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BIOEFFICACY OF COMMON BOTANICALS FOR THE MANAGEMENT OF ANTHRACNOSE DISEASE OF STRAWBERRY (*FRAGARIA ANANASSA*)

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ABSTRACT

An experiment was conducted during 2020-21 and 2021-22 in the Department of Plant Pathology, Biswanath College of Agriculture, Biswanath Chariali, Assam, to evaluate the efficacy of common botanicals for managing anthracnose disease of strawberries*in vivo*. In the *in vitro* experiment, six common botanicals *viz*., Hena, Holy basil, Garlic, Neem, Datura and Mint were evaluated against the pathogen (*Colletotrichum acutatum*) causing anthracnose disease of strawberries at different concentrations of 10%, 15%, 20% and 25%. Amongst all, the aqueous extracts of Datura resulted in maximum mean inhibition (58.17%) of radial mycelial growth of *C. acutatum* over control at all the concentrations followed by Garlic and Hena extract with 53.03% and 46.78% mean inhibition of radial mycelial growth, respectively. In the field experiment, the plot treated with Datura extract (25%) resulted in the highest reduction of disease incidence (74.86%) and disease severity (65.75%) over control with the highest yield (182.39 qha^{-1}) indicating27.25% increase of strawberry yield over control.

Key words : Anthracnose, Aqueous extract, Botanicals, Percent inhibition, Strawberry.

Introduction

Strawberry (*Fragaria ananassa*) is an important fruit crop grown commercially in the temperate and subtropical regions of the world. It is a low perennial creeping herb belonging to the family Rosaceae. The total production of strawberries in the world was 9.6 million tons in the year 2022. China was the highest producer in the world, with 40% of total production. In India, it is cultivated over 3000 ha with an annual production of 19840 MT during 2020-21. Strawberry cultivation in India gained traction in the late 1960s in Himachal Pradesh and the hills of then-Uttar Pradesh (now Uttarakhand).

Anthracnose is one of the most severe diseases of Strawberries (*Fragaria ananassa*) attacking all parts of the plants*viz.*, leaves, petioles, runner, crown, roots, flower, and fruits. Three species have been reported as causal agents of strawberry anthracnose; *viz*., *Colletotrichum acutatum*, *C. fragariae* and *C. gloeosporioides* (Maas, 1998; Smith, 1998). The primary cause of anthracnose fruit rot and irregular leaf spots is *C. acutatum*. *C. fragariae* and *C. gloeosporioides* can infect any above ground part of the plant and cause crown rot, fruit rot and leaf spots. *C. gloeosporioides* and *C. acutatum* are found on a wide variety of hosts around the world, whereas *C. fragariae* is found on a small number of hosts (MacKenzie *et al*., 2009). Anthracnose causes up to 80% death of plants in nurseries and more than 50% loss in the strawberry fields (Sreenivasaprasad and Talhinhas, 2005).

Synthetic fungicides are often applied for management of anthracnose disease. The frequent use of chemicals may harm human health as well as the environment. The indiscriminate use of synthetic fungicides and pesticides has created various typesof environmental and toxicological problems for human health (Varma and Saran, 2019). This necessitates a

needfor safe and effective alternatives. One of the feasible solutions is to use plant extracts as biofungicides. Biological control seems to be the best alternative to plant disease control (Svetlana *et al*, 2010). Plants contain thousands of constituents and are a valuable source of new and biologically active molecules with antimicrobial properties. Natural plant extracts are safe for human consumption as they are easily biodegradable leave no toxic residues on fruits and exhibit a stimulating effect on plant metabolism.

Materials and Methods

Isolation and purification of the causal organism

The diseased specimens showing typical symptomson fruits were collected from the farmer's field, Pabhoi, Biswanath Chariali and washed thoroughly with tap water first and then with distilled water. Small portions of infected parts containing both healthy and diseased tissues were cut into 0.5cm pieces with the help of a sterilized scalpel blade. These pieces were then surface sterilized with 1 percent sodium hypochlorite (NaOCl) for 1 minute rinsed aseptically in three changes of sterilized distilled water and dried in sterilized blotting paper. The surface sterilized pieces were then transferred aseptically to Petri dishes containing 2 percent sterilized Potato Dextrose Agar (PDA) with the help of a sterilized needle and incubated at 28±2°C for 7-8 days in BOD incubator. The Petri dishes were examined regularly for fungal growth and then transferred aseptically to potato dextrose agar slants. The fungal culture was purified by the hyphal tip method and identified based on the morphological characteristicsand by referring to the "Illustrated Genera of Imperfect Fungi" (Barnett and Hunter, 1972) and reference book (Ainsworth, 1971) and by sequencing the ITS region of fungal isolate followed byPhylogenetic analysis

