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COLLETOTRICHUM DISEASE OF *ELEPHANTOPUS SCABER* AND ITS EFFECT ON SECONDARY METABOLITES

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ABSTRACT

Elephantopus scaber is an important medicinal herb in India which is extensively used in the traditional system of medicine, for the treatment of various diseases and disorders in humans. A systematic study was carried out to determine the causal organism that caused foliar disease in Bhadra Wildlife sanctuary, the incidence and severity of foliar disease in *E. scaber* growing extensively in its natural habitat during 2006-09. The spatial distribution of foliar fungal disease in *E. scaber* was determined by modified Taylor's power law. The seedborne nature and transmission of the causal organism and its management by seed dressing with fungicides was also determined. The secondary metabolite content in infected foliages was determined. The present study indicated that *Colletotrichum dematium* is a major foliar disease causing pathogen in *E. scaber*. The foliar disease severity is high in Kagemanegiri forest region during November-December. The disease incidence is homogeneously distributed in all the nine forest regions of the study area. The causal pathogen is seedborne and seed transmitted and could be managed with Bavistin or Captra. The analysis of secondary metabolites in diseased foliages indicated that steroids decreased with increase in infection due to *C. dematium,* while alkaloids, phenols and flavonoids increased upon infection. The study also suggested that foliar infection by *C. dematium* might cause considerable damage to *E. scaber* plants and could alter the quality of secondary metabolites.

KEYWORDS:***Elephantopus scaber*, *Colletotrichum dematium*, disease severity, seedborne, Bavistin, secondary metabolites**

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INTRODUCTION

Elephantopus scaber L. (Family: Asteracae), a subscapigerous medicinal herb commonly called as prickly-leaved elephants foot, is popular in many countries of Southeast Asia, and Latin America[1,2]. Since 1970's, many chemical constituents of *E. scaber* and their pharmacological evaluations are reported. The major constituents include flavonoids, flavonoid esters, triterpenoids and sesquiterpene lactones in addition to elephantopin, stigmasterol, epifriedelinol and lupeol[3]. The whole plant is used in the treatment of nephritis, dampness, pain in the chest and arthralgia due to wounding[4,5], diseases of blood, skin and heart, piles, dysuria, urethrorrhea, swelling, filariasis, and heamorrhoids and possesses anti-diarrheal, hepatoprotective, anti-poison, alexipharmic, aphrodisiac, expectorant and febrifuge activities[6,7,8,9,10]. A survey of Bhadra Wildlife sanctuary in Karnataka indicated that *E. scaber* occurs in wild and is affected by a foliar fungal disease in mature plants during different seasons of the year in varying severity[11]. There are no comprehensive reports of fungal disease(s) in *E. scaber* plants althrough the year, or seed transmission of pathogen and its management. In view of this, an attempt was made to study the foliar disease, and to determine disease incidence and severity in the sanctuary through different growing stages of *E. scaber* and seasons, seedborne nature and transmission of pathogen and its management by seed treatment. The effect of foliar disease on certain secondary metabolite content was also determined.

MATERIALS AND METHODS

(i) Selection of study area

Bhadra Wildlife sanctuary (13^0 34^1 to 13^0 46^1 N lat. and 75^0 29^1 to 75^0 45^1 E long.), located in the southern central part of the Western Ghats region of Karnataka, is comprised of 12 state forest regions with an area of 492.46 sq km. The above forest regions were selected as the study regions (Table 1). The study area harbors the dry- and moist-deciduous as well as semi evergreen forests and receives an annual rainfall of 1600-2000 mm. The plant specimens were collected and identified based on the flora[12,13] and herbarial specimens in the Department of Applied Botany, Kuvempu University. The species identity was also authenticated by Prof. Dr. M. S. Sudarshana, University of Mysore. In each study region, three sites were identified randomly; each of the study site consisted of three quadrates (10x10 sq m) representing three replicates.

