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Original Research Article

Occurrence and Morphological Studies on Foliar Fungal Pathogens of Medicinal and Aromatic Grasses

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A B S T R A C T

lndia is a global leader in essential oil steam distilled from aromatic crops. Lemongrass oil, Citronella oil and Palmarosa oil are the names of commercially important essential oils extracted from the genus. The diseased samples of Lemongrass and Citronella were collected from Nagarjun garden and Centre of Excellence, Dr. PDKV, Akola. The fungi associated with foliar fungal diseases of lemongrass and citronella were isolated and identified on the basis of published literature and morphological characters which resembled with *Dresclera tetramera, Bipolaris sacchari, Nigrospora sphaerica* and *Alternaria alternata. Dresclera tetramera*, *Bipolaris sacchari*, *Nigrospora sphaerica* and *Alternaria alternata* were found pathogenic to lemongrass and citronella causing leaf spot/blight diseases. The pathogenicity exhibited typical symptoms of *Dresclera tetramera, Bipolaris sacchari, Nigrospora sphaerica* and *Alternaria alternata* after 12 to 20 days from the date of inoculation respectively and revealed their pathogenic association with the test host plants. In case of morphological studies of isolated fungi, conidiophores of *D. tetramera* were septate, dark brown at the base, sub hyaline at the tip. The conidia were borne singly in succession; they were light green, thick walled, obclavate, straight, 3 septate, measured 21.13-27.36 ×9.05-11.49 µm in size. Conidia of *N. sphearica* were black, spherical to subsphaerical and measured 18.11 to 21.61 × 15.16 to 19.08 µm. In case of *A.alternata* conidiophores were short to long, simple or branched arising singly. Conidia were borne in long chains (6-11) on conidiophores, they were thick walled, beaked and brown in colour measured 40.94 × 18.76 µm in size. Conidia of *B. saccchari* of size 80.58-102.49 × 17.92-19.15 µm were slightly curved, elliptic to elliptic fusiform, tapering towards rounded ends, pale brown to greyish brown, (5–9)-distoseptate.

Keywords

Dresclera tetramera, Alternaria alternata, Nigrospora sphearica, Bipolaris sacchari, Conidial measurement

Introduction

Aromatic grasses produce essential oils, perfumes and flavours are in use with our civilization since several thousand years. Lemongrass (*Cymbopogon flexuosus*) is a perennial aromatic tropical C4 grass that belongs to the family poaceae or gramineae. The genus *Cymbopogon* comprises about 140 spacies (kumari *et al*, 2007), of which three aromatic grasses (i.e. Lemongrass, Citronella & Palmarosa) considered economically important for the production of essential oils and aromatic herb (Eltahir and Abuereish,

2010). Palmarosa (*Cymbopogon martini*) also known as Indian Rosha or Motia or Tikhadi, This perennial grass is native to southeast Asia, especially India , and it is cultivated for its oil. Citronella was first introduced in India in 1959 from Indonesia (Java Island, hence the name Java citronella) (Kaul *et al.*, 1997). Citronella java (*Cymbopogon winterianus*) is perennial, aromatic grass, growing to a height of 90 - 180 cm. Light or medium, deep, well drained soils with good fertility are ideal for citronella. Disease is one of the major constraint in economic crop production as they inflict heavy losses. Like other plants, aromatic grasses are also attacked by many diseases during their growth. Few fungal and bacterial pathogens are known to attack these grasses resulting into substantial yield losses. Due to foliar diseases the major oil constituents were depleted in diseased leaf as compared to the healthy one. The recovery of oil was decreased along with the quantity of citronellal, citronellol, citronellyl acetate, geranyl acetate and limonene in the diseased leaf oil. The grass is affected by many foliar diseases like leaf blight (*Curvularia andropogonis*), leaf spot (*Colletotrichum graminicola* or *Drechslera victoria*), lethal yellowing (*Pythium aphanidermatum*) (Alam *et al*, 1992), Rust (*Puccinia nakanishikii*) (Mekonnnen *et al*, 2014), Little leaf (virus) (Anonymous, 2018).

