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# A comparative evaluation of fungicides and plant leaf extracts against the fungi associated with seeds of pulses

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## Abstract

The pulses seed is caused by various seed borne fungal pathogen during storage condition. In the present in vitro investigation a comparative evaluation of antifungal activity of four plant leaf extract and chemical fungicides against major seed borne fungi associated with seeds of pulses viz. *Aspergillus flavus, Aspergillus niger, Fusarium oxysporium, Rhizopus stolonifer and Alternaria alternate.* The antifungal activities of four plant leaf extracts were compared with commercially available four chemical fungicides. The antifungal activity of plant leaf extracts viz. *Datura inoxia* Mill., *Calotropis procera* Aiton., *Parthenium hysterophorus* L. and *Ipomoea carnea* L.. The antifungal activities of *Ipomoea carnea* L. was found most mycelia growth of inhibition of these test fungi; rest of the plant extracts *Datura inoxia* Mill., *Calotropis procera* Aiton., *Parthenium hysterophorus* L. were found effective inhibition of mycelia growth of fungi. Four chemical fungicides viz. Mancozeb (Indofil M-45), Copper oxychloride (Blitox 50WP), Carbendazim (Bavistin 50WP) and Hexaconazole (Contaf 5 EC) were evaluated against seed borne test fungi. The Systemic fungicides Hexaconazole and Carbendazim was found most efficient inhibitor which completely inhibited the radial mycelia growth of seed borne fungi followed by non-systemic fungicide Mancozeb and Copper oxychloride. The plant extracts as a biofungicides can possibly be effort in the management of seed borne fungi by an ecofriendly way.

**Key words:** Wild plant$_1$, Plant leaf extracts$_2$, Chemical fungicide$_3$, Seed borne fungi$_4$, Pulses seed$_5$, In vitro evaluation$_6$.

# 1. Introduction

The legume or pulses belongs to family fabaceae is the high protein content makes them desirable crop in agriculture. Pulses are of high economic value because of the rich source of protein and contain carbohydrate, dietary fiber and minerals [7]. India is largest producer, importer and consumer of pulses in the world. The persistent crop growing, demand and supply gap is putting pressure on prices. Pulses are an integral component of sustainable crop production as they have ability of biological nitrogen fixation and basic ingredient in the diet of vast majority of poor and vegetarian population in India [1]. The population of India increasing day by day so there is need in field of sustainable agriculture practices to improve the yield of pulse crop. The pulses like Gram (*Cicer arietium* L.), Red gram (*Cajanus cajan* L.) and Green gram (*Vigna radiate* L.) are the important crop grown in Marathwada region of Maharashtra during both kaharif and ruby season.

The seeds of pulses crop carry a mycoflora both in field and storage condition, field fungi gradually disappear and storage fungi then predominant. The storage seeds associated with many storage fungal species like *Aspergillus sp., Rhizopus sp., Penicillum sp., Fusarium sp., Alternaria sp., Cladosporium sp., and Rhizotinia sp.* The association of seed mycoflora adversely affects the health of seed and seedling. The storage fungi may cause discoloration of the seeds and germination failure [3]. These seed mycoflora especially grow vigorously and initiate grain spoilage. They also are tiring about several undesirable changes making them unfit for consumption and sowing. Many diseases of pulses are carried through seed and cause enormous losses to the crop. Seed borne fungi cause disease and play a major role in losses in crop production [6, 8]. Seed borne diseases can causes economic crop losses, reduction in plant growth and productivity of the crop [4]. The diseases of pulses and its control a lot of research has been done in many countries but information on storage mycoflora of pulses seed and its control are inadequate [10-12]. Presence or absence of seed borne fungi is one of the major aspects that reduce the quality of seed. The present investigation was aimed to estimate the fungi associated with seeds of selected pulses in stored condition. This paper also deals with the management of seed mycoflora of pulses seed.

