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Detection of seed associated mycoflora of sesame

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Abstract
Sesame (*Sesamum indicum* L.) or Til is an erect annual, flowering crop grouped under Pedaliaceae family. Sesame is known as queen of oil seeds because of its high quality polyunsaturated stable fatty acid, that restrains oxidative rancidity. In this study 45 genotypes of sesame were detected for the seed mycoflora using seed health testing methods *viz.*, paper towel method, standard blotter method and standard agar plate method and six distinct fungi *viz., Alternaria, Macrophomina, Fusarium, Aspergillus, Penicillium,* and *Rhizopus* spp. were detected. In the three methods employed to identify seed mycoflora the standard blotter method has identified the maximum number of seeds infected by fungi compared to paper towel method and standard agar plate method. After a few days of germination, it was observed that the majority of the seeds were infected with disease, which hampered normal seedling growth and resulted in abnormal and rotted seedlings.

Keywords: Seed mycoflora, seed testing methods, fungal incidence and germination percentage

Introduction
Sesame is one of the world's oldest spice and oilseed crop originated in Africa. It is cultivated in different parts of the world like India, China, Nigeria, Myanmar and Tanzania. In India, sesame crop is grown in all parts of country and occupies an area of 16.03 lakh ha with a total production of 7.08 lakh tones and productivity of 442 Kg/ha (Anon, 2021). It is a drought tolerant crop because of its extensive root system and grows in well drained light to medium soils with an optimum pH of 5.5 to 8.0. This crop is cultivated at a latitude of 1600 m in a wide range of atmospheres, extending from semi-arid tropics and subtropics to temperate areas of the world (Karibasappa *et al.*, 2020) [8]. *Sesamum* seed is rich in its edible oil content (50%), quality protein (20%), calcium (1.3%), oleic acid (47%) and linolenic acid (39%). sesame oil is used as a cooking oil and is renowned as the "king of oils" because of the presence of intrinsic antioxidants sesamol, sesamoline, and sesamin and used as ingredients in production of bread, candies, chips, and health foods and also in manufacturing soaps, paints and Insecticides (Nayyar *et al.*, 2013) [10].

Sesame crop is attacked by many phytopathogens like where most of them are seed borne, seed borne mycoflora are carried over by infected seeds and they cause deterioration of seed in soil-affecting germination, causing seedling mortality and further infection of foliage is observed at growth stage. Different fungi *viz.*, *Alternaria sesami, Curvularia species, Macrophomina phaseolina, Cercospora sesami, Fusarium oxysporum f. sp. sesami, Helminthosporium, Penicillium* and *Rhizopus* spp. were associated with sesame seed (ISTA, 1999) [7]. Some other fungus like *Aspergillus niger, A. nidulans, A. alba and A. flavus* (that produce a toxic secondary metabolite called Aflatoxin) were also isolated from the seeds of sesame. These mycoflora are causing qualitative and quantitative loses in sesame. Among them *M. phaseolina, A. sesami, Cercospora sesami and Curvularia* spp. were the most prevalent fungi ranging from 23.9 to 35.4% (Ranasingh *et al.*, 2019) [11]. Early identification of seed mycoflora is very important to ensure the production, quality and health. Therefore, to plan the management practices identification of these pathogens is very important (Nayyar *et al.*, 2013) [10].

Materials and Methods
Collection of seed samples: Sesame seeds of 45 genotypes (SET-I & SET-II) like TLT-06, RT-390, TLT-10, TKG-22(NC), RT-389, DS-19-56, SVT-451, GT-10(NC), DS-19-49, MT-2017-11, JCS-3593, DS-18-10, RT-346 and AT-470, RT-392, PCU-21-1, RT-394, PCU-21-2, RT-395, MT-2019-7, TKG-22(NC), VS19-036, MT-2019-11, VS19-064, GKVKS-1, VS19-045, GKVK-2, GT-10(NC), TLT-07, JLS-1217-3-1, TLT-12, TIOS-1102, OSM-19-07, TIOS-

3101, OSC-79-19-3, AT-467, SVT-460, AT-457, TKG-2021-3, JCS-4039, TKG-2021-6, BBT-12, TKG-2021-9, DS-68, RT-346 were taken from All India Coordinated Research Project on Sesame and Niger, College of Agriculture, JNKVV, Jabalpur.

