Open Access

Phytopathogenomics and Disease Control*., *2024, 3(2)233-239
ISSN (Online):2957-5842; ISSN (Print):2957-5834
DOI: https://doi.org/10.22194/Pdc/3.1042
https://societyfia.org/journal/PDC

Society for Innovative Agriculture

Integrated Management of Leaf Spot of Aloe Vera Caused by *Alternaria alternata* through Chemicals and Plant Extracts

Nimra Arshad[1], Nasir Ahmed Rajput[1,*], Muhammad Atiq[1], Abdul Rehman[1], Akhtar Hameed[2], Ghalib Ayaz Kachelo[1,3], Ayesha Batool[1] and Nasir Ahmed Khan[1,*]

[1]Department of Plant Pathology, University of Agriculture, Faisalabad[3]Institute of Plant Protection, Muhammad Nawaz Sharif University of Agriculture, Multan, Pakistan
[3]Crop Diseases Research Institute, PARC-SARC, Karachi, Pakistan
[*]Corresponding author's e-mail: nasirrajput81@gmail.com; nasir.ahmed@uaf.edu.pk

Aloe Vera (*Aloe Barbadenisis* Miller) plant used for centuries for its medicinal, beauty, health and skin care properties. It is a xerophytic, succulent, pea-green colored plant which contains vitamins, minerals, sugar, enzymes, lignin, salicylic acid, saponins and amino acids. Leaf spot of Aloe vera caused by *Alternaria alternata*, is the most significant fungal infection affecting commercial Aloe vera farming. Current study was designed to investigate the effects of chemicals and botanicals against leaf spot disease of Aloe vera. For this purpose, infected plants of Aloe vera were gathered Horticultural Research Area, UAF. The fungus was isolated, purified and identified at Phytopathology Laboratory. Five fungicides and plant extracts were used with three different concentrations under completely randomized design (CRD). The outcomes of current study indicated that, among tested plant extracts, Clove inhibited maximum mycelial growth (6.53 mm) followed by Garlic (9.52 mm), Turmeric (10.42 mm), Ginger (11.52 mm) and Black pepper (13.42 mm), whereas among fungicides Tilt showed significant inhibitory effects against pathogen with minimum mycelial growth (6.60 mm), while Champion was found least effective among fungicides having maximum fungal growth (13.71 mm). Moreover, under greenhouse conditions most effective phyto-extract (Clove), chemical (Tilt) and their amalgamation were applied on inoculated aloe vera plants and found that the combination expressed most effective results against disease with least disease severity percentage (9.92%), followed by solo application of Tilt (13.82%) and clove (23.82%). It was suggested that Tilt as solo application or in combination with clove extract could be used as effective remedy to control leaf spot disease of aloe vera, however for home gardens extract of clove is highly recommended due to non-toxic effects on human health.

Keywords: Clove, Turmeric, Fungicides, Phytoextracts, tilt, Fungal growth, Aloe vera, leaf spot disease, alternaria alternata, Plant extracts.

INTRODUCTION

Aloe vera (*Aloe Barbadenisis* Miller) belongs to family *Xanthorrhoeaceae* (Hekmatpou *et al*., 2019). It is a short stem, shrubby, pea green succulent plant that bears flowers in summer. Its leaves contains 18 (amino acids), 12 (vitamins), 20 (minerals), water, and 75 nutrients in leaves of Aloe vera (Magluria *et al*., 2019). It also contains sugars, salicylic acids having anti-fungal, anti-bacterial, anti-inflammatory, immune-enhancing and anti-cancerous properties. Due to its high nutritious value, healing and softening effects Aloe vera plays a vital role in pharmaceutical and cosmetic industry (Sultana *et al*., 2021). It is a wonderful most potent and well-known medicinal plant. Its broad use in cosmetics, particularly to cure burns and sunburn, wound healing, and cell ageing, has gained a strong global reputation. Aloe vera plants are also beneficial for the immune system and blood circulation. It is utilized in ayurvedic, homoeopathic, and allopathic treatment, not only in tribal communities but also throughout the world (Jadhav *et al*., 2020).

