



Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology

ISSN: 1019-763X (Print), 2305-0284 (Online)

<http://www.pakps.com>



OCCURRENCE OF ALTERNARIA LEAF SPOT DISEASE ON *ALOE VERA* AND ITS MANAGEMENT

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ABSTRACT

Aloe vera is a valuable medicinal crop of the world. It suffers from several diseases but among them alternaria leaf spot and dry rot is most serious fungal disease which effects the commercial production and quality losses to *Aloe vera*. A survey was conducted to estimate the prevalence of dry rot disease in different nurseries located in urban areas of Faisalabad. During this survey maximum disease incidence was recorded in Horti club nursery (100%) and minimum disease incidence recorded at Faiz baho nursery and Qadir baksh form (13.8%). Among *in-vitro* tested fungicides Score gives maximum mycelial growth inhibition (89.5%) and maximum disease control (17.7%) as compared to control and other tested chemicals. Among *in-vitro* tested plant extracts Neem gives maximum mycelial growth inhibition (41.2%) and in green house gives 14.2% disease control. Out of four tested fungicides and plant extracts Score and Neem gives best results against *A. alternata* mycelial growth and disease control.

Keywords: *Aloe vera*, Fungicides, Plant extracts, *A. alternata*

INTRODUCTION

Aloe vera (syn. *Aloe barbadensis* Miller) belongs to family Xanthorrhoeaceae (alternatively engaged in Aloaceae and Asphodelaceae). *Aloe vera* is playing vital role in cosmetic and pharmaceutical approaches and most applicable homeopathic plant worldwide (Gantait *et al.*, 2014). This plant is believed to be originated in African continent specifically in Egypt (Ayodele and Ilondu, 2008). *Aloe vera* is a drought resistant plant of warm and dry environment and is extensively distributed in Africa, Asia and tropical areas of the world.

Aloe vera is prone to different diseases all over the world. Leaf spot disease caused by *Alternaria alternata* was notice firstly from India and Pakistan. This

pharmaceutically important plant of India has facing heavy damages due to a leaf spot disease at harvested stages during 2006. The symptoms noted were small, round to oval dark brownish necrotic sunken spots placed mostly on the leaf end tip, with normal diameter of 1-3 mm. The fungal pathogen was isolated and identified as *A. alternata* and the pathogenicity was proven. The pathogen conidiophores were straight, branched, golden browning in colour, measuring 15 mm long and 2-6 mm width. The conidia were gold brownish in colour, formed in lengthy branched chains, obclavate in form, with small pointed flask. These finding of Kamalakannan *et al.*, 2008 considered as the principal report of an alternaria leaf spot disease of aloe vera in India. From North India, Abkhoo and Sabbagh, 2013, reported that *A. alternata* leaf spot problem is the most severe fungal disease disturbing the viable cultivation of *Aloe* plant. Meanwhile from Pakistan Bajwa *et al.*, 2010, was firstly reported this disease in *Aloe vera* fields of University of the Punjab Lahore. They also describe that during the months of December this diseased rapidly

Submitted: April 04, 2019

Revised: June 03, 2019

Accepted for Publication: June 22, 2019

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spread to healthy plants with showing sunken, oval shaped necrotic lesions having grey colour. These qualitative and quantitative losses can be manage by using different chemicals as their effects are so quick but not acceptable environmentally. So it is dare need to estimate the disease incidence on different locations of urban areas of Faisalabad, isolation and verify the pathogen and to check its pathogenicity on healthy plants in glasshouse. Integrated approach for disease management by using both chemical and plant extracts

could be a safe alternatives which already proven by Neeraj and Verma, 2010 and Gurjar *et al.*, 2012.

MATERIALS AND METHODS

Survey for Disease Incidence and Samples

Collection: A survey was conducted at different local nurseries of urban areas of Faisalabad to assess the disease incidence of *Alternaria* leaf spot of *Aloe vera*. Different observations were noted in each nursery to determine the incidence of black leaf spot disease by following this formula.

$$\text{Disease incidence (\%)} = \frac{\text{Infected plant in a nursery}}{\text{Total No. of Plants}} \times 100$$

From further pathological processing three diseased leaves were collected randomly from each nursery. These infected samples placed into separately

labeled plastic bags and were brought to the department laboratory for isolation and identification of pathogen.

