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Effect of resistance inducers on *in vitro* inhibition of mycelial growth and sporulation of *Fusarium solani* causing root rot of fenugreek

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Abstract

Fenugreek is a leafy vegetable plant known for its medicinal, pharmaceutical and nutraceutical properties. The root rot of fenugreek is more severe in recent years. Controlling this disease depends mainly on use of fungicides. The use of resistance inducers is an effective and non-fungicide alternative with antimicrobial properties and also induces resistance in plant. All isolates of *Fusarium solani* had potency to cause root rot disease but the isolate from Jaygaon locality was found highly virulent one and was selected for *in vitro* studies. The inhibitory effect of plant resistance inducers *viz*. chitosan (1-15 mg/ml), methyl jasmonate (1-20 mM) and salicylic acid (20-80 mM) on linear growth and sporulation of *F. solani* was evaluated by poisoned food technique. The obtained results showed that chitosan had significantly reduced linear growth and sporulation of *F. solani* with increase of concentrations when compared to control. The complete inhibition of both linear growth and sporulation was observed at 15 mg/ml and 10 mg/ml concentrations respectively. Methyl jasmonate (MeJa) had also significantly reduced linear growth in a concentration dependent manner. The highest fungal growth reduction (76.37 %) was observed with 20 mM concentration. It was interesting that MeJa had halted spore formation of fungus at10 mM concentration. The increasing concentrations (20, 40 and 60 mM) of salicylic acid (SA) were found to promote linear growth. However, 80 mM of SA had totally suppressed the growth of fungus. But, reduction of sporulation was found as SA concentrations increased over control. The results of current study showed the possibility of using resistance inducers to control *Fusarium* root rot of fenugreek.

Key words – antifungal – chitosan-methyl jasmonate – salicylic acid – fenugreek – root rot

Introduction

Fenugreek (*Trigonella foenum-graecum* L.) belongs to the family Fabaceae. It is an annual legume native to Mediterranean region, locally known as Methi, is cultivated not only as a vegetable but also for medicinal purposes (Som & Maity 1993). It is widely cultivated in India, China, northern and eastern Africa, parts of Mediterranean Europe, Argentina and Australia. Green methi is a good source of iron (Fe) as well as other minerals for human beings (Chhibba et al. 2000).The crop is gaining importance among seed spices because of its demand in the international market (Anonymous 2009). In 2010-11, the total area under methi in India was 81.2 thousand

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hectares with 1.18 lac tons production. The crop is attacked by several fungi, bacteria, viruses and nematodes causing diseases resulting in reduced yields (Khare et al. 2014). Diseases are the major constraints to the production of fenugreek. Among the diseases, *Fusarium* root rot in fenugreek is more severe in recent years and farmers are using fungicides to control it. Younger plants are more susceptible than older ones. At the seedlings stage, the recorded rot incidence was 50-75 per cent (Khokhar et al. 2012). Controlling this disease depends mainly on use of fungicides. Application of fungicides is a quick method to manage *Fusarium* root rot but fungicides are hazardous and toxic to both people and domestic animals and leads to environmental pollution (Ragab et al. 2012). The fungicides often lead to development of fungicide resistant strains of the pathogens. Hence, there is an urgent need for effective and safe non-fungicide means of controlling *Fusarium* diseases. One such environment friendly approach is stimulating the plant's own resistance mechanism by agents which can mimic natural inducers of resistance (Walters et al. 2005). Plant resistant inducers do not have any pesticidal and antibiotic activity.

The phenomenon of plant resistance to pathogens can be enhanced by the application of various abiotic agent (chemical inducers) in plants such as salicylic acid, ethephon, hydrogen peroxide (Akram & Anjum 2011), jasmonic acid, 2, 6, dichloroisonicotic acid and DL - 3 amino-n-butanoic acid (BABA) (Kessmann et al. 1994, Oostendorp et al. 2001), chitosan (Reddy et al. 1999). Some of these chemical inducers are commercially available in market and provide an easy and effective approach to the farmer for better disease control (Wisniewski et al. 2007). Many of these compounds claim additional benefits by increasing plant health and yields (Abdel-Monaim et al. 2012). The effectiveness of these inducers has been confirmed by a number of researchers. e.g. (Segarra et al. 2006) conducted a study on induction of resistance under the activity of chemical inducers against root rot diseases and found induced systemic resistance (ISR) rapidly developed in plants making the plant resistant. Chemical playing role behind this resistance induction were accumulation of phytoalexins, lignification of phenols and activation of chitinase, polyphenoloxidase and peroxidase. Chemical inducers and their effects on different plant species from around the world have enlisted (Thakur & Sohal 2013).

A few reports are available in India on use of plant resistant inducers to control tobamoviruses infection in bell pepper and tomato plants (Madhusudhan et al. 2011), *Fusarium* wilt in tomato (Ojha & Chatterjee 2012), root rot of medicinal *Coleus* (Bhattacharya & Bhattacharya 2012). Survey of literature reveals that there seems to be no report regarding effect of chemical inducers on mycelial growth and sporulation of *F. solani* causing root rot of fenugreek. Therefore, present investigation has been undertaken to control root rot pathogen of fenugreek under laboratory conditions using chemical inducers.

Materials & Methods

Collection of disease sample

Fenugreek plants showing typical wilt like symptoms were collected from different farmer's field's *viz*., Kharsinge, Chorade, Holichagaon, Kuroli, Jaygaon, Pusegaon, Umbarde and Aundh of Khatav Taluka, Satara District of Maharashtra State and brought to the laboratory in clean sterilized polythene bags and used for isolation within 24 hrs.

Isolation and identification of the pathogen

The infected plants showing typical wilt like symptoms were used for the isolation of pathogen. Isolation was made by tissue isolation technique. The infected roots were thoroughly washed with tap water to remove soil and cut into pieces of disease part along with healthy tissue. These pieces were surface sterilized with 70 % ethyl alcohol for 2 minutes and washed serially in sterilized distilled water and dried off with sterilized filter paper. The surface sterilized pieces were transferred aseptically to sterilized Petri plates containing Czapek Dox agar (CDA) medium fortified with 30 µg/ml streptomycin sulphate. The plates were then incubated at 27±1ºC and observed periodically for the growth of pure colonies.

