

Research Article**Seed Borne Mycoflora of Some Pulses from Nanded District, Maharashtra****¹Jadhav Nikita Madhav and ²P. V. Pawar****Article Info**

¹Research Scholar, N.E.S. Science College, Nanded.

²Associate Professor and Research Guide, Dept. of Botany, Madhavrao Patil ACS College, Palam, District Parbhani (M.S.)

Corresponding Author: P.V. Pawar
email-drpv pawar74@gmail.com

Received: 01/01/2026

Accepted: 15/02/2026

Published: 20/02/2026

DOI: 10.5281/zenodo.18707402

Publisher's Note: IJABR Press stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: ©2026 by the author(s). Licensee IJABR Press, India. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution-Share Alike (CC BY -SA) license.

ABSTRACT

The current study was conducted to identify pulse seed infection using various seed health testing techniques and to ascertain the impact of certain chemical fungicides, bioagents, and botanicals on seed germination and seed borne mycoflora. Five seed borne fungus, including *Aspergillus sp.*, *Penicillium sp.*, *Rhizopus sp.*, *Alternaria sp.*, and *Trichoderma sp.*, were found in five pulse seeds that were gathered from the local market in Nanded District, Maharashtra. The PDA plate approach outperformed the blotter paper method and the seed washates method among the seed health testing techniques used. With a mean incidence of 14.54%, the fungal occurrence was higher in the PDA approach. *Rhizopus sp.* was found to be the most common fungus. *Alternaria sp.* had the lowest fungal occurrence, with an incidence of 20.6%. (11.99%). Gram seeds had the lowest mycoflora incidence (10.22%), whereas lentil seeds had the highest (18.89%). Mancozeb at 0.2% was found to be the most effective chemical in lowering the fungal incidence of *Alternaria sp.*, *Rhizopus sp.*, *Aspergillus sp.*, and *Penicillium sp.* to zero. The effectiveness of each bioagent in lowering the incidence of fungus was comparable. In comparison to the other therapies, all of the leaf extracts were found to be less effective. The bioagents T. At 70%, *Harzianum* had the greatest pulse seed germination rate.

Keywords: Pulse seeds, seed borne fungi, fungicides, bioagents.

INTRODUCTION

The quality of seed, the fundamental input and foundation of agriculture, is significantly impacted by microorganisms. Fungi are the largest group of microorganisms that have an impact on seed health and can lead to seed borne illnesses. Numerous crop seeds are

known to harbor a variety of pathogenic and non-pathogenic fungus, which are referred to as seed mycoflora or seed borne fungi. Fungi create toxins that may be harmful to humans and domestic animals, discolor seeds, diminish seed weight, and decrease the germinability of seeds. An essential part of managing

agricultural diseases is evaluating seeds for the presence of seed borne pathogens [18]. Numerous workers have been found to carry a number of fungi both internally and externally that cause seed discoloration, spoilage, and various diseases in the field, including root rot (*Rhizoctonia solani*), white rot (*Sclerotinia sclerotiorum*), anthracnose (*Ascochyta pinodella*), powdery mildew (*Erysiphe pisi*), wilt (*Fusarium oxysporum f.sp. pisi*), and seed rot (*Aspergillus niger*). Given their significance as both toxin makers and degrading agents, more research is being done on the mycoflora of seeds. The organism carried by the seed and the degree of infection or infestation that will be introduced to another area or nation are revealed by seed health information. Such data is derived from surveys or studies conducted in the field where the seed is cultivated [6].

By giving the plants more vigor and resistance, seed treatment lessens the host's susceptibility to infections. The Fabaceae family of food plants includes pulses, which constitute the second most significant group after grains. Major vegetarians can obtain essential amino acids and protein from pulses [7]. According to Narayan and Kumar [9], green gram, pigeon pea, black gram, and lentil are the main pulses grown in India. Numerous researchers have tested the health of pulses seeds [1,8,12,13]. Fungicides [10,3] in conjunction with hot water treatment bioagents [2,3] and plant extracts [4,5,11,15]. The goals of this research project were to isolate and identify mycoflora from significant pulse seeds in Nanded District, Maharashtra, and investigate the effectiveness of seed treating agents for managing the seed mycoflora in light of the aforementioned developments and the dearth of information on seed mycoflora of pulses in Nanded District, Maharashtra.