Aromatic grasses suffer from many diseases caused by fungi, bacteria and viruses. Among the fungal diseases, leaf spot (eye spot) caused by *Bipolaris sacchari*, leaf spot caused by *Dresclera tetramera*, *Alternaria* leaf spot caused by *Alternaria alternata*, leaf blight caused by *Nigrospora sphaerica* etc. are important diseases. Demand and price of herbal products and essential oils are increasing consistently in national and international markets due to strong pro-consumer movement. Maharashtra state is one of the leading supplier of Aromatic plants and allied products to other states & to the industrialized countries of the West, where demand for natural drugs/herbal products has been on the increase in recent years.

The cultivation of Aromatic plants has now become an important area in the international agribusiness with an estimated growth rate of 15-18 % and thus the aromatic plants being a natural source of raw material for industrial products offers great scope to achieve higher net returns with the upliftment of rural economy (Lodha *et. al*, 2016). Being a divesting foliar fungal disease of these grasses resulting in yield loss, it is essential to generate the information on the foliar fungal pathogens. There is lack of sufficient information on the morphological studies. Hence studies need to be undertaken to assess the occurrence and morphological studies.

Materials and Methods

The present investigations on morphological studies of foliar fungal pathogens of medicinal and aromatic grasses i.e. Lemongrass, Citronella and Palmrosa in Vitro and their related aspects were studied during 2017-2018 at the Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The details of the material and methods used are as follows.

Material required

Collection of diseased samples

Diseased leaves of aromatic grasses showing leaf spot/leaf blight symptoms were collected from the Nagarjun Garden, Dr. P.D.K.V., Akola. Based on symptoms, microscopic examination of diseased samples, association of the pathogen as *Dresclera tetramera, Alternaria alternata, Nigrospora sphearica* and *Bipolaris sacchari* was recorded.

Preparation of culture media

Potato Dextrose Agar (PDA)

Potato dextrose agar (PDA) medium was used for isolation and maintenance of pure cultures of *Dresclera tetramera, Alternaria alternata, Nigrospora sphearica* and *Bipolaris sacchari* and also used for maintenance of biocontrol agents. The composition of PDA -Peeled potatoes -200 g, Dextrose - 20 g, Agar agar-20 g, Distilled water-1000 ml. Healthy peeled potatoes 200 g were cut into pieces and boiled in 500 ml of sterilized distilled water in sauce pan for 30min. The extract was strained through muslin cloth and quantity was measured. In remaining 500 ml water, 20 g agar-agar and 20 g dextrose were dissolved by heating. Then both mixed and volume was made to one litre. The medium was filtered through muslin cloth and poured into conical flasks and test tubes. Then plugged with non-absorbent cotton and autoclaved at 1.05 kg/cm² for 20min. Autoclaved tubes were kept in slanting position to obtain PDA slants for maintenance of cultures.

Disinfection / sterilization of laboratory materials

The glassware were washed with detergent powder under running water, dried and then sterilized in hot air oven at 180^{o}C for 1 hr. before use. The other material were disinfected with denature spirit.

Isolation of pathogen by tissue isolation method

Infected leaf samples were cut into small pieces with sterilized blade and disinfected with sodium hypochlorite solution for two minute. Pieces were washed with three changes of sterilized distilled water and bits after dried on sterilized filter paper and around flame of spirit lamp were placed on sterilized solidified PDA medium in plate. Each plate contained five bits. The plates were incubated at room temperature (28 ± 20^{O}C). All these operations were carried out aseptically. The plates were examined regularly. The fungus colonies growing around the each bit were examined and sub cultured. Based on morphological characters and published literature the fungi were identified as *Dresclera tetramera, Alternaria alternata, Nigrospora sphearica* and *Bipolaris sacchari*. The pure culture was transferred on PDA slants and maintained for further studies.