Purification of the resulting isolates was done by using hyphal tip method. A total of eight isolates of fungus were obtained. Culture tubes were preserved at 4ºC and used for further studies. The isolates of the pathogen were identified with the help of relevant literature (Leslie & Summerell 2008) as *Fusarium solani* (Mart.) Sacc. The identity of highly virulent isolate (Accession no. NFCCI 4501) was confirmed using sequencing of Elongation Factor 1-alpha (EF-1α) gene and Internal Transcribed Spacer (ITS) region by National Fungal Culture Collection of India, Agharkar Research Institute, Pune, India. The DNA sequences of EF- 1α (MN201580), ITS (MN243824) and RPB1 (MN264211) were deposited to NCBI gene bank. NCBI BLASTn sequence analysis of this isolate showed 100 % similarity with *Fusarium solani* isolates EF-1α (DQ247490, 473/473 base pairs), ITS sequences (KY910884, 557/557 base pairs) and RPB1 (JN985161, 670/670 base pairs).

Pathogenicity of isolates

The eight isolates of *Fusarium solani* were screened for their pathogenicity on fenugreek susceptible cultivar (Deepak) under net house conditions. The cultivar is widely grown by the farmers as vegetable due to its delicious leaves. The inoculum of each isolate was multiplied on sterilized sand maize meal medium (10:1). For preparation of sand maize meal mixture, 200 g riverbed sand, 20 g maize meal and 50 ml distilled water were taken in each 500 ml Erlenmeyer flask. The medium was autoclaved alternately for two consecutive days. Flasks were inoculated with the mycelial discs (8mm diameter) of each isolate growing actively on CDA in a Petri plate and incubated at 25ºC for 21 days. During incubation, the culture was mixed thoroughly to get uniform growth of each isolate of *F. solani*. These inoculums were used for pathogenicity using soil inoculation method (Radhakrishnan & Sen 1985) Sandy loamy soil collected from farmer's field was autoclaved for 20 minutes for two consecutive days. The pots (20 cm width × 20 cm depth) were surface sterilized by 2 per cent formalin solution and filled with sterilized soil after mixing with inoculums at 40 g per Kg soil. For each isolate, three pots were prepared. The pots without inoculums were served as control. These pots were placed for five days for fungal multiplication after watering. On sixth day, twenty fenugreek seeds of cultivar Deepak which purchased from the local market were surface sterilized with 70 % ethyl alcohol and sown in each pot. Watering was done as and when needed. Observations were taken for disease development at regular interval. The disease incidence was recorded at 30 days after sowing. Re-isolation was made from plants showing wilt symptoms and compared with the original cultures. Highly virulent isolate was selected based on per cent wilt incidence for *in vitro* studies. The per cent wilt incidence was calculated according to the formula:

$$\text{Wilt incidence (\%)} = (\text{Number of wilted plants} / \text{Total number of plants}) \times 100$$

Resistance inducers tested

Chitosan**,** methyl jasmonate (MeJa) 95 % liquid and salicylic acid (SA) as fungicide alternatives were tested *in vitro* are listed in Table 1.

Preparation of chitosan, methyl jasmonate and salicylic acid solutions

Chitosan of low molecular weight (from shrimp shells, degree of deacetylation >=75%) was purchased from Himedia Laboratories Pvt. Ltd., India. To prepare stock solution (20 mg/ml), 4 g of chitosan were dissolved in 100 ml of distilled water with 4 ml of glacial acetic acid (stirred for 24 h) using magnetic stirrer and volume was taken to 200 ml with distilled water. The pH of the solution was adjusted to 5.6 by adding1N NaOH (El Ghaouth et al.1991). Chitosan solution was autoclaved for 20 minutes. This was diluted to desired concentrations (1, 5, 10 and 15 mg/ml) with sterile distilled water before use. 4.48 gm of methyl jasmonate 95% (MeJa) was dissolved in sterilized distilled water and prepared as 40 mM stock solution. This was further diluted at concentrations (1, 5, 10, 15 and 20 mM) with sterilized distilled water. 100 mM stock solution of salicylic acid (SA) was prepared by dissolving 6.906 gm in 500 ml sterilized distilled water (40ºC)

and diluted with sterilized distilled water to obtain the desired concentrations (20, 40, 60 and 80 mM).

Effect of chemical inducers on growth and sporulation of wilt pathogen *in vitro*

The inhibitory effect of chitosan, MeJa and SA, was determined against highly virulent isolate of *F. solani* by poisoned food technique on CDA medium supplemented with streptomycin sulphate (30 µg/ml). The inducers were tested at different concentrations. Flask containing CDA medium was prepared and amended with each concentration of inducer and poured in sterilized Petri-plates (80 mm dia.) A 8 mm culture disc of *F. solani* from 7 day old colony was placed upside down in the centre. Five replications were maintained for each concentration. The plates were sealed with parafilm and incubated at 27±1ºC. The plates without inducer served as control. The linear fungal growth was measured at an interval of 2 days up to 8 days. The control plates reached full growth after 8 days. The percentage of inhibition of mycelial growth was calculated using the formula (Tiru et al. 2013):

$$I\% = (C2-C1) / C2\times100$$

Where: I% = Percentage of inhibition, C2 = Mean diameter of growth in the control, C1 = Mean diameter of growth in treatment

Sporulation was estimated from 14 days old culture using Neubauer haemocytometer. 5 ml sterile distilled water was added to each Petri plate and the surface was rubbed with sterile glass rod. Suspensions from five plates were pooled together and filtered and then diluted to 50ml with sterile distilled water (Ketabchi & Shahrtash 2011). About 10 µl suspension was pipetted into each side of haemocytometer and mounted for counting under the compound microscope (40X). The number of spores per ml suspension (five replicates/ treatment) was calculated by using formula (Aneja 2018)

Spores/ml = Average number of spores in one large square (I+II+III+IV+V) $\times10^4 cm^3$

Statistical analysis

All treatments were a completely randomised design with five replications. Test for significance differences among treatments was analysed using online WASP-Web Agri Stat Package 1.0.

