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Original Research Article

Detection of Sunflower Seedborne Mycoflora and their Effect on Seed and Seedling Parameters

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A B S T R A C T

Sunflower (*Helianthus annuus* L.), the most commercially grown oil seed crop is suffered by many phytopathogens, most of which are seed borne, causing accountable quantitative as well as qualitative losses. Therefore, present study was planned and conducted to estimate seed borne mycoflora of sunflower and their impact on seed and seedling parameters, at the Department of Plant Pathology, College of Agriculture, Latur (Maharashtra), during 2016-17. Results of the present study revealed that all of the five STH methods attempted were found efficient and reliable for the detection of major seed borne phytopathogenic and saprophytic fungi in sunflower Hyb. LSFH-171 and Var. Morden. However, Blotter paper, 2,4-D blotter and Modified PDA methods were most efficient. The major seed borne phytopathogenic fungi detected were *Alternaria alternata* (frequency: 67.33 - 78.25% and 70.75 – 76.83%), *Fusarium oxysporum* (frequency: 64.50 – 72.41% and 66.33 – 71.50%), respectively in Hyb. LSFH-171 and Var. Morden. Also three major saprophytic fungi *viz.*, *Aspergillus flavus*, *A. niger* and *Rhizopus stolonifer* were detected. All of these phytopathogenic as well as saprophytic seed borne fungi were found to influence the seed and seedling parameters in LSFH-171 and Var. Morden.

Keywords

Sunflower, Seed borne, Detection, Phyto pathogenic, Saprophytic, Frequency

Introduction

Sunflower (*Helianthus annuus* L.) is one of the commercial oilseed crops grown for edible oil all over the world. Sunflower crop is affected by more than 30 pathogens including fungi, bacteria and viruses. Among these, fungal pathogens are major one are reported to be seed borne, causing drastic reductions in seed yield and oil quality (Gulya *et al.*, 1994; Godika *et al.*, 1996; Nahara *et al.*, 2005).

The pathogenic fungi associated with the sunflower seeds are mostly *Alternaria helianthi*, *Rhizoctonia bataticola*, *Macrophomina phaseolina*, *Plasmopara halstedii* and *Alternaria alternata*; along with other saprophytic fungi *viz.*, *Aspergillus flavus*, *A. niger* and species of *Rhizopus*, *Penicillium* and *Curvularia* were reported earlier by several workers (Gulya *et al.*, 1994; Godika *et al.*, 1996; Nahara *et al.*, 2005; Rao, 2006). These pathogenic as well as saprophytic fungi cause quantitative and qualitative losses, besides reduction in seed germination, seed decay and mycotoxins production. (Gulya *et al.*, 1994; Afzal *et al.*,

2010). Therefore, present study was planned and conducted to estimate seed borne mycoflora of sunflower and their impact on seed and seedling parameters, at the Department of Plant Pathology, College of Agriculture, Latur (Maharashtra), during 2016-17.

Materials and Methods

Isolation, characterization and identification of seed mycoflora

Previous season harvested and stored seeds of sunflower hybrid LSFH-171 and variety Morden were collected from the Oilseeds Research Station, Latur and Seed Processing Unit (National Seed Project) VNMKV, Parbhani and subjected to isolations by employing various Seed Health Testing (SHT) methods (Khare, 1996; Annonymous, 2003).

For this purpose, 400 sunflower seeds each of Hyb. LSHF-171 and Var. Morden, per seed health testing method were selected and subjected to testing by various STH methods *viz.*, Blotter paper, 2, 4-D blotter, Agar plate, Modified PDA and Seed washing (Khare, 1996; Annonymous, 2003). The seeds (400 per method) were incubated for a week at 25 ± 2^0 C, under alternating cycles of light and darkness. After completion of incubation, fungi developed on seeds were examined under stereomicroscope. The fungi associated were distinguished by observing their growth habit and morphological characteristics, by referring identification key (Annonymous, 2003) and their per cent incidence frequency was calculated.

The seed mycoflora isolated were purified by single spore isolation technique (Tuite, 1969) on autoclaved and cooled (45 ^{0} C) Potato dextrose agar medium and incubated at room temperature. The pathogenicity of two predominant fungi *viz.*, *Alternaria alternata* and *Fusarium oxysporum* isolated from sunflower seeds was proved by seed inoculation and blotter paper techniques by using surface sterilized (2% Sodium hypochlorite solution) seeds of Hyb. LSFH-171 and Var. Morden.

