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Prevalence and Distribution of Seed Borne Fungi in Commercial Vegetable seeds

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Abstract: *Horticulture is a science of cultivation and management of fruit vegetable ornamentals. Their area is famous for cultivation of vegetable crops as well as fruitorchards. To understand what happens with vegetable seeds was the main aim and hence three vegetable from family Cucurbitaceae (like Chakki, Bhopala and Karale) were collected in the form of seeds from the local market or from the farm for study of agents of deterioration.In all three vegetables are selected from their area and they were studied by Blotter & Agar medium to understand seed deteriorating and seed borne fungi.. The seeds treated with fungicides generally most show less number of fungi but in some cases it was observed that their number was high. In general in seed deterioration saprophytes like Rhizopus, Mucor, Aspergillius, and Cladorsporium were common. Blotter had shown good number of fungi in most of the seeds under observation. Out of three seed grown vegetables Blotter or agar medium when the seeds were inoculated within one week fungi growth around the seeds was prominent and in few cases very less fungi growth was noticed on medium or well as on Blotter.*

Keywords: *Horticulture*.

I. INTRODUCTION

The science of seed pathology is relatively young having its beginning in seed health testing and control of seed borne pathogens. From 1970, there has been a wide interest in research out rich and training actively related to seed pathology. The study of seed borne fungal pathogen has special consideration in the areas like seed production and plant quarantine activities. Seed carry several destructive pathogens, which are causing several diseases on crops raise from them. The important seed borne pathogen in the field of agriculture is known from 1900 and it is science and technology dealing with seed borne plant diseases and seed diseases. This science has great appreciation because of its importance in development of agriculture. Basically, in understanding the seed borne pathogens, it helps to solve the problem of seed borne diseases. Seed harbors varieties of pathogen inside and outside which was a part of investigation of present survey seed crops.

Seed borne pathogens are of two types: Those adheres to the outer covering of seed and those borne inside the seed. In the present survey many of vegetables seed crops, seed have been studied. Some of such pathogens were parasitic or other saprophytic. Whatever, the materials, has been selected is extremely important. In such a case seeds are infected and they carry dangerous pathogen, if such materials are consumed by human beings it may cause diseases or problem in the health.

Materials and Methods:

The externally and internally seed borne fungi are identified or detected by two important method which are commonly used in laboratory and research institute. These methods are as Blotter paper method and Agar plate method.

A) Blotter Paper Method:

1) Procedure for untreated seeds.

Take three periplates and three blotters which are of the petriplates. Write sample number and data with pencil of ball pen. Dip them in sterilized water with the help of forcep. Keep blotters in vertical position till the excess of water is removed. Place the blotter in a pertriplates. Take 10 seeds at random with the help of forcep.Place 10 seeds at equal

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distance on the moist blotter, 8 seeds in a outer ring and 2 in the middle or at the center. Write sample's name, on dish with help of marking pencil. In such a manner prepare plates with 3 seeds of each sample. Keep these petriplates for one week at a fixed temperature at about 25^0c. in incubator chamber. Examine the seeds under stereoscopic binocular microscope at 50 X magnification after 8-10 days. Examine the seeds of outer ring first and then seeds in the center of the plates. Note the habit character of fungi. Prepare the slides and examine under compound microscope. Note the percentage of infection of the individual fungus and the number of fungus associated on each particular variety of seeds. After this record the result and observations.

2) Procedure for treated seeds.

Take three periplates and three blotters which are of the periplates. Write sample number and data with pencil of ball pen. Dip them in sterilized water with the help of forcep. Keep blotters in vertical position till the excess of water in removed. Place the blotter in a pertriplates. Take 10 seeds at random with the help of forceps. Place 10 seeds at equal distance on the moist blotter, 8 seeds in an outer ring and 2 in the middle or at the center. Write sample's name, on dish with help of marker pen. In such a manner prepare plates with 10 seeds of each sample. Keep these petriplates for one week at a fixed temperature at about 25^0c. in incubator chamber.