Testing procedure: Seed samples of sesame were tested by using Paper towel, Standard blotter and Standard agar plate method. In paper towel method 50 seeds are taken from each genotype of sesame and in blotter paper method 25 seeds were taken from each sesame genotype and kept in seed germinator at 25 °C and relative humidity was 89.4% and 10 seeds were taken from each genotype for Standard agar plate method and kept in BOD incubator. After seven days the mycoflora associated with sesame seed samples were observed under microscopy and number of infected seeds and seedlings were noted and fungal incidence in each genotype was calculated and germination percentage was also calculated using paper towel method.

Results and Discussions

Six distinct fungi like *Alternaria, Macrophomina, Fusarium, Aspergillus, Penicilium, and Rhizopus* spp. were detected using the three seed health testing methods (Plate 1). Table 1 revealed that maximum fungi were observed in the genotype (SET-I) RT-346 and followed by RT-389 and minimum were observed in RT-390 followed by TKG-22(NC) and in SET-II genotypes of sesame maximum fungi were observed in the genotype RT-346 followed by TKG-2021-6 and minimum fungi were observed in RT-394 followed by GT-10(NC).

In the comparative study of all the three methods Table 1 (paper towel method, standard blotter method and standard agar plate method) for the detection of mycoflora associated with 45 genotypes of sesame maximum number of seed infected with fungi were observed in the blotter method and it was observed that *Macrophomina phaseolina* was the predominant fungi identified through paper towel method and *Alternaria* spp. through standard blotter method and *Aspergillus* spp. was seen more in standard agar plate method.

Percentage of Fungal incidence

The fungal incidence on each genotype is calculated based on number of infected seeds & seedlings Table 2. It has ranged from 6 to 30% in SET-I and 8 to 28% in SET-II seeds of sesame under paper towel method and 8 to 44% in SET-I and 12 to 40% in SET-II under standard blotter method and from 10 to 40% in SET-I and 10 to 50% in SET-II seeds of sesame under standard agar plate method.

Genotypes (SET-I) with fungal incidence below 10% includes RT-390, TKG-22(NC), GT-10(NC) and above 10% includes TLT-06, MT-2017-11, SVT-451, DS-19-49, JCS-3593 and above 20% includes DS-18-10, TLT-10, DS-19-56, RT-389, RT-346 and in SET-II genotypes fungal incidence below 10% includes RT-394 and above 10% includes GT-10(NC), PCU-21-1, MT-2019-7, VS19-045, OSM-19-07, JCS-4039, PCU-21-2, MT-2019-11, TLT-12, SVT-460, DS-68, AT-470, TKG-22(NC), GKVKS-2, TIOS-3101, TKG-2021-3, VS19-036,G KVKS-1, TLT-07, TIOS-1102, AT-457, TKG-2021-9 and above 20% includes RT-392, AT-467, RT-395, VS19-064, JLS-1217-3-1, OSC-79-19-3, BBT-12, TKG-2021-6 and RT-346 under paper towel method.

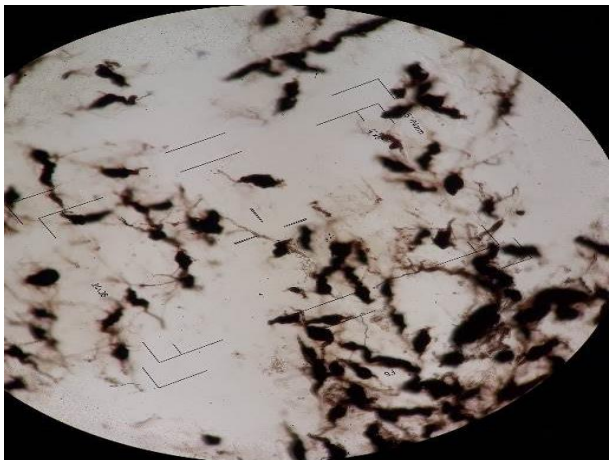
In standard blotter method, genotypes (SET-I) with fungal incidence below 10% includes RT-390 and above 10% includes TKG-22(NC), GT-10(NC) ,SVT-451, DS-18-10 and above 20% includes TLT-06, MT-2017-11 and 30% includes DS-19-49, JCS-3593, TLT-10, DS-19-56, RT-389 and 40% includes RT-346 and in SET-II genotypes fungal incidence above 10% includes RT-394, GT-10(NC), PCU-21-1, PCU-21-2, TKG-22(NC), VS19-045 and above 20% includes MT-2019-7, OSM-19-07, JCS-4039, MT-2019-11, TLT-12,SVT-460, DS-68,AT-470, OSC-79-19-3, VS19-064, GKVKS-2, TIOS-3101, TKG-2021-3, VS19-036, GKVKS-1, TLT-07, TIOS-1102, AT-467, TKG-2021-9 and above 30% includes RT-392, AT-457,RT-395, JLS-1217-3-1 , BBT-12, TKG-2021-6 and RT-346 (Fig 5 & 6).

