Report for the M. Sc. student

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Title of the research topic: ***"In vitro* evaluation of bio-agents and botanicals against *Fusarium* wilt of chickpea"**

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CERTIFICATE

This is to certified that this synopsis entitled "***In vitro evaluation of bio-agents and botanicals against Fusarium wilt of chickpea***" submitted in partial fulfillment of requirements for degree – Master of Science in Plant Pathology by **Komala Kanuri**, Registration no. 11719048 to Department of Plant Pathology, School of Agriculture, Lovely Professional University, has been formulated and finalized by the student himself on the subject.

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Finally, I would like to thank everybody who was important to the successful realization of thesis, as well as expressing my apology that I could not mention personally one by one.

Date: (***Komala Kanuri***)

Place: LPU, Phagwara

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Introduction

Chickpea (*Cicer arietinum* L.) is an annual grain legume, grown mainly for human consumption. Low yield of chickpea is primarily attributed to its susceptibility to several fungal, bacterial and viral diseases (Rehman *et al.* 2013). Among the economically important diseases, wilt (*Fusarium oxysporum* f. sp. *ciceri*), dry root rot (*Rhizoctonia bataticola*) and collar rot (*Sclerotium rolfsii*) are the major and widespread diseases affecting chickpea cultivation (Nene and Sheila, 1999). Biological control represents a viable alternative to the use of chemical fungicides and it is considered to be a safe, effective and eco-friendly method for plant disease management (Benitez *et al.* 2004). *Fusarium oxysporum* f. sp. *ciceri* is a soil and seed borne pathogen colonizing the xylem vessels and blocking them completely to cause wilting. Wilt (*Fusarium oxysporum* f. sp. *ciceri* (Pad.) Snyd and Hans) is one of the serious disease of Chickpea, causing heavy loss upto 10-100% depending on fungal inoculum and environmental condition (Sumitha and Gaikwad, 1995).

Fusarium sp. is a soil borne fungal pathogen that attacks plants through roots at all stages of plant growth, is considered as one of the main soil-borne systemic diseases and the major limiting factor in the production of tomato both in greenhouse and field-grown (Borrero *et al.* 2004; Srivastava *et al.* 2010). In India, chickpea is ranked first in terms of production and consumption in the world (Patole *et al.* 2017). Chickpeas provide high quality protein to large population sectors in South and West Asia, and the Mediterranean Basin and also playing a significant role in farming systems as a substitute for fallow in cereal crop rotations. *Fusarium* wilt caused by the soilborne fungus *Fusarium oxysporum* f. sp. *ciceri*, has become a major factor limiting chickpea production worldwide (Jiménez-Díaz *et al.* 2015).

Soil drenching with fungicides are generally used to control of this disease, however, frequent and indiscriminant use of it leads to ill effects on environment causing soil and water pollution and development of new strain with more virulence, hence Bio-control and Botanicals has been advocated as one of promising alternative strategy to overcome these problems. The present study was conducted to find out effective Bio-agents and Plant extracts for eco-friendly and economical management of Chickpea wilts (Kamdi *et al.* 2012).

Trichoderma has long been considered as one of the most promising biocontrol agent for several plant pathogens. It is also produced many antifungal secondary metabolites that

adversely affect the growth of different fungi phytopathogens (Barakat *et al.* 2014; Li *et al.* 2016). Several modes of action have been proposed to explain the bio-control of plant pathogens by *Trichoderma*; these include production of antibiotic and cell wall degrading enzymes, competition for key nutrients, parasitism, stimulation of plant defense mechanisms and combination of those possibilities (Cook, 1985). *Trichoderma* spp. generally grows in its natural habit on plant root surface and therefore it controls root diseases in particular (Monte, 2001; Faruk *et al.* 2002; Kamlesh and Gujar, 2002). The species of *Trichoderma* have been evaluated against the wilt pathogen and have exhibited greater potential in managing chickpea wilt under field condition (Podder *et al.* 2004).

So, by keeping the above views in mind, the present study has been undertaken to evaluate the effects of some ecofriendly bio-control agents and botanicals against *Fusarium oxysporum f. sp. ciceri*, by which we can improve the agricultural production of chickpea. The following objectives are as follows:-

Objectives-

1. To isolate and identify the pathogen (*Fusarium oxysporum f. sp. ciceri*) associated with the wilt of chickpea.
2. To evaluate the effect of the pathogen on the biochemical properties of chickpea.
3. To evaluate the *in vitro* effect of selected bio-control agents and botanicals on the test pathogen.

Review of Literature

Chickpea (*Cicer arietinum* L.) is an important pulse crop grown in India. This crop suffers from many fungal diseases, among them vascular wilt incited by *Fusarium oxysporum* f. sp. *ciceri* is the most serious and causing considerable yield loss in many chickpea growing areas. The present investigations included the isolation, identification and *in-vitro* evaluation of antagonistic organism isolated against *F. oxysporum* f. sp. *ciceri*, management of wilt through cultural and biological methods in field conditions. The literature pertaining to studies on these aspects with *F. oxysporum* f. sp. *ciceri* and other species of *Fusarium* are reviewed here under.