Disease rating scale:

Grade	% Disease Incidence	Level of Response	Disease Severity
0	0	Immune	Leaves free from infection
1	1-10	Resistant	Spots covering <5% Leaf area
2	10.1-25	Moderately Resistant	Spots covering 5.1-10% leaf area
3	25.1-50	Moderately Susceptible	Rings covering 10.1-25% leaf area
4	50.1-75	Susceptible	<i>Alternaria</i> symptoms covering 25.1 -50% leaf area
5	75 and above	Highly susceptible	<i>Alternaria</i> symptom covering > 50% leaf area

(Sangeetah and Siddaramaiah, 2007)

Evaluation of Plant Extract: Plant extracts were prepared by grinding them with manual plant extraction machine procedure. The solutions of plant extracts were prepared at 5, 10, 20 and 40 % concentration level. Then apply these plants extracts by poisoned food technique *in vitro* condition to check their response on % inhabitation in mycelial growth of *A. alternata* and in green house by spraying method to check disease control.

Evaluation of Fungicides: Fungicides inhibitory effect was tested against *A. alternata* in laboratory by poisoned food technique and in green house by spraying method to check % inhibition in mycelial growth and disease control. Four fungicides Copper oxychloride, Score, Aerosal, and Carbendazim were used for confirmation of efficacy against *A. alternata* under *in vitro* and in green house conditions on artificially inoculated plants. These apparently looking healthy aloe vera plants, of two months age were obtained from a local nursery. All selected fungicides were tested at 50, 100, 200 and 500 ppm concentration in lab. and 500ppm in green house. In vitro data of % inhibition in mycelial growth noted after 7 days and green house data of disease control was recorded after 15 days.

RESULTS

The comprehensive survey on occurrence and incidence of *Alternaria* leaf spot disease was recorded (Figure 1). The disease incidence percentage ranged between 13.88 to 100 % at different nurseries locations. The maximum incidence was (100%) recorded at Horti club nursery and minimum (13.88%) recorded at Faiz Baho nursery and Qadir Baksh form.

Morphological characters of the fungus *A. alternata*:

Morphological identifications of the fungus were noted by using slide culture technique. Fungus developed profuse growth of mycelium on PDA. Firstly mycelium was hyaline that changed to grey-brownish, septate, multi-celled, and branches are irregular. In initial developmental stage, hyphae were thin, narrow, and hyaline but became slightly thick 4.42 μm in diameter as they developed old. Conidiophores raised separately or in groups, usually 2-6 μm and were long or small. Conidiophores were pale olivaceous-brown, straight or curved, geniculate, slightly swollen at apex having lethal scars indicating the point of attachment of conidia. The conidiophores measured 42.26 μm in length and 4.29 μm in thickness. The length: width ratio was 9.85. Conidia were produced in chains up to 10 or more, light

olivaceous to dark brown in colour, different in shape from obclavate to mostly ellipsoidal, muriform having pointed apex. The length of the conidium was 3-5 times more than its width. The chlamydo spores were

produced in the old culture of *A. alternata*. They were intercalary, dense walled, circular to ovoid in shape, dark brown in color and calculating 7.2 μm (4.9 - 9.8 μm) in diameter (Nagrle *et al.*, 2013) (Figure 2).

