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PATHOLOGIACL STUDIES ON *Alternaria brassicicola* AND *Phoma lingam* LEAF SPOT DISEASES ON CANOLA PLANTS UNDER GREENHOUSE CONDITIONS

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ABSTRACT

Black leaf spot and blackleg diseases of canola were studied during three successive growing seasons (2010 /2011, 2011/2012 and 2012/2013) in four Governorates (Giza, Ismaillia, Gharbia and Sharkia). The causal organisms were isolated from infected leaf samples, identified as *Alternaria brassicicola* (Sch.) Wiltshire and *Phoma lingam* (Tode) Desm and inspected for their pathogenic potentiality. Pactol was the most resistant canola cultivar while, Serw 4 was very susceptible one. Radish (*Raphanus sativus* L.) was highly susceptible host to be infect with the tested fungal genera compared with the other tested ones. Level of $N_1P_1K_1$ fertilizer reduced disease incidence and severity. All tested bioagents reduced disease severity using attached leaf technique two days after inoculation. *Trichoderma harzianum* Rifai was the most effective in reducing severity of alternaria black spot disease. While *T. viride* Pers. reduced severity of blackleg disease. On the other hand, *Bacillus subtilis* reduced canola leaf spot disease caused by *A. brassicicola* and *P. lingam*. The tested fungicides reduced severity of canola disease using attached leaf. The disease severity caused by *A. brassicicola* was significantly reduced by Carbendazim. While, opposite results were obtained with *P. lingam*. The highest significant reduction percent was obviously noted when the higher Ridomil-Mancozeb concentration, was sprayed. On the other hand, Carbendazium was found to be the less effective fungicide in controlling *P. lingam* when the infected attached leaves examined under greenhouse conditions.

Key words: *Alternaria brassicicola*, *Phoma lingam*, canola, black spot, blackleg.

INTRODUCTION

Canola is a member of family Brassicaceae. There are two genera of *Brassica*; *B. nupus* L. and *B. rapa* L. are important as oil seed crops. These species are natives of Asia and Mediterranean countries. During the few last years, canola was introduced to Egypt as a new oil crop, grown in small areas in several Governorates, *e.g.* Giza, Ismaillia, Gharbia and Sharkia as well as in different agricultural experimental stations. Some fungal genera were isolated from naturally infected canola plants causing three major diseases namely black spot, blackleg and white rust. Isolates from diseased pods, shoots and leaves of canola plants revealed the presence of several pathogenic fungi, *i.e. Alternaria brassicicola*, *Phoma lingam* and *Albugo candida* Pers. These fungi were previously reported to be associated with foliar diseases of canola in other countries (Antonijevic and Mitrovic, 2007; Hood *et al*., 2007; Steed *et al*., 2007).

In Egypt, black spot disease symptoms was observed as leaf spots caused by *A. brassicicola*, started as small brown to black points which enlarge and varied in size. The disease symptoms appeared also on stems and pods of infected

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plants (El-Deeb *et al*., 1989). On the other hand, blackleg disease symptoms was observed as leaf spots caused by *P. lingam* started as lesions on cotyledons and leaves are appeared grayish in color, and vary in size and shape. Numerous black pinhead sized pycnidia are often observed in the dead leaf tissue at the center of lesions. Pycnidia appear as tiny round specks. The disease symptoms appeared also on stems and pods (Gugel and Petrie, 1992).

In Egypt, canola seed production is impaired by the occurrence of destructive diseases, in particular foliar diseases which affect the economical part of the plant (West *et al.*, 2001). However, Rouxel and Balesdent (2005) found that, *Leptosphaeria maculans* had a wide host range of cultivated brassicas such as *B. napus* (canola), *B. rapa, B. juncea* and *B. oleracea* with numerous wild crucifers species. Reis and Boiteux (2010) recorded that *Alternaria brassicae* and *A. brassicicola* are the major leaf pathogens on Brassicaceae throughout the world.

Fertilizers might play an important role in enhancing crop productivity through using nitrogen, potassium and phosphorous fertilizers and at least cause a reduction of various plant diseases. The highest seed yield was shown when a combination of the three fertilizers was applied at recommended doses (El-Korashy, 2003; Kutcher *et al*., 2005).

Many bioagents and/or their metabolites have been studied for their ability to reduce the growth, sporulation, spore germination and toxin production of *A. brassicicola* and *P. lingam* affecting canola and other brassicas, subsequently reducing leaf spot diseases. Pichard and Thouvenot (1999) isolated a strain of *Bacillus polymyxa* [*Paenibacillus polymyxa*], BP1, from cauliflower seeds, and indicated that the strain has potential as a biological control agent against *A. brassicicola* when it was applied to inoculated seeds. Sharma and Dureja (2004) used twelve isolates of *Trichoderma* spp. (eight of *T. harzianum* and four of *T. viride*) to develop suitable methods for screening antagonists against phytotoxic culture filtrates of ten pathogens, including *A. brassicicola* and *A. alternata*. They found that *Trichoderma* spp. produce phytotoxins exhibited mild symptoms development compared to the control phytotoxin, and their metabolites were effective in reducing the disease symptoms on leaves and seedlings.

