Advances in Research

22(4): 7-15, 2021; Article no.AIR.70554
ISSN: 2348-0394, NLM ID: 101666096

Management of Anthracnose Disease of *Aloe vera* in Bangladesh

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AIR/2021/v22i430306
Editor(s):
(1) Prof. Antonio Mastino, University of Messina, Italy.
Reviewers:
(1) Ngoh Dooh Jules Patrice, University of Maroua, Cameroon.
(2) Kedar Nath, Navsari Agricultural University, India.
Complete Peer review History: https://www.sdiarticle4.com/review-history/70554

Original Research Article

Received 10 May 2021
Accepted 15 July 2021
Published 10 August 2021

ABSTRACT

Aims: To identify the causal organisms of anthracnose disease of *Aloe vera* in Bangladesh and to manage this disease in field condition.
Study Design: The experiment was designed by Randomized Complete Block Design (RCBD) with three replications.
Place and Duration of Study: The field experiments were conducted in Natore, Bangladesh and the laboratory experiments were carried out at the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh from January, 2017 to December, 2018.
Methodology: The causal organism *Colletotrichum gloeosporioides* was isolated by tissue plating method and identified based on morphological and cultural characteristics and that was confirmed by pathogenicity test. Infested farmer's fields were selected in rainy season under natural epiphytic condition to evaluate the efficacy of eleven treatments.
Results: Among the treatments, Bordeaux mixture gave best result against this disease. Moreover, Tilt 250 EC and Folicur 25 EC and Garlic bulb extract showed better effect against the disease than the other treatments. Lime also has moderate effect against anthracnose disease of *A. vera*. In 2017, after 4th spray, the lowest incidence was recorded in Bordeaux mixture (58.33%) which was statistically identical with Folicur (64.58%), Tilt (64.58%) and Garlic bulb extract (66.67%). Similarly, the lowest disease severity was found in Bordeaux mixture (3.55) followed by Folicur (5.67%), Tilt (6.67%) and Garlic bulb extract (7.67%). Similar result also found in 2018. After 4th spray, the

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lowest incidence was recorded in Bordeaux mixture (38.58%) which was statistically identical with Lime (41.66%) and Garlic bulb extract (45.83%). Similarly, the lowest disease severity was found in Bordeaux mixture (0.20%) followed by Lime (0.25%) and Garlic bulb extract (0.36%).
Conclusion: Garlic bulb extract could be used as eco-friendly approach. Moreover, use of Bordeaux mixture is better than the traditional use of lime. From chemical pesticides, Tilt 250 EC and Folicur 25 EC could be used for controlling the disease as the last option.

Keywords: Medicinal plant; biological control; disease severity; leaf spot; bordeaux mixture.

1. INTRODUCTION

Anthracnose disease of *Aloe vera* is the most devastating disease, which is now wide spread problem of aloe growers in Bangladesh [1]. The most common symptoms of anthracnose are the circular or oval shape having deep sunken lesion. There are very limited research works on diseases of *A. vera* in Bangladesh and also in South Asia. Different study reported that the causal organism of this disease is *Colletotrichum gloeosporioides* [2,3,4,5].

The most important method of protecting the plants against the fungal attack is the use of fungicides. Use of chemical pesticides is discouraged in *A. vera* due to its residual effect in leaf. In most cases, its gel is used to prepare traditional juice in Bangladesh. Thus, spraying of lime solution in the leaves is a common practice to control leaf spot disease of *A. vera* in Bangladesh. But, sometimes lime produce heat and toxicity in the plant that reduce production of *A. vera* gel in plant. Considering this issue, application of botanical or plant extracts may be a good alternative. However, very limited findings are available regarding this disease and its management in Bangladesh. So, this is an urgent issue to detect and identify this disease. It is also essential to measure the severity and incidence of diseases to find out the yield loss. From the farmer interest, it is also need to develop farm applicable preventive measures to control this disease with minimal residual effect and reduce the yield loss of this medicinal plant. Considering the above facts and points this research work was designed to identify the causal organism and find out field management practices for anthracnose disease of *A. vera* in Bangladesh.

