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Evaluation of common botanicals against *Colletotrichum acutatum* causing anthracnose disease of strawberry (*Fragaria ananassa*)

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Abstract
Anthracnose is one of the most important fungal diseases of strawberry affecting almost all parts of the plant including leaves, petioles, runners, flower stalk and fruits. Chemicals are not encouraged to manage the disease as the fruit is consumed directly which may lead to various health hazards which demands for an eco-friendly alternative method of disease management. The present investigation was conducted to evaluate the six promising botanicals *viz*. Hena, Holy basil, Garlic, Neem, Datura and Mint against the pathogen (*C. acutatum*) at different concentrations *viz*. 10%, 15%, 20% and 25% *in vitro*. Among all the botanicals, the aqueous extract of Datura (25%) resulted maximum inhibition (88.37%) of radial mycelial growth of *C. acutatum* over control followed by Garlic and Hena extract with 85.01% and 81.38% inhibition of radial mycelial growth of the fungus over control, respectively.

Keywords: Strawberry, anthracnose, aqueous extract, botanicals, percent inhibition

Introduction
Strawberry (*Fragaria ananassa)* is one of the important fruit crops and is grown commercially mainly in the temperate and sub tropical regions of the world. It is a low perennial creeping herb belongs to the family Rosaceae. The total production of strawberries in the world was 9.2 million tons in the year 2017. China was the highest producer in the world, with 40% of total production. In India, it is cultivated over 2000 ha with an annual production of 18000 MT during 2019-20 (Anon, 2020) [2]. Strawberry cultivation in India gained traction in the late 1960s in Himachal Pradesh and the hills of then-Uttar Pradesh (now Uttarakhand).

The strawberry plant is infected by many plant pathogens *viz*. fungi, bacteria, and viruses. Among all, fungi cause some of the most serious diseases of strawberry. Anthracnose disease is the most serious fungal disease of strawberry attacking all parts of the plant like leaves, petioles, runner, crown, roots, flower and fruits. Three species have been reported as causal agents of strawberry's anthracnose: *Colletotrichum acutatum*, *C. fragariae,* and *C. gloeosporioides* (Maas, 1998, Smith, 1998). The primary cause of anthracnose fruit rot and irregular leaf spot is *C. acutatum. C. fragariae and C. gloeosporioides* can infect any part of the plant above ground and cause anthracnose crown rot, anthracnose fruit rot, and anthracnose leaf spots. *C. gloeosporioides* and *C. acutatum* is 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) [6]. Anthracnose causes up to 80% of death in nurseries and more than 50% loss of performance in the strawberry fields (Sreenivasaprasad and Talhinhas, 2005) [13].

The management of anthracnose disease of strawberry has been almost exclusively based on the application of chemical pesticides. The application of fungicides over a long period may result in developing resistance by the pathogen, making them ineffective and other fungicides must be used for effective control of the disease. The indiscriminate use of synthetic fungicides and pesticides has created various types of environmental and toxicological problems to the human health (Varma and Saran, 2019) [15].

Application of biological agents and botanicals against plant pathogenic microorganisms for the management of several plant diseases has grained importance in recent years. Biological control seems to be the best alternative to controlling of plant disease (Svetlana *et al*., 2010) [14]. Plants contain thousands of constituents and are a valuable source of new and biologically active molecules with antimicrobial properties. The use of natural plant extracts allows you to avoid chemical preservatives.

Further, these are safe and effective in view of their systemic action and lack residual effect, easily biodegradable and exhibit stimulating effect on plant metabolism.

Materials and Methods

Collection of disease specimens

The disease specimens were collected from the farmer's field, Pabhoi, Biswanath Chariali showing typical symptoms of anthracnose disease on strawberry fruits. The samples were brought to the laboratory for critical observation and investigations (study of symptoms, isolation and description of the pathogen) and for further studies.

Isolation and purification of the causal organism

The diseased specimens (infected fruits) showing typical symptom were first washed thoroughly with tap water and then rinsed with distilled water for further studies.

Small portion of infected parts containing healthy as well as diseased tissues were cut in to 0.5cm pieces with the help of sterilized scalpel blade. These pieces were then surface sterilized with 1 percent sodium hypochlorite (NaOCl) for 1 minute and 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 per cent sterilized Potato Dextrose Agar (PDA) with the help of a sterilized needle and incubated at 28±2 °C for 7-8 days in BOD. The petri dishes were examined at regular time intervals for fungal growth and then transferred aseptically to potato dextrose agar slants. The fungal culture was purified by single spore isolation method and the fungus isolated during the present study was identified based on the spore morphology and colony characters of the fungus by referring to the "Illustrated genera of Imperfect fungi" (Barnett and Hunter, 1972) [3] and reference book (Ainsworth, 1971) [1].