# 2. Material Methods

**Isolation of Seed-borne Fungi of Pulses seed:**
The present study is carried on storage seeds of pulses. Seed sample of Gram (*Cicer arietium* L.), Red gram (*Cajanus cajan* L.) and Green gram (*Vigna radiate* L.) was collected from different places of storage condition in Udgir region of Latur district, Maharashtra. Seed sample was placed in clean paper bag, labeled properly and preserved in laboratory. The fungi were isolated from seed sample on PDA and Blotter paper method as recommended by International Seed Testing Association [5]. One hundred seeds of each sample were placed on three layers of moist blotter paper in Petri plates. Fungi grown in the seeds were transferred to separate PDA slants for further studies. Identification of the isolates was determined based on morphological characteristics observed under compound microscope by slandered literature. The most predominant fungi identified were *Aspergillus flavus, Aspergillus niger, Fusarium oxysporium, Rhizopus stolonifer and Alternaria alternate*.

**Collection of Plant material:**
In the present studies four important plants were selected from local wild flora of Udgir region, Dist Latur (M.S.). The collected plants sample was *Datura inoxia* Mill. *Calotropis procera* Aiton. ,*Parthenium hysterophorus* L. and *Ipomoea carnea* L.

**Preparation of plant leaf extracts:**
Study of antifungal activity leaf extracts of four different plants was used. The fresh leaf samples collected and sterilized with 0.1% $Hgcl_2$ then wash with sterile D.W. sterilized leaves were crushed in mortar and pestle with sterile water at the rate of one ml/g. The extract was obtained by squeezing and filtered through muslin cloth and made to 10% concentration by adding distilled water.

The efficacy of non-systemic fungicide and systemic fungicide, four different fungicide viz. Mancozeb (Indofil M-45), Copper oxychloride (Blitox 50WP), Carbendazim (Bavistin 50WP) and Hexaconazole (Contaf 5 EC) at 0.2 percent of concentration were evaluated against seed borne fungi.

**In vitro evaluation of plant leaf extracts and chemical fungicides:**

Four plant leaf extracts and four fungicides were evaluated against seed borne fungi of pulses seed by poison food technique. The pure culture plate was inoculated with 5mm mycelia hyphae of pathogen taken from pure culture. In the center of the medium a cup cavity of 8mm diameter was made with sterilized cork borer. The cup was filled with 2ml of leaf extract, [9] and fungicides. The Petri plate was incubated at 25±2$^0$ c and radial mycelia growth of pathogen was measured at 6 to7 days after inoculation. The diameter of the colony of mycelium was measured and percent inhibition of growth of the test pathogen was calculated by using formula [13].

## 3. Results and Discussion

The antifungal activity of four plant leaf extracts and four chemical fungicides against seed borne fungi of pulses. A total 5 fungal species belong to 4 genera were found to be associated with storage seeds of pulses. The isolated fungi were *Aspergillus flavus, Aspergillus niger, Fusarium oxysporium, Rhizopus stolonifer* and *Alternaria. alternata.*

**Antifungal activity of plant leaf extracts and fungicides on mycelial growth of *Aspergillus flavus*:**

The antifungal activity of plant extract and chemical fungicide against *Aspergillus flavus* (Table-1) was show significant difference. In vitro condition the radial mycelia growth of *Aspergillus flavus* was found (85%) in inhibition in plant leaf extract of *Ipomoea carnea* L. and *Datura inoxia* Mill. shows (72%) growth of inhibition where as rest of the plant extracts shows less effective i.e. *Calotropis procera* Aiton. (49.2%) and *Parthenium hysterophorus* L. (30%). Among the chemical fungicides growth of inhibition was found (100%) in Carbendazim and Hexaconazole where as in Copper oxychloride (70.2%) and Mancozeb (69.4%). The effect of Carendazim + Mancozeb (100%) in growth of inhibition of *Aspergillus flavus* was reported by [14].

**Antifungal activity of plant leaf extracts and fungicides on mycelial growth of *Aspergillus niger*:**

There was significant difference due to various treatments of plant leaf extracts and fungicides against *Aspergillus niger* under in vitro condition. The radial growth of inhibition shows in leaf extracts of *Ipomoea carnea* L. (86%), *Datura inoxia* Mill. (71%), other two plant leaf extracts *Calotropis procera* Aiton. (50.2%) and *Parthenium hysterophorus* L. (29.2%) shows least effective. The chemical fungicides mycelia radial growth of inhibition was found (100%) in Carbendazim and Hexaconazole reaming two non-systemic fungicide, Mancozeb (70.3%) and Copper oxychloride (71%) shows least growth of inhibition. The antifungal activity of Carbendazim shows (100%) inhibition of colony growth of *Aspergillus niger* was earlier reported by [2].

