



Effect of fungal pathogens on morphological properties of *Aloe vera*

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Abstract

Aloe vera (L.) Burm. f. is an important medicinal plant with long time tradition of use by variety of cultures. This plant is suffered with various fungal diseases caused by variety of fungal pathogens in different seasons which affects its morphological characters and diminishes the quality, quantity and production of gel. The present study was aimed to study the alteration in the morphological characteristics of *Aloe vera* artificially infested with *Colletotrichum gloeosporioides* and *Fusarium proliferatum*; the leaf spot disease causing fungi in this plant. Artificially infested leaves were examined in terms of plant height, leaf characteristics, colour and consistency of gel. Results of artificial infestation revealed that both the fungal pathogens have the ability to alter the morphological properties of *A. vera* plants. Therefore, control of the fungal diseases by applying appropriate management strategies is essential to protect this medicinal plant of massive commercial value.

Key words – *Aloe vera* – fungal pathogens – gel texture – leaf spot – leaf length and width – plant height

Introduction

Aloe vera is well known herbal plant with a long and illustrious history. It is considered as an ancient herb with long time tradition of use by a variety of cultures. The popularity of *A. vera* can be predicted with the fact, it is being used across the world for its health, beauty, medicinal and skin care properties. It is an important gel bearing plant belonging to the family Aloeaceae. Though, it is distributed all over the globe; it is native of warm regions, especially North Africa and Mediterranean region of Southern Europe (Morton 1977, Pegu & Sharma 2019). The main portion of this plant is leaf gel which contains amino acids, enzymes, lignin, minerals, mono and polysaccharides, phenolics, salicylic acid, saponins and vitamins with potential biological activities useful in pharmaceutical and cosmetic industries (Boudreau & Beland 2006, Medhin et al. 2019). The consumption of its gel is supposed to improve digestive problems, prevent asthma and diabetes and to enhance immunity (Ravi et al. 2011). It stimulates uterine contraction (Ritchie 2001) and anti-pruritic activities (Das 2011). It also provides antioxidative, antimicrobial and hypoglycemic effects (Yang et al. 2005, Campos et al. 2006, Li et al. 2008). Numbers of products in the form of powders, creams, lotions, soaps, shampoos and facial cleansers are available in the market having *A. vera* gel as base material (Hamman 2008, Haque et al. 2012). Presently, the use of *A. vera* has

gained popularity with the industry size of about \$125 million dollars for aloe raw material. The volume of the industry for finished products containing aloe gel is supposed to be around \$110 billion dollars (Nazir & Ahsan 2017).

Aloe vera, being a medicinal plant possessed antimicrobial activities against number of phytopathogens. Its antimicrobial effects particularly against fungi have been credited to the plant's natural phenolics and anthraquinones present in its gel which have demonstrated *in vitro* inhibition of many plant pathogenic fungi like *Botrytis* sp., *Candida* sp., *Rhizoctonia* sp., *Heterosporium* sp. and *Penicillium* sp. (Sitara et al. 2011, Jain et al. 2017, Gautam et al. 2017). Despite of having antifungal properties, this medicinal plant is suffered with various fungal diseases in different seasons. Four types of diseases namely; leaf spot, leaf rot, collar rot and root rot are found associated with *A. vera* plants in Madhya Pradesh, India (Avasthi et al. 2019a). The fungal diseases are considered to be one the most destructive enemies of *A. vera* plantations as they cause alterations in morphological characters as well as diminishes the quality, quantity and production of gel. The phytopathogenic fungi also alter the production of several secondary metabolites of the host plant (Avasthi et al. 2019b). Among the all fungal pathogens, two important fungi namely *Colletotrichum gloeosporioides* (Avasthi et al. 2011) and *Fusarium proliferatum* (Avasthi et al. 2018a) have been reported to cause leaf spot disease in *A. vera* and to modify the concentration of biochemical constituents significantly (Avasthi et al. 2018a, 2019b). The present study was aimed to study the alteration in the morphological characteristics of *A. vera* artificially infested with *Colletotrichum gloeosporioides* and *Fusarium proliferatum*. In addition, changes in biochemical components due to fungal infestation (*C. gloeosporioides* and *F. proliferatum*) have also been discussed in context to compare with alteration in the morphological characteristics of *A. vera* plant on the whole.

