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Detection, location and seed transmission of *Sclerotium rolfsii* in Niger

O Nagaraja and M Krishnappa

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 stem-rot caused by *Sclerotium rolfsii*., the disease reduces the seed germination and yield up to 40-50%. The present study concentrated on detection, location and seed transmission of *S. rolfsii* in Niger seeds during kharif seasons-2010-2011. A total of 132 seed samples were collected from farmers, retail shops, fields and APMC markets and were subjected to SBM, PDA, Water agar and 2,4,D methods. Five seed samples showing higher incidence of *S. rolfsii* and other fungi in SBM were selected for location and transmission of the pathogen. The incidence of ranged between *S. rolfsii* in selective medium SBM (8-19%). The collected seed samples, fields and farmer's samples show a higher incidence of *S. rolfsii* and other fungi. The SBM method is more superior for isolating the *S. rolfsii* and other fungi than PDA, water agar and 2,4, D methods. The results revealed that the kharif - 2010 shows *S. rolfsii* (8-19%) in the SBM method. *S. rolfsii* ranged from (1-6%) in seed coat, (0-2%) in cotyledons, while (0-0%) in embryonic axis. In kharif - 2011, *S. rolfsii* (11-23%) in the SBM method. *S. rolfsii* ranged from (3-11%) in seed coat, (3-7%) in cotyledons, while (0-1%) in embryonic axis. The seeds tested during kharif 2010 - 2011 seasons harvested seeds favors the more number of *S. rolfsii* and other pathogens in the seed coat & cotyledons than in the other components. The transmission of *S. rolfsii* was (18.0%) in kharif 2010. In kharif 2011, the transmission was (29.4%) in all the five seed samples. The present study reveals that the disease transmission is more during kharif-2011 season than 2010. The *S. rolfsii* is a causal agent of stem-rot disease of Niger crop.

Keywords: Bird seed, location, transmission, *S. rolfsii*, SBM Test, stem-rot

Introduction
Niger [*Guizotia abyssinica* L (f) Cass.] is commonly called "*birdseed*" it belongs to the family Asteraceae/Compositae. 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 (Annonymous., 2010) [1]. It is mainly cultivated in tribal pockets of Gujarat, M.P., Orissa, Maharashtra, Bihar, Karnataka and Andhara 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 hactares with a production 6169 tonnes in Karnataka. (Annonymous., 2010) [1]. The crop is affected by number of fungal, bacterial, viral and nematodal diseases. The important fungal diseases are Alternaria blight - *Alternaria porii & 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* (Kandel., 2002; Saharan GS, Naresh Mehta and Sangavan MS., 2005; Rangaswamy G and Mahadevan., 2005). [14, 21, 25]. Stem rot disease is considered to be a major devastating disease to the Niger in India and also reduce the yield and oil quality. In the present work the occurrence, Location, seed to seedling transmission, their frequency of mortality, recovery of pathogens and its significance were studied.

Materials and Methods

Scope of the Study

The present experiment was carried out at Department of Applied Botany, Plant Pathology laboratory, Kuvempu university, Shankaraghatta, Shivamogga Karnataka during *kharif* season- 2010-2011. Niger seed samples (local variety) collected from different niger growing districts of Karnataka *viz*., Bellary, Bidar, Chitradurga, Chikmagalur, Davanagere, Dharwad, Gulbarga, Haveri, Mysore, Chamarajanagar, Chikmagalur, Tumkur, Bangalore-rural, Bangalore-urban, Kolar, Dharwad and Raichur districts.

Collection of Niger seed samples

The seeds of niger were collected from different locations of Karnataka state during *kharif*-2010. A total of one thirty two samples were collected from fields, farmers, retail shops and APMC markets of Bellary, Bidar, Chitradurga, Chikmagalur, Davanagere, Dharwad, Gulbarga, Haveri, Mysore, Chamarajanagar, Chikmagalur, Tumkur, Bangalore-rural, Bangalore-urban, Kolar, Dharwad and Raichur districts of Karnataka. The samples were collected and brought to the plant pathology laboratory of Applied Botany, Kuvempu University and stored in cloth bags room temperature for subsequent studies.

