



TRIGONELLA FONEUM-GRAECUML AND FUNGAL DISEASES

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Paper Received On: 20 July 2024

Peer Reviewed On: 24 August 2024

Published On: 01 September 2024

Abstract

The leafy vegetables viz Fenugreek (Trigonella foneum-graecum L.), was selected for the study. Commonly three diseases i.e. Leaf spot caused by Alternaria alternata wilt caused by Fusarium oxysporum and powdery mildew caused by Erysiphae polygoni are found with Fenugreek (Trigonella foneum-graecum L.). Fenugreek (Trigonella foneum graecum) Isolated pathogen viz. Alternaria alternata and Fusarium oxysporum were tested against Chlorothalonil, Mancozeb and Carbendazim in in vitro and in vivo studies. Out of which Mancozeb showed positive results in MIC i.e .ranged form 80 g/ml to 3100 g/ml in vitro and in case of in vivo studies it was ranged from 200 g/ml to 4500 g/ml of these fungi.

Key Words: Leafy vegetable, Disease, Fungi, Pathogenicity.

Material and Methods

The diseased samples were collected from various part of the Aligarh region of Uttar Pradesh State. Surveys which was commenced from June 2018 to July 2019 in growing season. Delicious leafy vegetable crops are attacked by many fungal diseases like, leaf spot, leaf blight, damping off, wilt, root rot, rot (post harvest) and powdery mildews. During the surveys the following diseases were found. Fenugreek (Trigonella foneum-graecum L.): It is found commonly three diseases i.e. Leaf spot caused by Alternaria alternata wilt caused by Fusarium oxysporum and powdery mildew caused by Erysiphae polygoni.

Symptomatology of pathogen

Alternaria alternata- The leaf spot disease occurs as a typical blackish spot on leaves, leaf spot vary in size from pin point up to one to two cm in diameter and dark ash colored spot with concentric rings appeared.

Fusarium oxysporum - It shows total leaves exhibiting wilting process

Erysiphae polygoni - Powdary white growth on both surfaces.

Pathogenicity test

Pathogenicity (on leaves)- For pathogenicity test isolates were grown on PDA for 7 days inoculation were done using detached surface sterilized on leaves. A single drop (5 l) of spore suspension (1×10^3 conidia/ml) was placed on each leaves. Leaves were incubated in humid growth chamber (80-90% relative humidity) for intensity with a photoperiod of 12h. After 8 days, leaf spots similar to the original symptoms were developed on all tested leaves and root was consistently reisolated fulfilling Koch's postulates (Tetarwal et al., 2008). Control leaves inoculated with sterilized distilled water remained symptomless.

Sensitivity of isolated pathogen viz. *Alternaria tenuissima*, *Fusarium proliferatum*, *Pythium* sp., *Alternaria spinaciae*, *Fusarium oxysporum* f. sp. *spinaciae* *Alternaria alternata*, *Fusarium oxysporum*, *Fusarium oxysporum* (1) and *Phytophthora colocasiae* were tested against Chlorothalonil (75% WP), Mancozeb (75% WP), Carbendazim (50% WP) and Copper oxychloride (50% WP) of in vitro and in vivo studied by food poisoning technique (Dekker and Gielinck, 1979). Czapek Dox Agar (CZA) plates were prepared containing different concentration (50-5000 g/ml) of fungicides. Mycelial mats (8mm disc) of the isolates were inoculated at the centre of plates in triplicates. The plates were then incubated at $28 \pm 2^\circ\text{C}$ in dark or BOD incubator and radial growth was measured at different intervals. Without fungicides of plates was served as control. Calculation as percentage method by Vincent (1947).

$$L = \frac{(C - T)}{C} \times 100$$

L = Percentage of inhibition,

C = Growth of fungus in control,

T = Growth of fungus in the treatment.

Isolation of fungal DNA

DNA was extracted from fully grown sporulated fungus (SDS Ammonium acetate method). Loopful of the tissue was ground in 1.5ml extraction buffer (50mM TrisHCl, 50mM EDTA), 250mM NaCl, 1.5% Sucrose). The cells were pelleted at 10000 rpm for 5 mins. The pellet was resuspended in suspension buffer (50mM TrisHCl, 10mM EDTA) and 80 l of SDS (20%) was added to it. The solution was vortexed and incubated for 30min at 65°C , 200 l of

7.5M Ammonium acetate was then added to the above solution and mixed by inverting. The tubes were incubated at RT for 15 minutes. DNA was extracted using 1 volume of chloroform: isoamyl alcohol mixture (24:1) and centrifuging at 10000rpm for 7mins. Equal or double volume of ethanol (96-100%) was added to the aqueous phase in a new tube, inverted twice and allowed to stand at 40°C for 1 hour. The mixture was then centrifuged at 10000 rpm for 15 minutes. After drying for few seconds pellet was dissolved in elution buffer (10mM TrisHCl, 1mM EDTA). The DNA was stored at 20°C for further use.

