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Location and seed transmission of *Alternaria alternata*, *Cercospora guizoticola* in Niger [*Guizotia abyssinica* L, (f) Cass]

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Abstract
Niger is commonly called "*birdseed*". It is important minor, edible, traditional oil seed crop in India. The crop is affected by number of diseases. Among them leaf blight caused by *Alternaria alternata* and leaf spot caused by *Cercospora guizoticola* these diseases are reduces the seed germination and yield up to 40-50%. Present study concentrated on location and transmission of *A. alternata*, and *C. guizoticola* in Niger seeds during kharif 2009-10 seasons in Karnataka. A total of 132 seed samples were collected from farmers, retail shops, fields and APMC markets and were subjected to SBM method. Five seed samples showing higher incidence of seed borne fungi in SBM were selected for location and transmission of the pathogen. The results revealed that kharif 2009 shows *A. alternata* (13-28%) and *C. guizoticola* (16-40%) in the SBM method. *A. alternata* ranged from 5-13% in seed coat, 0-8% in cotyledons, while 0-1% in embryonic axis. *C. guizoticola* ranged from 6-11% in seed coat, 0-6% in cotyledons, while 0-1% in embryonic axis. In kharif-2010, *A. alternata* (18-31%) and *C. guizoticola* (22-42%) in the SBM method. *A. alternata* ranged from 8-17% in seed coat, 3-6% in cotyledons, while 0-2% in embryonic axis. *C. guizoticola* ranged from 8-15% in seed coat, 2-6% in cotyledons, while 0-3% in embryonic axis. The seeds tested during kharif 2009-2010 season harvested seeds favors the more number of pathogens in the seed coat & cotyledons than in the other components. The transmission of *A. alternata* and *C. guizoticola* was 28.8% in kharif 2009. In kharif 2010, the transmission was 36.6% in all the five seed samples. The present study reveals that the disease transmission is more during kharif-2010 season than 2009. The above pathogens causes leaf spot and leaf blight diseases of Niger crop.

Keywords: Niger, location, transmission, SBM, *A. alternata* and *C. guizoticola*

Introduction
Niger [*Guizotia abyssinica* L (f) Cass.] is commonly called "*birdseed*" it belongs to the family Asteraceae. It is known by various names such as Ramtil or Kalatil in India. It is important minor, edible, traditional oil seed crop in India, cultivated over an area of 0.45 million ha with production of 0.11 million tones and productivity of 2.57 quintals/ha (Anon, 2009) [1]. It is mainly cultivated in tribal pockets of Gujarat, M.P., Orissa, Maharashtra, Bihar, Karnataka and Andhra Pradesh. Niger is a crop of dry areas grown mostly by tribal and interior places as life line of tribal segment. Niger is grown in marginal, intercropping and sub marginal lands. Niger is cultivated over on area of 32700 hectares with a production 6169 tonnes in Karnataka. (Anon, 2009). The crop is affected by number of fungal, bacterial, viral and nematodal diseases. The important fungal diseases are Alternaria blight - *Alternaria porri* & *A. alternata*, leaf spot - *Cercospora guizoticola*, Seedling blight - *Alternaria tenuis*, seed rot - *Rhizoctonia bataticola*, rust – *Puccinia guizotiae*, powdery mildew - *Sphaerotheca* sp, Downy mildew - *Plasmopara* spp, Tar spot - *Phyllosticta* spp, Root rot – *Rhizoctonia solani* & *Macrophomina phaseolina* and Ozonium wilt - *Ozonium texanum* (Saharan G.S *et al* 2005; Rangaswamy G. and Mahadevan. 2005) [9, 11]. Leaf blight & leaf spot of Niger caused by *Alternaria alternata* and *C. guizoticola* is considered to be a major devastating disease to the Niger in India (Govindu, H.C., and Thirumalachar M.J., 1956; Vyas S.C., 1981; Yirgou D., 1964;) [6, 16, 17]. In the present work the occurrence, Location, seed to seedling transmission, their frequency of mortality, recovery of pathogens and its significance were studied.

Methodology

Collection of Niger seed samples

In the present study occurrence of *Alternaria alternata* and *C. guizoticola*, Location, seed to seedling transmission, their frequency of mortality, recovery of pathogens and its significance were studied. The study was carried out for the period of two years 2009-2010. However, observations reported during the year 2009-2010 are discussed in the paper.

A total of 132 samples were collected from Niger during kharif, 2009-10. Seeds were harvested from mature Niger plants, farmers, fields, retail shops and APMC markets in different agro climatic regions of Karnataka state. The collected seed samples were dried in sunlight to bring down the safe storage seed moisture and were subjected to standard blotter method (SBM).

