Study of mycoflora associated with seeds obtained from farmers as compared to certified seeds

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Abstract
In India, soybean (Glycine max (L.) Merrill) is a significant legume widely accepted due to high protein and oil content and on account of nutraceutical and pharmaceutical values. The investigation was under taken to determine the seed health status of farmers own saved seeds and certified seeds of soybean, associated mycoflora, its impact on seed sowing quality and management of soybean. During the period 150 seed samples obtained from 09 districts spread over 4 agroclimatic regions were analyzed for the associated mycoflora through Standard blotter method and visual observations on Diaphanoscope.

Keywords: mycoflora, Macrophomina, Colletotrichum, Fusarium, Aspergillus, Cercospora, Soybean

Introduction
Soybean (Glycine max (L.) Merrill) a native of eastern Asia is a significant legume widely accepted due to high protein and oil content and on account of nutraceutical and pharmaceutical values. Soybean seeds contain protein (43%), oil (21%) and are good source of lysine (6.4%) which is a limiting factor in cereals. The contribution of India in the world soybean area and production is about 7.9 and 2.3%, respectively. In India during 2013-2014 the area under soybean was 108.834 lakh hectares with the production of 104.366 lakh tones and productivity being 959 kg/ha. (Anonymous, 2014) [6].

Material and Methods
The status of mycoflora associated with soybean seeds obtained from farmers of various agroclimatic zones of Madhya Pradesh was determined. The seed belonged to farmers and...
certified category. The impact of associated mycoflora on sowing seed quality was investigated. The effectiveness of chemical fungicides and biocides against seed borne mycoflora was investigated in the present study. Material used and methods followed are described herewith.

**General**

**Cleaning and sterilization of apparatus**
The glassware used during the course of investigation was of Corning and Borosil make. Prior to use, each glassware was cleaned with chromic acid solution.

**Preparation of chromic acid solution**
- Sulphuric acid: 300 ml
- Potassium dichromate: 80 g
- Distilled water: 400 ml

The glassware was cleaned with acid solution followed by thorough washing with detergent powder and finally rinsed with normal tap water and/or distilled water as per need. The air dried glassware was sterilized in an autoclave at 1.05 kg/cm² (15 lb per square inch) for 15 minutes. Whereas, sand and field soil at 1.05 kg/cm² for 180 minutes. Plastic trays were disinfested with 0.1% Copper sulphate solution and later washed by sterile water.

The inoculation needle, forceps and biological needle were surface disinfested by dipping in alcohol and there after heating over a flame. The inner surface of growth chamber and clean air flow system was disinfested by using exposing the Ultra violet rays through UV lamps and spray of formaldehyde solution. Prior to use, safety precaution were adopted while using ultra violet lamp and formaldehyde solution.

**Media**
The ingredients of media used during the course of investigation are as follow:

- **Potato sucrose agar (PSA)**
  - Peeled and sliced healthy potato: 200 g
  - Sucrose: 20 g
  - Agar-agar: 20 g
  - Distilled water: 1000 ml

**Incubation chamber**
The seeded petriplates were incubated under two set of Philips 40 Watt day tube light placed horizontally at the height of 40 cm. Alternate cycles of 12hr light and 12hr dark period were maintained.

**Collection of seed sample**
Seed samples of soybean variety JS 335 were obtained from farmers of 09 districts covering 4 agroclimatic zones of Madhya Pradesh. Certified seed sample from respective location were also procured and used for comparison. From each district 5 samples of certified seed and 5 samples of farmers own saved seed were obtained through Seed Technology Research Centre, JNKVV, Jabalpur. In all 90 seed samples were obtained. The samples were numbered and stored in paper envelops under low temperature condition to avoid further deterioration. The seeds were tested by dry seed examination and incubation techniques. Collection of farmers own saved and certified seed sample from seven agroclimatic zones covering 09 districts

| Agroclimatic zone        | District                  |
|--------------------------|---------------------------|
| Kymore plateau and Saipura Hills | Jabalpur, Katni, Seoni    |
| Satpura plateau          | Chhindwara, Betul         |
| Nimar valley             | Khandwa, Khargaon         |
| Central Narmada valley   | Hoshangabad, Narasinghpur |

**Dry seed examination**
The certified seed samples and samples of farmers own saved seeds were examined on diaphanoscope. The diseased seed samples were sorted and identified on the basis of symptoms.

**Standard Blotter method**
Seed collected from different sources were tested by standard blotter method (ISTA, 1996) [21] for the associated mycoflora. Three circular blotter papers of the size of petridishes (90mm) were cut and dipped in sterilized water. Excess of water was removed and placed in each sterilized petridish. In each petridish, 10 soybean seeds were placed with the help of pre-sterilized forceps (eight in outer circle and two in the centre). The seeded petridishes were incubated in the growth chamber. Seed were pretreated with 0.1% NaOCl for 20 seconds. Petridish were examined on fifth day of incubation. Mycoflora were identified on the basis of colony and habit character, subsequently confirming by making slides of fungal structure, fruiting bodies and spores.

