TOPIC APPROVAL PERFORMA

School of Agriculture

Program : P26B-NN6::M.Sc. Ag. (Plant Pathology)

COURSE CODE : PTH596 **REGULAR/BACKLOG :** Regular **GROUP NUMBER :** AGRRGD0295

Supervisor Name : Dr. Adesh Kumar **UID :** 19078 **Designation :** Assistant Professor

Qualification : _____ **Research Experience :** _____

SR.NO.	NAME OF STUDENT	REGISTRATION NO	BATCH	SECTION	CONTACT NUMBER
1	Syed Jilani	11718608	2017	H1727	8977288808

SPECIALIZATION AREA : Plant Pathology **Supervisor Signature:** _____

PROPOSED TOPIC : A study on evaluation of effective isolates of antagonists possessing high decomposition ability

Qualitative Assessment of Proposed Topic by PAC		
Sr.No.	**Parameter**	**Rating (out of 10)**
1	Project Novelty: Potential of the project to create new knowledge	8.00
2	Project Feasibility: Project can be timely carried out in-house with low-cost and available resources in the University by the students.	7.67
3	Project Academic Inputs: Project topic is relevant and makes extensive use of academic inputs in UG program and serves as a culminating effort for core study area of the degree program.	7.67
4	Project Supervision: Project supervisor's is technically competent to guide students, resolve any issues, and impart necessary skills.	8.33
5	Social Applicability: Project work intends to solve a practical problem.	7.67
6	Future Scope: Project has potential to become basis of future research work, publication or patent.	8.00

PAC Co mmittee Members		
PAC Member 1 Name: Dr. Vinit Pratap Singh	UID: 18630	Recommended (Y/N): Yes
PAC Member 2 Name: Dr. Adesh Kumar	UID: 19078	Recommended (Y/N): Yes
PAC Member 3 Name: Dr. Ankush Moreshwar Raut	UID: 19876	Recommended (Y/N): Yes
DAA Nominee Name: Manoj Kumar	UID: 22237	Recommended (Y/N): NA

Final Topic Approved by PAC: **Studies on Induced Systemic Resistance through different origin chemicals against Soil Borne Infections of Tomato (Solanum lycopersicum L.).**

Overall Remarks: Approved (with major changes)

PAC CHAIRPERSON Name: 19212::Dr. Ramesh Kumar Sadawarti **Approval Date:** 30 Mar 2018 5 /14/2018 4:08:22 PM

Studies on Induced Systemic Resistance through different origin chemicals against Soil Borne Infections of Tomato (Solanum lycopersicum L.)

SYNOPSIS FOR PRE DISSERTATION

PTH 596

A Project thesis submitted to the Lovely Professional University, Phagwara,

In partial fulfillment of the Requirement for the award of degree

Master of Science

In

Agriculture
(Plant Pathology)

By

Syed Jilani

Registration Number 11718608

Section H1727

Under the supervision and guidance of

Dr. Adesh Kumar

Assistant Professor

May, 2018

LOVELY PROFESSIONAL UNIVERSITY
Transforming Education Transforming India

LOVELY PROFESSIONAL UNIVERSITY

Transforming Education Transforming India

School of Agriculture

Date: 14 May 2018

CERTIFICATE

This is to certify that work embodied in this Thesis report entitled "**Studies on Induced Systemic Resistance through different origin chemicals against Soil Borne Infections of Tomato (Solanum lycopersicum L.)**" has been carried out by **Syed Jilani, Registration No.: 11718608** under my supervision and guidance. To the best of my knowledge, the present work is the result of his original investigation and study. No part of this thesis has ever been submitted for any other degree or diploma. The work has been carried out by him at School of Agriculture, Lovely Professional University, Phagwara, Punjab, India. He fulfilled the requirement for the award of the degree Master of Science in Plant Pathology.

