INCIDENCE OF SEED-BORNE FUNGI ON SESAME (*SESAMUMINDICUM* L.) AND THEIR EFFECT ON GERMINATION

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SUMMARY

Investigations were conducted in the teaching and research laboratory of Department of Crop protection, Bayero University, Kano, to detect seed borne fungi and their effect on the germination of some Sesame cultivars. Standard blotter method of seed health testing was used to detect fungi from the seeds of five sesame cultivars namely E8, Ex-Sudan, Yendev-55, Ex-Katsina and Ex-Bauchi; these fungi were sub cultured in potato dextrose agar amended with streptomycin for further growth and sporulation. The effect of associated fungi on germination was evaluated using rolled paper towel method. Fungi associated with the seeds were *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus niger* and *Rhizopus* sp. Fungal incidence varied among the cultivars and ranged from 0.00-26.00 % with E8 (0.00-4.00%), Ex-Sudan (5.00-25.50%), Yendev-55 (3.00-4.50%), Ex-Katsina (16.50-25.00%) and Ex-Bauchi (8.50-26.00%). The incidence of *Aspergillus niger* was higher on surface sterilised seeds while, *Rhizopus* sp. was more prevalent on unsterilized seeds. The germination percentage varied among cultivars, from 84.50-95.12% with E8 recording the highest.

Key words: Seed borne fungi, Sesame, Incidence and Germination.

SESAME (*Sesamum indicum*L.) also known as Benniseed, is one of the world's oldest spice (common ingredient in cuisines) and important oilseed crop in Saharan Africa (12). It is reported to have originated in Africa and is considered to be one of the primeval oil seed plant brought into cultivation in various parts of the world. Nigeria is the world's 6th largest producer of sesame, producing 461,000 metric tons

annually and generating over $700 million in export revenue (8). Sesame cultivation in Nigeria is traditional to the Guinea Savanna, Sudan and Sahel agro-ecological zones, comprising Benue, Kwara, Kogi, Niger, and Jigawa states (6).

Sesame seeds are good source of protein (20%) and edible oil (50%), and contain about 47% oleic acid and 39% linolenic acid (15). Sesame seeds are used as ingredients in bread, candies, chips and other health foods making whereas sesame oil is used as cuisine oil which has brilliant permanence due to the presence of innate antioxidants sesamol, sesamoline and sesamin hence known as the "king of oils" (14), it is also a raw material for the manufacturing of margarine, paints, varnishes, soaps, perfumes, pharmaceuticals, and insecticides.

Although sesame is extensively used for numerous purposes, the crop has very low yielding capacity (2). Numerous deteriorative microbes particularly fungi have created so many problems particularly seed rot and mortality, and poor germination in sesame production and storage hence presenting a major threat to crop establishment and yield (10). These seed borne fungi may be externally borne on the seed surface or within their tissues (13). Many authors reported the incidence of *Aspergillus flavus* in sesame seeds along with other fungi (11). Other fungi viz., *Aspergillus niger, A. viridus, A. alba*, *Fusarium* sp., *Alternaria redicina, A. brassicola*, *Drechslera* sp., *Curvularia* sp., *Cephalosporium* sp., *Penicillium* sp. have also been isolated from sesame (13) which have serious effects on this crop such as root rot, wilting and damping off (7).

In Nigeria, seeds for planting are often untreated and often obtained from local markets or from farmers' own reserve, sometimes from seed companies or from agricultural development programmes (ADPs). However, (17) indicated that, the first step towards the attainment of maximum crop yield is the use of high-quality seeds hence revealing seed borne pathogens as a factor in production. Thus, proper identification of these pathogens is vital in disease management strategies. The present work was planned to detect seed mycoflora of sesame cultivars grown in Kano (Sudan savannah ecological area of Nigeria) and their effect on germination.

MATERIALS AND METHODS

Experimental Site and Sesame Cultivars

The experiment was conducted at the Research and Teaching Laboratory of Crop Protection Department, Faculty of Agriculture, Bayero University, Kano (11^0 58^0N, 8^0 25^0 E and 475m above sea level). Five cultivars of sesame seeds namely E8, Yendev-55, Ex-Sudan, Ex-Katsina and Ex-Bauchi were used for this study. E8 and Ex-Sudan were obtained from Department of Agronomy, Faculty of Agriculture, Bayero University, Kano while, Yendev-55, Ex-Katsina and Ex-Bauchi were obtained from Dawanau grain market in Kano. These seeds were kept in polyethylene bags and stored in refrigerator until when needed.

