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# Efficacy of Fungicides on Seed Mycoflora of Groundnut at Different Storage Periods

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## ABSTRACT

The present study was conducted at Seed Research & Technology Centre, Professor Jayashankar Telangana State Agricultural University, Hyderabad, Telangana, India during January-June, 2016.The efficacy of 7 fungicides viz., captan (0.25%), mancozeb (0.25%), carboxin+thiram (0.3%), carbendazim (0.2%), benomyl (0.2%), tebuconazole (0.1%) and carbendazim+iprodione (0.2%) against seed mycoflora of groundnut at recommended dosages and at different storage periods (immediately after treatment, 1 day after treatment, 1 week after treatment, 2 weeks after treatment, 3 weeks after treatment, 1 month after treatment, 2 months after treatment and 3 months after treatment) were studied using standard blotter method. Treated seeds (of different storage periods) were incubated for 7 days and data on percent seed infection and frequency were recorded. Among the fungicides tested, seed treatment with carboxin+thiram (21.19%) was found significantly superior in reducing the percent seed infection followed by captan (24.00%) and the least effective was carbendazim (71.91%). A total of 13 seedborne fungi belonging to 10 genera viz., *Macrophomina phaseolina*, *Rhizoctonia* spp., *Rhizopus* spp., *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus ustus*, *Alternaria* spp., *Fusarium* spp., *Penicillium* spp., *Trichoderma* spp., *Chaetomium* spp. and *Cladosporium* spp. were recovered from untreated and treated seeds at different storage periods. The percent seed infection by different seed mycoflora increased with the increase in storage period. However, there was a gradual decline in field mycoflora viz., *Macrophomina phaseolina* and *Rhizoctonia* spp. and gradual increase in storage mycoflora viz., *Aspergillus* spp., *Cladosporium* spp., *Rhizopus* spp., *Penicillium* spp. etc. found with the increase in storage period.

**KEYWORDS:** Fungicides, groundnut, seed mycoflora, storage periods

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***Data Availability Statement:*** Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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## 1. INTRODUCTION

Groundnut (*Arachis hypogeae* L.) is one of the world's most popular oilseed crops of the tropics and it is considered as "King of oilseeds" (Anonymous, 2019). The groundnut seeds contain 47–53% oil, 26% protein, 11.5% starch, nutritional fibres, vitamins and some minerals (Nagpurne and Patwari, 2014). The total area of groundnut in India is 4.82 mha with a production of 9.95 mt. It occupies 3rd place among the oilseed crops grown in India in terms of production after soybean and rapeseed & mustard. The area under groundnut in Telangana is 0.11 mha with a production of 0.26 mt (Anonymous, 2019–20).

Seed health plays an important role in successful cultivation and yield exploration of a crop (Wimalasekera, 2015). Among various factors that affect seed health, the most important are the seed borne pathogens that not only lower seed germination, but also reduce seed vigor resulting in low yield. Fungi are the main component of microflora associated with seeds and are the main cause of deterioration and loss observed during storage (Tanaka et al., 2001, Amza, 2018). The associated microorganisms may be pathogenic or non pathogenic in nature.

Major seedborne diseases of groundnut include, stem rot (*Sclerotium rolfsii*), tikka leaf spot (*Cercospora arachidicola* and *Phaeoisariopsis personata*), pepper leaf spot (*Leptosphaerulina crassiasca*), aflaroot or yellow mold (*Aspergillus flavus*) and crown rot or collar rot (*Aspergillus niger*) (Anonymous, 2012). *Fusarium* spp., *Rhizoctonia solani*, *Physalospora rhodina* (*Diplodia gossypina*) also cause seed borne diseases in groundnut and *Aspergillus flavus*, *Botryodiplodia* spp. and *Cladosporium herbarum* were found to cause reduction in oil content altering its colour and making it smell rancid (Neergaard, 1977). In addition to these seedborne pathogens, seeds are also known to harbour several other fungi which may cause seed rot, seedling mortality, reduced seedling vigour and seed viability which leads to poor plant stand in the field (Srinivas, 2017, Amza, 2018). The seed quality also affects the rate and uniformity of emergence and the dynamics of initial plant growth. The seedborne fungi may also cause systemic or local infections, resulting in development of diseases at later stages of the crop growth. Seedborne diseases accounted for 30–40% yield loss in groundnut (Anonymous, 2012). Therefore, management of seedborne fungi is extremely important for realization of full yield potential of cultivars.