Signature of Student

Syed Jilani

Reg. No. 11718608

Signature of the supervisor

Dr. Adesh Kumar

UID: 19078

Assistant Professor

DECLARATION

I hereby declare that the project work entitled "**Studies on Induced Systemic Resistance through different origin chemicals against Soil Borne Infections of Tomato (Solanum lycopersicum L.)**" is an authentic record of my work carried out at Lovely Professional University as requirements of project work for award of degree of Master of Science in Plant Pathology, under the guidance of Dr. Adesh kumar Assistant Professor, Department of Plant Protection, School of Agriculture, Lovely Professional University, Jalandhar, Punjab India.

Syed Jilani

(Registration No.11718608)

TABLE OF CONTENTS

1. INTRODUCTION

Tomato crop (*Lycopersicon esculentum* Mill.) is one among the world's most widely grown vegetable crops for consumption as fruits and as various processed products **(**Giovanni et al 2004, Hariprasad et. al. 2009). The less yield of tomato is due to its susceptibility to various pathogenic bacteria, nematodes, viruses and fungi, which are major threat to tomato cultivation **(**Barone et. al. 2007). Wilt of fusarium caused by soil borne fungus, *Fusarium oxysporum*Schlectend.: Fr. f. sp. *lycopersici* (Sacc.) W.C. Snyder and H.N. Hansen, is one of the most destructive diseases of tomato **(**Sudhamoy et. al. 2009). It affects both greenhouse and the field grown tomatoes in warm crop production areas. It's characteristic symptoms are chlorotic leaves and wilting plants with very less or no crop yield. 30 to 40% loss of yield due to the disease and which may leads to 80% under severe conditions **(**Kirankumar et. al. 2008, Kapoor et. al. 1988). Higher incidence of Fusarium wilt in tomato of 25–55% has been recorded from different parts of India (Pandey et. al.. 2013, Asha et. al. 2011). Initially root epidermis invaded by the pathogen and extends into the vascular tissue. It produces mycelium and conidia which colonizes the xylem vessels. Severe water stress results the characteristic wilt symptoms, mainly due to vessel clogging **(**Beckman et. al. 1987). The three physiological races (1, 2, and 3) of this pathogen are illustrated by their specific pathogenicity to the cultivars of tomato **(**Kawabe et. al. 2005). The exchange of genetic material takes place through somatic fusion and hetreokaryon formation between vegetatively compatible strains as *F. oxysporum* f. sp. *lycopersici* (Fol) is an asexual fungus **(**Leslie et. al. 1993).

2. OBJECTIVES OF STUDY

However, due to above points the important role of this research is to find out the "**Studies on Induced Systemic Resistance through different origin chemicals against Soil Borne Infections of Tomato (Solanum lycopersicum L.)**" on the assumption of the following objectives:

Objectives :

1. Comparative evaluation of inducers against Fusarium wilt in Tomato.

2. To find out the efficacy of inducers on Tomato growth parameters.

3. To study biochemical changes in Tomato due to the effect of seedling treatment with inducers.

4. To study the defense response in Fusarium wilt in Tomato due to the effect of inducers.

3. SCOPE OF STUDY

Studying or evaluating the effect of different chemical inucers against fusarium wilt of tomato and growth parameters.We are evaluating for the best inducer of these chemicals. Because prevention is always better than cure as once the disease severity increases the chemical usage increases which leads to economic loss. Thus there is need to find out the alternate sources, which reduces the cost of input and promotion of growth of the plants.

4. REVIEW OF LITERATURE

4.1 ISR:

According to Ahmad *et al*., 2010 the phenomena of most induced resistance are based on a combination of direct induction and priming.

Probenazole is the first chemical resistance activator which was registered as Oryzemate in 1975 in Japan, and since then many other chemical and biological activators have been developed, including: ASM, registered as Milsana (*Reynoutria sacalinensis* extract; KHH BioScience), Messenger (harpin protein; Plant Health Care), Elexa (chitosan; SafeScience) and Bion and Actigard (Syngenta).

4.2 Germination test:

Each treatment with three replicates.The Seed germination was calculated by counting the number of completely germinated seeds per plate (Nejad and Johnson, 2000).

