Leaf extract + bioagents				
Xs+ Tv	78	26.6	19.1	3564.6
Sn + Tv	89	28.2	20.6	4343.2
As+ Tv	76	20.2	12.1	2454.8
C) Fungicides				
SAAF Pawder	65	20.8	18.1	2528.5
Dithane M-45	90	21.1	19.2	3627
Bavistin	87	20.4	18.6	3393

Xs = *Xanthium strumarium*, Sn = *Solanum nigrum* and As = *Alternanthera sessilis* and Tv = *Trichoderma viridae*

Table 2: Effect of some leaf extract and fungicides on seed germination, vigour index of Cabbage.

Treatment	Seed Germination %	Mean Root Length (mm)	Mean Shoot Length (mm)	Vigour Index
Control	70	11.5	9.9	1498
A) Leaf extract				
Xs	89	9.8	7.5	1539.7
sn	81	12.5	8.2	1676.7
As	72	10.6	6.8	1252.8
B) Leaf extract + bioagents				
Xs + Tv	89	15.5	9.5	2225
Pm + Tv	78	12.2	8.8	1638
As+ Tv	68	10.6	7.5	1230.8
C) Fungicides				
SAAF Powder	70	9.5	7.8	1211
Dithane M-45	83	14.4	10.3	2050.1
Bavistin	79	13.5	11.9	2006.6

Xs = *Xanthium strumarium*, Sn = *Solanum nigrum* and As = *Alternanthera sessilis* and Tv = *Trichderma viridae*

Table 3: Effect of some leaf extract, *Trichoderma* + leaf extract and fungicides on seed mycoflora isolated from Chilli and Cabbage Seeds.

Treatment	Percent incidence of mycoflora							
	Aspergillus flavus	***Rhizoctonia solani***	***Aspergillus niger***	***Fusarium solani***	***Fusarium oxysporum***	***Penicillium* sp.**	***Alternaria* sp.**	**Total Mycoflora**
Control	7	2	1	7	8	3	4	32
A) Leaf extract								
Am	3	1	-	-	-	-	6	10
Ph	2	3	-	1	3	-	5	14
Sa	1	-	-	-	2	3	2	08
B) Leaf extract + *Trichoderma*								
Xs + Tv	1	-	-	-	-	-	2	03
Pm + Tv	3	-	1	-	1	-	1	06
As+ Tv	3	1	-	1	2	-	-	07
C) Fungicides								
Benomyl	2	3	-	-	-	-	-	05
Dithane M-45	1	1	-	-	-	-		2
Bavistin	3	2	3	2	-	-	-	10

Xs = *Xanthium strumarium*, Sn = *Solanum nigrum* and As = *Alternanthera sessilis* and Tv = *Trichderma viridae*

Effect of plant extracts on seed germination and seed associate mycoflora

The various aqueous plant extracts employed had a significant impact on the germination of the seeds of both chilli and cabbage. When treated with *Solanum nigrum* leaf extract, the germination of chilli seeds was at its highest (92%), whereas *Alternanthera sessilis* had the lowest germination rates (Table 1). *Xanthium strumarium* leaf extract had the highest seed germination rate (89%) for cabbage, whereas *Alternanthera sessilis* extract had the lowest seed germination rate (78%) (Table 2). For increasing seed germination percentage, seedling growth, and seedling vigour of both vegetables, *Solanum nigrum* leaf extract in conjunction with *Trichderma viridae* shown the greatest efficacy among the evaluated plant extracts (Table 1 & 2).

In controlling the mycoflora associated with the seeds of both vegetables, plant extracts also exhibited antifungal activity (Table 3). *A. nigur* was successfully controlled in both vegetable seeds using extracts from every plant tested, and among other fungi, their numbers were reduced over control using extracts from every plant tested and *Trichderma viridae* in combination (Table 3). Under laboratory conditions, mean seedling vigour in Chilli ranged from 2136 (Xs) to 2750.8 (Sn) over the control 1872, and in combination with Trichoderma the maximum vigour index was recorded 4343.2 in Sn + Tv, while in Cabbage mean seedling vigour ranged from 1339.2 (25 mM) to 3924.9 (5mM) over the control (2592.5). Similarly in combination with *Trichoderma* the maximum vigour index was recorded 2225 Xs + Tv. At a proportionate rise in dosage or concentration, the seedling vigour declined. Harding *et al*. (2012) [12] and Cheema and Atta (2003) [13] reported similar findings, stating that seedling height dropped with increasing irradiation dose, however the decline was not proportionate to the rising dosage.