History of the causal organism *Fusarium oxysporum* f. sp. *ciceri*

The chickpea wilt disease was first mentioned by Butler in his book, ―The fungi and plant diseases (1918). In 1923, Mckerral from Burma considered the disease as a soil borne. He associated with Indian scientists and isolated the pathogen which yielded *Fusarium* sp. Narasimhan in 1929 reported the association of *Fusarium* sp. and *Rhizoctonia* sp. with wilted plants. Dastur (1935) observed association of *Rhizoctonia bataticola* (Taub.) in infected plants. Butler produced wilted plants by inoculating *Rhizoctonia* and he called it as *Rhizoctonia* wilt. Although, Butler isolated *Fusarium* from several wilted plants, but he could not produce the disease artificially. In view of the fact that his description of symptoms and field pattern of incidence was almost identical to that of typical wilt caused by *F. oxysporum* f. sp. *ciceri* and he failed to prove pathogenicity.

Symptomatology

The disease occurs at seedling and flowering stage of plant growth. The symptoms are drooping of petioles and rachis, yellowing and drying of leaves from base to upward, browning of vascular bundles, improper branching, withering of plants and finally death of plants (Prasad and Padwick *et al*. 1939; Westerlund *et al*. 1974).

Erwin (1958) reported that the foliage of the *F. oxysporum* f. sp. *ciceri* infected plant turns yellow before wilting and the xylem tissue shows light brown discoloration. Saxena and Singh (1987) reported that internal discoloration of pith and xylem can be seen if stem and root of the wilted plants split vertically. It produces enzymes that degrade the cell walls so that gels are formed that block the plant‘s transport system. Discoloration of the internal tissues progresses from the roots to the aerial parts of the plant, yellowing and wilting of the foliage occurs and finally to necrosis. The microconidia detach and are carried upward in the vascular system until movement is stopped, at which point they germinate and the mycelium penetrates the wall of the adjacent vessel.

Isolation and Identification of the pathogen

Wilt complex pathogen *Fusarium oxysporium* f. sp. *ciceri*, *Rhizoctonia bataticola, Sclerotium rolfsii* were isolated by plating the infected cut piece of roots and stem of chickpea plant on potato dextrose Agar (PDA) medium after surface sterilization with 0.1 per cent, mercuric chloride ($HgCl_2$) solution. After seven days pathogens were isolated and transferred on

PDA for growth. (More and Parate, 2016). Nikam *et al*. (2011) collected wilt infected chickpea plant samples from different locations and isolated *F. oxysporum* f. sp. *ciceri* on potato dextrose agar (PDA) in the laboratory. Isolation was also done by soil samples by preparing the different dilutions. One milliliter of each dilution was uniformly spread over PDA. The obtained colonies were sub cultured on PDA plates by transferring small mycelial from the colony margins. Pure culture was obtained by sub-culturing three times (Mohamed *et al.* 2016).

The fungus was identified based on morphology and colony characteristics using the method of Watanabe (2000). The identification of the fungus was based on visual culture characteristics, mainly the growth patterns and pigmentation. Furthermore, microscopic examinations were carried out for mycelial and conidia structure based on the methods of Booth's key (1977).

Maintenance of the Pathogen

Barnet and Hunter (1972) purified *F. oxysporum* f. sp. *ciceri* by single spore isolation method and maintained on PDA slants throughout the investigation by periodical transfer or sub-culturing on PDA. Kulkarni (2006) reported that *F. oxysporum* f. sp. *ciceri* was sub cultured on PDA slants and allowed to grow at 27 ± 1 ºC for ten days and such slants were preserved in a refrigerator at 5 ºC and revived once in 30 days. Pure culture of *F. oxysporum* f. sp. *ciceri* was prepared on Czapek dox agar medium and it was multiplied on Waksman's agar medium (Glucose 10 g, Peptone 5 g, Potassium dihydrogen phosphate 1 g, Magnesium sulphate 0.5 g, Distilled water 1000 ml) (Ahmad, 2010). *Fusarium* species were maintained on PDA slants and were stored at 4°C tillusage (Hend *et al*. 2012).

Biochemical changes in chickpea caused by *Fusarium oxysporium* f. sp *ciceri*

In interactions with invading pathogens, plants frequently activate defense-related genes that lead to expression of pathogenesis-related (PR) proteins (Liu *et al*. 2009). PR proteins are one of the important non-specific defense mechanisms of plants against pathogen (Van loon and Van strien, 1999).

The phenolic content was increased in the roots of susceptible and resistant cultivars of chickpea after inoculation with the virulent and hypovirulent isolates of *F. oxysporum*, f. sp. *ciceris* in fact the susceptibility or resistance of host appear to follow common pathways

involving the preexisting and induced expression of defense component s activated by a number of fungal and plant metabolites. They found the highest increase in phenol content against the highly virulent isolate in the roots of both cultivars, whereas least increase was found in less virulent isolates (Singh *et al*. 2003; Khan *et al*. 2005; Rathod and Vakharia, 2011).

