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IDENTIFICATION AND *IN VITRO* CONTROL OF CANOLA SPOT DISEASE PATHOGEN

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A B S T R A C T

Fungal spot disease is an emerging problem of canola, a major edible oil producing crop in Pakistan. During present study, *Alternaria* sp. was isolated and identified from the different infected tissues of diseased plant. Eighteen different commercially available fungicides; Defeater, Defeater Plus, Bloom, Wisdom, Trifort, Definite, Cordate, Flare, Benedict, Triton, Hiten, Pyranil, Flumax, Tebuconazole, Epic, Primacy, Vegard and Picoxystrobin were screened for their efficacy in controlling the identified pathogen. Maximum reduction of 90% in fungal growth was obtained by Wisdom, Benedict and Tebucanazole followed by Bloom, Flare and Flumax with 75-81% growth inhibition. Afterward by Epic, Defeater plus, Primacy and Picoxystrobin with upto 55 % fungal growth inhibition. Remaining fungicides caused 11-50% restricted fungal growth.

Keywords: Saturated fats, Pathogen, disease severity, chemical control.

INTRODUCTION

Canola (*Brassica napus* L.) a member of Family *Brassicaceae* is the edible oil plant of rapeseed group that has been growing in Europe since 13th century. Canola is traditional crop of Pakistan and is most popular in Punjab. Today canola oil is acknowledged as a premium vegetable oil due to low (7%) saturated fat content (Aminpanah *et al*., 2013). Canola contains both high oil (44%) and protein content (23%). Canola oil is low in saturated fat and contains both omega-6 (linoleic) and omega-3 fatty acids (α-linolenic acid) in a ratio of 2:1 making it as one of the healthiest cooking oils even better than olive oil. It has highest levels of plant sterols, especially β-sitosterol and campesterols that competitively inhibit cholesterol absorption in the gut and thereby can reduce cholesterol levels by 10% to 15%. The oil also contains valuable amounts of lipid soluble anti-oxidant vitamin E that help to maintain integrity of cell.

Alternaria blight diseases of crop plants have been reported from all the continents of the world, cause average loss of 5-47% in India (Kolte, 1985; Kolte *et al*., 1987; Sharma and Kolt, 1994); 5% in Canada (Tewari, 1991); 57% in Nepal (Shrestha *et al.*, 2005). In Pakistan, Alternaria black spot diseases has been reported on number of plants including sunflower (Mirza and Beg, 1983); tomato (Akhtar *et al*., 2004); okra (Jiskani, 2006); Aloevera (Bajwa *et al*., 2010) and Mango (Mohsan *et al*., 2011). Alternaria black spot (also called grey leaf spot) caused by the conidia of different species of fungal Genus Alternaria for example *A. brassicae* (Berk.) Sacc.,*A. brassicicola* (Schw.) Wiltsh., *A. raphani* Groves & Skolko, and *A. alternata* (Fr.) Kreissler (Meena *et al*., 2010; Nowicki *et al*., 2012). The pathogens infect their hosts by direct penetration or through wounds and natural openings. The disease is favored by warm, humid weather. These pathogens are disseminated as conidia within and among fields by splashing water and wind. The pathogens survive in infested crop debris and on seed (Conn *et al*., 1990).

Alternaria black spot symptoms appear as brown to black circular spots on leaves that enlarge under favorable conditions. Lesions become gray with concentric rings and develop a purple or black border. Lesions on stems and pods are black or black with gray centers. Under dry conditions, lesions remain small and black, and often develop a yellow halo. Severe defoliation can occur during disease-favorable weather. Pods with infected pedicels fail to develop and drop off

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the plant. Severely spotted pods dry, shrink, and may split open prematurely, allowing shrunken seeds to drop to the ground. The Alternaria black spot pathogens can also cause a seed rot or post emergence damping-off (Schwartz and Gent, 2004). Alternaria black spot diseases lead to reduction in photosynthetic area, defoliation and accelerated senescence (Conn *et al*., 1988).

Several management strategies are available to control black spot disease of crop plants like manual, mechanical cultural methods and chemical fungicides (Schwartz and Gent, 2004). One of the most effective and old method for disease control is the use of fungicides. There are several fungicides which are being commercially available while several others are being evaluated in different laboratories.

