



Full Length Article

Seed-Borne Fungi and their Effect on Seed Health of Green Gram

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ABSTRACT

Green gram seeds screened for their seed mycoflora, In all sixteen seed-borne fungi were reported from the test pulse. All fungi affected adversely to seed health of test pulse. Six seed-borne fungi; out of sixteen; were found to be dominant on the test pulse. Effect of six dominant seed-borne fungi on seed health of was studied by germination and seedling emergence methods. The dominant seed-borne fungi affected adversely to seed health; namely, seed germination, seedling emergence of the Green gram.

Key words: Green gram, seed-borne fungi

INTRODUCTION

Green gram (*Vigna radiate* L.) is an annual plant with herbaceous bushy appearance. Green gram is nutritious as it contains 24 g protein/100g seed, carbohydrates 56.7 g/100g of edible part of the seeds, thiamin (0.47mg/100 g seed), and riboflavin (0.27mg/100g seed), iron (7.3mg/100g seed) (Shakuntala Manay and Shadaksharaswamy, 1987). Various scientist studied seed mycoflora of Green gram and other pulses. Sinha (1979) studied seed mycoflora of Green gram and Black gram, and identified fungi belonging to genera *Aspergillus*, *Aschotrichina*, *Botrytis*, *Curvularia*, *Chaetomium*, *Colletotrichum*, *Cercospora*, *Drechslera*, *Fusarium*, *Helminthosporium*, *Nigrospora*, *Penicillium*, *Phoma*, *Pleospora* and *Trichothecium*. Deo Gupta (1980) showed that, *Aspergillus*, *Penicillium*, *Alternaria*, *Chaetomium*, *Curvularia*, *Drechslera*, *Cliocladium*, *Monilia* etc were present on seeds of Gram. Sinha *et al.*, (1980) identified some important seed-borne fungi of pulses which caused reduction in seed germination. Agrawal (1981) studied seed mycoflora of Green gram and found fungi like *Cercosporakikuchii*, *Colletotrichumtruncatum*, *Colletotrichum lindemuthianum*, *Curvularia lunata*, *Helminthosporium tetramera*, *Fusarium equiseti*, *Fusarium moniliforme*, *Fusarium semitectum*, *Myrothecium* and *Phoma*, *Alternaria porri*, *Cephalosporium spp.*, *Macrophomina phaseolina*,

Myrothecium roridum and *Periconia* spp. The predominant fungi were *Aspergillusflavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus luchuensis*, *Alternaria* spp. *Penicillium* spp., *Cladosporium* spp. and *Curvularia* spp. Patil *et al*, (1990) reported eighteen species of fungi belonging 14 genera on Green gram, of which 8 fungi *Alternariaalternata*, *Aspergillusfumigatus*, *A. niger*, *Curvularia geniculation*, *Fusarium moniliforme*, *F. oxysporum*, *Macrophomina phaseolina* and *Rhizoctonia bataticola* were pathogenic. Lal and Singh (1997) studied seed mycoflora of Green gram and 25 fungal species were isolated from seeds of cultivar pant-2 and T44. Murthy *et al.* (2003) studied Cowpea, Horse gram, Black gram and Green gram seeds and found that, *Macrophomina phaseolina*, *Fusarium semitectum*, *Fusarium moniliforme* and *Fusarium solani* were associated with seeds. Brau *et al.* (2007) recorded ten fungi of eight genera viz; *Acremonium strictum*, *Aspergillus flavus*, *A. niger*, *Curvularia lunata*, *Fusarium oxysporum*, *F. semitectum*, *Macrophomina phaseolina*, *Phoma medicaginis*, *Penicillium* spp. and *Rhizopus* spp. Similarly, Ali *et.al* (2010) found similar seed-borne fungi of Green gram. Chilkuri and Giri (2014) reported ten fungi belonging to eight genera on Green gram, among all *Macrophomina phaseolina* was dominant fungi.

MATERIALS AND METHODS

Collection of seed samples

The methods prescribed by Paul Neergaard (1977) have been adopted for the collection of seed samples. Seed samples of Green gram were collected from field, market places from Nanded. A composite seed sample from different sources of the pulse crop was made by mixing the individual seed sample together, preserved in gunny bags at room temperature during the studies.

Detection of seed mycoflora:

The seed-borne fungi of test pulse was detected by moist blotter (B) and agar (A) plate methods as recommended by ISTA (1966), De Tempe (1970), Neergaard (1977) and Agrawal (1981). The procedure of moist blotter (B) and agar (A) plate methods is described as below.