Seed treatment is one of the best methods to manage seedborne diseases. It has become a common practice to use fungicides as seed dressers for reducing the seedborne infections under field conditions. Fungicides form a zone of protection over the seed surface that reduces seed decay and seedling blight, resulting in healthy and vigorous seedlings (Marimuthu and Nakeeran, 2001, Begum et al., 2013). Treating the seeds with fungicides may eradicate pathogens in or on seeds and can also protect seeds and seedlings from soil-borne pathogens (Maude, 1996, Lamichhane et al., 2020, Ayesha et al., 2021). Effective seed treatments can eliminate the need for foliar application of fungicides later in the season (Mancini and Romanazzi, 2014).

Earlier works by different scientists reported the effectiveness of fungicide seed treatments *in vitro* and in field conditions. Effective management of root rot and significant yield benefit from groundnut was obtained through integrated use of mancozeb treatment (3 g $kg^{-1}$ seed) with properly cleaned seeds (Tarekegn et al., 2007). Other reports include, Seeds treated with carboxin 2 g $kg^{-1}$ completely controlled the flag smut of wheat disease (Shekhawat and Majumdar, 2011). Seed treatment with Tecto and Benlate at 2.5 g $kg^{-1}$ gave better performance in reducing the fungal population and increasing seed germination in sunflower (Bhutta et al., 2001). Nghiep and Gaur (2005) reported that, most of the seedborne fungi *viz., Bipolaris oryzae, Alternaria padwickii, Curvularia lunata* and other seedborne fungi were eradicated by vitavax followed by thiram and mancozeb. Field fungi decreased progressively, while storage fungi increased gradually during the increasing period of storage. Fungicides, carboxin+thiram, carbendazim+mancozeb and carbendazim were found most effective against seed mycoflora of sorghum viz., *Alternaria, Helminthosporium, Curvularia and Fusarium* (Ram et al., 2021). Thiram+carbendazim was also found effective in reducing seed mycoflora of soybean (Pawaret al., 2015).

In the current study 7 fungicides were tested for their efficacy against seed mycoflora of groundnut under *in vitro* conditions.

## 2. MATERIALS AND METHODS

The present study was conducted at Seed Research & Technology Centre, Professor Jayashankar Telangana State Agricultural University, Hyderabad, Telangana, India during January-June, 2016.

### *2.1. Efficacy of fungicides against seed mycoflora*

Seeds of groundnut variety K9 were procured from ARS, Kadiri, Andhra Pradesh and stored at ambient storage temperature of 28±2°C. The seeds were treated with fungicides viz., captan (0.25%), mancozeb (0.25%), carboxin+thiram (0.3%), carbendazim (0.2%), benomyl (0.2%), tebuconazole (0.1%) and carbendazim+iprodione (0.2%). The treated seeds were stored in butter paper bags along with untreated control for further use. The effect of fungicides on seed mycoflora was assessed by employing standard blotter method (Anonymous, 1996). The randomly selected 400 treated seeds were subjected to seed

health testing at different intervals viz., immediately after treatment, 1 day after treatment, 1 week after treatment, 2 weeks after treatment, 3 weeks after treatment, 1 month after treatment, 2 months after treatment and 3 months after treatment consecutively along with controls to estimate seed borne mycoflora. The data on number of seeds infected by different fungi and a specific fungus was recorded separately to calculate percent seed infection and frequency of a specific fungus.

*2.2. Detection of seed mycoflora by standard blotter method*

Sterilized blotting paper discs of 90mm diameter were placed in sterile Petri plates and moistened with sterile distilled water. The excess water was drained off from the plates. Seeds were transferred to the plates containing moist blotting paper discs. 10 seeds plate$^{-1}$ were placed at equidistance, 10 such plates were maintained under each replication. The experiment was conducted with 4 replications and under each replication 100 seeds were tested. The plates were incubated at 24±2°C for seven days in an incubator. The mycoflora observed on seeds were isolated and identified.

*2.3. Data recording*

On 8th day, the incubated seeds were examined under stereo binocular microscope. The mycelium and the fungal structures obtained from the seeds were further observed critically under 10x and then under 40x objective lens of a compound microscope by preparing water mount slides.

