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RESEARCH ARTICLE

SCREENING OF DIFFERENT OKRA (BHENDI/ LADY'S FINGER) SEED SAMPLES FOR THE OCCURRENCE OF PATHOGENIC MYCOFLORA.

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Abstract

Okra is widely cultivated in India, because of its quick growing nature and high yield potential. But, quality of seeds is the limitation for its wide spread cultivation. The seed-borne fungal pathogens in okra are responsible for the poor germination and low yield. At present, besides IARI research stations, private industries are also releasing various hybrid seed varieties in to the market. To evaluate the health status of newly released varieties, regular monitoring and screening of seed samples appear to be essential. Okra crop production is affected by various diseases caused by fungi, viruses, bacteria and nematodes. Among these, fungi are a major cause for the severe loss under field conditions. Accordingly in the present study 30 samples were evaluated showed varied species of pathogenic and saprophytic fungi. Macrophomina phaseolina and Fusarium verticilloides were observed at high incidence in many of the samples resulted in poor quality of developing seedlings with less vigor.

Introduction:-

Okra (Abelmoschus esculentus (L.) Moench) which is commonly known an bhendi or lady's finger fruit, is a common vegetable crop mainly grown in tropical arid and semi-arid zones in various parts of the world. Its fruits are green, rich in ascorbic acid and other minerals. Okra has a prominent position among vegetables due to its wide adaptability, year round cultivation, export potential and high nutritive value. Yield of the crop varies depending upon geographical region and cultivar used. Due to high humidity and temperature, fungi are able to colonize the seed and cause severe crop losses to the farmers. Plants parts are also being used in clarification of sugarcane juice in jaggery industries in India. Raw fruits and seeds are also rich in mucilage serves a good source of antioxidants. This crop is known to affect by various microbes in the field, which are basically carry through the seeds and disseminates throughout the field during growing on stages. The significance of seed-borne pathogens in causing seed rot, seedling infections and further, causing several crop epidemics is well known (De Tempo, 1964; Neergaard, 1977; Aulakh et al., 1976; Subbaiah et al., 1982). Hence, in the present investigation, an attempt has been made to record the seed-borne fungi in various seed samples of okra collected from different places in Karnataka state.

Materials and Methods:-

Seed samples of okra were collected from different sources such as Uniroyal chemicals, Bangalore; Nunhems Seed Company, Hyderabad; Mahyco Seed Company, Bangalore; Seed shops in Mysore and from farmers of Mandya, Mysore, Chamarajanagar, Hassan, Kolar, Davanagere, Chikkamagalore, Gadag, Raichur and Gulberga districts. Apart from these seeds of improved varieties such as Arka Anamika, Arka Abhay and Pusa Sawani were also

collected from Indian Institute of Horticulture Research, Bangalore and named as S_1 to S_{30} serially with respective order of their collection. All the collected seed samples were stored in plastic bags at 22+/-2^{o}C in BOD incubator.

Before subjecting the seed samples to incubation tests, they were subjected to dry seed examination, in which the seed samples were carefully examined for the physical appearance. The white or black discolorations on the seeds and other contaminants such as plant debris were also noticed along with the deformed, shriveled or under sized seeds. Considering all the samples three different incubation tests were conducted to assess the mycoflora of the seed samples.

400 hundred seeds per sample were drawn after thorough mixing and were surface sterilized with 2% NaOCl to minimize the saprophytic fungi. The seeds were then plated on three layers of wet blotters in the Perspex plates and incubated for a period of 8 days under alternate cycles of 12/12h Near Ultraviolet light and darkness at 22+/-^{o}C. On 8th of incubation observations were made with an aid of stereo-binocular and compound microscopes for the occurrence of fungi and their incidence was recorded and the data were tabulated.

Similarly, in the other set 400 seeds of each sample were randomly drawn and were plated equidistantly on moistened germination papers. After plating the seeds, the paper towels were rolled and incubated for a period of 21 days in the incubation chamber as per the conditions of ISTA rules. On 21st day of incubation the paper rolls were unrolled and the seedlings were examined. The normal seedlings were counted and root-shoot length was measured. From these data percentage seed germination was calculated and the vigor index was also determined based on the formula described by Abdul Baki and Anderson (1973).

Results and Discussion:-

The seed samples, based on the visual examination were classified into five following categories: yellow green, pinkish grey, gray green, blackish gray black, and black. Considering the seed density, samples with white and black discoloration were found lighter, comparatively. In the incubation tests, totally about 14 fungi, both pathogenic as well as saprophytic were recorded. Fungi recorded in the standard blotter method were Actinomycetous species, Alternaria alternata, Aspergillus columnaris, Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Botryodiplodia theobromae, Chaetomium globosum, Colletotrichum dematium, Fusarium verticilloides, Fusarium solani, Macrophomina phaseolina, Rhizoctonia solani, Rhizopus stolonifer and Trichothecium roseum. Data presented in Table 1 showed incidence of different fungi whose abundance varies with different samples of okra. Among the samples screened, Multiplex, showed higher incidence of fungi which also a showed percentage germination of 50% and vigor index of 390. Fungi like Fusarium vertilloides and Macrophomina phaseolina were recorded in all the samples (Table 1).

