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School of Agriculture

Synopsis of Thesis/Dissertation Report Work of Post-Graduate Study

M.Sc. (Agri.)

TITLE OF THE RESEARCH WORK : Study on bio-efficacy of various biocontrol agents and fungicides against the effective management of *Alternaria brassicae* (Berk.) *Sacc*. on Indian mustard (*Brassica juncea*)

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CERTIFICATE

I certified that this synopsis by **Kabrabam Suraj Jackson** with registration no: **11616948** has been formulated and finalized by the student on the subject, **"Study on bio-efficacy of various biocontrol agents and fungicides against the effective management of *Alternaria brassicae* (Berk.) *Sacc*. on Indian mustard (*Brassica juncea*)"**

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DECLARATION

I hereby declare that the project work entitle **(Study on bio-efficacy of various biocontrol agents and fungicides against the effective management of *Alternaria brassicae* (Berk.) *Sacc*. on Indian mustard (*Brassica juncea*))** 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.

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INTRODUCTION

Indian mustard (*Brassica juncea*) is an annual growing perennial herb and is one of the important oilseed crop that belongs to the family (*Brassicaceae*) *Crucifereae*. Indian mustard has 36 chromosomes (2n) and is amphidiploid in nature .

Brassica crops is mostly cultivated for edible vegetable oil production. They have a long list of history owing to their cultivation and varied use and has a great contribution in world's agricultural economy. They are widely cultivated as spices as condiments throughout the world both for human consumption and also for livestock feedings. Commonly cultivated species of Indian mustard (Brown mustard) are B. *juncea ssp. Integrifolia*, B. *juncea ssp. juncea*, B. *juncea ssp. napiformis*, and B. *juncea ssp. taisai*.

India comes as the third largest country in mustard production after China and Canada with an annual production of 7.36 million tonnes from 6.5 million ha area **(GOI, 2009)**. India accounts for 10.7 per cent and 20.2 per cent of the production and total area production globally **(USDA, 2012)**. Rajasthan, Uttar Pradesh, Jammu & Kashmir, Gujarat, Bihar, Assam, Haryana, Punjab, West Bengal, and Madhya Pradesh and are the major mustard growing states in india. In Punjab, it is grown in 31('000 hectares) and produces 38('000 tonnes) with an average yield of about 1226 kg/ha by both rai and mustard **(GOI, 2016)**. It is grown during September – December as catch crops in the state.

Among the most common pathogen of rapeseed-mustard, *Alternaria brassicae* (Berk.) *Sacc*. causing Leaf blight in *Brassica juncea* is found to be destructive to the crop in India **(S.J. Kotle, 1985).** The pathogen can infect all the growth stages of the crop and also affect the seed germination, and deterioration of both quality and quantity on oil **(Meena et. al., 2010)**. Usually the sign of pathogen presence can be seen on 45 days old crop, with severity increase in 75 days old plants **(Meena et. al., 2004)**.

The pathogen attacks on major parts like leaves, stem and pods and produces visual symptoms like brown to black spots with concentric rings, first on the lower side of the leaves which later enlarges to form prominent concentric spots and eventually results in defoliation of leaves in later stage, although the symptom and sizes of the spots may vary with the host and environment. Infection on siliqua can be identified by certain characteristics such as deep lesions on siliqua with a decreased seeds per pod, pod length, seed weight, and oil content **(S.J. Kotle, 1988)**. The yield loss of the crop may vary from 37 to 75% among different species of oil seed *Brassicae* and the region where its grown worldwide.

Considering the importance of the disease the present investigation aims in managing the pathogen by using various economically acceptable and easy market accessible bio-agents and fungicides.

OBJECTIVES

- Isolation, identification, and characterization of *Alternaria brassicae* (Berk.) *Sacc*. from soils and leaf of *Brassica juncea*
- Inoculation of isolated and pure cultured *Alternaria brassicae* (Berk.) *Sacc*. on the leaf of Brassica juncea for confirmation of pathogen
- Bio-efficacy testing of various bio-agents and fungicides against the pathogen

REVIEW OF LITERATURE

The brief resume of research works done in India and abroad on various aspects relevant to present investigation entitled "**Study on bio-efficacy of various biocontrol agents and fungicides against the effective management of *Alternaria brassicae* (Berk.) *Sacc*. on Indian mustard (*Brassica juncea*)** " has been reviewed and presented below.

Lacomi *et. al*. (2004) reported that flutriafol, difenoconazole and prochloraz reduced the mycelial growth of A. brassicicola , A. brassicae, and A. japonica equally. Iprodione belonging to dicarboximide group and fludioxonil of phenylpyrroles group also proved effective against the test pathogens.

Surviliene and Dambrauskiene (2006) demonstrated the efficacy of Zato 50 WG SC Signum 334 WG Amistar 250 , and Folicur 250 EW in inhibiting mycelial growth of different Alternaria spp..

Chahal (1986) applied four sprays of Baycor, Difolatan, Blitox, Daconil or Dithane M-45 under field conditions. Among the different fungicides, Blitox was the most economical giving the highest, 1:5.99 and 1:6.62 cost/benefit ratio respectively.