Trigonella foneum graecum L. Diseases:

Disease 1 - Leaf spot

Symptoms - The leaf spot disease occurs as a typical blackish spot on leaves, leaf spot vary in size from pin point up to one to two cm in diameter and dark ash colored spot with concentric rings appeared.

Casual organism - *Alternaria alternata*(Fr.) Keissler

Colony Characters: Colonies usually black conidia formed in long, often branched chain, overall length 2063(37) µm, 918 (13) µm thick in the broadest part; beak pale, 2.5 µm thick.

DISEASE 2 – Wilt

Symptoms - It shows total leaves exhibiting wilting process

Casual organism – *Fusarium oxysporum* (Schl ex fr)

Colony Characters: Colonies was cottony whitish, soft texture. .

DISEASE 3 – Powdery Mildew

Symptoms - Powdary white growth on both surfaces

Casual organism – *Erysiphe polygoni*

Colony Characters: -----

It was noted that the content of all parameters in the pathogen varied in sensitive and resistant strains. Moisture in the *Trigonella foneum graecum* infected with sensitive and resistant strains was decreased. Among them Crude protein was increased in healthy leaf, sensitive and resistant followed by Iron, Total ash, Phosphorus and Ascorbic acid, healthy leaf sensitive and resistant. In case of total sugar it was in reduced infected leaf. But Calcium and Fat however was decreased due to infection of both isolates.

Biochemical changes in Fenugreek (*Trigonella foneum-graecum*L.) due to fungal infections are evaluated in the given table-

Table 1: Biochemical changes in Fenugreek (*Trigonella foneum-graecum*L.) due to fungal infection

S No	Estimation	Fenugreek (<i>Trigonella foneum-graecum</i> L.)				
		Healthy	Aa(S)	Aa(R)	Fo(S)	Fo®
1	Moisture (%)	15.53	10.20	11.54	09.10	10.30
2	Fat (%)	0.55	0.39	0.41	0.25	0.35
3	Crude protein (%)	12.99	9.11	10.12	10.15	11.11
4	Crude Fiber	1.12	0.6	1.25	0.74	1.62
5	Total Sugar (mg/g)	4.10	2.55	2.66	2.54	2.82
6	Reducing Sugar (mg/g)	2.60	1.55	2.10	1.66	1.80
7	Non Reducing Sugar (mg/g)	1.55	0.77	0.86	0.89	0.95
8	Total ash	10.65	7.65	9.22	6.98	9.98
9	Ascorbic acid	9.90	7.86	9.21	5.96	8.99
10	Phosphorus	9.95	12.20	8.88	11.98	6.88
11	Iron (mg/100g)	1.66	9.55	10.22	9.85	10.05
12	Calcium	0.96	0.33	0.61	0.35	0.59

Aa = *Alternaria alternata*, Fo = *Fusarium oxysporum*

Synergistic effects of pathogens with other agrochemicals -

Fungicides

The synergistic effects of fungicides such as Mancozeb, Copper Oxychloride and Chlorothalonil were evaluated against *Alternaria tenuissima*, *Alternaria spinaciae*, *Alternaria alternata*, *Pythium* sp., *Fusarium proliferatum*, *Fusarium oxysporum*, f. sp. *spinaciae*, *Fusarium oxysporum*, *Fusarium oxysporum*(1) and *Phytophthora colocasiae*. In Mancozeb, 25 and 50 g/ml was showed synergistic effects against *Phytophthora colocasiae* and *Pythium* sp. but in *Fusarium proliferatum* was found completely inhibition. While it was completely inhibitory Mancozeb @ 100 g/ml against *Alternaria spinaciae*, *Alternaria alternata* and *Fusarium oxysporum*. The Copper Oxychloride exhibited synergistic effects against *Alternaria tenuissima*, *Alternaria alternata*, *Alternaria spinaciae* and *Fusarium oxysporum* at 100 g/ml but in *Fusarium proliferatum* was found completely inhibition. In Chlorothalonil against *Fusarium oxysporum* f. sp. *spinaciae*, *Fusarium oxysporum* and *Phytophthora colocasiae* were completely inhibited at 25 g/ml.

Insecticides

The synergistic effects of insecticides such as Phorate showed completely inhibited @ 25 g/ml on *Phytophthora colocasiae*, *Pythium* sp. and *Fusarium oxysporum* while Phorate was inhibitory @ 100 g/ml on *Fusarium oxysporum* f. sp. *spinaciae*. The Endosulphan showed

completely inhibited @ 25 g/ml on *Pythium* sp., *Alternaria alternata*, *Fusarium oxysporum*, *Fusarium oxysporum* (1) and *Phytophthora colocasiae*, while *Alternaria spinaciae* was inhibitory at @ 50 g/ml. In case of Chlorpyrifos only *Pythium* sp. and *Phytophthora colocasiae* were completely inhibitory @ 25 g/ml.