**Standard ragdoll method**
Standard Ragdoll method (ISTA, 1996) [21] was used for the testing and effect of the associated mycoflora on germination of soybean seed. In the method, four hundred seed of each category and sample were used. The towel (blotters) papers were moistened with sterilized water. Excess of water was removed, the paper were stretched over the flat surface and kept over clean surface of the working table. Fifty seed were arranged on the half portion of the towel paper. Seed were covered with the other half portion of the paper and rolled over. A wax paper was wrapped on the rolled paper towel and both ends were tightened with rubber bands. It prevented the run-off of water and helped in maintenance of the moisture required for seed germination process. The rolled towel papers were kept in a slanting position in a plastic tray. The seeded towel were placed in a seed germinator at 25 °C with RH around 85%. The seedlings were examined on the 10th day of incubation and germination per cent was calculated.

**Identification of mycoflora**
The mycoflora developing on incubated seeds were identified on the basis of nature of colony characteristics, fruiting bodies and habit character directly under stereoscopic binocular microscope.

**Detection of mycoflora associated with soybean seeds**

| Agroclimatic zone | District                  |
|-------------------|---------------------------|
| Kymore plateau and Saipura Hills | Jabalpur, Katni, Seoni    |
| Satpura plateau   | Chhindwara, Betul         |
| Nimar valley      | Khandwa, Khargaon         |
| Central Narmada   | Hoshangabad, Narasinghpur |

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microscope and subsequent preparation of microslides. Help of fungal identification key developed by Kulshreshtha et al. (1976), Ahmed and Reddy (1993) [3], Ram Nath et al. (1970) [25], Barnet (1965) [10], Tiffany and Gilman (1954) [35] and Booth (1971) [14], were used for the purpose.

Impact of seed associated mycoflora

On seed germination

The effect of seed associated mycoflora was determined by Standard Ragdoll method (between the blotters), Standard Blotter method (top of the blotters).

On seed emergence

In the method counted soybean seeds were sown in a plastic tray filled with sterilized soil and sand kept under laboratory condition. Seed emergence was recorded after 5 and 10 day incubation. The trays were irrigated with sterile water whenever required. Light was provided by horizontally hanging day tube light. Seed emergence was also determined under field conditions.

On seedling vigour index

Seedling vigour index was calculated based on the seedling length as per the formula recommended by Abdul-Baki and Anderson (1973) [1].

Seedling vigour index = (Mean root length + mean shoot length) × percent seed germination

To determine seedling vigour index, 10 seedling of each sample grown between towel papers were used. Shoot length was measured from the collar region to the point of attachment of cotyledons. Root length was measured from the collar region to the tip of main root.

On viability

The effect of seed mycoflora on seed viability was determined by tetrazolium salt test.

Reagent: 1% tetrazolium solution (2, 3, 5- triphenyl tetrazolium chloride)

Procedure: The seed of all the categories were completely immersed in distilled water for 18 hr to initiate activity of dehydrogenase enzyme and to facilitate penetration of tetrazolium solution. The testa (seed coat) of the seed was removed with the help of forceps and then remaining part of the seed immersed in 1% tetrazolium solution for 3 hr at 20 ℃ in complete dark. Then the seeds were rinsed with water and examined. Each seed was evaluated as viable or dead on the basis of staining pattern and intensity of the red colour.

On seed coat cracking

Influence of mycoflora on seed coat cracking was recorded by Ferric-Chloride (FeCl₃) test.

Chemical used: FeCl₃ solution 20%

Procedure: In the test, the selected seed samples of all the categories were soaked in 20% Ferric chloride solution placed in a beaker. After 15 minutes observation on the development of black stain on the seed surface was investigated. Observations were recorded by naked eye as well the help of hand lense (10x). Qualitative observation was taken.

Management of seed associated mycoflora

The seeds of soybean pre tested variety JS 335 having maximum natural infection of target pathogens was used. The seeds were treated with individual fungicides and observations were recorded on the associated mycoflora adopting standard blotter method (ISTA, 1996) [21], Standard Ragdoll method (ISTA, 1996) [21].

Standard blotter method

Fungicide treated seeds were used. Untreated seeds served as control. In the method, 3 circular blotter papers of the size of the petridish were cut and dipped in sterilized water. Excess water was removed and soaked sheets were placed in each petridish. Twenty five soybean seeds were placed in each petridish with the help of sterilized forceps under aseptic conditions of inoculation chamber. In the petriplate 16 seeds were placed in outer circle, 8 in the inner circle and 1 in the center so as to allow in the equal distance between the seeds. Seeded plates were kept for the incubation in the chamber. Fungi were identified by making slides and observing under microscope on eight day of incubation with the help of identification manuals.