2. MATERIALS AND METHODS

2.1 Isolation and Identification of Causal Organisms of Anthracnose Disease of *A. vera*

Diseased leaves of commercially cultivated *A. vera* were collected from Kholabaria and Kathalbaria villages of Natore district in Bangladesh. Diseased leaves (anthracnose) with some healthy portion were selected as sample for isolation and identification of the causal organism. The diseased leaves were cut into pieces (5 mm diameter) and surface sterilized with $HgCl_2$ (1: 1000) for 30 seconds. Then the cut pieces were washed in sterile water thrice and placed on to solidified PDA medium in Petri dish. The plates containing leaf pieces were incubated at room temperature for seven days. When the fungus grew well and sporulated, the organism was re-cultured by tip culture method to obtain pure culture. Then slides were prepared from pathogenic structures and observed under microscope and identified with the help of relevant literature and CMI description [6,7,8,9]. After purification the culture plates were kept at refrigerator at 4 °C.

2.1.1 Pathogenicity test of anthracnose disease of *A. vera*

Pathogenicity test was performed to confirm the pathogen as per prescribed procedure [9]. Pathogen was isolated from diseased leaves and cultured in PDA medium. Hyphal mat from 5 days old potato broth cultures of the pathogens were scraped aseptically on to a fine cheese cloth filtered and washed in several changes of sterile distilled water to remove traces of stalling materials. The mats were then transferred aseptically into 200 ml of distilled water containing 5 ml glucose solution in warring blender and homogenized for one minute at low speed in order to get the inoculum ready for pathogenicity test on test plant [10]. Plant leaves were inoculated with fungal spore suspension containing concentration of 10^5 spores ml^{-1} by pin prick method. Three leaves of each plant were inoculated. Plants were covered with polythene bag after inoculation for 24 hours to maintain suitable moisture condition.

2.2 Management of Anthracnose Disease of *A. vera*

In 2017, the experiment was conducted in heavily infested farmer's field of *A. vera* in Kholabaria and confirmation of the experiment

was done in 2018 at Kathalbaria in rainy season under natural epiphytic condition by Randomized Complete Block Design (RCBD) with three replications. Each plot size was 1.5 meter length with 1 meter width. Plot to plot distance was 0.5 meter and block to block distance was 1 meter. In 2017, eleven treatments were used to check their efficacy against anthracnose disease. The treatments were T_1 = Folicur 25 EC @ 0.1% (Tebuconazole), T_2 = Tilt 250 EC @ 0.1% (Propiconazole), T_3 = Autosin 50 WP @ 0.2% (Carbendazim), T_4 = Diathane M 45 @ 0.2% (Mancozeb), T_5 = Companion @ 0.2% (Mancozeb + Carbendazim), T_6 = Bordeaux mixture @ 1% ($CuSO_4$+CaO), T_7 = Lime @ 0.5 % (CaO), T_8 = *Trichoderma harzianum* (0.2 %), T_9 = Garlic bulb extract 1:1 (w/v) @ 1 % (*Allium sativum*), T_{10} = Neem leaf extract 1:1 (w/v) @ 1 % (*Azadirachta indica*) and T_{11} = Allamanda leaf extract 1:1 (w/v) @ 1% (*Allamanda cathartica*). However, nine treatments were used at Kathalbaria field experiment in 2018. Companion and Neem leaf extracts were excluded due their fewer efficacies against anthracnose disease of A. vera. The treatments were T_1 = Autosin 50 WP (0.2 %), T_2 = Diathane M 45 (0.2 %), T_3 = Tilt 250 EC (0.1 %), T_4 = Folicur 25 EC (0.1 %), T_5 = Bordeaux mixture (1%), T_6 = Lime (0.5 %), T_7 = *T. harzianum* (0.2 %), T_8 = Garlic bulb extract 1:1 (w/v) (1 %), T_9 = Allamanda leaf extract 1:1 (w/v) (1 %) and T_{10} = Control. Bordeaux mixture suspension was prepared at the day of spraying. Plant extracts were prepared by using the method of Ashrafuzzaman and Hossain [11]. *Trichoderma* suspension was collected from Ispahani Biotech Ltd. for this experiment. Two ml *Trichoderma* solution was mixed with one liter water to prepare 0.2% solution. The bio-agent suspension was prepared just before spraying. Spray was done in standing plant of the field. In 2017, four sprays of the selected treatments were done at afternoon at 10 days interval in rainy season. On the other hand, in 2018 three sprays with selected ten treatments were done at 10 days interval. Disease incidence and severity were recorded for each experiment before spray. Ten plants of a plot were considered for measuring plant incidence. Five plants for each plot were selected randomly for measurement of disease incidence (plant and leaf) and severity.

