FINAL REPORT

ON

MINOR RESEARCH PROJECT ENTITLED

"Use of Plant Resistant Inducers to Control with Pathogen of Fenugreek"

F. 47-1244 / 14 (WRO) dated 21 Dec., 2015

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DECLARATION AND CERTIFICATE

I hereby declare and certify that, the Minor research Project entitled **"Use of Plant Resistant Inducers to Control with Pathogen of Fenugreek"** F. 47-1244 / 14 (WRO) dated 21 Dec., 2015 is a bonafide record of research work carried out by me during the year 2017-2019. Further certify that the work presented in the report is original and carried out according to the plan in the proposal and guidelines of the University Grants Commission.

Principal Investigator

Annual Report on the Minor Research Project in First Year

Project Title: "Use of plant resistant inducers to control with pathogen of fenugreek"

Plant resistant inducers induce defence responses in plants, which can prevent or slow pathogen infection and enhance growth. The application of chemical inducers of resistance is an exciting new perspective to supplement the classical chemical means of disease control by providing both effective and ecologically-friendly plant protection. This study will be useful to control wilt disease of fenugreek, a leafy vegetable without use of fungicides.

Methodology:

1. 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. Isolation was made within 24 hrs after collection.

2. **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 on the basis of spore morphology and colony characters as *Fusarium oxysporum.*

3. Pathogenicity of isolates

The eight isolates of *Fusarium oxysporum* were screened for their pathogenicity on fenugreek cultivar Deepak under net house. The inoculum of each isolate was multiplied on sterilized sand maize meal medium (10:1). For preparation of sand maize meal mixture, 200g riverbed sand, 20g maize meal and 50 ml distilled water were taken in each 500ml 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. oxysporum*. These inoculums were used for pathogenicity using soil inoculation method.

Sandy loamy soil collected from the field was autoclaved for 20 minutes for two consecutive days. The pots (25 cm width × 23cm 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 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. The per cent wilt incidence was calculated. Highly virulent isolate was selected based on per cent wilt incidence for further studies.

4. Chemical inducers tested

The chemical inducers such as salicylic acid, chitosan (HiMedia) and methyl jasmonate 95 % liquid (Otto) were tested to evaluate their effect as inducers against wilt pathogen of fenugreek

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

The inhibitory effect of salicylic acid, chitosan and methyl jasmonate was determined against highly virulent isolate of *F. oxysporum* by poison food technique on CDA medium supplemented with streptomycin sulphate (30µg/ml). The inducers were

tested at different concentrations. Flask containing CDA medium was prepared was prepared and amended with each concentration of inducer and poured in sterilized Petri-plates (80mm dia.) A 8mm culture disc of *F. oxysporum* 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 were served as control. The linear fungal growth was measured at an interval of 2 days up to 8 days. Percent growth inhibition was calculated.

Sporulation was estimated using haemocytometer after 8 days. 5ml 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 diluted to 50ml with sterile distilled water. A drop of this solution was placed into the haemocytometer and mounted for counting under the compound microscope (40X). The number of spores per ml was calculated.

Annual Report on the Minor Research Project in Second Year

***Fusarium solani* causes root rot of fenugreek**

On morphological basis**,** pathogen causing root rot in fenugreek was identified as *Fusarium oxysporum*. However, pathogen was identified as *Fusarium solani* (Mart.) Sacc. on basis of sequencing of Elongation Factor-1α gene (partial) and sequencing of D1/D2 region of large sub-unit (LSU)- rDNA at Agharkar Research Institute, Pune.

Effect of seed soaking in inducers on disease severity development

Healthy fenugreek seeds of susceptible cultivar Deepak were used for this study. The surface sterilized seeds were soaked in different concentrations of Methyl jasmonate and salicylic acid for 8 hrs while chitosan for 5 hrs and then washed 3-4 times with sterile distilled water to remove traces of inducers. The plastic pots were sterilized with 2% formalin and wait for 2 hrs to dry and filled with autoclaved sterilized sand loamy soil. Twenty treated seeds were sown in plastic pots containing sand-loamy soil. Twenty seeds were sown in each pot and three pots were used as replicates for particular treatment. The seed soaked in water served as control. All the pots containing sterilized soil were infested with conidial suspensions (157×10^4) which adjusted with haemocytometer. 100ml of spore suspensions was poured in each pot except control 1 where 100 ml sterile water was poured. For control 2, water soaked seeds were infested

with spore suspensions. After 8 days, seed germination was noted and seedling height was measured using camel ruler (15cm). The disease severity was determined according to disease severity index scale after 30 days. The growth parameters such germination %, height of plant and no. of leaves per plant were also recorded.

Biochemical analysis of resistance response in plants under the activity of seed treatment

Analysis of biochemical changes in fenugreek plant due to seed treatment with chemical inducers was carried out. The enzyme activities of chitinase, polyphenoloxidase and peroxidase were determined and found significant results.

CONTENTS

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 (Acharya et al. 2006). 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 hectares with 1.18 lac tons production and 1.5 tons productivity per hectre (Khare et al.2014).

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 not eco-friendly and adversely affected human beings, microorganisms and environment with its toxic residues (Parker et al.1985). The fungicides often lead to development of fungicide resistant strains of the pathogens. It has been observed that *F. solani* has been developed fungicide resistance to benomyl (Ramteke & Kamble 2010). 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. Pathogens are unable to develop resistance because plant inducers don't have direct interaction with pathogens (Huang & Hsu 2003).

Systemic acquired resistance (SAR) or induction of plants to resist against pathogen is a promising approach for controlling plant diseases (Abdul-Monaim et al. 2012). Exogenous or endogenous factors could substantially affect host physiology, leading to rapid and coordinated activation of defense gene in plants expressing susceptibility to pathogen (Mandal et al.2000). A time interval (lag period) between the primary and "challenge" inoculation is a prerequisite for effective expression of induced resistance. Span of time is necessary for signals to be translocated to non-inoculated tissues and for triggering and development of defense potential in these tissues (Kuc 2001). Extent of induced resistance depends upon the type of treatment, dose of treatment, age of plant and method of reproduction (Shafique et al. 2011).