Dry seed treatment with fungicide and biopesticide

Each category of soybean seeds was taken in polythene bag and required quantity of fungicide and biopesticide was sprinkled over the seeds. The fungicides and biopesticides seeds were gently shake so as to get uniform coating on individual seed. The treated seeds were sown in sterile soil and sand media at equal distance in a plastic tray. The effect of fungicides and biopesticides on seed germination, seed emergence and seedling vigour was recorded by sowing the treated seeds in sterile soil and sand in a plastic tray. Observation was recorded on 6 and 9 days after sowing.

Table: Fungicides and biopesticides used for seed treatment

| Fungicide                          | % Conc. | Doses/kg seeds | Applications       |
|-----------------------------------|---------|----------------|--------------------|
| Thiram + Carbendazim              | 0.25    | 2.5 g          | Seed treatment     |
| Thiram + Carboxin                 | 0.20    | 2.0 g          | Seed treatment     |
| Copper oxychloride                | 0.25    | 2.5 g          | Seed treatment     |
| Carboxin                          | 0.20    | 2.0 g          | Seed treatment     |
| Carbendazim                       | 0.20    | 2.0 g          | Seed treatment     |
| Tebuconazole                      | 0.20    | 2.0 g          | Seed treatment     |
| Thiride                           | 0.25    | 2.5 g          | Seed treatment     |
| Trichoderma viride                | 0.60    | 6.0 g          | Seed treatment     |
| Trichoderma harzianum             | 0.60    | 6.0 g          | Seed treatment     |
| Pseudomonas Fluorescence          | 0.60    | 6.0 g          | Seed treatment     |
| Untreated control                 |         |                | -                  |

Result and Discussion

In the present investigation seed health status of farmers own saved seed was determined and compared with certified seed category. The impact of associated mycoflora on sowing seed quality was investigated and the observations on the effectiveness of fungicides and biopesticides were recorded and results are presented herewith.

Collection of seed sample

In all 90 seed samples of soybean variety JS 335 were obtained from farmers of 09 districts covering 04 agroclimatic zones of Madhya Pradesh (Table 1). The seed sample were numbered and stored in paper envelopes under low temperature condition. The seed samples were subjected for seed health by different standard technique (ISTA, 1996) [21].

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Table 1: Sources of soybean seed sample of variety JS 335 from different agroclimatic zones

| Agro climatic Zone | District   | Farmer seed | Certified seed | Total |
|--------------------|------------|-------------|---------------|-------|
| Kymore plateau & Satpura Hills | Jabalpur 05 | 05 | 10 |
|                    | Katni 05   | 05 | 10 |
|                    | Seoni 05  | 05 | 10 |
| Satpura plateau    | Chhindwara 05 | 05 | 10 |
|                    | Betul 05  | 05 | 10 |
| Nimar valley       | Khandwa 05 | 05 | 10 |
|                    | Khargoan 05 | 05 | 10 |
| Central Narmada valley | Hoshangabad 05 | 05 | 10 |
|                    | Narasinghpur 05 | 05 | 10 |

Table 2: Association of mycoflora with soybean seeds obtained from farmers of Kymore Plateau & Satpura Hills and certified seed samples as detected by Standard blotter method (ISTA, 1996) [21]

| Agro climatic Zone / District / Sample | Mycoflora                      | Farmer seed | Certified seed |
|---------------------------------------|--------------------------------|-------------|---------------|
|                                       |                                | Percent     | Percent       | Percent     | Percent       |
|                                       |                                | association | seed germination | association | seed germination |
| Kymore Plateau & Satpura Hills        |                                |             |               |             |               |
| Sample 01                             | Macrophomina phaseolina        | 2.0         | 77.0          | 3.0         | 81.0          |
|                                       | Colletotrichum dematium        | 5.0         | 4.0           |             |               |
|                                       | Fusarium oxysporum             | 11.0        | 7.0           |             |               |
|                                       | Aspergillus niger              | 7.0         | 6.0           |             |               |
|                                       | Aspergillus flavus             | 7.0         | 3.0           |             |               |
| Sample 02                             | Macrophomina phaseolina        | 1.0         | 75.0          | 0.0         | 81.0          |
|                                       | Colletotrichum dematium        | 3.0         | 0.0           |             |               |
|                                       | Fusarium oxysporum             | 10.0        | 5.0           |             |               |
|                                       | Aspergillus niger              | 6.0         | 2.0           |             |               |
|                                       | Aspergillus flavus             | 9.0         | 1.0           |             |               |
| Sample 03                             | Macrophomina phaseolina        | 2.0         | 70.0          | 0.0         | 80.0          |
|                                       | Colletotrichum dematium        | 5.0         | 3.0           |             |               |
|                                       | Fusarium oxysporum             | 9.0         | 4.0           |             |               |
|                                       | Aspergillus niger              | 7.0         | 6.0           |             |               |
|                                       | Aspergillus flavus             | 0.0         | 0.0           |             |               |
| Sample 04                             | Macrophomina phaseolina        | 2.0         | 72.0          | 0.0         | 73.0          |
|                                       | Colletotrichum dematium        | 5.0         | 1.0           |             |               |
|                                       | Fusarium oxysporum             | 8.0         | 0.0           |             |               |
|                                       | Aspergillus niger              | 7.0         | 0.0           |             |               |
|                                       | Aspergillus flavus             | 6.0         | 2.0           |             |               |
| Sample 05                             | Macrophomina phaseolina        | 5.0         | 75.0          | 2.0         | 75.0          |
|                                       | Colletotrichum dematium        | 5.0         | 1.0           |             |               |