Effect of seedborne mycoflora on seed and seedling parameters

Apparently healthy looking but suspected to be diseased seeds of sunflower Hyb. LSFH-171 and Var. Morden were selected and seeded (50 seeds / towel paper) on sterilized and moistened towel paper, rolled these papers separately, tied both of their ends with rubber bands and incubated at room temperature.

After a week of incubation, these were opened and recorded the observations on root / radical length (from collar region to the tip of the primary root), shoot / plumule length (from collar region to the point of junction of the cotyledons), computed average root and shoot length (cm) and seedling vigor index was calculated by applying following formula.

Vigor index = Seed germination (%) x (Shoot length + Root Length in cm)

Results and Discussion

Detection of seed mycoflora by various STH methods

The results (Table 1. Fig. 1A and B) revealed that all of the five SHT methods attempted were found to be efficient in detecting various mycoflora associated with the seeds of sunflower Hyb. LSFH-171 and Var. Morden. However, Blotter paper method was found to be most efficient, followed by the methods *viz.*, 2-4 D blotter

paper, Modified PDA, Agar plate and Paper towel.

With Blotter paper method, per cent frequency of the fungi evidenced was maximum of *Alternaria alternata* in both LFSH-171 (77.50%) and Morden (72.60%), followed by *Fusarium oxysporum* (72.41% and 69.75%), *Aspergillus niger* (71.83% and 62.83%), *A. flavus* (64.75% and 56.16%) and *Rhizopus stolonifer* (58.91% and 60.41%), in LFSH-171 and Morden, respectively.

With 2-4 D blotter method, per cent frequency of the fungi evidenced was maximum of *A. alternata* in both LFSH-171(78.25%) and Morden (76.25%), followed by *F. oxysporum* (70.25% and 66.33%), *A. flavus* (57.66% and 56.33%), *A. niger* (52.66% and 46.83%), and *R. stolonifer* (51.00% and 43.16%), in LFSH-171 and Morden, respectively.

With Modified PDA method, per cent frequency of the fungi evidenced was maximum of *A. alternata* in both LFSH-171(74.33%) and Morden (76.83%), followed by *F. oxysporum* (64.50% and 71.50%), *A. niger* (58.00% and 67.33%), *R. stolonifer* (51.50% and 46.08%) and *A. flavus* (50.08% and 50.41%), in LFSH-171 and Morden, respectively.

With Agar plate method, per cent frequency of the fungi evidenced was maximum of *A. alternata* in both LFSH-171(72.91%) and Morden (70.75%).

In LSFH-171, per cent frequency of *R. stolonifer* was second highest (72.25%), followed by *F. oxysporum* (66.50%), *A. flavus* (59.66%) and *A. niger* (54.91%). Whereas, in Morden, per cent frequency of *F. oxysporum* was second highest (67.83%), followed by *A. niger* (57.16%), *A. flavus* (56.66%) and *R. stolonifer* (52.41%). With Paper towel method, per cent frequency of the fungi evidenced was maximum of *R. stolonifer* in both LFSH-171 (69.33%) and Morden (75.41%) followed by *A. alternata* (67.33% and 71.50%), *F. oxysporum* (66.83% and 71.41%), *A. flavus* (57.25% and 50.83%) and *A. niger* (55.00% and 67.33%), in LFSH-171 and Morden, respectively.

In present study, among five seed health testing (SHT) methods attempted, the most efficient were Blotter paper, Agar plate and Rolled towel paper methods.

These methods of SHT were also reported as most efficient and reliable for detection of various seed borne mycoflora of the crops *viz.,* sunflower, sesame, niger, chickpea, pigeonpea, soybean *etc*, by several earlier workers (Nahar *et al.,* 2005; Mandhare *et al.,* 2009; Deshmukhand Kare 2010; Pradhan *et al.,* 2014; Radha *et al.,* 2015).

In sunflower, niger, sesame, groundnut and safflower, by applying various STH methods, the major seed borne pathogenic fungi *viz., Alternaria alternata, A. helianthi, Rhizoctonia bataticola, Macrophomina phaseolina* and *Fusarium oxysporum*; as well as saprophyutic fungi *viz., Aspegillus niger, A. flavus, R. stolonifer* etc. were reported earlier by several workers (Nagarjan and Krishnappa 2009; Abdullah and Al-Mosavi, 2010; Afzal *et al.,* 2010; Radha *et al.,* 2015)

Effect on seed and seedling parameters

Apparently healthy but disease suspected seeds of sunflower Hyb. LSFH-171 and Var. Morden were tested by employing rolled towel paper method and the results obtained on effect on seed and seedling parameters are presented in Table 2.

