**TOPIC APPROVAL PERFORMA**

School of Agriculture

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

**COURSE CODE :** PTH596 **REGULAR/BACKLOG :** Regular **GROUP NUMBER :** AGRRGD0296

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

**Qualification:**_____**ResearchExperiencence**_____

| SR.NO. | NAME OF STUDENT | REGISTRATION NO | BATCH | SECTION | CONTACT NUMBER |
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**SPECIALIZATION AREA** : Plant Pathology **Supervisor Signature:** _____

**PROPOSED TOPIC** : Studies on Induced Systemic Resistance through different origin chemicals against soil borne infections of Aubergine (*Solanum melongena.L)*

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| 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. | 8.00 |
| 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: | Recommended (Y/N): Yes |

| | | |
|---|---|---|
| | 19078 | |
| 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 Aubergine (Solanum melongena L.).**

**Overall Remarks:** Approved (with major changes)

**PAC CHAIRPERSON Name:** 19212::Dr. Ramesh Kumar Sadawarti **Approval Date:** 30 Mar 2018

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LOVELY PROFESSIONAL UNIVERSITY

**SCHOOL OF AGRICULTURE**

Synopsis of Thesis/Dissertation Report Work of post-graduate study

PTH 596

*M.Sc. Agriculture (Plant pathology)*

**TITLE OF THE RESEARCH WORK:**

Studies on Induced Systemic Resistance through different origin chemicals against soil borne infections of Aubergine (*Solanum melongena.L)*

**Name of the student :** P. Renuka devi sri

**Reg .No** **:**11717740

**Programme of study :** Master of science in Agriculture

**Major Disicipline** **:** Plant pathology

**Project Advisor** **:** Dr. Adesh kumar

Head of the department

School of Agriculture

Lovely professional university

## CERTIFICATE

I certified that this synopsis by P.Renuka devi sri with registration no: 11717740 has been formulated and finalized by the student on the subject**,** studies on induced systemic resistance through different origin chemical against soil borne infections of *solanum melongena.L*

**Signature of student:**

P.Renuka devi sri

**Signature of supervisor**

Dr.Adesh kumar

UID:19078

H O D

Department of agriculture

Lovely professional university

## DECLARATION

I hereby declare that the project work entitle: studies on induced systemic resistance through different origin chemical against soil borne infections of *solanum melongena.L*) is an authentic record of my work carried out at lovely professional university as requirements of project work for the award of degree of Master of Science in Plant Pathology, under the guidance of Dr. Adesh Kumar, Assistant professor, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India.

P.Renuka devi sri
11717740

# INTRODUCTION

Brinjal (*Solanum melongena* L. fam. Solanaceae) or eggplant or aubergine in an important and widely consumed nutritious vegetable crop in India or cultivated commercially throughout tropical and subtropical region of the world. The name brinjal is popular in India subcontinents and is derived from Arabic and Sanskrit. It is considered as native of India or major Asia where the major domestication of large fruited cultivars occurred. It has been cultivated in India for last 4,000 years, although it is often thought as a Mediterranean or mid-Eastern vegetable.

Brinjal grows in warm areas of Far East, being grown intensively in India, Pakistan ,Bangladesh, Philippines and China. It is also popular in Egypt, France, Italy and US. In India, it is the principal vegetable crops grown throughout the country incept higher altitudes. It is a perennial but grown commercially as an annual crop. The major producing states are West Bengal, Orissa, Bihar, Gujarat, Maharashtra, Karnataka, Uttar Pradesh and Andhra Pradesh. In Rajasthan, it is grown in Alwar, Kota, Jaipur, Sriganganagar and Bharatpur.

The global area under brinjal cultivation has been estimated at 1.85 million h with total production of brinjal fruit of about 32 million MTs (Anonymous, 2005). India accounts for about 8.7 million MTs with an area of 0.53 million h under cultivation. It is also exported as fresh or frozen form. In 2007-2008, 34 million kg worth of Rs. 19 million was imported mainly to UK, Netherlands, Saudi Arabia and Middle East countries4. Rajasthan accounts for about 20339 MTs with an area of 7 h.

