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Evaluation of seed health of Sesame cultivars

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Seeds of nine sesame cullivars collected from different parts of Nagaland and Assam were used for the evaluation of seed health. Maximum seed infestation was shown by Gowri cultivar while Local-1 showed the lowest infestation. The seed germination percentage was lowest in Gowri (13 %) and the highest (99 %) in Local-4. Moisture content was maximum in Local-3 (6.8 %) and lowest (1.15 %) in Local-1. A total of nine fungal species, viz.. *Aspergillus flavus*, *A. niger. A. terreus. Rhizoctonia* sp.. *Rhizopus* sp.. *Trichothecium roseum*, *Alternaria* sp.. *Mucor* sp. and *Penicillium* sp. were isolated from the seeds.

Key words : Evaluation of seed health, isolation of fungi, sesame

INTRODUCTION

Sesame (*Sesamum indicum* L.), known variously as til. tila. tal. ellu. khasa etc., is an important oil crop in India. It is called as "Queen of edible oils". Sesame seeds can be black, brown or white in colour and contains 50% oil, 21% protein, manganese, copper, calcium, Vit B, Vit E, phytoestrogens with antioxidant and anticancer properties. Among edible oils, sesame oil has the highest antioxidant content. Its medicinal properties make it a principal ingredient in the preparation of ayurvedic medicines.

Seed borne fungi are reported to cause reduction in the volume of storage tissue and amount of reserve food available to the developing seeds, which accounts for poor germination and poor seedlings growth (Vidyasekharan *et al*, 1972, Nandi *et al.* 1982). Hence, in order to obtain information on health status of seed lot and the mycoflora in different cultivars. seed health testing has been done.

MATERIALS AND METHODS

The present investigation was carried out in the laboratory of Department of Plant Pathology, SASRD, Nagaland University, Medziphema Campus in the year 2008. The approaches and procedures adopted during the course of investigation for seed health testing of sesame cultivars like AST-1. RT-1. Pd Til-1 .Gowri.Local 1. Local 2. Local 3. Local 4. Local 5 are described as follows.

Examination of dry seeds

The seed sample were first examined by naked eye and then under stereoscope binocular microscope. The mixture of seeds with other crop seeds, weed seeds, inert materials and broken seeds were recorded.

Moisture content (MC)

Four gram of seed lot was evenly distributed over the Petri plates and kept in an oven at a temperature of 103± 2°C for 17± I hr (according to International Seed Testing Association). At the end of the prescribed period, it was placed in a desiccator to cool for 30-45 min and then the moisture content was calculated using the following formula.

M.C (%) = $M_2 - M_3/M_2 - M_1 \times 100$ where M_1 = wt of Petri plates; M_2 = wt of seed lot with Petri plates (before drying) and M_3 = wt of seed lot with Petri plates (after drying).

Germination test

In order to get information with respect to field planting value, germination test was done by taking 20 seed lot using Top of Paper method (TP) where the first count was taken on the 2nd day and final count on the 6th day. Germination percentage was calculated using the following formula :

Germination % = No. of seed germinated / Total no. of seeds × 100

Seed mycoflora

The methods - Agar Plate method and Standard Blotter Method devised by ISTA (1966) was followed throughout the studies with certain modifications.

The experiment was placed in a Completely Randomized Design (CRD) and each treatment was replicated 5 times. The cultivars used in the present experiment are given Table 1.

Standard Blotter method

Seed mycoflora of sesame cultivars were studied using Standard Blotter Method given by Dover (1938). In this method 2 layers of sterilized blotting papers were placed in Petri plates and were moistened with sterile distilled water. 20 seeds were placed equidistantly in each plate and the seeded plates were kept in the incubation room for 8 days at room temperature (20-30°C) under natural light and darkness.

Agar Plate method

Seed mycotlora of sesame cultivars were studied using Agar Plate Method given by Muskett and Malone (1941). In this method. 20 seeds were placed equidistantly in Petri plates containing Potato-Dextrose Agar (PDA) medium and incubated at room temperature (20°C -30°C) under natural light and darkness.

Appearanee of seed-borne fungi on both Blotter and Agar Plate method was examined macroscopically and microscopically. The number and colonies appearing on the seeds were recorded at 48 his interval till the 8th day.

Fungal growth obtained from both Standard Blotter Method and Agar Plate Method were transferred to the PDA slants with the help of the inoculating needle and the isolated fungi were purified further by single spore or hyphal-tip technique.

