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STUDY OF SEED BORNE FUNGI OF SOME SEEDS FROM AHMADNAGAR MARKET

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ABSTRACT:
Identification of seed borne fungi of common Seeds from Ahmadnagar market was conducted. A total of nine fungi namely Aspergillus, Penicilium, Rhizopus, Verticilium, Alternaria, Monilia, Fusarium, Helmintosporium, Mucor, and there different species were isolated from the five different seed samples namely groundnut. (Arachis hypogea), maize. (Zea mays) gram. (Cicerarietinum), jawar. (Sorghum vulgare), pea. (Pisumsativum). In the present study we studied external and internal different fungus on the basis of their growth of the colony, color, spore and mycelium morphology of seeds from Ahmadnagar seed market. Occurance of common fungi like Aspergillus (four species), Penicilium (two species on the basis of colour), Mucor, Rhizopus etc. But Seed borne fungi like Fusarium, Verticilum, Gliocladium, Cladosporium as such have occurred on few seeds. On the other hand Alternaria, Drechslera, Helminthosporium, occurred on jawar seeds. These fungi have production of masses of conidiospores also and the study on seed borne fungi by blotter method has shown occurrence of few forms but culturing seeds on medium showed excellent response. On CD Amedium more forms have been recorded than PDA Medium. It may be due to balanced dose of nutrients in a medium. I n particular Alternaria, Helmithosporium, Fusarium occurred profusely on many cultured seed material

INTRODUCTION:

Seeds are vital role in associating micro-organisms which prove hazardous for the seed or the new plant created from it. The associated micro-organisms may be pathogenic, weak parasites or saprophytes. They may be associated internally and externally with the seed or as contamination as sclerotia, gall, fungal bodies, bacteria looze, infected plant parts soil partical etc. mixed with the seed. Seed borne pathogen may or may not be seed transmitted. Seed borne microorganism not only create problems in agricultural production but prove hazardous to animals and human being thus play generally a negative roll in human welfare. A heavy loss has been observed to caused by seed borne pathogen in various crops. Seed rots, seedling rots i.e. pre and post emergence losses disease at various stages of crop growth like leaf spot, stem, rot, wilt, root rot, fruit rot etc. Influenced the crop stand and ultimate yield. In number of leaf spot pathogen are also seed borne like *collitotrichum gramineum, Curvularia, lunata.* The term seed pathology denotes the science dealing with seed health and concern with the seed born microorganism which may be associated externally, internally or as contamination or physical condition, deficiency of element.

In India also seed pathology has attracted attention of agricultural as well as traditional universities. Many scientists have been trained in seed pathology at the Danish government institute of seed pathology for developing countries. The seed borne fungi may be present in the form of hyphae, conidia, oospore, chlamadospore, sclerotia. Seeds provide natural substrate for the growth of associated fungi. Moist blotter method with its various modifications is the most widely used method of seed health testing. It is very economic easy to perform and is suitable for the detection of wild variety of seed borne fungi. As the seed is the basic unit in crop production technology, people were conscious of seed quality and methods of seed treatment for improvement of seed germination and emergence. From the ancient times it has been confirmed that some seed get lost during germination so some seed born agencies may be present which cause disease. Now it has been confirmed that a number of disease are seed born and seed are accompanied by variety of fungal organisms. Some fungal diseases are contagious and carried through seed .Thus it was established by many scientists, that seed plays a vital role in associating micro-organism which proves hazardous for the seeds or for the new plant created from it.This knowledge of seed born nature of micro-organism launched a new era in plant pathology the term 'seed pathology' refers to the science dealing with seed health and is connected with seed born micro-organism or physical conditions, and controlling the seed borne diseases in the field and during storage seed carry several destructive pathogens and cause severe losses. Such disease spread from infected plant to healthy plant within a short

time and often takes a heavy toll. Affected seeds also act as important agents of transfer of diseases to vast areas of land. Some seed infections are such that if the infected seeds are consumed, they cause diseases to man and domestic animals, which become fatal in some cases. Seed born infections carried in the form of spores or spore producing structure .some pathogens are associated with seed just on the surface. Such seed are called infested seed. In other cases the pathogen may be within the seed tissues and in such cases the seeds are said to be infected seeds. So in the both cases, infested or infected, the seeds become part of the studies of seed pathologists. Seed pathologists take into consideration, identifying the attack by pathogen as well as how affected seeds show some biochemical changes because of presence of fungus on or in seed. For the survey of some internal and external seed borne fungi collected seeds of ground nut, maize, gram, pea, and Jawar from Ahmednagar area for the study of external and internal seed borne fungi.