4.3 Disease

F. o. f.sp. *lycopersici* (Sacc) W.C. Synder and H.N. Hansen (1940) of class Hypomycetes, causes tomato wilt, major disease occurring in nearly all the tomato grown areas of the world. Its incidence is almost every year in mild or severe form in India.

4.4 Disease status

Tomato wilt, caused by *F. o. f.sp. lycopersici* is a destructive disease worldwide in major tomato-growing regions and has been reported in at least 32 countries.

Wilt of tomato caused by *F. o.* f.sp. *lycopersici* (Sacc) W.C. Synder and H.N. Hansen (1940), an economically important disease which occurs in most of tomato growing countries, in the world like Australia, Brazil, China, India, Israel, Mexico, Netherlands, Turkey and United states (Livingston and Erwin, 1969).

In India, the disease is widely distributed through out the state especially in Assam, Karnataka, Orissa, Maharashtra, Bihar, Haryana, Punjab, West Bengal and Uttar Pradesh (Rao and Mukherji, 1972)

4.5 Host pathogen relationship

F.o. f.sp. *lycopersici* is host specific pathogen infecting only Tomato (Subramanian, 1971; Booth, 1971; Padwick, 1940; Upadhyay and Rai, 1979; Gerlach and Nirenberg, 1982). The fungus is a soil borne facultative parasite and enters into plant through fine roots wchich spreads to the whole plant via vascular bundle. Kaiser and Sengupta (1971) described the distribution of *F.o.* f.sp. *lycopersici* in host tissues particularly vascular bundles. As a result a very high level of its population was observed in the vicinity of infected plants which further increases in the infested plots with cultivation of Tomato in the same plot (Nene *et al*., 1979; Upadhyay and Rai, 1989). The pathogen spread more

rapidly along the roots than across the soil (Butler, 1910). Mohanty (1946) reported that the conidia produced on tissues in the wilted plant retained viability for a few months but it is still to be determined whether these can survive from one to another season under field conditions. The age of the plant has marked correlation with wilting (Mundkur, 1935; Kotasthane and Gupta, 1981; Reddy *et al*., 1988; Pawar *et al*., 1993). Butler (1906) recorded disease appearance in young seedlings during August and in November - December during flowering time mortality in mature plants is highest. The disease is initiated when the plants are 30 days old or slightly more (Pawar *et al*., 1993; Chaube, 1968) and continues to persist up to maturity. The pathogen has been reported to produce pectin methyl esterase, polygalacturonase and cellulase (Singh, and Husain, 1962, 1968; Hiremath *et al*., 1973; Kaiser and Sengupta, 1979) and a toxin fusaric acid (Singh and Husain, 1964; Prasad and Chaudhary, 1974) both *in vivo* and *in vitro*. A fast degradation of fructose, refinose, maltose, glucose and amino acids in the infected plants was observed (Chaudhary and Prasad, 1974; Prasad and Chaudhary, 1974). Kalyansundaram (1952) showed reduction of ascorbic acid and increase level of reducing sugars of infected host leaves. Murthy (1975) recorded the amount of phenols, total sugar, amino nitrogen, reducing sugars, amino acids, alkaloids and flavanols are higher in resistant varieties of tomato. These varieties also have higher amount of cysteine, tryptophan and xylose and lesser amount to phenyl alanine. Murthy (1975) and Kotasthane *et al*. (1983) noticed that the spore germination is influenced by root exudates, according to them higher spore germination take place in the root extracts of susceptible cultivars than that in the extracts of resistant cultivars. They found that there are some inhibitory compounds to spore germination and germtube growth present in the root extracts of resistant cultivars. These were identified as chlorogenic acid, caffeic acid and an unknown phenolic acid etc.

4.6 Disease cycle

The disease cycle of tomato wilt as earlier described by Mc Rae and Shaw (1933). The pathogen survives saprophytically for 3-5 years primarily on host residues. Other than host debris, pathogen also survives on organic matter for short span. Mycoparasitic survival on other fungi in soil also reported (Upadhyay *et. al.,* 1983). Chlamydospores plays a major role in disease cycle. They are formed both in parasitic and saprophytic phases from the hypha and conidial cells and helped in the survival during prolonged absence of host. Perithecia produced on collar region and exposed root also serve as resting structures under unfavorable conditions, ascospores produce somatic hypha either to cause infection or to produce conidia, which in turn cause infection (Updhayay and Rai, 1992).