METHODOLOGY

Survey and collection of plant samples: A visit of Kenzo Farm, Shiekupura was conducted in April 2013 for the collection of plant samples. At the farm, canola was grown over area of 2250 ft^2 and around 85% of the plants were found be infected with black, necrotic lesion on leaves, pods and stems. Both healthy and infected plants were uprooted carefully and brought to the laboratory in clean polythene bags for disease identification and pathogen isolation.

Isolation and identification of fungal pathogen: Malt extract agar medium (MEA) was for the culturing of fungal pathogen from infected plant parts. Leaves, stems and pods of canola plant that showed symptoms of the disease were cut into small and inoculated on growth medium after surface sterilization with 1 % sodium hypochlorite solution. The Petri plates were incubated for at 25ºC and observed regularly for fungal growth.

Pure culture of the pathogen fungus was obtained by transferring the mycelium from the actively growing fungus onto fresh growth medium plate. Fungus was identified on cultural and morphological basis (Simmons, 2007) and pure fungal cultures were stored at 4^{o}C for further experimentation.

Antifungal assays for fungicides screening: A broad range of fungicides having different active ingredients was used. Such fungicides were procured from KANZO Seeds, Evyol Group. Details of concentrations and recommended doses of fungicides used during present study are given in Table 1.

Table 1. List of fungicides evaluated for their potential to control the fungal pathogen.

Sr. No	Brand	Active Ingredient	Dose/(100 L)
1	Defeater	Flumorph	250 g
2	Defeater Plus	Flumorph Fosetly-aluminium	500 g
3	Bloom	Myclobutanil	40 ml
4	Wisdom	Fosetly-aluminium	250 g
5	Trifort	Triadimefon	100 g
6	Definite	Difenoconazole	300 g
7	Cordate	Kasugamycin	300 g
8	Flare	Streptomycin	100 g
9	Benedict	Iprobenfos	200 ml
10	Triton	Validamycin	100 g
11	Hiten	Fentin Hydroxide	250 ml
12	Pyranil	Pyrimethanil	300 ml
13	Flumax	Fluazinam + Metalaxyl-M	150 ml
14	Tebuconazole	Tebuconazole	750 ml
15	Epic	Epoxiconazole	160 ml
16	Primacy	Azoxystrobin	180 ml
17	Vegard	Highly bioactive plant-derived (*Rheum officinale* Baill)	100 ml/ 50-60 LOW
18	Picoxystrobin	Strobin	240-352 ml/A

Determination of antifungal activity: Fungal strain was maintained on MEA. The growth inhibition tests by were carried out on broth ME. Aqueous solutions of the fungicides were prepared fresh when required and added to provide the final concentration as required. Each treatment was done in triplicate.Malt extract (ME) (100 mL) broth amended with recommended dose of fungicide (Table 1) was prepared to evaluate their

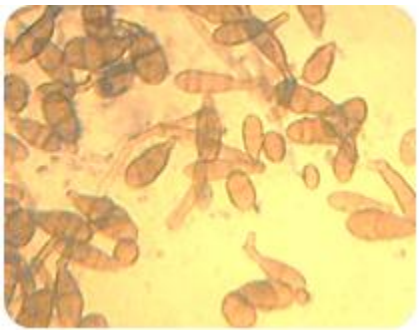
fungicidal activity against test pathogen. Control contained the same quantity of distilled water. After autoclaving, actively growing mycelium discs of test fungus were transferred to media flasks aseptically. Flasks were incubated at 25 °C and 200 rpm for 5 days. Mycelium was filtered on a pre-weighted filter paper and oven dried at 60 ºC for 24 hours to get the dry.

Data Analysis: Data obtained from different treatments were compared through mean values of three replicates. All means were tested for a significant difference utilizing Duncan's Multiple Range Test (Steel *et al*., 1997).

RESULTS & DISCUSSION

Isolation and identification of pathogenic fungus: Alternaria black spot symptoms were apparent in the field on leaves, stem and pod as elongated dark brown to black lesions of 0.1-0.5 cm in length with an irregular margin. Recovery of pathogen was about 95%. *Alternaria* sp. was identified based on following characteristics.