Among all the fungal diseases, leaf spot of Aloe vera caused by *Alternaria alternata* is one of the most significant fungal disease affecting commercial Aloe vera farming. When pathogen attack on plant initially spots remain small, oval in shape and pale brown to black in color. As disease progress, spots increase in size, in a concentric manner, leading to dead

Arshad, N., N.A. Rajput, M. Atiq, A. Rehman, A. Hameed, G.A. Kachelo, A. Batool and N.A. Khan. 2024. Integrated management of leaf spot of aloe vera caused by *alternaria alternata* through chemicals and plant extracts. Phytopathogenomics and Disease Control 3:233-239.
[Received 30 Apr 2024; Accepted 25 May 2024; Published 18 Jun 2024]

appearance. The disease starts from the lower leaves of plant and slowly transfer to the upper leaves (Blagojević *et al.*, 2020). *A. alternata* is an air borne fungus and has long survival ability in plant debris (Tozlu *et al.*, 2018). The pathogen can also survive the winter on tree, surface, and soil layers of disabled bodies that were 5, 10, 15, 20, and 30 cm thick, and the soil layer of 10-15 cm had the highest spore survival rates (Jin-yu *et al.*, 2018). In *A. alternata*, sexual spore conidia are produced in conidiophores that are almost 160-200µm in length. Spore dispersion occur through water, animals, tools and wind. After harvesting, plant debris remains in the field which act as primary source of infection (Mamgain *et al.*, 2013). There are different environmental factors that favor the growth of *A. alternata* like temperature, relative humidity, moisture and rainfall. In field conditions, favorable temperature for the pathogen is 25-30°C. If pH range is 6-6.50 and relative humidity is 90-100% then disease rate will be maximum (Gawai and Mangnalikar, 2018). Species of Alternaria behave like saprotroph, opportunistic pathogens which cause diseases in human and plants (Armitage *et al.*, 2020). Pathogenic capability of *A. alternata* depends on the production of host-selective toxins. This fungus also uses cell-wall-degrading enzymes to penetrate in host plant (Ma *et al.*, 2019).

A number of management strategies have been used to combat the disease. Natural disease resistance is environment friendly way of controlling plant disease and reduce the use of chemicals. Resistant varieties can prevent the spread of disease. If resistant varieties are not available and disease is in severe form then the use of fungicides is recommended (Nelson *et al.*, 2018). Chemicals are quick in action and they are easily available, they can slow or stop the development of new symptoms. When fungal infections spread quickly in the field, farmers have no choice except to use fungicides (Dukare *et al.*, 2022). Fungicides act in a number of ways, but the majority of them destroy fungal cell membranes or prevent fungal cells from producing energy. Due to this reason chemical fungicide Mancozeb was used against leaf spot of Aloe vera caused by *Alternaria alternata*. It controlled the pathogen growth at a very low concentration of 100µg/ml (Ghosh *et al.*, 2016). Horsfield *et al.* (2010) conducted an *In-vitro* experiment on four different fungicides azoxystrobin (Amistar 250 SC), trifloxystrobin (Zato 50 WG), tebuconazole (Folicur 250 EW) and Signum 334 WG (boscalid+pyraclostrobin) to check the growth of *A. alternata*. Signum (boscalid+pyraclostrobin) and Folicur 250 EW (tebuconazole) showed the most inhibitory effect against *A. alternata.*

But excessive use of chemicals has become threating to human health and are phytotoxic. That's why plants extracts are used as an alternative approach against different plant diseases. Plant extract has been used to implement a new way to controlling diseases that affect quality food production. Plant extracts contain a variety of bioactive components that can inhibit the growth of fungi (Choudhury *et al.*, 2018). Plant disease management through phytoextracts are environment friendly, non-hazardous, and non-polluting. Different studies showed suppressive effects of botanicals against *A. alternata* by using poisoned food technique. Sharma *et al.* (2021) found significant inhibitory effect of *Calotropis gigantea, Alstonia scholaris, Zingiber officinale, Allium sativum, Azadirachta indica, Datura stramonium* against *A. alternata*. Similarly, turmeric and neem extracts were found highly effective against the pathogen when used at 50% concentration (Nagaraju *et al.*, 2020). Keeping in view all the findings from different studies, contemporary study was designed to investigate the potential of synthetic fungicides and phytoextracts against *Alternaria alternata* causing leaf spot of aloe vera.

MATERIALS AND METHODS

***Sample collection, isolation and purification of pathogen*:** For isolation of *Alternaria alternata,* infected leaves of aloe vera were collected and brought to the Mycology Laboratory, Department of Plant Pathology, UAF for further study and diagnosis. Diseased samples rinsed with tap water for making samples dirt free. For isolation samples cut into small pieces by taking half diseased and half healthy portion. Using 1% sodium hypochlorite, small sample pieces were surface sterilized for 30 seconds and then rinsed two times in distilled water, after that samples were placed on sterilized blotter paper for drying purpose. PDA media was poured into Petri plates in laminar air flow chamber. After solidification of PDA, samples were transfer to petri plates with the help of sterilized forceps. Petri plates were wrapped, labeled and incubated at 25±2°C temperature for 48 hours in the incubator (Senanayake *et al.*, 2020). Single hyphal tip method was used for the purification of fungus. Small amount of mycelium was taken from the edge of cultural plate with the help of sterilized inoculating needle in laminar air flow chamber and transferred to the fresh PDA. The pure culture of isolated pathogen was studied under compound microscope for morphological characterization like colony shape, color, spore size and shape of the pathogen (Hassan *et al.*, 2022).