Herbicides

Experimental Results The synergistic effects of herbicides Atrazine was slightly inhibitory against *Alternaria tenuissima*, *Pythium* sp. and *Fusarium oxysporum* f. sp. *spinaciae* @100 g/ml but 2, 4, D sodium salts was also slightly inhibitory against *Fusarium oxysporum* f. sp. *spinaciae* and *Phytophthora colocasiae* @100 g/ml. Metribuzin was inhibitory against *Fusarium proliferatum* @100 g/ml. Herbicides are not complete significantly inhibitory against the pathogens .

Antibiotics

In case of antibiotic Penicillin was showed inhibitory @ 0.1% on *Phytophthora colocasiae*, *Pythium* sp., *Alternaria spinaciae*, *Fusarium oxysporum*, *Fusarium proliferatum*, *Fusarium oxysporum* f. sp. *spinaciae*, while complete inhibition was found against *Alternaria alternata* at 0.4%. While Streptomycin was showed inhibitory @ 0.1% on *Phytophthora colocasiae*, *Pythium* sp. and *Fusarium oxysporum*. Ampicillin was inhibitory *Phytophthora colocasiae*, *Pythium* sp., *Alternaria tenuissima* at @ 0.1% and @ 0.4% on *Alternaria alternata* and *Fusarium proliferatum* .

Salts

In salts, Mercuric Chloride @ 0.1 g/ml completely exhibited synergistic effects against all tested pathogens. The Sodium Chloride @0.4 g/ml on *Fusarium oxysporum* f. sp. *spinaciae*, *Pythium* sp., *Fusarium oxysporum* and *Fusarium proliferatum* inhibited the radial growth.

Fertilizers

The synergistic effects of fertilizers such as Urea showed inhibitory@ 0.1 g/ml against *Pythium* sp. and @ 0.4 g/ml on *Fusarium oxysporum* while DAP showed inhibitory @ 0.1 g/ml on *Phytophthora colocasiae*, *Fusarium proliferatum*, *Fusarium oxysporum* f. sp. *spinaciae* and *Pythium* sp. and @ 0.4 g/ml on *Fusarium oxysporum*. Completely inhibitory on *Alternaria tenuissima* *Alternaria spinaciae* and *Pythium* sp. and@ 0.4 *Phytophthora colocasiae*, *Fusarium proliferatum*, *Fusarium oxysporum* f. sp. *spinaciae* inhibited the radial growth.

Fenugreek (*Trigonella foneum-graecum*) **Experimental Results** Plant leaf extracts was tested against two pathogenic fungi to determine their antifungal activity. *Azadirachta indica* leaf

extract showed significant reduction of radial growth of *Alternaria alternata* (78.88%) and *Fusarium oxysporum* (68.88%) at 100% conc. respectively. There was no significant reduction of radial growth in case of *Ocimum gratissimum*, *Santalum album* and *Aegle mormelos*.

Findings:

There are found commonly three diseases i.e. Leaf spot caused by *Alternaria alternata* wilt caused by *Fusarium oxysporum* and powdery mildew caused by *Erysiphe polygoni* with Fenugreek (*Trigonella foneum-graecum* L.). Fenugreek (*Trigonella foneum graecum*) Isolated pathogen viz. *Alternaria alternata* and *Fusarium oxysporum* were tested against Chlorothalonil, Mancozeb and Carbendazim in in vitro and in vivo studies. Out of which Mancozeb showed positive results in MIC i.e. ranged from 80 g/ml to 3100 g/ml in vitro and in case of in vivo studies it was ranged from 200 g/ml to 4500 g/ml of these two fungi. Among the different Sulphate sources (0.1%), Magnesium Sulphate, Sodium Sulphate, Ammonium Sulphate and Copper Sulphate were more favorable for the growth. Among the five Vitamin sources (0.01%), Riboflavin, Ascorbic acid, Thiamine and Pyridoxine were tested with tested pathogens resistant strains of all test pathogens showed higher growth on all the Vitamin sources. Among these Vitamin sources, Riboflavin was inhibitory to the sensitive strains of test pathogens. Amino acid (0.3%) of the test fungi were evaluated by treating with four different Amino acid sources viz. Lysine, Leucine, Tyrosine and Tryptophan. Resistant strains of all test pathogens gave higher growth on all the Amino acid. Among these, Amino acid sources Tyrosine was inhibitory to the sensitive strains of test pathogens. Biochemical changes showed that there are significant variations between artificially inoculated and healthy leafy vegetables which served as control. It showed significant changes with respect to estimation of Moisture, Ash, Fat, Crude protein, Carbohydrates, crude fibre, Phosphorus, Iron, Ascorbic acid, Calcium, Reducing sugar, Non reducing sugar and total sugar.

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