Materials and Methods

This study was carried out at N.E.S. Science College Nanded, Department of Botany. Five pulses - Gram, Pigeon Pea, Green Gram, Lentil, and Black Gram - were sampled from local markets in Nanded District, Maharashtra. The

seeds were gathered, labeled appropriately, and transported to the lab for additional research in sterile polythene bags. To find out whether mycoflora was present, thirty seeds from each of the five samples were inspected under a stereo binocular microscope. Ten seeds were placed on moist sterilized filter paper (Whatman No. 1) on Petri plates and allowed to incubate at room temperature (25±2°C) in order to isolate fungi from various pulses. As a result, the fungal colonies on the seed surface were directly detected. On PDA plates, every fungus was isolated. Each plate's fungal colonies were counted, cleaned, and kept in a refrigerator.

Fungal infection percentages were calculated using the following formula:

$$\frac{\text{Number of seeds on which fungal species occurs}}{\text{Total number of seeds}} \times 100$$

$$\text{Infection \%} = \frac{\text{Number of seeds on which fungal species occurs}}{\text{Total number of seeds}} \times 100$$

A digital microscope was used to take pictures of each seed mycofloras characteristic distinguishing traits. In accordance with and with the assistance of pertinent literature, all identifications were based on physical traits and photographic descriptions of fungi.

Isolation of seed borne mycoflora

The PDA plate, blotter, and seed washates method were the three techniques used. Thirty seeds from each of the five pulse samples were treated with 0.1% mercuric chloride for two minutes in the PDA plate method, and then they were washed four times with sterile water. PDA filled Petri dishes with a diameter of nine centimetres were filled with uniformly spaced circles of surface sterilized seeds.

Similar surface sterilization was used in the blotter approach, and the seeds were evenly spaced on Petri plates with three layer sterile filter paper (Whatman no. 1) beds. In contrast, surface sterilized seeds were arranged equally in circles in Petri dishes filled with Seed Washes media. In a separate test, two grams of each seed sample were combined with ten millilitres of sterile distilled water, and the mixture was shaken for ten minutes on a mechanical shaker. Using a micropipette, 250

microliters of this suspension were transferred to PDA medium.

Three replications of each pulse sample were made. Following seed implantation, the plates were incubated at $28\pm 1^\circ\text{C}$. Using the above formula, the infection % was determined after seven days.

Seed treatment with fungicides, bioagent and plant extracts

The effects of five fungicides - Bavistin 0.1% (carbendazim), Blitox 0.3% (copper oxychloride), Indofil M-45 0.2% (mancozeb), Ridomil 0.1% (metalaxyl+mancozeb), and Captan 0.1% on the seed borne mycoflora of the pulses under investigation were examined. For 20 minutes, thirty seeds from each pulse sample were immersed in solutions containing each of the five chemical fungicides, while the control samples seeds were immersed in sterile distilled water. After that, seeds were incubated in three duplicates on PDA media in a Petri plate at $28\pm 1^\circ\text{C}$. Seed derived fungal colonies were separated, cleaned, and identified.

The effects of three bioagents - *Trichoderma viride*, *T. harzianum*, and *T. koningii* on the seed borne mycoflora of the five pulses under

minutes. Three duplicates of the treated seeds were cultured on PDA media in a Petri plate at $28\pm 1^\circ\text{C}$. Seed derived fungal colonies were separated, cleaned, and identified.

Plant samples of garlic (bulb), ginger (rhizome), and eucalyptus (leaf) were gathered, cleaned, and shade dried. They were crushed using sterile distilled water (1:1 w/v) and a sterile mortar and pestle. The resulting plant extract was handled at 100% concentration. Pulse seeds were individually dipped in 10% plant extract for 20 minutes, cleaned with sterile water, and then put on PDA medium in three repetitions. Plates were checked for fungal growth after being incubated at $28\pm 1^\circ\text{C}$. If any were found, they were extracted, purified, and identified.

The ANOVA approach was used to evaluate the data collected during this inquiry, and Microsoft Excel software was used for the analysis at the 5% significant level.

Results and Discussion

Three distinct techniques were used to separate the seed mycoflora from five distinct pulse species - Gram, Pigeon Pea, Green Gram, Black Gram, and Lentil that were gathered from local markets in Nanded District, Maharashtra.

