

MYCOFLORA ASSOCIATED WITH *PHASEOLUS VULGARIS* L. SEEDS AND ITS IMPACT ON SEED GERMINATION IN AZAD JAMMU & KASHMIR

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Abstract

Seed of *Phaseolus vulgaris* L. collected from various localities of Azad Jammu and Kashmir were evaluated for seed associated Mycoflora by Standard blotter paper method and Agar plate method. The seed samples were observed contaminated with fungi corresponding to eight genera including *Alternaria* Nees, *Aspergillus* Micheli, *Curvularia* Boedijn, *Drechslera* S. Ito, *Fusarium* Link, *Mucor* Fresen, *Penicillium* Link and *Rhizopus* Ehrenb. The highest infection percentage was recorded from Trarkhal, Bagh and Chikar samples, i.e. 24.75A, 18.25B and 13.25C respectively. Least infection percentage was observed on Lawaat 3.00H and Kail 11.8E samples. Impact of fungal presence on seed germination was elucidated by Standard rolled paper towel method. Lawaat and Kail samples showed lesser impact and better germination 93.66% and 92.33% respectively. Incidence of fungi was recorded high and low germination in Trarkhal and Palangi samples 43.33% and 22% respectively. Three treatments namely, heat, *Allium sativum* extract and Benomyl (fungicide) were used for Mycoflora management. These treatments were assessed through Standard rolled paper towel method. Use of different antifungal treatments found effective in increasing mean germination percentage 6-8% and health of seedlings. Efficacy of management was evaluated by using CRD2 Factorial test. The interaction between treatments and localities was significantly different at the level of 0.05. Benomyl was more effective in increasing seed germination with 76.00A mean. Locality of Lawaat showed the highest germination percentage with different treatments having 96.00A mean value. Best interaction was observed between treatment 3 and locality 2, i.e. 97.33 while least interaction was calculated between treatment 2 and locality 6 i.e., 22.33. All seed samples of *P. vulgaris* collected from different sites were found contaminated with seed associated Mycoflora. These fungi reduced the germination percentage and use of different antifungal treatments efficiently increased the germination percentage and reduced the fungal growth. Benomyl was found more effective seed treatment before sowing.

Key words: *Phaseolus vulgaris*, Seed associated fungi, Seed health, Seed treatment, Plant diffusate, Benomyl.

Introduction

Phaseolus vulgaris L. belongs to family Fabaceae. Documentation based on the history, linguistic, archeology and botany revealed that common beans have originated and domesticated in America (Gepts & Dpbouk, 1991; Papa & Gepts, 2003 and Papa *et al.*, 2005). In Pakistan, it is cultivated in northern parts of the country, mainly on hilly tracks. Similarly, in Azad Jammu and Kashmir its cultivation is restricted to hilly parts of the state including Kail, Lawaat, Bheri, Chikar, Bagh, Palangi, Hilaan, Abaspur and Trarkhal. *P. vulgaris* is put to use in all parts of the world, particularly by the low income population in the developing countries (Shimelis & Rakshit, 2005). Mostly beans are consumed as dry seeds, while some people utilized them as green pods or green seeds (Lin *et al.*, 2008). Beans have great social and economic importance, being source of proteins, micronutrients and minerals for the population. Major proteins in *P. vulgaris* are albumins and globulins, whereas lesser fractions are glutelins and prolamines (Imran *et al.*, 2014). Common beans also incorporate large amounts of starch, vitamins, dietary fiber and minerals (Kutos *et al.*, 2003; Costa *et al.*, 2006). In addition to these, *P. vulgaris* are rich source of variety of phytochemicals, an extensive array of flavonoids such as anthocyanins, flavonoids, flavonols, isoflavones and phenolic acids (Beninger & Hosfield, 2003; Lin *et al.*, 2008 and Granito *et al.*, 2008). Common bean utilization is

supported due to its health encouraging properties, like prevention of cancer, cardiovascular diseases, diabetes mellitus and obesity (Carlos *et al.*, 2010).

Detection of pathogenic Mycoflora associated with seeds is very crucial step for the treatment of crop diseases. Tseng *et al.*, (1995) comparatively investigated seed associated fungi and mycotoxins in *P. vulgaris* from Ontario and Taiwan. The isolated fungi were *Alternaria*, *Fusarium*, *Gliocladium*, *Mucor*, *Rhizoctonia*, *Rhizopus*, *Penicillium*, and *Sclerotinia*. Ontario samples were found contaminated with *Fusarium* toxins, while Taiwan samples contained Aflatoxins. Gerard *et al.*, (1997) examined the presence of seed associated Mycoflora pathogenic to *P. vulgaris*. He identified the pathogens according to symptoms, colony morphology, reproductive configuration, and disease causing ability. The prevalence of isolates varied in accordance with region and seasons. *Pythium* were most affiliated fungi, showing their importance for the root rot spread in Rwanda.

