



In Vitro Seed Treatment of Fungicides for the Control of Seed Borne Fungi of Soybean Variety Durga

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ABSTRACT

Soybean (Glycine max L.) are associated by several seed borne pathogens. These fungi were detected by the methods as recommended ISTA. Fungi such as Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Alternaria tenuis, Fusarium oxysporum, Penicillium notatum, Sclerotium rolfsii, Mucor mucedo Curvularia lunata Cladosporium herbarum and Drechslera oryzae, and Rhizopus stolonifer were isolated from the Soybean variety Durga. Effect of different fungicides like Thiram, captan and Copper oxychloride on the incidence of seed borne fungi and their effect on seed germination were evaluated. The seed treatment by the fungicides showed that Copper oxychloride increased the germination percentage and reduced seed mycoflora. Thus the seeds are treated with fungicides to eliminate the seed borne mycoflora of Soybean.

KEYWORDS : Soybean seed, Seed borne fungi, fungicides, blotter paper test, Agar- Agar plate method Seed washates

Introduction –

In a world facing problem of malnutrition oil rich crops assume special significance. Obtaining maximum production through all available avenues and protecting adequately what is produced would certainly alleviate the problem. In India, soybean estimated yield is 9.81 mt from 9.21 m ha. In developing countries like India, most of the crops yield is reduced due to pathogenic fungi (Agrios 2005). Soybean is an important host of the *Aspergillus flavus*, *Alternaria tenuis* and *Fusarium oxysporum* which causes Seed rot, Seedling blight, Root rot and wilt diseases. In past, several fungicides have been employed in the control of fungal diseases of crops. Agrochemical like Thiram, captan and Copper oxychloride were used to control a number of diseases caused by fungal pathogens of Soybean. Ibiem et al (2000) and (2006) observed that seed dressing fungicides- Benlate, Apron plus 50 Ds Fernasan- D, Dithane M-45 and Bavistin controlling seed borne fungi of Rice Mane et al. (2010). evaluated various fungicidal Seed Treatment on Seed Mycoflora and Seed Germination During Storage of Sorghum. Even through effective and efficient control of seed borne fungi can be achieved by the use synthetic chemical fungicides, the same cannot be applied to grains for reasons of pesticide toxicity (Harris et al. 2001). It is now realized that chemical fungicides cause serious environmental problems and are toxic to non target organisms. (Anon, 2005). The toxic effect of synthetic chemicals can be overcome, only by persistent search for new and safer pesticides accompanied by wide use of pest control method, which are eco-friendly and effective (Mohana et al. 2011).

Materials and Methods –

Collection of seed samples (Cultivars) -

The method described by Neergaard (1973) has been adopted for the collection of seed samples. Accordingly, three random samples of seeds (half Kg each) were collected from oil mills, market places, Oil Seeds Research Station, Latur and Pulses Research Center, Badnapur. (M. S.)

Detection of Seed Mycoflora -

The seed mycoflora was isolated by using different methods such as Standard blotter paper method, Agar plate method, and Seed washates as recommended by International Seed Testing Association ISTA (1966), De Tempe (1970), Neergaard (1997) and Agrawal (1976).

Agar plate method -

In Northern Ireland, Muskett (1948) first used this method for seed health management. In this method, pre sterilized petriplates were poured with 15 mL of autoclaved Potato Dextrose Agar (PDA). On cooling the medium, ten seeds per plate of the sample to be studied were equidistantly placed aseptically. Pair of sterile white blotter papers of 8.5 cm diameter were soaked in sterile distilled water and were placed in pre-sterilized petriplates of 90 mm diameter. Ten seeds of test sample per petriplate were then placed at equal distance on moist blotter. 400 seeds were used in each experiment. The plates were incubated at 28° ± 2°C under diurnal conditions. On seventh day of incubation, seeds

were first examined under stereoscopic microscope for determining the various fungal growths. The identification and further confirmation of seed borne fungi was made by preparing slides of the fungi.

The frequency of the fungus was calculated by the following formula

$$\frac{\text{No. of seeds containing a particular} \times 100}{\text{Total seeds used}}$$

Relative abundance of the fungi was calculated by the formula

$$\frac{\text{No. of seeds colonies of a fungus on seed} \times 100}{\text{Total no. of colonies of all fungus}}$$

Treatments –

Effect of seed treatment fungicide on seed mycoflora, seed germination and vigour index of Soybean seed was studied in - vitro condition. Different concentration of fungicides made were 0.1 to 0.5% and applied to the seeds of Soybean. The effect on seed mycoflora, seed germination and vigour index was recorded. The vigour index was determined by multiplying the percentage germination with the sum of the root and shoot length. Percent inhibition was recorded by using the formula given below by Vincent (1947).

$$\text{Vigour index} = (\text{Root length in cm} + \text{shoot length in cm}) \times \text{germination} (\%)$$

Result and Discussion -

Seed borne pathogens of Soybean are responsible for causing various fungi and also reducing yield from 20-22% if infected are planted in the field. Seeds play a vital role in the transmission of plant pathogens causing plant diseases. The pathogen may be externally or internally seed – borne or associated with seeds contaminant.