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; Oostendrop et al. 2001), chitosan (Bohland et al. 1997, 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 2007). Many of these compounds claim additional benefits by increasing plant health and yields (Abdul- 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. Peroxidases oxidize phenols to phenols to quinines, which are toxic to pathogens. Peroxidases participate in a broad range of physiological processes, such as the formation of lignin and suberin, the cross-linking of cell wall components and phytoalexin synthesis. Peroxidase also functions in the metabolism of Reactive Active Species (ROS) and Reactive Nitrogen Species (RNS), thus activating the Hypersensitive Response (HR), a form of programmed cell death at the infection site that is associated with limiting pathogen development (Almagro et al. 2009). Polyphenol oxidases participate in the oxidation of aromatic substrates and dihydroxyphenolic compounds in the presence of oxygen in host tissues, producing quinines that are toxic to pathogens (Mayer 2006). 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, Biswas et al. 2012), root rot of medicinal *Coleus* (Bhattacharya & Bhattacharya 2012). Survey of literature reveals that there seems to be no report on use of chemical inducers to control *Fusarium solani* causing root rot of fenugreek. Therefore, present investigation was undertaken to control root rot pathogen of fenugreek *in vitro* and *in vivo* using plant resistance inducers with the following objectives:

- To determine the efficacy of chemical inducers on growth and sporulation of pathogen *in vitro*
- To study the impact of chemical inducers on disease control *in vivo*

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 and brought to the laboratory in clean sterilized polythene bags and isolation was made.

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. CDA was prepared by dissolving 30gm of sucrose, 2gm of sodium nitrate,1gm of dipotassium phosphate, 0.5gm of magnesium sulphate, 0.5 gm of potassium chloride, 0.01gm of ferrous sulphate and agar-agar15 gm in 1000ml distilled water, final pH 7.3±0.2. All the chemicals were purchased from Himedia Laboratories Pvt. Ltd., India 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-1α gene (partial) and sequencing of D1/D2 region of large sub-unit (LSU) – rDNA by Agharkar Research Institute, Pune, India.

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, 200g riverbed sand, 20g maize meal and 50 ml distilled water were taken in each 500ml 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 × 20cm 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 after 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:

Wilt incidence (%) = (Number of wilted plants / Total number of plants) × 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, India. To prepare stock solution (20 mg/ml), 4g 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.

4.48gm of methyl jasmonate 95% (MeJa) was dissolved in sterilized distilled water and prepared as 40mM stock solution. This was further diluted at concentrations (1, 5, 10, 15 and 20mM) with sterilized distilled water.

100mM stock solution of salicylic acid (SA) was prepared by dissolving 6.906gm in 500ml sterilized distilled water (40ºC) and diluted with sterilized distilled water to obtain the desired concentrations (20, 40, 60 and 80mM).

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 (30µg/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 (80mm dia.) A 8mm 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):

$$I\% = (C2 - C1) / C2 \times 100$$

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. 5ml 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 et al. 2011). About 10µl 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) $\times 10^4 cm^3$

Table1. Resistance inducers tested for *in vitro* and *in vivo* 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

Effect of seed soaking in inducers on disease severity development

Healthy fenugreek seeds of susceptible cultivar Deepak were used for this study. The surface sterilized seeds were soaked in different concentrations of methyl jasmonate and salicylic acid for 8 hrs while chitosan for 5 hrs and then washed 3-4 times with sterile distilled water to remove traces of inducers. The plastic pots were sterilized with 2% formalin and wait for 2 hrs to dry and filled with autoclaved sterilized sand loamy soil. Twenty treated seeds were sown in plastic pots containing sand-loamy soil as mentioned earlier. Twenty seeds were sown in each pot and three pots were used as replicates for particular treatment. The seed soaked in water served as control. All the pots containing sterilized soil were infested with conidial suspensions (157×10^4) which adjusted with haemocytometer. 100ml of spore suspensions was poured in each pot except control 1 where 100 ml sterile water was poured. For control 2, water soaked seeds were infested with spore suspensions. After 8 days, seed germination was noted and seedling height was measured using camel ruler (15cm). The disease severity was determined according to disease severity index scale after 30 days. The growth parameters such germination %, height of plant and no. of leaves per plant were also recorded. Five random plants from each pot were chosen for height of plant and no. of leaves per plant parameters.

Disease Severity

The infected plants of each treatment (in triplicate) were removed from the soil after 30 days of infestation and washed them thoroughly to remove soil particles. The disease severity percentage (DS %) was estimated (Ali et al. 2018) as follows: DS (%) = $\sum$ [(1A+2B+3C+4D) / 4T] × 100 Where, A, B, C and D are the number of plants corresponding to the numerical grade, 1, 2, 3 and 4 respectively and 4T is the total number of plants (T) multiplied by the maximum discoloration grade 4 where T= A+ B+C +D.

To detect the different degrees of disease, plants were classified into four categories according to Abo-Elyousar et al. 2014; Dorrance et al. 2003; Ali et al. 2018 with slight modifications. The root rot rating scale was as follows:

0= no root rot; 1= 1 to 25% of roots with visible lesions or root rot; 2= approximately 26 to 50 % of the roots rot or damaged; 3= 51 to 75 % of the root rot and 4= 76 to 100 % root rot or completely damaged.

Biochemical analysis of resistance response in plants under the activity of seed treatment

Analysis of biochemical changes in fenugreek plant due to seed treatment with chemical inducers was carried out to determine the effect of different treatments on the activity of chitinse, polyphenoloxidase and peroxidise which responsible for disease resistance. The infected roots from pot experiments were used for biochemical analysis.

Chitinase extraction:

One gram of root sample from each treatment was homogenized in a pre-chilled mortar pestle in 4 ml of 0.1 M sodium citrate buffer, pH 5.The homogenate was filtered through two layers of cheese cloth pre-moistened in 0.1 M sodium citrate buffer, pH 5. The filtrate was centrifuged at 10,000 g in a cooling centrifuge for 20 min and the supernatant was collected and used for assay.

