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IMPACT OF PATHOGENIC FUNGI AND BACTERIA ON FENUGREEK *(TRIGONELLA FOENUM-GRAECUM* L) PLANT STAND QUALITY UNDER NATURAL CONDITION

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ABSTRACT

An investigation was carried out to assess the effect of fungal and bacterial pathogen levels on Fenugreek *(Trigonella foenum-graecum* L) seedlings grown in sand. Both the fungal and bacterial inoculum affected the mortality of crop seedlings. Crop responded significantly to inoculum as decreased number and size of leaf and also length of seedling but has over growth of root as bunching and elongation of root length by 32-78%. Small water-soaked lesions on leaf margins appeared which eventually turned brown with the increase of day interval. Leaves and petioles showed discoloration. Formation of adventitious root, wilting of leaves and young stems, defoliation, and marginal necrosis of remaining leaves was also observed.
Key words: discoloration, Fenugreek wilting, bunching and defoliation.

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum*), an annual legume native to the Mediterranean region, locally known as *Methi*, is cultivated in Pakistan as well as other parts of the world not only as a leafy vegetable but also for medicinal purposes (Som & Maity, 1993). In Pakistan Kasoori Methi, is known for its appetizing fragrance. It is cultivated in counties India, Argentina, Egypt, Southern France, Morocco and Lebanon. Green methi is a good source of iron (Fe) as well as other minerals for human beings (Chhibba *et al*., 2000). Seeds contain proteins 26%, Water-soluble polysaccharide (galactmannan) 20%, hemi-cellulose and cellulose 24.5%, Water 9%, Fat (fenugreek oil) 7%, Lignin 2.5%, and Saponin 8-10%.

It is best suited to neutral to alkaline soils, sandy loam to friable clay and not suited to acidic duplex clays in regions with 380-530 mm average annual rainfall. Salt tolerance is found to be moderate.

Little information exists concerning the response of this crop to applied nutrients, particularly, the micronutrients. In Punjab, *Methi* is very commonly grown as a leafy vegetable. Since the soils of this state are prone to Fe deficiency (Takkar *et al*., 1989), because of being alkaline, fenugreek raised on them may suffer from a short supply of this nutrient. In Pakistan its seed in vegetable markets is sold under highly contaminated environment, therefore, present analysis for the effect of seed associated pathogenic complex on early growth and plant stand quality was done.

Very little information on its susceptibility against fungal and bacterial pathogen is available. According to available literature this crop is subjected to the attack of number of fungal and bacterial pathogens. Among the fungal diseases Cercospora leaf spot and powdery mildew caused by *Erysiphe polygoni* , blight disease caused by *Cercospora traversiana* and wilt caused by *Fusarium oxsysporum* and *Rhizoctonia solani* can reduce crop yield and has the potential to affect biomass and seed yield in crops grown under a wide range of climatic variations (Jongebloed, 2004;Prakash and Sharma, 2000 and AAFRD, 1998). Other well known fungal diseases observed to be associated with fenugreek are collar rot, leaf spot and pod spot diseases (Petropoulos, 2002). Fungal pathogen isolated from fenugreek include *Rhizoctonia*, *Phoma*, *Erysiphe*, *Alternaria* and *Cercospora*. In most cases foliar fungal pathogens have not been yield limiting. Bacterial and fungal plant infections can have similar symptoms.

Among the bacterial diseases bacterial leaf spot in fenugreek caused by *Pseudomonas syringae* pv. syringae and *Xanthomonas alfalfa* leads to loss in productivity (Petropoulos, 2002).

Delayed sowing, reducing crop stress and sensible rotations will reduce the chance of bacterial blight. Clean seed will also help but there is no reliable commercial seed test currently available. Bacterial blight can flair up when the crop is under stress (due to frost, herbicide damage, fungal infection).