Okra cultivar Adhik showed fewer incidences of fungi and high percentage of seed germination as well as vigor index. Cultivar Sungro shown moderate incidence of fungi, as a result the percentage germination and vigor index remained moderate compared to Adhik. All the three samples Adhik(apparently healthy seeds), Sungro (Moderately infected) and Multiplex (highly infected seeds) showed varied response with respect to the occurrence of mycoflora, percentage germination and vigor index (Table 2).

Seed samples from Gulberga, Raichur and Gadag districts showed high incidence of fungi and poor germination and root-shoot length compared to those samples from Chikkamagalore and Kolar districts. Among these fungi dominant species such as Macrophomina phaseolina and Fusarium verticilloides were isolated and used for further studies. Seed samples of all the districts were found to have a couple of common fungi like Fusarium verticilloides and Macrophomina phaseolina. Seed discoloration is consistently known to have associated with one or more particular fungi (Patil et al., 1987). The seed coat color in okra also provides useful information for upgrading of bulk seed lots.

The variable response of sample with respect to the incidence of fungi and seed germination percentage is in accordance with the similar reports of Aulakh et al. (1976). The variation is probably due to the varied inoculums load with respect to the growing season and location. This also may be due to the varied ingredients present with the seeds. Many workers have reported about a variety of fungi present in okra (Gupta and Basu Chaudary, 1995; Pandit and Samajpati, 1988; Fernandes et al., 1990). The multiplicity of incidence of fungi in different samples may be due to the competition among different fungal species for nutrition. Present investigation is also in agreement with the findings of Pandit and Samajpati (1998), who has discussed the rate of infection of fungi in vegetable seed

samples. The reduction in germination under higher incidence of fungi is probably due to the pathogenic effect of competing ones, which might have played a role in hindering the germination through the production of some toxic metabolites. Higher incidence of Fusarium verticilloides in Multiplex resulted in poor germination of the sample might be due to the toxic metabolites released in to the host tissues. Similarly, toxicity of Fusarium verticilloides was reported by Brodnick (1975) in some maize samaples. Fungi are known to produce a variety of mycotoxins which paralyse the embryonal axis to a greater extent. Fungi are also known to produce certain growth inhibitory substances which are responsible for the reduction of seed germination.

Macrophomina phaseolina which is seed-borne in many crops is reported generally known to cause collar rot of okra (Dubey and Jha, 1999). M. phaseolina caused severe root rot of okra, at high humidity of 76%, which is the optimum for the growth of the fungus. Aspergillus and Rhizopus species are the storage molds, known to produce various mycotoxins (Christensen and Kaufmann, 1965). Trichothecium roseum, Alternaria alternata, Chaetomium globosum and Colletotrichum dematium were the fungi contaminated with the seed and were responsible for the reduction in the seed germination and vigor. This survey indicated the phytotoxicity of various pathogenic fungi to the growing plants which will trigger in the field variously with changing agro-climatic conditions.

Table 1: Occurrence of seed mycoflora in different seed samples of okra.

Seed Sample	% incidence of fungi in different seed samples of okra*														
	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o
S1	11	12	12	31	10	10	1	1	2	13	10	15	10	6	2
S2	40	10	0	53	14	0	1	1	2	12	2	1	2	6	13
S3	0	11	13	11	11	5	1	1	2	15	20	10	20	6	2
S4	0	0	0	2	0	0	0	0	0	0	0	10	0	4	4
S5	1	32	5	5	4	4	5	2	2	76	21	73	2	10	1
S6	0	11	0	10	0	0	0	0	0	5	10	8	10	2	0
S7	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
S8	8	0	0	16	0	0	0	0	0	21	0	21	0	0	0
S9	0	0	0	0	0	0	0	0	0	21	0	21	0	0	0
S10	0	0	0	0	0	0	0	0	0	10	0	11	0	0	0
S11	0	0	0	2	3	1	0	0	0	4	0	11	0	0	0
S12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S13	2	0	0	0	0	0	0	0	0	0	0	2	0	0	0
S14	2	0	0	0	0	0	0	0	0	0	0	2	0	0	0
S15	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
S16	3	1	0	12	7	8	0	1	0	1	0	0	0	3	0
S17	24	1	0	6	0	0	0	0	0	2	4	0	0	0	0
S18	0	0	0	18	0	59	0	0	0	0	0	0	0	6	0
S19	0	0	0	0	0	0	0	8	0	3	0	6	0	13	0
S20	6	0	0	6	3	0	0	0	0	4	0	0	0	0	0
S21	1	13	0	0	0	18	0	0	0	4	0	0	0	9	4
S22	18	23	0	23	0	8	0	4	0	19	2	3	0	0	0

Table 1. Contd.