Hossain and Mian (2004) investigated four fungicides, *i.e.*, Rovral 50 WP (Iprodione), Dithane M-45 (Mancozeb), Bavistin 70 WP (Carbendazim) and Tilt 250 EC (Propiconazole), alone or in different combinations against cabbage Alternaria blight caused by *A. brassicicola*. They found that all the tested fungicides reduced the disease severity and increased seed yield as well as yield components in a field trial. As well, Ayub *et al.* (1997) evaluated four fungicides Ridomil-MZ 72WP, Lirotect-M and Rovral 50WP against alternaria blight of cauliflower (*A. brassicae, A. brassicicola*) in field experiment, among the four fungicides tested, Rovral was effective against alternaria blight and maximized seed yield.

Thus, the main purpose of this work is to survey canola foliar diseases in different Egyptian locations. Isolation, purification, pathogenic potentiality and identification were undertaken. Host range of the isolated fungi and control the disease through different methods including resistant cultivars, NPK levels, biological and chemical treatments were put into consideration.

MATERALS AND METHODS

Survey, Frequency and Symptomatology

A survey study was carried out during three successive growing seasons 2010/2011, 2011/ 2012 and 2012/2013 to detect fungal genera associated with foliar parts of canola plants grown in different locations of four governorates (Giza, Ismaillia, Gharbia and Sharkia). Plants doubted to be infected were collected in paper bags and transferred to the Plant Patho. Lab., Plant Pathol. Res. Institute, Ismaillia Agric. Res. Station.

Isolation and Purification of the Causal Organisms

Infected leaves, shoots and pods were thoroughly washed in running tap water, then cut into small pieces before surface sterilized with 3% sodium hypochlorite solution for 3 min. Pieces were rinsed several times in sterilized

water and dried between two sterilized filter papers, then plotted on PDA medium in Petri dishes and incubated at 25°C. Cultures were purified using hyphal tip and/or single spore techniques according to Sinclair and Dhingra (1995).

Identification of the Causal Organisms

The isolated microorganisms from spotted leaves, stems and pods of canola were microscopically identified using the description of Barnett and Hunter (1998). Identification of the selected isolates was kindly confirmed by the Mycological Research and Diseases Survey Dept., Plant Pathology Research Institute, ARC, Giza, Egypt.

All the following experiments were conducted under greenhouse conditions of Ismaillia Agric. Res. Station.

Pathogenicity Tests

Pathogenicity of *A. brassicicola* and *P. lingam* were tested for their pathogenic potentiality under greenhouse conditions of Ismaillia Agric. Res. Station during 2012/ 2013 growing season.

Preparation of spore suspension and inoculation

Inoculum of *A. brassicicola* and *P. lingam* were prepared from cultures grown on potato dextrose broth medium and incubated at 21 ± 23°C for 10-14 days. Mycelial mats were picked up and washed for several times with sterilized distilled water then blended with water for 3 min. The spore concentration was adjusted microscopically with the aid of hemocytometer technique to be 10^6 cfu/ml of *A. brassicicola* (Brame and Flood, 1983) and 2×10^7 cfu/ml of *P. lingam* (Hammoudi *et al*., 2012).

Plastic pots (25 cm in diameter), were sterilized by immersing in 5% formalin solution and left in the open air in order to get rid of the remained poisonous formalin. Sterilized pots were filled with sterile autoclaved soil. This experiment was carried out in randomized complete block design with three replicates for each particular treatment. Canola seeds obtained from Field Crops Research Institute (FCRI), ARC, Giza, Egypt. Canola plants Serw 4 cultivar, 60-days old, were washed with sterilized water then atomized with the desired aforementioned spore suspension after kindly removing waxy layer away of canola leaves. Plants were sprayed only with water to serve as control. Inoculated and control plants were kept in a moist chamber for 48 hrs., then transferred to greenhouse and sprayed with water twice for a week, to maintain high relative humidity. Occurrence and symptoms development of the disease were recorded 10 days after inoculation on plant leaves. Three plants from each plot were randomly chosen and used to estimate the percentage of the affected leaves. Disease incidence (DI) and disease severity index (DSI) of *A. brassicicola* and *P. lingam*, were calculated for each pot by summing the scores of ten leaves and analyzing using rating scale of symptoms described by Conn *et al.* (1990) and Kutcher *et al*. (1993).

Reaction of canola plants to the pathogens

The reaction of canola plants to the pathogenic fungal genera were determined as visual symptoms doubted to be due to various infectious pathogens. Symptoms observed as leaf spot, were detected and photographed.

Disease assessment

Disease incidence (DI) and disease severity index (DSI) of *A. brassicicola* and *P. lingam*, were calculated as mentioned before.

Response of Canola Cultivars to Inoculate with *Alternaria brassicicola* and *Phoma lingam*

This experiment was conducted to test the susceptibility of the three most promising canola cultivars *i.e.* Serw 4, Serw 6 and Pactol to black spot and/or blackleg diseases incited by *A. brassicicola* and *P. lingam*, respectively. The tested canola cultivars were sown in clay pots (30 cm in diameter) filled with field soil (Table 1).

Nine kg/pot of the soil was seeded with 20 canola seeds on 20th of November 2013 growing season. After full germination, the plants were thinned to 5 seedlings/pot. In a randomized complete block design with three replicates for each particular treatment, plants of two months old were individually inoculated with *A. brassicicola* and/or *P. lingam* as mentioned before.

Plants sprayed with distilled water served as control treatment. The inoculated plants were kept under polyethylene bags for 48 hr., to ensure humid atmosphere then transferred to

greenhouse. Disease reaction was determined 10 days after inoculation as mentioned before in pathogenicity test.