In standard agar plate method, genotypes (SET-I) with fungal incidence of 10% includes RT-390 and 20% includes TLT-06, TKG-22(NC), GT-10(NC), MT-2017-11, DS-18-10 and 30% includes TLT-10, RT-389, DS-19-56, SVT-451, DS-19-49, JCS-3593 and 40% includes RT-346 and in SET-II genotypes fungal incidence with 10% includes RT-394, GT-10(NC) and 20% includes AT-470, PCU-21-1, PCU-21-2, RT-395, MT-2019-7, VS19-045, TLT-07, TIOS-1102, OSM-19-07, OSC-79-19-3, JCS-4039, BBT-12, TKG-2021-9 and 30% includes TKG-22(NC), VS19-064, GKVKS-2TLT-12, TIOS-3101, SVT-460, TKG-2021-3, TKG-2021-6, DS-68 and 40% includes RT-392, JLS-1217-3-1, AT-467, AT-457 and 50% includes RT-346.

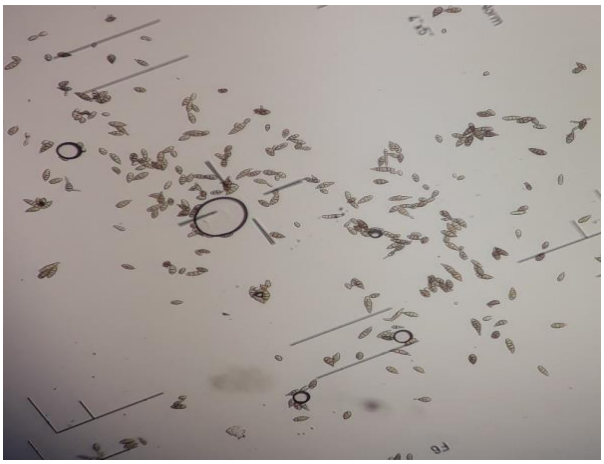
Table 1: Mycoflora associated with sesame genotypes (SET-I & SET-II) by three methods

S.no.	Genotype	Mycoflora																	
		Alternaria sesami			*Macrophomina phaseolina*			*Fusarium*			*Aspergillus* spp.			*Penicilium* spp.			*Rhizopus* spp.		
		P	B	A	P	B	A	P	B	A	P	B	A	P	B	A	P	B	A
1	TLT-06	1	3	0	2	1	1	1	0	0	1	1	0	1	1	1	0	1	0
2	RT-390	0	0	1	1	1	0	1	0	0	0	1	0	1	0	0	0	0	0
3	TLT-10	2	3	0	3	1	1	2	1	0	2	1	1	1	1	0	2	1	1
4	TKG-22(NC)	1	1	1	1	1	0	2	0	1	0	1	0	0	0	0	0	0	0
5	RT-389	3	2	0	3	2	0	2	2	0	2	2	1	1	1	1	3	1	1
6	DS-19-56	1	2	1	3	1	0	2	2	0	3	2	0	1	0	1	2	2	1
7	SVT-451	1	1	1	1	1	1	1	1	0	2	0	1	1	1	0	1	1	0
8	GT-10(NC)	1	2	0	0	0	1	1	0	0	2	2	0	0	0	0	1	0	1
9	DS-19-49	2	3	1	2	1	1	1	2	1	1	0	0	1	1	0	1	1	0
10	MT-2017-11	1	0	1	1	1	0	2	1	0	1	1	1	0	2	0	1	1	0
11	JCS-3593	2	2	0	2	3	1	2	1	1	1	1	1	1	1	0	2	1	0
12	DS-18-10	3	1	0	2	1	0	3	0	0	1	1	1	1	1	0	1	0	1
13	RT-346	2	2	0	3	2	0	3	2	1	2	2	1	2	1	1	3	2	1
14	AT-470	1	2	0	2	2	0	1	0	0	2	1	1	2	0	1	1	1	0
15	RT-392	2	2	1	1	1	1	2	2	1	2	1	1	2	1	0	2	1	0
16	PCU-21-1	1	1	1	2	1	1	0	0	0	1	1	0	2	2	0	1	0	0