Data on number of seeds infected by different fungi and a specific fungus were recorded separately to calculate percent seed infection and frequency, respectively. To calculate percent seed infection (Alemu, 2014) and frequency of the species (Vishunavat, 2016) the following formulae were used.

Per cent seed infection=(Number of infected seeds/Total number of seeds)×100 .....(1)

Frequency=(Number of seeds containing a specific fungus/ Total number of seeds)×100 .....(2)

*2.4. Identification of fungi*

Identification of various seed mycoflora was done using relevant keys given by Subramanian (1971), Booth (1971), Barnett (1965) and descriptions of CMI (Anonymous, 1970).

*2.5. Statistical analysis*

Four replications were maintained in the current experiment and the data was statistically analyzed using two factorial CRD as per the procedures suggested by Gomez and Gomez (1984). The data was transformed using angular transformation and the actual percent values along with their corresponding transformed values are given in tables.

## 3. RESULTS AND DISCUSSION

All the fungicides were found significantly effective in suppressing seed mycoflora when compared to control. Among the fungicides, carboxin+thiram (21.19%) was found significantly superior (Plate 1 and Table 1) followed by captan (24.00%), carbendazim+iprodione (35.72%) and the least effective (71.91%) was carbendazim.

Plate 1: Efficacy of carboxin+thiram against seed mycoflora of groundnut

A total of 13 seedborne fungi belonging to 10 genera viz., *Macrophomina phaseolina*, *Rhizoctonia* spp., *Rhizopus* spp., *Aspergillus flavus*, *Aspergillus niger, Aspergillus ochraceus*, *Aspergillus ustus*, *Alternaria* spp., *Fusarium*spp., *Penicillium* spp., *Trichoderma* spp., *Chaetomium* spp. and *Cladosporium* spp. (Table 2 and Plate 2) were recovered from untreated and treated seeds at different storage periods. All the fungi which were observed on untreated seeds were also observed on seeds treated with fungicides.

*Aspergillus niger* was recorded with highest abundance from all the fungicides tested followed by *Aspergillus flavus* and *Fusarium* spp., while *Rhizopus* spp. was found to be absent in seeds treated with carboxin+thiram. The fungi viz., *Macrophomina phaseolina, Rhizoctonia* spp., *Aspergillus ochraceus*, *Aspergillus ustus*, *Alternaria* spp., *Penicillium* spp., *Trichoderma* spp., *Chaetomium* spp. and *Cladosporium* spp. were rarely recovered from all the fungicides tested (Table 2).

It was observed that, the percent seed infection by different seed mycoflora increased with the increase in storage period. However, there was a gradual decline in field mycoflora viz., *Macrophomina phaseolina* and *Rhizoctonia* spp. and gradual increase in storage mycoflora viz., *Aspergillus* spp., *Cladosporium* spp., *Rhizopus* spp., *Penicillium* spp. etc.

Table 1: Efficacy of fungicides against seed mycoflora of groundnut at different storage periods

