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Characterization and Identification of Seed Mycoflora of Safflower

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Abstract: *The present investigation was carried out to detect seed mycoflora with oilseed crop like safflower (Carthamus tinctorius. L) seeds. A total five varieties of safflower viz Sharda, Nira, Nari-6, PBNS-12 and PBNS-40 screened by two methods standard Blotter paper method &Agar plate method. Among these two methods, the per cent incidence of seed mycoflora were found to be maximum in Agar plate method. Seeds were associated with 12 fungi isolated from five varieties of safflower. These fungi detected and identified based on their cultural and morphological characteristics. Thepercent incidence of mycoflora was noticed with Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus terreus, Fusarium oxysporium. Alternaria carthami.*

Keywords: Seed mycoflora, Standard blotter paper, Agar plate method

I. INTRODUCTION

Carthamus tinctorius L. is commonly known as safflower or kardi is one of the important oilseed crop belonging to family Asteraceae. In India, it is cultivated for it's valuable oil, protein., & orange dye obtained from flower. It contains high percentage of essential polysaturated fatty acid and linoleic acid which plays an important role in reducing cholesterol level in blood. The area under safflower is concentrated mainly in Maharashtra, Karnataka & Andhra Pradesh accounting to 99% of Indian hectarage (Krushnaprasad 1988). In Marathwada, safflower is a major rabi crop, occupying about 54 per cent of hectarage with 40% production (Anonymous 1996). In Marathwada, area under this crop was 1.95 lakh ha with the production of 0.982 lakh tonnes (Anonymous 1998). Safflower is known to suffer from many fungal, bacterial, viral diseases and nematodes at different stages of crop growth. Among these, wilt caused by *Fusarium oxysporiumf.sp.carthami*(Kliswicz and Houston,1962) and Alternaria leaf spot/blight caused by *Alternaria carthami* (Choudhary 1944) are Seed and soil borne diseases causing serve yield losses in Maharashtra and especially in Marathwada region.

II. MATERIAL AND METHODS

Collection of seed sample of Five varieties of safflower are Sharda,PBNS-12, Nira, Nari-6 and PBNS-40 collected from Safflower Research centre, Vasantrao Naik Marathwada Agriculture University, Parbhani. For the detection of seed-borne mycoflora, two methods were used such as Standard blotter paper method and Agar plate method recommended by International seed testing Association ISTA (1966). Observations were recorded in per cent incidence of seed borne fungi associated with sterilized and unsterilized seeds. The fungi which appeared on seed were isolated in pure culture for identification and for further studies.

1. **Standard blotter paper method:** This is very simple, most convenient and efficient of all the incubation methods. Doyer (1938) and DeTempe.J.(1953) were first to adopt blotter paper method in seed heath testing. In this method a pair of blotter paper 10cm diameter were soaked in sterile distilled water in pre-sterilized petri-plates. Ten seeds of test sample per petri plates were placed at equidistance of moist blotter, nine in the periphery and one in the centre. One hundred seeds were tested for each treatment. The plates were incubated at $25\ ^0C \pm 2^0C$ for 7 days.
2. **Agar plate Method:** In Northen Ireland, Musket (1948) first used this method for seed heath testing. In this method, pre-sterilized Petri plates were poured with 15 ml of autoclaved potato dextrose agar medium (PDA).

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On cooling the medium, ten seeds were placed in Petri-plates at equidistance aseptically. The plates were incubated at temperature $25\ ^0C \pm 2^0C$ for 7 days.

In both the methods, the incubated seeds were observed for eight days by using stereobinocular and compound microscope. The identification of fungi was done using key given by Barnett (2003) and Booth (1972).

III. RESULT AND DISCUSSION

The fungi were isolated with unsterilised and sterilised seeds of five safflower cultivars like Sharda,PBNS-12, Nira, Nari-6 & PBNS-40. It is observed that the per cent incidence of fungal mycoflora of unsterilized seed was more as compared to sterilized seed. Twelve fungal species namely *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus terrus, Fusarium oxysporium, Alternaria carthami, Penicillium spp, Rhizopus nigricans, deshleriaspp, Curvularialunata, Cladosporium &Myseliasterila*were found associated with five cultivars of safflower. These fungi detected and identified based on their cultural and morphological characteristics.(Table-1) Out of these fungal species the occurrence of the highest per cent incidence of fungal mycoflora was noticed with Aspergillus spp. in Agar plate method. *A.niger*was found predominantly followed by *A.flavus.* Aspergillus spp plays a vital role in seed deterioration & reduced fat content and also effect on seed germination (Prom 2004 Rajendraprasad 2021). Similar observation was reported by Gayatri et.al (2014), Rajendraprasad et.al (2021). The number of colonies of seed mycoflora recorded were less in blotter paper method than Agar paper method hence the agar plate method was proved to be superior for the growth of fungi as compared to blotter paper method. (Shovan et.al 2008). The per cent incidence of fungal mycoflora was higher in Sharda, PBNS-12 followed by Nira. Simillar results was found by Waghmare (2007), Murumkar et.al (2008), Rajeshwari et.al (2012). Sharda showed maximum infection of *Fusarium* species while Nari-6 showed maximum infection of *Alternaria carthami*in blotter paper method.*Penicillum*spp, *Rhizopus nigricans* and *Deshleriaspp* showed minimum per cent incidence of fungal mycoflora *while Curvularia, cladosprium& Mycelia sterila*was found to be least per cent incidence.(Table-2)