B) Agar plate method:

I. Procedure for untreated Seeds:

Prepare Glucose nitrate agar medium. Take pertriplates for each sample of seeds. Sterile the GNA medium and petriplates in autoclave. After sterilization cool the medium and pour 15 ml of GNA medium in each petriplate.Allowed the medium solidify for sometimes. Place 10 seeds per plate at equal distance. Incubate the petriplated at 250 c temp. With 12 hr. darkness and 12 hour light. Examine the plates after 6 days of incubation. Note characteristics of fungal colonies from periphery to the center of petriplates. Prepare the slides and examine then under compound microscope. Record % of incidence of infection of different fungi. Observed the changes takes place in time of infection of fungi on seed.

II. Procedure for treated seeds:

Prepare a GNA medium. Take petriplates for each sample. After sterilization pour 15 ml of nutrient agar medium in each petriplates. Allowed to solidify the medium for some time. Treated the seeds with 0.1% of $Hgcl_2$ for 2 min.Wash the seeds with sterilized water for removal of excess of mercuric chloride. Place 10 seeds at equal distance. Incubate these petriplates in incubating chamber for 6-8 days. Ultra violet light are bombarded for 5-10 min each day. Examine the plates after 8 days and note the characteristics fungal colonies associates with each seeds. Prepare the slides and examine them under microscope. Record% of infection of different fungi. Observe the changes taking place infection of seeded.

Compositions of 0.1 % $HgCl_2$:

It is prepared by dissolving 10 mg/ms, of $Hgcl_2$ in 100 ml of distilled water. Then it gives 0.1 % of $Hgcl_2$

Glucose Nitrate Agar Medium:

SR.NO.	CONTENTS	QUANTITY
1	**Agar**	**20gms**
2	**Glucose**	**10 gms**
3	KH_2PO_4	**1gm**
4	KNO_3	**2.5 gms**
5	$MgSO_4$	**0.5 gms**
6	D.W.	**1000ml**
7	pH	**6.5**

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Table: 1 The Vegetable seeds under studies were as below:

SR. NO.	FAMILY	COMMON NAME	BOTANICAL NAME
01	Cucurbitaceae	Bhopala	*Cucurbita maxima* Duch ex Lam.
02	Cucurbitaceae	Chakki	*Benincasahespida*(Thunb) Cogne.
03	Cucurbitaceae	Karle	*Momordicacharantia*L.

OBSERVATIONS

I) **V.N**. – **Pumpkin / Redsquashgourd .**
B.N. – *Cucurbitamaxima*Duch ex lam .
L.N. – Bhopala / TaambaddaBhopalla .
Family – Cucurbitaceae .

Herb annual prostrate or twining leaves arbicular or raniform in outline 5 lobed dentate flower reddish yellow or orange coloured , axillary solitary.
Flowering &fruiting : March – August, Cultivated in most part of the rainy season
Chemical composition –
Seed yield resin having vermisidal properties seed oil contains sterols &triterpenoids. Fruit contain Vitamin A also stigmatas 7.25 (28) – dien – 3 B. ol& stigmast – 7,25dienol acetate &euglobunin from seeds .
Fungi on seeds :*Rhizopus, Mucor, Aspergillus, Penicillium, Monilia, Alternariatenuis.*

II) **V.N.** – Ashgourd .
B.N. – *Benincasahispida*(Thunb) coge.
L.N. – Chakki .
Family – Cucurbitaceae .
Herb stem branched leaves are broadly ovate 5-7 lobed margins irregularly dentate , tendriler tender .
Flower – Yellow monoecius axillary solitary .
Fruit – Fleshy hairy , when young waxy bloom , when mature , seed – yellowish compressed ovoid .
Flowering & Fruiting – June to October .Fruit both raw tripe used as vegetable .
Chemical composition – Fruit contain B. Sitosterol ,orginine , aspartic acid , glutamic acid, glutamine , hydroxyproline , isoleucine , cystenine& source of vitamine B1
Fungi on seeds :*Aspergillus, Rhizopus,Mucor, Chaetomium, Monilia*.

