

## Seed Mycoflora in Kidney Bean (*Phaseolus vulgaris* L.) in Himachal Pradesh

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**ABSTRACT** Investigations on comparative occurrence of mycoflora on vegetable and pulse type of beans revealed that vegetable type varieties harboured more mycoflora as compared to pulse type beans. Agar plating of seeds showed the presence of 16 species of pathogenic and storage fungi belonging to ten genera. However, the frequency of fungi was more on unsterilized seeds (4.0-64.0 %) as compared to sterilized seeds (8.0-32.0 %) in both types of beans. Major storage and pathogenic fungi recorded were the species of *Alternaria*, *Aspergillus*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Penicillium*, *Rhizoctonia*, *Rhizopus*, *Stemphylium* and *Trichoderma*. Presence of mycoflora on seed had little effect on seed germination; however, higher germination was recorded in surface sterilized seeds of both types of beans. Seed smearing with fungal spore suspension revealed that almost all the storage fungi except *Cladosporium* sp. had least effect on seed germination. However, culture filtrates of these fungi reduced the germination drastically along with induction of variable symptoms.

**Keywords:** Seed mycoflora, vegetable and pulse type kidney bean, seed germination, spore suspension, culture filtrate

Kidney bean, popularly known as Rajmash, occupies a premier position amongst the various grain legumes grown in Himachal Pradesh. The pulse type of bean crop is cultivated in mid (900-1300 m asl) and high hills (1800-3000 m asl) of Chamba, Kangra, Kinnaur, Kullu, Mandi, Shimla and Sirmour districts [1] whereas, snap beans (vegetable type) are cultivated mainly in low lying areas of the state. Seed being one of the most important yield contributing factors in both the types, has been reported to be affected by various biotic and abiotic factors during maturation, harvesting and storage [2, 3]. Of these factors, micro-organisms cause bio-deterioration of seed during storage and in the field, some of them like *Colletotrichum lindemuthianum* (Sacc. and Magn.), *Sclerotinia sclerotiorum* (Lib) de Bary, *Sclerotium rolfsii* Sacc. and *Rhizoctonia solani* Kuhn [4, 5] are serious seedborne pathogens of beans. In the present investigations, an effort has been made to study the colonization behavior of seed mycoflora on different types of kidney bean varieties and effect of surface sterilization on seed germination and development of mycoflora.

### MATERIALS AND METHODS

The seeds of different types of kidney bean varieties viz. Contender, Hans, Kentucky wonder,

Laxmi, SVM-1 (vegetable type); Baspa, Him-1, Jawala, Kanchan and Triloki (pulse type) were collected from the farmer's stores immediately after harvest and kept in metallic bins for six months at ambient temperature (November-April 2000-2001). After six months storage 100 seeds/sample, both unsterilized and sterilized (with sodium hypochlorite 0.1%) were subjected to Agar plating to record the development of mycoflora. Ten seeds were placed in each plate equidistantly [3] on Malt Extract Agar (MEA) medium supplemented with 100 ppm streptomycin to avoid bacterial contamination. These plates were incubated at  $25 \pm 1^\circ\text{C}$  in BOD incubator with 12 hours alternating cycles of light and dark and thereafter shifted to deep freezer ( $-20^\circ\text{C}$ ) for 24 hours to prevent seed germination. The plates were then again incubated at  $25 \pm 1^\circ\text{C}$  for 7 days. Incubated seeds were examined visually and under stereoscopic binocular microscope for the associated mycoflora. Rolled paper towel method [6] was employed to record seed germination percentage of different varieties after six months of storage. In order to study the effect of isolated mycoflora on seed health, two cultivars one each of pulse type (Kanchan) and vegetable type (contender) were treated with spore suspension and culture filtrates of isolated fungi by employing seed smearing method [7]. Culture filtrates obtained from 15 days old culture were used

