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Original Article

Screening of fungicides and plant extracts in controlling leaf spot and anthracnose diseases of *Rauwolfia serpentina* (L.) Benth. ex Kurz.

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ABSTRACT: Field experiment was conducted to evaluate the efficacy of two fungicides and two plant extracts against leaf spot and anthracnose diseases of *Rauwolfia serpentina* Benth. ex Kurz in Botanical research garden, Department of Botany, University of Dhaka. Both the fungicides Autostin 50WPG and Tilt 250EC and leaf extracts shows effective management of the disease over untreated check. However, among the treatments Autostin and Tilt at 100 ppm concentration and *Azadiracta indica* and *Citrus limon* at 10% concentration was found significantly superior in controlling the disease severity, PDI (Percent disease index) and increasing number of healthy leaves. Number of healthy leaves which was highest per plant 79.55 in 2016 and 52.00 in 2017.

Keywords: Screening, Fungicide, Plant extract, Leaf Anthracnose, *Rauwolfia serpentina*

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INTRODUCTION

Rauwolfia serpentina (L.) Benth. ex Kurz is a medicinal shrub belongs to the family Apocynaceae. It is locally known as "Sarpagandha" as well as "Indian snakeroot". In Bangladesh it grows in Chittagong, Dhaka, Mymensingh and Sylhet . Root of this shrub is mostly used as a good antidote for high blood pressure . Seventeen different alkaloids have been extracted from the bark of the root of this shrub . Serpentine is one of those alkaloids[1]. Leaf spot and Anthracnose are

common fungal diseases of *R. serpentina.* The other diseases include Target leaf blotch, Cercospora leaf spot, Die-back, Powdery mildew, Fusarium wilt, Root-knot disease, etc[2]. Yasmin and Shamsi (2015)[3] reported diseases of *R. serpentina* from Bangladesh. Most of the research work carried out on *R. serpentina* under Phytochemical, Pharmacological, Biochemical and Antimicrobial disciplines, but research about its fungal diseases and control is inadequate[4-7]. Now-a-days, many inorganic and organic fungicides are used frequently to control plant diseases[8]. Use of chemical fungicides provides excellent control of the diseases and result in improved yield. In agriculture, fungicides are used to protect tubers, fruits and vegetables during storage as well as applied directly to ornamental plants, trees, field crops, cereals and turf grasses[9-10]. From India Thyagarthi *et al*. (2013)[11] reported that *R. serpentina* is affected in wild by *C. rauwolfiae* and *A. alternata*. The later pathogen is the minor leaf spot disease caused by *Alternaria alternata* occurred occasionally. *Alternaria alternata* is seedborne and seed transmitted

and could be managed by seed treatment with Captra or Hyzeb. Therefore, it is necessary to test the efficacy of the fungicides against the targeted pathogen.

Various workers in different countries of the world evaluated the efficacy of different fungicides against *Colletotrichum* spp., *Phomopsis vexans*, *Macrophomina phaseolina*, *Rhizopus nodosus*, *Fusarium* spp., *Phoma* spp., *Botryodiplodia theobromae*, *Colletotrichum gloeosporioides*, *Sclerotium rolfsii* and *Alternaria* spp., under laboratory and field conditions12-20. But fungicide's toxicity is not always restricted to the target pest organism, having also been demonstrated in mammals including humans[21]. Most fungicides can cause acute toxicity, and some cause chronic toxicity as well[22].

The World Health Organization (WHO) and the United Nations Environment Program (UNEP) estimates that each year, three million workers in agriculture in developing world experience severe poisoning from pesticides, about 18,000 of whom die[23]. However, care should be taken during the using of the fungicides.

MATERIALS AND METHODS

The field experiments were conducted in Botanical research garden, University of Dhaka, during the tenure of 2016 and 2017. Leaf spot and Anthracnose diseases susceptible *Rauwolfia serpentina* was selected to study the efficacy of fungicides and plant extracts. The field plot was prepared in January of each experimental year. Bio fertilizer was used during field preparation. The seedlings were transplanted after 3 weeks in 1.5 × 1 m plot using randomized block design with four replications. Spacing between sub- plots was 1 m. The plants were spaced 25 cm by 25 cm. Five plants from each plot were randomly selected and from each selected plants, fifteen leave were selected for recording disease severity. The disease severity was recorded by using 0–9 scale.

For visual estimation of severity, 0 – 9 point scale were used for rating of all foliar diseases studied (PDI=McKinney's Index)[29].

No infection – 0, 0 – 10% leaf area infected – 1, 10 – 20% leaf area infected – 2, 20 – 30%

leaf area infected – 3, 30 – 40% leaf area infected – 4, 40 – 50% leaf area infected – 5, 50

-60% leaf area infected – 6, 60 – 70% leaf area infected – 7, 70 – 80% leaf area infected -8, 80 –90% or more leaf area infected – 9.