Seed testing and detection of seed borne fungi

The inoculating chamber was washed with Dettol (Chloroxylenol B. PC. 4.8 % w/v; Oleum PiniAromaticum 8.38% w/w, Isopropyl Alcohol 9.43% w/w, SapoVegetalis 5.60% w/w, Saccharum Ustumq.s.,Aqua ad 100 vols) and fumigated with a spray of 80% ethanol. Sample processing was in accordance with the International rules for seed testing (9). Four hundred (400) randomly selected seeds per cultivar were divided into two subsamples. One subsample was surface sterilized in 1% sodium hypochlorite (NaOCl) for three (3) minutes and then rinsed in three changes of sterile distilled water (SDW) while the second which served as control was rinsed in three changes of sterile distilled water (SDW) and tested using the standard paper blotter methods (10). Twenty-five (25) seeds were equidistantly arranged from each other in three (3) concentric rings in 9 cm diameter sterile plastic Petri dishes lined with SDW moistened filter paper and covered to create high humidity and stimulate fungal growth. Filter papers in the Petri dishes were kept moist as when needed periodically, while care been taken not to completely open the plates when adding the distilled water so as to avoid contamination. The experimental design used was Completely Randomized Design (CRD) in which each variety serves as treatment and repeated eight (8) times (twenty-five seeds per repetition) respectively. The same procedures were followed also for the unsterilized subsamples. These treatments were randomly arranged to avoid bias.

The Petri dishes containing the seeds were then spread out on a laboratory bench for seven (7) days at 28±2^{o}C.

The incidence of the fungi growing on the sesame seeds was assessed between third and fifth day (however, the plates were left for additional two days before discarding) by counting infected seeds (with fungal growth) and expressed as percentage of the total seeds plated on the blotter. The frequency of isolation of a fungus was assessed by counting the number of times a particular fungus manifest per each treatment (cultivar) and expressed as per cent frequency of isolation. However, normal and abnormal seeds were counted separately and expressed as percentage of the total number of seeds per plate.

Isolation of fungi on Agar plate

Fungi growing on infected seeds based on their growth characteristics were cultured on Potato Dextrose Agar (PDA) medium amended with Streptomycin. The plates were incubated at 28± 2^{o}C for 7 days. Growth habits of the various fungi were observed, slides were prepared for microscopic examination, and identification using the illustrated genera of imperfect fungi as a guide (3).

Germination test

Germination test was carried out using standard rolled paper towel method (10). One hundred (100) seeds of each cultivar were randomly selected and allowed to germinate between two (2) rolled papers at ambient temperature of 28±2^{o}C in darkness for seven (7) days. After incubation, the numbers of germinated seeds were counted. The germinated seeds were graded as normal and abnormal. Normal seedlings were with developed roots and shoot and free from symptoms of infection while, the abnormal seedlings are those seedlings with either poorly developed short roots or shoot or both, and exhibiting disease symptoms.

Data Analysis

Data obtained in percentages were transformed using arc sine transformation and subjected to analysis of variance (ANOVA) using GENSTAT 17 Edition. Differences between means were separated using Student Newman Keuls (SNK) test at 5% level of probability.

RESULTS

The result shows the presence of four fungi belonging to three genera, this include *Fusarium oxysporum*, *Aspergillus flavus*, *A. niger* and *Rhizopus* sp. (Plate 1). Morphological characteristics of the fungi identified: (A) *Fusarium;* presence of macro and micro conidia. Macro conidia were several-celled, slightly curved or bent,

typically canoe shaped. Micro conidia were 1-celled, ovoid, borne singly. (B and C) *Aspergillus;* presence of upright conidiophores, simple, terminating in a globose ascus. Conidia were 1-celled and globose. (D) *Rhizopus;* presence of branching hyphae which is coenocytic. Sporangiospores are produced inside sporangium. Sporangiophores arise among distinctive, root like rhizoids.