The composition per 100g of edible portion of brinjal constitutes Calories (24.0), Sodium (mg) (3.0), and moisture content (%) (92.7), Copper (mg) (0.12),Carbohydrates (%)(4.0), Potassium (mg) (2.0), Protein(g) (1.4), Sulphur (mg) (44.0), Fat (g) (0.3), Chlorine(mg) (52.0), Fiber (g) (1.3), Vitamin A (I.U.) (124.0),Oxalic acid (mg) (18.0), Folic acid (µg) (34.0), Calcium(mg) (18.0), Thiamine (mg) (0.04), Magnesium (mg) (15.0), Riboflavin (mg) (0.11), Phosphorus (mg) (47.0), B-Carotene (µg) (0.74), Iron (mg) (0.38), Vitamin C
(mg) (12.0), Zinc (mg) (0.22) and Amino acid (0.22) 18.Botanically, it is an herbaceous prickly perennial herb or undershrub; flowers purple, solitary; berries large green or purple, globular or oblong, acrid-sweet in taste. The fruit contains arginin, aspartic acid,solanin, histidine, leucine, methionine, pipecolic acid,phenylanine, theonine, tryptophane, valine,

choline,nicotinic acid, riboflavin, vit- A & C, fructose, glucose,sucrose, anthocynine, lycoxanthin, caffic acid and chlorogenic acid24( Dilip Kumar Sharma et al) 2013Taxonomically there are 3 main botanical varieties under the species Melongena. The common brinjal, to which large, round or egg shaped fruited forms belong, or grouped under *Solanum melongena* var. *esculentum*. The long, slender types are included under *Solanum melongena* var. *serpentinum* and the dwarf brinjal plants are put under *Solanum melongena* var. *depressum* (Choudhury, 1976). It has been reported that on an average, the oblong-fruited egg plant cultivars are rich in total soluble sugars, whereas the long-fruited cultivars contain a higher content of free reducing sugars, anthocyanin, phenols, glycoalkaloids (solasodine), dry matter and amide proteins.

Plant diseases have been recognized as a major problem for centuries associated with agriculture and horticulture. Plant diseases need to be controlled to maintain the quality and quantity of food, feed and fiber produced by growers around the world.Different approaches may be used to prevent or control plant diseases. Soil borne pathogens are controlled by using resistant cultivars, chemical fungicides and following seed certification, crop rotation, soil fumigation etc. Indiscriminate use of various fungicides and fertilizers in agricultural fields in order to control diseases so as to increase productivity, however, affects non target beneficial micro organisms and consequently causes environmental degradation at various levels.

**Objectives :**

1.Comparative evaluation of inducers against Phomopsis blight in brinjal.
2.To find out the efficacy of inducers on growth parameter of brinjal.
3.To study the biochemical changes in brinjal due to the effect of seedling treatment with inducers.
4.To study the defense response in phomopsis blight in brinjal due to the effect of inducers.

**RIVIEW OF LITERATURE**

**ISR:**

It seems likely that most induced resistance phenomena are based on a combination of direct induction and priming (Ahmad *et al*., 2010).-

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

**Germination test:**

Each treatment had three replicates. Seed germination was measured by counting the number of fully germinated seeds per plate (Nejad and Johnson, 2000).

**Biochar:**

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

CaCl2:

Foliar application of CaCl2 showed significant reduction of wilt incidence after challenge inoculation. Increased production of defense and antioxidant enzymes was observed 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.

**Ferric chloride**:

Ferric chloride, di-potassium hydrogen phosphate and salicylic acid were tested for their capacity to suppress rice blast under greenhouse and field conditions. In greenhouse experiments, the chemicals significantly reduced disease severity when applied as a soil drench, thus demonstrating a systemic effect. In another experiment, t:lat included combinations of sprays at different growth stages, only ferric chloride significantly increased the grain yield. Previous studies suggest that blast control involves induced resistance from the applied chemicals. Manandhar, et al 1998

Salicylic acid (SA) has been used successfully to control some plant disease such as root rot/wilt of sesame (Abdou et al., 2001), root rot of wheat (El-Bana et al., 2002), root rot/wilt of lupine (Ali et al., 2007 and Abdel-Monaim, 2008), Fusarium wilt of tomato (Zgnen, 2001 and El- Khallal, 2007) and Fusarium wilt of chickpea (Nighat-Sarwar et al., 2005).