Detection of mycoflora by Standard Blotter method

Association of mycoflora with soybean seeds obtained from farmer and certified seed samples was detected by standard blotter method (ISTA, 1996) [21]. Results of association of mycoflora were detected from the seed samples belonging to 7 agroclimatic zone and 15 district of Madhya Pradesh are presented.

Kymore plateau & Satpura Hills

In all, 15 seed samples from 3 district (Jabalpur, Katni and Seoni) were analysed. Data presented in Table 02 indicate the association of 5 major mycoflora in variable proportions. Maximum association of Macrophomina phaseolina (10.0%) was recorded from the seed samples from Seoni. Seed samples from Katni had shown maximum association of Colletotrichum dematium (6.0%). Association of Fusarium oxysporum was maximum (14.0%) in the seed sample from Seoni and Jabalpur in the farmer seed samples. It was noticed that association of mycoflora was lesser in certified seed samples as compared to farmer seeds (10.0%). Incidence of Macrophomina phaseolina was only 4.0% in certified seed. While Colletotrichum dematium (4.0%) was recorded as compared to in certified seed. Association of Fusarium oxysporum was (10.0%) while Aspergillus flavus was 5.0% in certified seed. The germination percent ranged up to 77.0% in farmer seed as compared to 81.0% in certified seed (Table 02).
|                | Macrophomina phaseolina | Colletotrichum dematium | Fusarium oxysporum | Aspergillus niger | Aspergillus flavus |
|----------------|-------------------------|-------------------------|--------------------|------------------|-------------------|
| **Katni**      |                         |                         |                    |                  |                   |
| Sample 01      | 5.0                     | 70.0                    | 0.0                | 68.0             |                   |
|                |                         |                         |                    |                  |                   |
| Sample 02      | 3.0                     | 7.0                     | 2.0                | 68.0             |                   |
|                |                         |                         |                    |                  |                   |
| Sample 03      | 2.0                     | 71.0                    | 2.0                | 65.0             |                   |
|                |                         |                         |                    |                  |                   |
| Sample 04      | 4.0                     | 71.0                    | 2.0                | 75.0             |                   |
|                |                         |                         |                    |                  |                   |
| Sample 05      | 5.0                     | 73.0                    | 1.0                | 63.0             |                   |
|                |                         |                         |                    |                  |                   |
| **Seoni**      |                         |                         |                    |                  |                   |
| Sample 01      | 7.0                     | 75.0                    | 3.0                | 70.0             |                   |
|                |                         |                         |                    |                  |                   |
| Sample 02      | 6.0                     | 71.0                    | 2.0                | 71.0             |                   |
|                |                         |                         |                    |                  |                   |
| Sample 03      | 6.0                     | 76.0                    | 0.0                | 70.0             |                   |
|                |                         |                         |                    |                  |                   |
| Sample 04      | 8.0                     | 72.0                    | 2.0                | 72.0             |                   |
|                |                         |                         |                    |                  |                   |
| Sample 05      | 10.0                    | 76.0                    | 0.0                | 71.0             |                   |
| Agro climatic Zone / District / Sample | Mycoflora                  | Farmer seed | Certified seed |
|-------------------------------------|---------------------------|-------------|---------------|
|                                     | Percent association       | % seed germination | Percent association | % seed germination |
|                                     |                           |              |               |
| Central Narmada Valley              |                           |              |               |
| Hoshangabad                         | Macrophomina phaseolina    | 7.0          | 73.0          | 3.0          | 76.0          |
|                                    | Colletotrichum dematium   | 3.0          | 2.0           |              |               |
|                                     | Fusarium oxysporum        | 5.0          | 4.0           |              |               |
|                                     | Aspergillus niger         | 7.0          | 3.0           |              |               |
|                                     | Aspergillus flavus        | 3.0          | 0.0           |              |               |
| Sample 01                           | Macrophomina phaseolina    | 6.0          | 74.0          | 6.0          | 72.0          |
|                                    | Colletotrichum dematium   | 2.0          | 2.0           |              |               |
|                                     | Fusarium oxysporum        | 11.0         | 10.0          |              |               |
|                                     | Aspergillus niger         | 4.0          | 2.0           |              |               |
|                                     | Aspergillus flavus        | 2.0          | 0.0           |              |               |
| Sample 02                           | Macrophomina phaseolina    | 11.0         | 73.0          | 5.0          | 75.0          |
|                                    | Colletotrichum dematium   | 0.0          | 0.0           |              |               |
|                                     | Fusarium oxysporum        | 10.0         | 6.0           |              |               |
|                                     | Aspergillus niger         | 5.0          | 2.0           |              |               |
|                                     | Aspergillus flavus        | 5.0          | 0.0           |              |               |
| Sample 03                           | Macrophomina phaseolina    | 12.0         | 75.0          | 6.0          | 73.0          |
|                                    | Colletotrichum dematium   | 0.0          | 4.0           |              |               |
|                                     | Fusarium oxysporum        | 10.0         | 2.0           |              |               |
|                                     | Aspergillus niger         | 5.0          | 2.0           |              |               |
|                                     | Aspergillus flavus        | 0.0          | 0.0           |              |               |
| Sample 04                           | Macrophomina phaseolina    | 12.0         | 71.0          | 5.0          | 74.0          |
|                                    | Colletotrichum dematium   | 5.0          | 3.0           |              |               |
|                                     | Fusarium oxysporum        | 9.0          | 2.0           |              |               |
|                                     | Aspergillus niger         | 5.0          | 0.0           |              |               |
|                                     | Aspergillus flavus        | 5.0          | 0.0           |              |               |
| Narasinghpur                        | Macrophomina phaseolina    | 5.0          | 71.0          | 2.0          | 72.0          |
| Sample 01                           | Colletotrichum dematium   | 3.0          | 1.0           |              |               |
|                                    | Fusarium oxysporum        | 8.0          | 2.0           |              |               |
|                                    | Aspergillus niger         | 3.0          | 3.0           |              |               |
|                                    | Aspergillus flavus        | 2.0          | 0.0           |              |               |
| Sample 02                           | Macrophomina phaseolina    | 4.0          | 70.0          | 4.0          | 75.0          |