Detection of seed-borne *S. rolfsii* by Seed health test methods (ISTA, 1993)

a. SBM Method: 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 water and placed at the bottom of the Petri plates. One hundred seeds were sterilized in 0.2% sodium hypochlorite solution for 2 to 3 minutes and seeds taken randomly from each sample and were placed in ten Petri plates (20 seeds per plate). The Petri plates with seeds were then incubated at for seven days in the laboratory. The plates were kept under alternating cycles of 12 hrs light and 12 hrs darkness for seven days. After incubation, the distilled water was added every fourth day to the blotter so as to keep it sufficiently moist. The germination and fungi associated with the seeds were recorded during the incubation period. The incubated seeds were 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 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 than examined under Labomed vision 2000 microscope.

b. PDA Method: For potato dextrose agar method, 100 seeds were sterilized with 0.2% sodium hypo chlorite solution for 2 to 3 minutes. Then, the seeds were plated on sterile glass Petri plates containing PDA medium. Twenty seeds per petri plates and than the plates were incubated at 40 ºC in alternating cycles of 12 hrs light and 12 hrs darkness for seven days. After incubation eighth days the seeds were examined by stereo binocular microscope.

c. Water agar Method: For agar plate method, 100 seeds were sterilized with 0.2% sodium hypo chlorite solution for 2 to 3 minuets. Seeds were plated on sterile glass Petri plates containing (2.5%., i.e., 12.5 gms in 1000 ml of distilled water) water agar medium. These Petri plates were incubated at 25±2 ºC for seven days. After seven days these seeds were examined under stereo binocular microscope (Neergaard., 1977) [31].

d. 2, 4-D Method: In this method, 100 seeds were sterilized with 0.2% sodium hypo chlorite solution for 2 to 3 minuets. The three layers of blotter paper discs were dipped in 0.2% of 2,4-Dichloro Phenoxy acetic acid solution. Twenty seeds were placed equidistantly on moist blotter discs using sterilized forceps in laminar air flow wood under aseptic conditions. The plates were incubated room temperature for seven days. The observations were taken on the seventh day and then seeds were examined under stereo binocular microscope (Limonaard., 1968) [32].

Screening of *S. rolfsii* and other associated mycoflora

The incubated seeds were screened on eighth day using stereo binocular and labomed vision 2000 compound microscope. The germination, associated fungi were recorded and identified with the help of standard guides and manuals like (Barnett H L.., 1960; Booth C.., 1977; Sigourd and Funder., 1961; Subramanian C.V.., 1983; Van Arx J.A.., 1981) [5, 8, 27, 28, 30].

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) [6]. 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 *S. rolfsii* 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 July - 2010. 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 plants and leaves randomly in each plant. The severity of the disease was assessed by

using 0-9 scale (Mayee CD., and Datar VV., 1986) [9] and percentage of diseases index was calculated by using the formula. Seed to seedling transmission of *S. rolfsii* was studied.

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

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 2011 season in experimental plot for recovery of pathogens were studied. These seeds yielded the *S. rolfsii* and other fungi. The study shows that *S. rolfsii* are transmitted from seed to seedlings and to the seeds (Thippeswami B., *et al.,* 2006) [29].

Results and Discussion

Seed health testing

Results of four types of methods used to detect *S. rolfsii* and other mycoflora shown in (Table 1). The standard blotter method were more sensitive in detection of *S. rolfsii* than the PDA, Water agar and 2, 4, Dichloro phenoxy acetic acid mediums. Significant differences in occurrence of seed mycoflora were observed and the results indicated that irrespective of the locations and sources, a total of thirteen fungal species *viz., Alternaria porri. Fusarium oxysporum, Alternaria alternata, Cercospora guizoticola, Macrophomina phaseolina, Chaetomium guizotiae, Curvularia lunata, Verticillium dahlia, Cladosporium cladosporioides, Aspergillus flavus, Aspergillus niger*, and *Aspergillus ochraceus* belonging to two genera were detected from local variety of niger seeds. All the fungi decreased germination potential. Out of thirteen fungal species recorded, the occurrence of *S. rolfsii* was found predominant in the seed samples analyzed from seventeen districts (8 to 19.0%). The present study revealed that occurrence of seed borne *S. rolfsii* and other fungi may varied depending up on the location and sources of collection from different farmers and fields. Resume of literature reveals that voluminous work has been carried out all over the world. Some of the noteworthy and recent publications are (Sharma S.M., 1982; Rangeshwaran R., and Prasad RD., 2000; Rao Raghavendra NN., and Pavgi MS., 1975; Kulkarni JH, and Oblisami GH.., 1973; Kolte S.R. 1985; Caromona M.A, *et al*., 2006) [10, 18, 19, 22, 23, 26]. The present findings are in conformity with earlier reports of oil seed crops, who reported that variation in the occurrence of seed borne *S. rolfsii* and other fungi according to geographic location in niger crop (Choudary KCB, and Puttoo B.L., 1991; Cook A.A.., 1955; Bradley CA, and Del Rio LE..,2002; Basuchaudary KC, and Putto BL.., 1997; Hans Kendal., 2002). [7, 9-11, 14].