Host Range

This experiment was designed to investigate the susceptibility of the five different winter crops *i.e*. Linen (*Linum usitatissimum* L.), Bean (*Phaseolus vulgaris* L.), Lupine (*Lupinus polyphyllus* Lindl), Radish (*Raphanus sativus* L.) and Arugul (*Eruca sativa* L.) to black spot and/or blackleg diseases individually. The same method previously mentioned with canola cultivars test was used. Disease severity was determined 10 days after inoculation as mentioned before in pathogenicity test.

Percentage of Canola Foliar Disease Infection and Oil Seed Contents as Affected by NPK Fertilizer Levels

The effect of different levels of nitrogen, phosphorus and potassium on disease incidence, was carried out. Nitrogen, phosphorus and potassium were applied to each pot (30 cm. in diameter) with 9Kg soil.

Levels of NPK were added as ammonium nitrate (150 Kg/fad.), calcium superphosphate (15Kg /fad.) and potassium sulfate (24 Kg/fad.) according to the recommended dose. Solutions (50 ml per pot) in each treatment, was applied as mentioned in Table 1. Fertilizers were dissolved in distilled water and added to each pot. Fertilizers were added in three equal portions, the first on sowing time, the second after one month of sowing, and the third one at the flowering stage. Four pots were prepared for each particular treatment. Seeds of canola (cultivar Serw 4) were sown on November 20th, 2013 growing season at the rate of 20 seeds per pot. After full germination, the plants were thinned to 5 seedlings/pot.

One month later, plants were individually sprayed with the spore suspension of *A. brassicicola* and/or *P. lingam* as mentioned before. Plants were then placed in a moist chamber for 48 hr., then transferred to the greenhouse. Percentages of infection were recorded at harvest time. The seed oil content was determined according to the methods described by Association of Analytical Communities (AOAC) using soxhelt apparatus (Straccia *et al.*, 2012).

Biological Control

Testing of different antagonistic microorganisms against alternaria black leaf spot and blackleg diseases *in vivo*

The effect of *Bacillus subtilis*, *Trichoderma viride* and *Trichoderma harzianum* on canola black spot and blackleg diseases were investigated under greenhouse conditions. Bioagents used were kindly obtained from the stock cultures of Plant Pathology Dept., Fac. Agric., Zagazig Univ.

Trichoderma viride and *T. harzianum* were grown individually on 200 ml of Gliotoxin Fermentation Medium (GFM) in 500 ml Erlenmeyer flasks on a rotary shaker (100 rpm) for 7 days at 28 ±ºC in the dark as described by Brain and Hemming (1945). The liquid cultures of each *Trichoderma* spp. were mixed in a blender and adjusted to 10^6 cfu/ml. On the other hand, bacterial isolate, *Bacillus subtilis* was inoculated in flasks (250 ml in capacity) each containing 100 ml of King's B medium using a loop of 24 hr., old bacterial cultures. Flasks were incubated at 28±ºC on rotary shaker (100 rpm) for 24 hr. The liquid culture of each bacterial isolate was mixed in a blender and adjusted to 10^8 cfu/ml (King *et al.*, 1945).

The effect of spraying canola plants with antagonistic fungi (*T. viride* and *T. harzianum*) and bacteria (*Bacillus subtilis*) on canola black spot and blackleg diseases were investigated *in vivo*. This experiment was carried out in pots and designed at random complete block in three groups under greenhouse conditions of Ismaillia Agric. Res. Station. After 4-5 weeks from seedling, plants were sprayed with the aforementioned bacterial and fungal bioagents suspensions after kindly removing waxy layer away of canola leaves. Control treatment was sprayed with autoclaved GF or King's B media. Sprayed plants were kept under polyethylene bags for 24 hr. Three replicates were carried out of each particular treatment. The treated plants were kept under polyethylene bags for 48 hours to ensure the infection process then exposed to greenhouse conditions. Disease reactions were determined 10 days after inoculation of sprayed leaves. Disease severity recorded as mentioned before in pathogenicity test.

Table 1. Application rate of nitrogen, phosphorus and potassium under greenhouse conditions

Treatment (NPK)	Chemical fertilizer g/pot		
	Nitrogen	Phosphorus	Potassium
	Ammonium nitrate g/pot	Calcium superphosphate g/pot	Potassium sulfate g/pot
(Control) $N_0 P_0 K_0$	0.00	0.00	0.00
$N_1 P_0 K_0$	1.35	0.00	0.00
$N_0 P_1 K_0$	0.00	0.135	0.00
$N_0 P_0 K_1$	0.00	0.00	0.22
$N_1 P_1 K_0$	1.35	0.135	0.00
$N_1 P_0 K_1$	1.35	0.00	0.22
$N_0 P_1 K_1$	0.00	0.135	0.22
$N_1 P_1 K_1$	1.35	0.135	0.22

Chemical Control

The inhibitory effect of different systemic and contact fungicides being (Ridomil-Mancozeb 72%, Carbendazim 50% and Dithane M-45), were investigated against *A. brassicicola* and *P. lingam in vivo*.