(ii) Isolation and characterization of causal organism of foliar disease

The infected plants were studied for symptoms of disease on various plant parts during different growing stages and seasons. The infected foliages of *E. scaber* were collected in sterilized polypropylene bags, surface disinfected (sodium hypochlorite, 0.2%, 2 min), segmented into 1-sq-cm pieces and incubated on moistened blotter discs in Petri dishes under light-darkness cycle of 12/12 hr at 23±2°C for 5 days. The fungal species associated with incubated diseased foliages were identified based on their morphological characteristics by referring to identification manuals[14,15,16,17] and by visiting *Index Fungorum* (www.indexfungorum.org). The fungal species were cultured on PDA medium and identified by comparing with the characteristics of fungal colonies on incubated diseased parts. The fungal species associated with disease symptom was subjected to pathogenicity test by detached leaf bioassay technique[18], where in the surface disinfected apparently healthy leaves were prick-inoculated with sterile water or spore suspension ($4x10^6$ or $6x10^6$ spores ml^{-1}) of the fungal species cultured on PDA and incubated on moistened blotter discs as described previously.

(iii) Seasonal occurrence and distribution of foliar disease

The data of disease incidence (DI %) and severity (DS %) were recorded every month for a period of 36 months during 2006-09. The disease incidence and severity was determined as described[18]. The data of weather parameters of study area were collected from the Divisional Meteorological department, Shimoga, Statistical officer of Chikmagalur District, Karnataka State Natural Disaster Monitoring Centre website and Central Coffee Research Institute, Balehonnuru. The weather data, and foliar disease incidence and severity were subjected to Pearson's correlation[19]. The distribution of foliar disease in the study area during 2006-09 was analyzed by the binary form of Taylor's power law[20] using the data of disease incidence. The modified Taylor's power law[21] was used to determine the relationship between logarithum of the observed variance (V_{obs}) and expected variance (V_r) [log (V_{obs}) = log (A) + b log (V_r)], where 'A' and 'b' are parameters as suggested by Achar and Shivanna[22]. Regression analysis was used to estimate the intercept and slope parameters of Taylor's power law using MINITAB version 15.1.1.0, and slope parameter is used as an index of aggregation of foliar fungal disease in the study area.

(iv) Determination of seed mycoflora

Mature and drying seeds of *E. scaber* were collected from the study sites and dried under ambient conditions of the laboratory. Seeds of the same range were mixed to obtain three samples representing Lakkavalli, Hebbe and Muthodi forests regions. Four hundred seeds of *E. scaber* from each range were incubated (12 hr alternating cycles of fluorescent light/darkness at 22 ± 2^{o}C for 7 days) by standard blotter method[23]. Fungal species occurring on incubated seeds were identified as described previously and seed germination was recorded. Data of the seedborne fungal incidence were subjected to analysis of variance (ANOVA).

(v) Seed localization and transmission of fungal pathogen

Seeds were subjected to the component plating method for determining the localization of fungal pathogen[24]. Accordingly, seeds were dissected into seed coat, cotyledon and embryonic axis which were surface disinfected and incubated by the blotter method at room temperature. Seed transmission of the causal organism was determined by the sand method [25,26]. Seed sample (100 seeds) collected from study sites were sown in poly-pots (10 X 5 cm) containing the autoclaved potting soil (farmyard manure: red soil: sand; 2:1:1) and irrigated with sterile distilled water and cultured for a period of six weeks in the greenhouse. Data of pre- and post-emergence seedling mortalities and disease occurrence were recorded. Seedlings with disease symptoms were incubated by the blotter method for confirmation of the pathogen involvement. Dead and ungerminated seeds were also dug out from the soil for the pathogen presence. The data of seedling mortality and disease incidence and severity were subjected to ANOVA.

(vi) Management of seedborne fungal flora

All the three seed samples from their respective forest zones were mixed to obtain a single sample which was subjected to fungicide treatment by dusting with Bavistin (Carbendazim 50% WP), Mancozeb 75% WP (Hyzeb M-45), Antracol (Propineb 70% WP) or Captra (Captan 50% WP) @ 2% (mg g^{-1}). The untreated and treated seeds were incubated by the blotter method as described previously. The plates were arranged in randomized complete block design with four replications. The data of fungal colonies occurring on incubated seeds as well as seed germination were collected and subjected to ANOVA.

(vii) Assay of phytochemical compounds in infected foliages

Leaf samples of *E. scaber* were collected from the study sites during the maximum disease severity and graded into i). The leaf sample containing only apparently healthy leaves ii). A

sample containing infected leaves that are partially infected (≤50% DS) and iii). A sample containing totally infected leaves or totally diseased (100% DS) based on the blotter test. The dried leaf samples were powdered (0.5-mm size particles) and determined quantitatively for secondary metabolites like phenols (Folin Denis reagent method[27]), flavonoids[28], steroids[29] and alkaloids[30]. The data of secondary metabolites were subjected to ANOVA (Minitab 15.1.1.0 software).