Purification and maintenance of fungal culture

Culture was purified by following hyphal tip method and culture obtained was maintained on potato dextrose agar (PDA) medium slants by adopting subsequent sub culturing at periodical, regular intervals. Seven days old culture was used for further studies.

Preparation of spore suspension for pathogenicity test

Seven days old growth of the fungi was scrapped from lawn culture of PDA and mixed in distilled sterilized water separately. The bits of medium were removed by sedimentation and by filtering through three folds of muslin cloth and pure spore suspension was obtained.

Pathogenicity test by spray inoculation method

Three weeks old test plants were used for inoculation. Spore suspensions of the above fungal isolates were prepared separately as described earlier and spore count per ml was recorded with help of haemocytometer. In this spore suspension little amount of

carborandam powder was added as abrasive to fine injury to surface of the leaf. These spore suspensions were applied on the leaves of the plant with help of spray inoculation method. Pot containing the test plants was kept in the humidity chamber for 10-12 hrs. prior to inoculation and 48 hrs. after inoculation in order to provide sufficient humidity. After 48 hr. treated plants were removed from humidity chamber and kept on cage house benches under observation. After appearance of symptoms on artificially inoculated leaves, reisolation of the pathogen from infected tissues was done to confirm the identity of the pathogens, so as to prove the Koch's postulates.

Conidial measurement

Size of the conidia of test pathogens was measured using ocular micrometer. For the measurement of conidial size, ocular micrometre was first standardized with stage micrometer to obtain calibration factor. Measurements was taken at 10X and 40X depending upon the size of the conidia. Calibration factor was calculated by using following formula given below:

$$\text{One ocular division (in µm)} = \frac{\text{No. of divisions on stage micrometer}}{\text{No. of divisions on ocular micrometer}} \times 10$$

After caliberation, the stage micrometer was replaced with the freshly prepared slide of the microorganism. Then number of ocular micrometer divisions, a conidia occupies were counted. By rotating the ocular lens the divisions of several conidia was counted and length and width of the conidia was measured. Measurement of different conidia was carried out for 100 times to find the particular range of conidia. The size of conidia was measured by using following formula (Aneja, 2007):

Size of conidia = No. of ocular division occupied × Caliberation Factor

And the data was analysed by standard deviation.

Results and Discussions

Collection of disease samples, isolation and maintenance of fungal culture

The foliar diseased samples of Lemongrass, Citronella and Palmrosa were collected from Nagarjun Garden and Centre of Excellence, Dr. P.D.K.V., Akola. Isolation of pathogen from leaf samples were made on PDA by tissue isolation method. Fungi were found to be associated with the samples. The fungus was identified based on morphological characters and published literature as *Dresclera tetramera, Alternaria alternata, Nigrospora sphearica* and *Bipolaris sacchari* and was purified by hyphal tip method. The majority of isolation yielded the fungus *Dresclera tetramera, Alternaria alternata, Nigrospora sphearica* and *Bipolaris sacchari.* The isolated fungus culture was maintained on PDA slants for further studies (Ellis, 1971) (Ellis, 1976).

Similar leaf blight symptoms caused by *Dresclera spp.* was recorded by Magorzata *et. al* (2011) on Oat and Kiran (2011) on finger millet foxtail millet and respectively. Occurrence of Nigrospora leaf blight caused by *Nigrospora sphaerica* as one of the important disease of tea in Darjeeling district of West Bengal was reported by Dutta (2012). Occurance of *Alternaria alternata* causing Alternaria blight in pigeonpea in India was reported by Mamta *et al.,* (2013). Similar symptoms were recorded by Silva *et al.,* (2008) in case of *Bipolaris sacchari* on the leaves of Banana in Brazil and also by Elliot (2005) in case of *Bipolaris sacchari* from the host *Lygodium japonicum* and *L. microphyllum.*

Some other fungal species viz. *Fusarium semitectum, Curvularia lunata* and *Cladosporium spp.* were also recorded during the isolation. The identification of the fungus was confirmed based on morphological characters and published literature and used for further investigation. Similarly Shazia and Ahmad (2005) conducted survey for the assessment of foliar blight of wheat in main rice-wheat cropping areas of Punjab, Pakistan and from leaf samples isolated *Alternaria alternata* and *Cladosporium spp*.