Effect of three different fungicides on canola black spot and/or blackleg diseases incidence using attached leaf technique

Canola plants at 4-5 weeks old were sprayed with tested fungicides 1000 ppm of Ridomil-Mancozeb 72%, Carbendazim 50% and Dithane M-45. Control plants sprayed with distilled water. After 24 hr., plants were inoculated with 30 ml/plant of *A. brassicicola* and/or *P. lingam* as mentioned before. The sprayed plants were covered with plastic box and 10 days after inoculation, disease assessment was calculated as mentioned before under pathogenicity tests.

Statistical Analysis

All the previously investigated experiments were subjected to statistical analysis using ANOVA methods reported by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Survey, Frequency and Symptomatology

A survey study was carried out in three successive growing seasons as mentioned before in material and methods to detect different fungal diseases associated with canola growing plants. Disease incidence was recorded while symptoms were photographed. Data presented in Table 2 and Figs. 1 and 2 indicate that blackleg disease was the most frequently dominant (59.4%) from the expected diseased canola leaves followed by black spot disease in this respect (47.0%). It was also noticed that both previously mentioned diseases were dominant in the inspected localities caused by *A. brassicicola* and *P. lingam*. The isolated pathogenic fungi *P. lingam* was highly frequently isolated from diseased canola samples obtained from Ismaillia and Gharbia localities being 70.6% and 60.0%, respectively. Whereas, the least frequency percentage of the disease was recorded from El-Sharkia locality being 50.0% exhibiting the least disease incidence. However, black spot disease recorded the highest disease incidence of canola samples obtained from Ismaillia and Gharbia localities being 54.0% and 50.0%, respectively. Whereas, the least disease incidence of the black spot disease caused by *A. brassicicola* was recorded from Sherkia locality being 37.6%

Table 2. Percentage of black spot and blackleg diseases associated with canola plants detected from four Governorates in three successive winter growing seasons

Disease	Governorate	Diseases incidence (%)			Mean
		2012/2013	2011/2012	2010/2011	
Black spot disease	Giza	44.0	56.0	40.0	46.6
	Ismaillia	50.0	68.0	44.0	54.0
	Gharbia	39.0	76.0	35.0	50.0
	Sharkia	30.0	50.0	33.0	37.6
	Mean	40.7	62.5	38.0	47.0
Blackleg disease	Giza	60.0	55.0	56.0	57.0
	Ismaillia	77.0	65.0	70.0	70.6
	Gharbia	75.0	60.0	45.0	60.0
	Sharkia	65.0	45.0	40.0	50.0
	Mean	69.2	56.2	52.7	59.4

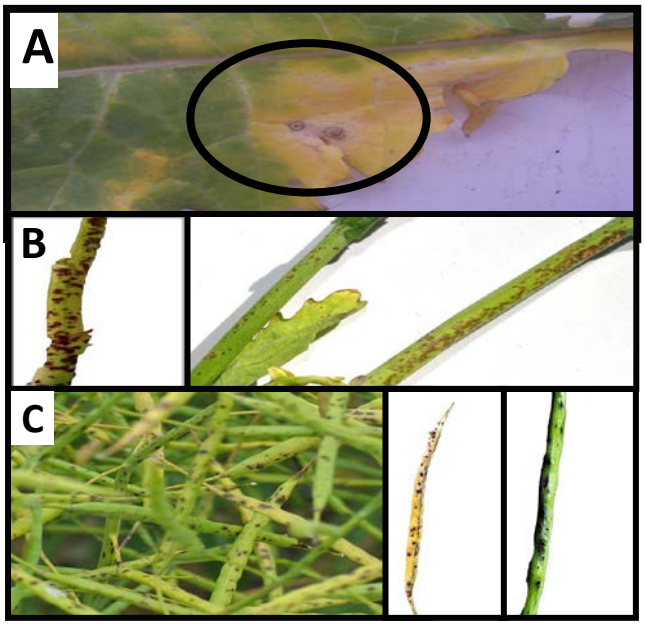

Fig. 1. Black spot disease on infected leaves A, stems B and pods C

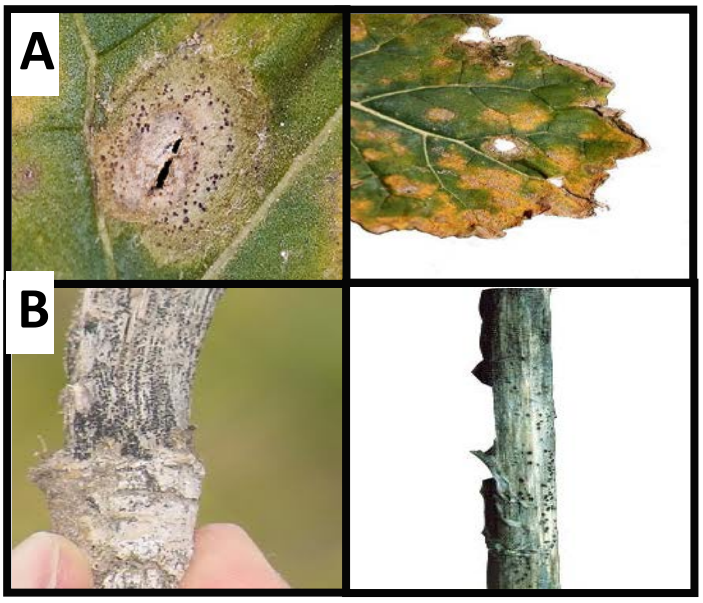

Fig. 2. Blackleg disease shown as spots and pycnidia on diseased leaves A and stems B

which exhibited the least disease incidence in survived fields. These results indicates the importance of this pathogens causing canola leaf spot disease appearance in different localities. The highest incidence of the black spot and blackleg diseases were occurred in Ismaillia Governorate. This might be due to the favorable environmental conditions in these locations for both *A. brassicicola* and/or *P. lingam*, that requires rainy periods, moist atmosphere, sunshine hours and duration of leaf wetness temperature around 18±20^{o}C for infection (Humpherson-Jones and Mauda, 1983; El-Deeb *et al.*, 1989; Gugel and Petrie, 1992).