Effect of fungicides on seed germination and reduction of seed mycoflora

The fungicide applied had a significant impact on both the germination of cabbage and chilli seeds. The different fungicide kinds also had an impact on seed germination (Table 1). In Chilli, seed germination was 90% when treated with dithane - M-45, followed by Bavistin, and 65% when treated with SAAF powder tested fungicides (Table 1). In the case of cabbage, seed germination was highest (83%) when treated with dithane-M 45, and lowest (79 and 70%) with the other fungicides tested. Dithane-M-45 was more efficient than other fungicides in increasing the percentage of seed germination of both vegetables (Table 1 & 2). Seed mycoflora of Chilli and Cabbage was remarkably reduced by the fungicide used. Seed mycoflora was also controlled with the fungicide types (Table 3). In blotter test isolated fungi were identified as *Aspergillus flavus, Aspergillus niger, Rhizoctonia solani, Mucor* sps *Fusarium oxysporum* and *Penicillium* sp. in Chilli and in case of Cabbage seeds, it was *A. flavus, F. oxysporum, Penicillium* sp. and *Fusarium semitectum*.

Discussion

Similar findings from the most recent studies corroborate numerous worker reports. On the mycoflora, germination, and vigour index of cowpea seeds, Mogle and Maske (2012) [14] studied the impacts of leaf extract, *Trichoderma*, and fungicides. According to the findings, seed vigour, germination, and seed-borne mycoflora were all enhanced by leaf extract and fungicides. Similar results have been observed by Anjorin *et al*. (2008), Anburani & Shakila (2008), and Signaboubo *et al*. (2015) [15-17].

Effects of bioagents in enhaning the percentage of germination as well as plant disease management in these study we found Increased root-shoot length and seedling vigour were seen as a result of the favourable impact that bioagents had on seed germination. Seeds that had been treated with *T. harzianum* were more effective (Table 3). Similar outcomes with the use of *T. harzianum* were also reported by Bunker and Mathur (2000) [18].

Fungicides may have operated through inducing metabolic shifts that prompted the creation of noxious compounds. Consequently, these substances rendered the internal milieu inhospitable for pathogenic proliferation and functionality. This sequence of events eventually culminated in the establishment of resistance mechanisms and fortification against infections. These findings align harmoniously with the conclusions drawn by Dubey and Patel (2001), Kumar and Dubey (2001), as well as Solorzano and Malvick (2011) [19-21]. The present findings are likewise in agreement with De and Chaudhary's (1999) [22] reports, which noted a reduction in wilt disease as a result of Bavistin, Mancozeb M-45, and Vitavax. In addition, the fungicide-treated

seedlings germinated better than untreated seeds. Singh *et al*. (2002) [23] conducted an *in vitro* investigation with fungicides such as Captan, Dithane M-45, Vitavax, and Bavistin. As well as testing various fungicide concentrations against various diseases, According to research by Singh *et al*., (2014), [24] the antifungal medications dithane M-45 and bavistin are effective at lowering seed-borne *Fusarium* sp. infection on maize seeds. Therefore, this study underscores the critical importance of employing optimal fungicides, bioagents, and plant extracts for seed treatment, effectively mitigating the prevalence of mycoflora within Chilli (*Capsicum annuum* L.) and Cabbage (*B. oleracea* var. *oleracea* L.) seeds. While fungicide treatments have demonstrated adept management of mycoflora, their non-environmentally friendly nature raises concerns regarding contamination. In contrast, the utilization of bioagents and aqueous leaf extracts from plants results in a notable enhancing the proportion of germinating seeds and reduction in the incidence of seed-borne mycoflora. This approach not only offers an environmentally conscious alternative but also exhibits the potential to enhance seed quality and growth outcomes.