Pathogenicity test

The pathogenicity of the pathogen causing anthracnose disease was confirmed by proving Koch's postulates. Spore suspension of 1ml was prepared from the 7 days old culture of already purified fungal isolate. The healthy leaves of strawberry plant were selected and surface sterilized 70% ethyl alcohol. Injury was made on leaves by gentle scraping with sand paper. The leaves were inoculated by the spore suspension with the help of cotton, and then the plant is covered with bell jar. When the typical symptoms of the disease were observed on the inoculated plants, the pathogen was reisolated on PDA and its identity was ascertained by comparing it with the original culture of the pathogen and Koch's postulates was established.

Preparation of plant extracts

The cold water extract method was used for preparation of plant extracts by following the procedure described by Shekhawat and Prasad (1971) [11] with certain modifications. 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 equal amount (100 ml) of sterilized distilled water (1:1 w/v). After grinding, the extract was filtered through muslin cloth before being centrifuged at 10,000 rpm for 20 minutes at room temperature. The supernatant was used as a standard plant extract solution (100%). The plant extracts were further filtered through bacterial membrane filter (Ran Disc, PVDF 0.22 µm) under aseptic condition.

Preliminary screening of botanicals against *Colletotrichum acutatum in-vitro*

Aqueous plant extracts of twenty selected botanicals were prepared and screened for their antifungal activity against the pathogen *C. acutatum* at 25 per cent concentration by following 'poison food technique' (Nene and Thapliyal, 2000). PDA medium was prepared and poured in 250 ml Erlenmeyer flasks and sterilized. 25 ml of plant extracts from 100 per cent aqueous plant extracts of each botanical were aseptically added to 75 ml molten PDA in flasks respectively to obtain the final 25 percent concentration of plant extracts in the medium and poured aseptically into sterilized Petri dishes at the rate of 20 ml per plate and allowed to solidify. Mycelial disc of 5 mm diameter from the edge of actively growing 10 days old culture of *C. acutatum* was separately cut with the help of a sterilized cork borer and simultaneously placed on the centre of the Petri dishes and incubated in the BOD at 28±2 °C for seven to ten days. Five replications were maintained for each treatment. The Petri dishes containing PDA medium inoculated with the *C. acutatum* alone served as control. The radial mycelial growth of the test fungus was measured and compared with radial growth of the (untreated) control. The percent inhibition of *C. acutatum* was calculated by adopting the following formula.

Inhibition (%) = C-T/C X 100

Where,
C = Diameter of fungus colony (cm) in control plate
T = Diameter of fungus colony (cm) in treated plate

Based on the percent inhibition of the radial mycelia growth of the target pathogen, six promising botanicals were selected for further studies.

Evaluation of botanicals at different concentrations against *C. acutatum in vitro*

Six most promising botanicals *viz.* Garlic (*Allium sativum*). Mint (*Mentha piperita*), Datura (*Datura stramonium*), Hena (*Lawsonia inermis*), Holy basil (*Ocimum sanctum*), Neem (*Azadirachta indica*) so selected from the preliminary screening were further tested against *Colletotrichum acutatum* in four different concentrations *viz*. 10, 15, 20 and 25 per cent, respectively by following poison food technique as described above. The radial mycelial growth of *C. acutatum* on treated plates were measured and compared with the untreated control plates. The percent inhibition of *C. acutatum* was calculated by adopting the formula mentioned above.

Result and Discussion

Pathogenecity test

The pathogenicity of the pathogen causing anthracnose disease was confirmed by proving Koch's postulates. The leaves inoculated with the pure culture of the fungus showed typical symptoms of the anthracnose disease as dark brown to black lesion on the leaves after seven days of inoculation. After appearance of symptoms the pathogen was re-isolated on PDA medium from the diseased portions and healthy leaves were reinoculated with the isolate grown on PDA.

Plate 1: Pathogenecity test conducted on strawberry plant

Effect of botanicals on mycelial growth of *Colletotrichum acutatum*

Preliminary screening of botanicals against *Colletotrichum acutatum in-vitro*

The results (table 1 and fig 1) revealed that aqueous extracts of all the botanicals significantly inhibited the mycelial growth of *Colletotrichum acutatum* at 25% concentration. Among all the twenty botanicals tested, *Datura stramonium* recorded highest inhibition (88.38 %) of radial mycelial growth of *C. acutatum* over control followed by *Allium sativum*, *Lawsonia inermis*, *Azadirachta indica*, *Ocimum sanctum* and *Mentha piperita* with 85.01%, 81.38%, 75.09%, 70.86% and 67.60% inhibition, respectively. The aqueous extracts of *Catharanthus roseus* and *Ricinus communis* resulted the lowest inhibition (10.86%) of radial mycelial growth of *C. acutatum in vitro*. Six numbers of most promising botanicals *viz. Datura stramonium*, *Allium sativum*, *Lawsonia inermis*, *Azadirachta indica*, *Ocimum sanctum* and *Mentha piperita* were selected based on their antifungal activity against *C. acutatum* at 25 percent concentration for further evaluation.