| Fungicide | percent seed infection | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | IAT | 1 DAT | 1 WAT | 2 WAT | 3 WAT | 1 MAT | 2 MAT | 3 MAT | Mean |
| Captan | 18.25* (25.27)** | 20.00 (26.54) | 21.50 (27.60) | 21.50 (27.60) | 22.50 (28.30) | 23.50 (28.98) | 24.75 (29.82) | 40.00 (39.22) | 24.00 |
| Mancozeb | 47.00 (43.27) | 48.25 (43.99) | 50.00 (45.00) | 51.50 (45.86) | 51.75 (46.00) | 51.75 (46.00) | 53.25 (46.86) | 66.75 (54.79) | 52.53 |
| Carboxin+thiram | 16.50 (23.94) | 16.50 (23.94) | 20.00 (26.54) | 20.00 (26.54) | 21.50 (27.61) | 21.75 (27.78) | 23.25 (28.81) | 30.00 (33.20) | 21.19 |
| Carbendazim | 66.50 (54.64) | 67.25 (55.09) | 70.00 (56.79) | 70.00 (56.79) | 71.50 (57.74) | 73.25 (58.86) | 76.75 (61.18) | 80.00 (63.45) | 71.91 |
| Benomyl | 48.25 (43.99) | 48.25 (43.99) | 51.50 (45.86) | 51.75 (46.00) | 56.50 (48.73) | 58.25 (49.75) | 76.75 (61.18) | 78.00 (62.04) | 58.66 |
| Tebuconazole | 43.25 (41.11) | 43.25 (41.11) | 45.00 (42.12) | 45.00 (42.12) | 46.50 (42.99) | 50.00 (45.00) | 66.75 (54.78) | 80.00 (63.45) | 52.47 |
| Carbendazim+iprodione | 33.25 (35.20) | 33.25 (35.20) | 35.00 (36.26) | 35.00 (36.26) | 36.75 (37.31) | 36.75 (37.31) | 37.25 (37.61) | 38.50 (38.34) | 35.72 |
| Control | 73.25 (58.87) | 73.50 (59.03) | 75.00 (60.02) | 76.00 (60.69) | 78.25 (62.23) | 85.00 (67.26) | 88.00 (69.81) | 92.00 (73.72) | 80.13 |
| Mean | 43.28 | 43.70 | 46.00 | 46.34 | 48.16 | 50.03 | 55.84 | 63.16 | |
| | Storage period | | | Fungicide | | | Storage period×Fungicide | | |
| SEm± | 0.22 | | | 0.22 | | | 0.64 | | |
| CD ($p$=0.05) | 0.63 | | | 0.63 | | | 1.79 | | |

IAT- Immediately after treatment; DAT- Day(s) after treatment; WAT- Week(s) after treatment; MAT- Month(s) after treatment; *Mean of 4 replications; **Figures in parenthesis are angular transformed values

Table 2: Seed mycoflora recovered from groundnut seeds treated with fungicides

| Storage period | Mp | Rhz | Rhi | An | Af | Fus | Alt | Pen | Tri | Ao | Au | Cha | Cla |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Captan | | | | | | | | | | | | | |
| IAT | - | - | + | + | + | - | - | - | - | - | - | - | - |
| 1 DAT | - | - | + | + | + | - | - | - | - | - | - | - | - |
| 1 WAT | - | - | + | + | + | - | - | - | - | - | - | - | - |
| 2 WAT | - | - | + | + | + | - | - | - | - | - | - | - | - |
| 3 WAT | + | - | + | + | + | + | - | - | - | - | - | - | - |
| 1 MAT | - | - | + | + | + | + | - | - | - | - | - | - | - |
| 2 MAT | + | - | + | + | + | - | - | - | - | + | - | - | - |
| 3 MAT | - | - | + | + | + | + | - | - | - | + | + | - | - |
| Mancozeb | | | | | | | | | | | | | |
| IAT | + | + | - | ++ | ++ | + | - | - | - | - | - | - | - |
| 1 DAT | + | + | + | ++ | ++ | + | - | - | - | - | - | - | - |
| 1 WAT | + | + | - | ++ | + | + | - | - | - | - | - | - | - |
| 2 WAT | + | + | + | ++ | + | + | - | - | - | - | - | - | - |
| 3 WAT | - | - | + | ++ | + | + | - | + | - | - | - | - | - |
| 1 MAT | - | - | + | ++ | ++ | + | - | - | - | - | - | - | - |

Table 2: Continue...