4.7 Disease management

Being primarily a soil borne disease, various practices like- cultural operations, resistance varieties, chemicals, biocontrol agents and soil amendments are suggested for the management of tomato wilt (Butler, 1953; Prasad *et al*., 1978; Walter and Hayslip, 1961; Mendal and Sinha, 1985; Amin, 1994; Reddy *et al*., 1990; Vishwa Dhar *et al*, 1994).

Silica

Kiirika et. al. (2013) reported that resistance induced against bacterial wilt caused by Ralstonia solanacearum with the treatments of Silicon (Si) in tomato. These Si treatments reduced the incidence of Bacterial wilt by 40% and 26.6% in King Kong 2 and in L390 varieties respectively.

Plants treated with silicon reduced the incidence of Bacterial wilt by 38.1% and 100% in King Kong 2 (moderately resistant genotype of tomato) and Hawaii 7998 (resistant genotype). 5 days after the inoculation, the pathogen density was drastically reduced in roots and stems of Hawaii 7998 genotype, and in stems of King Kong 2 in the plants treated with silicon compared to non-treated plants, indicating a silicon-induced resistance. **(**Diogo et. al. 2007)

Potassium Phosphite

Potassium phosphite was applied to seed potato tubers and foliage in a series of field experiments. The results indicate that the defense responses has been induced by the KPhi application to seed tuber and foliage against *Fusarium solani* in cortex and tuber periderm and that these reactions are related to biochemical and structural changes in these tissues. **(**Alexandersson et. al. 2016)

Ferric chloride:

The capacity of Ferric chloride tested to inhibit rice blast under field and greenhouse conditions. The chemicals considerably decreased disease severity by soil drench, thus establishing a systemic effect. Grain yield was significantly increased by all chemicals in one of the experiments which included five sprays from seedling to heading growth stages. Only ferric chloride significantly increased the grain yield. Studies suggest that the applied chemicals induced resistance involves the blast control. (Manandhar et. al. 1998)

CaCl2:

Foliar application of CaCl2 showed significant decrease in the incidence of wilt after inoculation. The production of defense and antioxidant enzymes increased in elicitor treated sets over control.(Chakraborty et. al. 2017)

Phosphates:

Solutions of K3PO4, K2HPO4, NA3PO4, and NA2HPO4 sprayed on the undersides of the first and second true leaves of cucumber induced systemic resistance in leaves 3 and 4 to anthracnose caused by Colletotrichum hydroxide.(Gottstein et. al. 1989)

Biochar:

Biochar applied to soil was demonstrated to induce systemic resistance to grey mould (Botrytis cinerea) on pepper, the broad mite pest (Polyphagotarsonemus latus) , and on pepper powdery mildew (Leveillula taurica) on tomato (Elad et al., 2010).

5. MATERIALS AND METHODS

5.1 Technical Programme of Work:

5.1.1 Location of the Field of Experiment

The present experiment will be conducted at main research field of the department of Agriculture of Lovely Professional University Phagwara (Punjab) situated at $31^0$33'00"N latitude and $75^0$56'99"E longitude and at an altitude of 252 m above sea level, which comes under the central plain zone of agro-climatic zones of Punjab.

5.1.2 Brief Introduction about the work

Crop: Tomato

Period of work: 2018-19

Design of Experiment: Randomized Block Design

Topic Under Discussion: Studies on Induced Systemic Resistance through different origin chemicals against Soil Borne Infections of Tomato (Solanum lycopersicum L.)

5.1.3 Experimental Details

Treatments: 13

Replications: 3

Total number of plots: 13 x 3 = 39

Design: on pot but RCBD

Total Plot size: 500m^2 Approx.

