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Screening of *Ocimum gratissimum* germplasms in natural field condition against leaf spot disease caused by *Alternaria alternata*

Ravi Pratap Singh and Mayank Singh

Abstract
A field experiment was conducted during *kharif* season (2016-2017) on Tulsi (*Ocimum gratissimum*) crop for screening of resistance in natural field condition against leaf spot disease caused by *Alternaria alternata* at Main Experimental Station, Department of Medicinal and Aromatic Plants, ANDUAT, Kumarganj, Ayodhya. The observed parameters of oil content percent, number of leaves per plant, leaf weight per plant (kg), leaf yield (q/ha) and disease severity percent were recorded in 90 days old plants. The results obtained revealed that germplasm NOB-7 showed maximum oil content (2.14%) while the maximum leaves yield recorded in NOB-1 (14.81 q/ha) with disease severity (15%).

Keywords: Germplasm, oil content, leaf yield, leaf weight, disease severity, *Ocimum gratissimum*

Introduction
The Basil is native of Asia and Africa and grows wild as a perennial on some pacific islands and was brought from India to Europe through the Middle East in sixteenth century, subsequently to America in the seventeenth century.
Three types of Tulsi are encountered with in cultivation, the green leafed (Sri or Rama Tulsi) is the most common, the second type (Krishna Tulsi) bears dark green-to-purple leaves, a third type is a forest variety Vana Tulsi (*Ocimum gratissimum*) that often grows wild. *Ocimum gratissimum* is a herbaceous plant of the *Lamiaceae* family. Tulsi meaning 'the incomparable one' is an important medicinal plant which is in demand. The medicinal properties of Tulsi were known since antiquity. It is used for the treatment of problems related to heart, blood, intestine and snake bite. Eugenol the important chemical constituent of Tulsi is useful for the synthesis of vanillin.
Ocimum gratissimum leaf extract is commonly used in traditional medical practice for the treatments of mental illness, epilepsy, high fever, diarrhoea, pneumonia, cough, and conjunctivitis [1]. It has been estimated that over 50% of medicines have their origins in this natural product [2].

Material and Methods
The experiment was conducted at experimental farm of Medicinal and Aromatic Plants of Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad. Randomized Block Design (RBD) was adopted with three replications. The percent disease intensity (PDI) was recorded during August to October 2017**.** Twenty-four germplasm obtained from the Department of Medicinal and Aromatic Plants bearing the IC number from IC- NOB-1 to IC-NOB-24 shown in (Table-1) were sown in July, 2016 at Main Experimental Station, Department of Medicinal and Aromatic Plant in Randomized Block Design. The crop suffers from leaf spot disease during different stages of crops but among all the leaf spot disease amounts heavy loss in leaf yield which ultimately effects the oil yield.
The twenty-four germplasms of *Ocimum gratissimum* were screened, disease severity will be recorded using 0-9 scale. Each germplasm was planted in well prepared field at row to row distance 60 cm and plant to plant distance 45 cm. Details regarding the experiment are described in Table-1.

Table 1: List of germplasms of *Ocimum gratissimum*

S. No.	Germplasms	S. No.	Germplasms
1	NOB-1	13	NOB-13
2	NOB-2	14	NOB-14
3	NOB-3	15	NOB-15
4	NOB-4	16	NOB-16
5	NOB-5	17	NOB-17
6	NOB-6	18	NOB-18
7	NOB-7	19	NOB-19
8	NOB-8	20	NOB-20
9	NOB-9	21	NOB-21
10	NOB-10	22	NOB-22
11	NOB-11	23	NOB-23
12	NOB-12	24	NOB-24

Results and Discussion

The present investigation has been carried during on 2016-17 "Screening of *Ocimum gratissimum* germplasms in natural field condition against leaf spot disease caused by *Alternaria alternata*".

The initial symptoms appeared at middle leaves as small light brown, circular to sub circular spots but in some spots coalesce to form necrotic spots and cover large area. Similar symptoms were also have been reported on Tulsi crop earlier [3, 4] that the disease affects 10% of 60-day old plants and 40% of 5-month-old plants. The brown-black lesions often surrounded by a yellow halo developed from the margins and tips of the upper side of older leaves, leading to the progressive defoliation of plants, followed by plant death (Fig A & B). In later stages leaves may turn brown and die. Although *Alternaria alternate* reported on *Ocimum sp.* From Kenya [5], *Alternaria sp.* on *Ocimum basilicum* from California [6] and Florida [7] and *Alternaria tenuissima* from Pakistan [8] and from India, *Alternaria alternata* has been reported on leaves of *Ocimum sanctum* from Poona [9], on seeds of *Ocimum sp.* from Solan, H.P. [10] and leaves of *Ocimum basilicum* from Nagarjun University, A.P [10].