Standard blotter method (ISTA.1993)

Seed samples were analyzed for the detection of seed-borne fungi by blotter method following ISTA, 1993 with some modifications. In this method three layers of blotter paper were soaked in sterilized and placed at the bottom of the Petri plates. 100 seeds were sterilized with 0.2% sodium hypo chloride solution for 2 to 3 minutes and seeds taken randomly from each sample and were placed in ten Petri plates (Twenty seeds per plate). The Petri plates with seeds were than incubated at room temperature for seven days in the laboratory. The plates were alternating cycles of 12 hrs light and 12 hrs darkness for seven days. Sterile distilled water was aseptically added to each Petri plates under incubation every third day in order to keep the blotter is sufficiently moist. Germination and fungi associated with the seeds were recorded during the incubation period. Each of the incubated seeds was examined under stereo binocular microscope to ascertain the presence of fungi. Some times were not apparent even after seven days of the incubation. In such condition, the Petri plates were allowed for further incubation. A temporary slide was prepared from each colony, which could not be identified stereo binocular microscope. Fungi were identified by preparing temporary slides and examined under labomed vision 2000 microscope. In fewer cases the fungi from the incubated seeds were transferred to PDA medium in Petri plates aseptically and incubated under controlled temperature (28±1 ºC) for 3 to 10 days and then examined under labomed vision 2000 compound microscope. The analysis of seed-borne *A. alternata* & *C. guizoticola* and other mycoflora was identified by using standard guides and manuals (Barnett H.L., 1960; Sigourd and Funder., 1961; Subramanian C.V., 1983; Van ArxJ.A., 1981) [3, 12, 13, 15]. Five seed samples showing higher incidence of *A. alternata* and *C. guizoticola* in Standard blotter method and were selected for location and transmission studies.

Location of the pathogen by component plating method

This method is adapted to know the location of the pathogen in different components of the seed (Basak A.B., 1998) [5]. The individual seed components were excised after soaking the surface sterilized seeds 0.2% sodium hypochlorite (NaOCl) for three min, in sterile distilled water for five hours. The seed coat, cotyledons and embryonic axis (Plumule and radicle) were dissected aseptically using forceps and needles on blotter. Each component was dipped separately in 0.2% sodium hypochlorite solution (NaOCl) for 50 to 90 seconds and was placed on SBM method. One hundred seeds were dissected in each sample and five replication were maintained. The plates incubated at 25±2 ^{0}C for room temperature. All the components plated individualy. After eight day observation of these plates under stereo binocular microscope. Fungal infection in different seed components was determined based on the appearance of the fungus on the SBM and the percentage of infection was calculated.

Disease transmission studies in the field

Among the total seed samples, five samples shows a higher incidence of *A. alternata* and *C. guizoticola* were selected for disease transmission in experimental plot. The seed samples were sterilized by 2% sodium hypochlorite solution (NaOCl) for 2-3 minutes and in the distilled water before sowing the seeds. Before sowing the seeds the experimental plot were prepared by 10 x 10 meter (row and columns) leveled and ploughed. Each sample selected 100 seeds in five replicates. Sterilized seeds were directly sowing in the fields in the month of August -2009. The proper agronomical practices were followed for raising the plants. All the seeds have germinated after 7-10 days. In experimental plots, 15 plants were randomly selected by selecting five leaves randomly in each plant. The severity of the disease was assessed by using 0-9 scale and percentage of diseases index was calculated by using the formula (Mayee C.D and Datar V.V., 1986) [8].

$$\% \text{ of disease Index (PDI)} = \frac{\text{Sum of individual ratings}}{\text{No. of leaves examined X Maximum disease grade (9)}}$$

Seed to seedling transmission of *A. alternata* and *C. guizoticola* of pathogens were studied.

Recovery of pathogens from diseased plants

Seeds were collected from experimental plots in rabi seasons, subjected for seed health testing methods. Again the seeds sown in kharif, August- 2010 season in experimental plot for recovery of pathogens were studied. These seeds yielded the *A. alternata* and *C. guizoticola*. The study shows that *A. alternate* and *C. guizoticola* are transmitted from seed to seedlings and to the seeds (Thippeswamy B *et al.,* 2006) [14].

Results

During the field survey the leaf spot, leaf blight and seedling blight of Niger was noticed in all visited fields during kharif and rabi seasons in 2009-2010. The severity of the leaf spot, leaf blights diseases was more in kharif-2010 then 2009.