Isolation, Purification and Identification of the Causal Organisms

The isolated microorganisms from spotted leaves and stems of canola were microscopically identified using the description of Barnett and Hunter (1998) as *Alternaria brassicicola* (Sch.) Wiltshire and *Phoma lingam* (Tode) Desm. According to (Ruchi *et al*., 2014; Hura *et al*., 2014).

Pathogenicity Tests

Results in Table 3 as well as Figs. 3 and 4 indicates that *A. brassicicola* was the most pathogenic appearing (61%) and (66%) as mean of both disease severity and incidence, respectively. However, *P. lingam* resulted (56.5%) and (63.3%) for both disease severity and incidence, respectively. Thus, *A. brassicicola* was significantly differed than *P. lingam* in its destructive pathogenic potentiality when examined under greenhouse conditions.

Alternaria black spot disease symptoms of infected canola leaves

Disease symptoms were appeared 3-4 days after inoculation, as brown to black necrotic lesion on leaves contact with soil. The most conspicuous symptoms were the black spots occurred on leaves, stems and pods. Most infected leaves were wilted and dropped, while spotted pods dried early before maturity (Fig. 3).

Phoma blackleg disease symptoms of infected canola leaves

Disease symptoms was appeared 7-8 days after inoculation, as brown to black necrotic lesions on leaves (Fig. 4). The most conspicuous symptoms were dirty white, round to irregularly shaped, and usually dotted with numerous small and black pycnidia, while the black spots occurred on leaves and stems. Most infected leaves were wilted and dropped, and spotted pods dried early before maturity.

Table 3. Pathogenic potentiality of different isolated fungi on attached leaves of Serw 4 canola cultivare under greenhouse conditions

Fungi	Disease incidence (%)	Disease severity (%)
A. brassicicola	66%	61%
P. lingam	63.3%	56.5%
LSD at 5%	24.84	17.61

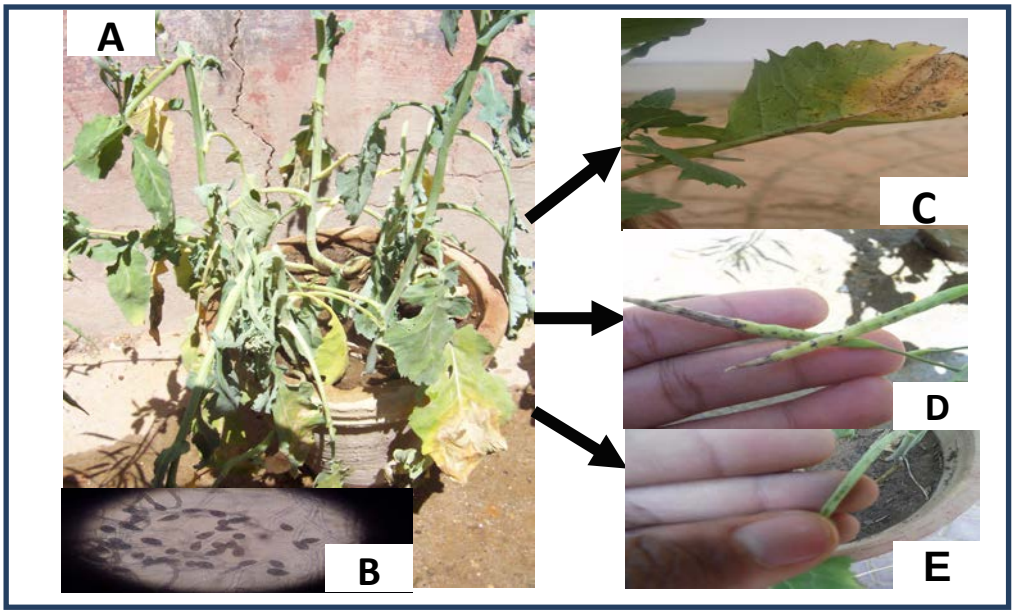

Fig. 3. Symptoms of black spot disease on infected canola leaves, stems and pods. A) Infected plant, B) *A. bracsicicola* conidiospore, C) Infected leaf, D) Infected pods, E) Infected stem