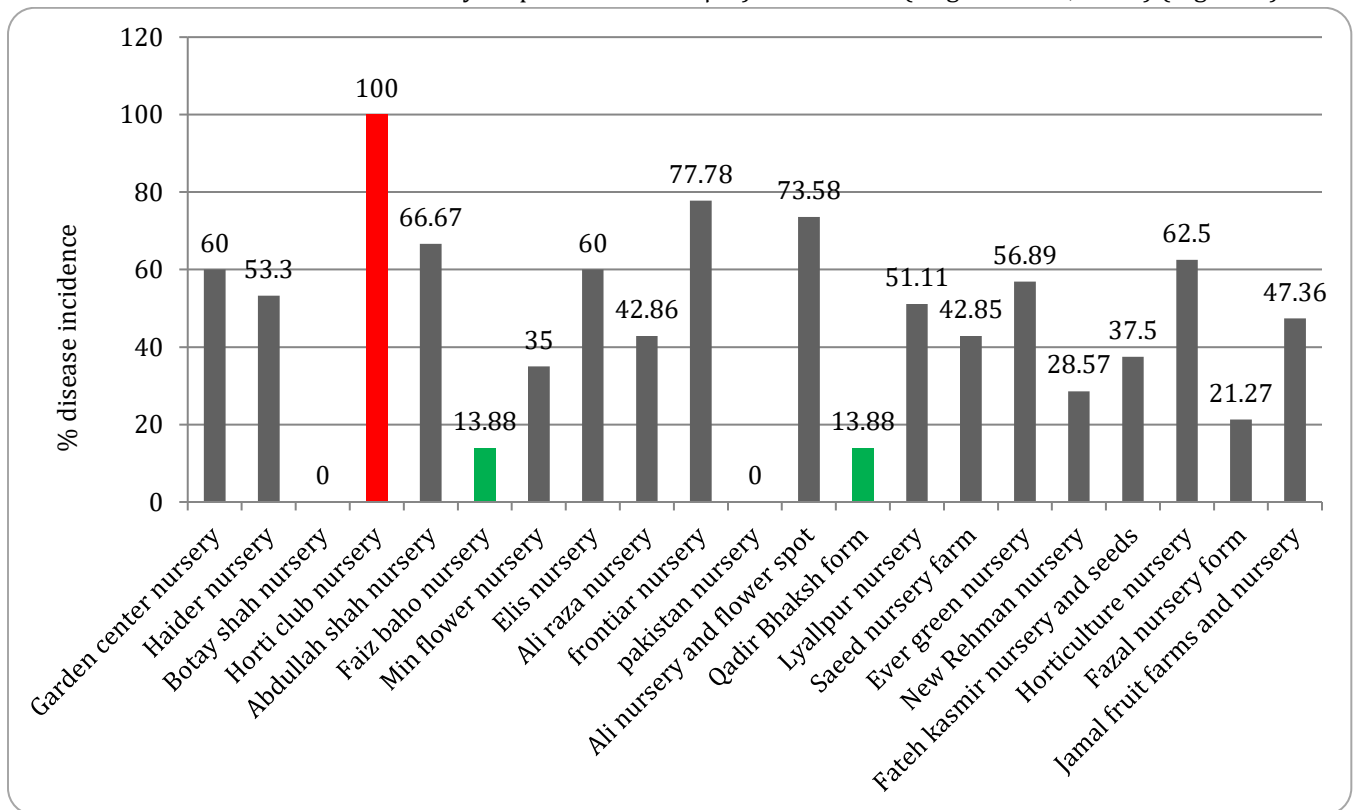


Figure 1. Graphical representation of Nurseries survey to check disease incidence at Faisalabad