Central Narmada Valley

In Central Narmada Valley, seed sample from Hoshangabad and Narasinghpur district were analysed. Association of Macrophomina phaseolina was 12.0% in farmer seed as compared to 6.0% in certified seed whereas Colletotrichum dematium 5.0% in farmer seed and 4.0% in certified seed. In certified seed association of Fusarium oxysporum was 10.0%, Aspergillus niger 5.0%, Aspergillus flavus 3.0% as compared to 11.0%, 7.0%, 5.0% in farmer seed, respectively (Table 03).

Maximum association of Macrophomina phaseolina (12.0%), 11.0% Fusarium oxysporum was recorded in the farmer seed samples from Hoshangabad district. The seed germination ranged up to 75.0% in farmer seed sample while it was 78.0% in certified seed. In certified seed samples the seed germination was higher and association was lesser as compared to farmer seed (Table 03).
Soybean seed samples obtained from Chhindwara and Betul districts were analysed for seed health. Association of *Macrophomina phaseolina* was maximum (15.0%) in the farmer seed sample from Chhindwara district. *Fusarium oxysporum* (13.0%) was also higher in the farmers seed of Chhindwara. Association of *Aspergillus flavus* was higher in certified seed as compared to farmer seed indicating the inappropriate threshing and harvesting techniques adopted. The germination percent of farmer seed samples ranged from 63.0 to 75.0% as compared 65.0 to 73.0% in the certified seed (Table 04).

Table 4: Association of mycoflora with soybean seeds obtained from farmers of Satpura Plateau and certified seed samples as detected by Standard blotter method (ISTA, 1996) [21]

| Agro climatic Zone / District / Sample | Mycoflora       | Farmer seed | Certified seed |
|--------------------------------------|-----------------|-------------|----------------|
|                                      | Percent association | % seed germination | Percent association | % seed germination |
| Satpura Plateau                      |                 |             |                 |
| Chhindwara                           |                 |             |                 |
| Sample 01                            | *Macrophomina phaseolina* | 13.0 | 75 | 10.0 | 69.0 |
|                                      | *Colletotrichum dematium* | 5.0 | 4.0 |         |     |
|                                      | *Fusarium oxysporum* | 11.0 | 6.0 |         |     |
|                                      | *Aspergillus niger* | 2.0 | 0.0 |         |     |
|                                      | *Aspergillus flavus* | 0.0 | 0.0 |         |     |
| Sample 02                            | *Macrophomina phaseolina* | 7.0 | 72 | 0.0 | 68.0 |
|                                      | *Colletotrichum dematium* | 5.0 | 2.0 |         |     |
|                                      | *Fusarium oxysporum* | 10.0 | 5.0 |         |     |
|                                      | *Aspergillus niger* | 2.0 | 1.0 |         |     |
|                                      | *Aspergillus flavus* | 4.0 | 3.0 |         |     |
| Sample 03                            | *Macrophomina phaseolina* | 4.0 | 73 | 4.0 | 65.0 |
|                                      | *Colletotrichum dematium* | 5.0 | 5.0 |         |     |
|                                      | *Fusarium oxysporum* | 11.0 | 10.0 |         |     |
|                                      | *Aspergillus niger* | 2.0 | 2.0 |         |     |
|                                      | *Aspergillus flavus* | 0.0 | 0.0 |         |     |
| Sample 04                            | *Macrophomina phaseolina* | 15.0 | 70 | 7.0 | 67.0 |
|                                      | *Colletotrichum dematium* | 3.0 | 2.0 |         |     |
|                                      | *Fusarium oxysporum* | 12.0 | 11.0 |         |     |
|                                      | *Aspergillus niger* | 0.0 | 2.0 |         |     |
|                                      | *Aspergillus flavus* | 4.0 | 4.0 |         |     |
Sample 05