Frequency of isolation on sterilized seeds was lower for all the fungi (Figure 1), *Fusarium oxysporum* had the least frequency of isolation in both sterilize and unsterilized seeds. When sterilized, frequency of isolation on seeds was highest for *A. niger* followed by *Rhizopus* sp. and *A. flavus*. On unsterilized seeds (Figure 2), however, *Rhizopus* sp. had highest frequency followed by *A. niger* then *A. flavus.*

Table 1 shows the incidence of seed borne fungi on normal and abnormal surface sterilized (1%NaOCl) and unsterilized seeds. The incidence significantly varied among cultivars both on sterilized and unsterilized, where E8 had the least fungal incidence, while Ex-Katsina and Ex-Bauchi recorded the highest. The normal seeds were significantly higher on surface sterilized E8 and Yendev-55 (22.50% and 21.50% respectively) than Ex-Sudan and Ex-Bauchi (20.50%) while, Ex-Katsina (20.12%) recorded the least normal seeds although it did not differ from Ex-Sudan and Ex-Bauchi too. The abnormal seeds (surface sterilized) were significantly higher on Ex-Katsina, Ex-Sudan and Ex-Bauchi while E8 recorded the least abnormal seeds but did not differed from Yendev-55. However, there was no significant difference observed on unsterilized seeds. The interactive effect of cultivar and treatment on the incidence of fungi is presented in Table 2. There was significant effect of treatments on sesame cultivar. The incidence was higher on unsterilized (0%NaOCl) treated Sesame cultivar than the sterilised (1%NaOCl) counterpart. Sesame cultivar shows significant differences on fungal incidence with E8 and Yendev-55 recording significantly the least incidence (2.00%) while Ex-Katsina which was statistically similar to Ex-Sudan and Ex-Bauchi had the highest (20.75%). The sterilant (1%NaOCl) used significantly reduced the incidence of the fungi.

Table 3 shows the effect of seed borne fungi on germination capacity of the Sesame cultivar, significant difference was observed among cultivars on germination, number of normal and abnormal seedlings. Cultivar E8 had the highest

germination percentage (95.12%) followed by Ex- Katsina and Ex-Bauchi while Ex-Sudan had the least germination. Yendev-55 had the highest number of normal seedlings (82.62) and least number of abnormal seedlings (9.50). This was followed by E8 while Ex-Katsina and Ex-Bauchi had the lowest number of normal seedlings and highest number of abnormal seedlings.

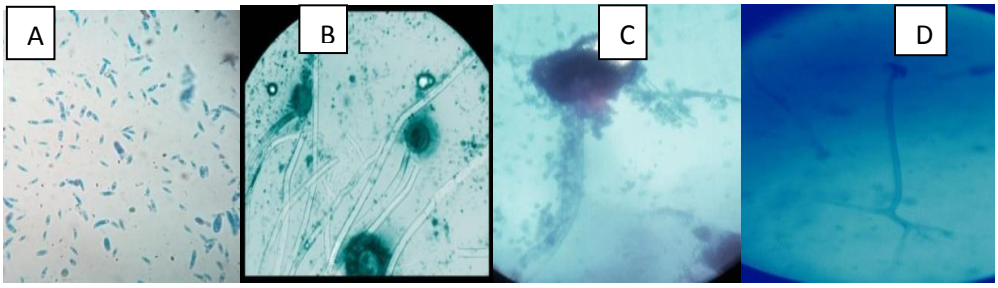

Plate 1: Photomicrographs of Seed Borne Fungi Detected on Seeds of five Sesame Cultivars. (A)*Fusarium oxysporium* (B) *Aspergillus flavus* (C) *A. niger* (D) *Rhizopus*sp