Materials & Methods

Plant Material

Healthy plants of *A. vera* were collected in sterilized paper bags along with soil around the roots. Soil from the roots of collected plants was removed and the plants were sterilized with distilled water. After that these plants were grown in sterilized earthen pots filled with sterilized soil and compost (Farm Yard Manure FYM) and maintained under greenhouse conditions until they were reached at 9 months old. The soil and farm yard manure (FYM) used in earthen pots was adjusted in (soil: FYM) 3:1 ratio.

Experimental Fungi isolation and inoculation

Two experimental fungi namely; *Colletotrichum gloeosporioides* and *Fusarium proliferatum* were isolated from the diseased leaves of *A. vera* collected during the survey of various nurseries and botanical gardens of Gwalior city, Madhya Pradesh, India. For isolation, collected leaves were surface sterilized with 2% sodium hypochlorite solution, washed with distilled water thrice, plated in potato dextrose agar (PDA) medium and incubated at 27°C for 5–7 days. Actively growing fungal hyphae were transferred to freshly prepared culture medium, purified by single spore technique and maintained on PDA. Isolated fungi were identified based on microscopic characteristics and confirmed by Indian Type Culture Collection (ITCC), Indian Agricultural Research Institute, New Delhi (*C. gloeosporioides* #ITCC accession no. 7800.10) and Fungal Culture Collection of India (NFCCI), Pune, Maharashtra (*F. proliferatum* # NFCCI Accession No. 3640).

Nine months old plants of *A. vera* were selected for artificial inoculation under glass house conditions. Total nine plants for each infestation with fungal pathogens (*C. gloeosporioides* and *F. proliferatum*) and control were maintained with single plant in each earthen pot. Six healthy leaves of each experimental plant were surface sterilized with 1% sodium hypochlorite (NaOCl) solution. The surface sterilized healthy leaves were pricked with sterilized needles and after that sprayed with conidial suspension of *C. gloeosporioides* and *F. proliferatum* (1×10^7 ml each). Leaves

sprayed with sterile distilled water were taken as control. To maintain high relative humidity (RH) required to promote growth of inoculated fungi, all the inoculated plants along with their healthy controls were covered with moisten polythene bags for 24 to 48 h. Relative humidity was maintained between 83–92% with a minimum and maximum temperature of 22°C and 28°C in greenhouse conditions to facilitate plant growth after inoculation. The changes in morphological characteristics of inoculated *A. vera* plants were observed at intervals of 16th and 32nd day for further analysis.

Analysis of morphological properties

To determine the effect of both fungal pathogens (*C. gloeosporioides* and *F. proliferatum*) on leaf characteristics, physical texture of leaves (colour, shape, length, width and total number), colour and consistency of gel and plant height were examined. The changes in shape and colour of infested leaves were examined on visual basis while length and width by using cm scale. The effect on plant height was also determined by using cm scale. The outer green rind of infested leaves was peeled off, colour and consistency of the gel was examined and compared with control. The infested and control leaves harvested on 16th and 32nd day were weighed out and compared to evaluate the effect on fresh weight.

Statistical analysis

Data obtained after analyses of each morphological characteristic were analyzed with the help of student's t-test for the comparison with control.

Results

Results of the present study were expressed in terms of *A. vera* disease symptomatology and variations in morphological characteristics viz. leaf colour, shape, length, width, fresh weight, total number of leaves, gel colour and consistency and plant height. It was observed that severity of infection directly affects the morphological properties of *A. vera* plants. After comparison of results, it was apparent that both the fungal pathogens (*C. gloeosporioides* and *F. proliferatum*) have a potential to alter the morphological characteristics, which reduce the growth and development of plant.

