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Evaluation of Mycoflora Associated with Pea Seeds and Some Control Measures

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Abstract: Three commercial pea varieties were studied for the presence of seed associated fungi and their impact on germination percentage of peas. Seed samples were also treated with Chlorox (1%), hot water (52°C for 12-13 min), fungicide (Vitvax @ 1 g kg^{-1}) to study their efficacy. Out of the 12 fungi isolated from pea seed samples, *Alternaria* sp., *Fusarium* sp., *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp., were found in high frequencies and among them *Alternaria* sp., had highest frequency i.e., 38, 27 and 67% in pea varieties, Matter Mateore Local, Matter Mateore Holland and Rondo, respectively under control. These mycoflora were found to cause an average decrease of 17.7% in germination of pea seeds. Pea variety Rondo showed highest infection percentage followed by Matter Mateore Holland and Matter Mateore local. Least infection percentage of local variety shows the increased resistance of local material as compared to exotic varieties and demands more research to utilize its potential. Fungicide treatment gave better results as compared to other treatments.

Key words: *Pisum sativum*, seed associated phytopathogens, seed treatments, *Alternaria* sp., vitavax

INTRODUCTION

Pea (*Pisum sativum* L., Family: Leguminosae) is an important vegetable crop of tropics[1]. It is grown all over the world for its fresh use, preservation, high level of digestibility which is more than most of the legumes. Dried peas have been found to contain 23.5% crude protein, 1.7% ether extract and 2.9% ash[2]. Owning to great nutritional importance, cultivation of peas in the world is increasing. In Pakistan, Mateore, Green feast, Rondo and Climax are the main commercial varieties of peas. These are grown on an area of 91038 ha with a production of 53957 tons[3]. National average yield of peas (576 kg ha^{-1}) is considered to be the lowest in the world and it does not support the demands of country. There are various factors of low yield such as inappropriate agronomic practices, misuse of chemicals, use of unhealthy seeds and continuous mono-cropping.

Besides other causes of low yield, seed associated mycoflora also play an important role. Economically important seed associated mycoflora of peas are *Alternaria* sp., *Aschochyta pisi*, *Fusarium* sp., *Aspergillus* sp., *Penicillium* sp., *Rhizoctonia* sp., Bean yellow mosaic virus, Seed borne mosaic potty virus (PSbMV) and *Pseudomonas syringeae* pv. pisi[4].

In Pakistan, peas are subjected to many seed associated diseases, but so far very little information is available on the role of seed associated mycoflora in poor seed germination and over all yield. In the light of above knowledge this study was planned with following objectives:

- To see the fungal mycoflora associated with different pea seed varieties.
- To note the efficacy of different types of seed treatments in increasing the germination %age and reducing infection %age of different pea varieties.

MATERIALS AND METHODS

Seed samples: Samples of three commercial varieties of peas (Matter Mateore Local, Matter Mateore Holland, and Rondo) were collected from vegetable seed stores and Horticultural Research Institute, National Agricultural Research Center, Islamabad. This experiment was conducted during 2002-2003.

Seed treatments: For each treatment a sample of 200 seeds of each variety was selected.

Treatment with chlorox: Seeds were first washed with distilled water then dipped in 1% chlorox solution for 2 min then again washed with distilled water and dried on blotter paper[5].

Hot water treatment: Seeds were soaked in sterilized distilled water for 5-10 min then placed in hot air oven at 53°C for 12-13 min. The seeds were then dried on blotter paper[6].

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Treatment with fungicide: Vitavax was selected for fungicide treatment. First of all fungicide solution was prepared and then applied to seeds @ 1g kg^{-1} seeds. Seeds were soaked in fungicide solution for 3-4 min then dried on blotter paper[6].

Treatment with sterilized distilled water (kept as control): For treatment with sterilized distilled water, distilled water was sterilized at 121°C and 20-25 lb for 15-20 min.

Testing procedures

Germination percentage: Germination percentage of all seed samples by giving different treatments was noted by using standard rolled paper towel method[7]. Hundred seeds of each seed variety were selected randomly, after giving different treatments seeds were dried on blotter paper and allowed to germinate between two layers of blotter papers at 25±2°C for twelve days under florescent day light tube. At the end of incubation, the number of ungerminated and germinated seeds and seeds with abnormal mortality were counted.