Chitinase assay:

Chitinase activity was measured by the release of N-Acetyl-D-glucosamine (NAG) using colloidal chitin as substrate; colloidal chitin was prepared according to Berger and Reynolds (1958). The reaction mixture consisted of 50 µl sample, 950 µl substrate buffer (1% colloidal chitin in 0.1M sodium citrate buffer pH 5). This reaction mixture was incubated for 1 h at 37 ºC. After centrifugation at10000 g for 10 min, the released NAG was estimated at 540 nm using the dinitrosalicylic acid reagent. The chitinase activity was calculated using the standard curve of NAG. Chitinase activity was expressed as of µmol min^{-1} mg^{-1} protein.

Polyphenoloxidase assay:

Polyphenoloxidase activity was determined as per the procedure of Sadasivam & Manickam (1992). A sample of one gram was homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) at 4ºC. The homogenate was centrifuged at 20,000 rpm for 15 min. The supernatant served as enzyme source and polyphenoloxidase activity was determined as given: the reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µl of the enzyme extract. To start the reaction, 200 µl of 0.1 M catechol was added and the activity was expressed as change in absorbance at 495 nm at 30 sec. intervals for 3 min. The enzyme activity was expressed as changes in absorbance min^{-1} mg^{-1} of protein.

Peroxidase assay:

Peroxidase activity was determined as per the procedure of Sadasivam and Manickam (1992) with some modifications. One gram of fresh root tissues was homogenized in 5 volumes of 0.1 M phosphate buffer (pH 7.0) in pre-cooled pestle and mortar. The homogenate was centrifuged at 18,000 g at 40 ºC for 15 min. The supernatant was used as enzyme source within 2- 4 h of extraction. The samples were in ice till the completion of assay. Enzyme activity was expressed as change in absorbance at 436 nm and values expressed as enzyme activity unit per litre.

Statistical analysis

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

Results

Fenugreek plants showing typical wilt like symptoms were collected from different farmer's field (Fig. 1A) of Khatav Taluka, Satara District of Maharashtra State. The pathogen was isolated from infected roots by tissue isolation technique and purified by hyphal tip method. The pathogen was identified as *Fusarium solani* using morphological features and molecular tools. The pathogenicity of eight isolates has been proved on susceptible cultivar of fenugreek. The fungus showed typical root rot symptoms under pathogenicity test and in field.

Identification of the pathogen

Colonies on Potato Dextrose Agar at 25±2ºC, fast growing, floccose, white (Fig.1B), reverse dull yellow. Conidiophore produces from lateral hyphae, simple to branched, 74.60 × 3 µm long. Phialides produced from lateral hyphae, slender, smooth, hyaline, septate, decreasing towards length 4950-108.95 x 2.65-4.42 µm. Macroconidia rarely produced, 2-3 septate, smooth walled, hyaline, foot cell absent, sickle shaped. Microconidia (Fig. 1C) produced in gleosporic mass from long slender phialides, 3.85-4.5 x 8.16-13.17µm.

The molecular identity of virulent isolate of the fungus was confirmed by a fragment of the translation elongation factor 1-alpha (EF- 1α) gene and large sub-unit (LSU)-rDNA partial gene at National Fungal Culture Collection of India (NFCCI), Agharkar Research Institute, Pune, India where pathogen was deposited (Accession No. NFCCI- 4501). Internal transcribed spaces (ITS) and RNA polymerase (RBP1) are also done. The isolate showed 100% sequence similarity with *Fusarium solani* based on Fusarium ID and BLAST results. The fungus was confirmed as *Fusarium solani* (Mart.) Sacc.

Elongation factor 1-alpha (EF- 1α) gene sequence of *Fusarium solani* isolate

CTCTGGCAAGTCGACCACCGTAAGTCAAACCCTCATCGCGATCTGCTTAT
CTCGGGTCGTGGAACCCCGCCTGGCATCTCCTCTGGCAAGTCGACCACC
GTAAGTCAAACCCTCATCGCGATCTGCTTATCTCGGGTCGTGGAACCCC
GCCTGGCATCTCGGGCGGGGTATTCATCAGTCACTTCATGCTGACAATC
ATCTACAGACCGGTCACTTGATCTACCAGTGCGGTGGTATCGAGGGCGG
GGTATTCATCAGTCACTTCATGCTGACAATCATCTACAGACCGGTCACTT
GATCTACCAGTGCGGTGGTATCGACAAGCGAACCATCGAGAAGTTCGAG
AAGGTTGGTGACATCTCCCCCGATCGCGCCTTGCTATTCCACAACGAATT
CCCTCCAAGCGAACCATCGAGAAGTTCGAGAAGGTTGGTGACATCTCCC
CCGATCGCGCCTTGCTATTCCACAACGAATTCCCTCCCTCGCGATACGCT
CTGCGCCCGCTTCTCCCGAGTCCCAAAATTTTTGCGGTCCGACCGTAATT
TTTTTTTTGGGGGGGCCCTCGCGATACGCTCTGCGCCCGCTTCTCCCGAG
TCCCAAAATTTTTGCGGTCCGACCGTAATTTTTTTTTTGGTGGGGCATTT
ACCCCGCCACTCGGGCGACGTTGGACAAAGCCCTGATCCCTGCACACAA
AAACACCAAACCCTCTTGGCGCGCATCATTTACCCCGCCACTCGGGCGA
CGTTGGACAAAGCCCTGATCCCTGCACACAAAAACACCAAACCCTCTTG
GCGCGCATCATCACGTGGTTCACAACAGACGCTAACCGGTCCAACAATA
GGAAGCCGCTGAGCTCGGTAAGGGTTCCTTCAAGATCACGTGGTTCACA
ACAGACGCTAACCGGTCCAACAATAGGAAGCCGCTGAGCTCGGTAAGG