MATERIALS AND METHODS

Earthen pots of 18x30 cm were filled with fine river sand sterilized at 60^{o} C for an hour. Pots were under natural conditions and irrigated as and when required. Fenugreek (*Trigonella foenum-graecum*L.) Seeds were procured from local market and were initially visually examined for its quality and purity. During the process of visual examination, inert matter, broken seed and mixing of other seeds were separated in accordance with the guidelines of Federal Seed Certifications and Registration Department (FSCRD), MINFAL Islamabad Pakistan. Pure healthy seeds were soaked in 1% NaOCl for few minutes. After soaking, these seeds were sown in the prepared, labeled pots. There were 10 seeds sown in each pot at a depth of 0.5cm. After sowing Plant stand was observed till vegetative growth stage and observations were recoded as per ISTA rules 1996. Key parameters were mortality %age,plant height and root shoot length at germination, three leaf, seedling and vegetative growth stages Seed germination data for each pot was started taking the next day after sowing for seven days. After seven days, the seedling data was started taking till the arrival of vegetative stage. When the seedlings had entered the vegetative stage, plants were inoculated with fungal and bacterial inoculum in seven treatments and a control. Each treatment including the control had five replicates. First five pots were referred as control. To the next five pots inoculum of *F. oxysporum* was inoculated and it was referred as treatment number 1

Control= no inoculum
T_1= *Fusarium oxysporum*
T_2= *Drechslera biseptata*
T_3= *Pseudomonas syringae*
T_4= *Xanthomonas campestris*
T_5= *Fusarium oxysporum + Drechslera biseptata*
T_6=*P. syringae + Xanthomonas campestris*
T_7= *Fusarium oxysporum + Drechslera biseptata + Pseudomonas syringae + Xanthomonas campestris*

The inoculum dose for each of fungal and bacterial treatment was maintained at 2%level as it was closer to its presence of soil microbes under natural conditions in Pakistani soils (Khan, 2002). Observations were recorded at 24 hours interval. The observations were recorded on plant mortality and systematic changes taking place in plant in response to inoculum addition. Harvest was taken two weeks after inoculation when plants in control set of treatments achieved vegetative growth stage. Number of leaves, number of roots, root and shoot length, biomass, and dry weight of each plant in each pot was recorded and represented graphically.

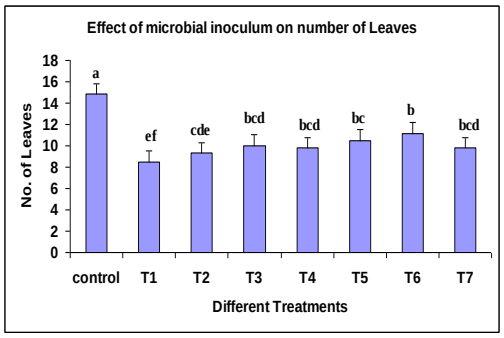
RESULTS AND DISCUSSION

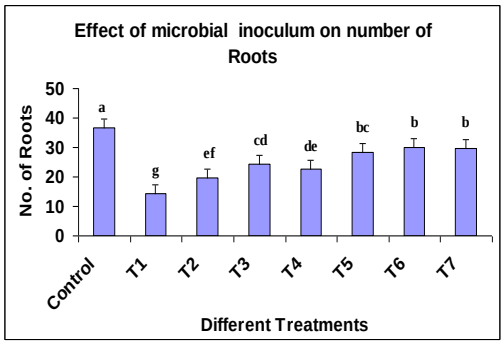
Very little information is available on seed pathology of spices and culinary crops because they are cultivated on smaller units and specified pockets in a particular agro ecological zone in a country. Hashmi (1988) reported association of fungi from 23 globally collected samples of *Trigonella foenum-graecum*. Nagerabi (2002) reported :*Aspergillus stellifer* and *Emericella variecolor*. The genus *Aspergillus* (15 species and 8 varieties) is the most prevalent, followed by *Drechslera* (3 species), *Rhizopus* (3 species), *Alternaria* and *Fusarium* (6 species each), *Emericella* (4 species and 2 varieties) *Cladosporium* and *Penicillium* (4 species each), *Chaetomium* (3 species) and *Curvularia*. Khan *et al*. (2006) pointed out in a survey conducted on carelessness in seed trade of medicinal plants that with the assumption of their toxic reaction against microbes they are safe for end-user ordinary unhygienic or contaminated environment of picking and marketing.