Conclusion

As a result, the current study emphasizes the significance of treating seeds with the best fungicides, bioagents, and plant extracts to lower the incidence of mycoflora in chili and cabbage seeds. According to reports, seeds treated with fungicides effectively controlled the mycoflora in the seeds, but they are not environmentally friendly and pollute our environment. The percentage of seeds that germinate and the occurrence of seed-borne mycoflora decrease when bioagents and plant aqueous leaf extract are used.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Jogi MG, Padule DN, Kamdi SR. Detection of seed mycoflora of chilli and its impact on seed germination and seedling vigour. Inter. J Plant Sci. 2010;5(2):502-504.
2. Sharma D, Rao DV. A field study of pest of cauliflower cabbage and okra in some areas of Jaipur. International Journal of Life Sciences Biotechnology and Pharma Research. 2012;1(2):2250-3137.
3. Argerich CA, Bradford KJ. The effects of priming and ageing on seed vigour in tomato. Journal of experimental botany. 1989;40(5):599-607.
4. Paul YS. Seed borne mycota of soybean and its control in Himachal Prodesh. Indian J of Mycol and Plant Pathol. 1989;119:235-257.
5. Bateman GL, Kwasna H. Effects of number of winter wheat crops grown successively on fungal communities on wheat roots. Appl Soil Ecol. 1999;13:271-282.
6. Hossain I, Mahamud H, Ashrafuzzaman H. Effect of plant extracts on fungi (*Bipolaris sorokiniana* and *Rhizoctonia solani*) and okra mosaic disease. Ecoprint. 1997;4(1):35-42.
7. Basak AB, Mridha MAU. Mycoflora associated with seeds of different varieties Aman rice collected from Chittagong and Chittagong Hill-tract districts of Bangladesh. Seed res. 1985;13(2):78-84.
8. Basak AB, Mridha MAU, Fakir GA. Mycoflora of Chilli seed as isolated from different types of fruit rot occurring in Chittagong district. Chittagong Univ stud Part II. 1991;15(2):11-17.
9. Gillman JC. A Manual of soil fungi. Lowa State Univ. Press; c1957.
10. Alexopoulos CJ, Mims CW. Introductory Mycolpogy. 3rd Ed. John Willey and Sons. Inc., U.S.A; c1979.
11. Abdul-Baki AA, Anderson JD. Vigor determination in soybean seed by multiple criteria 1. Crop science. 1973;13(6):630-633.
12. Harding SS, Johnson SD, Taylor DR, Dixon CA, Turay MY. Effect of gamma rays on seed germination, seedling height, survival percentage and tiller production in some rice varieties cultivated in Sierra Leone. American Journal of Experimental Agriculture. 2012;2(2):247.
13. Cheema AA, Atta BM. Radiosensitivity studies in basmati rice. Pak. J Bot. 2003;35(2):197-207.
14. Mogle UP, Maske SR. Efficacy of bioagents and fungicides on seed mycoflora, germination and vigour index of cowpea. Science Research Reporter. 2012;2(3):321-326.
15. Anjorin ST, Makun HA, Adesina T, Kudu I. Effects of Fusarium verticilloides, its metabolites and neem leaf extract on germination and vigour indices of maize (*Zea mays* L.). African Journal of Biotechnology; c2008, 7(14).
16. Anburani A, Shakila A. Influence of seed treatment on the enhancement of germination and seedling vigour of papaya. In II International Symposium on Papaya. 2008 Dec;851:295-298.
17. Signaboubo S, Noumbo GT, Aoudou Y, Fovo JD, Keuete E. Efficacy of three local plant extracts as seed treatment on the germination, infection and vigour index of two cotton seed varieties from Chad. International Journal of Applied Biology and Pharmaceutical Technology. 2015;6(2):39-47.
18. Bunker RN, Mathur K. Integration of biocontrol agents and fungicides for suppression of dry rot of *Capsicum frutescens*. J Mycol. Plant Pathol. 2000;31(3):330-334.
19. Dubey SC, Patil B. Determination of tolerence in *Thanatephores cucumeris*, *Trichoderma viride*, *Gliocladium virens* and *Rhizobium* sp. To fungicides. Indian Phytopathol. 2001;54(1):98-101.
20. Kumar M, Agarwal VK. Effect of fungicidal seed treatment on seed-borne fungi, germination and seedling vigour of maize. Seed Res. 1998;26(2):147-161.
21. Solorzano CD, Malvick DK. Effects of fungicide seed treatments on germination, population, and yield of maize grown from seed infected with fungal pathogens. Field Crops Research. 2011;122(3):173-178.
22. De KR, Chaudhary RG. Biological and chemical seed treatment against lentil wilt. LENS Newsletters. 1999;26(1&2):28-31.
23. Singh SD, Rawal P, Shekawat NS, Lodha PC. Management of mungbean (*Vigna radiata* (L.) Wikzek) seed mycoflora by seed dressing fungicides. J Mycol. Plant Pathol. 2002;23(1):149.
24. Singh S, Sinha A, Mishra J. Evaluation of different treatment on the occurrences of seed borne fungi of Mungbean *Vigna radiata* (L.) Wilczek seed. African Journal of Agricultural Research. 2014;9(44):3300-3304.