Observations:

Table 1: Fungal organism isolated from seeds of safflower

Sr.No	Name of Fugus	Morphological characters
1	*Aspergillus niger*	Colony dark, black in colour. On the medium growth and sporulation was abundant,conidiophore arising from long broad thick walled brownish foot cells. Conidia in large radiating heads mostly globous, Biseriate phialides.
2	*Aspergillus flavus*	Conidial heads radiating on larger conidiophores, conidia globose to sub-globus, colonies are yellowish green in colour, uniseriate or Biseriate phialides.
3	*Aspergillus fumigates*	Colony blue green to grey or olive green in colour, short, smooth, colourless, green conidiophores, uniseriate phialides, conidial head round.
4	*Aspergillus terrus*	Colony creamy to brown in colour, conidiophore was long smooth, colourless, brown,black, biseriate phialides, conidial head large .
5	*Fusarium oxysporium*	Colonies are fast growing, pale coloured with fully aerial mycelium. The conidiophore usually branched. The terminal branches were cylindrical. Macroconidia were fusiform to sickle shaped and many celled. One celled smaller macroconidia were produced. In older culture chlamydospores were visible.Greenish pink pigmentation was observed.
6	*Alternaria carthami*	Hyphae, conidiospores and conidia light to dark brown, Conidiospores mostly simple usually becoming geniculate by sympodial elongation formed singly or in acropetal chains, consisting of Ovide and ellipsoidal body with a broadly rounded base and an apical beak, muri form with several transverse and longitudinal septate.
7	*Penicillum spp.*	Conidiophores were macronematous penicillate. The phialides formed basipetal

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		chain of dry conidia. Colonies yellowish green.
8	*Rhizopus nigricans*	Dull white colony, Fastly growing and touching to lead , stolon and sporangiophores which arises about rhizoids, hyphae aseptate.
9	*Deshleriaspp*	Multicellular organism, composed filament, cells are long and thread- like and connected end -to-end.
9	*Curvularialunata*	Colonies on PDA formed compact black stomata. Conidiophore erect, pigmented, geniculate, formed sympodial elongation, producing single conidia through conspicuous spores, Conidia were smooth walled, curved brown. Conidia had three to more transverse septa. The cell of conidia was strongly curved.
10	*Cladosporium*	Colony olive greenish in colour, conidia variable, some typically lemon colour, conidia uniformly globose.
11	*Mycelia sterile*	Colonies on PDA blackish in colour, No sexual and asexual spore mycelium well developed. Hyphae prominent and septate.