Variety: Available composite/hybrid

5.1.4 TREATMENT DETAILS:

Chemical inducers	Concentrations	Treatments
1.Potassium phosphate	25mM 50mM	T1 T2
2.Sodium phosphate	25mM 50mM	T3 T4
3.Sodium chloride	25mM 50mM	T5 T6
4.Ferric chloride	25Mm 50mM	T7 T8
5.silicon dioxide	25mM 50mM	T9 T10
6.Biochar	25mM 50mM	T11 T12
7.Control		T13

6. PARAMETERS TO BE RECORDED

a) Soil Parameters

These parameters will be estimated in the soil laboratories and every parameter has different method of estimation.

1. Soil pH :- It should be estimated by Glass Electrode pH meter
2. N content in soil kg/ha :- Nitrogen should be estimated by Alkaline Potassium Permanganate Method.

b) Growth Parameters

1. Germination test (Blotter method)

The Blotter paper method was employed for the germination test. Petri dishes of 90 mm in diameter were used to conduct the experiment. Bottom and slide walls of Petri plate half way up and the under side of covers were laid down with thick sterilized blotting papers which were moistened with sterilized water. The 20 seeds were treated with each inducer to conduct the experiment *in vitro* condition. One plate was kept without seed treatment to serve as control. Three replications were kept for each treatment. All these plates were placed in a growth chamber at 20±1º C. Observations on the germination of the seed and growth of seedling were taken by measuring root and shoot length (cm) of seedling at every 24 hrs up to 10 days.

2. Germination percentage

Seed treatment with inducers was found responsible to increasing the germination percent instead of control (in which no inducers was applied). For measuring the germination percent, germinated seeds were counted at every 24 hrs in each treatment up to 10 days.

3. Root and shoot length

To considering the growth parameters (root and shoot length) of seedlings, observations on the growth of seedlings were taken by measuring root and shoot length (cm) of seedling at every 24 hrs up to 10 days.

4. Effect of seed treatment
5. Plant Height (cm)

c) Effect of inducers on disease severity

The disease severity of individual plants will be calculated by the following formula-

$$\text{Disease severity (PDI)} = \frac{\Sigma \text{ Class rating} \times \text{class frequency}}{\text{Total no. of leaves} \times \text{maximum class rating}} \times 100$$

d) Biochemical analysis

1. Soluble protein estimation

The method developed by Lowry *et al*., (1951) will be used with slightly modification to determine the soluble protein contents.

2. Phenol estimation

The phenols accumulation in tomato plants after treatment with different inducers followed by inoculation of pathogen will be estimated by procedure developed by Bray and Thorpe (1954). In this method the total phenol estimation was carried out with FCR, which was measured at 650 nm calorimetrically.

7. EXPECTED OUTCOMES

The experiment will be conducted at the Lovely Professional University, School of Agriculture, near the experimental farm of Phagwara, Punjab. By the use of various inorganic chemical inducers, it is expected that they will induce the resistance in tomato plants against fasarium wilt. Application of these chemical inducers will affect not only inducing the resistance, but also promote growth of the tomato plants.

8. REFERENCES

Ahmad S Gordon-Weeks R Pickett J Ton J . 2010. Natural variation in priming of basal resistance: from evolutionary origin to agricultural exploitation. Molecular Plant Pathology 11, 817–827.

Alexandersson, E., Mulugeta, T., Lankinen, Å., Liljeroth, E., & Andreasson, E. (2016). Plant Resistance Inducers against Pathogens in Solanaceae Species—From Molecular Mechanisms to Field Application. *International journal of molecular sciences*, *17*(10), 1673.

Asha, B. B., Chandra Nayaka, S., Udayashankar, A. C., Srinivas, C. & Niranjana, S. R. Biological control of Fusarium wilt of tomato. *Int. J. Microbiol. Res.* 3, 79–84 (2011).

Barone, A. & Frusciante, L. Marker-assisted selection: current status and future perspectives in crops, livestock, forestry and fish (eds Guimaraes, E. *et al.*) Ch. 9, 151–164 (Agriculture and Consumer Protection Dept., 2007).

Beckman, C. H. *The nature of wilt diseases of plants* 175–176 (American Phytopathological Society, 1987).

