ISSN No:-2456-2165

Screening and Isolation of Fungal Pathogens *Alternaria solani and Fusarium oxysporium*

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Abstract:- Agricultural production is depends on qualitative and quantitative of yeids . A productivity of agricultural plant is decide by the crop quality mostly depend on seed. If seed abnormal ,inferior quality it means the less productivity . Such inferior quality should be induce due to infection of seed borne pathogen result in seed rot, seedling decay and abnormalities, discolorations , seed size will be shorter and shriveled of seed.These symptom occurs when pathogen growing that causing the diseases ,when any diseased they shows such symptoms which reduced the productivity of crop .So that we have to study different types of pathogen . To study such pathogen and their widespread and effect crop . First we had to collection of plant material which infected by the pathogen and from pathogen we have to made culture then it should to be grown to be nutrient medium . The collection of plant material is maize and soybean seed .From that we identify which is pathogen by its morphological and reproductiove structure . On the basis of structure of conidia we identify the given pathogen are *Alternaria solani* and *Fusarium oxysporum.*They are isolated from plant material then culture are made they are grown on the nutrient media (potato dextrose agar) Then growth will occurred that preserved and maintained for different purpose.

Keywords:- *Alternaria solani and Fusarium oxysporum, Collection of material, isolation and maintainance*.

I. INTRODUCTION

The significance of sustainable Agricultural production is hidden in the use of quality seeds. Infection of seed borne pathogen result in seed rot, seedling decay, abnormalities, discolorations , seed size will be decrease and shriveled of seed. The role of seed borne pathogen has been defined and documented (Nene 1999). The seed should be infected or contaminated by the diseses they should have lower productivity , germination percentage will be lower down,crop growth lower down which overall impact on seedling yields . Seeds when infected by pathogen carries a primary source of inoculums for damaging seeds and plant with varying degrees.

Fungi are associated with seed internally or externally which cause disease to plant is called as seed borne fungi. Fungi are small, microscopic usually filamentous, branched. Spore bearing organism that lack of chlorophyll & have cell wall contain chitin, celluloses or both reproduce by means of sexual or asexual spores. In all 1,00,000spe of fungi have been described. More than 8000 spp. of fungi can cause diseases in plants. Fungi live on dead organic matter or parasite on living tissues. Seed abnormal size, seed quality will be reduced due to infection of pathogen such as eg. *Alternaria* ,seed necrosis ,seed color will change ,germination percentage will be reduced ,some physiological effect on seed will observed .

- *Alternaria solani: (EII&Mart) Jones & Grout.*

It is not large and extensive but is short, septate mycellium , branched, light brown but becoming darker with age .The colonies of *Alternaria* are wooly but more compact with the underside very dark colored. In the parasitic species, the hype are intercellular at first, but darker penetrate cells of the invaded tissues and thus become intracellular. The cells are the usually multinucleate. *Alternaria* is the most common contaminant of the laboratory culture. The cells are usually multinucleate. *Alternaria* have not sexual or perfect stage. They multiply asexually by the method of sporulation. The characteristic asexual spores which are produced exogenously are the conidia. The conidia are produce at the tip of ordinary hyphae which are comparatively short and dark coloured. Special hyphae termed conidiophores are not recognizable. The conidia are large, dark colored, several celled or beaked. The number of cells varies from 8-14 or even more. The Alternaria genus is found in the world .

- *Fusarium oxysporium :-(G. E .Massee)*

Legume seed carry various pathogenic fungi. It is of worldwide importance where at least 32 countries had reported the disease. At one time the disease nearly destroyed tomato production. Among those *Fusarium* spe cause harmful effect on the seeds of green gram *Fusarium* responsible for production of toxic substances. Described identification of *Fusarium* on the basis of their morphological character.(Henni et.al , 1994)

F. *oxysporum* causes banana wilt, wilt of cotton, wilt of sweet potato and stem rot, ginger wilt. *Fusarium* reproduces asexually by means of three kind of spores. Some author given the show the pictorial Guide to the identification of spp *Fusarium.(* Toussan T.A and Nelson P.E 1976) Viz. macro conidia, micro conidia, chlamydospores.Microconidia is avery small conidia abstracted form the tips of simple or branched conidiophores. The conidiophores are distinguish from the vegetative hyphae. The micro conidia vary in form the rounded to oval. In England described First, *Fusarium* wilt specially in Tomato (G.E. Massee 1895)