Location of the pathogen in different seed components

Location of the pathogen in the seed is important to control seed borne pathogens. Based on the location of the pathogen in the seeds, the chemicals are selected to prevent the seed borne pathogens. Majority of the seed borne pathogens are lodged on the seed coat, some pathogens are in the cotyledons and some are in embryonic axis (plumule and radical). In Niger, kharif 2009 shows *A. alternata* (13-28%) and *C. guizoticola* (16-40%) in the SBM method. *A. alternata* ranged from 5-13% in seed coat, 0-8% in cotyledons, while 0-1% in embryonic axis. *C. guizoticola* ranged from 6-11% in seed coat, 0-6% in cotyledons, while 0-1% in embryonic axis (Table 1). In kharif-2010, *A. alternata* (18-31%) and *C. guizoticola* (22-42%) in the SBM method. *A. alternata* ranged from 8-17% in seed coat, 3-6% in cotyledons, while 0-2% in embryonic axis. *C. guizoticola*

ranged from 8-15% in seed coat, 2-6% in cotyledons, while 0-3% in embryonic axis. The seeds tested during kharif 2009-2010 season harvested seeds favors the more number of pathogens in the seed coat & cotyledons than in the other components (Table 1 & 2).

Table 1: Location of *A. alternata* and *C. guizoticola* in different seed components of Niger in Kharif-2009

Place of collection	%infection of seed in SBM		In percentage					
			Seed coat		cotyledons		Embryonic Axis	
	A. alt	*C. gui*	*A. alt*	*C. gui*	*A. alt*	*C. gui*	*A. alt*	*C. gui*
Jalahalli	13.0	16.0	9.0	6.0	0.0	2.0	0.0	1.0
Anekal	28.0	20.0	13.0	7.0	5.0	6.0	1.0	0.0
Nelamangala	17.0	40.0	8.0	11.0	8.0	1.0	0.0	1.0
Vajarahalli	19.0	19.0	6.0	8.0	3.0	0.0	0.0	0.0
Basavanahalli	19.0	21.0	5.0	6.0	0.0	2.0	0.0	0.0
Mean	19.2	23.2	8.2	7.6	3.2	2.2	0.2	0.4
SD	4.915	8.565	2.785	1.854	3.059	2.039	0.4	0.489
SE	2.457	4.282	1.392	0.927	1.529	1.234	0.2	0.244

Table 2: Location of *A. alternata* and *C. guizoticola* in different seed components of Niger in Kharif-2010

Place of collection	%infection of seed in SBM		In percentage					
			Seed coat		cotyledons		Embryonic Axis	
	A. alt	*C. gui*	*A. alt*	*C. gui*	*A. alt*	*C. gui*	*A. alt*	*C. gui*
Jalahalli	18.0	22.0	10.0	12.0	5.0	3.0	0.0	2.0
Anekal	31.0	31.0	17.0	8.0	3.0	2.0	2.0	3.0
Nelamangala	21.0	42.0	11.0	10.0	6.0	6.0	0.0	0.0
Vajarahalli	29.0	23.0	9.0	15.0	3.0	5.0	1.0	1.0
Basavanahalli	21.0	25.0	8.0	13.0	5.0	2.0	0.0	1.0
Mean	24	28.6	11	11.6	4.4	3.6	0.6	1.4
SD	5.059	7.391	3.162	2.416	1.2	1.624	0.8	1.019
SE	2.529	3.543	1.543	1.543	0.6	0.321	0.4	0.021

. *****Data based on 100 seeds for each sample, each samples in five replicates.

Transmission studies in field

The present study results revealed that the seeds having 19.2% infection of *A. alternata* and 23.2% infection of *C. guizoticola* showed the transmission of 22.8% in Niger. (Average of five seed samples, Table, 3).

Table 3: Seed to Seedling transmission *A. alternata* and *C. guizoticola* in experimental plot during kharif-2009.

Place of collection	% of incidence in SBM		Germ %	Pre-emergence	Post-emergence	% of diseased plants	% of healthy plants	Recovery of pathogens	
	A. alt	*C. gui*						*A. alt*	*C. gui*
Jalahalli	13.0	16.0	81.0	19.0	3.0	23.0	55.0	18.0	20.0
Anekal	28.0	20.0	68.0	32.0	2.0	21.0	45.0	29.0	28.0
Nelamangala	17.0	40.0	77.0	23.0	3.0	33.0	42.0	31.0	24.0
Vajarahalli	19.0	19.0	66.0	34.0	5.0	18.0	43.0	32.0	25.0
Basavanahalli	19.0	21.0	69.0	31.0	3.0	19.0	47.0	30.0	22.0
Mean	19.2	23.2	72.2	27.8	3.2	22.8	46.4	28	23.8
SD	4.915	8.565	5.775	5.775	0.979	5.381	4.630	5.099	2.712
SE	2.457	4.282	2.887	2.887	0.489	2.690	2.315	2.549	1.356

Data based on 100 seeds for each samples and each sample in five replicates.