Chakraborty, N., Chandra, S., & Acharya, K. (2017). Biochemical basis of improvement of defense in tomato plant against Fusarium wilt by CaCl2. *Physiology and Molecular Biology of Plants*, *23*(3), 581-596.

Diogo, R. V., & Wydra, K. (2007). Silicon-induced basal resistance in tomato against Ralstonia solanacearum is related to modification of pectic cell wall polysaccharide structure. *Physiological and Molecular Plant Pathology*, *70*(4-6), 120-129.

Elad Y, David DR, Harel YM, Borenshtein M, Kalifa HB, Silber A, Graber ER. 2010. Induction of systemic resistance in plants by Biochar, a soil-applied carbon sequestering agent. Phytopathology 100, 913–921.

Giovanni, C. D. *et al.* Identification of PCR-based markers (RAPD, AFLP) linked to a novel powdery mildew resistance gene (l-2) in tomato. *Plant Sci.* 166, 41–48 (2004).

Gottstein, H. D., & Kuc, J. A. (1989). Induction of systemic resistance to anthracnose in cucumber by phosphates. *Phytopathology*, *79*(2), 176-179.

Hariprasad, P., Navya, H. M., Chandra Nayaka, S. & Niranjana, S. R. Advantage of PSIRB over PSRB and IRB to improve plant health of tomato. *Biol. Cont.* 50, 307–316 (2009).

Kapoor, I. J. Fungi involved in tomato wilt syndrome in Delhi, Maharashtra and Tamilnadu. *Indian Phytopathol.* 41, 208–213 (1988).

Kawabe, M. *et al.* Three evolutionary lineages of tomato wilt pathogen, *Fusarium oxysporum* f. sp. *lycopersici*, based on sequences of IGS, MAT1, and pg1, are each composed of isolates of a single mating type and a single or closely related vegetative compatibility group. *J. Gen. Plant Pathol.* 71, 263–272 (2005).

Kiirika, L. M., Stahl, F., & Wydra, K. (2013). Phenotypic and molecular characterization of resistance induction by single and combined application of chitosan and silicon in tomato against Ralstonia solanacearum. *Physiological and Molecular Plant Pathology*, *81*, 1-12.

Kirankumar, R., Jagadeesh, K. S., Krishnaraj, P. U. & Patil, M. S. Enhanced growth promotion of Tomato and nutrient uptake by plant growth promoting rhizobacterial isolates in presence of Tobacco Mosaic Virus pathogen. *J. Agric. Sci.* 21, 309–311 (2008).

Kumar, A. and Biswas, S. K. (2010). Biochemical evidences of induced resistance in tomato plant against Fusarium with through inorganic chemicals. *Journal of Mycopathological Research*, *48*(2), 213-219.

Leslie, J. F. Fungal vegetative compatibility. *Annu. Rev. Phytopathol.* 31, 127–150 (1993).

Manandhar, H. K., Jørgensen, H. L., Mathur, S. B., & Smedegaard-Petersen, V. (1998). Resistance to rice blast induced by ferric chloride, di-potassium hydrogen phosphate and salicylic acid. *Crop protection*, *17*(4), 323-329.

Nejad, P. and P.A. Johnson, 2000. Endophytic bacteria induce growth promotion and wilt disease suppression in oilseed rape and tomato. Biol. Control, 18: 208-215.

Pandey, K. K. & Gupta, R.C. Virulence analysis of *Fusarium oxysporum* f. sp. *lycopersici* causing tomato wilt in India. *J. Mycol. Plant Pathol.* 43, 409–413 (2013).

Sudhamoy, M., Nitupama, M. & Adinpunya, M. Salicylic acid induced resistance to *Fusarium oxysporum* f. sp. *lycopersici* in tomato. *Plant Physiol. Biochem.* 47, 642–649 (2009).

Van Bockhaven, J., De Vleesschauwer, D., & Höfte, M. (2012). Towards establishing broad-spectrum disease resistance in plants: silicon leads the way. *Journal of experimental botany*, *64*(5), 1281-1293.