Identification of fungi

The isolated fungi were studied under microscope and identified in the laboratory based on the morphological and cultural characters, and descriptions following. Gilman, (1967), Barnett and Hunter, (1972), Thorn and Raper, (1945), Raper and Thom, (1949) and Sharma. (1990).

RESULTS AND DISCUSSION

Observations recorded from Table 1 showed that cultivars showed variation in colour of seeds which

Table 1 : Seeds used for seed mycoflora.

Name of cultivar (cv)	Colour of seeds	1000 seed weight (g)	Duration of crops (days)	Place of collection
AST-1	Black	2.57	85—90	A.A.U., Jorhat
RT-1	Brownish black	2.52	85—90	A.A.U., Jorhat
Gowri	Brownish black	1.68	95—100	ICAR, Jharnapani
Pd til-1	Black	1.55	80—85	ICAR, Jharnapani
Local-1	Grey	1.30	90—120	Wokha
Local-2	Brownish black	1.32	90—120	Mopungchuket
Local-3	Grey	1.35	90—120	Chumukedima
Local-4	Brownish grey	1.40	90—120	Mokokchung
Local-5	Greyish brown	1.43	90—120	Changki

was black in case of AST-1 and Pd Til-1 while RT-1, Gowri and Local-2 seeds were brownish black in colour. Local-1 and Local-2 seeds were grey white Local-4 was brownish grey and Local-5 greyish brown in colour.

The test weight of seed (1000 seed lot) was found maximum (2.57 g) for AST-1 followed by RT-1 (2.52 g) while the other seed weight ranged from 1.30-1.68 g.

Seed health of sesame cultivars

All the nine sesame cultivars were first examined by naked eye and the data recorded on desirable parameters along with moisture content and germination percentage are presented in Table 2. The data from the table indicated presence of other crop seed in AST-1 and RT-1. while weed seeds were observed maximum (19 nos.) in RT-1 followed by Gowri (11 nos.). Pd Til-I (10 nos.) and AST-1 (4 nos.). However, rest of the cultivars recorded nil. Shrivelled seeds were found to be maximum (50 nos.) in Gowri whereas Local-1 recorded the lowest (5 nos.). Malformed seeds were recorded maximum in Gowri (32 nos.) followed by Local-4 (8 nos.). Local 5 and RT-1 (4 nos. each) while it was nil in rest of the cultivars. Broken seeds were recorded only from Local-5 (2 nos.) while inert matters recorded from AST-1. RT-1, Gowri and Pd Til-1 where 3, 150, 20, 5 nos. respectively.

The data on moisture content and percentage seed germination of different sesame cultivars has been tabulated in Table 2. The maximum (6.80%) moisture content was recorded from cultivar Local-3 followed by RT-1 (6.45%) while the lowest was recorded from Local-1 (1.15%). Maximum (99.0%) seed germination was recorded in Local-4 and the lowest (13%) was by Gowri. Germination percentage for rest of the cultivars ranged from 80.0-98.0 per cent.

Seed mycoflora of sesame cultivars

Seed mycoflora of sesame cultivars examined by Agar plate method is shown in Table 3 (a) and by Blotter method in Table 3 (b). A total of 9 fungal species distributed over seven genera were recorded from the nine sesame cultivars by both the

Table 2 : Examination of seed of Sesame cultivars for seed health

Cv	Wt. of seed lot (g)	Other crop seeds	Weed seeds	Shri-veled seeds	Mal-formed seeds	Broken seeds	Inert matters	MC (%)	Seed germination after 6th day (%)
AST-I	4	1	4	16	Nil	Nil	3	6.00 (2.53)	93.00 (9.66)
RT-I	4	1	19	7	4	Nil	15	6.45 (2.61)	92.00 (9.61)
Gowri	4	Nil	11	50	32	Nil	20	5.55 (2.18)	13.00 (8.93)
Pd til-1	4	Nil	10	33	Nil	Nil	5	4.60 (2.44)	80.00 (3.52)
Local-1	4	Nil	Nil	5	Nil	Nil	Nil	1.15 (1.28)	98.00 (9.92)
Local-2	4	Nil	Nil	28	Nil	Nil	Nil	3.05 (1.80)	97.00 (9.87)
Loeal-3	4	Nil	Nil	20	Nil	Nil	Nil	6.80 (2.69)	51.00 (7.16)
Local-4	4	Nil	Nil	36	8	Nil	Nil	2.15 (1.57)	99.00 (9.97)
Local-5	4	Nil	Nil	19	4	2	Nil	3.60 (1.98)	93.00 (9.66)
CD value at 5%								0.58	0.68

Figures in the parentheses are Square root transformed values.