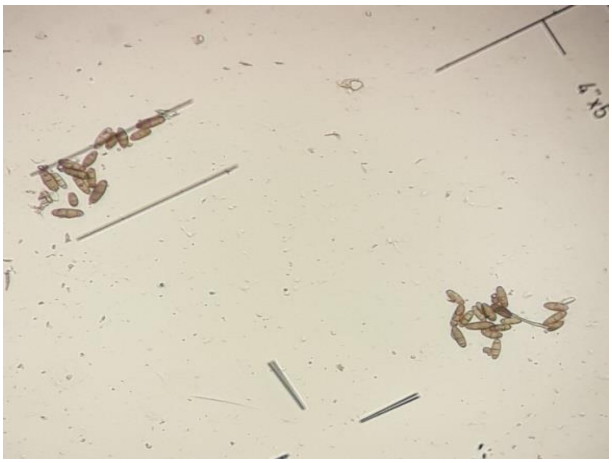
17	RT-394	0	1	0	1	1	1	0	0	0	1	0	0	1	1	0	1	0	0
18	PCU-21-2	2	1	1	2	1	0	0	1	1	2	1	0	2	0	0	0	1	0
19	RT-395	2	2	0	2	2	0	2	1	0	3	1	1	2	1	1	1	1	0
20	MT-2019-7	1	1	0	2	1	1	1	2	0	2	0	0	0	1	0	1	1	1
21	TKG-22(NC)	2	1	0	1	0	1	2	1	0	2	1	1	1	2	1	1	0	0
22	VS19-036	1	2	0	2	1	0	2	1	1	2	1	0	1	1	0	2	1	1
23	MT-2019-11	2	1	1	2	1	0	1	2	0	1	1	1	1	0	0	1	1	0
24	VS19-064	2	2	0	2	2	1	3	0	0	1	0	1	2	1	0	2	2	1
25	GKVKS-1	1	1	1	1	1	0	2	2	0	2	1	0	2	1	0	2	0	1
26	VS19-045	2	1	1	2	1	0	0	0	0	1	1	1	1	1	0	1	1	0
27	GKVKS-2	2	2	0	2	1	1	1	1	0	1	1	1	1	1	1	2	1	0
28	GT-10(NC)	1	1	0	2	1	1	1	0	0	0	1	0	1	1	0	1	0	0
29	TLT-07	2	2	0	2	1	0	1	1	0	1	1	1	2	0	0	2	1	1
30	JLS-1217-3-1	2	2	1	2	2	0	2	2	0	2	1	1	2	1	0	2	0	2
31	TLT-12	1	1	0	2	1	1	1	1	1	1	0	0	1	1	0	2	2	1
32	TIOS-1102	2	1	0	2	2	0	2	1	1	1	1	0	1	1	1	2	1	0
33	OSM-19-07	1	2	1	1	1	0	2	0	0	0	1	1	2	1	0	1	2	0
34	TIOS-3101	2	1	0	2	1	0	1	1	1	1	0	0	1	2	1	2	1	1
35	OSC-79-19-3	2	2	0	2	2	0	2	1	0	1	1	1	2	1	1	3	0	0
36	AT-467	1	0	1	2	1	1	2	2	1	2	2	0	2	1	1	2	1	0
37	SVT-460	2	1	0	1	1	1	1	1	1	1	0	1	1	2	0	2	1	0
38	AT-457	2	2	1	2	0	0	2	2	0	1	1	1	2	2	1	1	1	1
39	TKG-2021-3	1	2	1	1	1	0	1	1	1	2	1	0	2	0	1	2	1	0
40	JCS-4039	1	2	0	2	1	0	0	1	0	2	0	0	2	1	1	0	2	1
41	TKG-2021-6	2	2	0	2	2	1	2	1	0	2	2	0	3	1	1	2	1	1
42	BBT-12	1	1	1	1	2	0	2	1	0	3	1	0	2	2	1	3	1	0
43	TKG-2021-9	2	1	0	2	2	1	3	2	1	1	0	0	0	0	1	2	2	0
44	DS-68	1	2	1	1	1	1	1	0	0	2	1	1	1	2	0	2	1	0
45	RT-346	2	2	1	2	2	1	3	2	0	2	1	1	3	1	1	2	2	1