Table 1. Comparative mycoflora (%) on kidney bean seeds (unsterilised) of vegetable and pulse type cultivars

| Cultivars             | Germination (%) | <i>Alternaria</i> sp. | <i>Aspergillus fumigatus</i> | <i>A. niger</i> | <i>Cladosporium</i> sp. | <i>Colletotrichum lindemuthianum</i> | <i>Fusarium</i> spp. | <i>F. oxysporum</i> | <i>F. solani</i> | <i>Penicillium capsulatum</i> | <i>P. griseo-fulvum</i> | <i>P. simplicissimum</i> | <i>P. perpuragenum</i> | <i>Rhizoctonia solani</i> | <i>Rhizopus stolonifer</i> | <i>Stemphylium</i> sp. | <i>Trichoderma</i> sp. | Total mycoflora |
|-----------------------|-----------------|-----------------------|------------------------------|-----------------|-------------------------|--------------------------------------|----------------------|---------------------|------------------|-------------------------------|-------------------------|--------------------------|------------------------|---------------------------|----------------------------|------------------------|------------------------|-----------------|
| <b>Vegetable Type</b> |                 |                       |                              |                 |                         |                                      |                      |                     |                  |                               |                         |                          |                        |                           |                            |                        |                        |                 |
| Contender             | 88.00           | -                     | -                            | -               | -                       | 8                                    | -                    | -                   | -                | 24                            | -                       | -                        | 20                     | 28                        | -                          | -                      | -                      | 4               |
| Hans                  | 85.33           | -                     | 24                           | 32              | -                       | 24                                   | -                    | -                   | -                | -                             | -                       | 44                       | -                      | -                         | -                          | -                      | -                      | 4               |
| Kentucky Wonder       | 85.33           | 44                    | -                            | 32              | 24                      | 16                                   | -                    | -                   | -                | -                             | 12                      | -                        | -                      | -                         | -                          | 32                     | 20                     | 7               |
| Laxmi                 | 90.67           | -                     | -                            | 32              | -                       | -                                    | -                    | 24                  | -                | 16                            | -                       | -                        | -                      | 48                        | -                          | -                      | -                      | 4               |
| SVM-1                 | 90.67           | -                     | -                            | 48              | 8                       | 16                                   | 4                    | -                   | 32               | -                             | 32                      | -                        | -                      | -                         | -                          | -                      | -                      | 6               |
| CD (P=0.05)           | 3.28            |                       |                              |                 |                         |                                      |                      |                     |                  |                               |                         |                          |                        |                           |                            |                        |                        |                 |
| <b>Pulse Type</b>     |                 |                       |                              |                 |                         |                                      |                      |                     |                  |                               |                         |                          |                        |                           |                            |                        |                        |                 |
| Baspa                 | 86.67           | -                     | -                            | 44              | -                       | -                                    | -                    | -                   | -                | -                             | -                       | -                        | -                      | 36                        | 56                         | -                      | 32                     | 4               |
| Him I                 | 70.00           | -                     | 36                           | -               | -                       | 20                                   | -                    | -                   | -                | -                             | -                       | 20                       | -                      | 48                        | 64                         | -                      | -                      | 5               |
| Jawala                | 97.97           | -                     | -                            | -               | -                       | 24                                   | -                    | -                   | -                | -                             | -                       | -                        | -                      | 40                        | -                          | -                      | -                      | 2               |
| Kanchan               | 91.33           | 48                    | -                            | -               | -                       | -                                    | -                    | -                   | -                | -                             | 8                       | -                        | -                      | 8                         | 8                          | -                      | -                      | 4               |
| Triloki               | 95.32           | 32                    | -                            | -               | -                       | -                                    | -                    | -                   | -                | 8                             | -                       | -                        | -                      | 8                         | 12                         | -                      | -                      | 4               |
| CD (P=0.05)           | 5.48            |                       |                              |                 |                         |                                      |                      |                     |                  |                               |                         |                          |                        |                           |                            |                        |                        |                 |