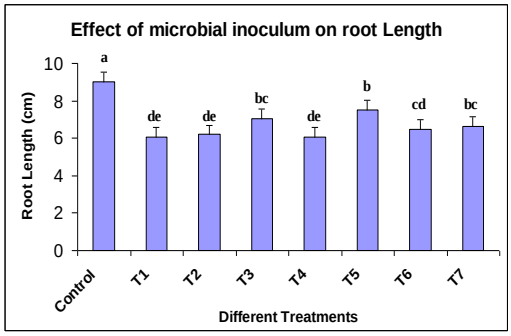
Number of Leaves: The maximum percentage of number of leaves observed was 73%, in the pots which were given the mixed inoculum of bacterial cultures of *Pseudomonas syringae* and *Xanthomonas campestris.*
This shows that minimum reduction in the number of leaves which was 27%, was done by the mixed bacterial inoculum. The minimum percentage of leaves was observed in *F. oxysporum* inoculum pots 55%, showing that *F. oxysporum* reduced the number of leaves to maximum which was 45%.
In the inoculum with *Drechslera biseptata*, percentage of number of leaves was 59%. In the treatments with *Pseudomonas syringae* and *Xanthomonas campestris*, the percentage of number of leaves was 67% and 59% respectively showing that bacterial cultures separately had not affected the number of leaves to a greater extent. In the pots with mixed inoculum of fungal cultures of *F. oxysporum* and *D. biseptata* the percentage recorded was 67% and 59% in those having the inoculum of all the four microbes.

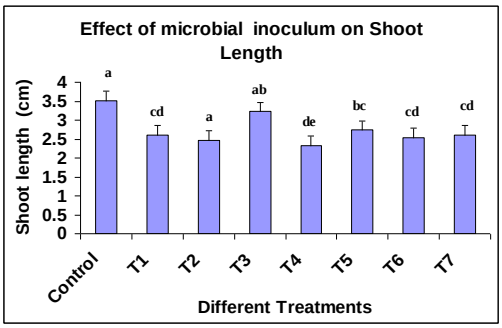
Effect of Microbial Inoculum on Number of Roots: The highest root development was recorded in the treatments T_5 (*Fusarium oxysporium+ Drechslera biseptata*) and T_6 (*Pseudomonas syringae+ Xanthomonas campestris*) for percentage of number

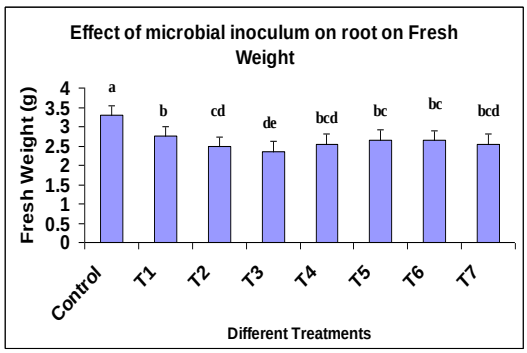
Effect of Fungal (*F. oxysporum and Drechslera biseptata*) and Bacterial (*Pseudomonas syringae and Xanthomonas campestris)* inoculums on Seedlings of Fenugreek (*Trigonella foenum-graecum*)

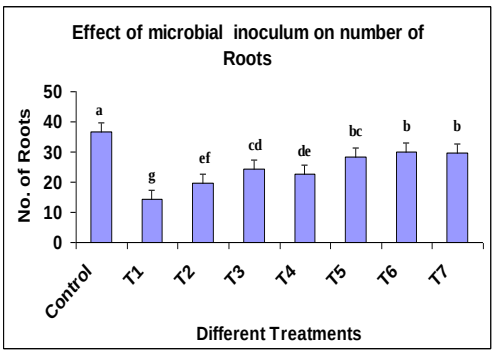












Note :