Similarly, visual sporulation of the fungus on the seed was generally heavier in the SBM methods than in Water agar, PDA and 2,4-D methods. However, the standard blotter method was the most effective and revealed a higher incidence of seed infection than the other methods. The collected seed samples, fields, farmers samples show a higher incidence of *S. rolfsii* and other fungi than in retail shops and APMC markets of Karnataka state. This method was also easy quick for recording the presence of *S. rolfsii* on the niger seeds.

Table 1: Incidence of *S. rolfsii* in seed health test methods of Niger.

Seed health testing methods	% seed infection								
	S. rol	***F. oxy***	***A. alt***	***M. pha***	***C. gui***	***A. por***	***A. nig***	***A. fla***	***A. och***
SBM	*19.0	11.6	19.2	3.0	23.2	36.6	18.2	2,8	3.4
PDA	2.4	5.8	9.4	0.4	8.4	14.0	5.0	7.0	0.0
Water agar	1.0	0.0	8.0	1.0	3.0	6.4	1.6	1.4	2.0
2,4-D	2.3	1.3	9.2	0.2	4.0	16.0	1.4	3.0	5.3
SD	8.573	5.243	5.203	1.279	9.332	12.91	7.940	2.884	2.238
SE	4.286	2.621	2.621	0.629	4.666	6.456	3.970	1.665	1.119

Average values of five samples and 100 seeds per method (five replicates of 100 seeds).

S.rol - *Sclerotium rolfsii*, F.oxy - *Fusarium oxysporum* A. por - *Alternaria porii,* A. alt -*Alternaria alternata,* M. pha - *Macrophomina phaseolina*, C. gui - *Cercospora guizoticola*, A. nig - *Aspergillus niger*, A.flav - *Aspergillus flavus*, A. och - *Aspergillus ochraceus*

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). Many researchers (Arya V K *et al*., 2004; Ashish Kumar Dubey and Tribhuvan Singh., 2005; Ashish Kumar Dubey and Tribhuvan Singh.., 2006; Basak A.B.., 1998; Ghasolia RP, and Jain SG.., 2004; Rout J.G.., 1985 [2-4, 6, 13, 24] reported the location of the pathogen in seed coat, cotyledons, endosperm and embryonic axis (plumule and radical) of various oil seed crops.

In Niger, The results revealed that the kharif 2010 shows *S. rolfsii* (8-19%) in the SBM method. *S. rolfsii* ranged from (1-6%) in seed coat, (0-2%) in cotyledons, while (0-0%) in embryonic axis. In kharif-2011, *S. rolfsii* (11-23%) in the SBM method. *S. rolfsii* ranged from (3-11%) in seed coat, (3-7%) in cotyledons, while (0-1%) in embryonic axis. The seeds tested during kharif 2011-2010 seasons harvested seeds favors the more number of *S. rolfsii* and other pathogens in the seed coat and cotyledons than in the other components (Table 2 & 3).

The expression of *S. rolfsii* was more percentage in seed coat than other seed components. The seeds were harvested during kharif-2011 season favored for the more number of pathogens in the seed coat than other components. The seeds harvested during kharif-2010 season shows a less incidence of mycoflora in the seed components when compare to the kharif 2011 season. This is due to the environmental factors like rainfall, temperature, humidity, P^H, aggressiveness of the pathogen and also in growth stages of the crop.

Table 2: Location of *S. rolfsii* in different seed components of Niger in Kharif-2010

Place of collection	% infection of seed in SBM		In percentage	
		Seed coat	Cotyledons	Embryonic axis
	S. rolfsii	*S. rolfsii*	*S. rolfsii*	*S. rolfsii*
Anekal	8.0	3.0	1.0	0.0
Pandavapura	18.0	6.0	2.0	0.0
Sondekolala	12.0	1.0	0.0	0.0
Vajarahalli	9.0	1.0	0.0	0.0
Basavanahalli	19.0	4.0	1.0	0.0
Mean	13.2	3	0.8	0.0
SD	4.5343	1.8973	0.7483	0.0
SE	2.2671	0.9486	0.3521	0.0

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

Table 3: Location of *S. rolfsii* in different seed components of Niger in Kharif-2011

Place of collection	% infection of seed in SBM		In percentage	
		Seed coat	Cotyledons	Embryonic axis
	S. rolfsii	*S. rolfsii*	*S. rolfsii*	*S. rolfsii*
Anekal	11.0	9.0	3.0	0.0
Pandavapura	23.0	6.0	7.0	0.0
Sondekolala	19.0	11.0	6.0	1.0
Vajarahalli	13.0	3.0	5.9	0.0
Basavanahalli	22.0	8.0	4.0	0.0
Mean	17.6	7.4	5.18	0.2
SD	4.8	2.7276	1.4593	0.4
SE	2.4	1.3523	0.7296	0.2