Colony grows rapidly on MEA at 25°C, reaching 4-5 cm diameter in seven days; radiate, floccose, no concentric growth ring observed. Colony color black without any pigmentation at the reverse of culture; exudates and odor not present. Under stereoscope, primary conidiophores arise directly from the agar surface sporulation abundant; *conidia* produced on long hyphae in short chains of 5-10. Conidiophores small, up to 50-60 x 2-3 µm; *conidia* shorter than *Alternaria alternata* ovoid, some are spherical, beak not very conspicuous. Mature conidia mostly with vertical septation, only 1-2longitudinal septa. Conidia brown or cinnamon in color when young and slightly darker upon maturity, smooth walled. Conidial size ranged from 10-30 x 3-4 µm.



Conidia of pathogen under lower magnification power

Conidia of pathogen under lower magnification power

Figure 1. Microphotographs of conidia of *Alternaria* sp.

Antifungal assays for screening of fungicides: In many previous findings fungicides were reported as an option to control *Alternaria* leaf spot diseases (Antonijevic *et al*., 2007; Bulajic *et al*., 2007; Mesta *et al*., 2011; Arain *et al*., 2012). During the present study, antifungal activity of commercially available 18 different fungicides against pathogen of canola leaf spot (*Alternaria* sp) was evaluated. Fresh and dry weight results showed similar pattern of growth inhibition (Figure 2 and 3). Results showed that all fungicides were effective in reducing fresh and dry biomass of fungus to variable extend. However maximum growth reduction was observed when the fungal cells were grown in the medium amended with Wisdom, Benedict and Tebucanazole. All these three fungicides inhibited more than 90% of fungal growth. Wisdom has active ingredient fosetyl-aluminium. It has been reported by Pérez Moreno and Chávez Hernández (1992) that fosetyl-aluminium is highly effective to control purple spot in onion by *Alternaria porri* while Tebucanazole was found to be very effective in controlling *Fusarium oxysporum* (Taskeen *et al.,* 2011). Flare 83 %, Flumax 81 %, and Bloom caused 76 % reduction in fungal dry biomass. 67% by Epic, 62 % by Defeater plus, 58% by Primacy and 55% by Picoxystrobin 55% was recorded. Remaining fungicides caused 11-50% reduction in fungal biomass. The combination of chemically active ingredients of fungicides was found to be effective in controlling the disease. For example Emisan-6 with Indofil M-45 of Indofil Z-78 in controlling Alternaria blight of potato (Singh *et al*., 1997). Mancozeb followed by Captafol were reported as potential fungicides against *A. solani* infecting tomato plants (Babu *et al*., 2000; Parsad and Neik, 2003). Similar to our study, Kumar *et al*. (2006) tested 17 fungicides *in vitro* against *A. brassicae*, cause of *Alternaria* blight of radish and recommended Iprodione, Mancozeb, Achook, Riodmil-MZ, Ziram and Captanto control the pathogen.