Table 1 Resistance inducers tested for *in vitro* studies.

Compound	Chemical formula	Molecular Weight	Company
Chitosan	$C_{12}H_{24}N_2O_9$	30.7 kDa	HiMedia, India
Methyl jasmonate	$C_{13}H_{20}O_3$	224.30 (g/mol)	Otto, India
Salicylic acid	$C_7H_6O_3$	138.12 (g/mol)	HiMedia, India

Results

The tested *F. solani* isolates showed variation in their ability to cause wilt symptoms in fenugreek under shade house conditions (Table 2). The root rot infected plants showed gradual yellowing of lower leaves, drooping, drying, shedding of leaves and stunted growth. Eventually, the inoculated plants wilted and died after 30 days. All the isolates had potency to cause root rot disease but the isolate (Fs-5) from Jaygaon location was found highly virulent one based on wilt incidence (83.33 %) and it was selected for *in vitro* studies.

Data shown in (Table 3 & Figs.1, 2) showed that chitosan inhibited mycelial growth and sporulation of *F. solani* in CDA medium, in concentration dependent manner. The linear growth of fungus and sporulation were decreased significantly with the increase of concentrations when compared to the control. Mycelial growth was inhibited 16.25, 29.00 and 60.37 % after 8 days of

treatment by 1, 5 and 10 mg/ml chitosan respectively. Chitosan had inhibited mycelial growth completely at 15 mg/ml concentration. Chitosan had halted sporulation at 10 mg/ml concentration.

MeJa had inhibitory effect on mycelial growth of *F. solani* after 8 days of incubation (Table 4 & Figs 3, 4). In the present investigation, MeJa significantly inhibited mycelial growth of *F. solani* as concentrations increased. Percent growth inhibition (76.37) was higher at 20 mM concentration of MeJa. The spore formation of fungus was increased at 1mM over control and decreased at 5 mM concentration. It was interesting that MeJa had halted spore formation of fungus at10 mM concentration.

The mycelial growth of *F. solani* was significantly affected by SA when added to CDA medium. The increasing concentrations of SA (20, 40 and 60 mM) were found to promote mycelial growth of *F. solani*. But, reduction of sporulation was found as SA concentrations increased over control. However, when tested at 80 mM concentration SA had totally suppressed mycelial growth of fungus due to fungitoxicity (Table 5 & Figs. 5, 6).

Table 2 Pathogenicity tests of *F. solani* isolates collected from different localities to fenugreek.

Isolates	**Locality**	**Wilt incidence (%)***
Fs-1	Kharsinge	55.00 (47.91)
Fs-2	Chorade	58.33 (49.83)
Fs-3	Holichagaon	43.33 (41.12)
Fs-4	Kuroli	66.66 (54.88)
Fs-5	Jaygaon	83.33 (65.95)
Fs-6	Pusegaon	51.66 (45.96)
Fs-7	Umbarde	63.33 (52.79)
Fs-8	Aundh	71.66 (57.85)
Control		0.00 (0.64)
SEM±		6.19
C.D. (P=0.05)		8.11

*mean of three replications (pots), figures in parentheses are arc-sine transformed values

Table 3 Effect of chitosan on linear growth and sporulation of F. solani causing root rot.

Chitosan (mg/ml)	**Linear growth (mm)***	**Percent Inhibition**	**Sporulation*×10^4**
1	67.00	16.25	132.00
5	56.80	29.00	114.80
10	31.70	60.37	0.00
15	0.00	100.00	0.00
Control	80.00	0.00	344.40
SEM±	14.19		62.97
C.D. (P=0.05)	3.46		14.16

*mean of five replications

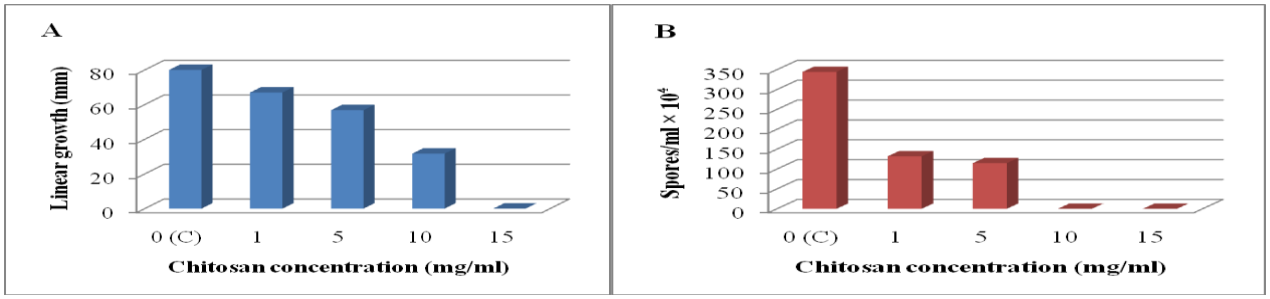

Fig. 1 – Effect of chitosan on linear growth (A) and sporulation (B) of *F. solani*.