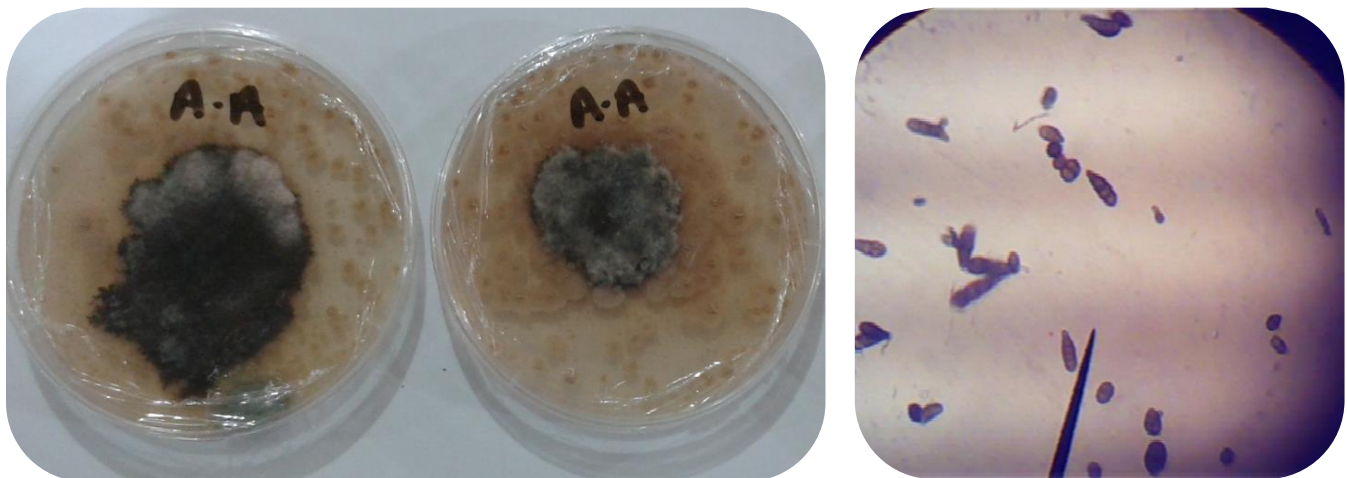


Figure 2. Identification of pathogen through colony of pure culture and structure of spores

In-Vitro Screening of Plant Extracts against *A. Alternata*: Four plants extract (*Syzygium cumini*, *Azadirachata indica*, *Datura strumarium* and *Eucalyptus comaldulances*) were evaluated against *A. alternata* growth. According to result represented in (Table 1) fungal mycelial growth inhibition ranged from 41.2% to 24.2%.

The highest mycelial growth inhibition (41.2%) was recorded by *Azadirachata indica*, followed by *Datura strumarium* (38.4%), *Syzygium cumini* (37.2%) and *Eucalyptus comaldulances* (34.2%). The smallest growth inhibition (27.2%) was recorded by *Azadirachata indica* followed by *Eucalyptus comaldulances* (24.2%).

Table 1. Data of Mean comparison of plant extracts against *In vitro* mycelial growth inhibition/control (mm) of *A. alternata* (After 7 days at 25 ± 2 °C)

Concentrations	Datura	Neem	Euclayptus	Jaman	Mean
5%	26.45j	27.25i	24.25l	25.72k	25.917D
10%	32.2g	34.53de	26.3j	30.25h	30.82C
20%	34.68d	38.2b	30.25h	33.63f	34.19B
40%	38.43b	41.25a	34.2e	37.27c	37.787A
Mean LSD = 0.19	32.94B	35.308A	28.75D	31.717C	LSD = 0.38

Means values sharing similar letters do not differ significantly from each other

Application of plant extracts in Green house condition: Four plants were selected viz., Jaman (*Syzygium cumini*), Neem (*Azadirachata indica*), Datura (*Datura strumarium*), Safeda (*Eucalyptus comaldulances*) for evaluation against alternaria leaf spot disease of *Aloe vera* plant. From the results data

(Table 2) it is evident that after fifteen days of spray, all the treatments significantly differ from untreated control. Highest per cent disease index of leaf spot disease in experiment of plant extracts was observed in control (53.29%) followed by *Eucalyptus comaldulances* (47.35%).