***Pathogenicity test*:** For the confirmation of pathogen association with host plant, Koch's postulates were followed. For this purpose, healthy aloe Vera plants were grown in earthen pots. One-month old plants were firstly surface sterilized with 1% sodium hypochlorite, then artificially inoculated by placing a 5 mm disc of one-week old pure culture of *A. alternata*. Disease symptoms were observed regularly for one week. From diseased leaves, re-isolation of the pathogen was performed and compared with the old one on the basis of morphological characters.

***In-vitro management of A. alternata through chemicals*:** Five different chemicals (Tilt, Cabrio top, Amistar top, Champion, Nativo) were evaluated against *A. alternata* at

three different concentrations (50ppm, 100ppm, 150ppm) with three replications of each treatment by using poisoned food technique. Stock solution of fungicides was prepared by following their active ingredients to make different concentrations. Required concentration of stock solution added in semi cooled PDA media in conical flasks. Then the amended media was transferred into Petri plates (90 mm) and every plate were inoculated with a 6 mm disc of mycelium obtained from one-week old pure culture of *A. alternata* using a Cork borer. While un-amended media was used for control plates. All the plates were swathed with parafilm and labelled with a undying marker. Data was recorded with 24 hours of intervals till one week (Akram *et al.*, 2018).

Table 1. Chemicals, their active substances and mechanism of action against *A. alternata*

Fungicides	Active Ingredients	Mode of Action	References
Cabrio Top	Pyraclostrobin	Blocks the energy supply of the fungus so it does not spread further in the plant	(Younas *et al.*, 2021)
Tilt	Propiconazole	The known targets of azoles are the demethylase enzymes involved in ergosterol biosynthesis	(Somani *et al.*, 2019)
Amistar Top	Azoxystrobin + difenoconazole	Reduces mycelial growth, inhibits mitochondrial respiration, prevents spore germination, and is effective against a variety of plant pathogenic fungus	(Mahoney *et al.*, 2015)
Champion	Copper hydroxide	Strong contact effect as well as cellular protein disruption	(Younas *et al.*, 2021)
Nativo	Trifloxystrobin + tebuconazole	Interferes with the fungus's ability to build its cell wall. It prevents fungus from reproducing and expanding further	(Shi *et al.*, 2020)

***In-vitro management of A. alternata through plant extracts*:** Five plant extracts (Ginger, Garlic, Clove, Turmeric, Black pepper) were used against *Alternaria alternata*. Ginger, garlic, clove, turmeric and black pepper were purchased from local market of Faisalabad. All the specimens were air dried and grinded to fine powder by using electric grinder machine. Three different concentrations (5, 10 and 15%) of each plant extract was prepared by adding 5, 10 and 15g of plant powder into 100 ml PDA media. Then, that amended PDA media was transferred into Petri plates (90 mm) and 6mm disc of mycelium from one-week old pure culture of *A. alternata* was taken by using cork borer and placed on the center of the petri plates containing treated PDA media. Then plates were wrapped with parafilm and labelled with permanent marker. Data of mycelial growth was recorded with 24 hours of interval till one week (Subedi *et al.*, 2019).

***Assessment of most effective synthetic chemical and phyto-extract, solely and in amalgamation against leaf spot of Aloe vera under greenhouse environment*:** For *in-vivo* experiment, aloe vera plants were grown in earthen pots (15×17 cm) containing sterilized soil (2 Kg/pot). Artificial inoculation was done by using spore suspension containing 1×10^8 spores/ml of water. After appearance of typical disease symptoms, the most effective treatments under lab conditions i.e. fungicide "Tilt" (Propiconazole) and plant extract "Clove" (*Syzygium aromaticum*) were sprayed by using hand sprayer, individually and with combination. Three concentrations (0.5, 1 and 1.5%) of each treatment was used. Distilled water was used as control treatment. The experiment was designed under completely randomized design (CRD) with three replications of each treatment to minimize the chance of error. Data regarding disease severity percent was recorded for 21 days with 7 days of interval.