RESULTS

1. Disease symptomatology, pathogen characterization and pathogenicity confirmation

Elephantopus scaber is distributed in the nine forest regions- Hebbegiri, Gangegiri, Kemmannugundi, Lakkavalli, Kakanahosudi, Aldhara, Madhuguni, Muthodi and Kagemanegiri state forest regions. The foliar disease symptom appeared initially as pale reddish-brown irregular spots with thick yellow margin on the upper surface of leaves; spots enlarged forming irregular large blotches extending into margins of the entire leaf lamina causing leaf blight; leaf lamina folded upward and dried. Foliage blight symptoms were commonly observed in most of the study sites. The incubated diseased leaf sample upon incubation produced *Colletotrichum dematium, C. lindemuthianum, Fusarium oxysporum, Myrothecium roridum* and *Macrophomina phaseolina,* and species of *Cercospora, Phoma* and *Phomopsis*. Amongst them, *Colletotrichum dematium* was observed in very high percentages (100) in leaf segments. Leaf bioassay test confirmed that the leaf spot disease in *E. scaber* is caused by *Colletotrichum dematium* (Pers.) Grove. The pathogen is characterized by the presence of round black bristly head of acervuli (100-600 µm), setae long, rigid, bristle-like, conidiophores that formed as a fringe around acervuli, septate, 14-28 x 3-6 µm and produced falcate, fusiform, apices acute, single-celled conidia, 18-30 x 2-2.7µ.

2. Incidence and severity of foliar disease and distribution of disease

The foliar disease incidence due to *C. dematium* ranged from 8.33 to 84.09%, which reached maximum during Nov-Dec (winter season) and decreased to as low as 9% during summer and started increasing gradually during rainy season. The disease severity ranged between 1.67 and 46.77% during the study period.The disease severity was highest in Muthodi region followed by Lakkavalli, Kemmannugundi and Kakanahosudi regions. In winter season, particularly during 2006, the disease severity was high (Fig 1).The logarithmic relationship between the observed variance (v) and the theoretical variance for binomial variance for the incidence of leaf spot disease caused by *C. dematium* in *E. scaber* is shown in Figure 2. The co-efficient value was low (0.2) in Gangegiri followed by Kemmannugundi (0.3), Hebbe (2.5), Muthodi (3.7), Madhuguni (5.5), Lakkavalli (5.9), Kagemanegiri (6.0), Kakanahosudi (9.1), and Aldhara (13.9) forest regions (Table 1). The value of slope parameters (b) was estimated as $b<1$ in all nine state forest regions ($p>0.05$), whereas intercept parameter estimates for log (A) were greater than zero level in all the forest regions ($p>0.05$). As for as the pooled data of entire sanctuary is concerned, the slope parameter was less than one.

3. Seed mycoflora of E. scaber

The seeds of *E. scaber* collected from Lakkavalli, Hebbe and Muthodi forest ranges were colonized by 14 fungal species of 10 genera (Table 2). Some of the seedborne fungal species recorded were - *Alternaria alternata* (2.71%), *Aspergillus candidus* (3.42%), *A. flavus* (6.25%), *A. niger* (9.42%), *A. versicolor* (8.59%), *Cladosporium cladosporioides* (8.21%), *Colletotrichum dematium* (24.29%), *Chaetomium globosum* (19.17%) and *Fusarium oxysporum* (9.59%), and species of *Cercospora* (4.55%), *Penicillium* (2.46%), *Phoma* (4.46%) and *Phomopsis* (5.54%).The percentage of seed germination was 44% in Muthodi

(maximum) and 36.13% in Lakkavalli region (minimum).