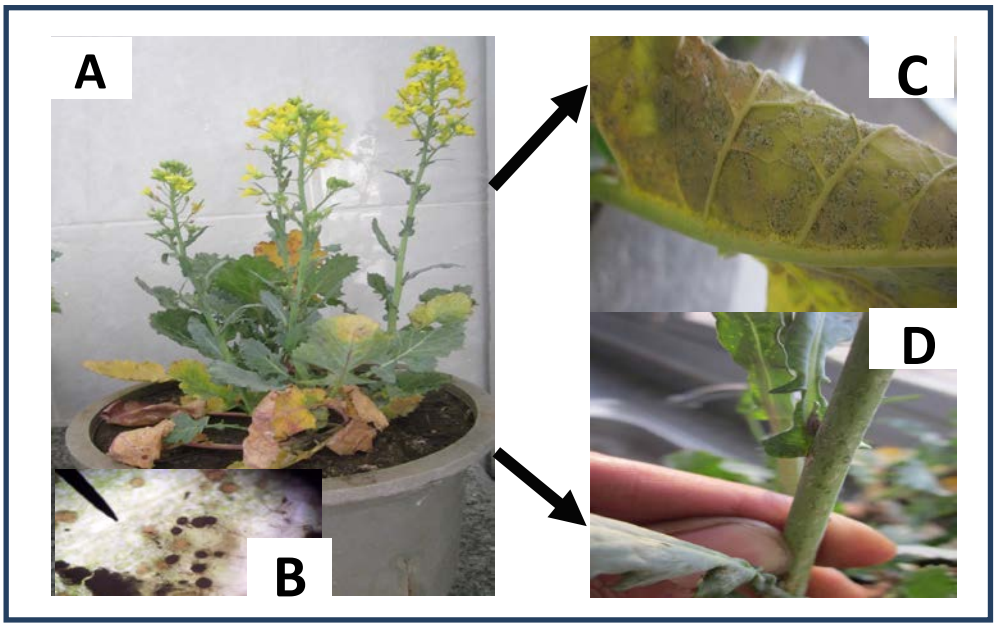

Fig. 4. Symptoms of blackleg diseases of infected canola leaves and stems. A) Infected plant, B) *P. lingam* conidiospores, C) Pycinidia on Infected leaf, D) Infected stem

Response of Canola Cultivars to Inoculate with *A. brassicicola* and *P. lingam*

Reaction of Serw 4, Serw 6 and Pactol canola cultivars to the infection with *A. brassicicola* and *P. lingam*, was conducted under greenhouse conditions. Results in Table 4 indicates that the three tested canola cultivars were significantly susceptible to infect with both fungal genera at various levels when compared with the control. Generally, Serw4 was the most infected one followed by Serw 6 without significant differences as affected by *A. brassicicola*. Meanwhile, Pactol proved to be the least infected one for both genera which it reveal 38.7% of both disease parameters with *A. brassicicola*, and 25% with *P. lingam.* However, Serw 4 proved to be highly susceptible when inoculated with *P. lingam* (where 88% of disease severity).

Sensitivity of the three canola cultivars to infect with both *A. brassicicola* and *P. lingam* indicated that Serw 4 followed by Serw 6 proved to be highly infected with *P. lingam* where 88% of disease severity, was recorded. However, *A. brassicicola* was also destructive on Serw 6 compared with *P. lingam*. The differences among cultivars in reaction might be due to their difference in genetic makeup and their genetic structure which might affect morphological and physiological characters of plants. From anther hand, environmental factors might also affect host pathogen relationship which might play a role in cultivars susceptibility (Dixelius and Wahlberg 1999; Thomas *et al.,* 1999; Hura *et al*., 2014). As well as, races distribution of the pathogens might cause destroying in cultivars resistance (Zala *et al*. 2012).

Host Range

Evaluation of five different winter crops *i.e*., linen, bean, lupine, radish and arugula against isolated fungi were conducted.

Results in Table 5 indicates that, radish was highly infected with the tested fungi compared with the other tested crops. *A. brassicicola* was the most destructive for radish resulting 85% and 100% for disease severity and incidence, respectively, when compared with *P. lingam* (63.7% and 71%) respectively. Lupine seemed to be the least affected one, while arugula showed moderate results. However, linen and bean plants were not affected. Generally, *A. brassicicola* was superior with high significant differences in inducing disease parameters on the crucifer plants *i.e.* radish and arugula rather than *P. lingam.*

Such differences between plant hosts could be explained on the basis that both fungal genera are not specialized compatible with the linen, bean and lupine hosts resulting no visible symptoms on such inoculated plant hosts (Voigt *et al.,* 2005).

Percentages of Disease Incidence, Severity, Oil Seed Contents and Seed Weight (g/pot) as Affected by NPK Fertilizer levels

Different levels of NPK fertilizers were tested for their effect on black spot and blackleg diseases. Results presented in Table 6 indicates that, disease severity caused by *A. brassicicola* and *P. lingam*, respectively was significantly increased at harvest time (85% and 91.7%) when N_1 P_0 K_0 was evaluated compared with control and other treatments. However, the disease severity was completely decreased when N_1 P_1 K_1 level was evaluated (17.3% and 9.7%) for *A. brassicicola* and *P. lingam*, respectively. The efficacy of the rest NPK levels recorded moderate values ranging from 21.7% to 66.0% with *A. brassicicola* and 11.7% to 48.3% with *P. lingam*, was inspected. Generally, disease severity percent caused by *P. lingam* was lower than those caused by *A. brassicicola* when evaluated with all the tested NPK levels or even if compared with the control one.

In consequence the lowest seed weight (g/pot) was found when N_1 P_0 K_0 (0.2 g) was applied showed the lowest oil percentage (12%). However, the highest seed weight was achieved (4.7g) accompanied with the highest oil percent (40%) when N_1 P_1 K_1 level, was added. Similar trend was obtained when plants were inoculated with *P. lingam* but with relatively higher values being 5.4 (g) and 48% for both seed weight (g) and oil percentage, respectively.