Disease symptomatology

In greenhouse experiment, inoculated plants showed initiation of leaf spot symptoms after fourth and fifth day of infestation with *C. gloeosporioides* and *F. proliferatum* respectively. The initial symptoms after artificial infestation with *F. proliferatum* were small circular spots with water soaked margins which became large, sunken, brown black in colour to form lesions of about 2.1 × 1.9 cm in diameter. Similarly, small circular, light maroon spots with water soaked margins were observed as initial symptoms after artificial infestation with *C. gloeosporioides*. As the infection progressed spots became large, sunken and turned into dark maroon colour. These spots were enlarged in size and coalesced to form lesions of about 1.9 × 1.7cm in diameter. Numerous acervuli were observed as dark concentric circles after 18th day of infection (Fig. 1).

Identification of fungal pathogens

The fungus *C. gloeosporioides* appeared on culture media as white to grey colony with puffy mycelium. Production of acervuli with 1–4 septate, brown setae (42–150 × 4–5 µm in size) after 2–3 weeks of inoculation is an important characteristic of this fungus. The conidia were hyaline, one celled, ovoid to oblong and dumbbell in shape (12.5–18×3–5 µm in size) borne on simple, short and erect conidiophores. On the other hand, colonies of *F. proliferatum* on culture media were white to light cream in colour with floccose aerial mycelium. Two types of conidia namely; microconidia and macroconidia were found to produce on loosely to densely branched typically sympodial and some irregularly verticillate conidiophores. Microconidia (aerial conidia) were oval to clavate, hyaline, 0–1 septate (7–10 × 2.5–3.2 µm in diameter) borne in chains on mono and polyphalides.

Similarly, macroconidia (sporodochial conidia) were observed as hyaline, smooth, slightly sickle shaped to almost straight (3–7 septate) with curved apical cells borne on monophialides. The size of conidia varied from $31\text{--}58 \times 2.7\text{--}3 \mu\text{m}$ in diameter. Chlamydospores were not observed. The macroconidia were observed few in number in comparison to microconidia.

Variations in morphological characteristics

Results of artificial infestation showed significant variations plant height, leaf length, leaf width and gel characteristics as compare to control. While, no difference was recorded in total number of leaves in infested and control plants. The detailed results of variations in morphological characteristics of artificial infestation of plants are presented in Fig. 2.

Plant height

It was recorded that the height of artificially infested plants was gradually reduced from 8th day onwards after infestation as compared to control plants. In healthy plants (control), the average height of 18 plants was recorded as $34.54\pm0.99 \text{ cm}$ and $36.61\pm0.99 \text{ cm}$ on 16th and 32nd day respectively. However, it was $33.48\pm0.99 \text{ cm}$ and $34.20\pm1.0 \text{ cm}$ in plants infested with *F. proliferatum* and $34.31\pm1.0 \text{ cm}$ and $35.20\pm1.0 \text{ cm}$ in plants infested with *C. gloeosporioides* on 16th and 32nd day respectively (Table 1). Although, gradual reduction in plants growth was initiated on 8th day after infestation, a significant difference between the height of control and infested plants was noted on 32nd day.

Leaf length

A gradual decrease was observed in the length of artificially infested leaves as compare to control. The length of leaves infested with *F. proliferatum* was $30.20\pm1.0 \text{ cm}$ and $30.83\pm1.02 \text{ cm}$ and leaves infested with *C. gloeosporioides* was $30.35\pm0.99 \text{ cm}$ and $31.19\pm1.02 \text{ cm}$ in comparison to control where it was $30.75\pm0.99 \text{ cm}$ and $32.54\pm1.0 \text{ cm}$ on 16th and 32nd day of infestation respectively (Table 1). Similar to plant height, a significant difference between the leaf length of control and infested plants was noted on 32nd day after infestation.