**Antifungal activity of plant leaf extracts and fungicides on mycelial growth of *Fusarium oxysporium*:**

The leaf extracts of *Ipomoea carnea* L. shows (100%) radial mycelia growth of inhibition. The antifungal activity of other three leaf extracts shows maximum growth of inhibition *Datura inoxia* Mill. (69%) where as *Calotropis procera* Aiton. (42.5%) and *Parthenium hysterophorus* L. (22%) less effective against *Fusarium oxysporium*. The radial growth of mycelia inhibition in fungicidal activity was significant difference. The systemic fungicide Carbendazim (100%), Hexaconazole (97.5%) and non-systemic fungicide Mancozeb (73.2%) and Copper oxychloride (66.3%) was found mycelia growth of inhibition.

**Antifungal activity of plant leaf extracts and fungicides on mycelial growth of *Rhizopus stolonifer*:**

The antifungal activity of plant leaf extract was determined against *Rhizopus stolonifer*. The radial mycelia growth of inhibition in plant leaf extract of *Ipomoea carnea* L. (96%) was found highest growth of inhibition, other plant leaf extract *Datura inoxia* Mill.

**Table: 1** Comparative analysis of antifungal activity of plant extracts / chemical fungicides against major seed borne fungi of pulses.

| Sr. No. | Plant extracts / Fungicides | Percent inhibition of radial growth concentration (%) | | | | |
|---|---|---|---|---|---|---|
| | | *Aspergillus flavus* | *Aspergillus niger* | *Fusarium oxysporium* | *Rhizopussto lonifer* | *Alternaria alternata* |
| **1.** | *Datura inoxia* Mill. | 72 | 71 | 69 | 62 | 70.03 |
| **2.** | *Calotropis procera* Aiton. | 49.2 | 50.2 | 42.5 | 47 | 51 |
| **3.** | *Parthenium hysterophorus* L | 30 | 29.2 | 22 | 21 | 24.3 |
| **4.** | *Ipomoea carnea* L. | 85 | 86 | 100 | 96 | 92.2 |
| **5.** | Mancozeb (Indofil M-45) | 69.4 | 70.3 | 73.2 | 65.3 | 60 |
| **6.** | Copper oxychloride (Blitox 50WP) | 70.2 | 71 | 66.3 | 63.2 | 54.3 |
| **7.** | Carbendazim (Bavistin 50WP) | 100 | 100 | 100 | 96.3 | 94.3 |
| **8.** | Hexaconazole (Contaf 5 EC) | 100 | 100 | 97.5 | 98.4 | 100 |

(62%), *Calotropis procera* Aiton. (47%) and *Parthenium hysterophorus* L. (21%) shows less effective. Among systemic and non-systemic fungicide Hexaconazole (98.4%) and Carbendazim (96.3%) recorded highest mycelia growth of inhibition followed by Mancozeb (65.3%) and Copper oxychloride (63.2%) shows least effective.

**Antifungal activity of plant leaf extracts and fungicides on mycelial growth of *Alternaria alternata*:** The leaf extract of *Ipomoea carnea* L. (92.2%) showed maximum radial mycelial growth of inhibition followed by *Datura inoxia* Mill. (70.3%), *Calotropis procera* Aiton. (51%) and *Parthenium hysterophorus* L. (24.3%) was reported least effective against *Alternaria alternata*. The radial mycelial growth of *Alternaria alternata* was found maximum inhibition in systemic fungicide Hexaconazole (100%) and Carbendazim (94.3%). The non-systemic fungicide Mancozeb (60%) and Copper oxychloride (54.3%) showed minimum growth of inhibition. Effectiveness of Carbendazim (100%) inhibition of colony growth of *Alternaria alternata* was earlier reported by [15].

## 4. Conclusion

The comparison of antifungal activity of plant leaf extract with chemical fungicide against seed borne pathogen the leaf extract of *Ipomoea carnea* L. and *Datura inoxia* Mill. was more active against pathogenic fungi. It is anticipated that this study would lead to the establishment of some compounds that could be used to formulate new and more effective antimicrobial drugs of natural origin for the treatment of plant pathogen. The plant extract was found most effective and ecofriendly in controlling seed borne fungi of pulses.

**Conflicts of interest:** The authors stated that no conflicts of interest.

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