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A comprehensive study on symptoms, occurrence and intensity of leaf blight disease of *Ocimum* sanctum in and adjoining areas of 24 Parganas(N), West Bengal

KEYWORDS: PDI, Devastating effect,Alternaria sp

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ABSTRACT *A destructive leaf blight disease of Ocimum sanctum L. is found in West Bengal. It's causal organism was isolated and phenotypically identified as Alternaria sp. Once in a week survey for two consecutive years of 2013 and 2014 in ten places of 24 Parganas(N) district,West Bengal establishes the presence of disease in all ten places. PDI was recorded by monitoring it's cultivation at 4 selected areas of and adjoining North 24 Parganas, West Bengal for the same time period.Maximum PDI (44.61%-76.66%) was between July to September and minimum (12.30-37.50 %) was recorded during December – January in all four studied areas. There was not only any significant report of fungal disease on Ocimum sanctum but also this is the first approach to the study of disease intensity of leaf blight disease of Ocimum sanctum all over the world. So our work is an entirely new one in this regard.*

Introduction

The plant *Ocimum sanctum,* belongs to family Lamiaceae, grows wild in the tropics and warm regions. The plant is distributed and cultivated throughout the world including India and it has international medicinal value. Different parts of plant are used in Ayurveda and Siddha Systems of Medicine for prevention and cure of many illnesses and everyday ailments like common cold, headache, cough, flu, ear ache, fever, colic pain, sore throat, asthma, hepatic diseases, malaria fever, as an antidote for snake bite and scorpion sting, flatulence, migraine headaches, fatigue, insomnia, arthritis, digestive disorders, night blindness, diarrhea and influenza. The leaves are good for nerves and to sharpen memory. Chewing of Tulsi leaves also cures ulcers and infections of mouth (Prajapati et. al., 2003 , Das and Vasudevan, 2006). It has also aromatic, stomachic, carminative, demulcent, diaphoretic, diuretic, expectorant, alexiteric, vermifuge and febrifuge properties (Gupta et. al., 2002) . The leaves of *Ocimum sanctum* contain 0.7% volatile oil comprising about 71% eugenol and 20% methyl eugenol. The oil also contains carvacrol and sesquiterpine hydrocarbon caryophyllene (Shah,1998). Fresh leaves and stem extract yielded some phenolic compounds (antioxidants) such as cirsilineol, circimaritin, isothymusin, apigenin and rosameric acid, and appreciable quantities of eugenol (Yanpallewar,et. al., 2004). Two flavonoids, viz., orientin and vicenin from aqueous leaf extract have been isolated (Gupta et. al., 2002). Ursolic acid, apigenin, luteolin, apigenin-7-O-glucuronide, luteolin-7-O glucuronide, orientin and molludistin have also been isolated from the leaf extract (Nair et. al., 1982) . The plant also contains a number of sesquiterpenes and monoterpenes viz., bornyl acetate, -pinenes, camphene,β- and α-elemene, neral, β -sitosterol (IDMA, 2002).

Recently the plant *Ocimum sanctum* was frequently reported with leaf blight disease caused by *Alternaria* sp from different other parts of the world (Taba et al., 2009, Gariboldi et. al., 2011, Dantoff et. al., 1997, Swart et. al., 2003).

Some fungal pathogens and non-pathogens produce mycotoxins in their infected hosts and substrates on which they grow (Anthony et al., 2009). WHO (1979) reported that mycotoxins are hazardous to human and animal health. As this medicinal plant is subjected to production of many important pharmaceutical drugs, fungal invasion to them with the production of mycotoxin is potent enough to interfere with the active principles of these plants.

Therefore, the main objectives of this work are to identify the causal organism of this disease phenotypically and record the occurrence and disease intensity of this disease in few selected areas of 24 – Parganas (N) district and neighbouring zones, West Bengal.