| Mycoflora         | Farmer seed | Certified seed |
|-------------------|-------------|----------------|
| Macrophomina phaseolina | 15.0        | 3.0            | 70.0 |
| Colletotrichum dematium | 4.0        | 5.0            |
| Fusarium oxysporum    | 13.0        | 6.0            |
| Aspergillus niger     | 0.0         | 0.0            |
| Aspergillus flavus    | 4.0         | 0.0            |

Betul

Sample 01

| Mycoflora         | Farmer seed | Certified seed |
|-------------------|-------------|----------------|
| Macrophomina phaseolina | 7.0        | 4.0            | 67.0 |
| Colletotrichum dematium | 4.0        | 4.0            |
| Fusarium oxysporum    | 11.0        | 2.0            |
| Aspergillus niger     | 2.0         | 0.0            |
| Aspergillus flavus    | 4.0         | 0.0            |

Sample 02

| Mycoflora         | Farmer seed | Certified seed |
|-------------------|-------------|----------------|
| Macrophomina phaseolina | 9.0        | 6.0            | 65.0 |
| Colletotrichum dematium | 7.0        | 3.0            |
| Fusarium oxysporum    | 9.0         | 3.0            |
| Aspergillus niger     | 3.0         | 4.0            |
| Aspergillus flavus    | 6.0         | 0.0            |

Sample 03

| Mycoflora         | Farmer seed | Certified seed |
|-------------------|-------------|----------------|
| Macrophomina phaseolina | 3.0        | 5.0            | 63.0 |
| Colletotrichum dematium | 2.0        | 2.0            |
| Fusarium oxysporum    | 9.0         | 4.0            |
| Aspergillus niger     | 3.0         | 1.0            |
| Aspergillus flavus    | 5.0         | 4.0            |

Sample 04

| Mycoflora         | Farmer seed | Certified seed |
|-------------------|-------------|----------------|
| Macrophomina phaseolina | 4.0        | 2.0            | 70.0 |
| Colletotrichum dematium | 3.0        | 0.0            |
| Fusarium oxysporum    | 6.0         | 0.0            |
| Aspergillus niger     | 2.0         | 0.0            |
| Aspergillus flavus    | 0.0         | 4.0            |

Sample 05

| Mycoflora         | Farmer seed | Certified seed |
|-------------------|-------------|----------------|
| Macrophomina phaseolina | 6.0        | 5.0            | 69.0 |
| Colletotrichum dematium | 3.0        | 0.0            |
| Fusarium oxysporum    | 2.0         | 0.0            |
| Aspergillus niger     | 2.0         | 0.0            |
| Aspergillus flavus    | 11.0        | 10.0           |

Maximum association

| Mycoflora         | Farmer seed | Certified seed |
|-------------------|-------------|----------------|
| Macrophomina phaseolina | 15.0       | 10.0           | 63.0-75.0 |
| Colletotrichum dematium | 7.0        | 5.0            |
| Fusarium oxysporum    | 13.0        | 11.0           |
| Aspergillus niger     | 3.0         | 4.0            |
| Aspergillus flavus    | 11.0        | 10.0           |

Nimar Valley

Data presented in Table 05 indicate that seed sample obtained from Khandwa and Khargone districts were analysed with maximum association of *Macrophomina phaseolina* (12.0%) from the farmer of Kharagavan district as compared to 7.0% in certified seed. Association of *Fusarium oxysporum* was 11.0%, *Colletotrichum dematium* 7.0%, *Aspergillus niger* 12.0% and *Aspergillus flavus* 10.0% in farmer seed sample as compared to 10.0, 6.0 and 7.0% in certified seed respectively (Table 05).

Table 5: Association of mycoflora with soybean seeds obtained from farmers of Nimar Valley and certified seed samples as detected by Standard blotter method (ISTA, 1996) [21]