III) **V.N**. – Bitter gourd .
B.N. – *Momordicacharntia* L.
L.N. – Karale .
Family – Cucurbitaceae .
Herb climbing or trailing leaves deeply 5 lobed membranous glabrous
Flower – Bright yellow solitary axillary, Berries fusiform tuberculate
Seed – Flat, yellowish brown.
Flowering & Fruiting – April- October.
Cultivated through the state, fruit are vegetable.
Chemical composition –
Contain alkaloids, mormoridicine, Saponine, Carotene, highly aromatic essential oil, fruit contains carotene& B. sitosteral – glucoside & stigmast 5,25 dinen – 3 B – 0 glucoside.
Fungi on seeds :*Rhizopus, Aspergillus,Mucor,Steriledematioushypomycetes, Cladosporium, Monilia, Sterile hypomycetes mycelium.*

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Table 2. Ash gourd seeds associated with Fungi on blotter paper

Sr. No.	Seed Sample	% Incidence of Mycoflora	% of Seed germination	Length of radicle (cm)	Length of plumule (cm)	Fungi associated
1.	Untreated	100	25	0.0	0.5	*Aspergillusnigur, Chaetomium spp., Mucor mucedo.*
2.	Treated	50	70	0.0	1.0	*Aspergillusnigur, Rhizopus spp.*

Table 3. Ash gourd seeds associated with Fungi on agar plate

Sr. No.	Seed Sample	% Incidence of Mycoflora	% of Seed germination	Length of radicle (cm)	Length of plumule (cm)	Fungi associated
1.	Untreated	100	00	0.0	0.0	*Rhizopus stolonifer, Mucor mucedo, Monilia spp.*
2.	Treated	100	00	0.0	0.0	*Aspergillusflavus, Rhizopus stolonifer.*

Table 4. Bitter gourd seeds associated with Fungi on blotter paper

Sr. No.	Seed Sample	% Incidence of Mycoflora	% of Seed germination	Length of radicle (cm)	Length of plumule (cm)	Fungi associated
1.	Untreated	60	80	8.0	2.0	*Rhizopus spp., Aspergilluscandidus, Mucor mucedo, Cladosporium.*
2.	Treated	40	100	7.0	2.0	*Rhizopus spp., Mucor mucedo.*

Table 5. Bitter gourd seeds associated with Fungi on agar plate

Sr. No.	Seed Sample	% Incidence of Mycoflora	% of Seed germination	Length of radicle (cm)	Length of plumule (cm)	Fungi associated
1.	Untreated	70	20	0.5	0.5	*Rhizopus spp., Aspergilluscandidus.*
2.	Treated	40	50	0.5	0.0	*Rhizopus spp., Fusariumoxysporum, Sterile hypomycetes mycelium.*

DISCUSSION:

Seed pathology is science of understanding fungi in and on the seed. It is of great significance for any agricultural production. There are number of crops of agriculture and horticulture that are cultivated by seed and thus seed is important propagative organ that continue next generation. Seed is thus perenating dormant hidden life of any plant.

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With this main objective in mind seed pathology emerged as special discipline of agriculture to understand and control seed deteriorating agents and seed born pathogen including fungi. When fungus remains or just adheres seed surface such pathogen are called seed infestant while those cause seed tissue infection or embryo infection are called seed infectants. Seed pathology is also one of the branch of post-harvest pathology were main aim is to minimize the losses caused by fungal pathogens. Horticulture is a science of cultivation and management of fruit vegetable ornamentals. Their area is famous for cultivation of vegetable crops as well as fruitorchards. To understand what happens with vegetable seeds was the main aim and hence three vegetable from family Cucurbitaceae (like Chakki, Bhopala, Karale) were collected in the form of seeds from the market or from the farm for study of agents of deterioration. The collected seeds were first observed for damage or occurrence of any agent and thus seed born organisms which may be parasite or saprophyte was detected by Blotter method and Nutrient agar method. The blotter is well known technique to understand superficial fungi coming out at relative high humidity and optimum temperature conducive for fungal development. To avoid bacterial growth blotter was deeped in dilute Benzyl penicillin which was pre sterilize. The seed were placed at equidistant incubated and then examine for any fungal infection. In nutrient agar method seeds which were surface sterilize were transferred and plates incubated about a week and then any kind of fungus growth noticed. Agar medium is specifically used in understanding any pathogenic fungus is associated with seed or not. Such plates were incubated at optimum temperature in incubator at about $25^{-+}1$ temperature.