Table 1: Preliminary screening of botanicals against *Colletotrichum acutatum* at 25%

Botanicals	Mycelial growth (mm)	% inhibition over control
T1: Parthenium	34.62i	58.19
T2: Ginger	41.23g	50.21
T3:Turmeric	64.24d	22.42
T4:Garlic	12.41n	85.01
T5: Pongam	61.43e	25.82
T6:Onion	52.23f	36.93
T7:Black pepper	41.16g	50.30
T8: Hena	15.42m	81.38
T9: Bael	64.24d	22.42
T10: Citronella	72.14bc	12.88
T11:Neem	20.63l	75.09
T12:Periwinkle	73.82b	10.86
T13: Cinnamon	52.26f	36.89
T14: Datura	9.62o	88.38
T15: Spanish flag	71.23c	13.98
T16:Castor	73.82b	10.86
T17:Clove	37.45h	54.78
T18: Mint	26.83j	67.60
T19:Pipoli	61.12e	26.19
T20: Holy basil	24.13k	70.86
T21: Control	82.81a	
S.Ed ±	0.91	
CD(P=0.05)	1.78	

Data are mean of five replications

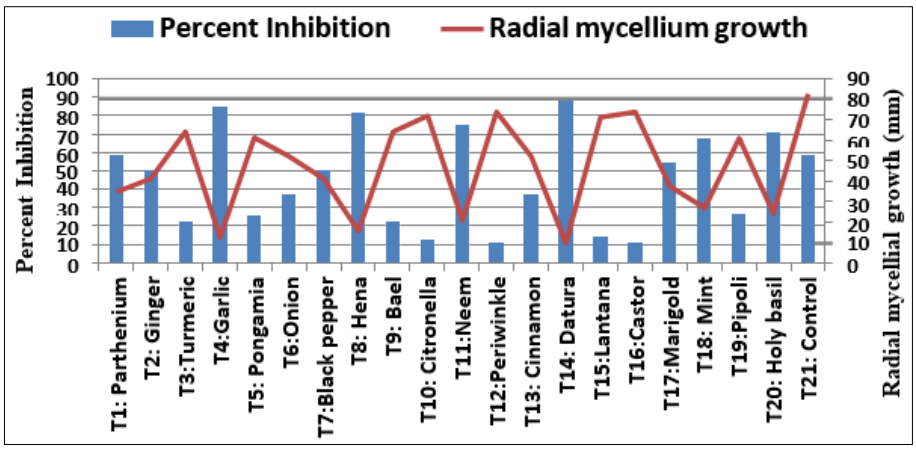

Fig 1: Preliminary screening of botanicals against *Colletotrichum acutatum* at 25%

Evaluation of botanicals at different concentrations *C. acutatum in vitro*

The data presented in Table 2 and Fig. 2 revealed that among all the botanicals at 10 per cent concentration, *Datura stramonium* was found most effective against *C. acutatum* in reducing highest inhibition (37.65%) of radial mycelial growth over control followed by *Allium sativum* and *Lawsonia inermis* with an inhibition of 31.64% and 21.97% of radial growth of *C. acutatum* over control, respectively.

At 15% concentration also all the botanicals significantly inhibited the radial mycelia growth of *C. acutatum*, highest being the *Datura stramonium* with 41.30% inhibition followed by *Allium sativum* and *Lawsonia inermis* with 36.23% and 30.21% inhibition, respectively.

In respect to percent inhibition at 20% concentration, aqueous extracts of *D. stramonium* showed the highest inhibitory effect on radial mycelial growth of *C.acutatum* followed by *Allium sativum* and *Lawsonia inermis* as compared to control. *D. stramonium* recorded the highest inhibition (65.91%) of radial mycelial growth of *C.acutatum* over control. This was followed by *Allium sativum and Lawsonia inermis* with 59.86% and 53.59% inhibition, respectively.

Among all the six botanicals, *Datura stramonium* was found to be most effective botanical amongst at 25% concentration. At 25% concentration, *D. stramonium* provided the highest percent inhibition (88.37%) over control which was followed by *Allium sativum and Lawsonia inermis* with 85.01% and 81.38% inhibition, respectively over control. Lowest percent inhibition (70.87%) was provided by *Mentha piperita* at 25% concentration.