| Agro climatic Zone / District / Sample | Mycoflora               | Farmer seed | Certified seed |
|---------------------------------------|-------------------------|-------------|----------------|
|                                       | Percent association     | Percent seed germination | Percent association | Percent seed germination |
|                                       | Nimar Valley            | Khandwa     | Sample 01      | Sample 02      |
|                                       |                         | Sample 01   | Sample 02      | Sample 05      |
|                                       | Macrophomina phaseolina | 8.0         | 73.0           | 3.0           | 75.0           |
|                                       | Colletotrichum dematium | 3.0         | 2.0            | 7.0           |
|                                       | Fusarium oxysporum      | 11.0        | 7.0            | 10.0           |
|                                       | Aspergillus niger       | 12.0        | 10.0           | 5.0            |
|                                       | Aspergillus flavus      | 10.0        | 5.0            | 2.0            |
| Sample 03 | Macrophomina phaseolina  | 6.0 | 75.0 | 3.0 | 72.0 |
| Sample 04 | Macrophomina phaseolina  | 3.0 | 76.0 | 2.0 | 72.0 |
| Sample 05 | Macrophomina phaseolina  | 5.0 | 77.0 | 0.0 | 77.0 |
| Sample 03 | Colletotrichum dematium  | 6.0 | 7.0 | 3.0 | |
| Sample 04 | Colletotrichum dematium  | 6.0 | 3.0 | 2.0 | |
| Sample 05 | Colletotrichum dematium  | 6.0 | 2.0 | 0.0 | |
| Sample 03 | Fusarium oxysporum  | 10.0 | 7.0 | |
| Sample 04 | Fusarium oxysporum  | 10.0 | 3.0 | |
| Sample 05 | Fusarium oxysporum  | 9.0 | 3.0 | |
| Sample 03 | Aspergillus niger  | 10.0 | 7.0 | |
| Sample 04 | Aspergillus niger  | 8.0 | 2.0 | |
| Sample 05 | Aspergillus niger  | 3.0 | 4.0 | |
| Sample 03 | Aspergillus flavus  | 10.0 | 7.0 | |
| Sample 04 | Aspergillus flavus  | 8.0 | 4.0 | |
| Sample 05 | Aspergillus flavus  | 5.0 | 0.0 | |

**Khargone**

| Sample 01 | Macrophomina phaseolina  | 4.0 | 72.0 | 5.0 | 71.0 |
| Sample 02 | Macrophomina phaseolina  | 7.0 | 73.0 | 7.0 | 72.0 |
| Sample 03 | Macrophomina phaseolina  | 12.0 | 75.0 | 5.0 | 72.0 |
| Sample 01 | Colletotrichum dematium  | 5.0 | 4.0 | |
| Sample 02 | Colletotrichum dematium  | 3.0 | 3.0 | |
| Sample 03 | Colletotrichum dematium  | 3.0 | 6.0 | |
| Sample 01 | Fusarium oxysporum  | 9.0 | 3.0 | |
| Sample 02 | Fusarium oxysporum  | 7.0 | 3.0 | |
| Sample 03 | Fusarium oxysporum  | 9.0 | 3.0 | |
| Sample 01 | Aspergillus niger  | 2.0 | 0.0 | |
| Sample 02 | Aspergillus niger  | 0.0 | 2.0 | |
| Sample 03 | Aspergillus niger  | 0.0 | 2.0 | |
| Sample 01 | Aspergillus flavus  | 0.0 | 0.0 | |
| Sample 02 | Aspergillus flavus  | 0.0 | 0.0 | |
| Sample 03 | Aspergillus flavus  | 0.0 | 0.0 | |

**Maximum association**

| Macrophomina phaseolina  | 12.0 | 72.0-78.0 | 7.0 | 71.0-77.0 |
| Colletotrichum dematium  | 7.0 | 6.0 | |
| Fusarium oxysporum  | 11.0 | 10.0 | |
| Aspergillus niger  | 12.0 | 10.0 | |
| Aspergillus flavus  | 10.0 | 7.0 | |
Seed samples obtained from 04 agroclimatic conditions were tested for the associated mycoflora

The results of association of mycoflora with soybean seed samples are summarized in Table 06. Among 4 agroclimatic zone, incidence of Macrophomina phaseolina was maximum up to 15.0% in farmer seed sample obtained from Satpura plateau while in certified seed samples. The incidence of Macrophomina phaseolina was comparatively low (10.0%) obtained from Satpura plateau as compared to farmer seed. In farmer seed samples Fusarium oxysporum was maximum up to 14.0% in seed samples from Kymore plateau and Satpura Hills as compared to the certified seed sample from Satpura plateau (11.0%).

Table 6: Association of mycoflora with soybean seeds obtained from seven agroclimatic zones and certified seed samples as detected by Standard blotter method (ISTA, 1996) [21]

| Agro climatic Zone /District / Sample          | Farmer seed     | Certified seed     |
|-----------------------------------------------|-----------------|-------------------|
|                                               | Per cent association | % seed Germination | Per cent association | % seed germination |
|                                               | Mp  | Cd  | Fo  | Mg  | Cd  | Fo  | Mg  | Cd  | Fo  |
| Kymore plateau & Satpura Hills                | 10.0 | 6.0 | 14.0 | 70.0-77.0 | 4.0 | 4.0 | 10.0 | 63.0-81.0 |
| Satpura plateau                               | 15.0 | 7.0 | 13.0 | 63.0-75.0 | 10.0 | 5.0 | 11.0 | 65.0-73.0 |
| Nimar valley                                  | 12.0 | 7.0 | 11.0 | 72.0-78.0 | 7.0 | 6.0 | 10.0 | 71.0-77.0 |
| Central Narmada valley                        | 12.0 | 5.0 | 11.0 | 70.0-75.0 | 6.0 | 4.0 | 10.0 | 72.0-78.0 |