Preparation of plant extracts

The cold-water extract method was used for the preparation of plant extracts by following the procedure described by Shekhawat and Prasad (1971). Fresh plant materials (*eg.*, Leaves, rhizomes, bulbs) of healthy plants are collected and washed thoroughly in tap water followed by sterile distilled water. Hundred grams of washed plant parts are ground in pre-chilled mortar and pestle by adding an equal amount (100 ml) of sterilized distilled water (1:1 w/v). After grinding, the extract was filtered through muslin cloth and centrifuged at 10,000 rpm for 20 minutes at room temperature. The supernatant was used as a standard solution (100%).

Evaluation of botanicals against *Colletotrichum acutatum in vitro*

Six promising botanicals *viz.*; Garlic (*Allium sativum*). Mint (*Mentha piperita*), Datura (*Datura stramonium*), Hena (*Lawsonia inermis*), Holy basil (*Ocimum sanctum*), Neem (*Azadirachta indica*) selected out of 20 botanicals based on preliminary screening at 25 % concentration were further evaluated against *Colletotrichum acutatum* at four different concentrations (10, 15, 20 and 25 percent) by following poison food technique (Nene and Thapliyal, 2000). The percent inhibition of *C. acutatum* was calculated by using the formula given by Vincent (1927)

Inhibition (%) = C-T/C × 100

Where,

C = Diameter of fungal colony (cm) in control plate

T = Diameter of fungal colony (cm) in treated plate

Evaluation of botanicals against *Colletotrichum acutatum in vivo*

Field trials were conducted during the rabi season of 2021-22 at the Post Graduate experimental field, Biswanath College of Agriculture, Biswanath Chariali with three replications to test the effect of six plant extracts against the anthracnose disease of strawberries. The experiment was conducted in Randomized Block Design (RBD) with a plot size of 2 × 1 m^2 with a spacing of 45 cm × 45 cm. The individual plots were prepared in a raised bed (15 cm high and 1.8 m wide) mulched with black plastic (30 micron thickness) by following all thepractices as recommended in Package of Practices for horticultural crops of Assam published jointly by AAU, Jorhat, and Department of Agriculture, Government of Assam. The following treatments were undertaken for the field experiments based on the performance of the *in vitro* experiments.

T_1: Hena extract (25%)

T_2: Holi basil extract (25%)

T_3: Garlic extract (25%)

T_4: Neem extract (25%)

T_5: Datura extract (25%)

T_6: Mint extract (25%)

T_7: Control

Method of application of plant extracts

The aqueous plant extracts of the six botanicals were prepared (25% concentration) and applied to the strawberry saplings first as a prophylactic spray, second spray at the initial appearance of the disease symptom, and third at 10 days after the second spray.

Assessment of disease incidence and disease severity

The anthracnose disease infection (DI) was assessed at the fruit maturity stage in each plot. Disease infection was measured by using the following formula.

$DI = n/N \times 100\%$

Where, DI = disease infection, n = number of fruits showing symptoms of anthracnose, N = number of fruits observed in each treatment.

Disease severity was measured by using the following formula

$DS = \Sigma (n\ xV)/Z\ xN \times 100\%$

Where, DS = Disease severity, n = number of fruits in the same category at each attack, V = score of each category of attack, N = number of fruits observed, Z = highest score attack. The disease score was measured as follows.

0 = There was no attack,

1 = 0 < x < 20 % of the fruit was attacked

2 = 20 < x < 40 % of the fruit was attacked

3 = 40 < x < 60 % of the fruit was attacked

4 = 60 < x < 80 % of the fruit was attacked

5= 80 < x < 100% of the fruit was attacked

Statistical analysis

The experimental data collected were analyzed statistically for its significance of difference by the normal statistical procedure adopted for Completely Randomized Design and Randomized Block Design and interpretation of data was carried out in accordance with Gomez and Gomez (1984). The observed data was analyzed by OPSTAT package of programs (Sheoran, 2006) after angular transformation. The treatment means were compared by Duncan's Multiple Range Test (DMRT).