GTTCCTTCAAG

LSU-rDNA partial sequence of *Fusarium solani* isolate

CCTAGGCTTCGTCACTGACCTCCACGTCCGCCTACTCCTCAGGGCATCGTTT
CTACCCTGAGGGCGAGGTATGGGTGAGACGCTTGAGCGCCATCCATTTTCA
GGGCTAGTACATTCGGCAGGTGAGTTGTTACACAGTCCTTAGCGGATTCCG
ACTTCCATGGCCACCGTCCTGCTGTCAAGATGTACTAACACCTTTTGTGGTG
TCTGATGAGCGTCTACTCTGGCACCTTAACCTCGCGTTCGGTTCATCCCGCA
TCGCCAGTTCTGCTTACCAAAAATGGCCCACTAGTGTTGATTCATTCGAATG
CCCACGTTCAATTAAGCAACAAGGGCTTCTTACATATTTAAAGTTTGAGAAT
GGATGAAGGCTAAATAGCGCCCCCGAGTCCCTAATCATTCGCTTTACCTCAT
AAAACTGAGTTCAACACTGCTATCCTGAGGGAAACTTCGGCGGAAACCAGC
TACTAGAAGGTTCGATTAGTCTTTCGCCCCCATGCCCATGTTTGACGATCGA
TTTGCACGTCAGAACCGCTGCGAGCCTCCACCAGAGTTTCCTCTGGCTTCAC
CCTACACAGGCATAGTTCACCTTCTTTCGGGTCCGGCCCCGTATGCTCTTAC
TCAAATCCATCCGATAACATCAGGATCGGTCGATGATGCGCCGAAGCTCTC
ACCTGCGTTCACTTTCATTTCGCGTAGGGGTTTGACACCCGAACACTCGCAT
ACGAAGACGACTCCTTGGTCCGTGTTTCAAGACGGGTCACTGATGACCATT
ACGCCAGCATCCTTGCGAATGCGCGAACCTCAGTCCGCCACAGGGTATTAC
GCAGCGGGCTATAACACTCCCCGGAGAGAGCCACATTCCCGAAGCCTTTAT
CCCCCAGGGCGAACTGATGCTGGCCTGAGCCGGAAGAGTGCACCGGGGAG
AACCCCGGATGATCAACCAAGCCCAAGTCTGGTCACAAGCGCTTCCCTTTC
AA

>*Fusarium solani* isolate FS internal transcribed spacer (ITS)

CGTTGGTGAACCAGCGGAGGGATCATTACCGAGTTATACAACTCATCAACC
CTGTGAACATACCTATAACGTTGCCTCGGCGGGAACAGACGGCCCCGTAAC
ACGGGCCGCCCCCGCCAGAGGACCCCCTAACTCTGTTTCTATAATGTTTCTT
CTGAGTAAACAAGCAAATAAATTAAAACTTTCAACAACGGATCTCTTGGCT
CTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAG
AATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCT
GGCGGGCATGCCTGTTCGAGCGTCATTACAACCCTCAGGCCCCCGGGCCTG
GCGTTGGGGATCGGCGGAAGCCCCCTGCGGGCACAACGCCGTCCCCCAAAT
ACAGTGGCGGTCCCGCCGCAGCTTCCATTGCGTAGTAGCTAACACCTCGCA
ACTGGAGAGCGGCGCGGCCACGCCGTAAAACACCCAACTTCTGAATGTTGA
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>*Fusarium solani*isolate FSpartial RPB1 gene for RNA polymerase II large subunit 1
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TGCAGTATCACGTTGCCACCTACATGG

Pathogenicity

The eight isolates of *F. solani* were screened for their pathogenicity on fenugreek susceptible cultivar Deepak under net house conditions. Pathogenicity of *F. solani* was observed for typical symptoms on root as well as on plant using soil inoculation and seed inoculation methods. Root rot symptoms were appeared after 16 days of infestation. The infected roots become shrivelled and resemble a thread. The cortical tissues of root decayed and showed a distinct brown discolouration. 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 (Fig.1 D & E). The control plants showed no symptoms. In case of seed inoculation method, symptoms appeared after 6 days as thin, dark brown radicle. The control radicle did not show any symptoms. The fungus was re-isolated from the artificially infected roots and was compared with the original culture of *F. solani*. The re-isolated fungus was identical in appearance with the original culture, thus Koch's postulates were fulfilled.

The tested *F. solani* isolates showed variation in their ability to cause wilt symptoms in fenugreek under shade house conditions (Table 2). All 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 and it was selected for *in vitro* studies.