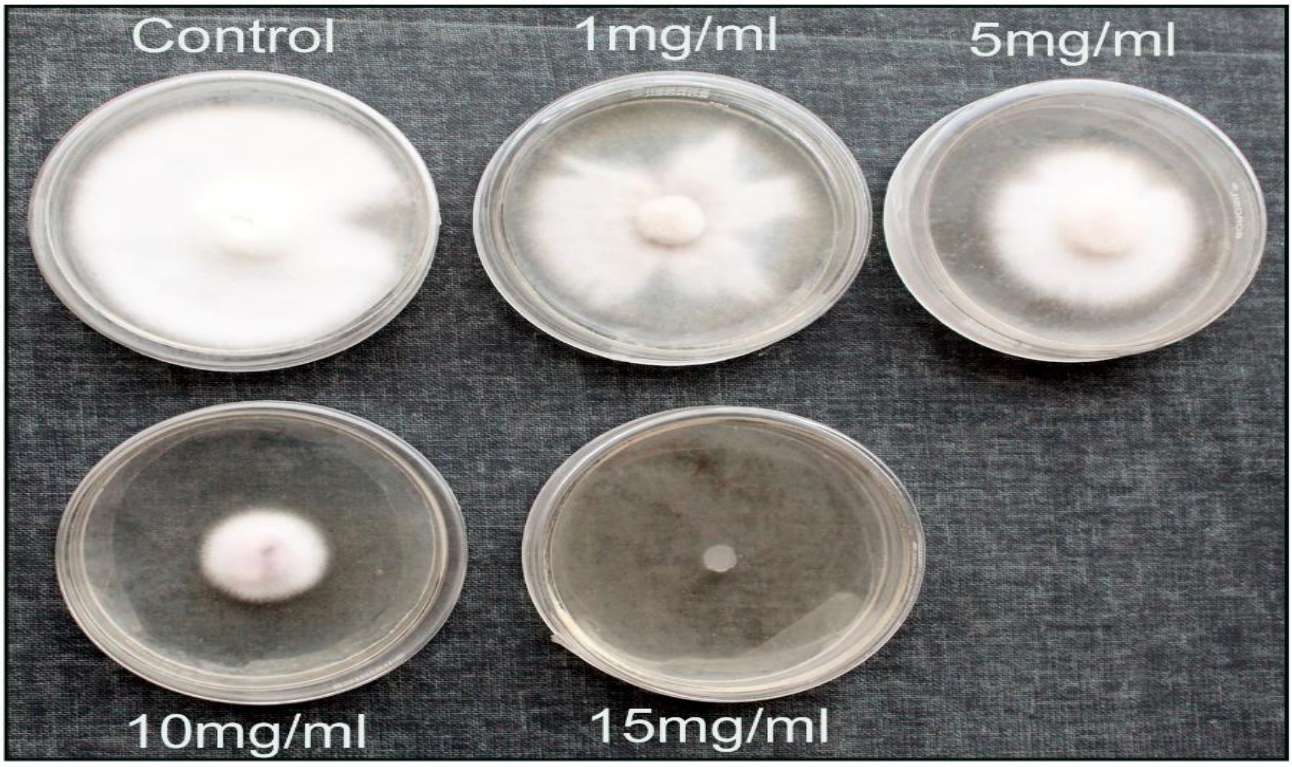

Fig. 2 – Effect of Chitosan on linear growth and sporulation of *F. solani*.

Table 4 Effect of methyl jasmonate (MeJa) on linear growth and sporulation of *F. solani* causing root rot disease of fenugreek.

MeJa (mM)	Linear growth* (mm)	Percent Inhibition	Sporulation*×104
1	64.04	19.00	721.00
5	43.06	45.50	298.40
10	26.50	66.87	0.00
15	21.70	72.87	0.00
20	18.90	76.37	0.00
Control	80.00	0.00	357.80
SEM±	10.24		118.43
C.D. (P= 0.05)	1.85		47.83

* mean of five replications

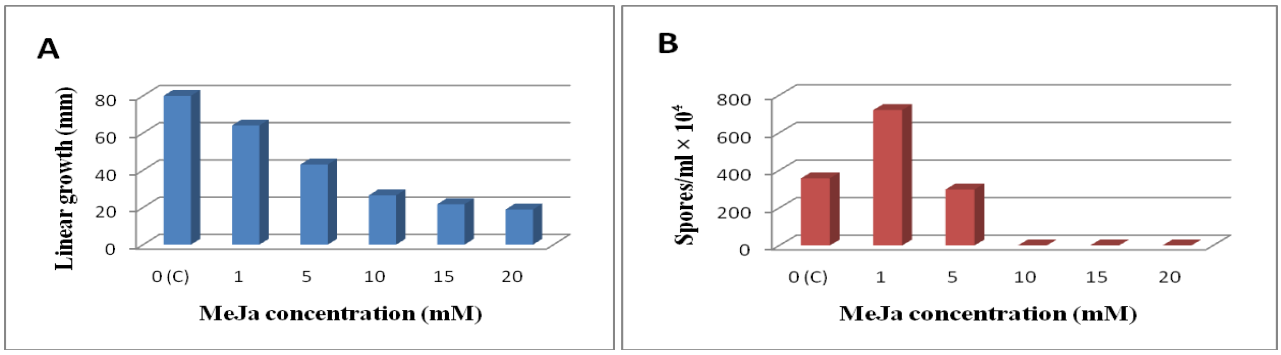

Fig. 3 – Effect of methyl jasmonate (MeJa) on linear growth (A) and sporulation (B) of *F. solani*.