RESULTS AND DISCUSSION

***Assessment of plant extracts against Alternaria alternata causing leaf spot of Aloe vera under lab conditions*:** All the treatments expressed significant effect on mycelial growth. Among all the phytoextracts maximum inhibitory effect was

Table 2. Plants, their scientific names, antifungal compounds and mode of action used against *A. alternata*.

Plant extract	Scientific name	Antifungal compound	Mode of action	References
Ginger	*Zingiber officinale*	Gingerone, dihydrogingerone and dehydroshogaol	Exhibit their anti-inflammatory effects in a variety of ways, including possibly by preventing the activation of signaling pathways.	(Saedisomeolia *et al.*, 2019)
Garlic	*Allium sativum*	Allicin	Disturb the activity of enzymes either stop bacterial and fungal growth or destroy cells	(Borlinghaus *et al.*, 2014)
Turmeric	*Curcuma longa*	Curcumin	It inhibits the growth of fungi & bacteria	(Zheng *et al.*, 2020)
Clove	*Syzygium aromaticum*	Phenolic compounds (eugenol, eugenol acetate)	It suppressed the disease index of fungus	(Jing *et al.*, 2017)
Black pepper	*Piper nigrum*	Alkaloids and phenols	It inhibits the enzyme activity	(Takooree *et al.*, 2019)

exhibited by clove with minimum mycelial growth 6.53mm followed by garlic (9.52), turmeric (10.42), ginger (11.53) and black pepper (13.47) mm (Table 3). The interaction between treatment and concentration showed that clove was found most effective at all three concentrations i.e. at 3% (10.18mm), 5% (9.53mm) and 7% (8.46mm) (Table 4). whereas the interaction between treatments and days expressed that black pepper was least effective at all the time intervals with 15.40, 14.60, 13.85 mm growth as compared to other treatments while minimum mycelial growth was found by using clove extract at 3rd day (5.76 mm) (Table 5).

Table 3. *Invitro* assessment of phyto-extracts against *A. alternata* causing leaf spot of Aloe vera.

Phyto-extracts	Mycelial growth (mm)
Cloves	6.53±0.58ad
Garlic	9.53±0.63b
Turmeric	10.42±0.87ab
Ginger	11.52±0.78c
Black pepper	13.42±0.39c
Control	25.53±0.48a
LSD	0.90

The column values sharing same letters do not differ significantly according to LSD test (P≤0.05)

Table 4. *Invitro* impact of interactions between phyto-extracts and concentrations on mycelial growth of *A. alternata*

Phyto-extracts	Mycelial growth		
	3%	5%	7%
Cloves	8.18±0.74c	7.13±0.89c	6.26±0.83b
Garlic	11.65±1.02c	11.23±1.02cd	10.17±0.85ab
Turmeric	12.96±1.30ac	12.37±0.84b	12.01±0.64c
Ginger	14.40±0.83b	13.49±0.49b	12.79±0.89c
Black pepper	15.40±0.94ad	14.60±0.84b	13.85±1.04ac
Control	20.64±0.74b	20.63±0.89c	21.63±1.95b
LSD		0.93	

The column values sharing same letters do not differ significantly according to LSD test (P≤0.05)

Table 5. *Invitro* Impact of interactions between phyto-extracts and days on mycelial growth of *A. alternata*.

Phyto-extracts	Mycelial growth		
	Day 1	Day 2	Day 3
Cloves	5.76±0.75a	6.92±0.72ac	8.62±0.58ac
Garlic	10.68±0.85c	12.28±0.98a	14.54±1.49b
Turmeric	14.53±0.49b	15.65±0.84ac	17.83±0.64ab
Ginger	11.58±0.85ab	13.14±1.03b	14.01±0.64a
Black pepper	17.32±1.05ac	20.25±1.49b	20.81±0.72ac
Control	33.04±1.03bc	38.05±0.85bc	39.01±0.75a
LSD		0.79	

The column values sharing same letters do not differ significantly according to LSD test (P≤0.05)

***Invitro* assessment of chemical fungicides against *Alternaria alternata* causing leaf spot of Aloe vera:** All the treatments exhibited significant mycelial reduction of *A. alternata.* Among all fungicides, minimum mycelial growth of *A. alternata* was showed by Tilt (6.60mm) followed by Amistar top (8.27mm), Cabrio top (10.19mm), Nativo (12.14mm) and Champion (13.71mm) (Table 6). While, the interaction between treatment and concentration expressed that Tilt showed maximum mycelial inhibition at 50ppm (8.12mm), 100ppm (6.42mm) and 150ppm (5.26mm) concentrations as compared to other treatments (Table 7). Impact of interaction between treatments and days indicated that Champion was found least effective with maximum mycelial growth at all three days (12.65, 13.83, 14.65 mm) whereas Tilt was found most effective with least mycelial growth (7.77 mm) at 3rd day of application (Table 8).