Fungicides and plant extracts were applied after eight weeks of transplantation at two weeks interval for five successive weeks

The incidence and severity of leaf spot and anthracnose of *R. serpentina* were recorded from the plants grown in gardens of Curzon Hall Campus, Dhaka University. Each plot was visited and data were recorded twice in a month. Data were expressed in percentage. Similarly per cent disease index (PDI) was recorded using the following formula: [30].

Additionally, the spread of plant diseases in natural ecosystems may preclude successful application of chemicals, because of the scale to which such applications might have to be applied[24].

Some pest management researchers have focused their efforts on developing alternative inputs to synthetic chemicals for controlling diseases[25-26]. One of them is the use of biological antagonists and plant extracts. Biological control presents a better alternative with relative low cost, no side effects and reduced resistance development in the pathogen [27-28]. Study of antagonist as biological control agent has now become one of the most exciting and rapidly developing areas in plant pathology because it has great potential to solve many agricultural and environmental sectors.

As control measures, chemical fungicides, leaf extracts are successfully reported to control leaf spot and anthracnose diseases. So, it is necessary to find out immediately the effective and sustainable control measure of the disease in our country.

The formula in calculating the disease incidence and severity is:

$$\% \text{ Leaf infection} = \frac{\text{Number of leaves / Plants infected} \times 100}{\text{Total number of leaves/plants counted}}$$

Evaluation of selected fungicides and plant extracts in controlling Leaf Spot and Anthracnose of *Rauwolfia serpentina* in field condition

The experiment was conducted in the field plots of Botanical Garden, DU. During 2016-2017 to evaluate two fungicides Autostin 50 WDG and Tilt 250 EC at 100 ppm concentration and two plant extracts *Azadirachta indica* and *Citrus limon* at10% concentration against leaf spot and anthracnose of *Rauwolfia serpentina.* Control plants were treat with tap water (Table 1 and 2).

Table 1. Particulars of the fungicides used in the experiment.

SL .No.	Trade name	Formulation	Recommended Dose (ppm)	Ten times lesser of Recommended Dose (ppm)	Manufacturer
1.	Autostin	50 WDG	1000	100	BASF Bangladesh Ltd
2.	Tilt	250EC	500	50	Syngenta (BD)Ltd

Table 2. Particulars of the plant extracts used in the experiment

Sl. No.	Plant species	Used part	Family
1.	*Citrus limon* L.	Leaf	Rutaceae
2.	*Azadirachta indica* A. Juss	Leaf	Meliaceae

A total of four sprays were done at 15 days interval. Experimental design was RBD, having four replications. Data were recorded after 15 days of each spray. Final data were recorded after 15 days of last spray.

Analysis of data

Data on different parameters were analyzed following computer package MSTAT-C and means were compared using DMRT. The data were collected and evaluated by analysis of variance (ANOVA) by using STAR statistical program.

RESULTS AND DISCUSSION

In 2016 Autostin showed lowest (5.72) severity and (18.07) PDI in leaf spot infected plants. The same fungicide showed maximum (9.00) severity and (25.49) PDI in case of anthracnose infected plants. Infected leaves per plant was (15.25) and healthy leaves per plant was (34.6) (Table 3).

Table 3. Screening of fungicides and plant extract to control Leaf spot and anthracnose diseases of *Rauwolfia serpentina*during 2016.

Treatment	Leaf spot		Anthracnose		No. of	No. of
	Severity	PDI	Severity	PDI	Infected Leaves/plants	Healthy leaves/plants
Autostin 50WPG	5.72c	18.07 b	8.67b	25.49 ab	15.25b	34.60 b
Tilt 250EC	6.73 c	14.47 b	7.95 b	20.97 c	17.30 a	72.00 a
Azadirachta indica	7.84 ab	13.91 b	8.02 b	23.44 bc	14.15b	79.55 a
Citrus limon	6.48 c	19.75 b	7.74 b	22.61 bc	14.67b	75.05 a
Control	8.73 a	34.35 a	9.00 a	28.58 a	19.80a	37.50 b

Means followed by the same letter within a column did not differ significantly at 5% level by LSD.

Tilt showed lowest (6.73) severity and (14.47) PDI in leaf spot infected plants. The same fungicide showed (7.95) severity and (20.97) PDI in anthracnose infected plants. Infected leaves per plant was (17.3) and healthy leaves per plant was (72) (Table 3).

Azadirachta indica showed (7.84) severity and (13.91) PDI in leaf spot infected plants. The same leaf extract showed (8.02) severity and (23.44) PDI in anthracnose infected plants. Infected leaves per plant was (14.15). and healthy leaves per plant was (79.55) (Table 3).