Table 3(a) : Seed mycoflora of Sesame cultivars—Agar Plate method

Cultivar used	No. of fungal colonies per Petri plate*									
Name of fungi	AST-1	RT-1	Gowri	Pd Til-1	Local 1	Local 2	Local 3	Local 4	Local 5	Mean
Aspergillus	0.60	0.80	1.20	0.80	0.00	0.20	0.40	0.20	0.20	0.48
flavus	(1.02)	(1.12)	(1.26)	(1.12)	(0.71)	(0.81)	(0.91)	(0.81)	(0.81)	(0.98)
Aspergillus	0.80	0.40	0.80	0.40	1.00	1.00	0.20	0.40	0.60	0.68
terreus	(1.12)	(0.91)	(1.12)	(0.91)	(1.03)	(1.03)	(0.81)	(0.91)	(1.02)	(0.99)
Aspergillus	1.20	1.20	2.20	0.40	2.60	1.20	1.40	1.00	1.40	1.64
niger	(1.26)	(1.26)	(1.62)	(1.32)	(1.67)	(1.26)	(1.32)	(1.16)	(1.24)	(1.35)
Rhizoctonia	0.40	0.40	0.60	0.60	0.00	0.20	0.40	0.60	0.40	0.40
sp.	(0.91)	(0.91)	(1.02)	(1.02)	(0.71)	(0.81)	(0.91)	(1.02)	(0.91)	(0.91)
Rhizopus	0.80	0.40	0.60	0.80	0.40	1.20	1.20	1.00	0.80	0.80
sp.	(1.12)	(0.91)	(1.02)	(1.12)	(0.88)	(1.26)	(1.26)	(1.16)	(1.12)	(1.12)
Trichothecium	0.60	0.4	1.00	0.60	2.20	0.80	0.80	0.80	1.60	0.98
roseum	(1.02)	(0.91)	(1.19)	(0.98)	(1.58)	(1.08)	(1.12)	(1.12)	(1.39)	(1.15)
Alternaria	0.60	0.80	1.20	0.8	1.00	1.00	0.80	0.60	0.60	0.82
sp.	(1.02)	(1.12)	(1.26)	(1.12)	(1.16)	(1.16)	(1.12)	(1.02)	(1.02)	(1.12)
Mucor	0.60	0.60	1.00	1.00	1.20	1.40	0.80	0.80	0.00	0.82
sp.	(1.02)	(1.02)	(1.22)	(1.22)	(1.26)	(1.36)	(1.12)	(1.12)	(0.71)	(1.12)
Penicillium	0.80	0.80	1.00	0.60	2.40	1.00	0.80	1.40	1.40	1.13
sp.	(1.12)	(1.12)	(1.22)	(1.02)	(1.67)	(1.22)	(112)	(1.37)	(1.37)	(1.34)
Mean	0.71	0.64	1.10	0.67	1.20	0.89	0.70	0.76	0.78	
	(1.07)	(1.03)	(1.21)	(1.09)	(1.18)	(1.13)	(1.07)	(1.07)	(1.07)	
Total no. of fungal species	9	9	9	9	7	9	9	9	8	

* Average of 5 plates
Figures in the parentheses are Square root transformed values.