Domijan *et al.*, (2005) investigated seed Mycoflora and ochratoxin A production from *P. vulgaris* in Republic of Croatia. Forty-five bean samples were analyzed for mycological and mycotoxin contamination. OTA was found in 17 out of 45 samples. *P. vulgaris* samples collected from south were OTA free and the least contaminated, whereas Mycofloral infection and OTA concentration was similar in all other samples. Youssef *et al.*, (2008) isolated and identified 143 species and 9 varieties belonging to 32 genera, from 15 samples for each species of broad bean, chickpea, kidney bean

and sweet pea collected from 8 sites of Libya. Fungal genera recorded with high prevalence were, *Aspergillus*, *Penicillium*, *Mucor*, *Alternaria*, *Fusarium*, *Rhizopus* and *Eurotium*.

Ghangaokar & Kshirsagar, (2013) reported legumes seed borne fungi associated with *Pisum sativum*, *Macrotyloma uniflorum*, *Lens culinaris*, *Phaseolus vulgaris*, *Vigna unguiculata*, *Cajanus cajan*, *Cicer arietinum* collected from Pune by using blotter paper method. The fungal species identified from these samples were *Alternaria*, *Chaetomium*, *Penicillium*, *Aspergillus*, *Rhizopus*, *Fusarium*, *Curvularia*, *Macrophomina*, *Monilia* etc. Ignjatov *et al.*, (2016) investigated seed quality of bean seeds. Morphological description of *Fusarium* spp. were performed through PDA and CLA. The amplification of 1-alpha portion of this gene was done through PCR by utilizing primers EF1 and EF2. Molecular and morphological traits of fungi, as well as sequencing results established that *Fusarium proliferatum* was the cause of seed rot.

Seed affiliated fungi are involved in deterioration of seed prior to germination, results seedling mortality and foliage infection at adult stage (Altaf *et al.*, 2004). The seed associated diseases are highly devastating as they weaken the seed strength and damage seedlings at early stage of growth (Maiti *et al.*, 1988). Diseases resulted by seed borne fungi are relatively difficult to control as fungal hyphae gets established and become dormant. Seed treatment is a valuable process that provides security against seed borne and soil borne plant pathogens and insects (Gwary *et al.*, 2007). Many researchers have analyzed the potency of different plant extracts as substitute seed treatments to synthetic fungicides against soil and seed borne pathogens on cereal crops (Shafique *et al.*, 2007 and Somda *et al.*, 2007) and legume crops (Pretorius *et al.*, 2002 and Masangwa *et al.*, 2013). Nasir (2003) investigated the efficacy of fungicides namely, Benomyl, Captan, Dithane, Thiram and Vitavax against fungi associated with Soybean. Benomyl shows best results. Umechuruba *et al.*, (2013) used physical techniques (dry heat and hot water) to reduce the seed associated Mycoflora of *Solanum gilo*. Both treatments at 60°C for 40min recorded the optimum germination percentage (83.26, 81.00 and 79.21, 70.12). Mahmoud *et al.*, (2013) reported that Seed borne fungi were cause of wilt and damping off diseases in *P. vulgaris* plants. Several fungi were isolated from seed samples. *Fusarium oxysporum* and *R. solani* were the most common fungi. Bean seeds treated with peppermint oil caused a reduction in the infection and reduced fungal transmission from seeds to seedlings.

Dube *et al.*, (2014) investigated that *P. vulgaris* seed can be hand sorted to reduce the level of contamination. The planting date effects the infection and discoloration of *P. vulgaris* seeds. The research represents that cropping date is considerably important for improvement of the seed quality. Kator *et al.*, (2016) conducted isolation and identification of seed associated fungi of common bean from selected markets in Makurdi. Percentage germination of the samples ranged from 33-53%.

Isolation of fungal pathogens from the bean seeds indicate that they should be treated before sowing to obtain good germination and healthy crop. Masangwa *et al.*, (2017) investigate the effect of crude plant extracts as seed treatments on *P. vulgaris*. Common bean and cowpea seeds were treated with crude water and acetone extracts of *Agapanthus caulescens*, *Allium sativum*, *Carica papaya* and *Syzygium cordatum* at 5 and 15 mg/ml concentrations. Cowpea seeds treated with *Carica* water extract had the better germination and emergence. *Syzygium* acetone was the exclusive extract with higher germination and emergence in both IT93K5132 and PAN 311 varieties. Therefore, *Carica* water and *Syzygium* acetone extracts can be considered as potential bean and cowpea seed treatments.

In AJ&K, certified healthy seeds of *P. vulgaris* are not available and seed borne fungi have gained little attention. Information on seed borne Mycoflora of common beans in state is lacking and needs to be investigated. Therefore, present research was organized to identify Mycoflora of *P. vulgaris* seeds, elucidate their impact on seed germination and evaluate management strategies.

Materials and Methods

Collection of samples: For present study, seeds were obtained in polythene bags from nine sites of Azad Jammu and Kashmir including 1 Kail, 2 Lawaat, 3 Bheri, 4 Chikar, 5 Bagh, 6 Palangi, 7 Hilaan, 8 Abasapur and 9 Trarkhal (Fig. 1). Seed samples were drawn according to the procedures laid down by ISTA, (1985). These samples were investigated at the plant pathology laboratory of the Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.