Verma and Saharan (1994) reported that iprodione (0.5 kg a.i./ha), procymidone(0.75 kg a.i./ha) , and prochloraz (0.5 kg a.i./ha) are effective against Alternaria spp. on oilseed rape in Denmark, India, England, and Poland.

Kumari *et. al*. (1999) sprayed seedlings of cauliflower (cv. Patna Early) infected with A. brassicae three times with Bavistin @ 1 kg/ha and resulted in 5 per cent disease intensity and maximum yield (166q/ha). Spraying with Rovral @ 2 kg/ha ranked second in efficacy, showing 8 per cent disease intensity and 161q/ha yield. Disease intensity was also reduced with sprays of Ridomil MZ, Indofil M-45, Kavach, Captaf and Blitox-50 in comparison with the control.

Kumar and Thakur (1996) reported that the six concentrations of iprodione (Rovral 50 WP) ranging from 0.05 to 0.3 per cent were effective in lowering the per cent disease intensity and increasing the crop yield. Three sprays of iprodione (0.2%) provided maximum net returns and cost benefit ratio.

Kumar and Singh (2003b) conducted pot experiments to determine the efficacy of captan, iprodione, Ridomil MZ (mancozeb + metalaxyl), Achook (Azadirachta indica), zineb, ziram and Blitox-50 (copper oxychloride) and reported that spraying of iprodine is the best control of the disease, followed by achook, mancozeb, and Ridomil-MZ.

Chattopadhyay *et. al.* (2003) reported Iprodine most efficacious against Alternaria leaf blight, followed by mancozeb. Significant increase in 1000 grain weight and highest seed yield was observed when iprodione was sprayed at post-flowering stage. But maximum economic return was obtained from two spraying of mancozeb at 45 DAS and 60 DAS.

Kumar (1996) conducted field trials on Indian mustard with four fungicides against Alternaria brassicae, Albugo candida and Peronospora parasitica infection and revealed the minimum Alternaria blight infection was recorded with Rovral (iprodione, 0.2%), followed by Difolatan (captafol, 0.2%), Indofil M -45 (mancozeb, 0.2%) and Ridomil MZ (mancozeb + metalaxyl, 0.25%). Maximum yield was recorded with iprodione but Indofil M-45 is recommended on the basis of the cost-benefit ratio.

Ayub *et. al.* (1997) conducted the experiment to evaluate the efficacy of some fungicides to control Alternaria blight. Iprodione reduced disease severity the most and increased seed weight and yield. Fentin hydroxide was the second best fungicide. Spraying of the fungicides at 40 days old plants showed maximum reduction and increase of yield.

Godika et. al. (2001b) conducted a field experiment on Indian mustard against White rust and Alternaria blight using various fungicides. All fungicides significantly controlled both the diseases, but their efficacy varied. Rovral showed highest efficacy against Alternaria blight with mean disease intensity on leaf and pod 8.75 and 5.6 per cent respectively. Yield was highest with Rovral (2.1 t/ha), followed by mancozeb and Ridomil MZ, each recording a yield of 1.9 t/ha.

Godika and Pathak (2002) conducted the experiment to check the efficacy of different fungicides in controlling blight disease and white rust and reported that the application of Antracol proved to be highly effective against Alternaria blight and Ridomil MZ against white rust. The highest yield (13.47 q/ha) and cost benefit ratio (3.14) were recorded with sprays of Ridomil MZ.

Kumar (2001) found that the four applications of mancozeb (at 75, 90, 105 and 120 DAS) resulted in the lowest disease intensity in leaves (25.6%) and pods (27.8%). Application of three sprays at 90, 105 and 120 DAS resulted in highest yield of 1759 kg/ha.

Yadav (2003) determined the efficacy of non-systemic and systemic fungicides and showed that all the fungicidal treatments were significantly superior to the control in reducing leaf or pod infection and in increasing grain yield. Two sprays of Ridomil MZ at 60 and 80 days after sowing reduced the disease incidence of white rust and Alternaria blight on the leaves from 62.7 to 17.1 per cent and from 57.3 to 41.4 per cent, respectively, and increased the yield from 1052 (control) to 1842 kg/ha. The highest grain yield (1900 kg/ha) was recorded from Antracol treatments.

Prasad *et. al.* (2003) treated the seeds with mancozeb and reported the reduction of disease incidence in all the genotypes of Indian mustard. Varuna under unprotected conditions showed the highest seed yield loss (20.8 and 21.9%) during the experiment.

Singh R and Singh V K (2007) reported that among the five fungicides, mancozeb was best followed by Bavistin and Blitox-50. Cuman L was least effective. The highest yield (18.66 and 16.50 q/ha) was obtained from mancozeb followed by Bavistin (16.43 and 14.40 q/ha) and Blitox-50 (15.68 and 13.00 q/ha) in both the years.

Narain *et. al.* (2006) reported that Indofil M-45 was the most effective treatment, recording the lowest per cent disease severity (18.0%) and the highest head yield (274 q/ha).