Leaf width

A minor change in leaf width in infested plants was recorded on 16th and 32nd day of infestation. The leaves infested with *F. proliferatum* were observed $3.07\pm0.11 \text{ cm}$ and $3.14\pm0.12 \text{ cm}$ in width whereas; it was $3.09\pm0.10 \text{ cm}$, and $3.16\pm0.11 \text{ cm}$ in leaves infested with *C. gloeosporioides*. In comparison, the leaf width of healthy leaves was recorded as $3.12\pm0.10 \text{ cm}$ and $3.27\pm0.10 \text{ cm}$ on 16th and 32nd day respectively (Table 1). However, the difference between width of healthy and infested leaves was found significant on 32nd day of infestation in comparison to previous days of infestation.

Total number of Leaves

No significant difference in total number of leaves was observed between infested and control plants. Total 146 and 153 leaves were recorded on plants infested with *F. proliferatum* on 16th and 32nd day of infestation. Emergence of new leaves was 6 to 18 (in infested plants) whereas; in control plants it was 5 to 23 up to 32nd day of infestation. Likewise, total number of leaves in *A. vera* plants infested with *C. gloeosporioides* was 154 and 161 on 16th and 32nd day of infestation. Total number of new emerged leaves was 7 to 18 (in infested plants) whereas; in control plant it was 7 to 22 up to 32nd day of infestation. In addition, the number of leaves in control plants was 148 and 158 on 16th and 32nd day after plantation (Table 1).

Leaf gel and leaf weight

The leaf gel of leaves infested with fungal pathogens was found light creamish in colour whereas, clear and transparent in healthy leaves. The texture of the gel was observed as viscous and transparent in leaves infested with *F. proliferatum* (mushy in healthy leaves) and gelatinous and

transparent in leaves infested with *C. gloeosporioides* (Soft and intact in healthy leaves). A significant change in leaf fresh weight of infested plants was observed as compare to control. The fresh weight of *F. proliferatum* infested leaves was 21.23 ± 1.18 g and 39.20 ± 1.22 g while it was 20.73 ± 1.19 g and 40.38 ± 1.19 g of *C. gloeosporioides* infested leaves. In addition, the leaf fresh weight in control plants was 25.39 ± 1.165 g and 50.06 ± 1.245 g on 16 and 32 days after plantation (Table 1).