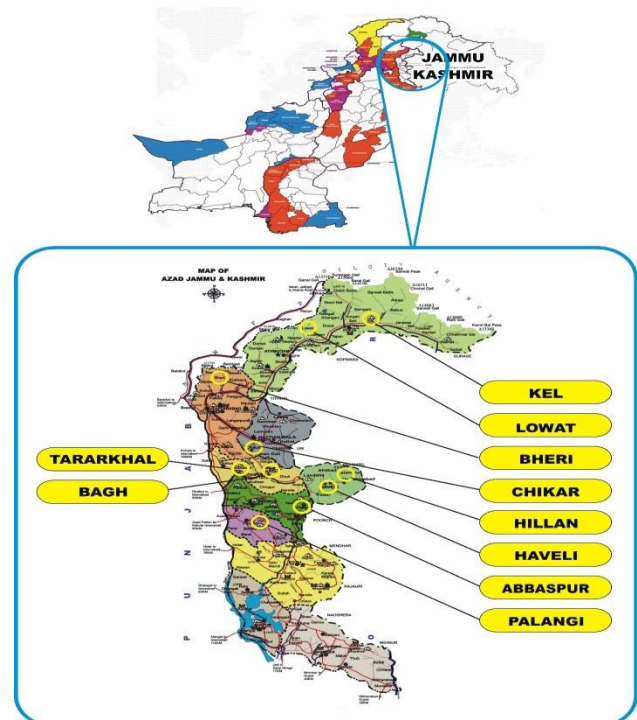


Fig. 1. Map showing sample collection sites of *Phaseolus vulgaris* seeds in Azad Jammu & Kashmir.

Isolation of seed associated fungi: Each sample containing 100 seeds were examined for their association of seed Mycoflora by standard blotter paper method (ISTA, 1976; Neergard, 1977; Khare, 1996). Experiments were done in three replicates. The seeds were decontaminated with 1% NaOCl for 2 to 3 minutes and washed with sterilized water before plating. Petri dishes were encrusted with two layers of filter papers soaked in distilled water. 10 seeds of common beans were plated in each petri dish. Filter papers in the Petri dishes were rehydrated at 24hrs interval. Replicates of all samples were arranged in completely randomized design (CRD). Plated seeds were nurtured at 25°C for 24hrs and then refrigerated for 24hrs to avoid germination (Limonard, 1968). Seeds were grown again at 25°C for 7 days. After incubation, fungi which grew out of the seeds were subcultures on sterile Potato Dextrose Agar (PDA) 20ml per plate added with Sulphate streptomycin (Rao & Bramel, 2000; Yesuf & Songchote, 2005).

Detection of seed borne fungi: To determine the fungal prevalence in Petri dishes visual and microscopic observation were made under microscope (Leitz Laborlux-d). For visual observation, growth and colony appearance were investigated every day. For microscopic detection, morphology of the isolated fungi was noted by using a microscope and identification was confirmed by using recommendations given by (Nelson *et al.*, 1983 and Barnett & Hunter, 1998).

Infection percentage: The plated seeds were classified as infected and non-infected. Infection percentage of associated Mycoflora of *P. vulgaris* was calculated by using the Standard blotter paper method (ISTA, 1976). Occurrence of different genera of fungi was calculated by using following formula:

$$\text{Infection (\%)} = \frac{\text{Seed infected by a particular fungus}}{\text{Total seeds of a sample}} \times 100$$

Impact of mycoflora on germination: Germination test was conducted by using standard paper towel method (Yaklich, 1985). 100 untreated seeds of each sample were randomly selected and allowed to germinate between two moistened blotter papers. Two rolled towels with the seeds in between placed in a sealed container to retain the moisture at 25°C for 7 days. All experiments were done in three replicates. After incubation, the germinated and ungerminated seeds were counted and percentage was calculated (Anwar *et al.*, 1994). The emerged seedlings were graded as abnormal and normal.

Seed treatment: Three treatments namely 1 heat, 2 plant defusate and 3 Benomyl were used in this experiment. For all treatments a sample of 100 seeds were taken from each locality. The experiment was done in three replications. The seeds after treatment were allowed to germinate by using standard rolled paper towel method (Yaklich, 1985). After 7 days rolled paper were opened and seedlings examined individually for the categories. Germinated seeds were graded as normal and abnormal seedlings and also counted ungerminated and rotted seeds.

Control: Seeds were soaked in sterilize water for 5min and then allowed to germinate. Infection %age was determined by grading seedlings.

Heat treatment: Seed were soaked in sterilize distilled water for 5min and then put into oven at 50°C for 15min. The incubated seeds were then allowed to germinate by using paper towel method. Seedlings were observed for grading into categories (Rahman *et al.*, 2008).

Plant defusate: Extract of garlic bulb (cloves) was evaluated for their antifungal activity against seed affiliated fungal pathogens. Forty-gram rhizome extract was dissolved in 400 ml sterilized distilled water. Seeds were soaked in filtrate of plant extract for 15min and dried on blotter paper. Seed were germinated by using standard rolled paper towel method. Seedlings were then examined for grading into categories (Hasan *et al.*, 2005).

Treatment with fungicide: Benomyl was used for seed dressing @ 2g/kg seeds (Nasir, 2003). Seeds were soaked in fungicide solution for 5min and then dried on blotter paper. Seeds were incubated at 25°C for seven days under fluorescent tube light. Seedlings were then analyzed for grading.