Table 6. Mean mycelial growth of *A. alternata* after application of different fungicides under laboratory environment.

Fungicides	Mycelial growth (mm)
Tilt	6.60±1.05ac
Cabrio Top	10.19±0.49b
Nativo	12.14±0.74d
Amistar Top	8.27±0.74b
Champion	13.71±0.85ab
Control	40.00±0.64c
LSD	0.82

The column values sharing same letters do not differ significantly according to LSD test (P≤0.05)

Table 7. *Invitro* impact of interactions of different fungicides and their concentrations on mycelial growth of *A. alternata*.

Fungicides	Mycelial growth		
	50ppm	100ppm	150ppm
Tilt	8.12±1.32c	6.42±0.85c	5.26±0.72ac
Cabrio Top	12.23±0.74b	9.97±0.59b	8.37±1.00b
Nativo	13.97±0.49c	11.79±1.42ac	10.67±0.07bc
Amistar Top	9.37±0.84d	8.50±0.73b	6.94±0.58ac
Champion	15.06±0.73b	14.50±0.75a	11.57±0.98a
Control	40.00±1.32a	40.00±0.58d	40.00±1.03b
LSD		0.79	

The column values sharing same letters do not differ significantly according to LSD test (P≤0.05)

***Assessment of phyto-extract and chemical fungicide, solely and in amalgamtion against leaf spot of Aloe vera under greenhouse environment*:** Findings showed that all the treatments expressed significnat effect on leaf spot of aloe vera under greenhouse conditions. Among all three treatments, Clove+Tilt showed minimum disease severity (9.92%) followed by Tilt (13.84%) and Clove (23.82%) as

compared to control (30%) (Table 9). The impact of treatment and concentration (T×C) expressed that Clove+Tilt showed minimum disease severity at all three concentrations i.e. 0.5%, 1% and 1.5% (11.48, 10.45, 7.83%) respectively followed by Tilt and Clove (Table 10).

Table 8. Invitro impact of interaction between fungicides and days on fungal growth of *A. alternata*.

Fungicides	Mycelial growth		
	Day 1	Day 2	Day 3
Tilt	5.41±1.32c	6.62±1.03b	7.77±1.30ac
Cabrio Top	9.12±1.49b	10.22±1.30d	11.24±0.72ac
Nativo	11.09±0.84ac	12.13±1.02c	13.20±0.85bc
Amistar Top	7.10±0.92b	8.27±0.64a	9.45±0.89a
Champion	12.65±0.74c	13.83±1.49b	14.65±0.83b
Control	40.00±0.64ab	40.00±1.03c	40.00±0.85ab
LSD		0.79	

The column values sharing same letters do not differ significantly according to LSD test (P≤0.05)

Table 9. Mean disease severity (%) after application of most effective phyto-extract and chemical.

Treatment	Disease severity (%)
Tilt+Clove	9.92±0.59b
Tilt	13.82±1.42ac
Clove	23.82±0.73b
Control	30.00±0.43a
LSD	0.90

The column values sharing same letters do not differ significantly according to LSD test (P≤0.05)

Table 10. The impact of treatments and their concentrations on disease severity (%).

Treatment	Disease severity (%)		
	0.5%	1%	1.5%
Tilt+Clove	11.48±0.48b	10.45±0.58ad	7.83±0.72ac
Tilt	16.47±0.52ab	13.49±0.63b	11.52±1.00b
Clove	26.47±0.58ac	23.49±0.87ab	21.52±0.31bc
Control	30.00±1.42ac	30.00±0.78c	30.00±0.58ac
LSD		0.83	

The column values sharing same letters do not differ significantly according to LSD test (P≤0.05)

Table 11. The impact of treatments and time interavls (weeks) on disease severity (%).

Treatment	Disease severity (%)		
	1st week	2nd week	3rd week
Tilt+Clove	8.78±1.42ac	9.91±0.94ad	11.06±0.87b
Tilt	12.81±0.73b	13.85±0.75ab	14.81±0.64c
Clove	22.81±0.75a	23.85±0.29bc	24.81±0.48ac
Control	30.00±0.58d	34.00±0.85d	39.80±1.49b
LSD		0.95	

The column values sharing same letters do not differ significantly according to LSD test (P≤0.05)

The interactions between treatments and days (T×D) showed that maximum disease severity percentage was expressed by the solo application of Clove (22.81, 23.85 and 24.81%) at 7th, 14th and 21st day, whereas least disease severity percentage (8.78, 9.91, 11.06%) was recorded by the application of combination of Clove+Tilt at same days (Table 11).