Table 3(b) : Seed mycoflora of Sesame cultivars—Blotter method

Cultivar used	No. of fungal colonies per Petri plate*									
Name of fungi	AST-1	RT-1	Gowri	Pd Til-1	Local 1	Local 2	Local 3	Local 4	Local 5	Mean
Aspergillus	0.60	0.40	0.20	0.20	0.00	0.20	0.40	0.20	0.00	0.24
flavus	(1-02)	(0.91)	(0.81)	(0.81)	(0.71)	(0.81)	(0.91)	(0.81)	(0.71)	(0.83)
Aspergillus	0.00	0.20	0.40	0.60	0.40	0.40	0.20	0.20	0.20	0.29
terreus	(0.71)	(0.81)	(0-91)	(1.02)	(0.91)	(1.12)	(1.02)	(1.02)	(0.81)	(0.92)
Aspergillus	0.40	0.80	0.60	0.40	0.40	0.40	0.20	0.40	0.60	0.51
niger	(0.91)	(1.12)	(1.02)	(0.91)	(0.91)	(0.91)	(0.81)	(0.91)	(1.02)	(0.94)
Rhizoctonia	0.20	0.40	0.40	0.80	0.20	0.20	0.40	0.00	0.00	0.29
sp.	(0.81)	(0.91)	(0.91)	(1.12)	(0.81)	(0.81)	(0.91)	(0.71)	(0.71)	(0.86)
Rhizopus	0.40	0.80	0.40	0.60	0.40	0.40	0.00	0.80	0.80	0.52
sp.	(0.91)	(1.12)	(0.91)	(1.02)	(0.91)	(0.91)	(0.71)	(1.12)	(1.12)	(0.97)
Trichothecium	0.20	0.00	0.60	0.60	0.00	0.40	0.20	0.00	0.20	0.31
roseum	(0.81)	(0.71)	(1.02)	(1.02)	(0.71)	(0.91)	(0.81)	(0.71)	(0.81)	(0.86)
Alternaria	0.20	0.40	0.80	0.40	0.00	0.40	0.00	0.60	0.20	0.33
sp.	(0.81)	(0.91)	(1.12)	(0.91)	(0.71)	(0.91)	(0.71)	(1.02)	(0.81)	(0.88)
Mucor	0.00	0.60	0.60	0.20	0.20	0.60	0.40	0.00	0.00	0.29
sp.	(0.71)	(1-02)	(1.02)	(0.81)	(0.81)	(1.02)	(0.91)	(0.71)	(0.71)	(0.86)
Penicillium	0.40	0.20	0.40	0.40	0.20	0.80	0.00	0.00	0.40	0.31
sp.	(0.91)	(0.81)	(0.91)	(0.91)	(0.81)	(1.12)	(0.71)	(0.71)	(0.91)	(0.89)
Mean	0.26	0.42	0.48	0.47	0.20	0.42	0.21	0.29	0.26	
	(0.84)	(0.93)	(0.96)	(0.94)	(0.81)	(0.94)	(0.83)	(0.86)	(0.84)	
Total no. of fungal species	7	8	9	9	6	9	6	5	6	

* Average of 5 plates
Figures in the parentheses are Square root transformed values.

methods. The fungal species recorded were : *Aspergillus flavus, A. niger, A. terreus. Rhizoctonia* sp.. *Rhizopus* sp.. *Trichothecium roseum Allenaria sp.. Mucor* sp. and *Penicillium* sp.

In Agar plate method (Table 3a) the maximum number of colonies were observed from *Aspergillus niger* (1.64) followed by *Penicillium* sp. (1.13) and the lowest number from *Rhizoctonia* sp. (0.40). Among the different test cultivars. the lowest number of fungal species association recorded was from Local-1 (7) where *Rhizoctonia* sp. and *A. flavus* were found absent followed by Local-5 (8) with absence of *Mucor* sp. The rest of the cultivars showed association of all the nine fungal species.

In Blotter method (Table 3b) the fungus *Rhizopus* sp. (0.52) colony was found to be maximum followed by *A. niger* (0.51) and the lowest was *A. flavus* (0.24). Among the cultivars. Local - 5 showed the lowest (5) number of fungal species with non representation of *Rhizoctonia* sp., *Trichothecium roseum, Mucor* sp. and *Penicillium* sp. In AST-1. Local-1 and Local-5. *A. flavus* was not recorded while *Allernaria* sp. was found to be absent in Local-1 and Local-3 where the number of fungal species associated were only 6. Cultivar AST-1 showed only 7 fungal species and RT-1, 8 fungal species association was found. However, in Gowri. Pd Til-1 and Local-2. all the nine fungal species were associated.