Statistical analysis

A completely randomized design was used for investigation and analysis of the results, the collected data was subjected to ANOVA followed by LSD test at the significant level of 0.05 according to Gomez & Gomez (1984).

Results and Discussion

Microorganism play major contributions in disturbing the quality of seeds, among them fungi are the important group (Butt *et al.*, 2011). The systematic work was carried out during present studies to detect the seed borne Mycoflora, their role in poor germination and elucidation of management strategies.

Detection of fungi: Eight fungal genera were found associated with seeds from different localities i.e. *Alternaria*, *Aspergillus*, *Curvularia*, *Drechslera*, *Fusarium*, *Mucor*, *Penicillium* and *Rhizopus* (Table 1; Figs. 2-9). No sample was found free from seed borne fungi. *Alternaria*, *Rhizopus*, *Fusarium*, *Penicillium* and *Aspergillus* were present almost in all localities whereas *Curvularia*, *Drechslera* and *Mucor* were recorded from few areas. To know the quantum of seed Mycoflora associated with *P. vulgaris* seeds, collection of samples was made from the nine hilly areas of AJ&K. The intention of testing was to screen out the number of seed borne fungi (Table 1; Fig. 1).

Infection percentage: The percentage incidence of all the fungi found varied from sample to sample and locality to locality (Table 2; Figs. 10 & 11). Mean percentage recorded for fungi associated with seed from different localities was relatively higher in case of *Penicillium*

40.3A, *Aspergillus* 29.00B and *Rhizopus* 14.1C, while lower in case of *Fusarium* 6.11D, *Mucor* 3.88E, *Alternaria* 3.77E, *Curvularia* 1.66F, *Drechslera* 1.55F. Seeds of Trarkhal community were highly infected having infection mean (24.75A) followed by localities of Bagh and Chikar with infection means (18.25B) and (13.25C) respectively. Least infection was recorded from Lawaat locality having infection mean (3.00H). The incidence of soil borne pathogens underscored the need for better seed production and improved storage conditions (Table 2; Figs. 10&11).

Impact of mycoflora on seed germination: Among the various factors responsible for low yield of *P. vulgaris*, one was fungal impact on seedlings before and after their emergence (Table 3; Fig. 12). From various localities total seed germination was calculated, seedlings were graded as normal and abnormal. Seeds were also counted as ungerminated and rotten. Highest impact of Mycoflora and low germination was recorded from Palangi (22.00H), Trarkhal (43.33G) and Bheri (75.66F) respectively whereas low impact and high germination

was analyzed from Lawaat (93.66A), Kail (92.33B) and Abasapur (83.66C) subsequently. Bhatti & Bhutta, (1990) reported that seed borne diseases alone reduce the production about 10 percent (Table 3; Fig. 12).

Management strategies for seed borne mycoflora: Three treatments i.e., Heat, Plant Defusate (Garlic extract) and antifungal Chemical (Benomyl) have been used in the present work. Efficacy of management strategies was evaluated by using CRD 2 Factorial test for treatments and localities. Variability was found among localities and different treatments and interaction between treatments and localities was also significantly different at the level of 0.05 (Table 5). Benomyl was more effective with increased germination (76.00A) mean followed by heat treatment (73.88B) and garlic extract (73.77B). Locality of Lawaat showed highest germination percentage with different treatments having (96.00A) subsequently Kail (86.75B) and Abasapur (85.25C) mean values. The highest interaction was observed between treatment 3 (Benomyl) and locality 2 (97.33). Least interaction was calculated between treatment 1 and locality 6 (22.66) (Table 4; Fig. 13).

Table 1. Detection of seed borne fungi.

| Name of fungi | Localities | | | | | | | | |
|--------------------|------------|----------|---------|----------|--------|-----------|----------|------------|------------|
| | 1 Kail | 2 Lawaat | 3 Bheri | 4 Chikar | 5 Bagh | 6 Palangi | 7 Hilaan | 8 Abasapur | 9 Trarkhal |
| <i>Alternaria</i> | + | - | + | + | + | + | + | + | + |
| <i>Aspergillus</i> | + | + | + | + | + | + | + | + | + |
| <i>Curvularia</i> | + | - | - | - | - | - | + | + | + |
| <i>Drechslera</i> | - | + | + | + | - | - | + | + | - |
| <i>Fusarium</i> | + | + | + | + | + | + | + | + | + |
| <i>Mucor</i> | - | - | - | - | - | - | + | + | + |
| <i>Penicillium</i> | + | + | + | + | + | + | + | + | + |
| <i>Rhizopus</i> | + | + | + | + | + | + | + | + | + |

Key: Present +, Absent -

Table 2. Fungal infection (%) age on seeds of all localities.