Table 2. Comparative mycoflora(%) on kidney bean seeds (sterilised) of vegetable and pulse type cultivars

| Cultivars             | Germination (%) | <i>Alternaria</i> sp. | <i>Colletotrichum lindemuthianum</i> | <i>Fusarium</i> spp. | <i>F. oxysporum</i> | <i>Rhizoctonia solani</i> | <i>Stemphylium</i> sp. | Total mycoflora |
|-----------------------|-----------------|-----------------------|--------------------------------------|----------------------|---------------------|---------------------------|------------------------|-----------------|
| <b>Vegetable Type</b> |                 |                       |                                      |                      |                     |                           |                        |                 |
| Contender             | 93.00           | -                     | -                                    | 48                   | -                   | 32                        | 24                     | 3               |
| Hans                  | 92.00           | -                     | -                                    | -                    | -                   | 24                        | -                      | 1               |
| Kentucky Wonder       | 88.00           | -                     | -                                    | 20                   | 44                  | 32                        | -                      | 3               |
| Laxmi                 | 94.00           | -                     | 12                                   | -                    | -                   | 16                        | -                      | 2               |
| SVM-1                 | 93.33           | 24                    | -                                    | 20                   | -                   | 20                        | 24                     | 4               |
| CD (P=0.05)           | 4.00            |                       |                                      |                      |                     |                           |                        |                 |
| <b>Pulse Type</b>     |                 |                       |                                      |                      |                     |                           |                        |                 |
| Baspa                 | 96.66           | -                     | -                                    | -                    | -                   | -                         | -                      | 0               |
| Him 1                 | 85.00           | -                     | 16                                   | -                    | -                   | 8                         | -                      | 2               |
| Jawala                | 97.97           | -                     | 12                                   | -                    | -                   | 12                        | -                      | 2               |
| Kanchan               | 92.00           | 32                    | -                                    | -                    | -                   | 12                        | -                      | 2               |
| Triloki               | 98.65           | 16                    | -                                    | -                    | -                   | 12                        | -                      | 2               |
| CD (P=0.05)           | 7.09            |                       |                                      |                      |                     |                           |                        |                 |

to treat the seeds by giving a dip of six hours and then placed on two layers of moistened germination sheets for growth [8, 9] and data were recorded on seed germination and seedling rot.

## RESULTS AND DISCUSSION

Storage studies on occurrence of mycoflora revealed the presence of 16 species of storage and pathogenic fungi belonging to 10 genera on both pulse and vegetable type of beans (Table 1). The vegetable type varieties harboured more number of fungi as compared to pulse type. Maximum number of fungi were recorded on Kentucky Wonder [7] followed by SVM-1 (6) whereas, other 3 varieties viz. Contender, Hans and Laxmi were colonized by 4 species each. *Aspergillus*, *Cephalosporium*, *Cladosporium*, *Penicillium* and *Rhizopus* were the dominating storage fungi whereas, *Colletotrichum* and *Rhizoctonia* constituted major pathogenic flora. Overall incidence of mycoflora ranged between 4 to 64 per cent. *Aspergillus niger* and *C. lindemuthianum* were detected from 4 vegetable type varieties showing 8.0-24.0 and 32.0-48.0 per cent infestation, respectively.

Among pulse type varieties, Him-1 harboured 5 fungi viz. *Aspergillus fumigatus*, *Colletotrichum*, *Penicillium*, *Rhizoctonia* and *Rhizopus* sp. followed by Baspa, Kanchan and Triloki. The incidence of storage and field fungi on pulse type beans ranged between 8.0-64.0 and 8.0-48.0 per cent whereas, it was 4.0-48.0 and 16.0-48.0 per cent, respectively, on vegetable type varieties. *Rhizopus* was prevalent on almost all the varieties showing 8.0-64.0 per cent incidence except Jawala.

Sterilized seeds of these two types of kidney beans yielded comparatively less fungi (Table 2). Cultivar SVM-1 was colonized by *Alternaria*, *Fusarium* spp., *Rhizoctonia* and *Stemphylium* showing 20.0-24.0 per cent incidence. *C. lindemuthianum* was detected only from variety Laxmi and *Rhizoctonia* was found to be prevalent on both the beans, though the incidence on pulse type was lower as compared to vegetable type. Cultivar Baspa was free of any fungal infestation. Cultivars like Kentucky Wonder and SVM-1 were the most colonized. Baspa variety of pulse type bean possessed minimum mycoflora. *Rhizoctonia* was prevalent on both the beans though the incidence was less on pulse type.