Fig.1 A Efficacy of various SHT methods in detecting seedborne mycoflora of Hyb. LSF171

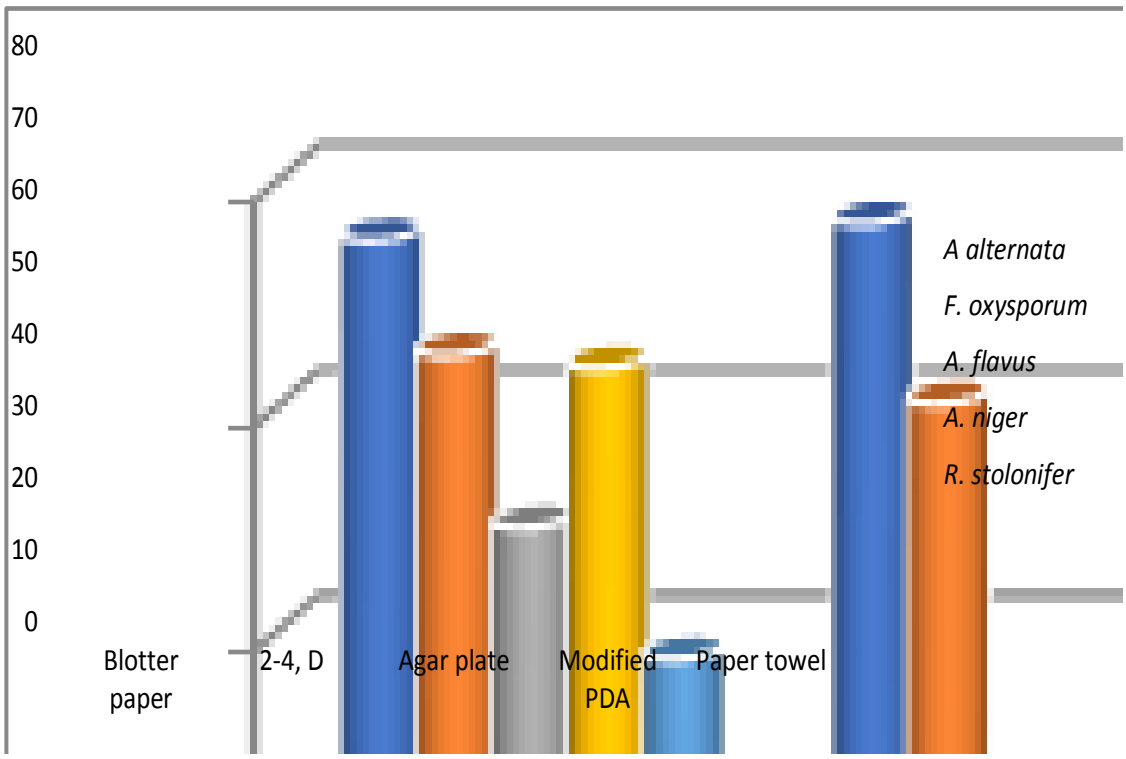

Fig.1 B Efficacy of various SHT methods in detecting seedborne mycoflora of Var. Morden