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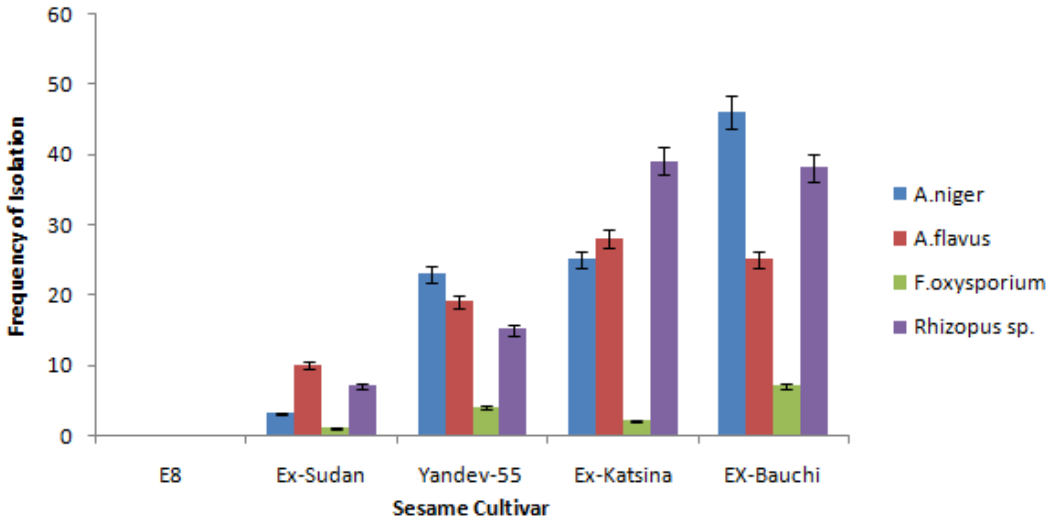

Figure 1: Frequency of isolation of fungi on sterilized seeds of sesame cultivar

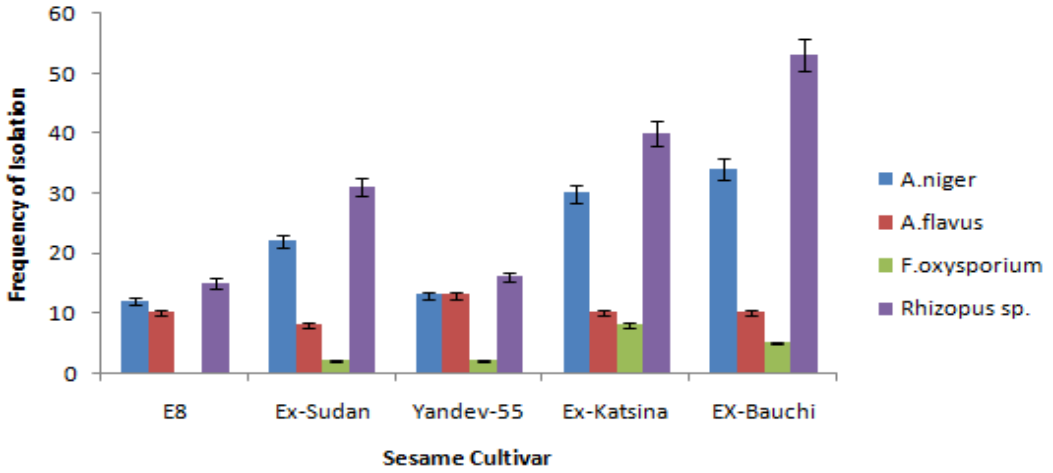

Figure 2: Frequency of isolation of fungi on unsterilized seeds of sesame cultivar

Table 1: Incidence of Seed-borne fungi on normal and abnormal seeds of surface sterilized (1%NaOCl) and unsterilized seeds of Sesame cultivar

Treatment	Incidence of Infected Seeds (%)				Fungal Incidence (%)	
	Normal		Abnormal			
	Sterilized	Unsterilized	Sterilized	Unsterilized	Sterilized	Unsterilized
E8	22.50a	21.38	2.50a	3.63	0.00(0.00a)	4.00(0.04a)
Ex-Sudan	20.50a-d	19.25	4.50b	5.75	3.00(0.03ab)	25.50(0.26b)
Yendev-55	21.50ab	19.50	3.50ab	5.50	5.00(0.05ab)	4.50(0.05a)
Ex-Katsina	20.12bd	19.88	4.86b	5.12	8.50(0.09b)	25.00(0.25b)
Ex-Bauchi	20.50a-d	19.89	4.50b	5.13	16.50(0.17c)	26.00(0.26b)
SED	0.79	1.08	0.79	1.08	0.026	0.043
LS	*	NS	*	NS	**	**

Figures in parenthesis are arc sine values to which SED are applicable. Means followed by the same letter(s) in a column are not significantly

different at 5% level of probability using Student-Newman Keuls Test (SNK). LS=level of significance, NS=not significant, *=Significant, **=highly significant.