4. Localization and seed transmission of C. dematium and its management

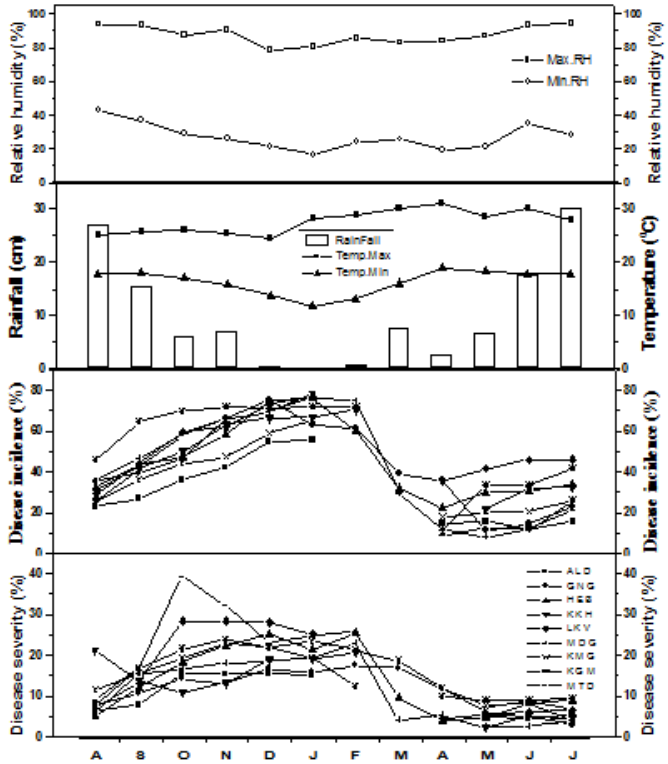
The component plating technique indicated that *C. dematium* was localized in the cotyledons (36.25%), embryonic axis region (24.0%) and also seed coat (7.88%). In pot experiment, the seeds showed 70.33% pre- and 2.33% post-emergence mortalities, however 6% of seedlings exhibited leaf spot disease symptom on cotyledons and leaves at 21days. The naturally infected seeds had 29.67% germination ability. All the seed dressing fungicides tested in the study inhibited fungal colony growth on incubated seeds. Bavistin and captra was highly effective in reducing the seedborne occurrence of *C. dematium.* Bavistin improved germination ability (45.75%) in seeds as compared to that in control (40%) (Table 3).

5. Assay of phytochemical compounds in infected foliages

The steroid content decreased ($p<0.005$) in both partially infected (79.15%) and completely diseased leaf samples (44.75%) when compared to the healthy leaf sample (1057.57 µg ml^{-1}) (Table 4). However, the alkaloids of *E. scaber* increased by 8.03% in partially infected and 22.26% in completely diseased samples as compared to the healthy samples (17.92 mg g^{-1}). The total phenol content increased by 701.31 and 307.59%, over control (34.25 µg ml^{-1}), in partially infected and completely infected leaf samples, respectively. Similarly, the flavonoids increased in both partially infected sample (16.12%) and completely infected sample (20.44%) as compared to the healthy sample (98.32 µg ml^{-1}).

Figure 1

Foliar disease incidence and severity in Elephantopus scaber due to Colletotrichum dematium in Bhadra Wildlife sanctuary, Karnataka



Note:ALD:Aldara; GNG:Gangegiri; HEB:Hebbegiri; KKH:Kakanahosudi; LKV:Lakkavalli; MDG:Madhuguni; KMG:Kemmangundi; KGM:Kagemanegiri; MTD:Muthodi. Average data based on the survey conducted during Aug 2006-June 2009.

Figure 2

Relationship between the log of the observed variance (v) and the log of the theoretical variance of the binomial variance for the incidence of foliar disease caused by Colletotrichum dematium in Elephantopus scaber.