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

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

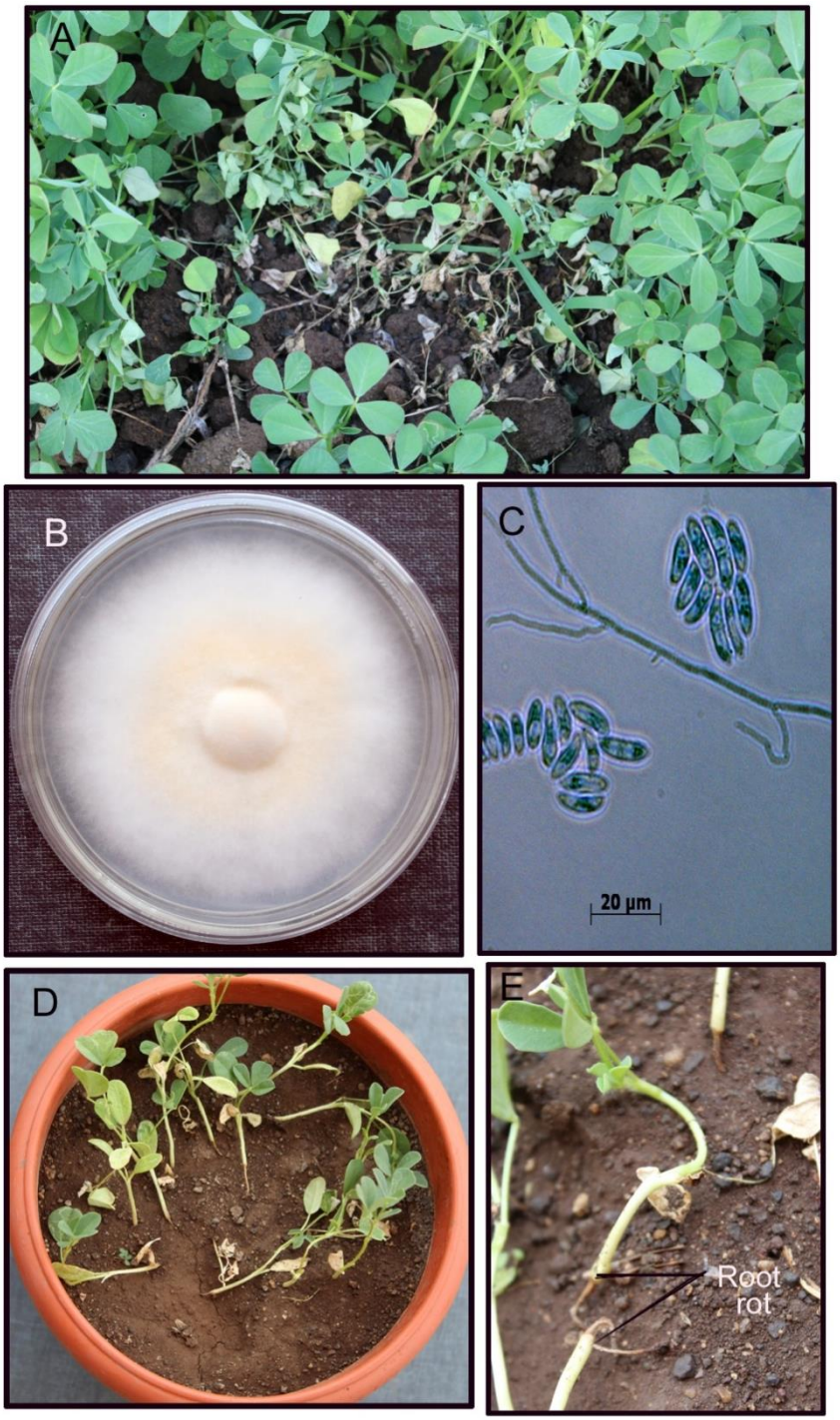


Fig. 1 A: Root rots symptoms fenugreek field; **B**: Colony growth and **C**: Conidial morphology of fungus isolated from infected part of fenugreek root; **D**: Pot study of fenugreek root rot pathogenicity caused by isolated fungus;**E**: Fenugreek root rot symptoms caused by isolated fungus

Data in Table 3 & Fig. 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.

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

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

MeJa had inhibitory effect on mycelial growth of *F. solani* after 8 days of incubation (Table 4 & Fig. 3). 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 5mM concentration. It was interesting that MeJa had halted spore formation of fungus at10mM concentration.

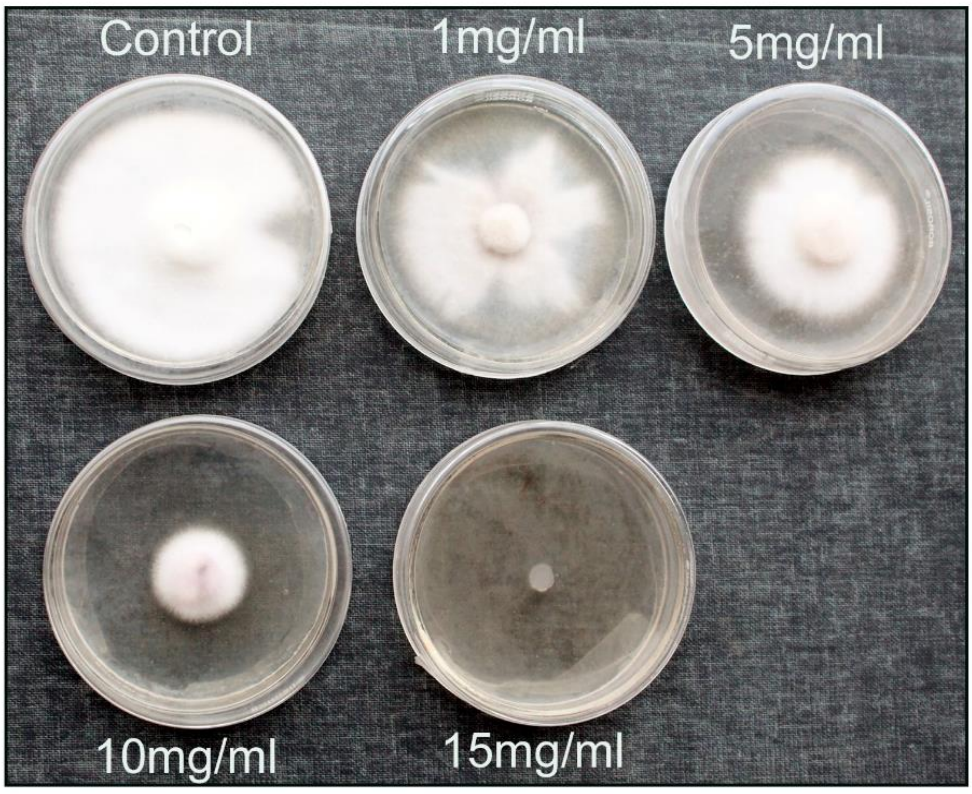
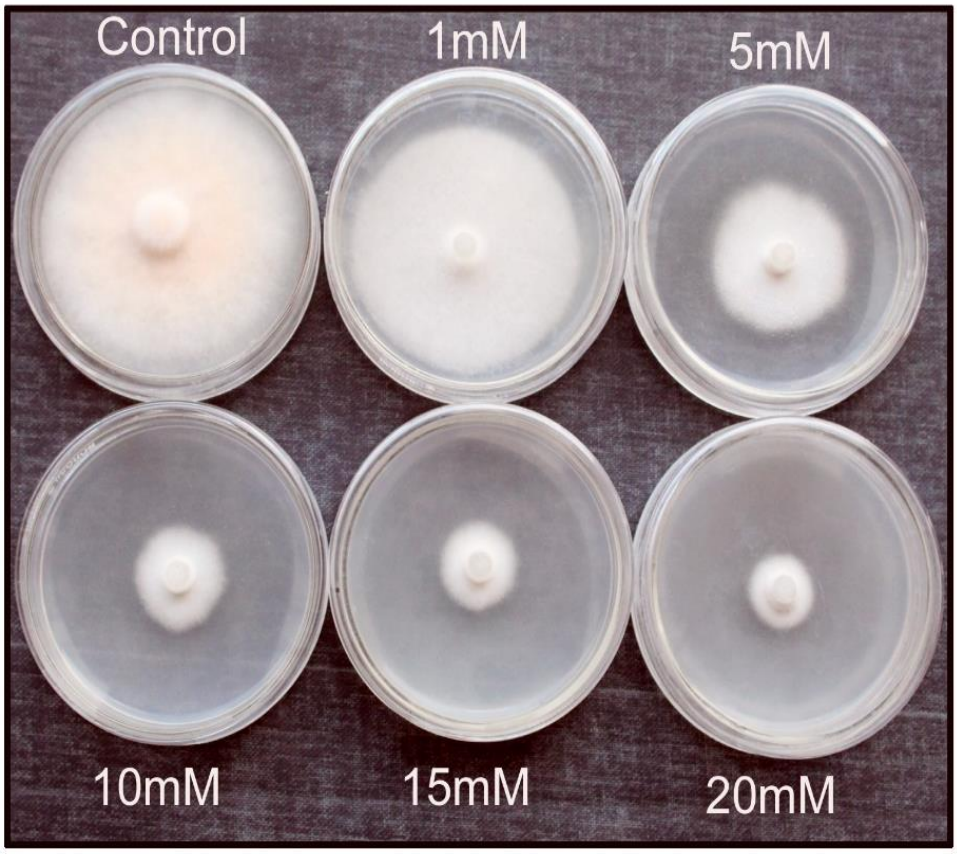