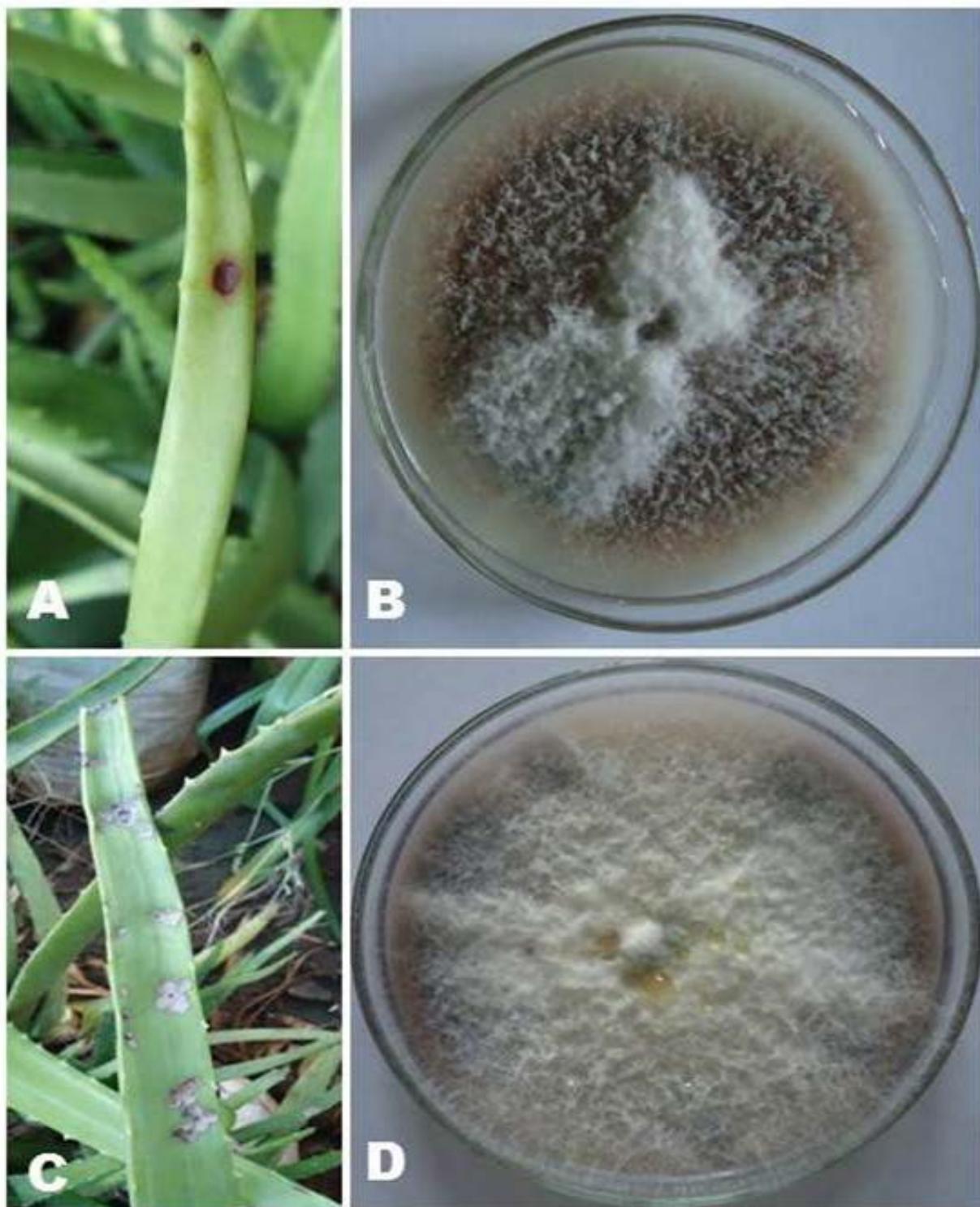


Fig. 1 – Symptoms of leaf spots on *Aloe vera* and culture characteristics: A–B disease symptoms caused by *C. gloeosporioides*; culture on PDA. C–D disease symptoms caused by *F. proliferatum*; culture on PDA.



Fig. 2 – Effect of fungal infestation on morphological attributes of *Aloe vera*: A) *F. proliferatum* (growth comparison between control and infested plant). B harvested leaves. C *C. gloeosporioides* (growth comparison between control and infested plant). D harvested leaves. E–F appearance of gel (control and infested) infested after 32 days of infestation.

Discussion

The present study revealed that *C. gloeosporioides* and *F. proliferatum* adversely affect the morphology of *A. vera* plants. In addition, both the fungal pathogens have the potential to alter the efficacy of phytochemicals which eventually nullify the quality and quantity of gel. Prolonged fungal infection in *A. vera* may affect the concentration of therapeutically important phytoconstituents. The alteration in morphological properties of infested *A. vera* plants viz. leaf colour, shape, length and width, total number, dry weight, gel colour, consistency and plant height are mainly due to the reduction in various physiological and biochemical properties of the plants. Fungal pathogens can cause significant decrease in the contents of chlorophyll-a, chlorophyll-b, total chlorophyll which leads the reduction in photosynthesis that could be attributed to the changes in green colour and reduction in energy creation which ultimately leads to lessening in length, width, weight, number of the leaves and even height of plants (Peru & Main 1970, Dai et al. 2004, Chen et al. 2005). Sugars are mainly required by plants at all growth stages from seed, to cotyledon stage, to leaf development, stem development and fruit development. The decreased synthesis of total soluble sugar in infested leaves might be responsible for reduction in growth of *A. vera* plants in terms all morphological attributes along with decreased chlorophyll synthesis. Moreover, the utilization of plant sugars by fungal pathogens for energy and synthetic reactions involved in multiplication also resulted in reduced plant growth and development (Nema 1989). Whereas, total phenolics, total anthraquinones, total flavonoid and vitamin E contents were significantly increased in infested leaves as compared to control. Changes in the concentration of biochemical were directly associated with the adverse effect of pathogen during the progression of infection on *A. vera* leaves. In comparison to *C. gloeosporioides*; *F. proliferatum* exhibited more potential effects on the major biochemicals in infested leaves (Avasthi et al. 2018a, Avasthi et al. 2019b).