	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o
S_{23}	13	7	0	0	0	0	0	0	0	0	0	0	0	5	2
S_{24}	35	36	0	8	5	0	13	1	16	81	6	0	2	0	6
S_{25}	15	0	0	0	0	0	1	2	0	8	1	10	2	5	0
S_{26}	2	0	0	0	0	0	0	0	0	10	3	20	1	0	1
S_{27}	2	1	0	0	0	0	0	0	0	20	0	16	1	0	2
S_{28}	5	2	2	1	4	1	1	1	1	44	6	20	4	1	1
S_{29}	10	6	6	2	1	2	2	1	1	40	2	51	2	1	4
S_{30}	15	36	1	5	1	2	1	1	16	70	2	60	1	2	5

*Data based on the examination of 400 seeds
S_1 to S_{30} are the okra seeds samples collected from different places and seeds companies in Karnataka.

S_1, S_2, S_3= Seed samples from Uniroyal chemicals, Bangalore; S_4= Solar seeds; S_5=Multiplex seeds; S_6=Sungro seeds; S_7=Adhik seeds; S_8=Green tech.; S_9=Sultan seeds; S_{10}=Sagar salani seeds; S_{11}=Quality seeds; S_{12}=Indo American hybrid seeds; S_{13}=Sun seed I; S_{14}=Sun seed II; S_{15}=Sun seed III; S_{16}=Arka anamika; S_{17}=Arka abhay IIHR; S_{18}=Local Mandya; S_{19}=Local Bangalore; S_{20}=Arka Abhay State Seed Corporation, Bangalore; S_{21}=Local Chamarajanagar; S_{22}=Davanagere; S_{23}=Mahyco seeds; S_{24}=Hassan; S_{25}=Mandya; S_{26}=Kolar; S_{27}=Chikkamagalore; S_{28}=Gadag; S_{29}=Raichur; S_{30}=Gulbarga

a to o : refers to the fungi as follows;
a= Actinomycete spp.; b=Alternaria alternaria; c=Aspergillus flavus; d=Aspergillus fumigatus; e=Asspergillus columnaris; f=Aspergillus niger; g=Botryodiplodia theobromae; h=Chaetomium globosum; i=Colletotrichum dematium; j=Fusarium verticilloides; k=Fusarium solani; i=Macrophomina phaseolina; m=Rhizoctonia solani; n=Rhizopus stolonifer; o=Trichothecium roseum.

Table 2: Evaluation of different seed samples of okra for seed germination and seedling vigor.

Seed Sample	Seed germination (%)	Mean Root length (cm)+/- S.E.	MeanShoot length (cm)+/- S.E.	Vigor Index (V.I.)
S_1	0	-	-	-
S_2	0	-	-	-
S_3	0	-	-	-
S_4	4	2(0.6)	8(0.6)	41
S_5	51	2(0.0)	5(0.0)	393
S_6	81	4(0.0)	10(0.1)	1093
S_7	94	5(0.0)	12(0.0)	1575
S_8	91	4(0.1)	11(0.0)	1328
S_9	96	5(0.0)	13(0.1)	1670
S_{10}	96	5(0.0)	11(0.0)	1517
S_{11}	90	4(0.0)	10(0.0)	1246
S_{12}	98	5(0.2)	14(0.2)	1754
S_{13}	98	6(0.5)	14(0.3)	1917
S_{14}	88	5(0.0)	13(0.3)	1565
S_{15}	88	5(0.0)	14(0.3)	1660
S_{16}	70	3(0.2)	11(0.2)	966
S_{17}	64	3(0.7)	13 (0.1)	806
S_{18}	46	3(0.3)	10(0.2)	507
S_{19}	58	4(0.2)	15(0.2)	1013
S_{20}	73	3(0.2)	10(0.2)	854
S_{21}	32	2(0.2)	9(0.2)	339

Table 2: Contd….

Seed sample	Seed germination (%)	Mean Root length (cm)+/- S.E.	Mean Shoot length (cm)+/- S.E.	Vigor Index (V.I.)
S_{22}	46	4(0.2)	15(0.5)	657
S_{23}	74	3(0.2)	9(0.2)	819
S_{24}	31	3(0.2)	7(0.2)	266
S_{25}	46	4(0.02)	7(0.0)	469
S_{26}	50	3(0.1)	7(0.01)	486
S_{27}	53	4(0.2)	6(0.01)	510
S_{28}	41	3(0.0)	6(0.02)	336
S_{29}	31	2(0.0)	5(0.01)	225
S_{30}	34	3(0.2)	5(0.03)	252

Data based on the average of 25 seedlings/sample, S.E. =Standard Error, Data represented in parenthesis indicate +/- standard error.

S_1 to S_{30} are the seed samples with the description as in the previous table (Table 1).

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