A large number of fungal association with sesame seed have been reported by many workers. Sulochana and Balakrishna (1997) have reported seed borne fungi isolated from 10 varieties of sesame using standard Blotter method and Agar plate method of 100 seed lot. The predominant fungi were: *Rhizopus nigricans, Aspergillus flavus, Mucor hiemalis, Aspergillus niger, Penicillium chrysogenum* and *Alternaria sesami.* Krishna *et al.* (2007) have also reported that from seeds of five sesame varieties, the fungi isolated were: *Allerrnaria* sp.. *Aspergillus niger, A. terreus, Curvularia* sp., *Fusarium* sp. *Penicillium* sp. and *Sclerotium* sp. However, in the present investigation, a total of nine fungal species distributed over seven genera were found associated with the seeds of nine sesame cultivars (Tables 3a and b). These fungi were: *Aspergillus flavus. A. niger, A. terreus, Rhizoclonia* sp., *Rhizopus* sp., *Trichothecium roseum, Alternaria* sp., *Mucor* sp. and *Penicillium* sp. which were also found to be the common fungi isolated and recorded by the other workers. Thus, the present findings are in confirmation with the reports of Sulochana and Balakrishna (1997), Krishna *et al.* (2007) and Tini Pillai *et al.* (2003) that these fungi were the most common fungi isolated from the sesame cultivars. However, the fungus causing leaf spot and blight was not observed either by Agar or Blotter method.

Maximum seed infestation was observed in Gowri cultivar sample with maximum number of shrivelled seeds (50). malformed seed (32), inert matter (20) while the lowest was observed in Local-1 cultivar with only 5 shrivelled seeds. Moisture content was found maximum in Local-3 cultivar (6.8%) while the lowest was recorded in Local-1 (1.15%). The seed germination was found to be lowest in Gowri (13%) while cultivar Local-4 showed the highest (99%) germination.

Observations on seed mycoflora from nine cultivars examined by Agar plate and Blotter method showed a total of nine fungal species, viz., *Aspergillus flavus. A. niger. A. terreus. Rhizoctonia* sp., *Rhizopus* sp., *Trichothecium roseum, Alternaria* sp.. *Mucor* sp. and *Penicillium* sp. Maximum number of *Aspergillus niger* were recorded from Agar plate method while *Penicillium* sp. from Blotter method. Lowest number of fungal species (7 nos.) were recorded in Local-1 by Agar plate method, while 5 nos. of fungal species from Local-4 by Blotter method. Gowri cultivar showed the maximum number of fungal species 9 nos. by both Agar and Blotter method. However, *Cercospora* causing leaf spot and blight and having seed borne nature was not observed either by Agar or Blotter method.

REFERENCES

Barnett. H.L. and Hunter, B.B. 1972. *Illustrated genera of imperfect fungi.* 3rd Ed. Burgess, *Mineapolis, Minnessola.*

Dover. L.C. 1938. *Manual for the Determination of seed-home Diseases*; Wageningen: International Seed Testing Association. 59 pp.

Gilman, J.C. 1967. *A Manual of soil fungi.* 2nd Ed. Oxford and IBH Publishing Co., New Delhi.

ISTA. 1966. International rules for seed testing. *Proe. Int. Seed Test. Ass.* **31:** 1-152.

Krishna. M.K.; Krishna Murthy: Rao. S.M and Reddy. G.I..N. 2007. Isolation of seed mycoflora from sesamum varieties. *The Andhra Agric J.* **54** (1 & 2): 72 -74.

Muskett. A.E. and Malone. J.P.1941. The Ulster method for the examination of flax seed for the presence of seed-borne parasite. *Ann. appl. Biol.* **28:** 8-13.

Nandi. D.; Mondal.G.S. and Nandi.B. 1982. Studies on deterioration of some oil seeds in storage III. Effects of different storage temperatures and relative humidities on seeds moisture.germinability and infection. *Seed Science and Technology.* **10:**141-150.

Rapper. K.B. and Thorn. C. 1949. *A manual of Penicillin.* Williams and Wikkins Co., Baltimore.

Sharma. O.P. 1990. *Text Book of Fungi*, Tata M.C. Graw-Hill Publishing Co., New Delhi.

Sulochana. K.K.. and Balakrishnan. S. 1997. Seed borne mycoflora of sesame (*Sesumum indicum* L). *Journal of Tropical Agriculture.* **35** (1): 64-66.

Thorn and Raper. 1945. *A manual of Aspergillus.* Williams and Wikkins Co.. Baltimore.

Tini Pillai; Raut: B.T.; Paulkar. P.K.; Agarkar. G.L). and Thakar. A.R. 2003. Assessment of Different Methods for Detection of Mycoflora of sesamum. *Annals of Plant Physiology.* **17** (2): 153-156

Vidhyasekharan, P., Lalithakumari, D. and Govindaswamy, C.V. 1972. Role of seed-borne fungi on the deterioration of quality of gingelly. *Indian Journal of Microbiology.* **12:**104-109.

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