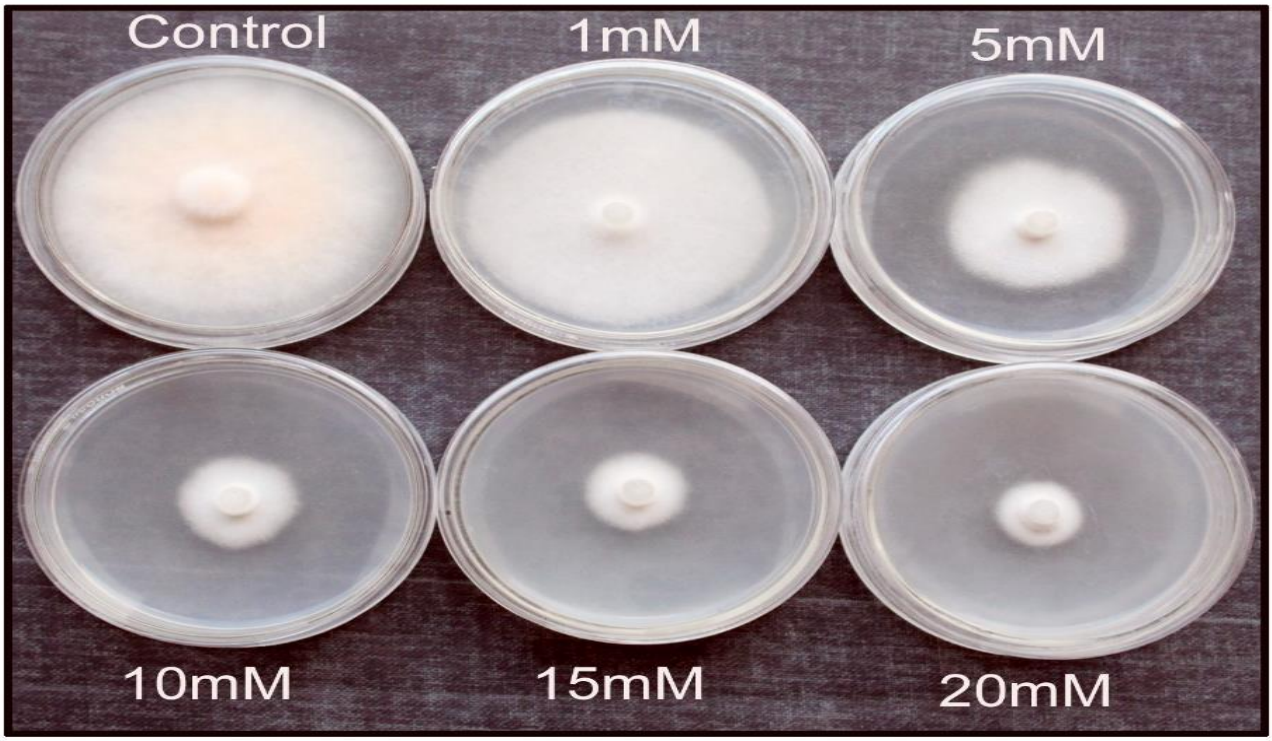

Fig. 4 – Effect of methyl jasmonate on linear growth and sporulation of *F. solani*.

Table 5 Effect of salicylic acid (SA) on linear growth and sporulation of *F. solani* causing root rot disease of fenugreek.

SA (mM)	Linear growth* (mm)	Percent Inhibition	Sporulation*×10^4
20	68.04	14.50	252.40
40	70.60	11.75	260.60
60	72.70	09.12	277.20
80	0.00	100.00	0.00
Control	80.00	0.00	351.00
SEM±	14.71		59.66
C.D. (P= 0.05)	0.38		28.15

*mean of five replications

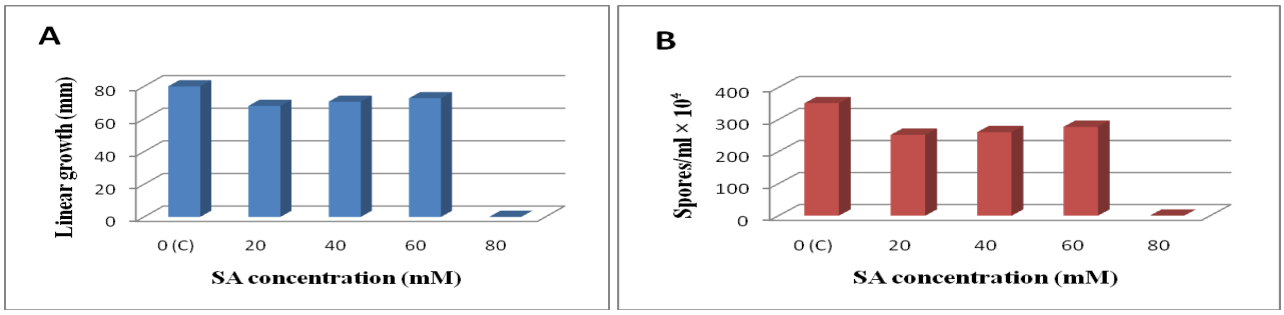

Fig. 5 – Effect of salicylic acid (SA) on linear growth (A) and sporulation (B) of *F. solani*.