Aloe vera (*Aloe barbadenisis* Miller) belongs to *Xanthoraceae* family. Aloe vera is grown all over the world because of their nutritional value. The aim of study was to find out the pathogen associated with leaf spot disease of aloe vera and its management through synthetic fungicides and phytoextracts. Whereas management trials through phytoextracts was carried under lab and greenhouse conditions by followed Complete Randomized Design (CRD). Isolated pathogen was recognized as *Alternaria alternata* through morphological characters and comparison with literature.

The findings of contemporary study expressed that among botanical extracts clove showed effective control with minimum mycelial growth (6.53 mm) against *A. alternata* under lab conditions, whereas black pepper was found least effective with mycelial growth 13.42 mm as compared to control. The results of current study are supported by Sharma *et al*. (2021) who conducted the experiment *In-vitro* conditions. Six phyto-extracts (*Calotropis gigantea, Alstonia scholaris, Zingiber officinale, Allium sativum, Azadirachta indica* and *Datura stramonium*) were tested using the poisoned food method against *A. alternata* at 5, 10, and 15% concentration. Garlic extract at a dose of 10% suppressed mycelial growth 100 percent in plants.

Similarly, Shingne *et al*. (2020) evaluated five different plant extracts (garlic, tulsi, eucalyptus, neem and turmeric) against *A. alternata* through poisoned food technique, where findings expressed that turmeric rhizome significantly inhibited mycelial growth (54.92%) followed by neem leaf extract, garlic bulb extract, tulsi leaf extract, and eucalyptus leaf extract. The findings of current study are also supported by the work of Rizwana *et al*. (2016), they conducted an experiment to check the effectiveness of Ginger against *A. alternata*, ginger dry powder was extracted from ethanol and treated in lab conditions at various doses ranging from 0.5 percent to 5.5 percent. The pathogen's mycelial growth was inhibited at all dosages. At the highest concentration of 5.5 percent, mycelial growth was inhibited around 90.4%.

In current study five synthetic fungicides viz Tilt (propiconazole), Amistar top (azoxystrobin and difenoconazole), Champion (coper hydroxide), Nativo (trifloxystrobin+tebuconazole) and Cabrio top (pyraclostrobin) were also evaluated against *A. alternata* using three different concentrations, where findings showed that Tilt and Amistar top exhibited significant inhibition with least mycelial growth (6.60 and 8.27 mm), while Cabrio top,

Nativo and champion expressed minimum effect on fungal growth under *invitro* conditions.

Results of the current study are supported by Horsfield *et al*. (2010), they assessed four different fungicides i.e. Amistar (Azoxystrobin), Zato (Trifloxystrobin), Folicur (Tebuconazole) and Signum (Pyraclostrobin) against *A. alternata*, however, Signum and Folicur suppressed maximum mycelial growth, additionally, Bavaji *et al*. (2012) used eleven fungicides such as Captan, Blitox 50, Chlorothalonil. Dithane Z-78, Thiram, Aureofungin, Monoceren, Ridomil MZ. Tilt and Difoltan at 50, 100, 250 and 500µg/ml concentrations and found most significant results by using Tilt and Blitox-50 at 500µg/ml. Whereas, the mixture of clove extract and tilt fungicide indicated most effective results against leaf spot of Aloe Vera under greenhouse conditions. The same has been discussed by Kachelo *et al*. (2022), where they used combination of tilt fungicide and moringa extract against leaf spot of spinach caused by *A. alternata* and found prominent decrease in disease incidence percentage.

***Conclusion*:** Results of contemporary study showed effective inhibition of *Altrenaria alternata* causing leaf spot of aloe Vera by using botanical extracts and fungicides. Among botanical extracts clove showed noticable results under invitro conditions which recommended that use of phytoextracts could be a better management approach instead of hazardous fungicides. However, in the case of sudden disease outbreak Tilt fungicide can be a suitable option for significant disease control.

***Conflict of interest*:** The authors declare no conflict of interest.

***Authors contribution*:** Nimra Arshad prepared the original draft, Nasir Ahmed Rajput and Muhammad Atiq conceived the idea; Abdul Rehman curated the data, Akhtar Hameed and Ghalib Ayaz Kachelo and Nasir Ahmed Khan reviewed and edited the manuscript and Ayesha Batool helped in conducting experiments.