| Name of fungi | Localities | | | | | | | | | |
|--------------------|------------|-----------|-----------|----------|------------|----------|------------|------------|------------|--------|
| | Kail | Lawaat | Bheri | Chikar | Bagh | Palangi | Hilaan | Abasapur | Trarkhal | Means |
| <i>Alternaria</i> | 4.33±1.23 | 0±0 | 1±0 | 1.33±0.3 | 3±2.66 | 6±2.3 | 4±0.57 | 4.33±1.45 | 10.33±2.6 | 3.77E |
| <i>Aspergillus</i> | 24.66±2.9 | 5±1.15 | 29.33±2.6 | 24±2.88 | 45±4.04 | 21.33±2 | 21.33±2.02 | 20±2.3 | 70.33±3.75 | 29.0 B |
| <i>Curvularia</i> | 3.66±1.2 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 3.66±1.2 | 4.33±0.88 | 3.33±0.88 | 1.66F |
| <i>Drechslera</i> | 0±0 | 2.66±1.2 | 1±0 | 6.33±1.4 | 0±0 | 0±0 | 1.66±0.66 | 2.33±0.88 | 0±0 | 1.55F |
| <i>Fusarium</i> | 6.33±0.88 | 3.33±0.88 | 5±1.73 | 2.66±1.2 | 12±1.15 | 2.66±0.3 | 8.33±0.88 | 7±1.15 | 7.66±1.2 | 6.11D |
| <i>Mucor</i> | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 | 3.66±1.2 | 6±0.57 | 25.33±3.17 | 3.88E |
| <i>Penicillium</i> | 47.66±1.76 | 7±1.73 | 23.33±2.0 | 53±4.04 | 68.33±2.6 | 43±1.73 | 24.33±2.6 | 38.33±3.75 | 58±2.88 | 40.3A |
| <i>Rhizopus</i> | 8±2.3 | 7.66±0.66 | 9.33±1.45 | 18±2.30 | 15.33±1.45 | 19.33±2 | 9±1.73 | 18.33±3.17 | 22±2.88 | 14.1C |
| Means | 11.8E | 3.00H | 8.50G | 13.25C | 18.25B | 11.50E | 9.25F | 12.63D | 24.75A | 0.00 |

Table 3. Impact of mycoflora in Germination of *P. vulgaris*.

| Localities | Germinated seeds | Normal seedlings | Abnormal seedlings | Ungerminated seeds | Rotted seeds | Associated fungi |
|------------|------------------|------------------|--------------------|--------------------|--------------|--|
| Kail | 92.33±1.45B | 85±2.3A | 7.33±0.33G | 7.66±1.66G | 3.33±0.88EF | <i>Alternaria, Aspergillus, Curvularia, Fusarium, Penicillium, Rhizopus</i> |
| Lawaat | 93.66±2.9A | 64.33±2.02D | 29.33±3.17A | 6.33±1.45H | 2.66±0.33F | <i>Aspergillus, Drechslera, Fusarium, Penicillium, Rhizopus</i> |
| Bheri | 75.66±2.33F | 52±2.88F | 24.66±1.76C | 24.33±2.02C | 8.33±0.88C | <i>Alternaria, Aspergillus, Drechslera, Fusarium, Penicillium, Rhizopus</i> |
| Chikar | 78.33±2.6E | 54.33±1.45E | 24±2.3B | 21.66±2.33D | 7.33±0.88C | <i>Alternaria, Aspergillus, Drechslera, Fusarium, Penicillium, Rhizopus</i> |
| Bagh | 81.33±3.17D | 66.33±2.6C | 15±2.88D | 18.66±2.9E | 13±2.88B | <i>Alternaria, Aspergillus, Fusarium, Penicillium, Rhizopus</i> |
| Palangi | 22±2.83H | 12±2.3H | 10±1.15F | 78±2.3A | 53.33±2.02A | <i>Alternaria, Aspergillus, Fusarium, Penicillium, Rhizopus</i> |
| Hilaan | 77.33±2.02F | 63±2.88D | 13.33±1.45E | 22.66±2.9C | 4.66±0.66DE | <i>Alternaria, Aspergillus, Curvularia, Drechslera, Fusarium, Mucor, Penicillium, Rhizopus</i> |
| Abasapur | 83.66±1.76C | 68.33±1.45B | 15.33±1.45D | 16.33±2.6F | 4.33±0.88DE | <i>Alternaria, Aspergillus, Curvularia, Drechslera, Fusarium, Penicillium, Rhizopus</i> |
| Trarkhal | 43.33±2.6G | 35.33±3.75G | 8±1.73G | 56.66±2.33B | 5.33±0.88D | <i>Alternaria, Aspergillus, Curvularia, Fusarium, Mucor, Penicillium, Rhizopus</i> |