Khan *et. al.* (2007b) assessed three fungicides: Bavistin, Ridomil MZ and Topsin-M alone and in mix with four non-foundational fungicides Captaf, Thiram, Indofil M-45,and Indofil Z-78 for their viability at different fixations. All fungicides decreased the severity when contrasted with untreated check however Ridomil MZ was best.

Husby *et. al.* (1998) demonstrated high efficacy of FolicurReg.EW in managing the disease with significant increase in the yield of oilseed rape, efficacy of FolicurReg.EW 250 against Botrytis cinerea, Sclerotinia sclerotiorum and Alternaria brassicae is similar to or better than known standards. FolicurReg.EW 250 protects the plants longer because of a homogeneous distribution of tebuconazole in the treated plant parts.

Sidlauskiene (2001) demonstrated the efficacy of Amistar 25 SC fungicide which reduced the disease prevalence (90.8%) and intensity (88.8%), and increased the yield (0.89 t/ha).

Brazauskiene and Petraitiene (2004) applied prochloraz and tebuconazole on all dates of sowing which resulted in the decrease in severity. The fungicides showed highest efficacy when sprayed after the appearance of first symptoms of Alternaria blight on siliques. Tebuconazole proved more effective than prochloraz. The fungicides decreased the seed infection per siliquae with an increase in the 1000-seed weight.

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

Experimental Details :

1. Year of experiment : 2017
2. No. of treatments : 8 (with control)
3. No. of replication : 3
4. Total no. of plants : 1000 nos.
5. Plot size : 3m X 2m
6. Experimental design : Randomized Block design (RBD)
7. Crop : Brassica juncea (Varuna T-59)

Layout design :

R1	IRRIGATION CHANNEL	R2	IRRIGATION CHANNEL	R3
T1		T8		T5
T2		T7		T4
T3		T6		T3
T4		T5		T2
T5		T4		T1
T6		T3		T8
T7		T2		T6
T8		T1		T7

Isolation and raising of pure culture of the pathogen

Isolation will be made from infected mustard crop from the Lovely agricultural field and its adjoining mustard growing areas. The leaves will be washed under clear running water and shade dried. Small bits of about 5 mm size from the infected part will be cut along with some healthy portion. The bits will be transferred to a petriplate containing 0.1 per cent Sodium hypochlorite, then pass to two other petriplates containing Sterile distilled water consecutively for about 1 minutes each and transfer to autoclaved, poured and cooled Potato Dextrose Agar (PDA) slants under aseptic condition inside the lamkinar air flow and incubate at 25 degree celcius in the BOD incubator.The obtained isolate will be purified by using single spore technique.

Chemical and Bioagents

The various bioagents and fungicides used in the present studies are: *Trichoderma Harzianum* (Niprot 0.50%), *Trichoderma Viride* 1% (Ecolife), *Pseudomonas fluorescens* 1.0% (Phasal Rakshak), Carbendazim 50% (Dhanustin 50%), Mancozeb 75% (Indofil M-45), Carbendazim 12%+ Mancozeb 63% (Colt), Azoxystrobin 23% (Godiwa), Propaconazole 25% EC (Partap 25).

In-vitro evaluation of fungicides and bioagents

In-vitro evaluation will be done using Poison Food Technique (PFT) in which 1 mm mycelial disc of pure cultured pathogen will be taken in sterile petriplates containing autoclaved and cooled PDA medium and different concentrates of the bioagents and chemicals taken for study will be incorporated along with a control (PDA alone). 3 replications for each of the concentrations will be maintained along with the controls and incubated inside the BOD incubator at about 25 degree Celsius for about 7 days until the mycelial growth covers the petriplate in the control treatment. The growth on each concentration will be measured and the data will be pooled for each replication and efficacy will be studied. Least concentration of a chemical or bioagent showing maximum restriction to the growth and spread of the fungal mycelium will be considered most efficacious.

In-vivo evaluation of fungicides and bioagents

Susceptible variety of Brassica juncea, Varuna 59 will be sown in micro test plots of 2x 3 m with 8 treatments of different chemical with 3 replications of each treatment. Recommended dosage of fertilizers will be supplied and normal agronomic practices will be carried out. Spore suspension of the pathogen will be sprayed at 45 days after sowing (DAS). First treatment of the chemicals and bioagents will be given at the first sign of pathogen appearance on the crop. The pathogen spore suspension will be sprayed at 10 days interval and the treatment chemicals and bioagents will be sprayed at 10 to 15 days interval until the maturity of the crop. Disease severity will be noted before each spray of the treatment from the same 5 random plants from each treatment every time. The plants taken for severity count will be tagged for easy identification for further counts.

Disease observation

Disease severity will be recorded from the lower, middle and upper leaves of individual plants using a 0-5 rating scale. Formulation of wheeler (1969) will be used to calculate the percent disease index (PDI)

$$\text{Per cent disease index (\%)} = \frac{\text{Sum of numerical rating}}{\text{Total no. of sample x Maximum rating grade}} \times 100$$

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