**Silica**

Silicon (Si) and chitosan (Chi) treatments induced resistance in tomato against bacterial wilt caused by Ralstonia solanacearum. Gene expression analysis conducted at 72 h post inoculation via TOM2 microarray revealed regulation of 204 and 126 genes in genotypes King Kong 2 and L390, respectively, with their majority classified into the categories defense-related, signal transduction and transcription. In the microarrays, translationally-controlled tumor protein homolog involved in stress reaction of plants, the defense genes chitinases and peroxidases were highly up-regulated in combined Si and Chi treatment. Bacterial wilt incidence was reduced by 40% and 56.6% in Si and Chi treatment, respectively, in King Kong 2, and by 26.6% and 33.3% in Si and Chi treatment, respectively, in L390, and by 74.7% in King Kong 2 and 46.6% Kiirika et al 2013.

**potassium phosphite**

In a series of field experiments, potassium phosphite (KPhi) was applied to seed potato tubers and foliage. After harvest, several variables were analyzed in tubers obtained from these plants. An increase in pectin content was observed in both periderm and cortex tissue in tubers originating from KPhi-treated plants. After wounding and infection with *Fusarium solani*, a higher amount of pectin accumulation in cortical tissues was observed in tubers following treatment with potassiumphosphate. These results suggest that KPhi applied to seed tuber and foliage induces defense responses in tuber periderm and cortex and that these reactions are associated with structural and biochemical changes in these tissues. Alexandersson et al 2016.

Kavroulakis,et al (2007)Reported that an endophytic fungal isolate was able to colonize root tissues and subsequently protect plants against the root pathogen Fusarium oxysporum f.sp. radicis-lycopersici (FORL), and elicit induced systemic resistance against the tomato foliar pathogen Septoria lycopersici.

Pieterse et al., 1996.ISR in Arabidopsis triggered by Pseudomonas fluorescens strain WCS417r is effective against different types of pathogens .

Cornelius S.et al (2001) Barry suggest that a subset of ethylene responses controlling vegetative growth and development may be constitutively activated in *epi*. In addition, the *epi* locus has been placed on the tomato RFLP map on the long arm of chromosome 4 and does not demonstrate linkage to reported tomato *CTR1* homologs.

José Díaz et al (2002)Ethylene and wound signaling acted independently on resistance. Salicylate and ethylene acted synergistically on defense gene expression, but antagonistically on resistance.

Saikia *et al.* (2003) examined 5 isolates of *P fluorescens* and chemical inducers for the growth promotion and induced systemic resistance against *Fusarium* wilt of chickpea. The isolates of/? *fluorescens* induced resistance against the wilt disease by 26-50% as compared to the control.

Tahat *et al.,* 2010-The establishment of AM fungi in the plant root has been shown to reduce the damage caused to plants by soil-borne plant pathogens with the enhancement of resistance. AM fungi generally reduce the severity of plant diseases in various crops suggesting their potential role in disease management .

Van Loon *et al.,* 1998 ISR is generally mediated by jasmonic acid (JA) and /or ethylene produced by non pathogenic rhizobacteria .
Conrath *et al.,* 2006 In development of ISR, no major change occurs in gene expression, instead the induced plant show a greater activation of defense responses after infection with challenged pathogen which is known as ‘potentiation’ or ‘priming’. But according to some eminent workers the complex mechanism of ISR development is thought to be regulated by multiple genes.

Pozo *et al.* (2002) observed die involvement of the signaling molecules- salicylic acid, jasmonic Acid and ethylene for induction of ISR in *Arabidopsis thaliana.*
Bhagawat, 1997.Antibiotics and phenols released during the decomposition of lignin containing materials induce resistance on the root surface as well as in the tissues when absorbed.