At all the concentration *D. stramonium* was found most effective against *C. acutatum*, however 25% concentration

provided highest inhibition against the pathogen. It may be due to presence of antifungal property in *D. stramonium*. Gurjar *et al.* (2012) [4] also reported similar observation and they mentioned that the highest inhibition exhibited by *D. stramonium* extract against the pathogen could be due to presence of alkaloid called Hyoscymine, Scopolamine. Aqueous extract of *D. stramonium* has insecticidal properties, while ethanol extract has antimicrobial properties. Leaves extracts have traditionally been used for injuries, wounds, bleeding, and pain. Flower petal juice is used to treat ear pain, and the seeds are used to treat cough, fever, and asthma (Sayyed and Shah, 2014) [10].

In the present investigation *A. sativum* was found second most promising botanical next to *D. stramonium* amongst all the six selected botanicals. Bulb extract of *Allium sativum* contains Allicin which belongs to class solfoxide that can be use as antifungal and antibacterial product (Gurjar *et al.*, 2012) [4]. Similar observation was also reported by Mukherjee *et al.* (2011) [8] who found that garlic extract at 70 per cent concentration significantly inhibited the radial mycelial growth *C. gloeosporioides*, the causal agent of anthracnose of mango.

The results of present investigation were in agreement with the findings of Jayalakshmi *et al.* (2018) [5] who reported that *D. stramonium* leaf extract caused 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.

Table 2: Evaluation of botanicals (10, 15, 20 and 25%) on inhibition of radial mycelial growth of *Colletotrichum acutatum in vitro*

Treatments	Inhibition of mycelia growth over control (%)				Mean
	10	15	20	25	
T1: Hena	21.953	30.162	53.621	81.398	46.783
	(27.917)	(33.256)	(47.058)	(64.463)	(43.173)*
T2: Holy basil	13.650	19.309	38.653	73.918	36.383
	(21.649)	(26.022)	(38.422)	(59.268)	(36.34)
T3:Garlic	30.963	36.228	59.904	85.018	53.028
	(33.788)	(36.987)	(50.693)	(67.226)	(47.173)
T4:Neem	18.041	22.697	46.124	75.121	40.496
	(25.081)	(28.432)	(42.759)	(60.062)	(39.084)
T5: Datura	37.078	41.295	65.931	88.401	58.176
	(37.491)	(39.965)	(54.274)	(70.088)	(50.454)
T6: Mint	10.982	15.194	32.353	71.012	32.385
T7:Control	(19.23)	(22.822)	(34.641)	(57.404)	(33.524)
	Plant extracts (P)		Concentrations (C)		Interaction (P X C)
S.Ed ±	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 mean of five replications

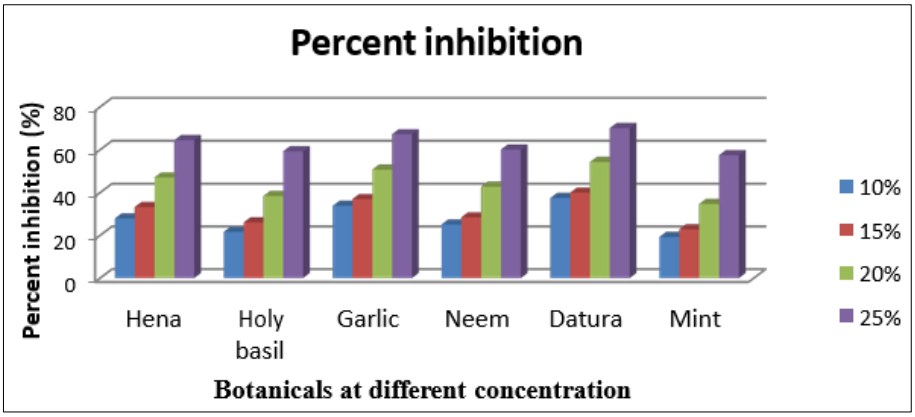

Fig 2: Evaluation of botanicals (10, 15, 20 and 25%) on initiation of radial mycelial growth of *C. acutatum in vitro.*

Conclusion

Aqueous extract of *D. stramonium* was found to be the most efficient in inhibiting the mycelial growth of *C. acutatum in vitro* at all the concentration (10, 15, 20 and 25%), however, highest inhibition (88.37%) over control was observed at 25% concentration. *D. stramonium* extract can be a very effective alternative approach for the management of anthracnose disease of strawberry which is eco-friendly and safe to human.

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