In all three vegetable crops seeds were taken for. Seeds selled in market were treated with fungicides while local seeds collected from farmers called untreated. It was noted that all treated seeds transferred on nutrient agar medium the occurrence of *Rhizopus*, while on blotter there was good growth of *Alternariatenuis*. Untreated farmers seeds have occurrence of good number of fungi like *Rhizopus, Mucor ,Penicillium, Alternaria* and *Sterile dematious mycelium* indicating that untreated seeds have more number of fungi.

Red squash gourd or Bhopala is one of the most valued Cucurbitaceous vegetable. The treated seeds are shown growth of fungus like *Rhizopus* has common saprophyte while untreated seeds have growth of several fungi like *Rhizopus, Mucor, Aspergillus, Penicillium, Monilia*indicating that treatment of seeds reduces population of fungi on seed.

Ash gourd or white gourd pumpkin which is extensively cultivated. Its seeds are yellow compressed and untreated seeds have shown over growth of *Rhizopus, Aspergillus, Mucor, Chaetomium* and*Monilia* on the other hand treated seeds have inhabitance of fungi like *Rhizopus*, on P.D.A. *Rhizopus* and *Aspergillus* on Malt while Blotter shows growth of a *Aspergillusflavus*. Showing growth of fungi like *Chaetomium*, *Aspergillusniger, Monilia* extensively on untreated seeds.

Bitter gourd is one of the most valuable climber vegetable of the family Cucurbitaceae with yellow brown flat seeds. The treated seeds on P.D.A. and Malt show growth of *Rhizopus* only while Blotter shows extensive growth of *Cladosporium* on the other side untreated seeds get deteriorated by fungi like *Aspergillus, Mucor, Rhizopus, Sterile hypomycetes mycelium, Monilia* and *Dematioushypomycetes*growth on Blotter or P.D.A. and Malt medium.

II. SUMMERY AND CONCLUSION

In all three vegetables are selected from their area and they were studied by Blotter & Agar medium to understand seed deteriorating and seed borne fungi.. The seeds treated with fungicides generally most show less number of fungi but in some cases it was observed that their number was high. In general in seed deterioration saprophytes like *Rhizopus, Mucor, Aspergillius, and Cladorsporium* were common. Blotter had shown good number of fungi in most of the seeds under observation.

Out of three seed grown vegetables Blotter or agar medium when the seeds were inoculated within one week fungi growth around the seeds was prominent and in few cases very less fungi growth was noticed on medium or well as on Blotter.

Following list shows a summarized account of fungi noted on different seeds during deterioration by seed borne organism or by common saprophytes.

1. Cucurbita maxima Duch ex Lam:

Rhizopus stolonifer, Mucor mucedo, Aspergillus, Penicillium, Monilia, Alternariatenuis.

2. Benincasahispida (Thunb) cogn*: *Aspergillus,Rhizopus,Mucor,Chaetomium, Monilia.

***3. Momordicacharntia* L.**

Rhizopus, Aspergillus, Mucor, Aspergillusflavus,Chaetomium, Pilblous .

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Table 6. Red squash gourd seeds associated with Fungi on blotter paper

Sr. No.	Seed Sample	% Incidence of Mycoflora	% of Seed germination	Length of radicle (cm)	Length of plumule (cm)	Fungi associated
1.	Untreated	100	50	6.0	2.0	*Rhizopus stolonifer, Mucor mucedo, Alternariatenuis.*
2.	Treated	50	70	8.0	2.0	*Mucor mucedo, Aspergillusflavus.*

Table 7. Red squash gourd seeds associated with Fungi on agar plate

Sr. No.	Seed Sample	% Incidence of Mycoflora	% of Seed germination	Length of radicle (cm)	Length of plumule (cm)	Fungi associated
1.	Untreated	100	00	0.0	0.0	*Rhizopus stolonifer, Mucor mucedo, Monilia spp.*
2.	Treated	80	30	0.5	0.0	*Aspergillusflavus, Alternariatenuis.*