Table 2- Fungi isolated from seeds of Safflower (*Carthamus tinctorius L.)*

Sr. No.	Name of the Fungi	Percent (%) incidence of mycoflora																			
		Cv.Sharda				Cv. PBNS-12				Cv. Nira				Cv. Nari-6				Cv. PBNS-40			
		Standard blotter paper		Agar plate		Standard blotter paper		Agar plate		blotter paper		Agar plate		blotter paper		Agar Plate		blotter paper		Agar Plate	
		USS	SS	USS	SS	USS	SS	USS	SS	USS	SS	USS	SS	USS	SS	USS	SS	USS	SS	USS	SS
1	*Aspergillus niger*	55	50	58.6	52	36	33.3	38.3	35.3	45	40	50	45	42	40	50	45	32	28	36	32
2	*Aspergillus flavus*	51	46	55	50	31.6	30	35	33.6	40	35	45	42	40	40	44.3	40	30	28.6	35	30.6
3	*Aspergillus umigatus*	45	40	50.6	44.3	24.6	21.3	27	25.3	38	25	42	40	34.6	25	37.6	34.3	28	25	32	29.6
4	*Aspergillus terrus*	38	35	42	40	18	14.6	22.3	20.3	32	22	40	36.6	32	20	34.3	30	22	20	29.6	28
5	*Fusarium xysporium*	32	25	38	34.6	15.6	12.3	12.6	10.6	28	10	40	32.3	28	18	25.3	20	15.3	14	20.3	18
6	*Alternaria carthami*	28	22	26	21.6	12.3	9.6	10	09	28	10	30	22	32.3	20	30	18	10.3	09	15.6	11.6
7	*Penicillumspp*	28	20	25	20	9.3	7.6	08	06	24	08	20	16	14	10.3	15	11.6	08	7.6	11.3	9.66
8	*Rhizopus nigricans*	24	18	19.6	15	09	8.3	05	05	14	05	15	12	9.6	5.6	12	9.6	7.3	06	8.3	7.66
9	*Deshleriaspp*	15	12	15.6	12	06	05	05	2.3	13	05	12	08	7.6	06	10	5.6	8.3	5.6	7.6	06
10	*Curvularialunata*	12	08	13	10	04	00	04	02	10	04	10	06	06	04	7.6	5.3	3.6	03	5.6	4.0
11	*Cladsoporium*	10	05	7.66	07	02	02	3.3	02	10	00	4.66	02	05	02	05	2.6	2.3	00	2.6	1.6
12	*Mycelia sterile*	05	00	05	02	01	00	02	01	2.33	00	03	00	04	01	05	1.6	00	00	2.0	01
	SE+--	1.18	1.15	1.29	1.29	0.92	0.90	0.87	0.75	1.00	0.84	1.25	1.15	0.81	0.93	1.06	0.97	1.19	1.17	1.17	1.51
	CD at 5%	3.45	3.36	3.76	3.79	2.70	2.63	2.63	2.19	2.92	2.47	3.65	3.35	2.36	2.71	3.11	2.84	3.48	3.42	3.42	4.42

REFERENCES

[1]. Anonymous (1996) Districtwise general statistical information of Agricultural department 1995-96, Epitome of Agriculture in Maharashtra.

[2]. Anonymous (1998) Annual progress Report, safflower, ICAR.All India co-originated Research Project on safflower, DOR Rajendranagar,Hydrabad,pp:82.

[3]. Barnett H.L.& Hunter B.B.(2003) Illustrated genera of imperfect fungi. Fourth edition APS press st.paul, Minnestova 92-94

[4]. Booth.C.(1971) The genus Fusarium common wealth mycological Institute, Kew Surrey,England 271

[5]. Chaudhary.S(1944) An Alternaria diseases of safflower. J.Indian Bot. Soc. 23:59-65

[6]. De Tempe. J (1953) The blotter paper method of seed health testing pro. Int. seed testing Assoc.28:32-36

[7]. Doyer .L.C(1938) Manual for the determination of seed borne diseases. Washington ,Netherland: International seed testing Association P-59.

[8]. Gayathri A, Krishna Rao V, Rajeswari B, Ramesh Babu T. Detection and Identification of Seed Mycoflora of Safflower. International journal of current research and academic review 2014;21:41-45 5.

[9]. ISTA (1966) International rules of seed testing: proc.Int.seed test.Association.32:565-589

[10]. Kilisiewicz,J.M.andHouston.B.R(1962) Fusarium wilt of safflower.Pl.Dis.Report,46(10):748-749

[11]. Krishnaprasad N.V (1988) Studies on the Alternaria leaf blight of safflower. Ph.D Thesis, Barsana,Hindu University.

[12]. Murumkar.D.R,M.A.Gud And A.N.Deshpande (2008) Screening of released varieties of hybrid of safflower against wilt (Fusarium oxysporiumf.sp.carthami)J.pl.Dis,3(1):114-115

[13]. Musket.A.E (1948) Techniques for examination of seeds for the presense of seed borne fungi. Trans.Britt. Mycol.Soc.,30: 74-83

[14]. Prom.L.K(2004) The effect of Fusarium spp. curvularialunata and their combination on sorghum germination and seed mycoflora. New seeds.6:39-49.

[15]. Rajeswari B, Keshavulu K, Krishna Rao V. Management of seed mycoflora of safflower. Journal of Oil Seeds Research 2012;29:332-335.

[16]. Shovan.L.R,BhuiyanMKA,Sultana N, Begum J.A, Pervez Z(2008) Prevalence of fungi associated with soyabean seeds and pathogenicity tests of the major seed borne pathogens. Int.J. Sustain.crop prod.3:24-33.

[17]. M.Rajendraprasad, B vidya sagar, G Uma Devi, C Narendra Reddy & S. Triveni (2021) Detection & Identification of seed mycoflora associated with safflower seed sample. The pharma Innovation Journal :10(4):51-56