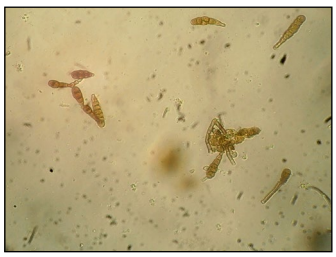
| Fungicide | Mp | Rhz | Rhi | An | Af | Fus | Alt | Pen | Tri | Ao | Au | Cha | Cla |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 MAT | + | - | + | ++ | ++ | + | - | - | - | - | - | - | - |
| 3 MAT | + | - | + | +++ | ++ | + | | - | + | + | + | - | - |
| Carboxin+thiram | | | | | | | | | | | | | |
| IAT | - | - | - | + | + | + | - | - | - | - | - | - | - |
| 1 DAT | - | - | - | + | + | + | - | - | - | - | - | - | - |
| 1 WAT | - | - | - | + | + | + | - | - | - | - | - | - | - |
| 2 WAT | - | - | - | + | + | + | - | - | - | - | - | - | - |
| 3 WAT | - | - | - | + | + | + | - | - | - | - | - | - | - |
| 1 MAT | - | - | - | + | + | + | - | - | - | - | - | - | - |
| 2 MAT | - | - | - | + | + | + | - | - | - | - | - | - | - |
| 3 MAT | + | - | - | + | + | + | - | - | - | + | + | - | - |
| Carbendazim | | | | | | | | | | | | | |
| IAT | + | + | + | +++ | ++ | + | - | - | - | - | - | - | - |
| 1 DAT | + | + | + | +++ | ++ | + | - | - | - | - | - | - | - |
| 1 WAT | - | - | + | +++ | ++ | + | - | + | - | - | - | - | - |
| 2 WAT | - | - | + | +++ | ++ | + | - | + | - | - | - | - | - |
| 3 WAT | - | - | + | +++ | ++ | - | - | + | - | - | - | - | - |
| 1 MAT | - | - | + | +++ | ++ | + | - | - | - | - | - | - | - |
| 2 MAT | - | - | + | ++++ | +++ | + | + | + | + | - | - | + | - |
| 3 MAT | - | - | ++ | ++++ | ++++ | + | + | - | + | + | + | - | + |
| Benomyl | | | | | | | | | | | | | |
| IAT | + | + | ++ | + | + | + | - | - | - | - | - | - | - |
| 1 DAT | + | + | + | + | + | + | - | + | - | - | - | - | - |
| 1 WAT | + | + | + | + | + | + | - | - | - | - | - | - | - |
| 2 WAT | + | + | + | ++ | + | + | - | + | - | - | - | - | - |
| 3 WAT | - | - | ++ | +++ | + | + | - | + | - | - | - | - | - |
| 1 MAT | - | - | ++ | +++ | + | + | - | - | - | - | - | - | - |
| 2 MAT | - | - | ++ | +++ | + | + | - | + | + | - | - | - | + |
| 3 MAT | - | - | +++ | ++++ | + | + | - | - | - | + | + | + | + |
| Tebuconazole | | | | | | | | | | | | | |
| IAT | - | - | - | ++ | ++ | + | - | + | - | - | - | - | - |
| 1 DAT | - | - | - | ++ | ++ | + | - | - | - | - | - | - | - |
| 1 WAT | - | - | - | ++ | ++ | + | - | - | - | - | - | - | - |
| 2 WAT | - | - | - | ++ | ++ | + | - | - | - | - | - | - | - |
| 3 WAT | - | - | + | ++ | ++ | + | - | - | - | - | - | - | - |
| 1 MAT | - | - | - | ++ | ++ | + | ++ | - | - | - | - | - | - |
| 2 MAT | + | - | + | ++ | ++++ | ++ | ++ | + | + | - | - | + | - |
| 3 MAT | + | - | + | ++ | ++++ | ++ | ++ | + | + | + | + | + | + |

Table 2: Continue...

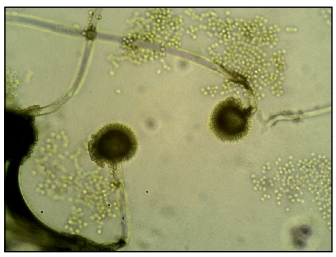
| Fungicide | Mp | Rhz | Rhi | An | Af | Fus | Alt | Pen | Tri | Ao | Au | Cha | Cla |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Carbendazim+iprodione | | | | | | | | | | | | | |
| IAT | - | - | + | + | + | + | - | - | - | - | - | - | - |
| 1 DAT | - | - | + | + | + | + | - | - | - | - | - | - | - |
| 1 WAT | - | - | + | + | + | + | - | - | - | - | - | - | - |
| 2 WAT | - | - | + | + | + | + | - | - | - | - | - | - | - |
| 3 WAT | - | - | + | + | + | + | - | - | - | - | - | - | - |
| 1 MAT | - | - | + | + | + | + | - | - | - | - | - | - | - |
| 2 MAT | - | - | + | + | + | + | - | - | - | - | - | - | - |
| 3 MAT | - | - | + | + | + | + | - | - | - | - | - | - | - |
| Control | | | | | | | | | | | | | |
| IAT | + | + | + | +++ | ++ | + | - | - | - | - | - | + | - |
| 1 DAT | - | - | + | +++ | ++ | + | - | - | - | - | - | - | - |
| 1 WAT | - | - | + | +++ | ++ | + | - | - | - | - | - | - | - |
| 2 WAT | - | - | + | +++ | ++ | + | - | - | - | - | - | - | - |
| 3 WAT | + | - | + | +++ | +++ | + | + | + | - | - | - | + | - |
| 1 MAT | - | - | + | +++ | +++ | + | + | - | - | - | + | - | + |
| 2 MAT | + | + | + | ++++ | ++++ | + | + | + | + | + | - | + | + |
| 3 MAT | + | + | ++ | ++++ | ++++ | + | + | + | + | + | + | + | + |