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

Transmission studies in field

During the field survey the stem rot, seedling blight of Niger was noticed in all visited fields during kharif and rabi seasons in 2010-2011. Symptoms of stem rot shows, The tissues of collar region become soft and depressed. White fungus grows on the diseased part and forms mustard seed like sclerotia. The diseased plants turn yellow and dry. The perfect stage of the fungus is *S. rolfsii*. The disease is seed borne as well as soil borne. As the plant number is reduced, the disease causes yield losses. The severity of stem rot, seedling blight diseases was more in kharif-2011 then 2010.The present study results revealed that the seeds having (13.2%) infection of *S. rolfsii* showed the transmission of (18.0%) in Niger. (Average of five seed samples, Table, 4).

Table 4: Seed to Seedling transmission *S. rolfsii* in experimental plot during kharif-2010.

Place of collection	% of incidence in SBM	Germ %	Pre-emergence	Post-emergence	% of diseased plants	% of healthy plants	Recovery of pathogens
	S. rolfsii						*S. rolfsii*
Anekal	8.0	72.0	28.0	2.0	17.0	53.0	11.0
Pandavapura	18.0	81.0	19.0	5.0	19.0	57.0	23.0
Sondekolala	12.0	69.0	31.0	1.0	21.0	47.0	19.0
Vajarahalli	9.0	76.0	24.0	3.0	15.0	58.0	13.0
Basavanahalli	19.0	71.0	29.0	3.0	18.0	50.0	22.0
Mean	13.2	73.8	26.2	2.8	18	53	17.6
SD	4.5343	4.2614	4.2614	1.3266	2	4.1472	4.8
SE	2.2671	2.1304	2.1304	0.6633	1.0023	2.0736	2.4

*Data based on 100 seeds for each sample 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 2011, infection having (17.6%) of *S. rolfsii* showed the (29.4%) transmission (Average of five seed samples, Table, 5).

Table 5: Seed to Seedling transmission *S. rolfsii* in experimental plot during kharif-2011.

Place of collection	% of incidence in SBM	Germ %	Pre-emergence	Post-emergence	% of diseased plants	% of healthy plants	Recovery of pathogens
	S. rolfsii						*S. rolfsii*
Anekal	11.0	68.0	32.0	1.0	33.0	34.0	36.0
Pandavapura	23.0	73.0	27.0	2.0	28.0	43.0	31.0
Sondekolala	19.0	79.0	21.0	5.0	31.0	43.0	27.0
Vajarahalli	13.0	63.0	37.0	3.0	26.0	34.0	28.0
Basavanahalli	22.0	77.0	23.0	1.0	29.0	47.0	33.0
Mean	17.6	72.0	28.0	2.4	29.4	40.2	31
SD	4.8	5.8651	5.8651	1.4966	2.4166	5.2687	3.2863
SE	2.4	2.9325	2.9325	0.7483	1.2083	2.6343	1.6431

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

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 like rainfall, temperature, humidity, P^H and also in growth stages of the crop. Current study revealed that the transmission of the pathogens were more during kharif 2011 than kharif 2010 harvested seeds. But disease transmission is more in kharif 2011 than kharif 2010 seasons. The disease appeared in the first fortnight of July and gradually increased up to November, decline in disease severity with lowering the temperature and relative humidity up to December. Many researchers ((Arya V K *et al*., 2004; Ashish Kumar Dubey and Tribhuvan Singh., 2005; Ashishkumar Dubey and Tribhuvan Singh.., 2006; Basak A.B., 1998; Ghasolia RP, and Jain SG.., 2004; Rout JG..,1985; Thippeswamy B *et al*., 2006) [2-4, 6, 13, 24, 29] have recorded the transmission of disease on different oil seed crops, like sesame, safflower, sunflower, soyabean, mustard, ground nut and chilli seeds etc.

The present study reveals that the disease transmission is more during kharif-2011 than 2010 kharif season. The results shows that the kharif-2011 season favors more percentage of pathogens have transmission from the seed to seedling and to the seeds. Because this is environmental factors like rainfall, temperature, humidity, P^H and also in growth stages of the crops and aggressiveness of the pathogens.

Conclusions

Results from the present investigation indicated that there was variation in *S. rolfsii* from one locality to another. Mycoflora of seed varied from place to place due to change in conditions prevailing during seed development, harvesting and storage. *S. rolfsii* was found predominant in the samples analysed from seventeen districts of Karnataka. Detection of seed-borne *S. rolfsii* 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. 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. *S. rolfsii* the causal agent of stem rot disease of Niger crop.

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