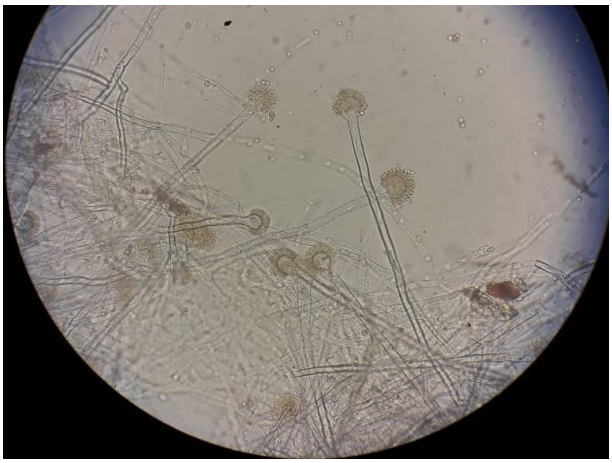
a) Sclerotia bodies of *Macrophomina phaseolina*



b) Conidia of *Alternaria* spp.



c) Conidia of *Fusarium* spp.



d) conidia of *Aspergillus* spp.

e) Sporangia of *Rhizopus* spp.

f) conidia of *Penicillium* spp.

Plate 1

Table 2: Percentage of Fungal incidence

S.no	Genotype	Percentage of Fungal incidence		
		Paper towel method	Standard Blotter method	Standard Agar plate method
SET-I Genotypes				
1	TLT-06	12	28	20
2	RT-390	6	8	10
3	TLT-10	24	32	30
4	TKG-22(NC)	8	12	20
5	RT-389	28	40	30
6	DS-19-56	24	36	30
7	SVT-451	14	20	30
8	GT-10(NC)	10	16	20
9	DS-19-49	16	32	30
10	MT-2017-11	12	24	20
11	JCS-3593	20	36	30
12	DS-18-10	22	16	20
13	RT-346	30	44	40
SET-II Genotypes				
14	AT-470	18	24	20
15	RT-392	22	32	40
16	PCU-21-1	14	20	20
17	RT-394	8	12	10
18	PCU-21-2	16	20	20
19	RT-395	24	32	20
20	MT-2019-7	14	24	20
21	TKG-22(NC)	18	20	30
22	VS19-036	20	28	20
23	MT-2019-11	16	24	20
24	VS19-064	24	28	30
25	GKVKS-1	20	24	20
26	VS19-045	14	20	20
27	GKVKS-2	18	28	30
28	GT-10(NC)	12	16	10
29	TLT-07	20	28	20
30	JLS-1217-3-1	24	32	40
31	TLT-12	16	24	30
32	TIOS-1102	20	28	20
33	OSM-19-07	14	28	20
34	TIOS-3101	18	24	30
35	OSC-79-19-3	24	28	20
36	AT-467	22	28	40
37	SVT-460	16	24	30
38	AT-457	20	32	40
39	TKG-2021-3	18	24	30
40	JCS-4039	14	28	20
41	TKG-2021-6	26	36	30
42	BBT-12	24	32	20
43	TKG-2021-9	20	28	20

44	DS-68	16	28	30
45	RT-346	28	44	50

*Out of 50 seeds from each genotype under paper towel method (Khare, 1996) [9] and 25 seeds from each genotype under standard blotter method and 10 seeds from each genotype under standard agar plate method Samaiya *et al.* (2015) [12].

Percentage of germination

Among the thirteen genotypes of SET-I Table 3 and Fig.1 germination percentage has ranged from 82 to 96% in these genotypes where minimum germination percentage was found in RT-346 with 82% followed by RT-389 with 84% and maximum germination percentage was found in TKG-22(NC) with 96% followed by GT-10(NC) and RT-390 with 94%.