Table 4. Response of canola cultivars to *Alternaria brassicicola* and *Phoma lingam*

Canola cultivar	*Alternaria brassicicola*		*Phoma lingam*		Control	
	Disease incidence (%)	Disease severity (%)	Disease incidence (%)	Disease severity (%)	Disease incidence (%)	Disease severity (%)
Serw 4	100.0	85.3	100.0	88.0	22.0	18.0
Serw 6	86.7	86.7	80.0	57.0	15.3	12.3
Pactol	38.7	38.7	25.0	25.0	13.3	10.0
LSD 5%	17.4	21.5	20.7	24.9	3.76	4.0

Table 5. Response of different plant hosts to inoculate with *Alternaria brassicicola* and *Phoma lingam*

Crop	*A. brassicicola*		*P. lingam*		Control	
	Disease incidence (%)	Disease severity (%)	Disease incidence (%)	Disease severity (%)	Disease incidence (%)	Disease severity (%)
Linen	0.0	0.0	0.0	0.0	0.0	0.0
Bean	0.0	0.0	0.0	0.0	0.0	0.0
Lupine	13.3	25.0	0.0	0.0	0.0	0.0
Radish	100.0	85.0	71.0	63.7	15.3	44.6
Arugula	62.0	73.0	71.0	55.3	18.6	28.6
LSD5%	7.51	14.0	4.1	4.8	3.1	4.8

Table 6. Percentages of disease incidence, severity, oil seeds contents (%) and seed weight (g/pot) in canola plants artificially inoculated with *Alternaria brassicicola* and *Phoma lingam* as affected by NPK fertilizer levels of during 2014 winter growing season

Treatment	*Alternaria brassicicola*				*Phoma lingam*				Control			
	Disease incidence (%)	Disease severity (%)	Seed weight (g/pot)	Oil (%)	Disease incidence (%)	Disease severity (%)	Seed weight (g/pot)	Oil (%)	Disease incidence (%)	Disease severity (%)	Seed weight (g/pot)	Oil (%)
$N_1 P_0 K_0$	81.6	85.0	0.2	12.0	86.6	91.7	0.04	14.0	4.6	3	1.1	20.0
$N_0 P_1 K_0$	21.6	21.7	2.4	29.0	8.6	11.7	1.6	30.0	14.0	11.3	2.3	49.0
$N_0 P_0 K_1$	28.3	28.3	1.4	16.0	10.0	15.0	1.1	20.0	12.5	16.6	2.8	33.6
$N_1 P_1 K_0$	72.6	66.0	2.4	25.0	51.6	46.7	2.3	29.1	10.0	13	4.3	45.0
$N_1 P_0 K_1$	62.0	64.7	1.3	19.0	35.0	48.3	1.2	25.0	23.7	22.3	2.0	34.6
$N_0 P_1 K_1$	37.6	31.7	1.0	15.0	26.6	16.7	0.9	20.0	11.7	6.3	1.3	23.0
$N_1 P_1 K_1$	22.6	17.3	4.7	40.0	6.6	9.7	5.4	48.0	20.0	22.2	6.2	55.0
$N_0 P_0 K_0$	59.6	63.0	1.6	20.0	60.3	13.6	1.1	22.0	14.2	12.6	3.1	31.3
LSD 5%	5.91	6.7	0.5	2.3	11.1	5.1	0.6	3.2	1.7	2.9	0.2	1.7

Thus, increasing the disease severity with the increase of nitrogen fertilizer level resulted in copious vegetation and consequently increase the disease severity. On the other hand, nitrogrn fertilization increased the potential of sporulation ability on the leaf surface.

Potassium and phosphorous fertilizer application decreased disease incidence and this might be due to reduction in activities of protopectianse, polygalacturonase, transaminase, pectin trans-eliminase and cellulases after inoculated with thereby inhibiting spots development and increased host resistance to pathogens. Also, their effects might be attributed to contribution in thickening of plant cell wall and this might retard the penetration of fungal pathogen (Atia, 2000). Thus, these fertilizers might played an important role in enhancing crop productivity through application of potassium and phosphorous fertilizers and led to control at least cause a reduction of various plant diseases. In consequence, weight of seeds (g) resulting the lowest oil percent (20%). However, our results revealed that the highest seed yield was achieved (6.1g) followed with the highest oil percent (46%) when N_1 P_1 K_1 level, was applied, similar results was obtained by (Vach *et al.,* 2005).

Biological Control

Data presented in Table 7 indicate that, *Bacillus subtilis* have significant suppressive effect on the disease severity caused by *A. brassicicola* recording 58.6% on the artificially inoculated plants. On the other hand, *P. lingam* proved to be more sensitive to *B. subtilis*. In this respect significant low percent of disease severity (23.3%), was obtained resulting 62.2% reduction in the disease incidence. Effect of bacterial isolate might be due to secreting antibiotics, effective metabolites producing extracellular toxins and/or lytic enzymes that affect the fungal growth and sporulation (Rajesh *et al.,* 2011).

Disease severity percent was significantly high (57.0%) with *T. viride* against *A. brassicicola* comparing with the other bioagents without significant differences with *T. harzianum*. The results show rates ranging between 57% and 51% of severity when both *Trichoderma* isolates were tested resulting the lowest reduction percentages of disease incidence being 31.3% and 39.6%, respectively.