REFERENCE

Akram, S., S.M. Khan, M.F. Khan, H.U. Khan, A. Tariq, U.U. Umar and A. Gill. 2018. Antifungal activity of different systemic fungicides against *Fusarium oxysporum* f.sp. *lycopersici* associated with tomato wilt and emergence of resistance in the pathogen. Pakistan Journal of Phytopathology 30:169-176.

Armitage, A.D., H.M. Cockerton, S. Sreenivasaprasad, J. Woodhall, C.R. Lane, R.J. Harrison and J.P. Clarkson. 2020. Genomics evolutionary history and diagnostics of the *Alternaria alternata* species group including apple and Asian pear pathotypes. Frontiers in Microbiology 10:3124.

Bavaji, M., M. Khamar and M.M. Nath. 2012. In vitro evaluation of fungicides and plant extracts on the incidence of leaf blight on sesame caused by *Alternaria alternata* (FR) Keissler. Intern. Journal of Food, Agriculture and Veterinary Sciences 2:105-107.

Blagojević, J., J. Vukojević, B. Ivanović and Ž. Ivanović. 2020. Characterization of *Alternaria* species associated with leaf spot disease of *Armoracia rusticana* in Serbia. Plant Disease 104:1378-1389.

Borlinghaus, J., F. Albrecht, M.C. Gruhlke, I.D. Nwachukwu and A.J. Slusarenko. 2014. Allicin: chemistry and biological properties. Molecules 19:12591-12618.

Choudhury, D., P. Dobhal, S. Srivastava, S. Saha and S. Kundu. 2018. Role of botanical plant extracts to control plant pathogens-A review. Indian Journal of Agricultural Research 52:341-346.

Dukare, A.S., R.K. Singh, R.K. Jangra and B. Bhushan. 2022. Non-fungicides-based promising technologies for managing post-production penicillium induced spoilage in horticultural commodities: a comprehensive review. Food Reviews International 38:227-267.

Gawai, D. and S. Mangnalikar. 2018. Effect of Temperature and pH on growth of *Alternaria alternata*, leaf spot pathogen of soyabean. Bioscience Discovery 9:162-165.

Ghosh, R., S. Barman, J. Khatun and N.C. Mandal. 2016. Biological control of *Alternaria alternata* causing leaf spot disease of Aloe vera using two strains of rhizobacteria. Biological Control 97:102-108.

Hassan, R.A., S. Ali, M.S. Zaheer, H.H. Ali, J. Iqbal, A. Habib, M.A. Nadeem and M.Z. Mumtaz. 2022. In-vitro and in-vivo evaluation of different fungicides against leaf blight causing fungus *Alternaria cucumerina* in bitter gourd. Journal of the Saudi Society of Agricultural Sciences 21:208-215.

Hekmatpou, D., F. Mehrabi, K. Rahzani and A. Aminiyan. 2019. The effect of Aloe vera clinical trials on prevention and healing of skin wound: A systematic review. Iranian Journal of Medical Sciences 44:1.

Horsfield, A., T. Wicks, K. Davies, D. Wilson and S. Paton. 2010. Effect of fungicide use strategies on the control of early blight (*Alternaria solani*) and potato yield. Australasian Plant Pathology 39:368-375.

Jadhav, A.S., O.A. Patil, S.V. Kadam and M.A. Bhutkar. 2020. Review on Aloe Vera is used in medicinal plant. Asian Journal of Research in Pharmaceutical Science 10:26-30.

Jin-Yu, L., J. Dilnur and J. Ping. 2018. Study on Overwintering Survival of Mycelia and Conidia of Walnut Leaf Spot Pathogens. Xinjiang Agricultural Sciences 55:1870.

Jing, C., J. Gou, X. Han, Q. Wu and C. Zhang. 2017. In vitro and in vivo activities of eugenol against tobacco black shank caused by *Phytophthora nicotianae*. Pesticide Biochemistry and Physiology 142:148-154.

Kachelo G.A., N.A. Rajput, M. Atiq, S.T. Sahi, N.A. Khan, A. Hameed, N. Muhammad and M.S. Mushtaq. 2022. Antifungal efficacy of plant extracts and chemicals against alternaria leaf spot disease of spinach. Pakistan Journal of Agricultural Research 35:380-387.

Ma, H., B. Zhang, Y. Gai, X. Sun, K.-R. Chung and H. Li. 2019. Cell-wall-degrading enzymes required for virulence in the host selective toxin-producing necrotroph *Alternaria alternata* of citrus. Frontiers in Microbiology 10:2514.

Magluria, A., S. Rawat and S. Mitra. 2019. Phytochemical review of aloe vera with emphasis on its cosmetic applicability. International Journal of Research in Medical Sciences and Technology 8.