Material and methods

Study of the disease occurrence and intensity (PDI)

We have carried out an intensive survey in order to record the Percentage of Disease Intensity (PDI) by monitoring cultivation of *Ocimum sanctum* from January 2013 to December 2014 at 4 selected areas of West Bengal namely Kalyani (BCKV), Nilgunj, North 24-Parganas, State Pharmacopical Laboratory and Pharmacy for Medicine, Govt. of W.B., Nadia and The Agri Horticultural Society of India, Alipore. Beside that an extensive survey was also carried out in few selected areas of North 24 Parganas to record disease occurrence of this novel medicinal plant for the same time period**.** The fields were visited once in a week regularly for the entire time duration to study the Percentage of Disease Intensity (PDI) and occurrence of disease. For the study of PDI, three plots from each of the four areas were taken and per plot 50 plants were randomly selected and tagged. Tagged plants were visited regularly, once in a week from January 2013 to December 2014 to record the PDI by using the following formula.

PDI= A/B X 100

A=Total Number of infected plant; B=Total Number of selected plants.

The temperature (Minimum &maximum) and humidity (minimum & maximum) of every day were recorded in the study area, they were averaged month wise and tabulated in the Table -1.

Study of Symptoms

The infected leaves were carried to the laboratory in sterilized biodegradable polythene bags and the symptoms studied under hand lens and simple microscope.

Volume : 5 | Issue : 8 | August 2015 | ISSN - 2249-555X

Isolation and purification of pathogen from diseased plant parts

The collected leaf samples were washed in sterile distilled water and soaked in alcohol to remove the surface impurities. After that the leaf samples were cut into small pieces of 3-5 mm in size from the diseased portion. Then they are passed through 0.1% of $HgCl_2$ solution for one minute for surface sterilization and washed three times in three changes of sterile distilled water. These leaf cuttings were blotted between sterile filter papers and aseptically plated on Potato Dextrose Agar (PDA). In each plate a single piece was placed and incubated at BOD (28± 1°C) for 7 days. After appearance of mycelial growth it was transferred on to fresh PDA slant. For purification of isolated pathogen, single hyphal tip method was taken. The entire procedure for isolation of the disease was done under laminar air flow.

Pathogenecity test of the pathogen

Pathogenecity test was done following the Koch postulate.

Characterization and Identification of the pathogen

The characterization and identification of the pathogen was done phenotypically by following Domsch et.al (1980), Nagamani *et al*, (2006) and Simmons (2007)

Results & Discussion

Symptoms of the disease

The symptom first appears as brown coloured spots mainly on the tips and sometimes on margins of leaves with 1-2 mm in diameter (Fig1A). Then with the moist condition these spots enlarge and coalesce together giving a dark brown appearance covering an area of 15-25 mm (Fig1B). In more severe condition the diseased portion of the leaves, sometimes the whole leaf becomes dry, shrivel and brittle. Finally breaks off from the plant. In most extensive condition of the disease all the leaves drop off leaving bare branches (Fig1C).

Fig 1A. initiation of blight on *Ocimum* leaf **Fig1B**. progression of blight **Fig 1C**. complete defoliation of *Ocimum* plant

Phenotypical characterization and identification of the pathogen

Pure culture was analysed both microscopically and macroscopically. The growth pattern is slow (around 2-3mm) per day. The pathogen looks greenish white at first. Then gradually turns dark green and finally dark brown to black with a yellowish margin at the plate. The fungal colony covers full plate in 7-9 days with a puffy growth (Fig 2A). The reverse plate shows completely black appearance (Fig 2B). Slide was prepared from the margin of the colony (Fig 2C). Size, shape and feature of spore, hyphal patterns were recorded by preparing numerous slides from different stages of the colonial growth. Conidiophores pale brown, cylindrical with monopodial branching, hyphae 10.5-17.5×5.25-10.5µm. Conidia solitary, straight or curved, obclavate to ellipsoidal, 4-6 fragmented, tapering gradually into paler beak. Size of conidia ranges from 8.75-28×3.5-14.87µm, generally with a beak attaining a size of 0-3.5×8.75µm. With all the macroscopical and microscopical characters the causal agent was identified as *Alternaria* sp. The entire procedure of identification was carried out following Domsch et.al (1980), Nagamani *et al*, (2006) and Simmons (2007).