Table 2: Effect of Surface Sterilizations (1%NaOCl) on the Incidence of Seed Borne Fungi on five Sesame Cultivars

Treatments	Incidence (%)
Sesame cultivar (SC)	
E8	2.00(0.02a)
Ex-Sudan	15.25(0.16b)
Yendev-55	3.75(0.04a)
Ex-Katsina	20.75(0.21b)
Ex-Bauchi	17.25(0.17b)
SED	0.025
Sodium Hypochlorite (SH)	
1% NaOCl	6.60(0.07a)
0% NaOCl	17.00(0.17b)
SED	0.016
Interaction SC X SH	**

Figures in parenthesis are arc sine values to which SED are applicable.Means followed by the same letter(s) in a column are not significantly different at 5% level of probability using Student-Newman Keuls Test (SNK). **=Highly significant. NaOCl=Sodium hypochlorite, SC=Sesame cultivar, SH=Sodium hypochlorite.

Table 3: Effect of seed-borne fungi on germination capacity of Sesame cultivar

Treatment	Germination (%)	Number of Normal seedlings	Number of Abnormal seedlings
E8	95.12(1.26a)	71.12b	24.00b
Ex-Sudan	84.50(1.01c)	57.00c	27.50c
Yendev-55	91.62(1.16bcd)	82.62a	9.50a
Ex-Katsina	92.50(1.18bc)	56.25c	35.62d
Ex-Bauchi	92.62(1.18b)	56.25c	36.38d
SED	0.031	0.93	1.55
LS	**	**	**

Figures in parenthesis are arc sine values to which SED are applicable, Means followed by the same letter(s) in a column are not significantly different at 5% level of probability using Student-Newman Keuls Test (SNK). **=Highly significant.

DISCUSSION

The four fungal species identified have also been reported earlier in Sesame seeds in countries like Nigeria, Pakistan and India (1, 13and 5). Most of these species identified are reputed seed borne pathogen of sesame (17). The frequency of isolation of these fungi in the samples tested revealed that *Rhizopus* sp. and *A. niger* were the most frequently detected fungi on both surface sterilized and unsterilized samples. This agrees with (5) who reported that *A. niger, A. flavus* and *Rhizopus* sp. were the most frequently isolated fungi from sesame seeds in Sialkot, Pakistan. A similar study by (13) found *A. niger* in high frequencies on almost all the cultivars of sesame tested. The genera *Aspergillus* have been reported to be present on all sesame samples and produce toxic substances in addition to deteriorative effect on seeds (11). The incidence was found to be higher on unsterilized seeds of sesame than the sterilized counterpart, with E8 having the lowest fungal incidence. This is in line with findings of (1) that reported that variety E8 has the lowest incidence of fungi. Surface sterilization of seeds with 1% NaOCl might have eliminated some of the

fungi on the seed surface, thereby reducing the fungal incidence. Report from (4) showed that 1%NaOCl treated seeds reduces the incidence of *Aspergillus* spp. On the effect of fungi on seed germination, the result showed that, the germination capacity of Ex-Sudan and Yendev-55 was slightly reduced. The slight reduction in germination capacity might be as a result of the deteriorative effect of some fungi present on or in the seeds samples. In a study by (16), it was reported that associated fungi decrease germination potential. Similarly, (13) reported that seed borne fungi are most disastrous as they reduce seed vigour and weaken the plant at initial stage of growth. However, this study agrees with the findings of other authors that had worked on seed health of Sesame seeds and other crops, that seeds are the major source of plant infection and disease transmission.

CONCLUSION

The results of this study indicated that there was significant variation in the incidence of the seed borne fungi associated with the different varieties tested, and the variety E8 had the lowest fungal incidence. The fungi isolated were also among the commonly reported seed borne fungi on Sesame. However, there is need for routine checks in order to find out if there are new fungal discoveries. Also, from this study, seeds that were surface sterilized with 1% NaOCl had significantly lower incidence of seed borne fungi than the unsterilized ones, hence indicating its effectiveness in reducing the seed borne fungi present on the seeds.

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