Disease incidence was calculated as a ratio between the numbers of infected leaves or plant and the numbers of inspected leaves or plant and was expressed in percentage. Similarly, disease severity was calculated as a ratio between area of tissues infected and area of tissues inspected and was expressed in percentage [9,12,13,14].

Disease severity was classified into following rating scales on the basis of percentage of leaf area covered by infection (infected spot) [16].

2.3 Statistical Analysis

The data were analyzed statistically by Statistics 10 computer package program by subjecting them to one way analysis of variance (ANOVA). The mean comparisons were carried out using Fisher's Least Significant Difference (LSD) test, where $P \leq 0.05$ was considered significant.

3. RESULTS AND DISCUSSION

3.1 Pathogenicity Test

The result of pathogenicity test revealed that the pathogen showed typical symptoms (Fig. 1A) and after re-isolation gave the same colonial color texture (Fig. 1B), conidiophores, acervuli (Fig. 1C) and conidia (Fig. 1D) as what these was inoculated. The identified causal organism was *Colletotrichum gloeosporioides*. The fungus produces spores within an acervulus (fungal fruiting structure). The disk or cushion shaped acervuli break through the surface of host tissue. Short, simple, colorless conidiophores produce abundant conidia. Long, black setae may be produced among conidiophores (Plate 6. B). Conidia are hyaline when viewed alone, but it may appear pink or salmon colored en mass. Spores are short, ovoid to cylindrical, and single celled. Based on the symptoms, mycelia and conidial characters, the fungus was identified as *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. Therefore, these seem to be pathogenic.

Table 1. Rating description for infected area

Rating Scale	Percent Area of Infection
0	No symptoms
1	0-10% leaf area covered by spots
2	11-25% leaf area covered by spots
3	26-50% leaf area covered by spots
4	51-75% leaf area covered by spots
5	More than 75% leaf area covered by spots

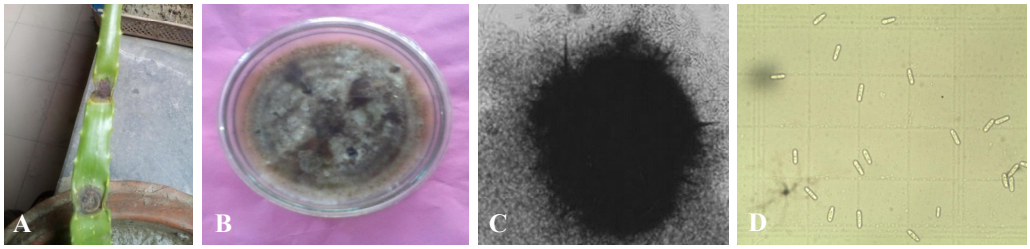

Fig. 1. Pathogenicity Test, A. Leaves of *A. vera* plant showing anthracnose disease; B. Pure culture of *C. gloeosporioides*; C. Acervulus and conidia of *C. gloeosporioides*; D. Conidia of *C. gloeosporioides* (40X)