They are often held in small masses. At times they are elongated or crescent shaped. Macroconidia is a large, malt cellular usually 2-4 celled conidia. They are elongated sickle shaped or crescent shaped. The *Fusarium* spp. cause harmful effect on the seed of Green-gram.(Mathar & Neergaard 1970) They are produced at the tip of simple or sparingly branched conidiophores. They are distributed by a wing on falling an a suitable substratum they germinate and initiate new infections. Chlamydospores are the are rounded, oval, thick- walled cells formed in the hyphae. They may be formed singly or in chains of two or more. F. formae and races of F. *oxysporium* causing wilt disease.(Armstrong, G.M, Armstrong J.K 1981) They become separated from the parent hyphae after maturing and function as resting spores under suitable condition, it germinates by means of germ tubes to form a fresh mycelium

Several method have been evolved for fasting seed borne associated microorganism (Neergaard 1945) while selecting a method, it is necessary to consider that it is reliable, less time consuming in obtaining result. Many times more than one method is used for testing a pathogen. Agar plate method is used for identification of micro-organism based on growth and colony characteristics on nutrient medium. The potato dextrose Agar are suggested for seed health testing by ISTA.

II. MATERIAL AND METHODS

A. Material

Seed sample of Soybean ,Maize ,Gram was collected from Local farmers. Seed selected from fields then brought immediately to the laboratory for further study.PDA medium, were used for the cultural studies of the pathogen.Inoculating needles, forceps, blotting paper, mortar and pestle, filter papers, blotter papers, towel paper (germination paper), spirit lamp, cork borer, thread, distilled water, cotton wool, muslin cloth, ethanol, agar-agar powder, dextrose etc. were used during the experimentation.Petriplate, conical flask, culture tube, measuring cylinder, glass rod ,beaker etc.Autoclave, laminar air flow, digital balance, Incubator etc.

B. Methods

- *Isolation of pathogen*

Pathogen was isolated by standard tissue isolation technique, Tatiana *et al*., (2010). Infected seeds showing brown to dark spots were collected from the susceptible variety . The infected seeds along with some healthy portions were surface sterilized using 0.1% mercuric chloride solution for 2 minutes. The bits were then washed thoroughly in sterilized distilled water for three times to remove traces $HgCl_2$. Then PDA medium plates incubated at room temperature and observed the growth of fungus

- *Single spore isolation*

The clear nutrient media of agar should be filter and solidify in sterile petriplate .then its allowed to solidify .Spore suspension prepared by diluting the culture by distilled water After preparation of suspension that is spread on the 1ml suspension spread on the culture agar plate .that plate should be incubated and examined the growth and identify the conidia

- *Cultural studies and maintainance of PDA*

200 grams of peeled potatoes were cut into small pieces and boiled in 250 ml distilled water and then filtered to get the extract. 20 grams of agar powder was boiled separately in 100 ml distilled water. Both were mixed together and to it 20 grams of dextrose was added and final volume was made up to 1000 ml by distilled water. The medium was then sterilized in autoclave at 15lbs pressure for 20 minutes. pH of the above medium was maintained between 7 to 7.5.

III. RESULT AND DISCUSSION

The Isolattion will be done more times gate pure of pathogen so that isolation done more times . after the Isolation we study the growth of fungus on PDA medium for that we prepare spore suspension culture .When we do inoculation culture spread on solidify media we got some growth that growth are examined under microscopic study in that structure of conidiophore is sometime found in singly or group brown in colur. The conidia brown ablong tapering at both ends which having broader 8-10 mm and longitudinal septa 0-4 in number the different author discuss the description of A.solani and we recognized the character or description of conidia will see

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A. Micro Conidia:-

It is very small conidia abstracted from the tips of simple or branched conidiophores. The conidio phores are contain micro conidia vary in form the rounded to oval. They are borne an simple phialides arising laterally and are abundant, oval-ellipsoid straight to curved, 5-12 x2.2-3.5 mm and non – septate.