The data presented in the Table -2 showed that this disease was found to present in all studied zones (Naihati, Halishahar, Hasnabad, Hingalganj, Badu, Nilganj, Duttafulia, Gopalnagar, Rajarhat, and Mohishbathan) all through the year for 2013 & 14. The disease was predominant in hot and humid weather from March to October during 2013 where as in 2014 the disease has it's maximum effect from April to September in all of our survey areas.

Similarly Chauvan and Korekar (2011) conducted a survey on fungal diseases of medicinal plants in Maharashtra state. They observed leaf spot disease of *Aloe vera*, *Withiania somnifera* and *Datura metel* in Osmanabad district of Maharashtra (2011). They reported three pathogens were associated with the disease namely *Alternaria alternata*, *A. tenuissima and Fusarium* sp. According to them the disease was found during rainy season and winter but absent in summer. According to our survey the occurrence of *Ocimum* leaf blight disease caused by *Alternaria* sp is also predominant from the early summer covering the rainy season and present in few areas during the winter and summer. So to some extent our result is as per with the results of Chauvan and Korekar (2011). The minute difference among our survey and their work is may be due to the differences in geographical and climatic factors among these two distantly located survey areas.

Table 2. Occurrence of disease *Ocimum sanctum* in different zones of North 24 Parganas during 2013 & 14:

Places	Occurrence of leaf blight disease (Alternaria sp.)in 2013 &14																					
	Jan		Feb		Mar		Apr		May		Jun		Jul		Aug		Sep		Oct		Nov		Dec	
	13	14	13	14	13	14	13	14	13	14	13	14	13	14	13	14	13	14	13	14	13	14	13	14
Naihati	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Halishahar	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
Hasnabad	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Hingalganj	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Badu	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Nilganj	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Duttafulia	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Gopalnagar	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Rajarhat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Mohishbathan	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-

Volume : 5 | Issue : 8 | August 2015 | ISSN - 2249-555X

The data presented in Table 3 shows that the disease intensity was recorded maximum in Nilgunj during the month of September. It was 74.16% in 2013 and the percentage rises upto 76.66% in the next year. After Nilgunj the effect of the disease intensity was recorded most at BCKV. It was 70% for the month of September (2013) which increased upto 72.50% in 2014 at the same time of the year. Agri Horticultural Society, Alipore was recorded with 71.95 % and 64.63% PDI during Spetember at 2013 & 2014 respectively. In State Pharmacopical Laboratory and Pharmacy for Medicine, Govt. of W.B., Nadia the effect of disease intensity was not that much in comparison to other areas. Here the disease intensity was recorded maximum as 44.61 in July, 2013 & September, 2014. Where as the intensity of disease was recorded lowest during the month of December-January. It was recorded as 12.30% & 16.92% for the month of December (2013) & January (2014) respectively in State Pharmacopical Laboratory and Pharmacy for Medicine, Govt. of W.B., Nadia . At Nilgunj (24.16% & 29.16% in December for 2013 &14 respectively). In Agri-Horticultural Society Alipore the PDI was 23.17% during December, 2013 & 26.82% during January 2014. BCKV also exhibited minimum disease intensity during December, recorded as 32.50% (2013) & 37,50% (2014).

This study indicated that maximum disease intensity range (44.61%-76.66%) was between July to September in all four studied areas. It was probably due to high temperature and moisture (26-38°C & humidity 85-100%) as per record obtained from Alipore Metereological Data Station (West Bengal). Moreover minimum percentage of disease intensity (12.30-37.50 %) was recorded during December – January in all four studied areas. During this time temperature and moisture are low (10-26°C & humidity 65-84%) and not so favorable to the fungal pathogen.

The plant *Ocimum basilicum* with leaf blight disease was first reported from Japan (Taba et al., 2009). In Italy the same disease was reported by (Gariboldi et. al., 2011). *Fusarium* wilt is a production constraint in basil and its occurrence is reported from different parts of the world. The disease is caused by *Fusarium oxysporum* f. sp. *basilica* (Fob) and it was reported from Florida (Dantoff et. al., 1997), Africa (Swart et. al., 2003).