The fungus *Alternaria alternata* was isolated from the diseased leaves of *Ocimum gratissimum*, which has some healthy portion on PDI slants. The growth of fungus was observed after five days of incubation at 25 ±2 °C. The fungal colony was olivaceous black with dark olive-green margins, and abundant branched septate, golden brown mycelium (Fig C & D). The conidiophores were branched, straight, golden-brown and smooth walled. The conidia were obpyriform, muriform produced in long branched chains, with a short pale beak [4] have observed that a fungus, consistently isolated on PDA from symptomatic leaves, formed conidia singly or in short chains (2-8 elements), dark brown, with 3-7 transverse and 0-4 longitudinal septa, 23.7-73.4×8.8-15.1 µm in size, and with a conical or cylindrical beak 3.5-19.4 µm long. The pathogen was identified as *Alternaria* sp. based on morphology.

In the month of August, the disease severity was in negative correlation (-0.988) with minimum temperature while the maximum temperature was significantly positive (0.487) in relation to disease severity followed by month of September where disease severity was in negative correlation (-0.997) with the minimum temperature and maximum temperature was also negatively correlated (-0.783) to disease severity while in the month of October the disease severity was positively correlated (0.323) with minimum temperature and negative correlated (-0.199) with maximum temperature, whereas the disease severity was positive correlated with rain fall, relative humidity (RH) in rest of the months, while September and October month were significantly positive towards rain fall and relative humidity (RH).

Table 2: Effect of meteorological data on development of disease on *Ocimum gratissimum*

Date of sowing	Months in which data was recorded	Standard week	Rainfall (mm)	Temperature °C		R.H. (%)	Diseases severity (%)
				Min.	Max.		
27 July 2017	August 2017	31	20.6	26.9	32.6	85.7	35.12
		33	1.0	26.8	31.9	84.4	37.44
		34	38.6	26.3	32.9	84.7	42.53
	September 2017	35	100.6	25.6	33.0	81.3	48.23
		36	49.4	26.0	34.0	80.1	44.82
		38	88.2	25.8	32.7	84.8	46.75
	October 2017	40	0.0	24.8	33.8	79.9	67.55
		42	0.0	21.4	33.7	71.7	63.78
		43	0.0	16.7	32.7	67.5	66.00

Table 3: Correlation co-efficient of disease severity in relation to meteorological data:

Months	Rain fall (mm)	Temperature °C		Relative Humidity (%)	Disease severity (%)
		Min.	Max.		
August 2017	0.653	-0.988	0.487	-0.574	38.36
September 2017	0.488	-0.997	-0.783	0.318	46.60
October 2017	0.0	0.323	-0.199	0.569	65.77

Table 4: Screening of *Ocimum gratissimum* germplasm s in natural field condition against leaf spot disease caused by *Alternaria alternata*

S. No.	Entries	Disease severity (%)	No. of leaves /plant	Green leaf weight /plant (Kg)	Green leaf yield (Q/ha)	Oil content (%)
1.	NOB-1	15	2897	0.144	14.81	0.44
2.	NOB-2	13	3245	0.142	8.51	1.49
3.	NOB-3	62	1380	0.032	3.33	0.58
4.	NOB-4	44	2176	0.053	5.46	0.62
5.	NOB-5	66	2348	0.028	2.96	0.86
6.	NOB-6	37	2762	0.064	6.66	1.53
7.	NOB-7	40	2480	0.103	10.67	2.14
8.	NOB-8	20	1541	0.062	6.38	0.91
9.	NOB-9	58	2010	0.041	4.25	1.05
10.	NOB-10	30	1835	0.082	8.51	0.63
11.	NOB-11	23	1752	0.091	9.44	1.86
12.	NOB-12	35	1642	0.070	7.24	0.54
13.	NOB-13	40	1376	0.062	6.38	0.50
14.	NOB-14	45	1862	0.051	5.27	1.20
15.	NOB-15	50	1754	0.045	4.62	1.02
16.	NOB-16	55	1840	0.040	4.16	1.54
17.	NOB-17	40	2044	0.062	6.38	1.02
18.	NOB-18	18	1665	0.104	10.74	0.66
19.	NOB-19	32	1488	0.074	7.68	1.08
20.	NOB-20	38	1932	0.090	9.25	1.75
21.	NOB-21	36	2110	0.068	7.03	1.55
22.	NOB-22	60	1738	0.037	3.88	1.52
23.	NOB-23	42	2052	0.055	5.74	1.33
24.	NOB-24	26	2246	0.063	6.48	0.56