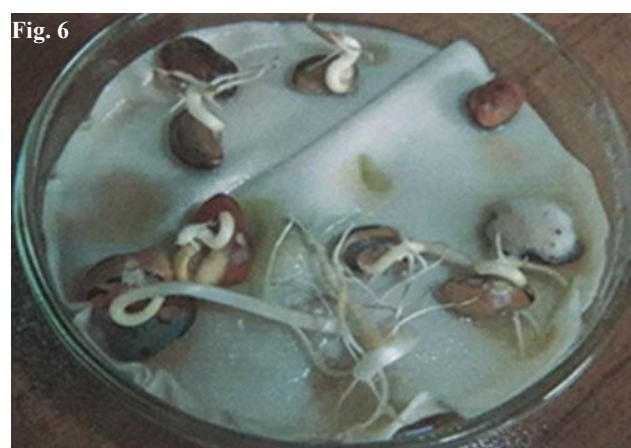
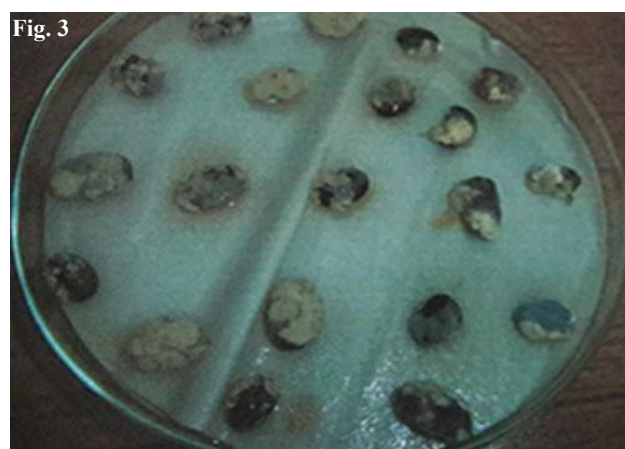


Fig. 2-9. *Alternaria*, *Aspergillus*, *Curvularia*, *Drechslera*, *Fusarium*, *Mucor*, *Penicillium* and *Rhizopus* species on seeds of *P. vulgaris*.

Table 4. Efficacy of different treatments against Mycoflora associated with *Phaseolus vulgaris* seeds.

| Localities | Sterilize water (control) | 1 Heat treatment | 2 Garlic extract | 3 Benomyl | Means |
|------------|---------------------------|------------------|------------------|--------------|---------|
| Kail | 84.33 ± 2.02 | 84.66 ± 2.9 | 91 ± 2.88 | 86.33 ± 3.17 | 86.75B |
| Lawaat | 91.66 ± 4.05 | 97 ± 2.6 | 96.33 ± 2.02 | 97.33 ± 2.02 | 96.00A |
| Bheri | 79.33 ± 3.17 | 78.33 ± 2.6 | 81.66 ± 2.33 | 82.33 ± 3.17 | 80.25DE |
| Chikar | 74.33 ± 4.33 | 81 ± 2.88 | 75.66 ± 2.9 | 83.66 ± 3.48 | 78.75E |
| Bagh | 80.66 ± 2.33 | 78.66 ± 1.76 | 84.33 ± 2.02 | 85.33 ± 2.6 | 82.00D |
| Palangi | 15.33 ± 3.75 | 22.66 ± 2.33 | 22.33 ± 3.17 | 24.33 ± 1.45 | 20.75G |
| Hilaan | 79 ± 3.46 | 86.33 ± 2.02 | 86.33 ± 2.6 | 83.66 ± 2.33 | 84.16C |
| Abaspur | 82.33 ± 3.17 | 88.33 ± 3.17 | 83 ± 2.3 | 85.33 ± 3.17 | 85.25C |
| Trarkhal | 25 ± 2.3 | 47.66 ± 2.9 | 42.33 ± 2.02 | 55.66 ± 2.33 | 42.41F |
| Means | 67.88C | 73.88B | 73.77B | 76.00A | 00.00 |

Table 5. Analysis of variance.

| K-value | Source | Degrees of freedom | Sum of squares | Mean square | F-value | Prob. |
|--------------|----------|--------------------|------------------|-------------|-----------|-------|
| 2 | Factor A | 3 | 1056.630 | 352.210 | 49.7888 | 0.00 |
| 4 | Factor B | 8 | 58149.630 | 7268.704 | 1027.5131 | 0.00 |
| 6 | AB | 24 | 1310.370 | 54.599 | 7.7182 | 0.00 |
| -7 | Error | 72 | 509.333 | 7.074 | | |
| Total | | 107 | 61025.963 | | | |