In Agar plate method the fungi isolated identified were *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Alternaria tenuis*, *Fusarium oxysporum*, *Penicillium notatum*, *Sclerotium rolfsii*, *Mucor mucedo Curvularia lunata Cladosporium herbarum* and *Drechslera oryzae*, and *Rhizopus stolonifer*. These 11 fungi were isolated from the untreated seeds of variety (Table 1). Similarly from the PDA and seed washates 8 -11 were isolated and identified. Similarly from the untreated seed of Blotter paper method, Agar plate method and Seed washates method highest frequency 46.00, 50.00 & 40.00 and relative abundance 25.00, 30.00 & 20.00 were recorded from *Aspergillus flavus* and *Aspergillus niger*.

Cultivar Durga shows the response to the Copper oxychloride treatment in relation to its seed mycoflora, seed germination and vigour index. The results are given in table 2. It was found that as concentration of Copper oxychloride increases, there was decrease in seed mycoflora where as increase in seed germination and vigour index. At 0.5% of

Copper oxychloride seed treatment it shows minimum seed mycoflora with 00% as compared with control with 70%. At the same concentration there was increase in seed germination which was 90% as com-

pared with control with 55% . At the concentration vigour index was 800 as compared with control with 150.

Table 1. Seed mycoflora of Soybean (*Glycine max* L.) variety Durga on Blotter, Agar plate and seed washates

| Isolated fungi | Blotter paper | | Agar plate | | Seed washates | |
|------------------------------|---------------|--------------------|------------|--------------------|---------------|--------------------|
| | Frequency | Relative abundance | Frequency | Relative abundance | Frequency | Relative abundance |
| <i>Aspergillus flavus</i> | 46 | 25 | 50 | 30 | 40 | 20 |
| <i>Aspergillus niger</i> | 42 | 21 | 46 | 28 | 35 | 30 |
| <i>Aspergillus fumigatus</i> | 21 | 14 | 25 | 17 | 25 | 15 |
| <i>Fusarium oxysporum</i> , | 15 | 10 | 20 | 12 | 13 | 08 |
| <i>Alternaria tenuis</i> | 09 | 05 | 10 | 07 | 08 | 03 |
| <i>Rhizopus stolanifer</i> | 07 | 4.25 | 09 | 06 | 05 | 02 |
| <i>Penicillium notatum</i> | 06 | 04 | 08 | 05 | 03 | 01 |
| <i>Mucor mucedo</i> | 04 | 03 | 05 | 04 | 05 | 00 |
| <i>Curvularia lunata</i> | 03 | 02 | 04 | 03 | 00 | 00 |
| <i>Cladosporium herbarum</i> | 02 | 01 | 03 | 01 | 00 | 00 |
| <i>Drechslera oryzae</i> , | 01 | 0.5 | 0.2 | 00 | 00 | 00 |
| S.E. ± | 4.68 | 2.40 | 16.40 | 9.83 | 13.84 | 9.70 |
| C. D at 5% | 10.43 | 5.35 | 36.57 | 21.92 | 30.88 | 21.63 |

00 = absence of fungi

Table No. 2: Effect of Copper oxychloride seed treatment on seed mycoflora, seed germination and vigour index of Soybean (Cv. Durga)

| Conc. (%) | Seed mycoflora (%) | Seed germination (%) | Vigour index |
|----------------|--------------------|----------------------|--------------|
| 0.00 (Control) | 70 | 55 | 150 |
| 0.1 | 67 | 65 | 180 |
| 0.15 | 65 | 67 | 198 |
| 0.2 | 50 | 70 | 245 |
| 0.25 | 40 | 78 | 290 |
| 0.3 | 25 | 80 | 300 |
| 0.35 | 15 | 83 | 510 |
| 0.4 | 10 | 85 | 650 |
| 0.45 | 05 | 88 | 700 |
| 0.5 | 00 | 90 | 800 |
| S.E ± | 8.15 | 3.42 | 72.32 |
| C.D. at 5% | 18.41 | 7.72 | 163.44 |

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