Karban *et al*., 1999 Any form of induced resistance can be of selective advantage only if the eliciting attack has a predictive value and thus can be used as a cue to indicate future attack by a given enemy .

Barbara Thuerig (2006) Reported that Penicillin triggers early defense-related responses in numerous plant species and provides resistance against several pathogens in A. thaliana.

Salicylic acid (SA) has been used successfully to control some plant disease such as root rot/wilt of sesame (Abdou et al., 2001), root rot of wheat (El-Bana et al., 2002), root rot/wilt of lupine (Ali et al., 2007 and Abdel-Monaim, 2008), Fusarium wilt of tomato (Zgnen, 2001 and El- Khallal, 2007) and Fusarium wilt of chickpea (Nighat-Sarwar et al., 2005).

Bhupendra Kumar Singh, Saurabh Singh Bijendra Kumar Singh and Sanwar Mal Yadav, 2014. Some Important Plant Pathogenic Disease of Brinjal (*Solanum melongena* L.) and their Management. *Plant Pathology Journal, 13: 208-213*.Reported that The pathogen attacks leaves but older ones are more susceptible. Lesions are epically circular, gray to brown and develop a light center.

## TECHNICAL PROGRAMME OF THE RESEARCH WORK

**Location of the experiment site:**

The experiment is conducted at Lovely professional University's agricultural farm.

Geographical location:

Latitude- 31 degree 24 minutes and 31.81 seconds north

Longitude- 75 degree 69 minutes and 4.06 seconds east

Altitude- 252 above mid sea level

Agro-climatic zone- Trans-Gangetic plain region, Punjab

## METHODOLOGY OF RESEARCH WORK DESIGN AND LAYOUT OF THE EXPERIMENT

### 1. Technical Programme:

**Experimental Details**

1. Year of experiment : 2018
2. No. of treatments : 13
3. No. of replication : 3
4. Crop : Brinjal(*solanum melongena.L*)

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

**Collection of diseased sample**

The samples were collected from Vegetable Research Farm of Lovely professional university, phagwara Punjab. The infected fruit apparently showing poor germination, seedling blight to fruit rot like symptoms were collected and brought to the laboratory for initial examination. The entire specimen were collected and examined in the laboratory for the presence of the causal organism and virulence study.

Potato dextrose agar (PDA)-2%

**Isolation, Purification and Identification of pathogen**

**Preparation of culture media**

**Composition**

| | |
|---|---|
| Agar –agar | 20.0g |
| Dextrose | 20.0g |
| Potato (peeled and sliced) | 200.0g |
| Distilled water | 1000 ml |
| Beaker (1 lt. capacity) | 1 |
| Saucepan | 1 |
| Measuring cylinder (1lt. capacity) | 1 |
| Conical flasks (250 ml) | 8 |

Knife

Muslin cloth/Cheese cloth

Non-absorbent cotton

Autoclave

**Preparation**

250 gm of potato was peeled and cut into small pieces/slice. Exactly 200 gm of potato slice was weight and kept it into saucepan. Add 500 ml of water into and boiled gently for 30 min or until they are easily penetrated by a glass rod. Filtered the contents of saucepan with muslin cloth and squeezed out all the liquid in measuring cylinder and discarded potato slices. Then add 500 ml water again in saucepan and heat it and mixed pre-weighted agar (20g) bit by bit to the hot water to dissolve it. At the same time dextrose (20g) is also added in boiled water (melted with agar) and made up the final volume to 1 litre. It was dispensed at about 200 ml in each of four conical flasks and 10 ml per culture tube up to 10 culture tubes. Both flasks and culture tubes were plugged with non-absorbent cotton and wrapped their mouth with butter paper and rubber band placed the culture tubes vertically (mouths up) in wire baskets. Then autoclaved the media of flasks and culture tubes at 15 lb. /sq. pressure for 18 min at 121.6º