In the 32 genotypes of SET-II Table 3 and Fig.2 germination percentage has ranged from 80 to 96% and minimum germination percentage was found in TKG-2021-6 with 80% followed by RT-392 with 82% and maximum germination percentage was found in GT-10(NC) with 96% and RT-394 with 94%.

Table 3: Percentage of germination in genotypes (SET-I & SET-II) of sesame under paper towel method

S.no	Genotype	Germination percentage
SET-I Genotypes		
1	TLT-06	92
2	RT-390	94
3	TLT-10	86
4	TKG-22(NC)	96
5	RT-389	84
6	DS-19-56	84
7	SVT-451	90
8	GT-10(NC)	94
9	DS-19-49	88
10	MT-2017-11	90
11	JCS-3593	88
12	DS-18-10	86
13	RT-346	82
SET-II Genotypes		
14	AT-470	88
15	RT-392	82
16	PCU-21-1	92
17	RT-394	94
18	PCU-21-2	92
19	RT-395	82
20	MT-2019-7	92
21	TKG-22(NC)	88
22	VS19-036	86
23	MT-2019-11	90
24	VS19-064	82
25	GKVKS-1	84
26	VS19-045	92
27	GKVKS-2	86
28	GT-10(NC)	96
29	TLT-07	84
30	JLS-1217-3-1	82
31	TLT-12	90
32	TIOS-1102	84
33	OSM-19-07	90
34	TIOS-3101	86
35	OSC-79-19-3	84
36	AT-467	82
37	SVT-460	88
38	AT-457	84
39	TKG-2021-3	86
40	JCS-4039	90
41	TKG-2021-6	80
42	BBT-12	82
43	TKG-2021-9	84
44	DS-68	88
45	RT-346	82