B. Macro Conidia :-

It is sparse to abundant are borne on branched conidiophores or an the surface of sporodia and thin walled 3-5 septate, sub-nucleate and pointed at both ends have pedicellate base. Three septate conidia measue27-46x3.5 mm while 5 septate conidia measures 35-60x 3-5 m.m.three septate spores are more common.

C. Chlamydospores :-

Rounded, oval, thick-walled cells formed in the hyphae. They may be formed singly or in chains of two or more. The *Fusarium oxysporium* species obtained on cultural media showed conidiophores of the fungus macro conidia thin walled, 3-5 septate, pointed at both ends have pedicel late base. Whereas micro conidia are very small condia abstracted from the tips or branched conidiophores. Conidia rounded to oval- ellipsoid straight. Where chlamydospores rounded oval thick walled cells formed in the hyphen. They may be formed singly or in chains of two or more

ISSN No:-2456-2165

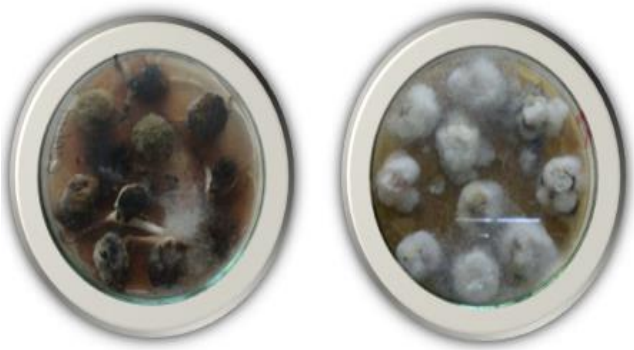

Fig 1:- Seed born pathogen Asocited with seed

Fig 2:- Fusarium oxysporium on PDA medium

Fig 3:- *AlternariAlternaria solani* on PDA Medium

Fig 4:- *Fusarium oxysporium* on PDA slant

Fig 5:- *AlternariAlternaria solani* on PDA slant

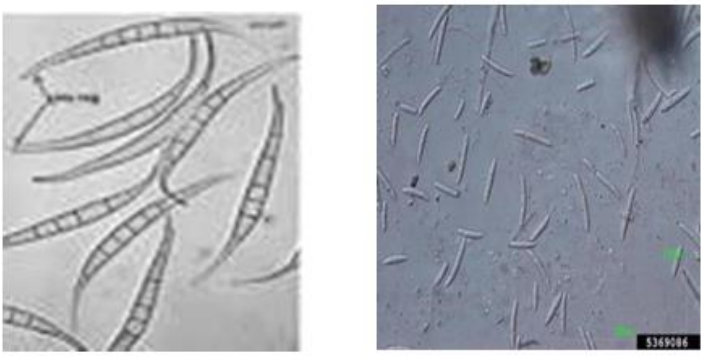

Fig 6:- (A,B)conidia of *Fusarium oxysporium*

ISSN No:-2456-2165

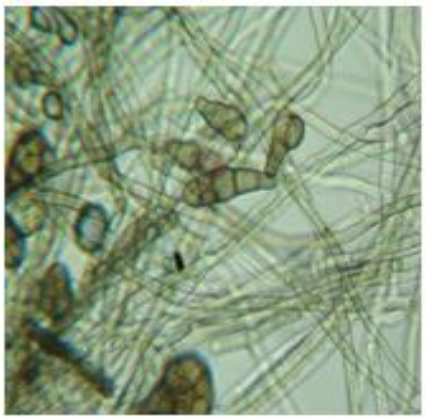

Fig 7:- Conidia of *AlternariAlternaria solani*

IV. CONCLUSION

The results are revealed through photographs. The pathogen, *A. solani* was isolated from the seeds and its identity was established and confirmed. Further work was carried out to study growth characteristics of pathogen on culture media. The PDA medium was found to be the best for its growth.The *A. Solani* and *F. oxysporum* isolated from solid media. After 9 day of inoculation which may which may be attributed to complex nature of natural media supporting good fungal growth.*A. Solani* and *F. oxysporum* P.D.A. showed good growth with excellent sporulation after 10 days of inoculation. The survivability of an organism depends an it's capacity to survive in an adverse environmental condition & it's host range for it's fitness to survive in the absence of main host.The maximum growth of Mycelium was obtained when exposed to alternate 12 hour light & 12 hour darkness.

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