Mp: *Macrophomina phaseolina*; Rhz: *Rhizoctonia* spp., Rhi: *Rhizopus* spp.; Af: *Aspergillus flavus*; An: *Aspergillus niger*, Ao: *Aspergillus ochraceus*; Au: *Aspergillus ustus*; Alt: *Alternaria* spp. Fus: *Fusarium* spp.; Pen: *Penicillium* spp.; Tri: *Trichoderma* spp.; Cha: *Chaetomium* spp.; Cla: *Cladosporium* spp.; IAT: Immediately after treatment; DAT: Day (s) after treatment; WAT: Week(s) after treatment; MAT: Month(s) after treatment; -: Absence of seed mycoflora; +: <15% Frequency, ++: 15–30%; +++: 30–45%; ++++: >45%

was found with the increase in storage period. Efficacy of carboxin in inhibiting seed mycoflora was also reported by Nghiep and Gaur (2005) in rice.



*Alternaria* spp.



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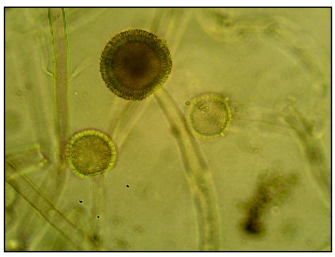

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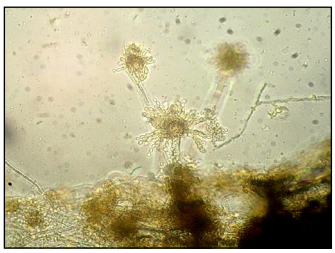

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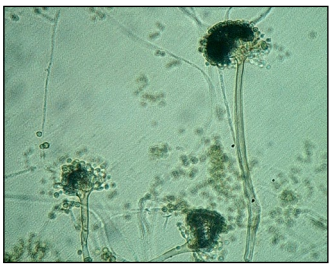
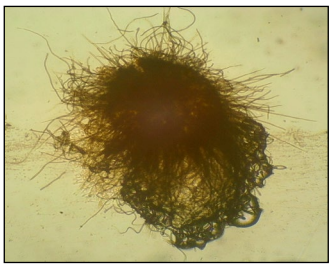
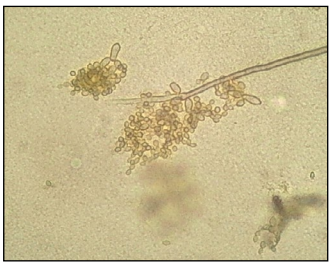


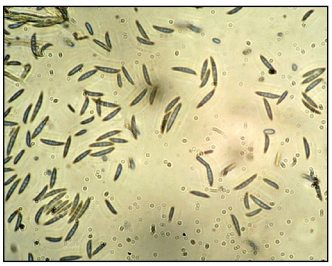
*Aspergillus ustus*



*Chaetomium spp.*

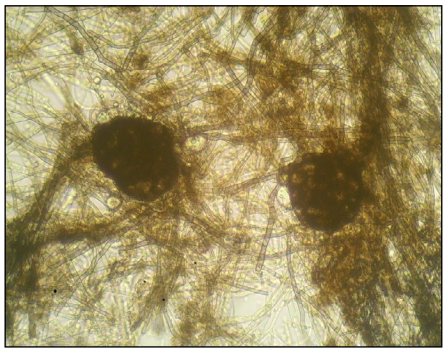


*Cladosporium* spp.