Methods	<i>Penicillium sp.</i>	<i>Aspergillus sp.</i>	<i>Rhizopus sp.</i>	<i>Alternaria sp.</i>	<i>Trichoderma sp.</i>	Mean
PDA	18.00	18.67	20.67	04.67	10.67	14.54
Blotter	17.33	16.67	19.33	03.33	06.67	12.66
Seed washates	14.67	17.33	19.33	01.33	07.33	11.99
Mean	16.66	17.55	19.77	03.11	08.22	
SEm±	02.82	03.56	03.26	01.20	02.85	
CD(P=0.05)	08.16	10.27	09.42	03.46	08.22	
PULSES	<i>Penicillium sp.</i>	<i>Aspergillus sp.</i>	<i>Rhizopus sp.</i>	<i>Alternaria sp.</i>	<i>Trichoderma sp.</i>	Mean
Gram	14.44	14.44	14.44	00.00	07.78	10.22
Pigeon Pea	18.89	25.56	18.89	00.00	02.22	13.11
Green Gram	21.11	11.11	20.00	00.00	11.11	12.66
Black Gram	06.67	04.44	16.67	15.56	08.89	10.44
Lentil	22.22	32.22	28.89	00.00	11.13	18.89
SEm±	03.65	04.59	04.21	01.55	03.67	
CD(P=0.05)	10.53	13.26	12.16	04.47	10.61	

investigation were examined. While the control seeds were immersed in sterile distilled water, thirty seeds from each sample were treated with talc powder formulations of each bioagent at 8 g/kg seeds in the form of slurry for two

Table-1 Percent incidence of fungi associated with pulse seeds under different isolation methods.

The data in Table-1 makes it clear that the PDA method had a higher mean incidence of fungal occurrence (14.54%), followed by the blotter paper method (12.66%) and the seed washates method (11.99%). This could be because of the nutrients in the medium, which may have been more significant than the blotter approach in initiating the growth of pulse fungus (Shaker et al. 2010). As advised by ISTA, seed borne fungus of pulse seeds were typically found using the PDA plate and blotter method [5,16]. Three distinct isolation techniques were used to separate five distinct fungus species - *Penicillium sp.*, *Aspergillus sp.*, *Rhizopus sp.*, *Alternaria sp.*, and *Trichoderma sp.*- from the pulse seeds. With a mean incidence of 19.77%, *Rhizopus sp.* was found to be the most common fungus on the pulse seeds, followed by *Aspergillus sp.* (17.55%) and *Alternaria sp.* (3.11%).

The highest occurrence of different seed mycoflora was observed in lentil seeds (18.89%) (Table-1), with the exception of *Alternaria sp.*, which was only isolated from black gram seeds (15.56%). Gram (10.22%) and black gram (10.44%) had the lowest mean incidence of mycoflora. While researching on pulse seeds gathered from Jaipur, Rajasthan, India, Agarwal et al. [1] identified six fungal species from pulse seeds, some of which are similar to our investigation, including *Penicillium sp.*, *Aspergillus sp.*, and *Rhizopus sp.*.

The highest incidence of *Penicillium sp.* among the seed mycoflora was found in lentil seeds (22.22%) (Table-1), which differs significantly from the incidence found in black gram seeds (6.67%). Lentil seeds had the highest incidence of *Aspergillus sp.* (32.22%), followed by Pigeon Pea (25.56%) and Gram (14.44%), whereas black gram seeds had the lowest incidence (4.44%). Similarly, lentil (28.89%) and green gram (20.00%) seeds were found to have the highest incidence of *Rhizopus sp.* Only black gram seeds yielded *Alternaria sp.* as a seed mycoflora, while *Trichoderma sp.* had the highest prevalence on lentils (11.13%), closely followed by green gram (11.11%). In all of the

pulses, *Rhizopus sp.* and *Aspergillus sp.* were the most common fungi. Previous researchers have obtained similar results about mycoflora associated with various pulse seeds. [14] Shaker et al.