Pathogenicity test

The pathogenicity of isolated fungi was proved by artificially inoculating three week old seedlings of Lemongrass and Citronella with their respective monosporic culture. Typical symptoms appeared on the inoculated plants after 10-15 days of inoculation. The symptoms expressed were similar to naturally infected plants. Fungus was reisolated from the infected leaves and colony morphology of the fungus with respect to mycelium, conidia, conidiophores was similar to the one used for inoculation.

Dresclera tetramera

In present pathogenicity test, *Dresclera tetramera* has produced symptoms on leaves of three weeks old Lemon grass and Citronella plant within 10-15 days from date of inoculation in the form of minute, oval, light brown lesions, as the seedling developed, these lesions enlarged and become dark brown, and several such lesions coalesced and formed large patches of infection on leaf blade (Plate. 8). The lesions were found on leaf blade and leaf sheath. Similar types of observations were recorded by Magorzata *et al.*, (2011) on Oat and Kiran (2011) on Finger millet and Foxtail millet respectively while studying pathogenicity of *Dresclera tetramera.*

Nigrospora sphearica

In case of *Nigrospora sphearica* symptoms were observed as brown, semi-circular or irregular shaped lesions, often surrounded by pale yellow on the young leaf margins and apices, distinct blight spots are seen delineated by the midrib of the leaves. Similar type of symptoms were recorded by Dutta (2012) on Tea leaves in Darjeeling and by Wright (2008) on Blueberries.

Alternaria alternata

In present investigation, *Alternaria alternata* has produced symptoms on lemon grass and citronella plant within 10-12 days from date of inoculation in form of small, circular, light brown necrotic spots (Plate. 10). Similar symptoms were observed on Pigeonpea leaves by Mamta *et al.,* (2013) and Devappa and Thejakumar (2016) on Chilli leaves.

Bipolaris sacchari

In case of *Bipolaris sacchari* symptoms were observed as small, water-soaked lesion. Within 10-15 days, this lesion turns to varying shades of yellow, reddish-brown, brown. The colored lesions may be surrounded by a chlorotic halo and these spots on leaves run parallel to veins (Plate. 11). Similar symptoms were recorded by Silva *et al.,* (2008) in case of *Bipolaris sacchari* on the leaves of Banana in Brazil and also by Elliot (2005) in case of *Bipolaris sacchari* from the host *Lygodium japonicum* and *L. microphyllum*.

Morphological studies

In the present study the isolates were studied for their morphological characters on PDA at room temperature of 28 ± 20^{O}C and the data are presented in table 1 and 2.

a) *Dresclera tetramera*

Seven days old culture shown mycelium of grey to olivaceous green, profusely branched and septate. Conidiophores were septate, dark brown at the base, sub hyaline at the tip. The conidia were borne singly in succession, they were light green, thick walled, obclavate, straight, 3 septate, measured 21.13-27.36 ×9.05-11.49 µm in size, tapering at both the ends with the hilum at the base (Plate. 12) (Ellis, 1971). Similar results were observed by Kiran (2011) in case of *D. nodulosa* and *D. setariae.*

b) *Nigrospora sphearica*

Seven days old culture shown the fungal colonies was fast growing, initially white, becoming light to dark grey with the onset of sporulation with black, spherical to subsphaerical conidia that measured 18.11 to 21.61 × 15.16 to 19.08 µm and were borne on a hyaline vesicle at the tip of each conidiophore (Plate. 13) (Ellis, 1971). These characteristics agree with published descriptions of *Nigrospora sphaerica* by Wright (2008) on Blueberries and by Dutta (2012) on Tea leaves.