MATERIAL & METHODS :

The study of some internal and external seed borne fungi refers the following crop seeds Ground nut (*Arachis hypogea*), Maize(*Zea mays*), Gram (*Cicerarietinum*), Jawar (*Sorghum vulgare)*, Pea (*Pisumsativum*).

Chemicals and Glassware : PDA medium, CDA medium, 1% sodium hypochlorite solution, blotter paper, sterile distilled water, petridishes, illuminating growth chamber with constant temperature.

Seeds are complex biologically dormant entities containing a living embryo. Methods for detecting seed born micro-organisms requires analyzing of large number of seed samples, and some simple to advanced methods are now available for detection of seed-born agencies. They include direct examination of seeds where seed coat colour, reduction of seed size, seed shape, and production of some fruiting structures can be detected.

The detection of seed- born pathogens was carried out by well known incubation methods. Incubation methods are of two types-

(1)Blotter Paper Method and

(2)Agar Plate Method it includes two methods (A) PDA (Potato Dextrose Agar Medium) and (B) CDA (CzapekDox Agar Medium)

These methods are recommended by (ISTA) for rooting examination of crop seed for fungal infections. These are suitable for infections accompanied by hyphae production, fruiting structures or spores. The tests are effective for detecting most seed-borne fungi. Identification is based on fungal morphology developed during incubation on seed surfaces on blotters or on colony characteristics on an agar medium.

(1) Blotter paper method

This is the most commonly followed technique which is a simple and inexpensive means for detecting fungal pathogens associated with the seeds. The basic principle in this method is to provide high levels of relative humidity and optimum light and temperature for fungal development. Blotters are soaked in distilled or tap water and placed in culture plates, after draining off the excess water as excess of water stimulates bacterial growth which is antagonistic to fungal development. The number of seeds used depends upon seed size and type of infections. In case of large seeds like maize, gram, ground nut and pea 10-15 incubated while 20 seeds are incubated smaller size like jawar. After placement of the seeds, culture plates are incubated. Seeds are usually examined within 10 days and fungal identification is based on mycelium growth, colour, arrangement of conidiophores, size, septation and arrangement of conidia on conidiophores. The blotter technique provides conditions for the evaluation of the severity of infection on each seed and seedling.

1) Procedure of blotter paper method-

Prepared at least 2-4 of blotter and cut according to diameter of petriplate, blotter made up moist by putting in water. Extensive water drain out then transferred in petriplate This petriplates in sterilized in autoclaved Inoculate the petriplates with at least 10 seed / plates placed on blotter at equal distance. Incubate this plates should be incubated 12 hours in light and 12 hours in dark for continuous 8 days.

2) Procedure Agar plate method

This method is useful in identification of fungi associated with the seeds, based on growth and colony characteristics on a nutrient medium. For this ISRA has suggested use of potato dextrose agar medium (PDA) for seed health testing. Usually about 10 seeds are used per 9cm culture plate and seeds are examined within 10 days. Colony characteristics are noted and then microscopic examination is carried out.

Agar method is used to detect superficial infections of fast growing fungi and hence widely followed for the cereal, millets and nut also which are fast growing plants.

The identification of fungi is carried out on the basis of colure and rate of mycelial growth, sporulation and spore morphology with the help of standard key

Procedure of agar plate method: The seeds were collected from stored container or any seed sample. Divided the seed into 2 lots.1st part or externally seed born fungi or 2nd for internally seed born fungi. Seed are surface sterilized 1% NaOCl solution for 2-8 min to analysis only internally seed borne fungi. Externally seed borne fungi are isolated directly plating they are PDA/CDA medium. At least 10seeds should be per plate. Internally seed born fungi, surface sterilized seeds are washed 2-3 times with sterile distilled water and then seeds may be placed in medium prepare at least 6 plates per crop/ seed. The plates should be incubated for 12 hours in light (near U.V. Light) and 12 hours dark for 6-8 days.