In order to lower the mycoflora of pulse seeds, several seed treatment agents, including fungicides, bioagents, and plant extracts, were investigated. Mancozeb was one of the fungicides that totally eradicated *Penicillium sp.* from the pulse seeds. *T. harzianum* was one of the bioagents that totally stopped *Penicillium sp.* from growing from the seeds of all the pulses. Eucalyptus leaf extract had the lowest *Penicillium sp.* incidence among plant extracts. On Green Gram seeds, however, the growth of *Penicillium sp.* was encouraged by garlic and eucalyptus extracts, with an incidence of 6.67% as opposed to 3.33% in the control. There have previously been reports on the impact of mancozeb on the control of pulse seed mycoflora. Dithane M-45 was found to have an impact on *Penicillium sp.* Carbendazim was shown to be less successful in lowering *Penicillium sp.* in the current trial. *T. harzianum* has been shown to have antifungal effect against Gram seed mycoflora by Mahamune and Kakde [8]. The outcome of our experiment is consistent with their findings. Ashwini and Giri [3] reported that *T. viride* was effective in reducing seed borne mycoflora in both green and black gram seeds.

Aspergillus sp. mancozeb treatment totally eradicated the fungus from all pulse seeds in terms of seed mycoflora. According to reports, applying mancozeb to Gram seeds eradicated every species of *Aspergillus*. *Aspergillus sp.* was most inhibited by *T. koningii* among bioagents. The plant extracts were generally the least effective, even though all of the treatments considerably decreased the *Aspergillus sp.* when compared to the control.

Rhizopus sp. on the pulse seeds was greatly decreased by all treatments, but it was totally eradicated by seed treatment with mancozeb and *T. koningii*. Even while the incidence of *Rhizopus sp.* from all the pulse seeds was lower with all the plant extracts than with the control,

the incidence of the fungus was still high, ranging from 18.66 to 21.99%. Only in the current study was the seed mycoflora *Alternaria sp.* identified from black gram seeds (Table-1). Comparable to *T. viride*, all of the fungicides had eradicated *Alternaria sp.* from black gram seeds. Among all the treatments, plant extracts had the least effect on *Alternaria sp.* control. When black gram seeds were treated with ginger, the incidence of *Alternaria sp.* rose from 16.67% in the control group to 20.00%. Plant extracts have been shown to have an inhibitory effect on seed mycoflora incidence, but they have also been shown to occasionally increase the incidence of some seed mycoflora [17].

All of the pulses under investigation have been found to contain *Trichoderma sp.* as seed mycoflora. By applying captan to the seeds, the incidence of *Trichoderma sp.* was eliminated in the current study. The incidence of *Trichoderma sp.* had been considerably decreased by all previous treatments. However, *Trichoderma sp.* of black gram seeds was unaffected by garlic or eucalyptus. The generation of chitinase, glucanase, antibiotics, and other modes of action are some of the ways that *Trichoderma sp.* have been shown to lower the incidence of seed mycoflora. The impact of various seed treatments on the germination rate of pulse seeds was noted in the current study (Table-1). With the exception of the eucalyptus seed treatment, it is clear that every treatment greatly raised the germination percentage of pulse seeds. Interestingly, the *T. harzianum* treatment had the highest mean germination percentage (70.66%), while the control group had a mean germination percentage of 46.66%. Treatment with mancozeb (70.00%), captan (68.66%), and *T. koningii* (67.33%) comes next. Bioagents have an impact on seed germination comparable to that of the fungicide mancozeb. Ashwini and Giri (2014) reported that seed treatment with *T. viride* increased the germination of pulse seeds, such as green gram and black gram. Additionally, fungicide has been shown to enhance seed germination. Generally speaking, improved seed germination

following fungicidal treatment results from the removal of fungi since they release mycotoxins, which are responsible for the decrease in seed germination.

According to Dhole and Gurme [5], *Acacia nilotica* leaf extract has been shown to boost seed germination % and decrease the occurrence of legume seed mycoflora. When *T. harzianum* is applied, the maximum mean percentage of pulse seed germination in response to seed treating agents is 70.66% (Table 1). *Eucalyptus odoratum* has the lowest mean percentage at 52.00%. *E. odoratum* has the greatest germination rate of 100% among Gram seeds. The germination rate of pigeon peas is unaffected by any seed treatment. For Green Gram, the treatments containing mancozeb, metalaxyl, captan, *T. harzianum*, and garlic showed the highest germination percentage (100%). The treatment with *T. harzianum* showed the maximum percentage (83.33%) for black gram, whereas the treatment with metalaxyl showed the lowest percentage (56.67%). The maximum germination rate for lentils is 96.67% when using the captan treatment, as opposed to 46.67% when using the control.