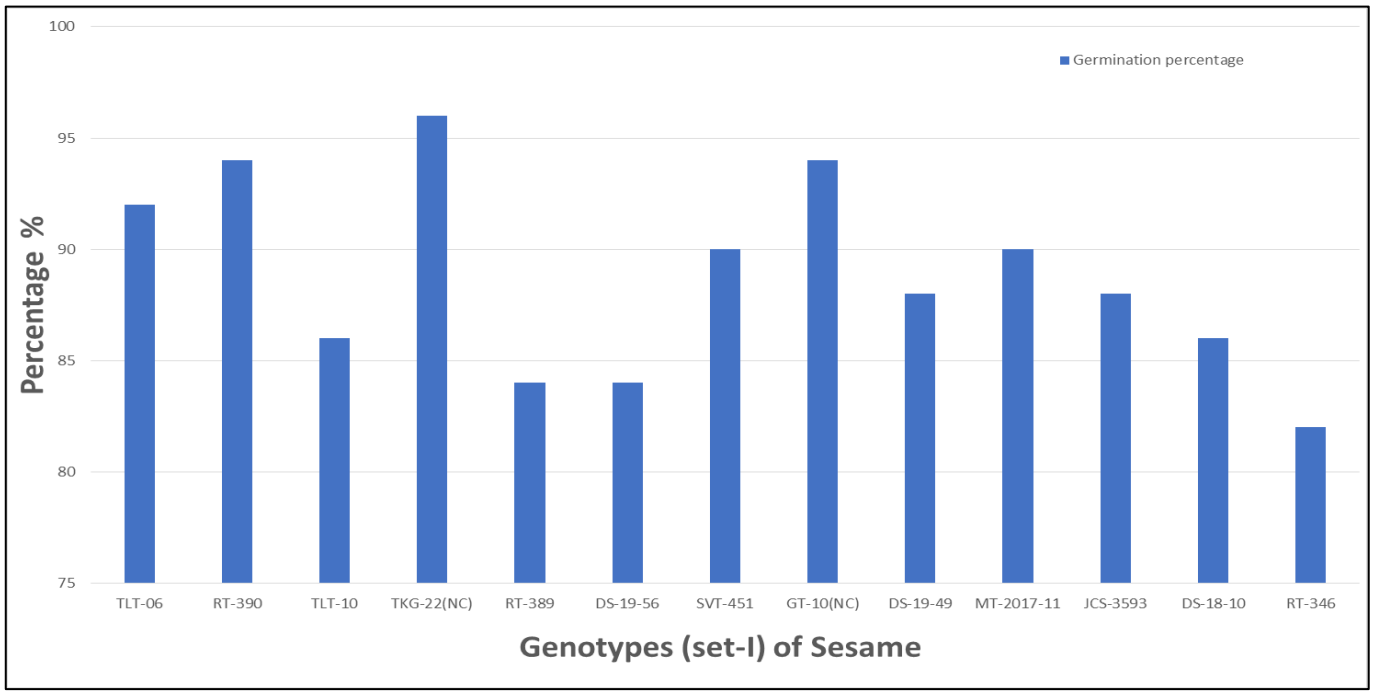


Fig 1: Percentage of germination in genotypes (SET-I) of sesame

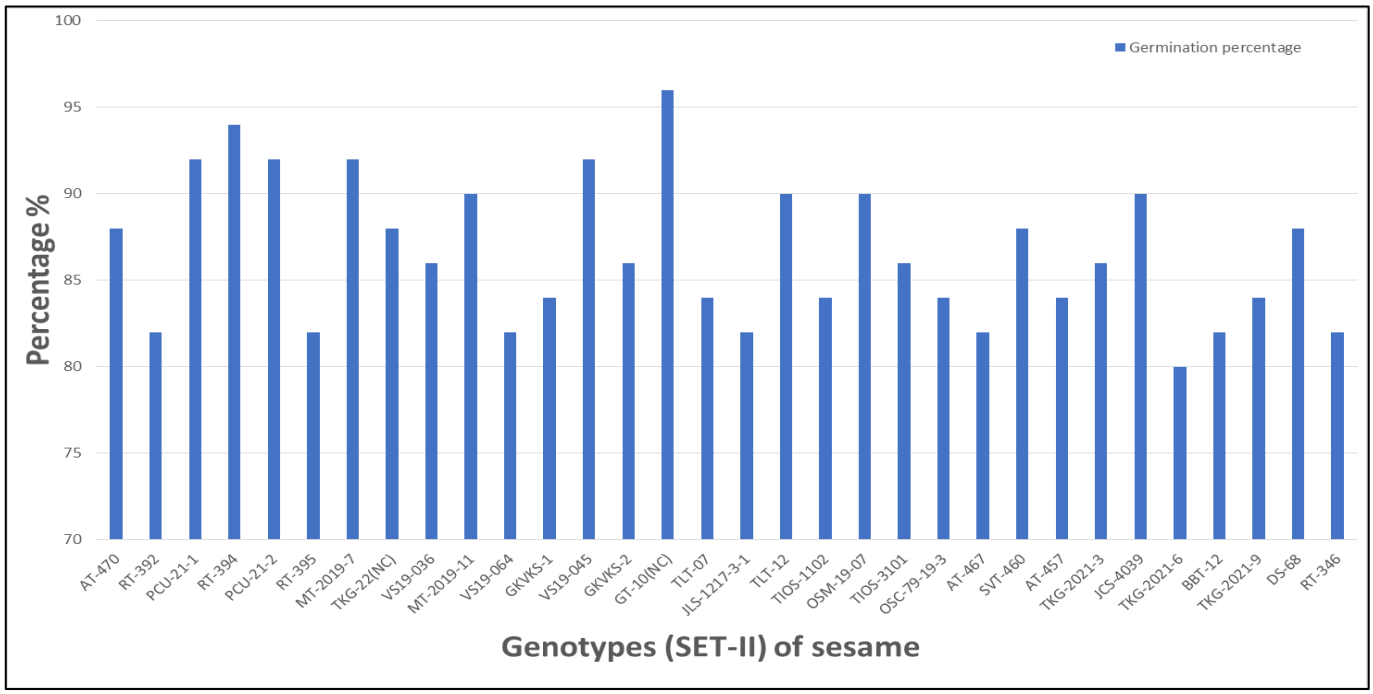


Fig 2: Percentage of germination in genotypes (SET-II) of sesame

Conclusion

- From the three seed health testing methods *i.e*., paper towel method, standard blotter method and agar plate method sesame seed samples of forty-five genotypes are found to be associated with six fungi *viz*., *Alternaria, Aspergillus, Fusarium, Macrophomina, Penicillium and Rhizopus* spp., presence of these mycoflora has affected the normal growth of sesame seedling and resulted in abnormal and infected growth of seedlings.
- Standard blotter method is found to be the best method for the identification of seed mycoflora as a greater number of the seeds infected with fungi were identified in this method and it is the simple method than paper towel method and standard agar plate method.

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