Moist blotter plate method

In moist blotter plate method; a pair of white blotter papers of 8.5 cm diameter was jointly soaked in sterile distilled water and placed in pre-sterilized borosil glass Petri-plates of 10 cm diameter. Ten seeds were placed at equal distance aseptically on the moist blotter paper. The plates were incubated at room temperature for ten days. On eleventh day the seeds were examined under microscope for the preliminary determination of seed mycoflora. The seed-borne fungi found on each and every seed were isolated and identified, brought into pure cultures and maintained on PDA (Potato Dextrose Agar) slants.

Agar plate method:

In agar plate method; 25 ml of sterilized PDA medium of pH 5.6 was poured in pre-sterilized borosil glass Petri-plate of 10 cm diameter. The Petri-plates were allowed to cool at room temperature; then ten seeds of test pulses were placed at equidistance under aseptic condition. The plates were incubated at room for ten days. On eleventh day the seeds were examined under microscope for the preliminary determination of seed mycoflora. The seed-borne fungi found on each and every seed were isolated and identified, brought into pure cultures and maintained on PDA (Potato Dextrose Agar) slants.

Preparation of spore suspension:

Spore suspension of dominant seed-borne fungi of pulse were prepared separately by adding 10 ml of sterile distilled water into the sporulating pure cultures of seed-borne fungi maintained on PDA slants for seven days at room temperature.

The slants were shaken and content filtered through muslin cloth to separate mycelium and spore. The filtrate thus obtained was used as spore suspension.

Seed germination method

In order to evaluate the effect of seed-borne fungi on percent seed germination, shoot and root length, the seeds of the test pulse was infested separately with spore suspension of dominant seed-borne fungi. These seeds were incubated in sterilized moist blotters at room temperature for ten days. After incubation period, percent seed germination, shoot and root length of each seeds of pulse was recorded.

Seedling emergence method

In order to evaluate the effect of seed-borne fungi on percent seedling emergence, shoot and root length, the seeds of the test pulse were infested separately with spore suspension of dominant seed-borne fungi. The seeds were sown in earthen pots (25 cm diameter) filled with sterilized soil. After ten days of sowing, on eleventh day percent seedling emergence, shoot and root length of each seedling was recorded. In order to record root length and shoot length seedlings were uprooted washed in distilled water, placed on filter paper and measurements were taken using meter scale.

RESULTS AND DISCUSSION

Seed mycoflora of Green gram (*Vigna radiata* L.):

The results in table 1 show that, total sixteen fungi were reported from Green gram. The incidence seed mycoflora was more on agar plate compared to moist blotter plate. Considering over all seed-borne fungal mycoflora of the seed, *Aspergillus flavus* showed maximum percent incidence on agar (70%), followed by *A. fumigatus* (61%) *A. niger* (60%), *Rhizopus stolonifer* (60%), *Drechslera tetramera* (55%), *Fusarium moniliforme* (45%) and *Alternaria tenuis* (42%). *Chaetomium globosum*, *Colletotrichum truncatum*, *Aspergillus carbonarius* and *Cladosporium* spp. showed no incidence on blotter and very less incidence on agar plates. Remaining fungi showed minimum incidence both on blotters and agar plates. Out of sixteen seed-borne fungi six were dominant; these are *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Drechslera tetramera*, *Fusarium moniliforme* and *Rhizopus stolonifer*.

Table 1: seed mycoflora of Green gram (*Vigna radiata* L.)

| Sr. No. | Seed mycoflora | Incidence of seed mycoflora (%) | |
|---------|---------------------------------|---------------------------------|------|
| | | Blotter | Agar |
| 1 | <i>Alternaria alternata</i> | 10 | 15 |
| 2 | <i>Alternaria tenuis</i> | 25 | 42 |
| 3 | <i>Aspergillus carbonarius</i> | 00 | 12 |
| 4 | <i>Aspergillus flavus</i> | 67 | 70 |
| 5 | <i>Aspergillus fumigatus</i> | 25 | 61 |
| 6 | <i>Aspergillus nidulans</i> | 28 | 25 |
| 7 | <i>Aspergillus niger</i> | 55 | 60 |
| 8 | <i>Chaetomium globosum</i> | 00 | 03 |
| 9 | <i>Cladosporium spp.</i> | 00 | 20 |
| 10 | <i>Colletotrichum truncatum</i> | 00 | 05 |
| 11 | <i>Curvularia lunata</i> | 12 | 23 |
| 12 | <i>Drechslera tetramera</i> | 25 | 55 |
| 13 | <i>Fusarium moniliforme</i> | 20 | 45 |
| 14 | <i>Fusarium oxysporum</i> | 15 | 35 |
| 15 | <i>Penicillium spp.</i> | 06 | 15 |
| 16 | <i>Rhizopus stolonifer</i> | 18 | 60 |