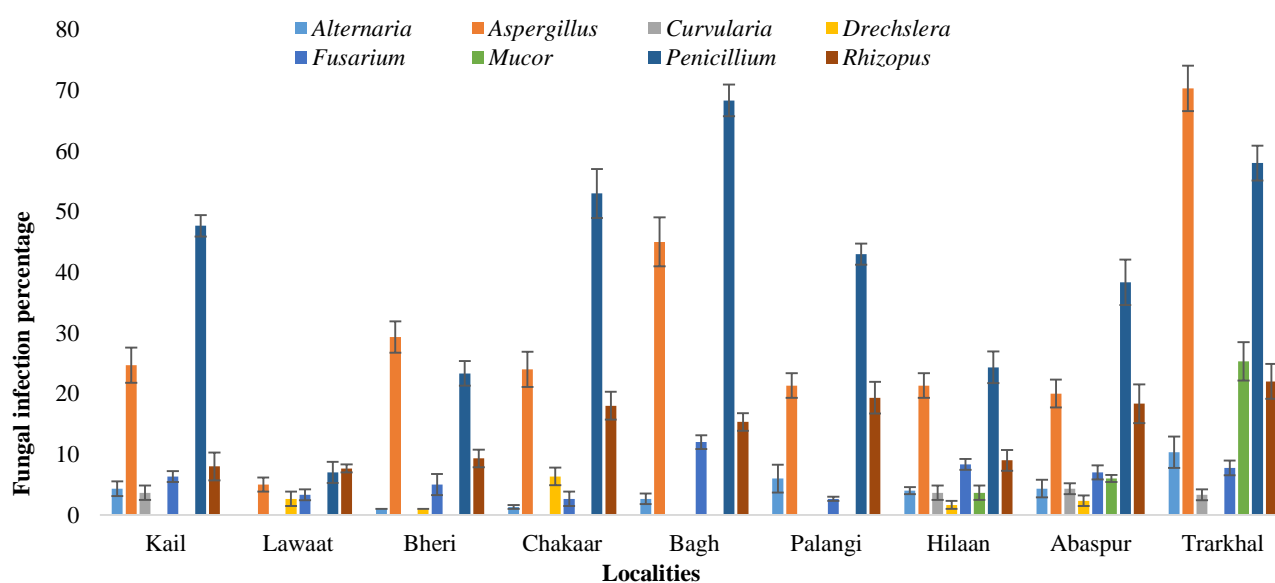


Fig. 10. Fungal infection percentage from all localities.

Discussion

Seeds of *P. vulgaris* collected from various localities of Azad Jammu and Kashmir were found infected with eight different genera of fungi. Our results are in agreement with (Youssef *et al.*, 2008 and Ghangoakar & Kashirsagar, 2013). *Drechslera* is the only genus not found associated with *P. vulgaris* seeds in the literature. Highest incidence of storage fungi was observed, whereas field fungi were in lesser frequency. Infection of fungi ranged from (3.00-24.75) on germinated seeds in different localities.

Viability of seeds varying from (22.00-93.66) indicated various level of fungal prevalence in different communities. Pathogenic Mycoflora is responsible for low germination and low yield of *P. vulgaris* in the state. Low infection and better germination was recorded from the locality of Lawaat (93.66A) followed by Kail and Abaspur (92.33 and 83.66) respectively,

whereas high prevalence and low germination was reported from Palangi (22.00) and Trarkhal (43.33). Mean germination recorded from various localities was (71.95), which was very low. Dominant fungal association was recorded for *Penicillium* (40.3A) and *Aspergillus* (29.00B), whereas lesser for *Drechslera* (1.55F) and *Curvularia* (1.66F) mean. Ismael *et al.*, (2004) described the incidence of fungi and its role in poor seed germination in safflower. Youssef *et al.*, 2008 reported the presence of seed associated mycoflora ranges from 0.2 to 14.5% and varies along with locations and growing conditions. Seed affiliated fungi are involved in deterioration of seed prior to germination, results seedling mortality and foliage infection at adult stage (Altaf *et al.*, 2004). The seed associated diseases are highly devastating as they weaken the seed strength and damage seedlings at early stage of growth (Maiti *et al.*, 1988).

■ *Alternaria* ■ *Aspergillus* ■ *Curvularia* ■ *Drechslera*
 ■ *Fusarium* ■ *Mucor* ■ *Penicillium* ■ *Rhizopus*

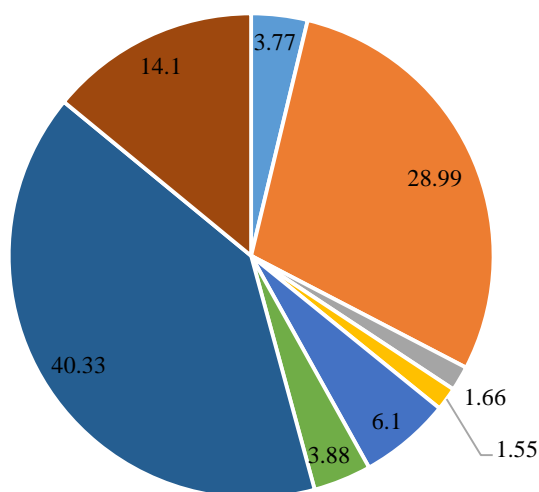


Fig. 11. Mean infection of fungal species from all samples.

Seed treatment is a valuable process that provides security against seed and soil borne plant pathogens and insects (Gwary *et al.*, 2007). In the present study use of different antifungal treatments found effective in increasing the germination (6-8%) and reducing the fungal growth. These strategies were more effective in the localities with poor germination like Trarkhal where increase in germination was 22-30% with different treatments. Benomyl was more effective with mean germination (76.00A) followed by heat treatment (73.88B) and garlic cloves extract (73.66B) respectively. Nasir (2003) stated the efficacy of five systematic fungicides against seed associated Mycoflora of Soybean. Benomyl found more effective. Many investigators have reported the potency of different plant defusates as substitute treatments to synthetic fungicides against soil and seed borne pathogens on cereal crops (Shafique *et al.*, 2007 and Somda *et al.*, 2007), legume crops (Pretorius *et al.*, 2002 and Masangwa *et al.*, 2013). Umechuruba *et al.*, 2013 used physical techniques (dry heat and hot water) to reduce the seed associated mycoflora of *Solanum gilo*. Both treatments were found effective for increasing germination percentage. Pathogen free seeds are foundation for better crops and ultimate requirement for good yield.