Mahoney, K., R. Vyn and C. Gillard. 2015. The effect of pyraclostrobin on soybean plant health, yield, and profitability in Ontario. Canadian Journal of Plant Science 95:285-292.

Mamgain, A., R. Roychowdhury and J. Tah. 2013. Alternaria pathogenicity and its strategic controls. Research Journal of Biology 1:1-9.

Nagaraju, K., J.P. Mishra, R. Prasad, J. Chandra, V.P.R. Sekhar and S. Kumar. 2020. Isolation and in vitro evaluation of different botanicals on mycelia growth of *Alternaria alternata* (Fr.) Keissler causing leaf spot of brinjal. Journal of Pharmacognosy and Phytochemistry 9:889-891.

Nelson, R., T. Wiesner-Hanks, R. Wisser and P. Balint-Kurti. 2018. Navigating complexity to breed disease-resistant crops. Nature Reviews Genetics 19:21-33.

Rizwana, H. 2016. Exploiting antifungal potential of ginger for the management of *Alternaria alternata*, the cause of leaf spot disease of spinach. Mycopath 13.

Saedisomeolia, A., M.M. Arzati, M. Abdolahi, M. Sedighiyan, A. Rangel, G. Muench, M. Zarezadeh, A. Jafarieh and N.M. Honarvar. 2019. Mechanisms of action of ginger in nuclear factor-kappaB signaling pathways in diabetes. Journal of Herbal Medicine 16:100239.

Senanayake, I., A. Rathnayaka, D. Marasinghe, M. Calabon, E. Gentekaki, H. Lee, V. Hurdeal, D. Pem, L. Dissanayake and S. Wijesinghe. 2020. Morphological approaches in studying fungi: Collection, examination, isolation, sporulation and preservation. Mycosphere 11:2678-2754.

Sharma, R.L., R. Ahir, S.L. Yadav, P. Sharma and R. Ghasolia. 2021. Effect of nutrients and plant extracts on Alternaria blight of tomato caused by *Alternaria Alternata*. Journal of Plant Diseases and Protection 128:951-960.

Shi, N., H. Ruan, L. Gan, Y. Dai, X. Yang, Y. Du and F. Chen. 2020. Evaluating the sensitivities and efficacies of fungicides with different modes of action against *Phomopsis asparagi*. Plant Disease 104:448-454.

Shingne, A.W., G. Giri and A.R. Bagade. 2020. In vitro evaluation of fungicides, botanicals and bio-agents against *Alternaria alternata* causing leaf spot disease of niger. International Journal of Chemical Studies 8:3360-3364.

Somani, D., R. Adhav, R. Prashant and N.Y. Kadoo. 2019. Transcriptomics analysis of propiconazole-treated *Cochliobolus sativus* reveals new putative azole targets in the plant pathogen. Functional & Integrative Genomics 19:453-465.

Subedi, S., S. Neupane, B. Surendra and L. Oli. 2019. In-vitro evaluation of botanicals, fungi-toxic chemicals and bio-control agent for efficacy against turcicum leaf blight of maize. Journal of Nepal Agricultural Research Council 5:73-80.

Sultana, T., A.H. Chowdhury, B.K. Saha, A. Rahman, T. Chowdhury and R. Sultana. 2021. Response of Aloe vera to potassium fertilization in relation to leaf biomass yield, its uptake and requirement, critical concentration and use efficiency. Journal of Plant Nutrition 44:2081-2095.

Takooree, H., M.Z. Aumeeruddy, K.R. Rengasamy, K.N. Venugopala, R. Jeewon, G. Zengin and M.F. Mahomoodally. 2019. A systematic review on black pepper (*Piper nigrum* L.): from folk uses to pharmacological applications. Critical Reviews in Food Science and Nutrition 59:S210-S243.

Tozlu, E., N. Tekiner, R. Kotan and S. Örtücü. 2018. Investigation on the biological control of *Alternaria alternata*. Indian Journal of Agricultural Sciences 88:1241-1247.

Younas, M., M. Atiq, N.A. Rajput, W. Abbas, M.R. Bashir, S. Ahmad, M.S. Ullah, W.A. Bhatti, N. Liaqat and I. Ahmad. 2021. Induction of resistance in onion against purple leaf blotch disease through chemicals**.** Asian Journal of Agriculture and Biology 3:

Zheng, D., C. Huang, H. Huang, Y. Zhao, M.R.U. Khan, H. Zhao and L. Huang. 2020. Antibacterial mechanism of curcumin: A review. Chemistry & Biodiversity 17:e2000171.