c) *Alternaria alternata*

The culture of the fungal colony was initially white, cottony with profuse aerial mycelium which gradually turned grey colour. Aged culture appeared completely greyish with aerial mycelium and distinct concentric rings was formed on medium. Conidiophores were short to long, simple or branched arising singly. Conidiophores were golden to brown coloured. Conidia were borne in long chains (6-11) on conidiophores, they were thick walled, beaked and brown in colour (Plate. 14) (Ellis, 1976). Based on the characters of the colony and morphological characters of conidiophores and conidia the fungus was identified as *Alternaria alternata*. Similar results were recorded by Vakalounakis (1990) and Devappa and Thejakumar (2016).

d) *Bipolaris sacchari*

Seven days old fungal culture on PDA was slow growing initially white then turns into green in colour. Conidiophores arising singly or in groups, simple, septate, usually straight, sometimes flexuous, geniculate at upper part, frequently swollen at base, pale brown to olivaceous brown.

Conidiogenous nodes dark brown, distinct. Conidia of size 80.58-102.49 × 17.92-19.15 µm, slightly curved, elliptic to elliptic fusiform, tapering towards rounded ends, pale brown to greyish brown, (5–9)-distoseptate (Ellis, 1976) which agrees with the descriptions of *Bipolaris sacchari* given by Manamgoda *et al.,* (2014) and by Silva *et al.,* (2008) Ellis and Holiday (1971).

In conclusion, *Dresclera tetramera, Nigrospora sphaerica, Alternaria alternata and Bipolaris sacchari* were found pathogenic to the leaves of Lemongrass and Citronella. In case of morphological studies conidial size of *D. tetramera* and *N. sphearica* were measured as 21.13-27.36 ×9.05-11.49 µm and 21.61 × 15.16 to 19.08 µm in size. Conidia of *A. alternata* were 40.94 × 18.76 µm in size and conidia of *B. saccchari* were 80.58-102.49 × 17.92-19.15 µm in size.

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Table.1 Particulars of the diseased samples collected for isolation

Sr. no	Date of Isolation	Types of spots / symptoms	Fungi obtained in isolation	Host
1	12/07/17	Minute, oval, light brown to dark brown lesions, and several such lesions coalesced and formed large patches of infection on leaf blade (Plate. 4)	*Dresclera tetramera*	Lemongrass, Citronella and Palmrosa
2	22/08/17	Brown, semi-circular or irregular shaped lesions, often surrounded by pale yellow on the young leaf margins and apices, distinct blight spots are seen delineated by the midrib (Plate. 5)	*Nigrospora sphearica*	Lemongrass and Citronella
3	15/09/17	Small, oval, necrotic lesions irregularly scattered on the leaves (Plate. 6)	*Alternaria alternate*	Lemongrass and Citronella
4	07/12/17	Spots on leaves run parallel to veins, reddish-brown to yellow-brown streaks, extending towards the leaf tips (Plate. 7)	*Bipolaris sacchari*	Lemongrass and Citronella

Table.2 Spore characters of *Dresclera tetramera, Alternaria alternata, Nigrospora sphearica* and *Bipolaris sacchari*

Sr. no	Spore characters	***Dresclera tetramera***	***Nigrospora sphearica***	***Alternaria alternata***	***Bipolaris sacchari***
1	Color	Light green	Black	Brown	Pale brown
2	Size* (L × B range)	21.13-27.36 ×9.05-11.49 µm	18.11-21.61 ×15.16-19.08 µm	33.95-47.92 × 17.59-19.93 µm	80.58-102.49 × 17.92-19.15 µm
3	Average (L × B)	24.24 × 10.27 µm	19.86 × 17.12 µm	40.94 × 18.76 µm	91.54 × 18.53 µm
4	SD	± 3.11(L) ± 1.21(B)	± 1.96(L) ± 1.74(B)	± 6.98(L) ± 1.17(B)	± 10.95(L) ± 0.61(B)
5	Septations	3	-	4-6	5-9

*= mean of 100 spores, L= Length, B= Breadth, SD= Standard deviation

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