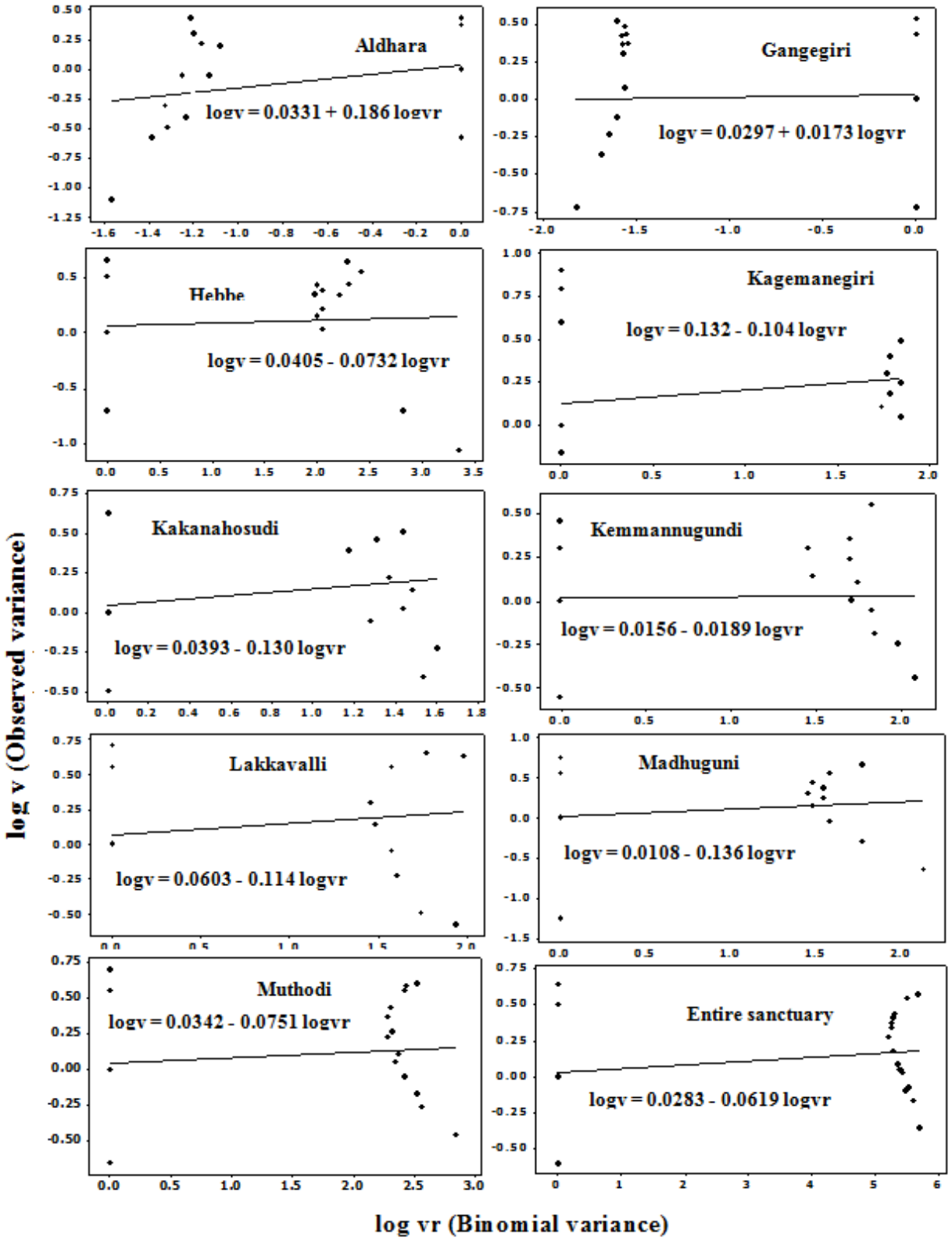


Table 1

Mean disease incidence, slope and intercept parameter estimates of the binary power law for the incidence of foliar disease in Elephantopus scaber caused by Colletotrichum dematium in the sanctuary.

Sl. no	State forest regions	P[1]	SE[2] (P)	A[3]	SE[4] (A)	b[5]	SE[6] (b)	[7]R[2]
1	Aldhara	0.188	0.036	0.033	0.064	0.186	0.079	13.9
2	Gangegiri	0.227	0.042	0.029	0.075	0.017	0.071	0.2
3	Hebbe	0.260	0.047	0.040	0.076	-0.073	0.078	2.5
4	Kagemanegiri	0.306	0.054	0.131	0.056	-0.104	0.070	6.0
5	Kakanahasudi	0.261	0.046	0.039	0.053	-0.129	0.070	9.1
6	Kemmannugundi	0.214	0.038	0.015	0.048	-0.018	0.056	0.3
7	Lakkavalli	0.267	0.052	0.060	0.062	-0.144	0.078	5.9
8	Madhuguni	0.266	0.049	0.010	0.079	-0.135	0.096	5.5
9	Muthodi	0.251	0.046	0.034	0.067	-0.075	0.066	3.7
Sanctuary (Pooled)		0.249	0.044	0.028	0.056	-0.061	0.036	7.9

Note: [1]Average mean Disease Incidence (Aug 2006 to July 2009): [2, 4, 6]Standard Error: [3]Intercept parameter of the binary power law: [5]Slope parameter of the binary power law: [7]Co-efficient of determination, higher the value the better fit of the data to the analysis.