Table 3. Effect of isolated mycoflora on germination (%) of kidney bean seeds

| Fungi                                | Spore/mycelial suspension |                  |                 |                 | Culture filtrate |                  | Sterilized distilled water |         | Incubation period# (Days) | Effect on germinated seed                                          |                                                                                |
|--------------------------------------|---------------------------|------------------|-----------------|-----------------|------------------|------------------|----------------------------|---------|---------------------------|--------------------------------------------------------------------|--------------------------------------------------------------------------------|
|                                      | Contender                 | Kanchan          | Contender       | Kanchan         | Contender        | Kanchan          | Contender                  | Kanchan |                           | Spore/mycelial suspension                                          | Culture filtrate                                                               |
| <i>Alternaria</i> sp.                | 90.0<br>(71.59)*          | 95.0<br>(77.22)  | 85.0<br>(67.38) | 90.0<br>(71.59) | 95.0<br>(77.22)  | 100.0<br>(89.96) |                            |         | 8                         | Discoloration of hypocotyl region                                  | Seed and seedling rot, reddish brown lesions covered with creamish fungal mass |
| <i>Aspergillus fumigatus</i>         | 75.0<br>(59.99)           | 80.0<br>(63.43)  | 10.0<br>(18.37) | 7.0<br>(15.23)  | 92.0<br>(73.62)  | 100.0<br>(89.96) |                            |         | 9                         | Extensive necrosis on cotyledons and stem                          | Seed rot                                                                       |
| <i>A. niger</i>                      | 93.0<br>(75.29)           | 100.0<br>(89.96) | 10.0<br>(18.37) | 10.0<br>(18.37) | 93.0<br>(75.29)  | 100.0<br>(89.96) |                            |         | 9                         | Extensive sporulation on cotyledons, Slight necrosis on hypocotyls | Slight necrosis on hypocotyls & root rot                                       |
| <i>Cladosporium</i> sp.              | 65.0<br>(53.71)           | 70.0<br>(56.77)  | 72.0<br>(58.09) | 80.0<br>(63.53) | 94.0<br>(76.66)  | 100.0<br>(89.96) |                            |         | 10                        | Root rot, Light brown patches on hypocotyls                        | Root rot, Light brown patches on stem                                          |
| <i>Colletotrichum lindemuthianum</i> | 55.0<br>(47.85)           | 68.0<br>(55.53)  | 42.0<br>(40.38) | 55.0<br>(47.85) | 95.0<br>(77.22)  | 100.0<br>(89.96) |                            |         | 7                         | Seed and seedling rot                                              | Seed and seedling rot                                                          |
| <i>Fusarium</i> spp.                 | 92.0<br>(73.62)           | 98.0<br>(83.41)  | 85.0<br>(67.21) | 95.0<br>(77.22) | 96.0<br>(78.69)  | 100.0<br>(89.96) |                            |         | 16                        | Slight necrosis on hypocotyls                                      | Slight necrosis on hypocotyls                                                  |
| <i>F. oxysporum</i>                  | 42.0<br>(40.38)           | 50.0<br>(44.98)  | 40.0<br>(39.21) | 42.0<br>(40.38) | 90.0<br>(71.59)  | 100.0<br>(89.96) |                            |         | 8                         | Hypocotyls having sunken light brown lesions with dark margins     | Partial sunken light brown lesions with dark margins                           |
| <i>F. solani</i>                     | 50.0<br>(44.98)           | 55.0<br>(47.85)  | 42.0<br>(40.38) | 50.0<br>(44.98) | 92.0<br>(73.62)  | 100.0<br>(89.96) |                            |         | 10                        | Inhibition of seed germination and radical necrosis                | Inhibition of seed germination                                                 |
| <i>Penicillium capsulatum</i>        | 90.0<br>(71.59)           | 100.0<br>(89.96) | 7.0<br>(15.23)  | 9.0<br>(17.38)  | 93.0<br>(75.29)  | 100.0<br>(89.96) |                            |         | 7                         | Seed rot                                                           | Seed rot                                                                       |
| <i>P. griseo-fulvum</i>              | 95.0<br>(77.22)           | 99.0<br>(85.34)  | 10.0<br>(18.37) | 12.0<br>(20.21) | 95.0<br>(77.22)  | 100.0<br>(89.96) |                            |         | 9                         | No effect                                                          | Seed rot                                                                       |
| <i>P. simplicissimum</i>             | 93.0<br>(75.29)           | 98.0<br>(83.41)  | 10.0<br>(18.37) | 8.0<br>(16.34)  | 95.0<br>(77.22)  | 100.0<br>(89.96) |                            |         | 12                        | No effect                                                          | Seed rot                                                                       |
| <i>P. perpuragenum</i>               | 92.0<br>(73.62)           | 100.0<br>(89.96) | 8.0<br>(16.34)  | 10.0<br>(18.37) | 95.0<br>(77.22)  | 100.0<br>(89.96) |                            |         | 8                         | No effect                                                          | Seed rot                                                                       |
| <i>Rhizoctonia solani</i>            | 50.0<br>(44.98)           | 55.0<br>(47.85)  | 40.0<br>(39.21) | 42.0<br>(40.38) | 90.0<br>(71.59)  | 100.0<br>(89.96) |                            |         | 10                        | Decreased seed germination                                         | Stem necrosis and root rot                                                     |
| <i>Rhizopus stolonifer</i>           | 92.0<br>(73.62)           | 98.0<br>(83.41)  | 93.0<br>(75.29) | 97.0<br>(80.42) | 92.0<br>(73.62)  | 100.0<br>(89.96) |                            |         | 15                        | Root necrosis (traces)                                             | Root necrosis (traces)                                                         |
| <i>Stemphylium</i> sp.               | 90.0<br>(71.59)           | 92.0<br>(73.62)  | 80.0<br>(63.43) | 90.0<br>(71.59) | 95.0<br>(77.22)  | 100.0<br>(89.96) |                            |         | 10                        | Discoloration of hypocotyl region                                  | Seed and seedling rot, reddish brown lesions covered with creamish fungal mass |
| <i>Trichoderma</i> sp.               | 82.0<br>(65.01)           | 93.0<br>(75.29)  | 80.0<br>(63.43) | 95.0<br>(77.22) | 90<br>(71.59)    | 100.0<br>(89.96) |                            |         | 7                         | No effect                                                          | No effect                                                                      |
| CD(0.05)                             | 4.58                      | 3.22             | 4.24            | 5.52            | NS               | NS               |                            |         | 1.66                      |                                                                    |                                                                                |