Results and Discussion

Identification of the causal organism

The causal organism was identified to be *Colletotrichum acutatum* based on the morphological characteristics and by referring to the "Illustrated Genera of Imperfect Fungi" (Barnett and Hunter, 1972) and reference book (Ainsworth, 1971) and by sequencing the ITS region of fungal isolate and Phylogenetic analysis (Fig. 1).

Evaluation of botanicals at different concentrations *Colletotrichum acutatum in vitro*

The Datura extractwas found most effective against *C. acutatum* at all the concentrations, however, the efficacy increased with the increase of concentration. Datura extract resulted in maximum inhibition (88.40%) of radial mycelial growth of *C. acutatum* over control at 25 % concentration followed by Garlic and Hena extract with 85.02 % and 81.39 % inhibition of radial mycelial growth, respectively (Tables 1 and 2).

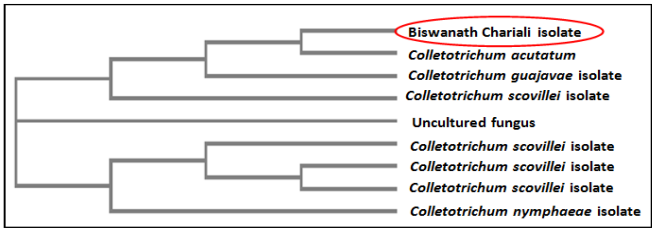

Fig. 1 : Phylogenetic tree showing genetic relationship of *C. acutatum* causing anthracnose disease of strawberries.

The inhibition of the pathogen may be due to the presence of antifungal properties in Datura. Gurjar *et al.* (2012) also reported similar observations and mentioned that the highest inhibition exhibited by Daturaextract against the pathogen could be due to the presence of alkaloids called Hyoscymine and scopolamine. Aqueous extract of Daturahas insecticidal properties, while ethanol extract has antimicrobial properties.

In the present investigation, Garlicwas found second most promising botanical next to Daturaamongst all the six selected botanicals. Bulb extract of Garliccontains Allicin, which belongs to the class solfoxide and can be used as an antifungal and antibacterial product (Gurjar *et al.*, 2012). A similar observation was also reported by Mukherjee *et al.* (2011), who found that garlic extract at 70 percent concentration significantly inhibited the radial mycelial growth *C. gloeosporioides*, the causal agent of anthracnose of mango.

The results of the present investigation were in agreement with the findings of Jayalakshmi *et al.* (2018) who reported that *D. stramonium* leaf extract caused the highest inhibition (61.70%) of the pathogen causing anthracnose disease of pomegranate at 30 percent concentration which was followed by *Allium sativum* bulb extract with 50% inhibition of the pathogen at 30 percent concentration.

Evaluation of botanicals against anthracnose disease of strawberries *in vivo*

Effect of botanicals on Disease incidence (DI) and disease severity (DS)

All the botanicals significantly reduced the disease incidence and disease severity of anthracnose disease at 25 % concentration under field conditions. The plot treated with aqueous extract of Datura (25%) recorded the

Table 1 : Effect of botanicals at different concentrations (10, 15, 20, 25%) on radial growth of mycelia of *Colletotrichum acutatum in vitro*.

Treatments	Radial growth of mycelia (mm)				Mean
	10	15	20	25	
T_1: Hena	53.112	49.474	38.276	23.06	40.98
T_2: Holi basil	57.27	54.8	45.44	27.68	46.297
T_3: Garlic	48.774	46.587	35.168	20.588	37.779
T_4: Neem	55.045	53.11	41.882	26.975	44.253
T_5: Datura	45.899	44.18	32.058	18.018	35.039
T_6: Mint	58.69	56.904	48.428	29.32	48.335
T_7: Control					
	Plant extracts (P)		Concentrations (C)		Interaction (P X C)
SEd ±	0.323		0.264		0.646
CD (p=0.05)	0.642		0.524		1.284
CV (%)					2.421

Data are the mean of five replications.

recorded in the plot treated with aqueous extract of Daturafollowed by Garlic (62.87%) and Hena (44.92%,), respectively. A similar trend was also observed for disease severity where the plot treated withthe aqueous extract of Daturarecorded a maximumreduction (65.75%) of DS over control. Botanicals found effective next to Datura were Garlic and Henawith (54.11%) and (45.89%) reduction of severity over control, respectively (Table 3).