Seed infection percentage: Seed infection percentage of three different pea varieties was counted by Blotter Paper method[8,9]. The seeds were sown in Petri dishes on moistened absorbent paper (blotter paper) after giving different types of treatments i.e., control, chlorox, hot water and fungicide treatment. Two hundreds seeds of each variety were placed at a fixed distance according to their size @ 10-16 per plate and incubated for a week at 20±2°C, the day and night cycle of 12/12 h was used under fluorescent light. The infection percentage was based on presence of the pathogenic signs of disease in/on the seedlings which may have germinated during incubation. Three successive readings were taken for 3 weeks.

Isolation, purification and identification of various fungi: To identify and purify fungal micro flora, fungi from seeds were cultured on potato dextrose agar medium (PDA). Fungal associations were examined under stereoscope. Identification of fungi was done with the aid of lab manual[10].

RESULTS

Efficacy of various treatments: Seed germination and infection percentage pea seed samples was recorded with different treatments i.e., Clorox (1%), hot water (52°C for 12-13 min), fungicide (Vitvax @ 1 g kg^{-1}). Germination percentage was highest in case of fungicide treatment in all pea seed samples i.e., 95.33% as compared to control i.e., 75%. Seed infection percentage successfully increased from 1st to 3rd week. Least infection percentage was observed in case of fungicide treatment (Vitvax @ 1 g kg^{-1}) i.e., 46.7% as compared to control i.e., 61.7% (Table 1). Seed associated mycoflora had significant impact on germination percentage of

Table 1: Efficacy of various treatments in increasing germination % and decreasing infection %

Treatments	Germination %	**Infection %
Control	75a*	61.77a
Chlorox	93c	57.55b
Hot water	89.67b	55.28bc
fungicide	95.33cd	46.70d

*Any two means not sharing a common letter(s) differ significantly (p = 0.05), **Average of three weeks data

Table 2: Comparison of different verities of peas

Varieties	Germination %	Infection %
MML**	92a	44.25a*
MMH	87.25b	55.32b
RONDO	85.50bc	78.03c

*Any two means not sharing a common letter(s) differ significantly (p =0.05)
**MML stands for Matter meteor local and MMH stands for Matter Meteor Holland.

Table 3: Impact of various microflora on germination %age of peas

Treatments	MML	MMH	Rondo
Treatments*	96.7%	91.3%	90%
Control**	78%	75%	72%
Decrease in Germination	18.7%	16.3%	18%

Average decrease in germination %age of 3 varieties=17.7%
*Chlorox, Hot water and fungicide treatments.
**Just washed with distilled sterilized water.