In vitro* evaluation of bioagents against *F. oxysporum* f. sp. *ciceri

Application of *B. subtillis* and *T. harzianum* either singly or in combination in both seed and liquid inoculation methods protect chickpea from *F. oxysporum* f.sp. *ciceris* infection indicating that the importance of application of biocontrol agents (Moradi *et al.* 2012). Singh *et al.* (2003); Misra (2006); Gupta *et al*. (2009) and Srivastava *et al.* (2011) reported that bioagents like *Aspergillus niger*, *Trichoderma* sp. and *Penicillium citrinum* and some bio-dynamic antagonists have shown their effectiveness towards the control of wilt pathogens of guava. Misra *et al*. (2004) was also tested these fungi for the control of wilt pathogen in laboratory conditions, these were found quite effective. When relative growth of the three bioagents was studied by Misra and Prasad (2003) then they were found that *Aspergillus niger* was fastest growing and most effective.

In vitro* evaluation of botanicals against *F. oxysporum* f. sp. *ciceri

Use of many plant extracts and botanical fungicides have been found to be effective and gaining importance in crop production in the view of their selective action, low cost, environment friendly, long lasting effect, etc. to control of many plant diseases (Oros and Ujvary, 1999; Mamatha and Rai, 2004). Singh et al. (1993), Shivpuri et al. (1996), Bansal and Gupta (2000) and Dwivedi and Shukla (2000) studied and reported that aqueous leaf extracts of *Azadirachta indica, Lantana camera, Ocimum sanctum, Datura fastosa, Ficus religiosa, Vitea megendo, Atropa belladonna, Calotropis procera, Eucalyptus amygdalina, Alianthus excels, Vinca rosea*, etc. were tested against *Fusarium oxysporum*. Extracts of *Allium sativum, Allium cepa* and *Mentha arvensis* were evaluated for their effect on the inhibition of mycelial growth and spore germination of *F. oxysporum* (Nisa *et al*. 2011).

Detailed Technical Programme

1. Isolation and identification of the pathogen (*Fusarium oxysporum f. sp. ciceri*) associated with the wilt of chickpea.

Isolation:

Isolation of *Fusarium oxysporum* f. sp. *ciceri* was carried out with the help of serial dilution technique in PDA (Aneja, 2004) and also by using the Agar Plate Method (Muskett, 1948).

Preparation of Permanent Slides and Identification of the Fungi:

Permanent mount of each fungus was made in glycerin. Cotton blue and lactophenol stains were used in the study. Small amount of fungal material was placed on slides and then mounting medium was added to the slides and a cover slip was placed over it. The preparation was sealed with the nail polish.

The following literature was consulted principally to identify the fungi isolated from *Jatropha* seeds:

- The Genus Fusarium (**C. Booth, 1971**)
- A Manual of Soil Fungi **(Gilman, 1975)**
- Hyphomycetes **(Subramaniam, 1971)**
- Illustrated Genera of Imperfect Fungi **(Barnett and Hunter, 1972)**
- More Dematiaceous Hyphomycetes **(Ellis, 1971)**
- The Genera of Fungi **(Clements and Shear, 1954)**
- The Genera of Hyphomycetes from Soil **(Barron, 1968)**
- www.mycobank.org

2. Evaluation of the effect of pathogen on the biochemical properties of chickpea.

   A. Protein Estimation: The protein content of seeds was determined by the method developed by Lowry et al. (1951).

   B. Phenolic content: Phenolic content has been estimated by Bray and Thorpe (1954). Phenols, the aromatic compounds with hydroxyl groups, are widespread in plant kingdom. Phenols are said to offer resistance to disease and pests in plants. Total phenol estimation can be carried out with the Folin-Ciocalteau reagent.

3. Management

Effect of Antagonists on *Fusarium oxysporum* f. sp. *ciceri*:

Bio-efficacy test of antagonists have been done against *Fusarium oxysporum* f. sp. *ciceri* with the help of dual culture technique (Aneja, 2004).

Formula to calculate I.O.C. % is:

$$\text{I.O.C. } (\%) = \frac{\text{Radial growth in control plate} - \text{Radial growth in treated plate}}{\text{Radial growth in control plate}} \times 100$$

Effect of Botanicals on *Fusarium oxysporum* f. sp. *ciceri*:

Effect of selected botanicals *viz., Allium* extract, neem leaf extract, *Datura* leaf extract, *Lantana* leaf extract at 5%, 10% and 15% with the help of poisoned food technique (Nene and Thapliyal, 2000).

Expected duration of work: Two years

Facilities needed for work: Every facility needed for work is available in the Department of Plant Pathology, Lovely Professional University, Phagwara (Punjab).

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