Fig. 2. Effect of Chitosan on linear growth and sporulation of



F.solani

Fig. 3. Effect of methyl jasmonate 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) Sporulation*×104	Linear growth* (mm)	Percent Inhibition	Sporulation*×10^4
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

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 & Fig. 4).

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

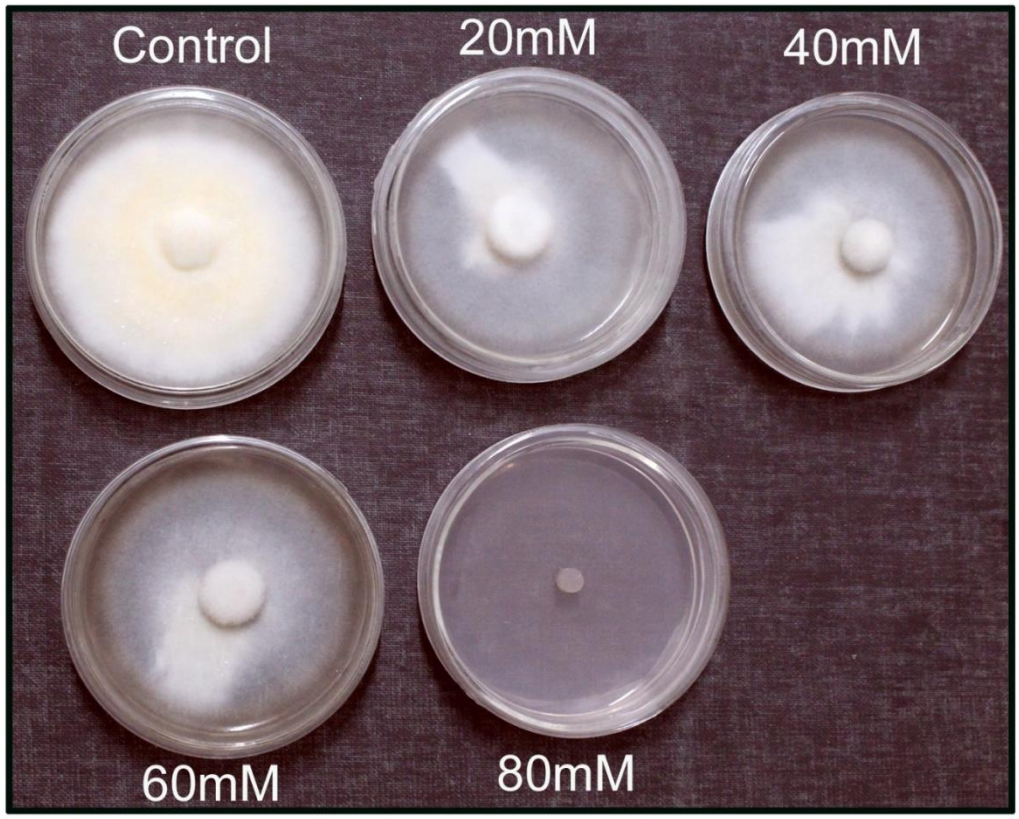


Fig. 4. Effect of salicylic acid on linear growth and sporulation of *F.solani*

1 mM 5mM 10mM 15mM 20mM

Fig. 5. Effect of methyl jasmonate on seed germination and length of seedlings after 8 days.

Influence of resistance inducer treatments on various physiological characteristics of fenugreek plant

None of the resistance inducers increased the germination percentage of fenugreek seeds as compared to untreated control and inoculated control. (Table 6). It was observed that when concentration of inducer increases, germination percentage decreases. Seed germination was found highest in chitosan (0.25mg/ml) treatment (88.30%) followed by MeJa (1mM) treatment (85%) followed by SA (1mM) treatment (80%). Chitosan treatment had inhibited seed germination beyond 0.50mg/ml concentration. It was due to entire covering over seed prevented germination. All of the tested resistance inducers increased seedling height when compared with the control (Table 6) but, seedling height decreases as concentration of inducer increases (Fig.5).