Table 2

Seed mycoflora of E. scaber in different state forest regions of the sanctuary

Sl.no	Range forest	Fungal incidence (%)													
		Aa	Ac	Af	An	Av	Cc	Cd	Csp	Cg	Fo	Psp1	Psp2	Pho	Phm
01	Muthodi	3.75	4.50	7.75	14.38	11.13	10.88	32.63	3.88	24.38	14.38	1.38	3.00	6.88	8.00
02	Lakkavalli	1.63	1.88	4.0	8.63	4.38	5.75	23.25	6.13	17.88	7.88	1.88	1.75	3.5	3.25
03	Hebbe	2.75	3.88	7.0	5.25	10.25	8.0	17.0	3.63	15.25	6.25	4.13	1.25	3.0	5.38
SEM±		0.19	0.23	0.43	0.59	0.59	0.3	0.54	0.24	0.86	0.61	0.14	0.12	0.27	0.39
CD at 5%		0.57	0.7	1.29	1.78	1.78	0.89	1.65	0.72	2.62	1.85	0.44	0.37	0.82	1.17
CV		19.53	19.03	19.28	17.67	19.3	10.17	6.33	14.81	12.75	18.14	16.61	17.25	17.22	19.74

Aa: Alternaria alternata, Ac: Aspergillus candidus, Af : A. flavus, An: A. niger, Av: A. versicolor, Cc: Cladosporium cladosporioides, Cd: Colletotrichum dematium, Csp: Cercospora sp., Cg: Chaetomium globosum, Fo: Fusarium oxysporum, Psp1: Penicillium sp.1, Psp2: Penicillium sp.2, Pho: Phoma sp., Phm: Phomopsis sp. *Data based on 400 seeds.

Table 3

Effect of fungicides (@ 2%) on seed mycoflora of Elephantopus scaber

Sl.no	Treatments	Fungal incidence (%)													
		Aa	Ac	Af	An	Av	Cc	Cd	Csp	Cg	Fo	Psp1	Psp2	Pho	Phm
01	Control	14.25	12.0	14.75	11.25	12.75	9.50	33.0	10.25	23.0	15.75	13.75	8.25	17.0	16.5
02	Bavistin	0.25	1.5	0.0	0.0	1.0	0.0	1.5	1.0	3.5	0.0	0.0	0.0	1.0	0.0
03	Hyzeb	0.0	0.0	0.0	0.0	7.25	3.25	3.25	4.0	2.5	2.0	0.0	0.0	1.75	0.25
04	Antracol	11.75	1.25	0.0	0.0	0.0	2.75	3.75	4.0	4.25	3.5	0.0	0.0	2.75	0.0
05	Captra	0.0	0.0	0.0	0.0	11.75	2.25	2.25	2.0	4.25	4.25	0.0	0.0	0.0	0.0
SEM±		0.28	0.27	0.28	0.21	0.62	0.33	0.78	0.41	0.73	0.42	0.11	0.11	0.5	0.29
CD at 5%		0.86	0.84	1.28	0.66	1.91	1.0	2.4	1.27	2.25	1.28	0.34	0.34	1.10	0.89
CV		10.58	18.57	19.08	19.03	18.96	18.36	17.8	20.54	19.51	16.31	8.13	13.55	15.84	17.23

Aa: Alternaria alternata, Ac: Aspergillus candidus, Af : A. flavus ,An: A. niger, Av: A. versicolor, Cc: Cladosporium cladosporioides, Cd: Colletotrichum dematium, Csp: Cercospora sp., Cg: Chaetomium globosum, Fo: Fusarium oxysporum, Psp1: Penicillium sp.1, Psp2: Penicillium sp.2, Pho: Phoma sp., Phm: Phomopsis sp., Data is based on 400 seeds/treatment.