Table 1 Effect of *Fusarium proliferatum* and *Colletotrichum gloeosporioides* on the morphological attributes of *Aloe vera*.

Morphological characteristics	<i>F. proliferatum</i>		<i>C. gloeosporioides</i>		Control	
	After 16 Days	After 32 Days	After 16 Days	After 32 Days	After 16 Days	After 32 Days
Leaf colour/ texture/ shape	dark green, dried with twisted margins	dark green, dried with twisted/ distorted margins	light green, dried with twisted margins	light green, dried with Twisted/ distorted margins	light green, thick and fleshy	dark green, thick and fleshy
Leaf length (cm)	30.20±1.0*	30.83±1.02**	30.35±0.99	31.19±1.02*	30.75±0.99	32.54±1.0
Leaf width (cm)	3.07±0.11*	3.14±0.12*	3.09±0.10	3.16±0.11*	3.12±0.10	3.27±0.10
Total number of leaves	146	153	154	161	148	158
Gel colour	clear	clear	clear	clear	light creamish	light creamish
Gel consistency	viscous and transparent	viscous and transparent	gelatinous and transparent	gelatinous and transparent	mushy, Soft and intact	mushy, Soft and intact
Leaf fresh weight (g)	21.23 ± 1.18***	39.20 ± 1.22***	20.73 ± 1.19***	40.38 ± 1.19***	25.39± 1.16	50.06± 1.24
Plant height (cm)	33.48 ±0.99	34.20±1.0	34.31±1.0	35.20±1.0	34.54±0.99	36.61±0.99

Mean ± standard deviation of nine replicates

*Marginally significant ($p \leq 0.10$)

**Significant ($p \leq 0.05$)

***Highly significant ($p \leq 0.01$)

A number of fungal pathogens are also found to be associated with leaf spot, leaf rot, collar rot and root rot disease of *A. vera* such as *Fusarium phyllophilum* (Kishi et al. 1999), *Colletotrichum gloeosporioides* (Avasthi et al. 2011), *Fusarium oxysporum* (Kawuri et al. 2012), *Nigrospora oryzae* (Zhai et al. 2013), *Phoma betae* (Avasthi et al. 2013), *Alternaria alternata* (Abkho & Sabbagh 2014), *Sphaeropsis sapinea* (Kamil et al. 2014), *Curvularia lunata* and *C. ovoidea* (Avasthi et al. 2015a), *Penicillium purpurogenum* (Avasthi et al. 2015b); *Cladosporium sphaerospermum* (Avasthi et al. 2016a), *Phomopsis* sp. (Avasthi et al. 2016b) *Polytrochata indica* (Avasthi et al. 2017a) and *Phoma eupyrena* (Avasthi et al. 2017b), *Fusarium fusaroides*, *F. moniliforme*, *F. solani* (Avasthi et al. 2018b), *Fusarium proliferatum* (Avasthi et al. 2018a), and *Pythium aphanidermatum*, *Helminthosporium* sp. (Avasthi et al. 2019a). Out of all reports, two pathogens namely; *Colletotrichum gloeosporioides* and *Fusarium proliferatum* have been evaluated for their effects on biochemical properties of *A. vera* (Avasthi et al. 2018c, 2019b). As per these studies, the contents of chlorophyll-a, chlorophyll-b, total chlorophyll and total soluble sugar decreased significantly in infested leaves as compared to control. In contrast, concentration of total phenolics, total anthraquinones, total flavonoids and vitamin E contents significantly increased in infested leaves as compared to control. Based on these studies, a comparative account on effects of both fungal pathogens on the major biochemicals in infested *A. vera* leaves has been prepared and provided (Table 2). Soni et al. (2011) have also reported the qualitative and quantitative loss in the mucilaginous gel of *A. vera* due to *Uromyces aloes* infection during a pathological survey in Jabalpur, Seoni, Balaghat and Chhindwara of Madhya Pradesh. Remarkable changes in the leaves of *A. vera* affected with *Alternaria alternata* has been reported earlier as yellowing and dried from the tip downwards with loss of leaf texture and mucilaginous jelly (Abkho & Sabbagh 2014).