Table 2: Effect of dominant seed-borne fungi on seed germination, shoot and root length of Green gram (*Vigna radiata* L.) by blotter method (After ten days of incubation).

| Sr. No. | Infestation by dominant seed-borne fungi | Seed health | | |
|---------|--|----------------------|-------------------|------------------|
| | | Seed germination (%) | Shoot length (cm) | Root length (cm) |
| 1 | <i>Aspergillus flavus</i> | 40 | 2.2 | 3.1 |
| 2 | <i>Aspergillus fumigatus</i> | 50 | 2.0 | 2.2 |
| 3 | <i>Aspergillus niger</i> | 30 | 1.5 | 3.0 |
| 4 | <i>Drechslera tetramera</i> | 60 | 4.0 | 3.4 |
| 5 | <i>Fusarium moniliforme</i> | 70 | 3.0 | 3.8 |
| 6 | <i>Rhizopus stolonifer</i> | 80 | 2.2 | 4.2 |
| 7 | Control | 100 | 6.3 | 5.1 |

Table 3: Effect of dominant seed-borne fungi on seedling emergence, shoot and root length of Green gram (*Vigna radiata* L.) by pot sowing method (After ten days of incubation).

| Sr. No. | Infestation by dominant seed-borne fungi | Seed health | | |
|---------|--|------------------------|-------------------|------------------|
| | | Seedling emergence (%) | Shoot length (cm) | Root length (cm) |
| 1 | <i>Aspergillus flavus</i> | 50 | 10 | 9 |
| 2 | <i>Aspergillus fumigatus</i> | 60 | 10 | 10 |
| 3 | <i>Aspergillus niger</i> | 40 | 5 | 5 |
| 4 | <i>Drechslera tetramera</i> | 40 | 7 | 7 |
| 5 | <i>Fusarium moniliforme</i> | 70 | 13 | 12 |
| 6 | <i>Rhizopus stolonifer</i> | 80 | 12 | 10 |
| 7 | Control | 90 | 14 | 10 |

Effect of dominant seed-borne fungi on seed health – seed germination and seedling emergence of Green gram (*Vigna radiata* L.):

Seed germination:

Effect of six dominant seed-borne fungi was observed on seed germination of Green gram, results in table 2 show that, all dominant seed-borne fungi of Green gram retarded percent seed germination shoot and root length in the test seed of pulse.

Aspergillus niger caused maximum reduction in seed germination (30 %) followed by *Aspergillus flavus* (40 %) and *A. fumigatus* (50 %). On the contrary *Rhizopus stolonifer* and *Fusarium moniliforme* showed better seed germination (80 % and 70 % respectively).

Similar studies were carried by Sinha and Prasad (1981); they reported adverse effects on seed germination of Mung due to *Alternaria alternata*, *Bortyodiplodiathoe brome*, *Curvularia lunata*, *Fusarium moniliforme* and *Macrophomina phaseolina*. Reddy and Shanker and Rao (1995) studied effects of soaking of *Vigna radiata* L. seeds for six hours in culture filtrates of *Aspergillus niger* and found that it caused reduction in seed germination. Howlett (2006) reported toxins of the seed-borne fungi responsible for inhibition of normal growth of seedlings in different crops. Swami and Alane (2013) reported that Green gram is infested with variety of fungi that deteriorate the seed contents. Chilkuri and Giri (2014) observed *Macrophomina phaseolina*, *Fusarium moniliforme*, *F. semitectum*; *Aspergillus flavus* were responsible for seedling blight of Green gram.

Seedling emergence:

Effect of six dominant seed-borne fungi was observed on seedling emergence, shoot and root length of Green gram (Table 3). The fungi *Aspergillus niger* and *Drechslera tetramera* affected most adversely to seedling emergence (40 % each, control 90 %), shoot length (5 cm, control 14 cm), and root length (5 cm, control 10 cm) respectively. The fungus *Rhizopus stolonifer* affected less adversely to seedling emergence compared to rest of the fungi (seedling emergence 80 %, control 90 %), shoot length (12 cm, control 14 cm) and root length (10 cm, control 10 cm). Shoot length was less affected in case of seeds infested with *Fusarium moniliforme* (13 cm, control 14 cm). Root length was not affected in case of seeds treated with *Rhizopus stolonifer* and *Aspergillus fumigatus*

but more root length (12 cm) was recorded in case of seeds infested with *Fusarium moniliforme* over control.

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