Citrus limon showed (6.48) severity and (19.75) PDI in leaf spot infected plants. The same leaf extract showed (7.74) severity and (22.61) PDI in anthracnose infected plants. Infected leaves per plant was (14.67) and healthy leaves per plant was (75.05) (Table 3).

Control showed (8.73) severity and (34.35) PDI in leaf spot infected plants. Untreated control showed (9.00) severity and (28.58) PDI in anthracnose infected plants. Infected leaves per plant was (19.8).and healthy leaves per plant was (37.5).

In 2016 after final application of fungicides and plant extracts Autostin significantly reduced disease severity of leaf spot (5.72) over control (6.73). PDI of leaf spot was significantly reduced (13.91) by *A. indica* over control (34.35). Whereas disease severity of anthracnose was significantly reduced ((7.74) by *C. limon* over control (9,00). PDI of anthracnose was significantly reduced (20.93) by Tilt over control (28.58). Number of infected leaves per plant were significantly reduced ((14.15) by *A. indica* over control (19.8). by *A. indica*. Moreover poduction of healthy leaves per plant was significantly increased (79.55) by *A. indica* followed by *C. limon* (75.05) and Tilt (72.00) over control (37.50) (Plate 1. A-B).

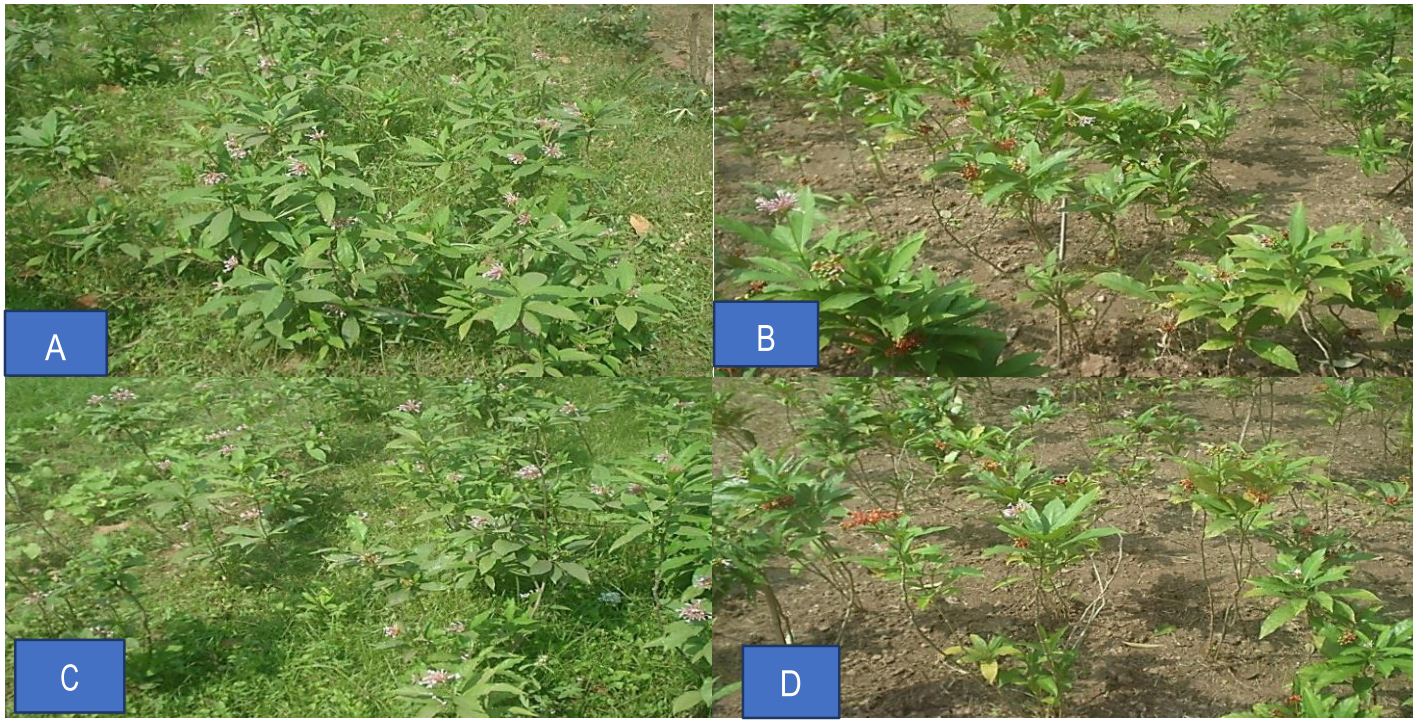

Plate 1. *Rauwolfia serpentina*: A-B. Field sub plots in 2016.
C-D. Field sub plots in 2017.