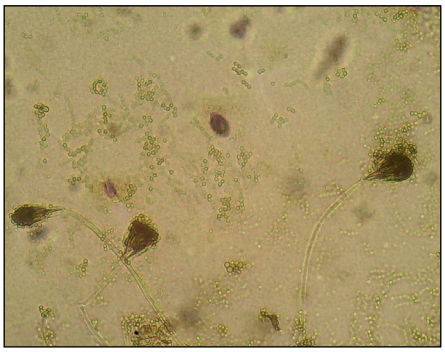


*Fusarium* spp.

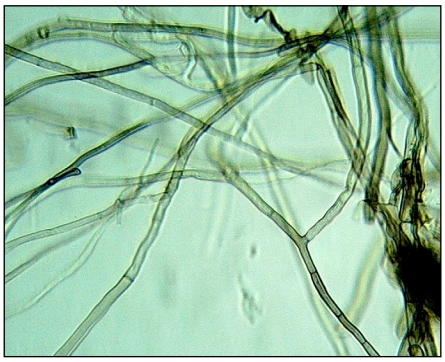
Plate 2: Continue...



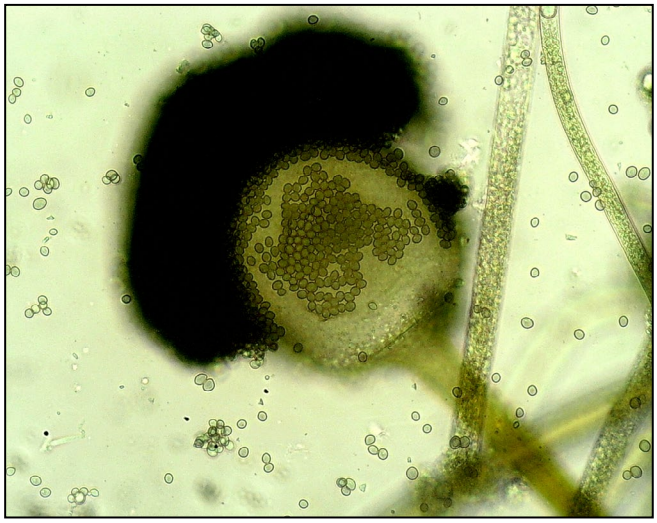
*Macrophomina phaseolina*



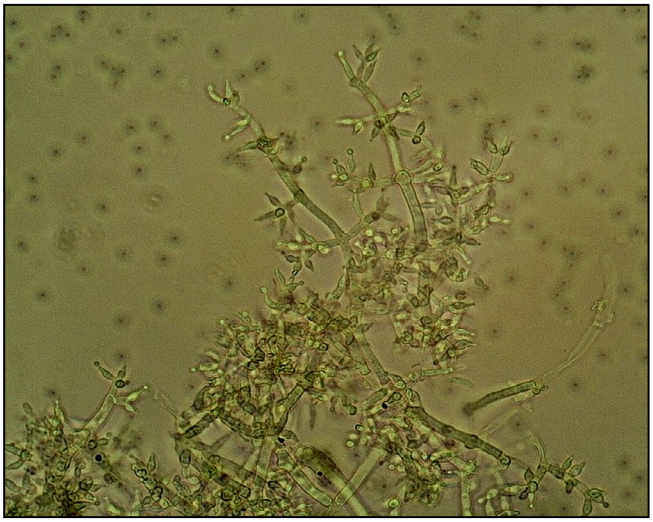
*Penicillium* spp.



*Rhizoctonia* spp.



*Rhizopus* spp.



*Trichoderma* spp.

Plate 2: Photomicrographs of seed mycoflora observed on groundnut

## 4. CONCLUSION

Among the different fungicides tested, seed treatment with carboxin+thiram (21.19%) was found significantly superior in reducing the percent seed infection by different seed mycoflora at different storage periods tested. A total of 13 seedborne fungi belonging to 10 genera were recovered from untreated and treated seeds. The percent seed infection by different seed mycoflora increased with the increase in storage period. However, there was a gradual decline in field mycoflora and gradual increase in storage mycoflora found with the increase in storage period.

## 5. FURTHER RESEARCH

Though vitavax power (carboxin+thiram) is not recommended in groundnut crop, based on the *in vitro* studies, its effectiveness in controlling seed borne diseases can be tested in field conditions. Since the chemical proved to be best in reducing seed borne mycoflora in groundnut, it can be tested in other crops also.

## 6. ACKNOWLEDGEMENT

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