However, either *T. viride* and / or *T. harzianum.* were highly effective against *P. lingam.* While, *T. harzianum* was the least effective bioagent between them where it recorded 57.0% for disease severity.

The effect of *T. viride* might be due to the ability to excrete toxic or inhibitory metabolites and / or mycoparasitism or lysis of the pathogen hyphae and thus might affect the pathogen growth resulting in decrease of disease severity.

Thus, it could be concluded that Such differences between the activity of the bioagents might be also due to the execrations of the plant leaves that might played an important role in microorganisms reproduction on the leaf surface and played an important role in bio-control mechanisms. These results are consistent with the prominent protective control activity of the beneficial microorganisms that might affect the foliar spot and blackleg diseases of plants.

Chemical Control

Effect of three different fungicides on canola black spot and blackleg diseases incidence under greenhouse conditions

Data presented in Table 8 indicate that *A. brassicicola* black spot disease was significantly reduced especially when Ridomil-Mancozeb 72%, was investigated. However, Dithane M-45 caused higher percentage of severity. Results in Table 9 were also obtained when plants inoculated with *P. lingam* were investigated. Complete reduction percent was obviously noted when Redomil-Mancozeb was investigated showing (100%) for tested concentration. On the other hand, Carbendazim was found to be less effective fungicide in controlling the disease caused by *P. lingam* of the attached examined leaves.

The obtained results indicate that the tested fungicides reduced effectively disease severity when canola plants were sprayed (4-5 weeks old) curative treatment. The obtained results demonstrate that, the action of such fungicides might be due to their direct toxic effect to the fungus resulting a reduction in spore adhesion to the leaf surface of canola plants and in consequence influenced the initiation of infection structure or even destroy the mycelial growth. Also, a fungicide might be improves the ability of canola plants defense mechanism to respond to infection.

Table 7. Reduction percentage of disease severity caused by *Alternaria brassicicola* and *Phoma lingam* as affected by different bioagents *in vivo* (winter 2014)

Disease severity (%) / Bioagent	*A. brassicicola*			*P. lingam*		
	Inoculated with bioagent	Non-inoculated with bioagent	Reduction (%)	Inoculated with bioagent	Non-inoculated with bioagent	Reduction (%)
Trichoderma viride	57.0	83.3	31.3	28.0	58.0	51.5
Trichoderma harzianum	51.0	84.6	39.6	57.0	61.0	6.5
Bacillus subtilis	35.0	84.6	58.6	23.3	62.0	62.2
LSD 5%	7.7	7.3	11.0	7.1	3.4	13.1

Table 8. Effect of three fungicides on canola black spot disease severity and disease reduction percentage under greenhouse conditions

Disease severity (%) / Fungicide (1000 ppm)	Treated with fungicide	Un-treated with fungicide	Reduction (%)
Ridomil Mancozeb	10.3	48.0	78.54
Dithane M-45	38.0	86.0	55.8
Carbendazim	18.8	84.0	77.62
LSD 5%	4.2	5.6	4.4

Table 9. Effect of three fungicides on canola blackleg disease severity and disease reduction percentage under greenhouse conditions

Disease Severity (%) / Fungicide (1000 ppm)	Treated with fungicide	Un-treated with fungicide	Reduction (%)
Ridomil Mancozeb	0.0	62.3	100.0
Dithane M-45	50.3	64.0	21.4
Carbendazim	7.6	62.6	87.8
LSD 5%	3.5	2.4	4.8

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دراســات مـرضيــة علـى تبقعـات فطــرى ألتـرنــاريا براسيسكولا وفـومـا لينجم على نباتات الكانولا تحت ظروف الصوبة

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تم عزل فطر ألترنايا وفطر فوما المسببين لتبقعات الأوراق على نبات الكانولا وذلك خلال مواسم الزارعة ٢٠١٠/ ٢٠١١، ٢٠١١/ ٢٠١٢ و ٢٠١٢/٢٠١٣ من أربعة محافظات وهم: (الجيزة، الإسماعيلية، الغربية والشرقية) وتم عزل تلك الفطريات من الأوراق والسوق، وتم تعريفها كفطرى ألترناريا براسيسكولا وفوما لينجم، ولقد وجد أن صنف الكانولا باكتول هو أكثر صنف مقاوم للإصابة بهذين الفطرين، وقد تبين أن الجرجير والفجل من النباتات القابلة للإصابة بالفطريات السابقة، ووجد أيضا أن التسميد بالعناصر الكبرى (النيتروجين والفسفور والبوتاسيوم) بمعدل ن$_1$بو$_1$فو$_1$ أعطى أفضل النتائج فى مقاومة المرض وتقليل نسبة وشده الإصابة، وقد تبين أن فطر تراكيودرما هارزيانم هو أكثر الفطريات تأثيراً لمقاومة فطر ألترناريا براسيسكولا بينما فطر ترايكودرما فيردى كان أكثر مقاومة لفطر فوما لينجم، وقد كان لزيادة تركيز مبيد الكاربندزيم دور فى تقليل نسبة وشده الإصابة بفطر ألترناريا براسيسكولا دون تأثير ملحوظ على فطر فوما لينجم أما زيادة تركيز مبيد الريدوميل قد أدت إلى تقليل نسبة وشدة الإصابة بفطر فوما لينجم دون تأثير ملحوظ على فطر ألترناريا براسيسكولا.

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