The genus *Alternaria* belongs to division Deuteromycota with several species. A great number of species were recorded for the genus *Alternaria* infecting different crops causing world-wide economic loss (Kirk, 2008). Among the medicinal plants *Alternaria alternata* causes leaf spot disease in *Withania somnifera* (Pati *et al.*, 2008), Chavan and Korekar (2011) reported leaf spot disease of *Aloe vera* caused by *Alteraria alternata*, *Alternaria tenuissima* & *Fusarium* sp. in Osmanabad district, Maharashtra. Ghosh and Banerjee (2014) found leaf spot disease in *Aloe vera* caused by *Alternaria brassicae* in West Bengal. A downy mildew namely *Peronospora lamii* has also been reported as one of the destructive disease of sweet basil (Belberi et. al.,2005; Gariboldi et. al., 2004; Gariboldi et. al., 2005; Hansford, 1933). Leaf spot disease of French basil (*Ocimum basilicum*) caused by *Corynespora cassicola* has been reported from Odakali, Kerala (Devi et. al., 1979). Root knot nematode (*Meloidogyne incognita* & *M. javanica*) are the serious problem for the cultivation of *Ocimum basilicum*, *O. sanctum*, *O. gratissimum* and *O. kilimandscharium* in different parts of the county (Balsubramanium and Rangaswami, 1964, Haseeb and Pandey, 1987, Rangaswami, et. al., 1961). Although there are a number of available disease reports on different species of *Ocimum* but there was no significant report on the disease of *Ocimum sanctum* and it's disease intensity all through the world. So our work is an entirely new one in this regard. The pathogen *Alternaria* is causing enormous crop loss by blight disease but how much they minimizing the medicinal properties of this important crop should be analyzed further. In this perspective it is urgent to control this disease immediately.

Conclusion

Our work establishes that even the plant *Ocimum sanctum,* inspite of having enormous medical importance, getting invaded by the pathogen *Alternaria sp* very frequently and exhibiting devastating effect. This work indicates that leaf blight of *Ocimum sanctum* occurs in few places of 24 Parganas, West Bengal throughout the two consecutive years 2013 & 14 where as in other areas of our survey they are reported mainly during the summer and rainy season. The disease intensity was at it's peak in July - September (44.61%-76.66%) in four places of our intensive survey. Where as the minimum percentage of disease was recorded in December-January (12.30-37.50 %) in all four study areas. This is the first approach to the study of disease intensity of leaf blight disease of *Ocimum sanctum* all over the world. It is expected that this work may encourage other workers to study this disease, its severity, crop loss and it's proper management to combat it. Infection of *Ocimum sanctum* by *Alternaria* sp not only reduces the crop loss and market value but it may reduce medicinal efficacy of this shrub.

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Table 1. Month wise report of temperature and humidity in our study area

Month	Temperature		Humidity	
	2013	2014	2013	2014
January	13-26	10-26	71-84	70-78
February	18-29	16-27	75-86	72-82
March	25-34	19-33	79-88	78-90
April	32-42	30-41	76-90	79-96
May	30-43	33-43	75-94	80-98
June	28-38	28-39	78-96	77-100
July	28-37	26-38	96-100	94-100
August	30-36	25-36	89-92	86-96
September	30-35	25-35	85-92	89-95
October	25-35	22-31	80-9	86-92
November	20-25	20-25	70-80	70-80
December	10-20	10-20	65-70	65-70

*****(+) indicates occurrence of disease and (–) indicates absence of disease.

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Table 3. Percentage of disease incidence (PDI) in *Ocimum sanctum* at four selected areas