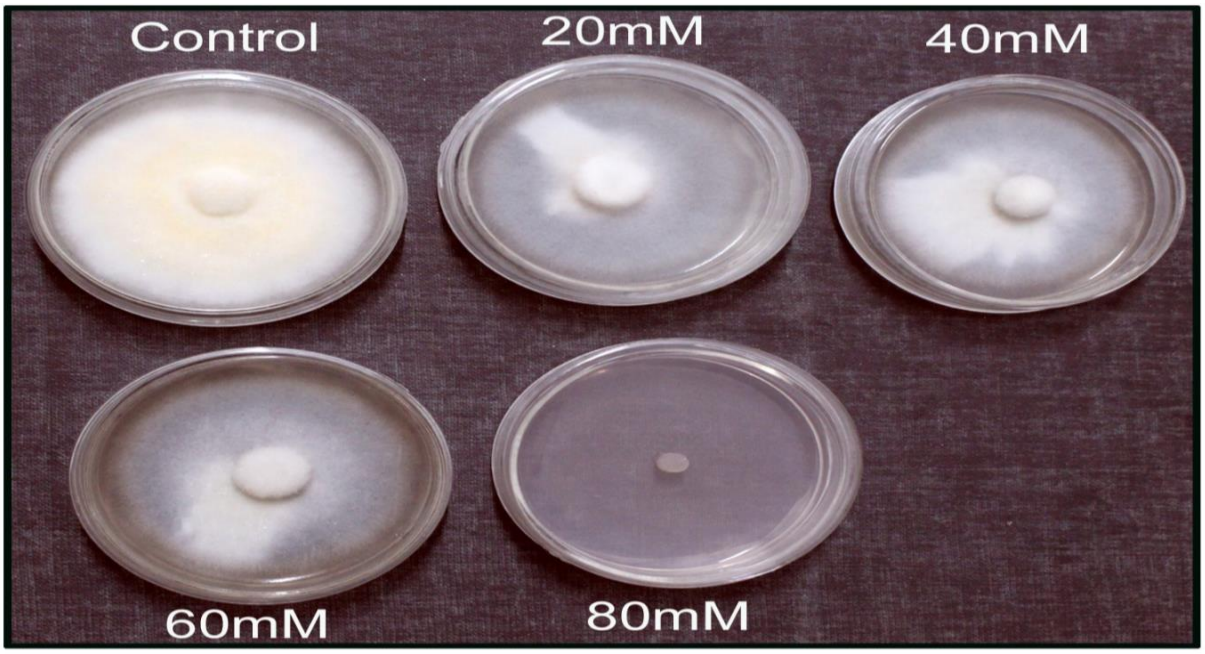

Fig. 6 – Effect of salicylic acid on linear growth and sporulation of *F. solani*.

Discussion

In the present study, an antifungal activity of chitosan with different concentrations against *F. solani* causing root rot in fenugreek was observed. Chitosan reduced mycelial growth and sporulation of *F. solani* as concentrations increased. The complete inhibition of both mycelial growth and sporulation was observed at 15 mg/ml and 10mg/ml concentrations respectively. Similar results were recorded by many researchers with different crops. Mycelial growth of *F. solani* f. sp. *phaseoli* and *F. solani* f. sp. *pisi* was inhibited at the minimum concentrations of 12 and 18 mg/ml respectively (Kendra & Hadwiger 1984). A complete mycelial growth and sporulation inhibition of fungi such as *F. oxysporum, Penicillium digitatum* and *Rhizopus stolonifer* isolated from infected papaya found at 3 % chitosan concentration (Bautista-Banos et al. 2004). Bhattacharya (2013) reported that maximum inhibition of mycelial growth and sporulation of *F. solani* causing root rot of *Coleus forskohlii* by chitosan was achieved at 0.20 % concentration. In general, sporulation of fungi treated with chitosan is reported to be lower than in untreated fungi. Moreover, in some reports no spore formation was observed after chitosan treatment. Abd-El-Kareem et al. (2006) reported complete inhibition of all tested fungi against tomato root rot disease at 6 g/L chitosan. Also, the mycelial growth of *F. oxysporum* the causal of wilt disease of pepper (*Capsicum annum* L.) was completely inhibited at 4.5 g/L chitosan (Ragab et al. 2012). The inhibitory effect of chitosan against pathogenic fungi was also reported by numerous authors (Abd-El-Kareem 2002, El-Mohamedy et al. 2013, Jabnoun-Khiareddine et al. 2015, Ramos-Guerrero et al. 2018). Many explanations have been postulated for mode of action of chitosan against fungi. The anti-fungal activity of chitosan is related to its ability to interfere with the plasma membrane function (Leuba & Stossel 1986) and the interaction of chitosan with fungal DNA and RNA (Hadwiger & Loschke 1981). Chitosan's anti-fungal and anti-microbial activities are believed to originate from its polycationic nature (Roller & Covill 2000). Recent studies showed that chitosan is not only effective in halting the growth of the pathogen, but also induces marked morphological changes, structural alterations and molecular disorganizations of the fungal cells (El Ghaouth et al. 1999, Ait Barka et al. 2004)

MeJa was found to reduce linear growth and sporulation in a concentration dependent manner. However, MeJa had halted spore formation of fungus at10 mM concentration. These results are in agreement with the earlier studies that Methyl jasmonate had inhibitory effects on mycelial growth, sporulation and metabolism of fungi (Goodrich-Tanriculu et al. 1995, Pczy ska &

Pczy ski 2005, Bhattacharya & Bhattacharya 2012, Kepczynska & Krol 2012). In present study, MeJa did not completely inhibit mycelial growth of *F. solani* at 20 mM concentration. Similar result was obtained by Serife et al. (2013) in case of *F. oxysporum* f.sp. *lycopersici* and *F. oxysporum* f.sp. *radicis-lycopersici* using jasmonic acid.

It was observed that SA did not possess direct antifungal activity at lower concentrations. At higher concentration, SA acts as toxic chemical and totally suppressed the mycelial growth of the fungus. This strengthens the hypothesis that SA activates the signal transduction pathway, thus leading to the expression of systemic acquired resistance, rather inhibiting the fungus directly (Metraux et al. 2002). The findings obtained in this study were in agreement with those reported by Spletzer & Enyedi (1999), Jendoubi et al. (2015) who found that SA promoted the mycelial growth of *Alternaria solani* and *F. oxysporum* f. sp. *radicis- lycopersici* at lower concentrations. Kumar & Bains (2018) also found that the exogenously applied SA promoted mycelial growth at low concentration (0.5 mM) but concentration higher than 0.5 mM decrease growth of two isolates of *F. mangiferae* under laboratory conditions. Yang et al. (2019) showed that there was no significant difference between the different concentrations of SA on the colony growth of the BAS1- over expressing strain or the WT strain compared with the control plants of each that were pre-treated with only DMSO. Abdel-Monaim et al. (2012) showed that SA had significantly inhibited radial growth and spore formation of *F. oxysporum* f. sp. *lycopersici* but at different degrees depending on concentrations. The finding was in accordance with those of Yao & Tian (2005) who reported that SA at 270 mg/ml exhibited fungitoxicity toward *Monilinia fructicola* and significantly inhibited mycelial of the pathogen *in vitro*. Similar results were also reported by Nebbache et al. (2018) against *Fusarium verticillioides* in garlic (*Allium sativum*). However, Jabnoun-Khiareddine et al. (2015) found that SA inhibited mycelial growth of phytopathogenic fungi grown in amended PDA medium in concentration dependent manner (1-25 mM) as compared to the untreated control. To the best of my knowledge, this is the first work reporting the effect of chitosan, MeJa and SA on mycelial growth and sporulation of *F. solani* causing root rot of fenugreek.