3.2 Management of Anthracnose Disease of *A. vera* by Selected Treatments

3.2.1 Efficacy of different treatments on anthracnose disease of *A. vera* at Kholabaria in 2017

3.2.1.1 Incidence & severity of anthracnose disease of A. vera

Before spraying, the leaf incidence and disease severity were varied from 59.26 to 81.48% and 12 to 13%, respectively (Table 2). At ten days after 1st spray, the effect of different treatments in terms of disease incidence and severity was not differed significantly in most cases comparison to control. However, Bordeaux mixture and Tilt 250 EC showed better effect than the other treatments (Table 2). After 2nd spray (Table 2) leaf incidence was varied significantly to different treatments from 46.92% to 80.24%, where the lowest leaf incidence was recorded in Bordeaux mixture (46.92%) that was statistically identical with Tilt 250 EC (44.44%). Similarly, disease severity was ranged from 8 to 12.33% and the lowest severity was observed in Bordeaux mixture. The highest incidence and severity was recorded in control plot. Similarly, at 10 days after 3rd spray (Table 2) the highest effect against the disease was observed in case spraying of Bordeaux mixture followed by Tilt 250 EC, Folicur 25 EC and Garlic bulb extract. The highest disease incidence and severity was recorded in Control treatment followed by neem leaf extract and *T. harzianum*. The lowest leaf incidence and disease severity was found in spraying of Bordeaux mixture that was 33.33% and 4.33%, respectively. After 4th spray (Table 2) the highest effect against leaf incidence was also observed in case of Bordeaux mixture (27.15) which was statistically similar with Tilt 250 EC (34.56%). Similarly, the lowest disease severity was found in Bordeaux mixture (3.55) followed by Folicur (5.67%), Tilt (6.67%) and Garlic bulb extract (7.67%). In all cases, the highest disease incidence and severity were recorded in Control treatments which were statistically similar with neem leaf extract and *T. harzianum*.

3.2.2 Efficacy of different treatments on anthracnose disease of *A. vera* at Kathalbaria in 2018

3.2.2.1 Incidence and severity of leaf anthracnose of A. vera

Before spraying, leaf incidence and disease severity were measured and found varied from 90.41 to 98.03% and 1.33 to 1.63%, respectively (Table 3). Ten days after 1st spray, the effect of different treatments in terms of disease incidence and severity was not differed significantly in most cases comparison to control (Table 3). However, Bordeaux mixture and Garlic bulb extract showed comparatively better effect than the other treatments. At 10 days after 2nd spray (Table 3) leaf incidence was varied significantly to different treatments ranged from 66.01 to 92.37%, where the lowest leaf incidence was recorded in Bordeaux mixture (66.01%) that was statistically similar with spraying of Garlic bulb extract (67.31%). The lowest disease severity was observed in spraying of Bordeaux mixture (1%) that was statistically similar with Lime (1.03%). At 20 days after 3rd spray the effect of treatments were differed quite significantly in terms of disease incidence and severity (Table 3). The highest effect against leaf incidence was also observed in case of Bordeaux mixture (18.51%) which is statistically similar with Lime (18.75%) and Garlic bulb extract (24.61%) too. Similarly, the lowest disease severity was found in Bordeaux mixture (0.20%) followed by Lime (0.25%) and Garlic bulb extract (0.36%). The highest disease incidence and severity were recorded in Control plot which is statistically similar with Autostin 50 WP and Dithane M 45.