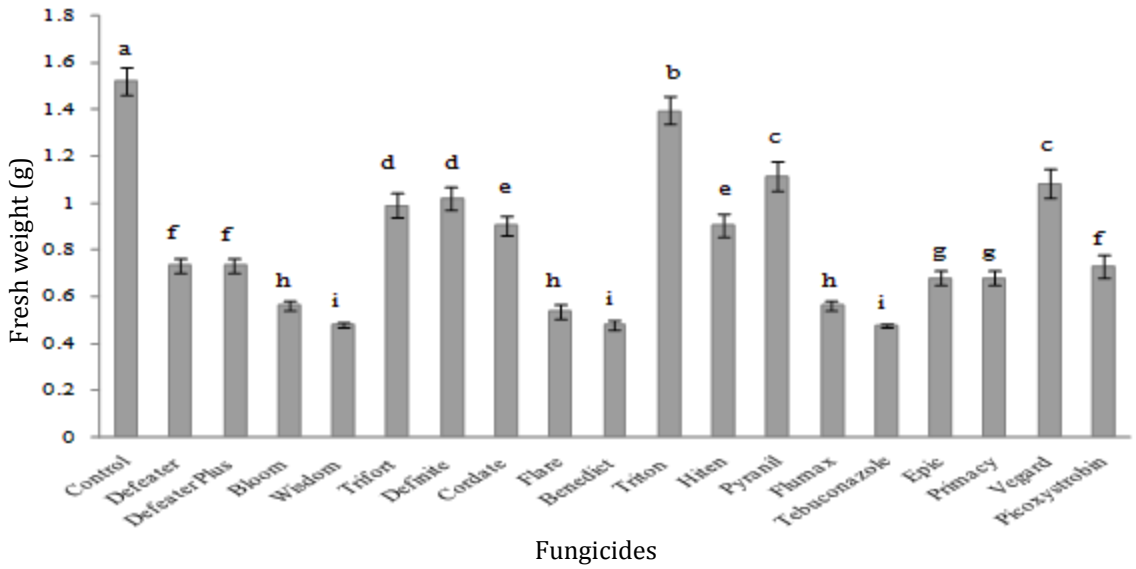


Figure 2. Comparative effect of fungicides on fresh biomass production of fungal pathogen.

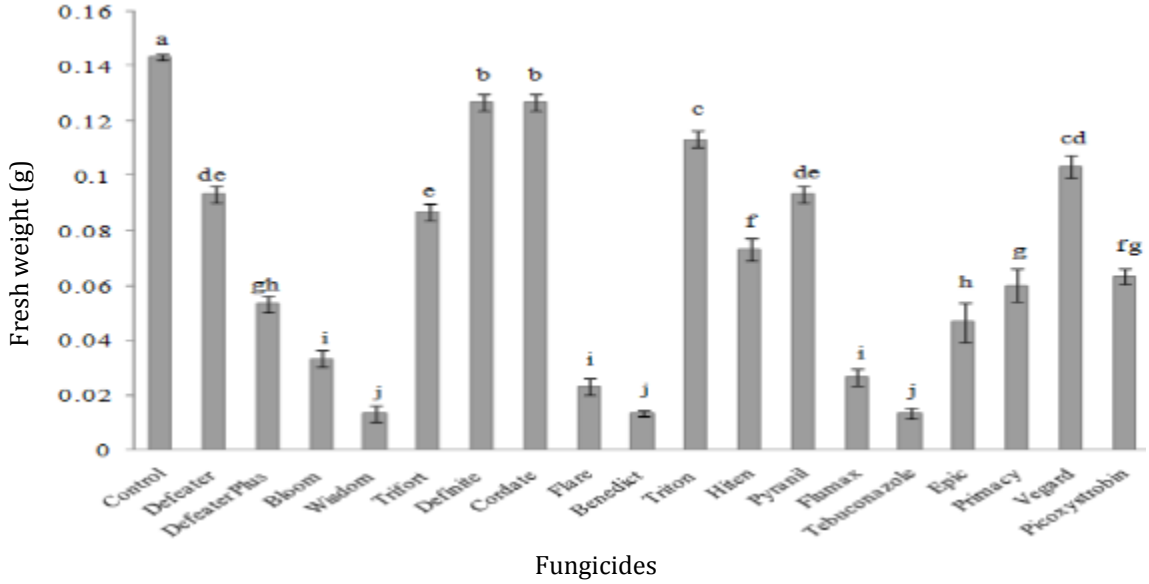


Figure 3. Comparative effect of fungicides on dry biomass production of fungal pathogen.

Table 2. Inhibition of fungal growth by fungicides.

Fungicide	% inhibition of fungal biomass		Fungicide	% inhibition of fungal biomass	
	Fresh	Dry		Fresh	Dry
Defeater	51.75	34.88	Triton	8.33	20.93
Defeater Plus	51.75	62.79	Hiten	40.57	48.84
Bloom	62.94	76.74	Pyranil	26.75	34.88
Wisdom	68.42	90.70	Flumax	62.94	81.40
Trifort	34.87	39.53	Tebuconazole	68.64	90.70
Definite	32.89	11.63	Epic	55.26	67.44
Cordate	40.57	11.63	Primacy	55.26	58.14
Flare	64.69	83.72	Vegard	28.73	27.91
Benedict	68.42	90.70	Picoxystrobin	51.97	55.81

These percentage inhibitions are based on the data presented in the form of Figure 2 and 3.

Meena et al. (2011) during the survey of fungicides found calcium sulphate, borax and sulphur can cause significantly reduced the Alternaria disease severity of mustard crop. Figure 2 and 3 show fresh and dry biomass production of *Alternaria* sp. on ME broth amended with different fungicides. Fungus was grown at 150 rpm and 25°C±3 for 7 days. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined Duncan's Multiple Range Test.

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