**Isolation of pathogen-**

Infected plant showing typical symptoms was selected for isolation of pathogen. Initially the diseased fruit of Brinjal was washed thoroughly with distilled water to remove dust particles. Then diseased portion of fruit were cut out into small pieces by a sterilized knife and each piece is having small bits of diseased and healthy tissues. These pieces were dipped in 0.1% mercuric chloride ($Hgcl_2$) solution for 30 seconds and then thoroughly washed thrice in distilled water to remove the traces amount of $Hgcl_2$ solution. Excess moisture was removed by putting these pieces between two folds of sterilized blotting paper under aseptic conditions. The pieces then transferred to sterilized Petri plates containing 2% potato dextrose Agar (PDA) medium in inoculation chamber with the help of sterilized forceps. Two pieces were placed aseptically in each Petri plates and incubated at room temperature (20±1ºC).The Petri plates used for isolation were previously sterilized at 165ºC for at least three hours in Hot Air Oven and poured with 2% PDA medium prepared and sterilized in 15 lb./sq.cm at 121.6ºC for 30 minutes. The Petri plates were observed daily to take notice of the presence of mycelial growth around the bits.

**Purification and identification**

The pathogen was purified by the transfer of hyphal tip in Petri plates which was previously poured with sterilized PDA in aseptic condition. The purified culture was then maintained at 25º±1ºC in refrigerator. On appearance of the colony in Petri plate, the pathogen was

examined under compound microscope and identified on the bases of its morphological and cultural characteristics, as described by (Sacc) W.C. Synder and H.N. Hansen, 1940.

**Pathogenicity test**

The pathogenicity test of the isolated fungus was conducted on the healthy host (Brinjal) plants in order to establish the pathogenic nature of the fungus. The pathogenicity was tested according to "Koch postulates (1882)".

**Effect of seed treatment with inducers on germination and growth parameter of brinjal (Blotter method).**

**Seed treatment**

Brinjal seeds were treated with the different concentration of these chemicals, which are shown as below

| Chemicals | Concentrations |
|---|---|
| 1.Potassium phosphate | 25mM<br>50Mm |
| 2.Sodium phosphate | 25mM<br>50Mm |
| 3.Sodium chloride | 25mM<br>50mM |
| 4.Ferric chloride | 25mM<br>50mM |
| 5.Silicon dioxide | 25mM<br>50mM |
| 6.Biochar | |

**Procedure**

For preparation of different concentrations of inducers , the required quantity of inducing agents are weighed separately and 100 ml of sterilized water was added to each conical flask , and shaken until become dissolved. Then 5 ml of solution was pipette out and placed into Petri plates separately. Exactly 5g of seeds of Brinjal were weighted and put in each Petri plates to soak in solution. Kept the Petri plates in dark place for over night and next day seeds are dried in shade and used for testing the germination percent and growth parameter of Brinjal seedlings.

**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.

**Growth parameters**

**Germination percent**

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.

**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.

**Effect of seed treatment with inducers on growth parameters of Brinjal plants**

The experiment will be conducted in the lab, Department of Plant Pathology, Lovely professional university,Punjab (Phagwara). The treated seeds with inducing agents of brinjal

were sown in 30 cm earthen pots, which was previously filled with a mixture of sandy loam and farm yard manure in the ratio of 21. In each pot 20 properly spaced seeds were sown and watered regularly. Three replications per treatment and three pots were sown with untreated seeds served as control. Observations pertaining to effect of different treatments on the growth of shoot and root were recorded at 10 days interval up to 30 days age of plants.

**Shoot and Root length**

**Shoot length**

Brinjal seeds treated with inducers were sown in earthen pots in the glasshouse and shoot length was measured at every 10 days interval up to 30 days age of Brinjal plants with the help of scale.

**Root length**

Prior to measure the root lengths of Brinjal plants, pots were irrigated and the seedlings were uprooted carefully, roots of the seedlings were separated from the shoots and washed with water to remove soil particles and then root length (cm) were measured with the help of scale.