In 2017 Autostin showed lowest (8.73) severity and (26.94) PDI in leaf spot infected plants. The same fungicide showed maximum (6.55) severity and (25.49) PDI in case of anthracnose infected plants. Infected leaves per plant was (15.25) and healthy leaves per plant was (44.60) (Table 4).

Tilt showed lowest (7.95) severity and (20.97) PDI in leaf spot infected plants. The same fungicide showed (4.28) severity and (20.97) PDI in anthracnose infected plants. Infected leaves per plant was (17.30) and healthy leaves per plant was (47.45) (Table 4).

Table 4. Screening of fungicides and plant extract to control Leaf spot and anthracnose diseases of *Rauwolfia serpentina* in 2017.

Treatment	Leaf spot		Anthracnose		No. of	No. of
	Severity	PDI	Severity	PDI	Infected Leaves/plants	Healthy leaves/plants
Autostin	8.73a	26.94 ab	6.55b	25.49 ab	15.25b	44.60 b
Tilt	7.95b	20.97c	4.28b	20.97 c	17.30a	47.45b
Azadirachta indica	7.74b	23.44 bc	6.28 b	23.44 bc	19.80a	52.00a
Citrus limon	6.48 c	22.61 bc	6.37 b	22.61 bc	14.67b	40.80b
Control	9.0a	28.58 a	9.0 a	28.58 a	24.15b	41.45 b

Azadirachta indica showed (7.74) severity and (23.44) PDI in leaf spot infected plants. The same leaf extract showed (6.28) severity and (23.44) PDI in anthracnose infected plants. Infected leaves per plant was (19.80). and healthy leaves per plant was (52.00) (Table 4).

Citrus limon showed (6.48) severity and (22.61) PDI in leaf spot infected plants. The same leaf extract showed (6.37) severity and (22.61) PDI in anthracnose infected plants. Infected leaves per plant was (14.67) and healthy leaves per plant was (40.80) (Table 4).

Control showed (9.0) severity and (28.58) PDI in leaf spot infected plants. Untreated control showed (9.00) severity and (28.58) PDI in anthracnose infected plants. Infected leaves per plant was (24.15).and healthy leaves per plant was (41.45) (Table 4).

In 2017 after final application of fungicides and plant extracts *C. limon* significantly reduced disease severity of leaf spot (6.48) over control (9.0). PDI of leaf spot was significantly reduced (20.97) by Tilt over control (28.58). Whereas disease severity of anthracnose was significantly reduced (4.28) by Tilt over control (9.00). PDI of anthracnose was significantly reduced (20.97) by Tilt over control (28.58). Number of infected leaves per plant were significantly reduced (14.67) by *C. limom* over control (24.15). Moreover Production of healthy leaves per plant was significantly increased (52.00) by *A. indica* followed by Tilt (47.45) and Autostin (44.60) over control (41.45) (Plate1. C-D and Plate 2).

Rauwolfia serpentina

Rauwolfia serpentina has long being used in India for the treatment of snakebites, hypertension, high blood pressure and mental illness. Eextensive work has been undertaken in recent years at different parts of the Indian subcontinent to explore the use of this plant in the treatment of different ailments by the tribals or the aboriginals as a part of their ethnomedical system. Different ethnic groups use this plant to treat snake, insect and animal bite, mental illness, schizophrenia, hypertension, blood pressure, gastrointestinal diseases, circulatory disorders, pneumonia, fever, malaria, asthma, skin diseases, scabies, eye diseases, spleen diseases, AIDS, rheumatism, body pain, veterinary diseases etc. This plant is also being used to prepare fermented food products. Similarly in Bangladesh the plant is used by ethnic people and also used as herbal medicine.

A. In vivo experiment indicates that Autostin and Tilt at 100 ppm concentration and plant extracts of *indica* and *C. limon* at 10% concentration significantly reduced disease severity and PDI of leaf spot and anthracnose of *Rauwolfia serpentina* and subsequently increase the healthy leaf production in the field condition both the years of studied.

CONCLUSION

International Union for the Conservation of Nature and Natural Recourses (IUCN) has placed this plant under endangered status. Considering the importance of the plant it is necessary to save the plat immediately. Leaf spot and anthracnose drastically damaged the leaves of *R. serpentina*. Present investigation is the first approach of controlling the diseases of the aforesaid plant in Bangladesh. Findings of this research work will be helpful for designing a proper management of leaf spot and anthracnose diseases of *R. serpentina*. Application of Autostin and Tilt at 100 ppm concentration may be commercially used for managing leaf spot and anthracnose of *Rauwolfia serpentina*. For more confirmation the above mentioned fungicides also need to 2-3 years trial in field condition. In small scale gardening or those person who want to maintain the plants in the yard as medicinal purposes, *Azadirachta indica* and *Citrus limon* at 10% concentration.

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