Table 2. Disease incidence and severity of anthracnose disease of Aloevera at Kholabria in 2017

Treatments	Before Spray		1st Spray		2nd Spray		3rd Spray		4th spray	
	DI (%)	DS (%)	DI (%)	DS (%)	DI (%)	DS (%)	DI (%)	DS (%)	DI (%)	DS (%)
T_1 (Folicur 25 EC)	70.37 ab	12.67 a	66.66 a-d	10.67 ab	56.78 cd	9.00 de	46.91 cd	7.00 c	37.03 de	5.67 e
T_2 (Tilt 250 EC)	59.26 b	12.33 a	50.61 d	10.67 ab	44.44 d	9.67 c-e	37.03 d	8.33 bc	34.56 ef	6.67 de
T_3 (Autostin 50 WP)	66.67 ab	12.67 a	60.49 b-d	11.67 ab	55.55 cd	11.33 a-c	73.15 ab	10.33 ab	45.678 cd	10.33 ab
T_4 (Dithane M 45)	77.78 ab	12.67 a	70.37 a-c	11.67 ab	64.19 bc	11.33 a-c	69.52 ab	10.00 ab	51.85 bc	10.00 ab
T_5 (Companion)	66.67 ab	13.00 a	64.19 b-d	12.00 ab	59.26 cd	11.00 a-d	54.31 b-d	10.17 ab	48.14 c	9.50 a-c
T_6 (Bordeaux mixture)	70.37 ab	12.00 a	58.02 cd	9.67 b	46.92 d	8.00 e	33.33 d	4.33 d	27.15 f	3.50 f
T_7 (Lime)	81.48 a	12.67 a	75.31 ab	11.33 ab	70.37 a-c	10.00 b-e	64.19 a-c	9.33 ab	58.02 b	9.00 bc
T_8 (*Trichoderma harzianum*)	81.48 a	12.33 a	82.71 a	12.33 ab	80.24a	12.33a	77.78 a	11.33 a	72.83 a	11.33 a
T_9 (Garlic bulb extract)	70.37 ab	12.67 a	62.96 b-d	12.00 ab	57.89 cd	10.33 a-d	45.67 cd	8.33 bc	38.27 de	7.67 cd
T_{10} (Neem leaf extract)	81.48 a	12.67 a	76.54 ab	12.67 a	75.31 ab	12.00 ab	70.70 ab	11.33 a	67.89 a	11.00 a
T_{11} (Control)	81.48 a	12.67 a	82.71 a	12.67 a	80.24a	12.33a	80.24a	12.33a	77.78 a	12.33a
LSD (0.05)	19.27	3.10	16.56	2.76	15.09	2.31	22.18	2.15	9.24	1.84
CV (%)	15.47	14.39	14.45	14.01	14.40	12.80	22.58	13.85	11.19	12.70

**DI = Disease Incidence (leaves); DS = Disease Severity*

Table 3. Disease incidence and severity of anthracnose disease of Aloevera at Kathalbaria in 2018

Treatments	Before Spray		1st Spray		2nd Spray		3rd Spray	
	DI (%)	DS (%)	DI (%)	DS (%)	DI (%)	DS (%)	DI (%)	DS (%)
T_1 (Autostin 50 WP)	92.59 a	1.36 a	85.18 a-c	1.26 a	81.48 b	1.20 ab	34.18 ab	0.50 ab
T_2 (Dithane M 45)	94.45 a	1.53 a	88.89 ab	1.43 a	83.33 ab	1.33 ab	29.48 bc	0.53 ab
T_3 (Tilt 250 EC)	94.44 a	1.33 a	90.74 ab	1.26 a	84.96 ab	1.16 ab	30.28 bc	0.43 bc
T_4 (Folicur 25 EC)	98.03 a	1.46 a	92.26 a	1.36 a	79.30 bc	1.16 ab	22.65 c-e	0.37 cd
T_5 (Bordeaux mixture)	92.48 a	1.53 a	81.15 bc	1.33 a	66.01 d	1.00 b	18.51 e	0.20 de
T_6 (Lime)	92.59 a	1.46 a	81.47 bc	1.26 a	70.37 cd	1.03 b	18.75 de	0.25 e
T_7 (*Trichoderma harzianum*)	96.29 a	1.43 a	90.73 ab	1.36 a	85.18 ab	1.30 ab	28.21 bc	0.50 ab
T_8(Garlic bulb extract)	90.41 a	1.56 a	78.86 c	1.46 a	67.31 d	1.26 ab	24.61 c-e	0.36 cd
T_9 (Allamanda leaf extract)	96.29 a	1.63 a	84.96 a-c	1.43 a	77.77 bc	1.26 ab	26.47 b-d	0.38 cd
T_{10} (Control)	96.30 a	1.43 a	94.33 a	1.43 a	92.37 a	1.40 a	41.39 a	0.56 a
LSD (0.05)	8.76	0.33	9.78	0.33	9.10	0.35	7.83	0.13
CV (%)	5.41	13.22	6.56	13.90	6.73	16.61	16.63	19.22