Table 4
The secondary metabolite contents in foliages of Elephantopus scaber infected with Colletotrichum dematium

Disease category (% infection)	Secondary metabolites			
	Alkaloids (mg g^{-1})	**Flavonoids** (µg ml^{-1})	**Phenolics** (µg ml^{-1})	**Steroids** (µg ml^{-1})
Apparently healthy (control)	17.92	98.32	34.25	1057.57
Partially diseased (<50% DS)	19.36(8.03)*	114.17(16.12)*	274.45(701.31)*	220.5(79.15)**
Completely diseased (100% DS)	21.91(22.26)*	118.42(20.44)*	139.6(307.59)*	584.26(44.75)**
SEm±	0.05	0.5	0.38	0.59
CD $_{(p=0.05)}$	0.14	1.55	1.18	1.83
CV	0.61	1.21	0.68	0.25

**** Values in parenthesis indicate the per cent increase of secondary metabolites over healthy category; ** Values in parenthesis indicate the per cent decrease of secondary metabolites over healthy category; Data of secondary metabolites was subjected to ANOVA (P≤0.005, P≤0.001)***

Table 5
Simple correlation co-efficient between weather data and foliar disease incidence and severity due to Colletotrichum dematium on Elephantopus scaber in Bhadra wildlife sanctuary, during 2006 to 2009.

Weather data		ALD	GNG	HEB	KKH	LKV	MDG	KMG	KGM	MTD	BWLS
Rainfall	DI	-0.499*	-0.478*	-0.378*	-0.562**	-0.632**	-0.368	-0.462	-0.306	-0.491	-0.486
	DS	-0.566	-0.626**	-0.397*	-0.303	-0.454	-0.439	-0.362	-0.333	-0.514	-0.473
Max.Temp	DI	-0.205	-0.39*	-0.041	0.099	0.065	-0.17	-0.168	-0.078	-0.285	-0.192
	DS	-0.054	-0.007	-0.021	-0.116	-0.411*	-0.121	0.152	0.112	-0.188	-0.053
Min.Temp	DI	-0.755**	-0.518**	-0.799**	-0.80**	0.769**	-0.77	-0.798	-0.723	-0.715	-0.794
	DS	-0.68	-0.567**	-0.755**	-0.491*	-0.513*	-0.69**	-0.55**	-0.46*	-0.351	-0.69**
Max.RH	DI	-0.453*	-0.408*	-0.236	-0.328	-0.560*	-0.239	-0.347	-0.047	-0.433	-0.326
	DS	-0.469*	-0.532**	-0.216	-0.202	-0.406*	-0.206	-0.169	-0.154	-0.267	-0.266
Min.RH	DI	-0.323	-0.258	-0.237	-0.262	-0.542*	-0.207	-0.233	-0.093	-0.293	-0.263
	DS	-0.376	-0.259	-0.146	0.068	-0.239	-0.108	-0.181	-0.163	-0.245	-0.134

Note:ALD:Aldara; GNG:Gangegiri; HEB:Hebbegiri; KKH:Kakanahosudi; LKV:Lakkavalli; MDG:Madhuguni; KMG:Kemmannugundi; KGM:Kagemanegiri; MTD:Muthodi; DI: Disease incidence, DS: Disease severity, BWLS: Bhadra Wildlife sanctuary *=P≤0.005, **=P≤0.001

DISCUSSIONS

Elephantopus scaber is an important ethnomedicinal herb used by people living around the sanctuary[31]. The present survey of the study area for fungal diseases in *E. scaber* indicated that the leaf spot disease is caused by *C. dematium* in Hebbegiri, Gangegiri, Kemmannugundi, Lakkavalli, Kakanahosudi, Aldhara, Madhuguni, Muthodi and Kagemanegiri forest regions of the sanctuary. Incubated diseased leaf segments of *E. scaber* was associated with *C. dematium* incidence in high percentage; the pathogen produced circular to semi-circular leaf spot symptoms in mature plants. The pathogenicity test confirmed that *C. dematium* as the foliar disease causing pathogen in *E. scaber.* The study also indicated that the incidence of *C. dematium* foliar disease was initiated during the rainy season that reached high during the winter and decreased during summer. Since the spores of *C. dematium* are dispersed by rain splashes[32], the rainy season favoured a gradual increase of disease incidence[33]. During summer, particularly in dry-deciduous forest regions, the