Declarations:

Acknowledgement

The authors gratefully acknowledge the facilities and other assistance provided by the HoD, Dept. of Botany, N.E.S. Science College, Nanded for carrying out this research work.

Funding: none stated.

Conflict of Interest: The authors declare no conflict of interest.

Declaration of Non-Use of AI: The authors confirm that no artificial intelligence tools were used in this study.

References

1. Agarwal T, Malhotra A and Trivedi P C. 2011. Fungi associated with chickpea,

- lentil and black gram seeds of Rajasthan. International Journal of Pharma and Biosciences 2: 478-483.
2. Amin M, Teshele J and Tesfay A. 2014. Evaluation of Bio-agents seed treatment against *Colletotrichum lindemuthianum*, in Haricot bean anthracnose under field condition. Research in Plant Sciences 2: 22-26.
 3. Ashwini C and Giri G K. 2014. Control of seed-borne fungi in green gram and black gram through bioagents. International Journal of Applied Biology and Pharmaceutical Technology 5: 168-170.
 4. Bhardwaj A, Verma S C, Bharat N K and Thakur M. 2013. Effect of vegetable oil seed treatment on seed mycoflora of pea, *Pisum sativum* L. International Journal of Farm Sciences 3: 46-51.
 5. Dhole A C and Gurme M K. 2013. Effect of leaf extract of *Acacia nilotica* on seed mycoflora of legumes. Research Journal of Recent Sciences 2: 83-95.
 6. Habib J A, Sahi NST and Waheed. 2012. Detection of seed-borne mycoflora of different course and fine rice varieties and their management through seed treatment. Pakistan Journal of Phytopathology 24: 133-136.
 7. Kandhare A S. 2014. Effect of common and dominant seed-borne fungi on fat content of pulses. Indian Journal on Advances in Plant Research 1: 7-18.
 8. Mahamune SE and Kakde R B. 2011. Incidence of seed-borne mycoflora on Gram mutants and its antagonistic activity against *Trichoderma harzianum*. Recent Research in Science and Technology 3: 62-67.
 9. Narayan P and Kumar S. 2014. Changing scenario of pulses in India- an analytical view. Bharatiya Krishi Anusandhan Patrika 28: 192-9.
 10. Pan S, Khalko and Das A. 2010. Effect of some fungicides on seed mycoflora, germination, viability and their persistence in treated seeds. The Journal of Plant Protection Sciences 2: 59-64.
 11. Patil B J and Madane E N 2014. Effect of *Hyptis suaveolens* (L.) Poit and *Eupatorium odoratum* L. leaf extracts on seed mycoflora of legume plants. Bioscience Discovery Journal 5: 237-240.
 12. Ramesh B V, Hireath S V, Naik MK, Amaresh Y S, Lokesh B K and Vasudevan S N. 2013. Study of seed mycoflora of soybean from north-eastern Karnataka. Journal of Agricultural Sciences 26: 58-62.
 13. Saleem A and Ebrahim MK H. 2013. Production of amylase by fungi isolated from legume seeds collected in Almadinah Almunawwarah, Saudi Arabia. Journal of Taibah University for Sciences 8: 90-97.
 14. Shaker M, Momin R K and Hashmi S. 2010. Isolation and identification of some pulses mycoflora. Bionano Frontier 3: 321-324.
 15. Singh S, Srivastava S, Mishra J, Raaj R and Sinha S. 2014. Evaluation of some plant extracts against predominant seed mycoflora of Mungbean *Vigna radiata* (L.) wilczed seed. An International Research Journal 51: 83-89.
 16. Singh V K. 2014. Detection of mycoflora associated with *Cicer arietinum* seeds by agar plate method with PDA. Weekly Science Research Journal 1: 1-4.
 17. Telang S M. 2010. Effect of extracts of various plant parts on seed mycoflora and seed germination of chilli. An Asian Journal of Soil Science 5: 24-25.
 18. Zaidi R K and Pathak N. 2014. Antifungal activity of plant extracts and *Trichoderma* sp. against seed fungi of cowpea. International Journal of Botany and Research 4: 51-59.