**DI = Disease Incidence (leaves); DS = Disease Severity*

4. DISCUSSION

Colletotrichum gloeosporioides were successfully isolated by tissue planting method from infected leaf of anthracnose disease of Aloevera collected from different fields of Natore district. However, pathogenicity test was successful for *C. gloeosporioides*. The previous literatures indicate that several pathogens are associated with this complex disease. Similar result was also found by some researcher's viz. Shutrodhar and Shamsi [5], Avasthi et al. [3] and Alam et al. [2]. Shutrodhar and Shamsi [5] recorded 8 fungal species viz. *Alternaria pluriseptata*, *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium oxysporum*, *C. gloeosporioides*, *Nigrospora oryzae*, *Penicillium* sp. and *Pestalotiopsis guepinii* associated with anthracnose and leaf spot disease of *A. vera* in India. However, Ahmmed and Rahman [16] found *Colletotrichum* sp. from anthracnose disease of aloevera in Bangladesh in 2015. They also reported *Alternaria* spp., *Curvularia* spp. from leaf spot and tip blight disease of aloevera in Bangladesh. The frequency of *C. gloeosporioides* was the maximum. Pathogenicity test revealed that *C. gloeosporioides* causes anthracnose disease in *A. vera*. Avasthi et al. [3] reported anthracnose symptom on the leaf surface of *A. vera* in India and identified the causal organism as *C. gloeosporioides.* Some instances of anthracnose disease of *A. vera* caused by *Colletotrichum* sp. was also reported from Lucknow [2].

In 2017, the experiment was conducted in heavily infested farmer's field of *A. vera* in Kholabaria in rainy season under natural epiphytic condition. To confirm the findings, the experiment was again re-tested in 2018 at Kathalbaria in another highly infested farmer's field in rainy season under natural epiphytic condition. *Aloevera* is commercially cultivated in Natore from last 25 years. Kathalbaria and Kholabaria villages of Laxsmipur Union of SadarUpazila of Natore district are very popular for commercial cultivation of aloevera in Bangladesh. Moreover, rainy season is very favorable for anthracnose disease of *A. vera*. Thus, disease incidence and severity is comparatively high in rainy season [17,18]. In addition, due to continuous monoculture, amount of disease is gradually increasing day by day in that region. Considering this point, the field experiment was conducted in Kathalbaria and Kholabaria villages in rainy season for management of anthracnose disease of aloevera. The experiments were conducted in farmer's field under natural epiphytic condition.

Eleven treatments including five commercial fungicides, one homemade fungicide (Bordeaux mixture), one traditional practice (Lime), one bio agent (*T. harzianum*) and two botanicals were tested against the disease in field condition in 2017. However, nine treatments including four commercial fungicides, one homemade fungicide (Bordeaux mixture), one traditional practice (Lime), one bio agent (*T. harzianum*) and two botanicals (Garlic bulb extract and Alamanda leaf extract) were tested against the anthracnose disease of *A. vera* in field condition in 2018.