\* Values in parentheses are arcsin transformed values

# Incubation period depicts the appearance of fungal species

The occurrence of more mycoflora on vegetable types of beans may be due to soft seeds and tender nature of the plants. More carbohydrate content in vegetable type varieties may be ascribed for more mycoflora in these varieties. Similar observations were recorded on vegetable type beans like Contender, Kentucky Wonder and SVM 1 by Gupta *et al* [10, 11].

After six months of storage it was observed that seed germination was higher in the sterilized seeds of both types of beans. The germination percentage ranged between 85.33-90.67 % and 88.00-94.00 % in unsterilized and sterilized seeds of vegetable type beans, however, it ranged between 70.00-95.32 % and 85.00-98.65 % among pulse type varieties which was higher than that of recommended standards in both unsterilized and sterilized seeds in two types of beans. Occurrence of mycoflora on bean seeds though had little effect on seed germination but could affect the seed adversely under field condition after sowing.

Seed smearing with fungal spore suspension was found to reduce the germination by 5.0-55.0 per cent after 7-13 days of incubation and with culture filtrates 4.0-48.0 per cent reduction was observed with pulse type variety. However, these values were much lower in vegetable type variety. Treated seeds showed blight, necrosis, seed/seedling rot and several other abnormalities (Table 3). It was also noticed that spore suspension of storage fungi had least effect on seed germination. However, culture filtrates of these fungi reduced the germination drastically along with induction of variable symptoms. Chisholm [12] observed maximum reduction in seed germination of three types of bean by culture filtrates of *A. flavus*, *A. niger* and *A. terreus*. Similar type of seed and seedling abnormalities in number of legume crops due to these fungi have also been reported by many workers [13-17].

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