Isolation and Identification of *Aspergillus sp* from vegetable crop field

Helen Sheeba D.K ,Sundar S.K*

Noorul Islam College of Arts and Science, Kumarakoil,Tamil nadu, India-629175.

* Assistant professor, PG Department of Microbiology ,MR Govt College of Arts and science, Mannarkudi,Tamil Nadu,India- 614001.
Affliated to Manonmanium Sundaranar University, Abishekapatti, Trinelveli, Tamil nadu, India-627012.

Abstract

Okra (Abelmoscus esculentus (L) of the malvaceae) is an important vegetable crop attacked by several bacterial,fungi,virus,nematodes, it leads to contamination of okra fruit.Hence study was carried out for the isolation of pathogenic fungi by plating technique and the pathogen was identified as Aspergillus sp through microscopic observation .Pathogenic test was performed in Okra fruit. It reproduce rot in artificially inoculated fruit okra. Aspergillus sp spread by spores which are produce in abundance.The spores can be carried and desseminate by wind current ,water(splashing of rain)(,soil(dust),insects.

Keywords-Okra,Aspergillus sp,fruit ,Rosebengal Agar.

I.INTRODCTION

Okra (Abelmoschus esculentus (L) is an economically important, tall growing, warm season, Vegetable crop grown in tropical pans of the world. It is cultivated throughout the tropical and warm temperate regions of the world for its green edible fibrous [12].Okra plays an important role in the human diet by supplying fats ,proteins,carbohydrates, minerals and vitamins in the right proportion to maintain growth and develpment .Moreover, its mucilage is suitable for certain medical and industrial applications [6]and contain moderate levels of vitamins A and C [18].Okra starts yielding about 60 days after planting time and allowed to mature on the, flowering and further production will be reduced.Okra is susceptible to several diseases in the field .Some varieties are highly susceptible to root decaying/root rot organisms while some are associated with deterioration of the fruits. Reported causal agents of okra fruit rot include *Fusarium solani,Rhizopus stolonifera,Rhizoctonia solani* .Micro-organisms that cause rots do so at high relative humidity RH) and temperature of 25-30°C [1]with some being more aggressive at high temperature of 35°C.

Crop suffers from a number of phytopathogenic fungal and bacterial species causing severe losses,reduces plating and market value[2,7,10,11,14,15,16,17]. The total loss vegetable okra on this account has been estimated up to 20-30%,but if the pathogensare allowed to develop,this loss may increase upto 80-90%[3,5]So the present study deals with the isolation and identification of pathogenic fungi from vegetable crop field.

II.Materials and Methods

Source of plant materials

Diseased parts of okra fruit was collected from okra field then put in sterile polythene bags and brought to the laboratory.

Enmeration of phytopathogenic fungi

Fruits were aseptically extracted with double distilled water placing the samples in laminar air flow chamber.The extracts were allowed to settle down and clear supernant were serially diluted sterile distilled water from 10-1 to 10^{-5} dilution. From 10^{-3} dilution ,0.1ml spread plate technique was made on solidified ,Rose Bengal Agar.The fungal plates were incubated at 28^0C $+2^0$C in the laboratory. Colony forming units(CFU) were counted.

$$\mathbf{CFU} = \frac{\textbf{Average number of colonies /plate}}{\textbf{volume plated in millilitres x dilution factor}}$$

Microbial Screening

Abundant colonies were subsequently subjected to pure culture.The most abundant fungal strains were preserved in slants for further identification.

Identification of Phytopathogenic fungi by lactophenol mounting technique

Preparation of lactophenol cotton blue (LPCB) slide mounts

1. A drop of seventy percent alcohol was placed on a clean microscopic slide.
2. Flame the wire by holding it upright in the hottest part of the Bunsen flame, just above the blue cone, until the whole length of the wire glows red hot.
3. Then ensure that the inoculating wire has cooled before placing it in a fungal culture – it should have cooled sufficiently after approximately ten seconds.
4. The cap from the tube remove but do not put it on the bench. Flame the neck of the tube,inorder to kill contaminating microorganisms which adhere the neck of the tube.
5. Remove a small amount of the culture with little of the agar medium together with the fungus..
6. Neck of the tube was flame once more and cap was replace
7. Immerse the fungal material in the drop of seventy percent alcohol. This drives out the air trapped between the hyphae.
8. Tease out the material very gently with mounted needles.
9. Sterilised the inoculating wire and the needles after use by heating to red heat in a Bunsen flame
10. Fungal structures are readily visualised after staining with a lactophenol cotton blue dye preparation.
11. Before the alcohol dries out , one or two drops of the stain was added .Holding the coverslip between our index finger and thumb, touch one edge of the drop of stain with the edge of the coverslip.

12. Lower the coverslip gently onto the slide, trying to avoid air bubbles. Preparation is now ready for examination.
13. Make the initial examination using a low power objective lens. The thinner parts of the preparation, generally around the edges of the mounted material, for the best images.
14. Switch to a higher power 40X objective for more detailed examination of spores and other structures

Sterilization of soil

Field soil were sterilized in autoclave at 15lb psi for one hour in three consecutive days.

Virulent /Pathogenecity test

1 ml of conidial suspension was injected into the inner tissues of the okra using the sterile syringe.Inoculated fruits was incubated for 7 days along with the uninoculated fruits as controls.All inoculated and uninoculated fruit was kept in an individual sterile container,with the cover and incubated at room temperature.

III.Results and Discussion

Enumeration of Fungal colony

The most abundant fungal colonies was enumerated in Rose Bengal Agar**.**Observation of the cultural characteristics of the isolated organism in the plate showed luxuriantly in the potato dextrose agar**.**Colony forming units(CFU) were calculated ,

$$\text{CFU/ml} = \frac{10}{0.1 \times 10^{-3}}$$

$$= 100 \times 10^{3} \text{ colonies /ml.}$$

Identification of Phytopathogenic fungi by lactophenol mounting technique

Colony morphology

After incubation, Potato dextrose agar plates showed Greenish blue colonies.

Microscopic appearance

When the phytopathogenic fungal culture mounded with lactophenol cottonblue stain the microscopic observation showed single-celled spores (conidia) in chain develop at the end of the sterigmata arising from the terminal bulb of the conidiophore.The vesicle and the long conidiophore arise from a septate mycelium.Hence the fungal strain was identified as *Aspergillus* sp.Theses organisms are commonly implicated in the post harvest deterioration of many crops reported severly[4,13]

Pathogenicity test

Results of the pathogenicity test revealed that fungal isolate namely *Aspergillus flavus* .It reproduce rot in artificially inoculated fruit okra .Therefore,these organisms are pathogenic to

Okra (*Abelmoschus esculentus L.*).It was considered to be the responsible pathogen of okra cause fruit rot in the crop field [9]have reported *R.Solani* in okra fruit.

IV.Conclusion

Based on the above results and discussion,the present study was clear that the okra field were contaminated by phytopathogenic fungi . Disease severity is due to weather,host resistance,inoculum load, and so on. The presence of this *Aspergillus* sp fungi in the crop field ,spread primarily by spores which are produced in abundance, leads to production of toxin aswell as yield lose. The present study which revealed the presence of phytopathogenic fungi ,which strongly emphasized the unhealthy condition of the crop okra ,so there is a need for taking urgent measure to protect crop

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