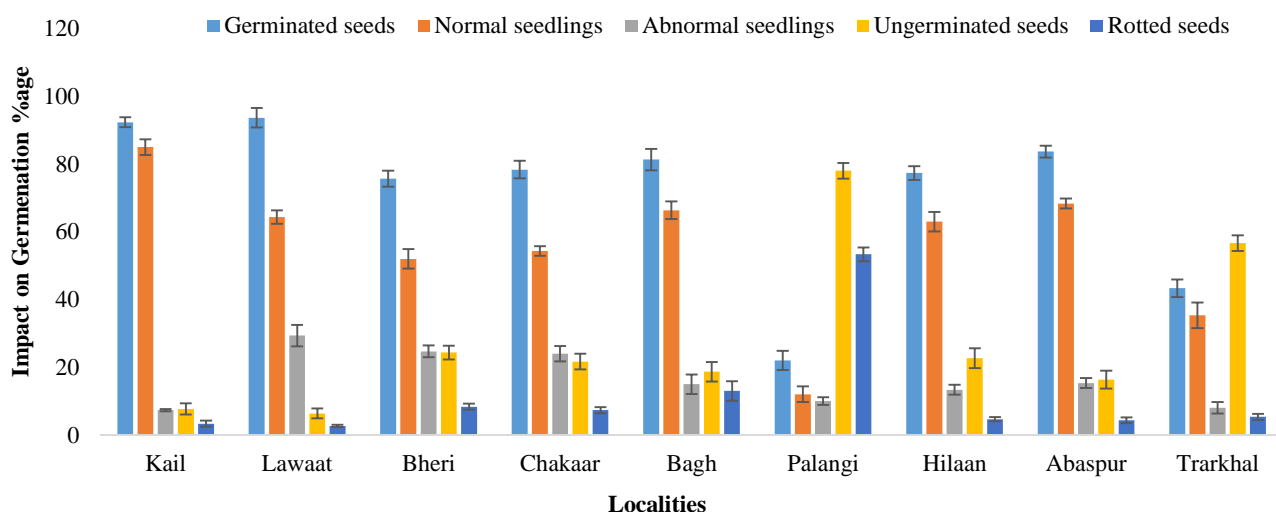


Fig. 12. Impact of Mycoflora on germination percentage in all localities.

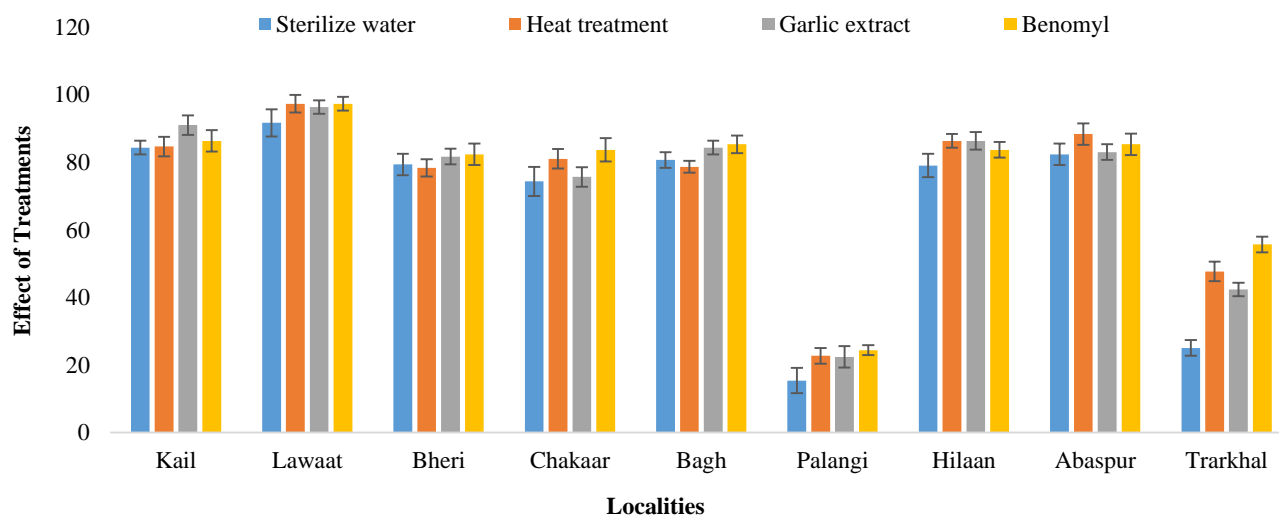


Fig. 13. Efficacy of different treatments against seed associated Mycoflora of *P. vulgaris*.

Conclusion

Prevalence of Mycoflora is responsible for low germination and low yield of *P. vulgaris* in the state. The uses of different antifungal treatments were found effective in increasing the germination percentage and reducing the fungal growth. Seeds should be treated before sowing to obtain maximum germination and better crop production.

Recommendations

Farmers of *P. vulgaris* should treat their seeds with Benomyl solution @2g/L/Kg for 5min, before sowing. They may use 100g/L/Kg garlic cloves extract for 15min. These treatments will increase the germination percentage of *P. vulgaris*.

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