foliar disease incidence in *E. scaber* was less. Maximum foliar DS was found in Muthodi forest region. This suggested that the pathogen is capable of causing disease in this plant, to a considerable extent in the wild. In the study area, weather parameters like rain fall, temperature (minimum and maximum) and maximum RH negatively correlated ($p<0.05$) with disease incidence and severity (Table 5). An average temperature of 23-26^{0}C was favorable for disease development, while an average RH of 75-90% and rain fall (20-25 cm) were found to favour disease spread. Power law analysis based on the data of leaf spot disease incidence caused by *C. dematium* in *E. scaber* indicated homogeneous distribution of disease in all the nine forest regions of the sanctuary. A similar condition was also observed for the pooled data of the entire sanctuary. On the other hand, Siddiqui and Shaukat[34] found that the root disease in egg plants is heterogeneous in distribution. Achar and Shivanna[22] showed that *Colletotrichum dematium* leaf spot disease of *Clitoria ternatea* in Lakkavalli region of the above sanctuary is homogeneously distributed, while that in Kemmannugundi region is heterogeneously distributed. They opined that such variation in spatial distribution depended on the type of forest and prevailing weather conditions. The incidence of fungal species in *E. scaber* was high ($p<0.05$) in seed sample collected from Lakkavalli range forest as compared to those collected from Hebbe and Muthodi ranges. *Colletotrichum dematium* is reported to be the major pathogen in field and seedborne[35,36]. The present study showed that cotyledons and the embryonic axis were highly colonized by *C. dematium.* Reports on the *C. dematium* internal infection of chilli seeds[37] is concurrent with the present observation. The internal seed infection might suggest the pathogen transmission from mother plant to the embryo through the connective tissue. Further, the seed infection due to *C. dematium* resulted in the expression of seedling disease suggesting its seed to seedling transmission. This pathogen is also shown to be seed transmitted in other plants[38]. The significant reduction in seedborne occurrence of *C. dematium* and other fungal species due to seed treatment with Bavistin @ 2% could have possibly lead to an improvement in seed germination and seedling stand. Captra is also effective which is also shown to manage seed infection by *C. dematium* in other crops[39]. Among the secondary metabolites, the alkaloid content increased in both partially infected and completely diseased foliar samples of *E. scaber.* Similarly, Shivanna and Mallikarjunaswamy[18] reported increase in alkaloids as the disease progressed in *Terminalia* species. In this study, the flavonoid content also increased in partially infected and completely infected leaf samples. Flavonoids in this plant are reported to possess nephroprotective and antioxidant activities[40]. Flavonols have also been shown to protect plants from UV-light and pathogens[41]. Vidyasagar *et al.*[42] showed a significant increase in the content of flavonol in infected leaves than in the healthy leaves. Total phenol content has been also shown to increase in blight infected tea plants than in healthy plants[43] as found in the present study. This suggested that phenolic compounds might be accumulated in diseased plants to protect plant systems in response to infection by plant pathogens[44]. However, the steroid content was shown to be decreased in infected leaf samples. A similar observation was reported by Prasad and Sah[45]. These steroids identified in *E. scaber* is shown to possess antidiabetic activity[46]. *Elephantopus scaber* is known to produce sesquiterpene lactones, which are derived from steroids and have antitumor activity when used as herbal medicine[3].

CONCLUSION

Results of the present study indicated that leaf spot in *E. scaber* is incited by fungal pathogen *C. dematium* which caused considerable damage to foliages through different seasons in different forest regions of the sanctuary. *Colletotrichum dematium* pathogen is seedborne and transmitted to seedlings. The

seedborne inoculum of *C. dematium* could be managed by seed treatment with Bavistin, Captra or Hyzeb. Foliar infection of *E. scaber* with *C. dematium* resulted in the reduction in steroid content, while increasing the alkaloids, phenolics and flavonoids. This alteration of pharmaceutically important components like alkaloids, flavonoids and steroids could possibly decrease their quality, which could become the cause of concern since the phytochemicals in *E. scaber* are therapeutically important compounds used in treating various diseases and disorders in humans. Results of the present study helped in the generation of baseline data which could be used while compiling the package of practices for intensive cultivation of *E. scaber* and production of disease-free crude drugs by pharmaceutical industries. At the same time, the transformed products of secondary metabolite(s) could become the source of novel chemicals in pharmaceutical industry.

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