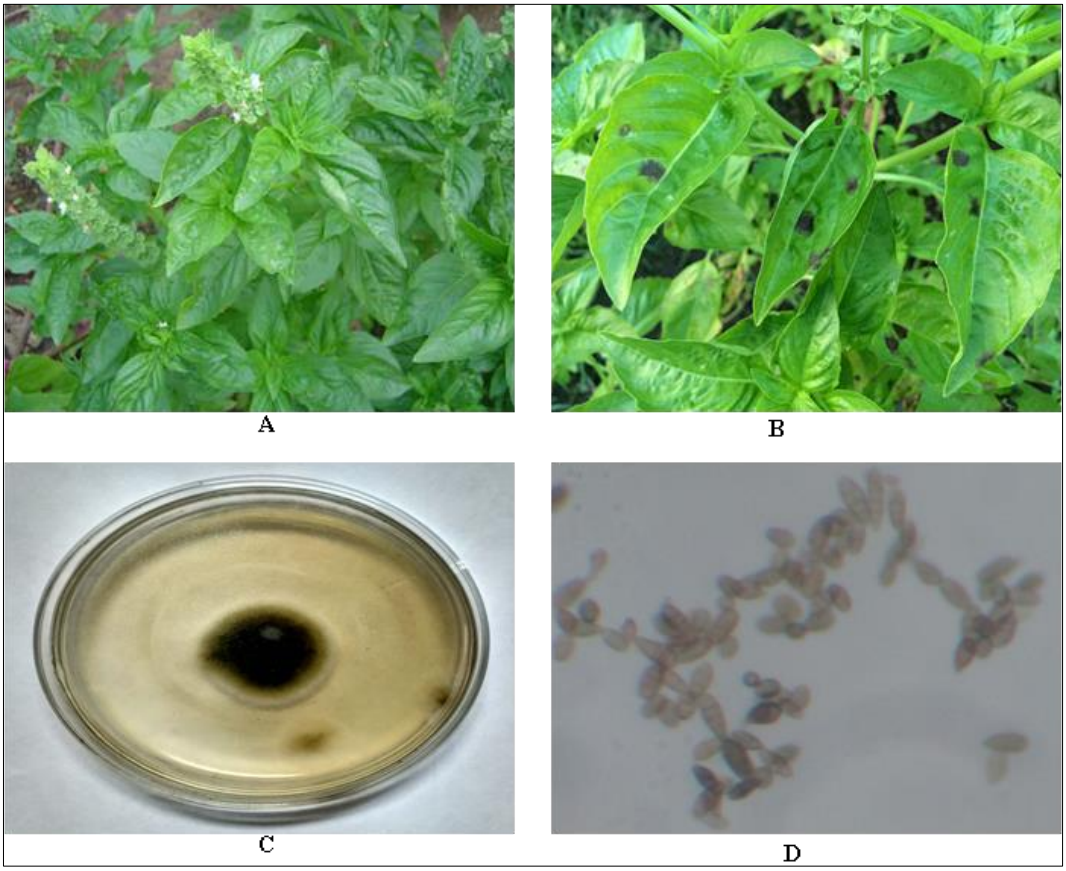

Fig A: Healthy plant of *Ocimum gratissimum* **B:** Spots of *Alternaria alternata* on *Ocimum gratissimum* **C:** Fungus of *Alternaria alternata* shown in the plate and **D:** Conidia of *Alternaria alternata* on *Ocimum gratissimum*

Conclusion

The minimum percent disease intensity was recorded in month of August followed by September. The maximum percent disease intensity was noted in month of October. In the month of August, the disease intensity mean has negative correlation with minimum temperature while, the maximum temperature was significantly positive. Similarly, in month of September the disease intensity was negative correlated with minimum temperature as well as maximum temperature. In the October month disease intensity mean have significantly positive correlation with minimum and negative with maximum temperatures. On the final note keeping in view the

importance of experimental crop *Ocimum gratissimum* having high medicinal value specially in Ayurveda as the herbs was known for its antiquity.

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