Recovery of the pathogen from seeds

Seed samples were collected from the experimental plot were subjected for seed health testing methods for recovery of diseases transmission. The seeds collected from disease transmitted plants, sown in again during kharif season, infection having (28.0%) of *A. alternata* and *C. guizoticola* (23.8%) showed the (33.6%) transmission (Average of five seed samples, Table, 4).

Table 4: Seed to Seedling transmission *A. alternata* and *C. guizoticola* in experimental plot duringkharif-2010.

Place of collection	% of incidence in SBM		Germ %	Pre-Emergence	Post-Emergence	% of diseased plants	% of healthy plants	Recovery of pathogens	
	A. alt	*C. gui*						*A. alt*	*C. gui*
Jalahalli	18.0	20.0	76.0	24.0	3.0	36.0	37.0	27.0	23.0
Anekal	29.0	28.0	81.0	19.0	5.0	28.0	48.0	38.0	31,0
Nelamangala	31.0	24.0	78.0	22.0	2.0	41.0	35.0	46.0	34.0
Vajarahalli	32.0	25.0	80.0	20.0	5.0	34.0	41.0	45.0	31.0
Basavanahalli	30.0	22.0	79.0	31.0	2.0	29.0	48.0	42.0	34.0
Mean	28	23.8	78.8	23.2	3.4	33.6	41.8	39.6	33
SD	5.099	2.712	1.720	4.261	1.356	4.758	5.414	6.887	1.414
SE	2.549	1.356	0.860	2.130	0.678	2.379	2.709	3.421	0.231

*****Data based on 100 seeds for each sample, each samples in five replicates

Discussion

The expression of *A. alternata* and *C. guizoticola* was more percentage in seed coat than other seed components. The seeds were harvested during kharif-2010 season favored for the more number of pathogens in the seed coat than other components. The seeds harvested during kharif-2009 season shows a less incidence of mycoflora in the seed components when compare to the kharif 2010 season. This is due to the environmental factors like rainfall, temperature, humidity, P^H and also in growth stages of the crop. Some studies revealed that location activities of some fungal pathogens (Arya *et. al.*, 2004; Ashish Kumar Dubey and Tribhuvan Singh., 2005; Basak A.B., 1998; Rout J.G.,1985; Thippeswamy B., *et al.,*2006;) [2, 3, 5, 10, 14].

Reduction of the seed yield is based on the environmental conditions and the severity of disease symptoms. The mode of seed to seedling transmission of the pathogen is depends on the aggressiveness of the pathogen and environmental conditions. Current study revealed that the transmission of the pathogens were more during kharif 2010 than kharif 2009 harvested seeds. But disease transmission is more in kharif 2010 than kharif 2009 seasons. The disease appeared in the first fortnight of July and gradually incrased up to November, decline in disease severity with lowering the temperature and relative humidity up to December. Resume of literature reveals that voluminous work has been carried out all over the world. Some of the noteworthy and recent publications are (Arya *et. al.*, 2004; Ashish Kumar Dubey and Tribhuvan Singh., 2005; Rout J.G., 1985; Thippeswamy B., *et al.*2006) [2. 3, 10, 14].

The present study reveals that the disease transmission is more during kharif-2010 than 2009 kharif season. The results shows that the kharif-2010 season favors more percentage of pathogens have transmission from the seed to seedling and to the seeds. Because this is environmental factors are influenced for the transmission of the pathogens.

Conclusion

- *A. Alternata* and *C. guizoticola* the causal agents of leaf spot and blight diseases of Niger crop.
- Detection of Seed-borne *A. alternata* and *C. guizoticola* and other mycoflora plays an important role in determining the quality and longevity of seeds. Microbial invasion can lead to the rotting, loss of seed viability, germination, quality productivity and yield.
- It suggests that seeds are major agent of fungal transmission. Seeds should be treated with suitable chemical before sowing to reduce the fungal infection.
- This is due to the environmental factors like rainfall, humidity, temperature, P^H and also in growth stages of the crop and aggressiveness of the pathogens.
- Seed pathology involves the study of living entities, environmental factor affecting adversely to the seed production and utilization, as well as disease management practices applied to seed.

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