**Effect of inducers on disease severity**

In order to ascertain in the effect of inducing agents on disease development, experiment will be performed in the lab with two replications for every treatment. Plants were treated with inducers at 48 hrs before root inoculation with pathogen. After inoculation with pathogen plants were covered with polythene bags for 48 hrs to give suitable moisture and humidity for pathogen development. During the course of this experiment two controls are kept, in one case plants were sprayed with water and in second case plants were inoculated with conidial suspension of *Phomopsis vexans*. After 7 days of inoculation, observations were taken on disease severity

**Measurement of Disease severity**

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

**Biochemical analysis**

Analysis of biochemical changes in Brinjal plants due to pre-inoculation with different inducers was conducted. Brinjal leaves were collected from different treatments and changes in the contents of soluble protein and phenol in the leaves were estimated. The protocol of different biochemical test is mentioned as under.

**Soluble protein estimation**

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

**Reagents needed**

Solution A (20% sodium carbonate in 0.1 N NaOH); Solution B (0.5% copper sulphate $CuSo_4$. $5H_2O$ in sodium potassium tartrate); Solution C (Alkaline copper solution prepared by mixing 50 ml of solution A with 1 ml of solution B just prior to use); Folin-Ciocalteu Reagent (FCR); stock standard protein solution (prepared by dissolving 50 mg of Bovine serum albumin/50 ml of water) and working standard solution (prepared by diluting 10 ml of the stock solution to 50 ml with water to obtain 200 micron gram protein/ml).

Brinjal leaves from different treatments were harvested, washed with distilled water several times and blotter dried before protein extraction. A quantity of 1.0 gm of each sample was cut into small pieces and grinded in pastel and mortar using 15 leaves extraction buffer. The suspension was centrifuged at 10,000 rpm for 30 minutes at 4ºC. The supernatant was collected. A quantity of 7.5 ml of the supernatant was transferred in a tube and mixed with 2.5 ml of sample buffer and used for protein estimation. The working standard solution was pipette out and 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the solution was put into series of test tubes. A quantity of 0.2 ml, 0.4, 0.8 and 1.0 ml of the sample extract was also pipette out and kept into other test tubes separately. Then volumes in all the tubes were made up to 1 ml with water. A tube with 1 ml of water served as a blank. Later on, 5 ml of solution C was mixed well and incubated at room temperature for 10 min. Thereafter, 0.5 ml of FCR was mixed well immediately and incubated at room temperature for 30 min at dark place. The absorbance at 660 nm against the blank was read and standard graph was drawn to calculate the amount of soluble protein in sample and represented as mg/g of fresh sample.

**Phenol estimation**

The accumulation of phenols in Brinjal plants after treatment with different inducers followed by inoculation of pathogen was estimated following 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.

**Reagents needed**

Ethanol 80%, FCR, 20% $Na_2CO_3$ and Standard (100 mg catechol in 100 ml of water), which was diluted 10 times for a working standard. For estimations, 1.0 gm of leaf sample of tomato was grind in a pestle and mortar in 10 times volume of 80% ethanol. It was then centrifuged to homogenate the suspension at 10,000 rpm for 30 minutes at room temperature. Supernatant was separated and re-extracted for 5 times with required volume of 80% ethanol, centrifuged and the supernatant were pooled. It was then evaporated to dryness and residues were dissolved in 5 ml of distilled water. Different aliquots (0.2 to 1.0 ml) were pipette out into test tubes and the volume in each tube was made to 3 ml with water. Subsequently 0.5 ml of FCR was added and after three minutes, 2 ml of 20% $Na_2CO_3$ solution in each tube was thoroughly mixed. Then tube were placed in boiling water for 1 min and then cooled. Then absorbance at 650 nm against blank was measured using Ultra Violet Visible (UV-VIS) spectrophotometer and the standard curve using different concentration of phenols was prepared. From the standard curve the concentration of phenols in the test sample was determined and expressed as mg phenols per gm of sample materials.

## 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 brinjal plant against phomopsis blight .Application of these chemical inducers will affect not only inducing the resistance, but also the growth of the brinjal plants.

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