Table 2. Mean comparison of plant extracts against leaf spot disease greenhouse trials

Plant Extract	BS(PDI)	AS(PDI)	AS(PDC)
Control	51.223	53.29	0d
Datura	50.75	46.81	12.277b
Euclayptus	50.06	47.35	11.143c
Jaman	48.703	46.783	12.21b
Neem	50.617	45.697	14.247a
LSD		0.554	

Means values sharing similar letters do not differ significantly

BS(PDI): Percent Disease Incidence Before Spray

AS(PDI): Percent Disease Incidence After Spray

AS(PDC): Percent Disease Control After Spray

In vitro screening of fungicides against A. alternata: Four fungicides (Score, Copper oxychloride, Aerosal and Carbendazium) were evaluated against *A. alternata* mycelial growth. According to result represented in (Table.3), fungal

mycelial growth inhibition ranged from 89.5% to 41.4%. The highest mycelial growth inhibition (89.5%) was recorded by Score, followed by Copper oxychloride (84.6%), Aerosal (79.3%) and Carbendazium (74.3%).

Table 3. Mean comparison of all Fungicides (*In vitro*) against mycelial growth inhibition/control (mm) of *A. alternata* (After 7 days at 25 ± 2 °c)

Conc.	Score	Carbendazium	Score	Copper oxychloride	Mean LSD = 0.1651
50ppm	43.48l	41.45m	49.42j	49.21j	45.89D
100ppm	49.75i	46.33k	60.25g	57.73h	53.52C
200ppm	64.68f	60.23g	74.58d	69.65e	67.29B
500ppm	79.3c	74.33d	89.5a	84.68b	81.95A
Mean	59.30C	55.59D	68.44A	65.32B	LSD = 0.3301
LSD = 0.165					

Means values sharing similar letters do not differ significantly

Application of fungicides in Greenhouse condition: Fungicides, viz. Score, Copper oxychloride, Aerosal and Carbendazium were evaluated against alternaria leaf spot and dry rot diseases. From the results (Table 4) it is evident

that at fifteen days after spray, all the treatments significantly differ from untreated check. Highest per cent disease index of leaf spot in experiment of fungicides was observed in control (51.5%) followed by Carbendazim (46.5%).

Table 4. Mean comparison of Fungicides against leaf spot disease in Green house

Fungicides	BS(PDI)	AS(PDI)	AS(PDC)
Carbendazium	50.137	46.56	9.606d
Copper oxychloride	47.137	43.67	15.21b
Score	48.37	42.41	17.07a
Aerosal	48.733	45.173	12.27c
Control	50.157	51.51	0e
LSD		0.5728	

Means values sharing similar letters do not significantly differ from each other

DISCUSSION

Survey of nurseries shows that maximum disease incidence recorded in Horti club nursery (100%) while minimum disease incidence recorded at Faiz baho nursery and Qadir baksh form (13.88%). Similar observations also recorded by Bajwa *et al.*, 2010, gives the evidence of *Alternaria alternata* pathogen causing the leaf spot disease of aloe vera plant in Pakistan.

In vitro evaluation of fungicides by poison food technique shows that Score shows maximum mycelial inhibition against *A. Alternata* at 500ppm. Kamble *et al.*, 2000 also conducted the same experiment and stated that Copper oxychloride and Score has been found effective against *A. alternata* *in vitro*. Singh *et al.*, 2003 and Taskeen-Un-Nisa, *et al.*, 2011 also performed the same experiment and proved that all tested fungicides significantly inhibit the mycelial growth of fungus *A. alternata*. Green house evaluation of fungicide by spraying method shows that Score provides maximum disease control (17.71%). Current studies were also approved with the results found by Timmer and Zitko 1997. Related experiment were conducted and proved by Brazauskiene and Petraitiene 2003.

In vitro evaluation of plant extracts shows that neem shows maximum (41.25%) mycelial inhibition against *A. Alternata*. Similar results on the efficacy of plant extracts against *Alternaria spp.* have been reported by Bajwa *et al.*, 2005, Shinde *et al.*, 2011, Gupta and Bhadauria, 2012, Gurjar *et al.*, 2012, Gupta *et al.*, 2014 and Regmi *et al.*, 2014. Green house evaluation of plant extracts shows that neem gives maximum disease control (14.25%) over control. Current studies were also in line with the results of Dellavalle *et al.*, 2011 and Anamika and Sobita Simon, 2011 and Gupta, *et al.*, 2014.

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