The present investigation was undertaken to determine the seed health status of farmers own saved seed of soybean and its management. Certified seeds were also collected to determine the difference with farmers seed. Globally about 130 diseases have been reported affecting the soybean crop at various stages of crop growth (Hartman et al., 1999) [19], of which 35 diseases are economically important in India. There are about 13 diseases that are transmitted through seeds. Several pathogen have been observed that are responsible for causing diseases of soybean (Hartman et al., 1999) [18] and many of these are reported to be transmitted through seeds (Kilpatrick, 1952; Wu et al., 1964; Hussain et al., 1989; Poharkar, 1992; Vishwadhar and Sarbhoy, 1987) [23, 38, 19, 29, 31]. Number of the pathogens have been observed on seed (Singh and Thapliyal, 1999; Arya et al., 2004; Begum et al., 2007; Shovan et al., 2008) [23, 8, 11, 32]. Investigations were made to determine the current status of seed mycoflora associated with seed obtained from farmers own saved seed sample as compared to certified seeds. Seed samples of soybean variety JS 335 were procured from the farmers of 09 districts covering 4 agroclimatic zones of Madhya Pradesh. Certified seed sample from respective location were also procured for comparison. From each district 5 samples of certified seed and 5 samples of farmers own saved seed were obtained through Seed Technology Research Centre, JNKVV, Jabalpur.

A number of mycoflora have been observed on soybean seeds. A good deal of recording has been made from Madhya Pradesh, India and global level. Incidence of mycoflora in variable proportions was noticed, however, the incidence of major mycoflora was comparatively less in certified seeds as compared to farmers own saved seeds. Major mycoflora found associated with soybean were Macrophomina phaseolina, Colletotrichum dematium, Fusarium oxysporum, Aspergillus flavus, Aspergillus niger and Cercospora kikuchii. Standard Blotter method was reported to be superior over Standard agar plate method for detection of associated mycoflora by Agarwal et al. (1972), Tripathi and Singh (1991), Vishunawat (2003) [3, 36, 37] also recorded its suitability. The incidence of seed and seeding diseases have been recorded at caused maximum reduction of seed germination (Hypperly et al., 1983) [20]. The seed samples having natural infection of Macrophomina phaseolina were tested for seed germination by top of the paper and between the blotter paper method. In the sample having no infection, Macrophomina phaseolina has resulted in 89 and 95% maximum seed germination in top of the paper and between the blotter paper. Seed having maximum infection of Macrophomina phaseolina had shown 72% germination in top of the paper and 70% in between the blotter paper. The seed samples having no infection of Colletotrichum dematium obtained from Hoshangabad district have exhibited maximum 91% germination in top of the paper method and 95% in between the blotter paper method. Seed sample from Sagar having association of 10.0% Colletotrichum dematium resulted in 96 to 75% germination as compared to 75 to 80% in between the blotter paper method.

Influence of target pathogens on the seed emergence was investigated through sowing of seeds in sterile and unsterile soil and sand. Reduction of 12% in seed emergence was noticed when seeds were sown in unsterile soil and infected with Macrophomina phaseolina. Whereas 7% reduction due to Colletotrichum dematium and 5% due to Fusarium oxysporum was noticed. Mortality was higher in sterile soil, indicating the influence of the particular mycoflora. Seed samples having no infection of Fusarium oxysporum, Macrophomina phaseolina and Colletotrichum dematium resulted in better seed germination as compared to seed sample having variable infection of the selected mycoflora reduction in seed germination was recorded due to seed infection. Pre and post emergence losses have been reported by Chauhan and Gupta (2005) [17]; Anuja et al. (2000) [7]. Results indicate that there has been significant role of seed mycoflora associated with seed of soybean that caused seed and seeding diseases (Hartman et al., 1999) [18], Bhale et al., 2003) [12].

In the present investigation, influence of seed treatment with biopesticide and chemical fungicide was determine in selected seed samples. In seed samples having maximum infection Macrophomina phaseolina (15.0%) elimination was observed in seed treated with Copper oxychloride, Carboxin. The association of Macrophomina phaseolina was 1 to 3 % in fungicide treated seed as compared to untreated seed 15.0%.
Association of *Colletotrichum dematium* was 1 to 3% in fungicide treated seed as compared to 9% in untreated seed. Complete elimination of *Colletotrichum dematium* was recorded in seeds treated with Thiram + Carbendazim, Thiram + Carboxin, Copper oxychloride, Carboxin, Carbendazim, Tebuconazole, Thiride.

In untreated seed association of *Fusarium oxysporum* was 14% while it ranges from 1 to 3% in treated seed. Association of *Fusarium oxysporum* was higher in seed treated with biopesticide as compared to chemical fungicide.

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