The reduction of disease incidence and severity of anthracnose in the case of treatment with Datura extract might be due to the presence of antimicrobial chemicals like alkaloids (Hyoscymine, Scopolamine) and due to the induction of systemic resistance in the plant.

The present observations were in conformity with the findings of Vijayan (1989) who also reported that the inhibitory effect of plant extracts on the reduction of disease incidence and disease severity may be due to their direct toxic effect on the pathogen. Amadioha (2000) mentioned that the active principles present in plant

Table 2 : Effect of botanicals at different concentrations (10, 15, 20, 25%) on inhibition of mycelia growth of *Colletotrichum acutatum in vitro*.

Treatments	Inhibition of mycelia growth over control (%)				Mean
	10	15	20	25	
T_1: Hena	21.953(27.917)	30.162(33.256)	53.621(47.058)	81.398(64.463)	46.783(43.173)*
T_2: Holi basil	13.650(21.649)	19.309(26.022)	38.653(38.422)	73.918(59.268)	36.383(36.34)
T_3: Garlic	30.963(33.788)	36.228(36.987)	59.904(50.693)	85.018(67.226)	53.028(47.173)
T_4: Neem	18.041(25.081)	22.697(28.432)	46.124(42.759)	75.121(60.062)	40.496(39.084)
T_5: Datura	37.078(37.491)	41.295(39.965)	65.931(54.274)	88.401(70.088)	58.176(50.454)
T_6: Mint	10.982(19.23)	15.194(22.822)	32.353(34.641)	71.012(57.404)	32.385(33.524)
T_7: Control					
	Plant extracts (P)		Concentrations (C)		Interaction(P X C)
SEd ±	0.469	0.383	0.938		
CD (p=0.05)	0.932	0.761	1.864		
CV (%)			3.56		

*Data within the parenthesis are arcsine transformed data. Data are the mean of five replications.

lowest disease incidence (9.64%) and disease severity (16.67%) among all the botanicals which was followed by Garlic with 14.24 % DI and 21.13% DS and Hena with 22.33% DI and (26.33%) DS, respectively. The control plot recorded the highest disease incidence (38.36%) and disease severity (48.67%).

In regards to the percent reduction of disease incidence and disease severity over control, the maximum reduction of disease incidence (74.86%) over control was

extracts may act on the pathogen directly. The plant extracts may induce systemic resistance in host plants resulting in reduction of disease development (Kagale *et al.*, 2004).

Effect of botanicals on plant height, Fruit number, Fruit length and Fruit diameter

The botanicals have no significant effect on plant height; however, significant effects of botanicals were

Table 3 : Effect of botanicals on percent disease incidence (DI) and disease severity (DS) under field conditions.

Treatments	DI (%)	Reduction of DI over control (%)	DS (%)	Reduction of DS over control (%)
T_1: Hena	21.13(27.34)^{d}	44.92	26.33(30.86)^{d}	45.89
T_2: Holi basil	28.25(32.09)^{bc}	26.34	36.33(37.06)^{b}	25.34
T_3: Garlic	14.24(22.14)^{e}	62.87	22.33(28.19)^{e}	54.11
T_4: Neem	25.28(30.176)^{c}	34.08	32.33(34.64)^{c}	33.56
T_5: Datura	9.64(18.084)^{f}	74.86	16.67(24.07)^{f}	65.75
T_6: Mint	31.64(34.218)^{b}	17.52	38.67(38.44)^{b}	20.54
T_7: Control	38.36(38.26)^{a}		48.67(44.23)^{a}	
SEd ±	1.26		0.98	
CD (P=0.05)	2.75		2.16	

*Data within the parenthesis are arcsine transformed data. Dataare the mean of three replications.

Table 4 : Effect of botanicals on the growth and yield attributing parameters of strawberries under field conditions.