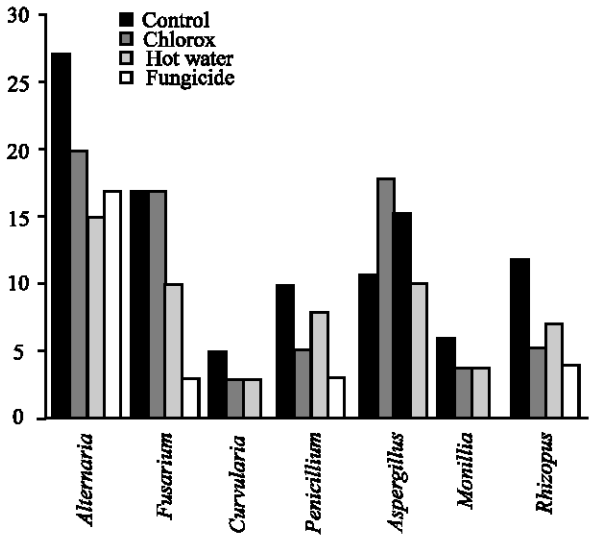

Fig. 1: Mutter meteor Holland

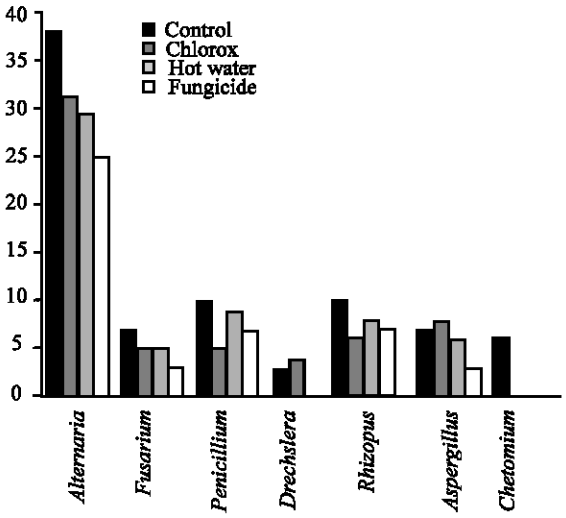

Fig. 2: Mutter meteor local

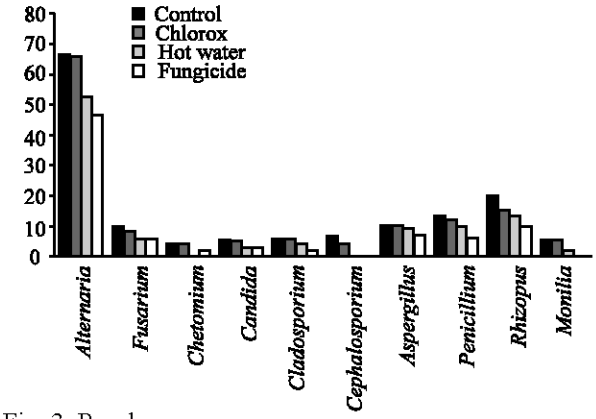

Fig. 3: Rondo

peas and were found to cause an average decrease of 17.7% in germination percentage of different pea varieties (Table 3).

Incidence of various mycoflora: Twelve species of various mycoflora were found to be associated with pea seed samples of different varieties. These include *Alternaria* sp., *Aspergillus* sp., *Candida* sp., *Chetomium* sp., *Cladosporium* sp., *Curvularia* sp., *Drechslera* sp., *Fusarium* sp., *Monilia* sp., *Penicillium* sp., *Rhizopus* sp., *Cephalosporium* sp., (Fig. 1-3). Out of these, five fungi i.e., *Alternaria* sp., *Fusarium* sp., *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp., were found in high frequencies and among them *Alternaria* sp., had highest frequency i.e., 38, 27 and 67% in pea varieties Matter Mateore Local, Matter Mateore Holland and Rondo variety, respectively in control (Fig. 1-3).

Comparison of different pea varieties: Among three varieties of peas, Matter Mateore Local proved to be more resistant by showing highest germination percentage i.e., 92% and least infection percentage i.e., 44.25% and it showed least incidence of various mycoflora as compared to other two varieties. Rondo variety showed highest infection percentage i.e., 78.03% and highest incidence of various mycoflora with minimum germination percentage i.e., 855% (Table 2).

DISCUSSIONS

All treatments were found to be significant in increasing germination percentage but not so much significant in reducing infection percentage.

Treatment with fungicide i.e., Vitavax @ 1 g kg^{-1} proved to be most effective. Vitavax is a systemic fungicide. Ahmad *et al.*[5] found that treatment with Vitavax @ 2 g kg^{-1} give 87.50% germination with 5724.45 kg ha^{-1} dry grain yield in maize as compared to control with 15.2% germination and 4835.56 kg ha^{-1} dry grain yield. It has also been reported to increase germination percentage upto 25.37% in sorghum[11] and 53% reduction in seed associated *Fusarium solani* in onion[12]. Chlorox and hot water treatment were not found to be effective as fungicide (Vitavax 1 g kg^{-1} of seed) because chlorox is only surface disinfectant[5], while hot water treatment[6] is effective mostly for nematodes, most of the fungal spores are above to survive even after hot water treatments.

Efficacy of Vitavax @ 1g kg^{-1} confirmed the findings of Ahmad *et al.*[5], Javed *et al.*[12], Haq and Khan[11].

High incidence of *Alternaria* sp., *Fusarium* sp., *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp. shows the economic importance of these mycoflora and confirm the findings of Hagedorn[4], Czyzewka[13] and Anwar *et al.*[14]. *Alternaria* sp., is one of the most economically important mycoflora associated with pea seed and plays a significant role in reducing over all vigor and yield of peas[15]. *Fusarium* sp., also has a significant role in reducing germination percentage and over all yield of peas[14]. While saprobes like *Aspergillus* sp., *Penicillium* sp., *and Rhizopus* sp., associated with pea seeds also play an important role in seed germination by producing toxins[16]. Seed associated mycoflora had significant impact on germination percentage of peas and were found to cause an average decrease of 17.7% in germination percentage of different pea varieties (Table 2). This confirmed the findings of Susuri *et al.*[15] and Anwar *et al.*[14].

Highest germination percentage, least infection percentage and incidence of various mycoflora in pea variety Matter Mateore Local showed increased resistance of local varieties as compared to exotic verities and this demands need of more research to utilize the potential of local material.

This study shows the impact of various mycoflora on germination of pea seeds and efficacy of various treatments for its control and it will be helpful in doing more research regarding seed pathology of peas.

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