Table 6 Seed germination and length of seedlings after 8 days subjected to the fenugreek seed treatment with chitosan, methyl jasmonate (MeJa), salicylic acid (SA) and the inoculation of *F. solani*

Treatment	Germination (%)*	Seedlings length (cm) #
Chitosan 0.25mg/ml	88.30	3.53
Chitosan 0.50mg/ml	76.65	2.90
MeJa 1mM	85.00	4.53
MeJa 5mM	71.65	4.19
MeJa 10mM	55.00	4.01
MeJa 15mM	51.65	3.63
MeJa 20mM	36.65	3.07
SA 1mM	80.00	4.10
SA 5mM	56.65	3.48
Un-inoculated control	91.65	3.22
Inoculated control	88.30	3.20
SEM±		0.15
C.D. (P= 0.05)		0.70

* mean of three replications (pots), #mean of fifteen random seedlings

The height of plant, no. of leaves/plant and root rot disease severity after 30 days were recorded and shown in table 7 & (Figs. 6,7, 8). The height of plant of tested inducers decreased after 30 days when compared with inoculated control (C2) except MeJa 1mM concentration. Number of leaves per plant was increased in SA treatments (1mM and 5 mM) among other inducers when compared to inoculated control. The root rot disease severity also reduced in SA treatments. In MeJa treatment, when

concentration increases, disease severity also increases. Among all tested inducers. SA was found superior in growth parameters and also reduced disease severity.

Table 7 Height of plant, no. of leaves / plant and root rot disease severity after 30 days subjected to the fenugreek seed treatment with chitosan, methyl jasmonate (MeJa), salicylic acid (SA) and the inoculation of *F. solani*

Treatment	Height of plant (cm) [#]	No. of leaves /plant[#]	Disease severity (%)
Chitosan 0.25mg/ml	7.79	7.46	26.56
Chitosan 0.50mg/ml	6.18	6.75	18.75
MeJa 1mM	9.48	7.86	32.00
MeJa 5mM	8.18	6.60	47.72
MeJa 10mM	7.49	7.40	57.69
MeJa 15mM	7.06	6.33	60.00
MeJa 20mM	6.54	6.46	57.35
SA 1mM	8.58	8.73	15.62
SA 5mM	8.18	8.60	14.70
Un-inoculated control	10.18	9.93	00.00
Inoculated control	8.74	4.80	84.21
SEM±	0.36	0.42	
C.D. (P= 0.05)	0.94	1.27	

[#]mean of fifteen random seedlings

Fig.6.Effect of chitosan on growth and disease A: C1, B: C2, C: 0.25, D: 0.50mg/ml

Fig.7. Effect of MeJa on growth and disease A: 1, B: 5, C: 10, D: 15, E: 20mM

Fig.8. Effect of salicylic acid on growth and disease A: C1 (Uninoculated control), B: C2 (Inoculated control), C: 1mM, D: 5mM

Induction of defense enzymes due to effect of resistance inducers under seed treatments

The activity of defense enzymes like peroxidise, polyphenoloxidase and chitinase in fenugreek plants due to resistance inducers against *F. solani* under seed treatments was studied after 30 days. Results presented in Table 8 showed that all the treatments increased the activity of peroxidase, ploy phenoloxidase and chitinase over uninoculated control and inoculated control. The highest increased activity of peroxidase was found in SA treatment at 5mM concentration (0.524) followed by MeJa at 20mM concentration (0.423) followed by SA at 1mM concentration (0.418). Overall, SA treatment found superior to induce peroxidase enzyme under seed treatment. Polyphenoloxidase (0.106) and chitinase (0.488) activities were also found superior among other treatments.

Table 8 Contents of chitinase, polyphenoloxidase and peroxidase / ml in roots after 30 days subjected to the fenugreek seed treatment with chitosan, methyl jasmonate (MeJa), salicylic acid (SA) and the inoculation of *F. solani*

Treatment	Chitinase	Polyphenoloxidase	Peroxidase
Chitosan 0.25mg/ml	0.506	0.084	0.341
Chitosan 0.50mg/ml	0.519	0.079	0.339
MeJa 1mM	0.478	0.065	0.340
MeJa 5mM	0.483	0.079	0.361
MeJa 10mM	0.487	0.084	0.385
MeJa 15mM	0.486	0.076	0.405
MeJa 20mM	0.487	0.074	0.423
SA 1mM	0.479	0.103	0.418
SA 5mM	0.488	0.106	0.524
Un-inoculated control	0.447	0.042	0.220
Inoculated control	0.459	0.054	0.320

Discussion

On the basis of morphological and molecular characteristics, *F. solani* was identified as causal agent of root rot in fenugreek. Young plants are more susceptible than older ones. At the seedlings stage, the recorded rot incidence was 50-75 per cent (Khokhar et al. 2012). Many fungi species have already been reported as the causal agent of root rot from fenugreek plants. Shivpuri & Bansal (1987) have first reported *F. oxysporum* Schle. as wilt pathogen in fenugreek from Rajasthan, India. *Fusarium solani* has already been reported associated with damping-off and root rot of fenugreek in Egypt (Hegazy et al. 2013, Ali et al. 2018). However, root rot of fenugreek caused by *F. solani* . *F. solani* produces root rot and wilt symptoms in many agricultural crops and ornamental plants. *F.solani* is a well- known fungus causing root rot in temperate and tropical soils. It causes serious root rot of French bean (Phaseolus) in the USA (Burke, 1965). In India, *F. solani* is reported to cause root rot in chickpea, soybean, fennel, sunflower, ginger, cucurbits and onion (Haware, 1993)

In the present study, I have observed an antifungal activity of chitosan with different concentrations against F. solani causing root rot in fenugreek. Chitosan reduced mycelial growth and sporulation of *F. solani* as concentrations increased. The complete inhibition of both mycelial growth and sporulation was observed at 15mg/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 (Baustista- 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 6g/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 (Hirano et al. 1990, 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 (Leuca 1986) and the interaction of chitosan with fungal DNA and RNA (Hadwiger 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 at10mM 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 20mM 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* s. *pradicis lycopersici* at lower concentrations. Kumar & Bains (2018) also found that the exogenously applied SA promoted mycelial growth at low concentration (0.5mM) but concentration higher than 0.5mM 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 S. 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 (1-25mM) as compared to the untreated control.