OBSERVATION TABLE :

S. N	Crop Name	Medium Name	Plate number	Colour of Colony Respectively	No.of Colony Respectively	Identification of fungi Respectively
1	Groundnut	PDA	1	Black,Green,White Brown	4,5,3,1	*RhizopusAspergillus,mucorAspergillus*
			2	Black,Green,White	2,6,2,3,	*Aspergillus, Aspergillus,Rhizopus, Mucor*
			3	Black green ,White	4,7,2.	*Mucor, Aspergillus,Rhizopus*
		CDA	1	Black,Greenish,Yellow,brown	2,2,2.	*Mucor,Penicillum ,Rhizopus.*
			2	Green ,Black	5,6.	*Penicillum, Mucor*
			3	Green ,Black,Brown	4,8,2.	*Aspergiluus,Mucor,Aspergillus*
		Blotter	1	Black ,White Yellow	2,1,1.	*Mucor, Rhizopus, Aspergillus.*
			2	Absent	Absent	*Absent*
2	Maize	PDA	1	Black,Green,Yellow,White Brown	3,4,1,1,1,	*Mucor,Penicillum ,Aspergillus,Rhizopus, Aspergillus*
			2	Black,Green,Yellow	8,4,1.	*Mucor,Penicillum ,Aspergillus.*
			3	Yellow,Green,Brown	2,6,1.	*Aspergillus, Aspergillus,Penicillum.*
		CDA	1	Green ,Brown, White,Black	2,1,1,2	*Penicillum ,Aspergillus,Rhizopus,Mucor.*
			2	Yellow,Green,Black.	4,6	*Monilia,Mucor,*
			3	Greenish,Black,Brown	3,4,2.	*Penicillum,Mucor,Aspergillus*
		Blotter	1	Black, Yellow, Greenish Black,	2,3,5.	*Aspergillus, Aspergillus,Mucor.*
			2	Greenish Black	4,2.	*Aspergillus,Mucor.*
3	Gram	PDA	1	Black, Yellowish white Green, Yellow,	5,2,1,1	*Mucor,Fusarium,AspergillusPenicillum.*
			2	Black, , Green, Yellow.	5,1,1.	*Mucor,AspergillusPenicillum.*
			3	Black, , Green, Yellow,White	5,1,2,2	*Mucor,AspergillusPenicillum,Fusarium.*
		CDA	1,2,3,	Absent	Absent	Absent
		Blotter	1	Black,Green.	4,2.	*Aspergillus, Aspergillus.*
			2	Absent	Absent	Absent
4	Sorghum	PDA	1	Brown , Black,White	2,3,4.	*Penicillum ,Aspergillus,Fusarium*
			2	Black,Green,Brown	5,1,1	*Mucor,Aspergillus,Helminthosporium*
			3	White,Black,Green	3,2,2,	*Fusarium,Mucor,Aspergillus.*
		CDA	1	Grren,GreenishBlack,White	2,2,3.	*Penicillum, verticillum ,Sterile mycelium*
			2	Absent	Absent	Absent
			3	Absent	Absent	Absent
		Blotter	1	White, Black, Green	2,5,2,	*Fusarium,Mucor,Aspergillus*
			2	Absent	Absent	Absent
5	Pea	PDA	1	Black,White,	2,2	*Mucor,Rhizopus*
			2	Absent	Absent	Absent
			3	Absent	Absent	Absent
		CDA	1	Green,Yellow,	1,1.	*Monilia,Rhizopus.*
			2	Black,White Green	1,1,2.	*Mucor,Rhizopus,Aspergillus.*
			3	Absent	Absent	Absent
		Blotter	1	Absent	Absent	Absent
			2	Absent	Absent	Absent

RESULT & DISCUSSION:

Seed borne fungi of five crops seeds was carried out by blotter method and culture method(PDA & CDA Medium) for about three months.Whatever 10 Seeds were transferred on medium / blotter showed occurance of common fungi like *Aspergillus* (4species), *Penicilium* (2 species on the Basis of colour), *Mucor, Rhizopus* etc. But Seed borne fungi like *Fusarium Verticilum, Gliocladium, Cladosporium*as such have occurred on few seeds. On the other hand *Alternaria, Drechslera, Helminthosporium*, occurred on jawar seeds. These fungi have

Production of masses of conidio spores also. The study on seed borne fungi by blotter method has shown occurrence of few forms but culturing seed son medium showed excellent response. On CD Amedium more forms have been recorded them PDA medium. If may be due to balanced dose of nutrients in a medium. Inparticular *Alternaria, Helmithosporium, Fusarium* occurred profusely on many cultured seed material.

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REFERENCES:

1.Agarwal, V. K. (1981). Assessment of seed borne infection and treatment of wheat seed for the control of loose smut.

2.Heperly. P. R. (1993). chemical control of seed borne fungi of sorghum and their association with seed quality and germination pureto rico plant dis. 66:902

3.Jha. D. K. (1995). Seed Pathology Vikas publishing house, New Delhi

4.Kamat. M. M ; (1971). *Practical plant pathology*, Prakash Publication Pune.