Conclusion

Chitosan had significantly inhibited linear growth and sporulation of *F. solani* at 15 and 10 mg/ml concentrations respectively. MeJa had significantly reduced linear growth as concentration increased but sporulation halted at 10 mM. SA had promoted linear growth but reduced sporulation in increasing concentrations (20, 40 and 60 mM). At highest concentration (80 mM), SA acts as toxic for pathogen.

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References

Abd-El-Kareem F, Nehal S El-Mougy, Nadia G, El-Gamal, Fotouh YO. 2006 – Use of chitin and chitosan against tomato root rot disease under greenhouse conditions. Research Journal of Agriculture and Biological Sciences 2(4), 147–152.

Abd-El-Kareem F. 2002 – Integrated treatments between bio agents and chitosan on root rot diseases of pea plant under field conditions. Egyptian Journal of Applied Science 17, 257–279.

Abdel-Monaim MF, Abdel Gaid MAW, Halium Armanious HA. 2012 – Effect of chemical inducers on root rot and wilt diseases, yield and quantity of tomato. International Journal of Agricultural Sciences 2(7), 211–220.

Ait Barka E, Eullaffroy P, Clement C, Vernet G. 2004 – Chitosan improves development, and protects *Vitis vinifera* L. against *Botrytis cineria*. Plant Cell Report 22, 608–614.

Akram W, Anjum T. 2011 – Use of bio agents and synthetic chemicals for induction of systemic resistance in tomato against diseases. International Research Journal of Agricultural Science and Soil Science 1(8), 286–292.

Aneja KR. 2018 – Experiments in Microbiology, Plant Pathology, Tissue Culture and Microbial Biotechnology (5th Edn.), New Age International Publishers, New Delhi.

Anonymous. 2009 – Spice Board India, Ministry of Commerce and Industry, Govt. of India.

Bautista-Banos S, Hernandez-Lopez M, Bosquez-Molina E. 2004 – Growth inhibition of selected fungi by chitosan and plant extracts. Mexican Journal of Phytopathology 22, 178–186.

Bhattacharya A, Bhattacharya S. 2012 – An environment friendly approach for controlling pathogenic *Fusarium solani* (Mart.) Sacc., the causal agent of root rot of medicinal *Coleus* by methyl jasmonate. Journal of Plant Pathology and Microbiology 3(1), 1–4.

Bhattacharya A. 2013 – Fungicidal potential of chitosan against phytopathogenic *Fusarium solani*. Journal of Experimental Biology and Agricultural sciences 1(4), 259–263.

Chhibba IM, Kanwar JS, Nayyar VK. 2000 – Yield and nutritive values of different varieties of fenugreek (*Trigonella* Spp.). Vegetable Science 27, 176–179.

El Ghaouth A, Arul J, Ponnampalam R. 1991 – Use of chitosan coating to reduce water loss and maintain quality of cucumbers and bell pepper fruits. Journal of Food Processing and Preservation 15, 359–368.

El Ghaouth A, Smilanick JL, Brown GE, Wisniewski M, Wilson CL. 1999 – Application of *Candida saitoana* and glycol-chitosan for the control of postharvest diseases of apple and citrus fruit under semi-commercial conditions. Plant Disease 84, 243–248.

El-Mohamedy RSR, Abdel-Kader MM, Abd-El-Kareem F, El-Mougy NS. 2013 – Inhibitory effect of antagonistic bio-agents and chitosan on the tomato root rot pathogens *In vitro.* International Journal of Agricultural Technology 9(6), 1521–1533.

Goodrich-Tanriculu M, Mahoney NE, Rodriguez SB. 1995 – The plant growth regulator methyl jasmonate inhibits aflatoxin production by *Aspergillus flavus*. Microbiology 141, 2831–2837.

Hadwiger LA, Loschke DC. 1981 – Molecular communication in host-parasite interactions: Hexosamine polymers (chitosan) as regulator compounds in race-specific and other interactions. Phytopathology 71, 756–762.

Jabnoun-Khiareddine H, El-Mohamedy RSR, Abdel-Kareem F, Aydi Ben Abdallah R et al. 2015 – Variation in chitosan and salicylic acid efficacy towards soil-borne and air borne fungi and their suppressive effect of tomato wilt severity. Journal of Plant Pathology and Microbiology 6(11), 325–334.

Jendoubi W, Harbaoui K, Hamada W. 2015 – Salicylic acid-induced resistance against *Fusarium oxysporum* f. sp. *radicis- lycopercisi* in hydroponic grown tomato plants. Journal of New Sciences, Agriculture and Biotechnology 21(5), 985–995.

Kendra DF, Hadwiger LA. 1984 – Characterization of the smallest chitosan oligomer that is maximally antifungal to *Fusarium solani* and elicits pisatin formation by *Pisum sativum*. Experimental Mycology 8, 276–281.

Kepczynska E, Krol P. 2012 – The phytohormone methyl jasmonate as an activator of induced resistance against the necrotroph *Alternaria porri* f. sp. *solani* in tomato plants. Journal of Plant Interactions 7(4), 307–315.

Kessmann HT, Staub C, Hofmann Maetzke T, Herzog J et al. 1994 – Induction of systemic acquired disease resistance in plants by chemicals. Annual Review of Phytopathology 32, 439–459.

Ketabchi S, Shahrtash M. 2011 – Effects of methyl jasmonate and cytokinin on biochemical responses of maize seedlings infected by *Fusarium moniliforme*. Asian Journal of Experimental Biological Sciences 2, 299–305.

Khare MN, Tiwari SP, Sharma YK. 2014 – Disease problems in fennel (*Trigonella-foenum graceum* L.) cultivation and their management for production of quality pathogen free seeds. International Journal of Seed Spices 4(2), 11–17.