The findings of present study concluded that *C. gloeosporioides* and *F. proliferatum* causes leaf spot disease in *A. vera* has significant effects on the morphological properties like plant height, quality and texture of leaves including leaf colour, shape, length and width, total number, fresh weight and mucilaginous gel resulted into the qualitative and quantitative loss of leaf gel. It also

modifies the concentration of biochemical constituents significantly. As *A. vera* is a highly valuable medicinal plant with pharmaceutically important constituents. The extent of fungal infection in this plant was enhanced due to use of non-scientific, improper agricultural practices and environmental factors. Therefore, it is very essential to apply suitable management strategies to control fungal diseases of *A. vera* to protect the plant of an enormous medicinal and industrial value. In addition, efforts should also be made to aware and encourage farmers to use scientific methods in *A. vera* cultivation and disease control.

Table 2 Comparative account on the effect of *Fusaroum proliferatum* and *Colletotrichum gloeosporioides* on the phytochemical attributes of *Aloe vera*.

Phytochemicals	<i>F. proliferatum</i>		<i>C. gloeosporioides</i>		Control	
	After 16 Days	After 32 Days	After 16 Days	After 32 Days	After 16 Days	After 32 Days
Chlorophyll-a (mg g ⁻¹ dr wt)	0.0280 ± 0.01	0.0218 ± 0.00**	0.0311 ± 0.00082***	0.0268 ± 0.00077***	0.0383 ± 0.0056	0.0448 ± 0.0061
Chlorophyll-b (mg g ⁻¹ dr wt)	0.0176 ± 0.01	0.0132 ± 0.01*	0.0241 ± 0.00063***	0.0204 ± 0.00083***	0.0256 ± 0.00527	0.0294 ± 0.0055
Total chlorophyll (mg g ⁻¹ dr wt)	0.0508 ± 0.01	0.0382 ± 0.01***	0.0577 ± 0.00386***	0.0460 ± 0.00301***	0.0649 ± 0.00576	0.073 ± 0.00576
Carotenoid (mg g ⁻¹ dr wt)	2.156±0.1 2 ***	1.948±0.1 4***	2.502 ± 0.12***	2.416 ± 0.13***	3.942 ± 0.12 39.27 ± 0.88	3.415 ± 47.23 ±
Total soluble sugar (mg g ⁻¹ dr wt)	35.55 ± 0.85***	41.51 ± 0. 90***	36.66 ± 0.85**	42.94 ± 0.88**		0.13 0.86
Total flavonoids (mg g ⁻¹ dr wt)	8.45 ± 0.15***	14.40 ± 0.16	8.89 ± 0.21**	14.7 ± 0.22	8.28 ± 0.16	10.545 ± 0.18
Total phenols (mg g ⁻¹ dr wt)	10.53 ± 0.15***	15.66 ± 0.14	10.57 ± 0.20***	15.52 ± 0.25	8.92 ± 0.19	11.81 ± 0.18
Total anthraquinones (mg g ⁻¹ dr wt)	0.075 ± 0.01	0.094 ± 0.01	0.074 ± 0.00125**	0.093 ± 0.00163***	0.068 ± 0.00553	0.078 ± 0.0056
Vitamin E (mg g ⁻¹ dr wt)	57.11 ± 0.87	73.78 ± 0.89*	57.39 ± 0.90***	73.33 ± 0.85	50.72 ± 0.86	60.195 ± 0.84

Mean ± standard deviation of nine replicates

*Marginally significant ($p \leq 0.10$)

**Significant ($p \leq 0.05$),

***Highly significant ($p \leq 0.01$)

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