5.Melhotra. R. S. (1994). *plant pathology. Tata. mcgraw hill new Delhi* pg.606-614

6.Nagar. M. L. et. al. (1976). chemical composition of healthy and diseased kernel of maize infected by *Fussarium moniliforme* var. *subglutinansand Mucorhiemalis Indian phytopath 29* (4): 441

7.Pandey. K. & Grover. S. K. (1978). "Seed mycoflora of different cultivres of wheat" (*Triticumastivum.*) abs. ing. ind. dot.

8. Ramnath. and Lal. S. P. (1963). Seed borne infection of stalk burn disease of rice, if extends and control *indian phytopath*, 16: 232-233

Vidyasekaran. P*,* 1967, Role of seed borne microflora in paddy seed spoilage, *Indian phytopath*,19(1):333-341

9.Jidda M. B. and ,F. Benjamin, (2016) .Identification of Fungi Associated with Storage Bulb Rot and Seed of Onion (*Allium cepa* L.) in Maiduguri, Northeastern Nigeria, *International Journal of Modern Botany* 6 (2): 26-30

10.Oladimeji Ahmed, Olusegun S. Balogun, Oluyemisi B. Fawole Oluwatoyin A. Fabiyi, Aliyu T. Hussein and Kassoum O. Kassoum (2016). Seed-Borne Fungi Of Soybeans (*Glycine Max* [L.] Merr) In The Guinea Savannah Agroecology Of Nigeria Journal of Agricultural Sciences Vol. 61, No. 1, Pages 57-68

11.Liamngee Kator, Aondo Terkaa Ogo-Oluwa, Akinyemi Bosede Kemi (2016). Isolation and Identification of Seed Borne Fungi of Common Bean (*Phaseolus vulgaris L.*) from Selected Markets in Makurdi, *International Journal of Applied Agricultural Sciences*. 2(5): 75-78

12.Faraj A. Abushaala, Adel O. Ashour Abdalla R. Ben Ramadan (2016). "Investigation of seed-borne fungi associated with cucurbit seeds in two different regions of Libya" *Al-sati l* Vol. 10 P. No. 15

13.Bokhary H. A. (2010). "Seed-borne fungi of date-palm, Phoenix dactylifera L. from Saudi Arabia" *Saudi Journal of Biological Sciences* (2010) 17, 327–329

14.Serferbe Signaboubo1, G.R. Tsopmbeng Noumbo1 & Kuiate Jules Roger2 (2016). Seed-Borne Fungi Associated with Rice Seeds Varieties in Bongor, Chad Republic *International Journal of Current Microbiology and Applied Sciences* Volume 5 Number 12 (2016) pp. 161-170

15.Pathak Neha, Razia. K. Zaidi (2013). Fungi associated with wheat seed discolouration and abnormalities in *in-vitro* study *Agricultural Sciences* Vol. 4, No. 9, 516-520.

16.Fakhrunnisa, M. H. Hashmi & A. Ghaffar (2006). Seed-borne mycoflora of wheat, sorghum and barley *pak. J. Bot.,* 38 (1): 185-192

17.Warude S.N., Mane S. S. & G. K. Giri (2016). Detection of Seed Borne Myco- Flora Associated with Chickpea ISSN: 2320 – 7051 *Int. J. Pure App. Biosci.* 4 (4): 309-315

18.Ghangaokar Narayan M. and Ayodhya d. Kshirsagar "Study of seed borne fungi of different legumes "*TLS volume 2 issue 1 (2013)* ISSN: 2319–4731 (print); 2319–5037 (online)

19.Kandhare Ashok S. (2016). Effect of Common and Dominant Seed-Borne Fungi on Protein Content of Pulses *American Journal of Biological and Environmental Statistics;* 2(4): 41-43

20.Naikoo Abbas, Wani Mubashir, Nazir Ahemad Bhat, Waheed-U-Zmeena, Md. Suliman Dar and Mohamood Ahmad Tak, (2013). "Effect of seed-borne mycoflora on the quality of three varieties of *Arachis hypogea*", *American Journal of Biological and Environmental Statistics Vol.* 3(1), pp. 35-42.

21.Margaret Elizabeth, Neeraja P. V. and B. Rajeshwari (2013). "Screening of Seed BorneMycoflora of *Cicerarietinum*" *Int. J. Curr. Microbiol. App. Sci* ISSN: 2319-7706 Volume 2 Number 8 (2013) pp. 124-130

22.Kandhare Ashok Sadhu (2014). "Seed-Borne Fungi and their Effect on Seed Health of Green Gram" *Bioscience Discovery*, 5(2):251-255