At Kholabaria, four spay was done with selected treatments in an interval of 10 days. After first spray no significant difference was found among the treatments. However, Bordeaux mixture (disease severity - 9.67%) showed better effect than the other treatments. After second spray, statistically no significant difference was found for disease incidence and severity. Bordeaux mixture (disease severity 8.00%) gave better performance similar as previous result. After third spray, significant difference was observed in terms of disease incidence and disease severity. In case of disease severity, the best treatment was again Bordeaux mixture followed by Folicur 25 EC and Garlic bulb extract with disease severity 4.33%, 7.00%, and 8.33% respectively. After the fourth and final spray at Kholabaria field of *A. vera*, a remarkable effect of the treatments was observed against the disease. In case of disease severity, the best treatment was again Bordeaux mixture followed by Folicur 25 EC, Tilt 250 EC and Garlic bulb extract with disease severity 3.50%, 5.67%, 6.67% and 7.67% respectively. The highest effect against leaf incidence was also observed in case of Bordeaux mixture (27.15%) which is statistically similar with Tilt 250 EC (34.56%).

On the other hand, at Kathalbaria three sprays of the selected treatments with an interval of 10 days were done in the field of *A. vera*. After first spay no significant difference was found among the treatments. Moreover, statistically no significant difference was also observed after second spray. But Bordeaux mixture and Garlic bulb extract showed better result. After third and final spray at Kathalbaria field experiment, the highest effect against leaf incidence was observed in case of Bordeaux mixture (18.51%) which is statistically similar with Lime (18.75%) and Garlic bulb extract (24.61%) too. Similarly,

the lowest disease severity was found in Bordeaux mixture (0.20%) followed by Lime (0.25%) and Garlic bulb extract (0.36%). The highest disease incidence and severity were recorded in control plot which is statistically similar with Autostin 50 WP and Dithane M 45. Lime also has moderate effect against anthracnose disease of *A. vera*.

Similar results were also found by Sohag et al. [19] and Regmi et al. [20]. Sohag et al. [19]. Sohag et al. [19] conducted a survey to evaluate the efficacy of Garlic bulb extract, Bion, Bavistin DF (Carbendazim) and proud for controlling leaf spot disease of Taro (*Colocasia esculenta*). Garlic bulb extract showed enhanced result against leaf spot disease of Taro (*C. esculenta*). Islam and Faruq [21] was reported that neem leaf extract, garlic clove extract and allamanda leaf extract were effective against damping off diseases of tomato, eggplant and chilli. Regmi et al. [20]. Regmi et al. [20] evaluated leaf extracts of six plants viz, *Jatropa curcas*, *Datura strumarium*, *Azadirachata indica*, *Moringa oleifera*, *Calotropis gigantean* and *Morus alba* @ 50% by food poison techniques against the fungus *Alternaria alternata* causing leaf spot disease of *A. vera*. Botanicals inhibited the mycelia growth of the fungus.

5. CONCLUSION

The fungi *C. gloeosporioides* were successfully isolated and pathogenicity test was successful conducted. Bordeaux mixture gave best result against this disease. Moreover, Tilt 250 EC and Folicur 25 EC and Garlic bulb extract showed better effect against the disease than the other treatments. Lime also has moderate effect against anthracnose disease of aloevera. Considering the overall performance of the treatments, garlic bulb extract could be used as eco-friendly approach. However, cost benefits analysis of garlic bulb extract required before advice to the farmers. Use of Bordeaux mixture is better than the traditional use of lime. From the chemical fungicides, Tilt 250 EC and Folicur 25 EC could be used for controlling the disease as the last option.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support provided by Ministry of Education (GARE, BANBEIS), Govt. of People's Republic of Bangladesh under the project entitled "Surveillance, monitoring and development of prescription package against major diseases of commercial medicinal crops of Bangladesh" and University Grant Commission Bangladesh under the project entitled "Identification of causal agents of leaf spot disease of aloevera (*Aloe vera* L.) and its economic management" executed in the Department of Plant Pathology, Sher-e- Bangla Agricultural University, Dhaka to conduct the study. The authors also wish to thank Mr. Dewan Kabiraj and Mr. Kader Kabiraj, the aloevera growers of Natore, Bangladesh for their help throughout the work of this experiment.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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