Control	T4=B2
T1 = F1	T5=F1+F2
T2 = F2	T6=B1+B2
T3 = B1	T7=F1+F2+B1+B2

FI= *Fusarium oxysporum*
F2= *Drechslera biseptata*
B1= *Pseudomonas syringae*
B2= *Xanthomonas campestris*

of roots recorded was 98% in two treatments. These were pots which had a bacterial and fungal complex e. g *F.oxysporum*, *D. bisepte*a, *P. syringaean and*. *X. campestris* .it also shows that both bacterial inoculum together did not affect the number of roots. The percentage was lowest at 49% in the treatment with *F. oxysporum* indicating maximum inhibition of root growth. 65% root growth was observed due to *D. biseptata* and 68% was observed due to *F. oxysporum* ans *D. bisepte*a. *P syringae* provided 81% roots to grow while *X. campestris* had allowed 75% roots.

Effect of Microbial Inoculum on Root Length: Root length was most affected upto 32% in treatments with *F. oxysporum* and *X. campestris.* Both inoculum allowed only 68% long roots. Root length was maximum at in the mixed fungal inoculum refering to the fact that both fungal together had least effect on root length. Root length in *D biseptata* treatment was 69% and in the *P. syringae* inoculum, it was 78%. The mixed bacterial inoculum had allowed 73% root length while all the four microbes together allowed 75% root length.

Effect of Microbial Inoculum on Shoot Length: Shoot length was recorded highest as 78% in pots where both fugal inoculum were present together as well as those which were treated with *Pseudomonas syringae.* It was minimum at 67% in pots of *X.campestris* indicating a reduction of 33%. *F. oxysporum,* mixed bacterial inoculum as well as all four microbes, all three treatments gave a 75% shoot length. Shoot length was 69% in treatment with *Drechslera biseptata.*

Effect of Microbial Inoculum on Fresh Weight: Fresh weight when measured was maximum at 83% in pots with *F. oxysporum* . This meant that *F. oxysporum* had deteriorated only 17% of the seedling biomass. Both fungus together and both bacterias together had a biomass of 77%. The mixed inoculum of all four microbes, and treatment with *X.campestris* had a biomass percentage of 74%. *D.biseptata* had a 71% biomass and fresh weight was minimum at 62% in the pots with *Pseudomonas syringae* showing a reduction in the biomass upto 38%.

Effect of Microbial Inoculum on Dry Weight: Dry weight was recorded at a maximum of 13.79% showing least loss of dry weight where as, it was minimum at 3.4% showing highest loss of dry weight of 96.6%. It was almost equal in all other treatments, showing dry weight of about 6.89%.

In pots with fungal inoculums, decreased number and size of leaf and also length of seedling was observed but over growth of root had occurred as bunching and elongation of root length took place. Small water-soaked spots on leaf margins appeared which eventually turned brown as the days passed. (*Wasnikar et al. (1991*). Leaves and petioles showed discoloration. Formation of adventitious root, wilting of leaves and young stems, defoliation, and marginal necrosis of remaining leaves was also observed. (Mushtaq and Hashmi (1997)

No significant difference was observed among the sets of treatments in germination of seed to three leaf stage it was perhaps due to the potential energy of seed. Inoculum of the fungus within the plant root and penetrating to the stem, the plant's water supply is greatly affected. This lack of water induces the leaves to shrink, the leaves wilt, and the plant eventually dies. *(*Ahmed *et al.*, 1994; Dawar, 1994). It is at this point that the fungus invades the plant's parenchymatous tissue, until it finally reaches the surface of the dead tissue, where it sporulates abundantly (Agrios, 1988). Bacterial strains of *Pseudomonas syringae* had an antifungal effect. They through the release of allelochemicals, inhibit the spore germination of *F. oxysporum*. There was reduced effect on seedlings of *F. oxysporum* inoculum where *Pseudomonas campestres* inoculum was also given. On the basis of results obtained, it was assessed that the severe symptoms were expressed when the two pathogen were inoculated simultaneously. The mechanism involved in disease expression through toxin production was complex for which molecular techniques are required to explain a host-pathogen relationship (Dangl, 1994; Ryley, 1989).

F.

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