Months	Nilganj			Agri Horti, Alipore			State pharmacopical, nadia			BCKV		
	2013	2014	Pull data	2013	2014	Pull data	2013	2014	Pull data	2013	2014	Pull data
Jan	54.16 (47.35)	36.66 (37.23)	45.41 (42.36)	35.36 (36.45)	26.82 (31.18)	31.09 (33.83)	27.69 (31.69)	16.92 (24.27)	22.30 (28.18)	51.25 (45.69)	38.75 (38.47)	45.00 (42.13)
Feb	57.50 (49.31)	40.83 (39.70)	49.16 (44.46)	45.12 (42.19)	32.92 (35.00)	39.02 (38.65)	33.84 (35.55)	23.07 (28.66)	28.45 (32,20)	52.50 (46.43)	40.00 (39.23)	46.25 (42.82)
Mar	58.33 (49.78)	46.66 (43.05)	52.49 (46.38)	47.56 (43.56)	40.24 (39.35)	43.90 (40.92)	36.92 (37.41)	27.69 (31.69)	32.30 (34.63)	55.00 (47.87)	42.50 (40.69)	48.75 (44.25)
Apr	60.83 (51.24)	52.50 (46.43)	56.66 (48.79)	50.00 (45.00)	42.68 (40.74)	46.34 (42.88)	38.41 (38.29)	29.23 (32.71)	33.82 (35.55)	57.50 (49.31)	45.00 (42.13)	51.25 (45.69)
May	62.50 (52.24)	57.50 (49.31)	60.00 (50.77)	54.87 (47.75)	46.34 (42.88)	50.60 (45.34)	40.00 (39.23)	32.30 (34.63)	36.15 (36.93)	60.00 (50.77)	47.50 (43.57)	53.75 (47.12
Jun	64.16 (53.19)	65.83 (54.21)	64.99 (53.67)	57.31 (49.20)	51.21 (45.69)	54.26 (47.41)	43.07 (40.98)	38.46 (38.29)	40.76 (39.64)	63.75 (52.95)	51.25 (45.69)	57.50 (49.31)
Jul	66.66 (54.70)	70.00 (56.79)	68.33 (55.73)	60.97 (51.30)	57.31 (49.20)	59.14 (50.24)	44.61 (41.90)	41.53 (40.11)	43.07 (40.98)	65.00 (53.73)	57.50 (49.31)	61.25 (51.47)
Aug	70.00 (56.79)	73.33 (58.89)	71.66 (57.80)	65.85 (54.21)	60.97 (51.30)	63.41 (52.77)	32.30 (34.63)	36.92 (37.41)	34.61 (36.03)	68.75 (55.98)	65.00 (53.73)	66.87 (54.82)
Sep	74.16 (59.41)	76.66 (61.07)	75.41 (60.27)	71.95 (57.99)	64.63 (53.49)	68.29 (55.67)	27.69 (31.69)	44.61 (41.90)	36.15 (39.93)	70.00 (56.79)	72.50 (58.37)	71.25 (57.54)
Oct	33.33 (35.24)	54.16 (47.35)	43.74 (41.38)	40.24 (39.35)	47.56 (43.57)	43.90 (40.92)	29.23 (32.71)	32.30 (34.63)	30.76 (33.65)	52.50 (46.43)	60.00 (50.77)	56.50 (48.73)
Nov	28.33 (32.14)	42.50 (40.69)	35.41 (36.51)	29.26 (32.71)	34.14 (35.73)	31.70 (34.27)	16.92 (24.27)	24.61 (29.73)	20.76 (27.06)	43.75 (41.38)	48.75 (44.25)	46.25 (42.82)
Dec	24.16 (29.40)	29.16 (32.65)	26.66 (31.05)	23.17 (28.73)	28.04 (31.95)	25.60 (30.40)	12.30 (20.53)	18.46 (25.40)	15.38 (23.03)	32.50 (34.76)	37.50 (37.76)	35.00 (36.27)
CD(p=0.05)	15.47	4.95	13.04	15.73	10.77	15.13	17.33	9.23	22.96	15.82	6.55	19.98
Sm±	7.47	2.41	27.00	7.60	5.20	7.30	8.37	4.46	11.09	7.64	3.16	9.16

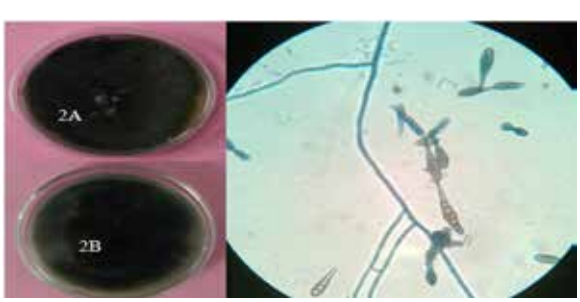

Fig 2A. normal view, 2B. reverse plate view &2C. microscopic view of Alternaria sp

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