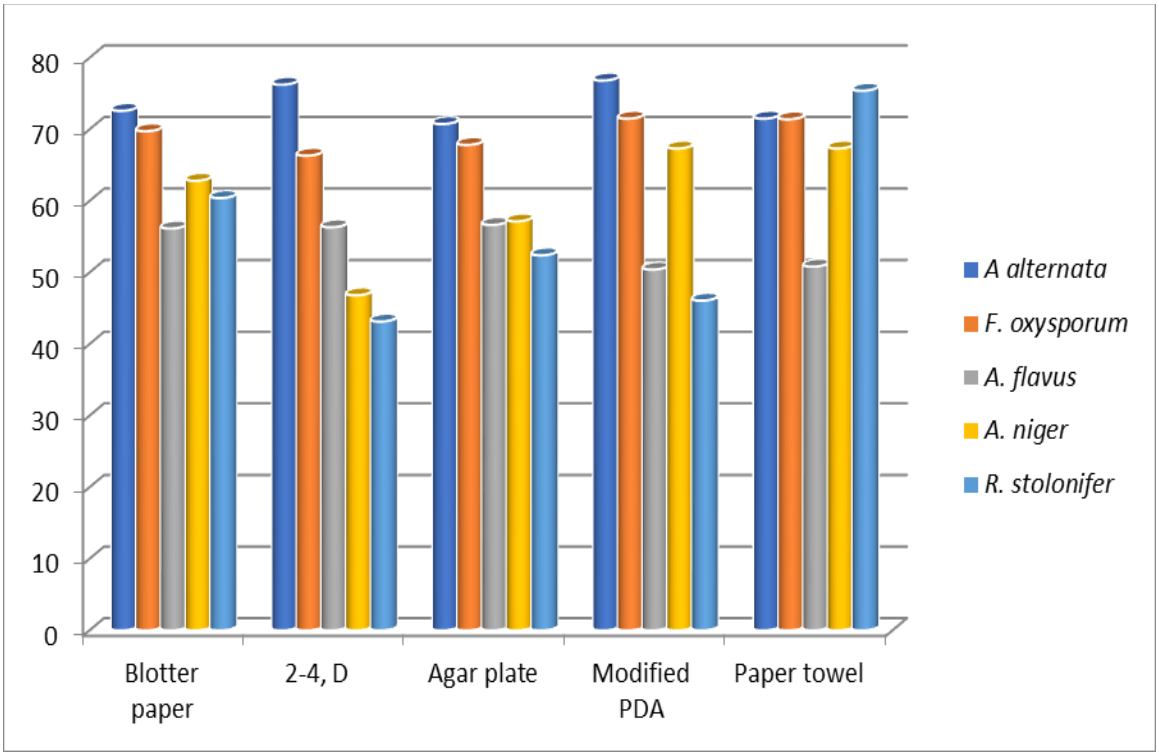

Table.1 Comparative performance of various SHT methods in detecting sunflower seedborne mycoflora

Sr. No.	Mycoflora	SHT Methods and % Frequency*				
		BP	2-4D	AP	MPDA	PT
		Hyb. LSFH-171				
1	*A. alternata*	77.50	78.25	72.91	74.33	67.33
2	*F. oxysporum*	72.41	70.25	66.50	64.50	66.83
3	*A. flavus*	64.75	57.00	59.66	50.08	57.25
4	*A. niger*	71.83	52.66	54.91	58.00	55.00
5	*R. stolonifer*	58.91	51.66	72.25	51.50	69.33
		Var. Morden				
1	*A. alternata*	72.60	76.25	70.75	76.83	71.50
2	*F. oxysporum*	69.75	66.33	67.83	71.50	71.41
3	*A. flavus*	56.16	56.33	56.66	50.41	50.83
4	*A. niger*	62.83	46.83	57.16	67.33	67.33
5	*R. stolonifer*	60.41	43.16	52.41	46.08	75.41

* Average of 400 seeds tested.
BP: Blotter paper, MPDA: Modified PDA, AP: Agar plate, 2-4 D: 2-4, D blotter,
PT: Paper towel

Table.2 Effect on seed and seedling parameters of sunflower, Hyb. LSFH-171 and Var. Morden

Sr. No.	Parameters*	Sunflower Hyb. / Var.	
		Hyb. LSFH-171	Var. Morden
1	Seed Germination (%)	76.66	50.00
2	Plumule / Shoot Length (cm)	14.45	14.08
3	Radical / Root Length (cm)	10.35	10.65
4	Seedling Vigour Index	1901.1	1236.5

*: Average of 100 seeds tested

Results (Table 2) revealed that seed and seedling parameters in sunflower Hyb. LSFH-171 and Var. Morden were influenced by the seed borne fungi. However, seed germination in LSFH-171 was comparatively higher (76.66%) than Morden (50.00%).

Similarly, in both LSFH-171 and Morden there were no rigorous differences in respect of plumule / shoot length (14.45 and 14.08cm, respectively) and radical / root length (10.35 and 10.65 cm, respectively); but, seedling vigour index (SVI) was maximum in LSFH -171 (1901.1) than the Morden (1236.5). In several cultivated crops, number of pathogenic and saprophytic seed borne fungi were reported to affect adversely the seed germination, root and shoot length and seedling vigor index as well as optimum plant population per unit area. In this context, results of the present study are in consonance with earlier reports of many workers (Singh *et al.,* 2003; Ahammed *et al.,* 2006; Nagaraja and Krishnappa 2009; Afzal *et al.*, 2010).

Thus, from the ongoing results and discussion, it is concluded that among the five seed health testing methods employed,

Blotter paper followed by 2,4-D, Agar plate and Modified PDA were found most efficient and reliable for detection of sunflower seed borne fungi *viz., Alternaria alternata, Fusarium oxysporum, Aspegillus niger, A. flavus* and *Rhizopus stolonifer*. Further, these seed borne fungi are also found to affect adversely the seed and seedling parameters in sunflower.

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