Treatments	Plant height (cm)	No of fruits/ plant	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Yield (g/plant)	Yield (q/h)
T_1: Hena (*Lawsonia inermis*)	30.54	10.38^{c}	2.97^{c}	2.19^{c}	24.87^{bc}	332.67^{c}	164.28^{c}
T_2: Holy basil (*Ocimum* spp)	29.98	8.17^{de}	2.42^{d}	1.72^{d}	20.34^{d}	308.67^{d}	152.43^{d}
T_3: Garlic (*Allium sativum*)	30.58	14.02^{b}	3.24^{b}	2.32^{b}	26.63^{b}	352.68^{b}	174.16^{b}
T_4: Neem (*Azadirachta indica*)	30.33	9.76^{cd}	2.48^{d}	1.81^{d}	21.52^{cd}	298.67^{de}	147.49^{de}
T_5: Datura (*Datura stramonium*)	29.81	16.81^{a}	3.46^{a}	2.54^{a}	32.73^{a}	369.34^{a}	182.39^{a}
T_6: Mint (*Mentha piperita*)	29.97	7.91^{e}	1.79^{e}	1.46^{e}	19.16^{d}	287.33^{e}	141.89^{e}
T_7: Control	30.51	6.13^{f}	1.48^{f}	1.28^{f}	15.52^{e}	268.67^{f}	132.68^{f}
SEd ±		0.764	0.05	0.05	1.64	6.38	3.15
CD(P=0.05)	NS	1.66	0.11	0.10	3.58	13.92	6.87

Table 5 : Economic on the management of anthracnose disease of strawberries (1 ha).

Treatments	Cost of cultivation (Rs)	Yield (q/h)	Gross income (Rs)	Net Return (Rs)	CBR
T_1: Hena (*Lawsonia inermis*)	1171327	15.42^{d}	3285600	2114273	1:2.81
T_2: Holy basil (*Ocimum* spp)	1171327	21.62^{c}	3048600	1877273	1:2.60
T_3: Garlic (*Allium sativum*)	1194326	12.41^{e}	3483200	2311873	1:2.92
T_4: Neem (*Azadirachta indica*)	1174248	20.62^{c}	2949800	1778473	1:2.51
T_5: Datura (*Datura stramonium*)	1171327	9.63^{f}	3647800	2476473	1:3.12
T_6: Mint (*Mentha piperita*)	1184628	24.12^{b}	2837800	1666473	1:2.39
T_7: Control	1168946	82.81^{a}	2653600	1483600	1:2.27

Data are the mean of three replications.

observed forthe number of fruitsplant^{-1}, fruit length and fruit diameter. In regards to yield attributes, the plot treated with Datura extract (25%) recorded the highest numbers of fruit plant^{-1}, fruit length, and fruit diameter (16.81, 3.46 cm, 2.54 cm) followed by Garlic (14.02, 3.24 cm, 2.32 cm) and Hena (10.38, 2.97 cm, 2.19 cm), respectively (Table 4).

Effect of botanicals on Fruit weight and fruit yield

The plot treated with aqueous extract of Datura (25%) recorded the highest fruit weight (32.73 g) and fruit yield (369.34 g plant^{-1}), which was followed by Garlic (26.63 g, 352.68 g plant^{-1}) and Hena (24.87 g, 332.67 g plant^{-1}), respectively. However, with respect to fruit weight, the result of Hena was statistically at par with that of Garlic (Table 4).

The yield data presented in Table 4 displayed that the plot treated with Daturaextract (25%) recorded the highest yield (182.39 qha^{-1}) followed by Garlic (174.16

qha^{-1}) and Hena (164.28 qha^{-1}), respectively. In regards to the percent increase of yield over control, the Datura extract (25%) treated plot recorded the highest increase of yield (27.25%) over control with a net profit of Rs 2476473 and B: C of 3.12, respectively (Tables 4 and 5).

The highest yield attributes and yield obtained in the *D. stramonium* extract treated plot could be due to reduction in disease incidence and severity, and may be due to induction of systemic resistance in the plants.

Conclusion

Aqueous extract of Datura was found to be the most efficient in inhibiting the mycelial growth of *C. acutatum in vitro* at all the concentrations (10, 15, 20 and 25%), however, the highest inhibition (88.40%) over control was observed at 25% concentration. In the field experiment also, 25 % Datura extract resulted in the highest reduction of disease incidence (74.86 %) and disease severity (65.75%) over control with the highest yield (182.39 qha^{-1}) and 27.25 % increase in strawberry yield over control. Aqueous extract of Datura could be considered an effective, eco-friendly alternative to a chemical approach for themanagement of anthracnose disease of strawberries.

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