Khokhar MK, Gupta R, Parrek D. 2012 – Efficacy of fungicides and plant extracts against *Fusarium* wilt in fenugreek. International Journal of Plant Protection 5(2), 417–419.
Kumar V, Bains G. 2018 – Effect of salicylic acid on mycelial growth and conidial germination of two isolates of *Fusarium mangiferae*. International Journal of Current Microbiology and Applied Sciences 7(10), 3704–3710.
Leslie JF, Summerell BA. 2008 – The *Fusarium* Laboratory Manual. Blackwell Publishing, Oxford, UK.
Leuba JL, Stossel P. 1986 – Chitosan and other polyamines: Antifungal activity and interaction with biological membranes. In Muzzarelli R and Goody GW (Eds.), Chitin in Nature and Technology, Plenum Press, New York, USA, 215–222.
Madhusudhan KN, Vinayarani G, Deepak SA, Niranjana SR et al. 2011 – Antiviral activity of plant extracts and other inducers against tobamoviruses infection in bell pepper and tomato plants. International Journal of Plant Pathology 2, 35–42.
Metraux JP, Nawrath C, Genoud T. 2002 – Systemic acquired resistance. Euphytica 124, 237–243.
Nebbache S, Sennoussi MM, Oufroukh A. 2018 – *In vitro* and *in vivo* evaluating the efficacy of salicylic acid and gibberellic acid against *Fusarium verticillioides* in garlic (*Allium sativum*). World Journal of Environmental Biosciences 7, 1–8.
Ojha S, Chatterjee NC. 2012 – Induction of resistance in tomato plants against *Fusarium oxysporum* f. sp. *Lycopersici* mediated through salicylic acid and *Trichoderma harzianum.* Journal of Plant Protection Research 52(2), 220–225.
Oostendorp M, Kunz W, Dietrich B, Staub T. 2001 – Induced disease resistance in plants by chemicals. European Journal of Plant Pathology 107, 19–28.
Pczy ska EK, Pyczy ski JK. 2005 – Inhibitory effect of methyl jasmonate on development of phytopathogen *Alternaria alternata* (Fr.) its reversal by ethepon and ACC. Acta Physiologiae Plantarum 27(4), 491–496.
Radhakrishnan P, Sen B. 1985 – Efficacy of different methods of inoculums of *Fusarium oxysporum* and *Fusarium solani* for inducing wilt in muskmelon. Indian Phytopathology 38(1), 70–73.
Ragab MMM, Ashour AMA, Abdul-Kader MM, El-Mohamady R, Abdel-Aziz A. 2012 – *In vitro* evaluation of some fungicides alternatives against *Fusarium oxysporum* the causal of wilt disease of pepper (*Capsicum annum* L.). International Journal of Agricultural and Forestry 2(2), 70–77.
Ramos-Guerrero A, Gonzalez- Estrada RR, Hanako-Rosas G, Baustista- Banos S et al. 2018 – Use of inductors in the control of *Colletotrichum gloesporioides* and *Rhizopus stolonifer* isolated from soursop fruits: *in vitro* tests. Food Science and Biotechnology 27, 755–763.
Reddy MVB, Arul J, Angers P, Couture L. 1999 – Chitosan treatment of wheat seeds induces resistance to *Fusarium graminineārum* and improve seed quality. Journal of Agriculture and Food Chemistry 47(3), 1208–1216.
Roller S, Covill N. 2000 – The antimicrobial properties in mayonnaise and mayonnaise based shrimp salads. Journal of Food Protection 63, 202–209.
Segarra G, Tauregui O, Casanova E, Trillas I. 2006 – Simultaneous quantitative LC- ESIMS/MS analysis of salicylic acid and jasmonic acid in crude extracts of *Cucumis sativus* under biotic stress. Phytochemistry 67(4), 395–401.
Serife EA, Gamze B, Ibrahim A. 2013 – Investigation of potential biological control of *Fusarium oxysporum* f.sp. *radicis-lycopersici* and *Fusarium oxysporum* f.sp. *lycopersici* by essential oils, plant extract and chemical elicitors *in vitro*. Pakistan Journal of Botany 45(6), 2119–2124.
Som MG, Maity TK. 1993 – Leafy vegetables. In: Bose TK, Som MG, Kabir J. (eds.), vegetables crops in India, 753–77. Naya Prokash, Culcutta, India.
Spletzer ME, Enyedi AJ. 1999 – Salicylic acid induces resistance to *Alternaria solani* in hydroponically grown tomato. Phytopathology 89, 722–727.

Thakur M, Sohal BS. 2013 – Role of elicitors in inducing resistance in plants against infection: a review. ISRN Biochemistry http://dx.doi.org/10.1155/2013/762412 (accessed 15 April 2019).
Tiru M, Meleta D, Bercha G, Adugna G. 2013 – Antagonistic effect of rhizobacteria against coffee wilt disease caused by *Gibberella xylarioides*. Asian Journal of Plant pathology 7, 109–122.
Walters D, Walsh D, Newton A, Lyon G. 2005 – Induced resistance for disease control: maximizing the efficacy of resistant elicitors. Phytopathology 95, 1368–1373.
Wisniewski ME, Wilson CL, Droby S, Chalutz E et al. 2007 – Postharvest Biocontrol: New concepts and Applications. In: Vincent C, Goettel MS, Lazarovits G Editors. Biological control: a global perspective. CAB International, 262–273.
Yang J, Wang Y, Liu L, Liu L, Wang C, Wang C, Li C. 2019 – Effect of exogenous salicylic acid and pH on pathogenicity of biotrophy-associated secreted protein1 (BAS1) – over expressing strain, *Magnaporthe oryzae*. Environmental Science and Pollution Research 26(14), 13725–13737.
Yao HJ, Tian SP. 2005 – Effects of pre-and post harvest application of salicylic acid or methyl jasmonate on inducing disease resistance of sweet cherry fruit in storage. Postharvest Biology and Technology 35, 253–262.