A convenient means of applying crop protection treatments involves treating the seed. Seed treatments can be particularly useful since they can provide protection to young plants during vulnerable stage in their development. Pre-treatment with MeJa resulted in enhanced levels of resistance in potato and tomato against *Phytophthora infestans* (Cohen et al. 1993). Resistance inducers provide an additional option to manage plant diseases while maintaining Sustainable production. Treating seeds with resistance inducers may represent a novel approach to control diseases in crop plants, particularly diseases caused by soil-borne pathogens (Maria PA et al. 2010).

All the treatments increased the activity of peroxidase, ploy phenoloxidase and chitinase over uninoculated control and inoculated control under seed treatments after 30 days. The highest increased activity of peroxidase was found in SA treatment at 5mM concentration (0.524) followed by MeJa at 20mM concentration (0.423) followed by SA at 1mM (0.418). Overall, SA treatment found superior to induce peroxidase enzyme under seed treatment. Polyphenoloxidase (0.106) and chitinase (0.488) activities were also found superior among other treatments. The increase activity of defense enzymes in seed treated plants might be responsible for defense response in fenugreek plants. Pre-treatment of plant with resistance inducers induce plant defense against subsequence attacked (Jendoubi et al. 2015).

Summary and Conclusion

Fenugreek plants showing wilt like symptoms were collected from different farmer's field of Khatav Taluka, Satara District of Maharashtra State. The pathogen was isolated from infected roots by tissue isolation technique and purified by hyphal tip method. The pathogen was identified as *Fusarium solani* (Mart.) Sacc. using morphological features and molecular tools. The pathogenicity of obtained eight isolates has been proved on susceptible Deepak cultivar of fenugreek. The fungus showed typical root rot symptoms under pathogenicity test and in field. Root rot symptoms were appeared after 16 days of infestation. The infected roots become shrivelled and resemble a thread. The cortical tissues of root decayed and showed a distinct brown discolouration. 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. The control plants showed no symptoms. All isolates had potency to cause root rot disease but the isolate (Fs-5) from Jaygaon locality was found highly virulent one and was selected for *in vitro* and *in vivo* studies.

The inhibitory effect of plant resistance inducers *viz*. chitosan (1-15mg/ml), methyl jasmonate (1-20mM) and salicylic acid (20-80mM) on linear growth and sporulation of *F. solani* was evaluated by poisoned food technique. Chitosan had significantly inhibited linear growth and sporulation of *F. solani* at 15 and 10 mg/ml concentrations respectively. Methyl jasmonate had significantly reduced linear growth as concentrations increased but sporulation halted at 10mM. Salicylic acid had promoted linear growth but reduced sporulation in increasing concentrations (20, 40 and 60 mM). At highest concentration (80mM), salicylic acid acts as toxic for pathogen.

It was observed that salicylic acid did not show direct antifungal activity against *F. solani* and root rot disease resistance in fenugreek plants was found as a result of plant defense mechanism rather than direct inhibitory effects of salicylic acid on the fungus. This means that SA activates the signal transduction pathways, thus leading to the expression of systemic acquired resistance (SAR) than direct inhibitory activity of the fungus. The other plant resistance inducers *viz*., methyl jasmonate and chitosan showed direct antifungal activity against *F. solani in vitro* but did not possess systemic acquired resistance as compared to salicylic acid. Present findings showed that direct antifungal activity and systemic acquired resistance in fenugreek plant using resistance inducers under seed treatments could not be influenced simultaneously.

None of the resistance inducers had increased the germination percentage of fenugreek seeds as compared to untreated control and inoculated control. It was observed that when concentration of inducer increases, germination percentage decreases. Seed germination was found highest in chitosan (0.25mg/ml) treatment (88.30%) followed by MeJa (1mM) treatment (85%) followed by SA (1mM) treatment (80%). Chitosan treatment had inhibited seed germination beyond 0.50mg/ml concentration. It was due to entire covering over seed prevented germination. All of the tested resistance inducers increased seedling height when compared with the control but, seedling height decreases as concentration of inducer increases.

The height of plant, no. of leaves/plant and root rot disease severity after 30 days were recorded under seed treatment of inducers. The height of plant of tested inducers

decreased after 30 days when compared with inoculated control except MeJa 1mM concentration. Number of leaves per plant was increased in SA treatments (1mM and 5 mM) among other inducers when compared to inoculated control. The root rot disease severity also reduced in SA treatments. In MeJa treatment, when concentration increases, disease severity also increases. Among all tested inducers. SA was found superior in growth parameters and also reduced disease severity.

All the inducers had increased the activity of peroxidase, ploy phenoloxidase and chitinase over uninoculated control and inoculated control under seed treatments. Salicylic acid treatment found superior to induce peroxidase, polyphenoloxidase and chitinase activities among other treatments. Thus, salicylic acid could be an eco-friendly alternative under seed treatment to control root rot of fenugreek without using toxic fungicides.

Significance of the Study

The application of chemical inducers of resistance is an exciting new perspective to supplement the classical chemical means of disease control by providing both effective and ecologically-friendly plant protection. Plant resistant inducers induced defence responses in fenugreek plants under seed treatments against *F. solani* pathogen. In this study, *F. solani* causes root rot disease on fenugreek was investigated. Salicylic acid was found superior in induction of disease resistance in fenugreek against *F. solani* under seed treatments. This study is very useful to control root rot disease of fenugreek, a leafy vegetable without use of fungicides.

Future prospects

Other resistance inducers will be screened against root rot of fenugreek

Experiments will be conducted in farmer's field using salicylic acid seed treatments

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