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Protease Activity of Common and Dominant Vegetable Mycoflora of Bhindi (*Abelmoschus esculentus L.*)

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Abstract: *Okra is an excellent source of Vitamin C and K_1.The Okra is low in saturated fat, cholesterol and sodium. High dietary fibre vitamin A, C, K, thiamine, Vitamin B_6, Folate, Magnesium, Phosphorus, Potassium, Manganese. Bhindi is known for its high soluble and insoluble fibre content. Numerous fungi affected vegetable adversely causing reduction in seed content and seed health. The objective of present investigation is to study effect of enzyme metabolites of common dominant test vegetable on seed health of Bhindi are evaluated. Total 13 fungi were found to be associated with the fruit. Out of these thirteen seed borne fungi, six Alternaria tenuis Auct, Aspergillus flavus, link ex.fr., Aspergillus niger van Teigh.,Curvularia lunata (wakker) Boedijn.,Dreschsler tetramera subram.&Jain., Fusarium moniliforme Sheldon, found to be common and dominant on Bhindi fruit.These common and dominant seed born fungi produced protease enzyme in variable quantity, which helped the fungi degrade the seed and ultimately affected seed quality yield.*

Keywords: Bhindi, Mycoflora, Protease activity

I. INTRODUCTION

Okar also known Bhindi is a flowering plant that's belongs to the family .It is mainly found in places like Africa, America and India .Okra is very low in calories and dense with nutrients it is high in fibre vitamin A,C and folate content. Okra has high nutrition content and economic crop grow in tropical and subtropical areas (Kimberly George 2020). The vegetable is known to improve and benefit health in a number of ways. Such as preventing and treating constipation , lowering cholesterol improving symptoms of depression helping to treat sore throat and lung inflammation reducing the risk of certain cancer (Salome P.2019).Okra contain a protein called lectine. Which is being studied for its role in cancer prevention and treatment (Natalie R.2019).

Total Thirteen fungi were found to be associated with the Bhindi. Out of these six found to common and dominant, these are *Alternaria tenuis Auct,Aspergillus flavus, link ex.fr.,Aspergillus niger van Teigh.,Curvularia lunata(wakker) Boedijn.,Dreschsler tetramera subram.&Jain., Fusarium moniliforme Sheldon.* Protease enzyme is important metabolite of seed born fungi necessary for pathogenesis. All the common and dominant seed borne fungi produce enzyme in variable quantity. Protease enzyme cause degradation of protein content of the seed reduces its protein content affecting seed quality. These common and dominant seed born fungi produced protease enzyme in variable quantity, which helped the fungi degrade. The seed and ultimately affected seed quality yield.

Danai (1994) Reported comparatively studies on the species of *Aspergillus* occurring on different plant seeds. She has observed more incidence of *Aspergillus flavus* link ex.fr. and *Aspergillus niger van Tiegh*. on the seed of Bhindi, Tomato and onion. Neeti et.al. (1982) Studies isolated *Fusarium moniliforme Sheldon. Aspergillus flavus link ex.fr Aspergillus sulpherens &Aspergillus niger van Tiegh*. from the seed of Bhindi. Nager and Chauhan (1977) reported loss in protein content due to seed borne fungi of Groundnut. Vijay Kumari et.al.(1981)studied pigeon pea and observed amylolytic activity due to *Aspergillus flavus van Tiegh*. Chary and Reddy (1982) Studied amylase production by seed born fungi of Green gram and its adverse effects on seed content. Protein is an important constituent of pulses its degradation due to seed borne fungi has been reported by Sinha and Prasad (1977). Prasad (1979) Studied seed borne fungi and found proteolytic activity of coriander seeds borne fungi responsible for seed biodeteroration.

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Similar study carried out by Biligrami K.S.et.al(1976),Bodke(2000),Dhake S.B(1995),Fashim M.M, Barakate F.M et.al(1985), Ivenov Pet.al(1989), Ismail & Medy (2001),Sexena N.et.al(1982), Sexana RM and Karan D.(1991),Sandhikar S.N.and Mukadam D.S.(1990),Vidyasekaran P. and Kandyaswamy(1972),Wadje S.S. and Deshpande K.S.(1977)

II. MATERIAL AND METHOD

2.1 Preparation of Spore Suspension

Spore suspension of common and dominant vegetable mycoflora were prepared separately by adding 10 ml of sterile distilled water into the sporulating pure cultures vegetable mycoflora namely *Alternaria tenuis* Auct., *Aspergillus flavus* Link ex, Fr., *Aspergillus niger* van Tiegh, *Curvularia lunata* (Wakker) Boedijn, *Drechslera tetramera* Subram. & Jain and *Fusarium moniliforme* Sheldon maintained on PDA slants for seven days at room temperature. The slants were shaken and content was filtered through muslin cloth to separate mycelium and spore. The filtrate thus obtained was used as spore suspension.

2.2 Bhindi Fruit Powder Nitrate (BN)

- Bhindi fruit powder -10gm
- KNO_3-2.5gm
- KH_2PO_4-1gm
- $MgSO_4.7H_2O$- 0.5gm
- Distilled water-1000ml

2.3 Basal Medium for Protease Assay

- Glucose-10gm
- Agar - 2gm
- Gelatine- 1gm
- Distilled water-100ml

2.4 Protease Production

Protease production by some common and dominant vegetable mycoflora such as *Alternaria tenuis Auct, Aspergillus flavus link*, *Aspergillus niger*, *Curvularia lunata (wakker) Boedijin., Dreschslera tetramera Subram&Jain., Fusarium moniliforme Sheldon*., was studied by growing them on liquid vegetable powder nitrate (VN) medium twenty five ml of the VM medium was poured in 100 ml conical flask separately autoclaved at 15lbs pressure for 20 minutes. The flask on cooling were inoculated separately with spore suspension of test vegetable prepare for seven day old culture grown on PDA slants. These flasks were incubated square into for 10 days at room temperature. After incubation the contents were filtered through Whatman filter paper No.1 to remove fungal mat and the liquid part was collected in pre sterilized bottles and used as crude enzyme preparation for the protease essay by cup plate method and the results are presented in table.

2.5 Protease assay by Cup Plate Method

Determination of protease activity was done with the help of cup plate method, adopted by Hislop et.al., (1982) and Rajamoni et.al.,(1988).A basal medium was prepared containing 2% (w/v) agar and 1% (w /v) gelatin.PH of the medium was adjusted at 5.6. The medium was sterilized at 15 lbs pressure for 20 minutes. 15 ml of medium was poured in presterilized Petriplates under aseptic conditions. 6 mm diameter cavity (cups) were made in the centre of the solidified agar plate with No. 4 cork borer .About 0.5 ml of culture filtrate (crude enzyme preparation) was poured in the cavity .The plates were incubated at room temperature for 24 hrs.15%$HgCl_2$ in 7HCL was headed to the plate after 10 minutes a transparent zone indicating hydrolysis of gelatin by extra cellular proteolytic enzymes was observed. The diameter of the transparent zone was used as a measure (mm) of proteas activity and non appearance of clear zone considered absence of protease in the culture filtrate (crude enzyme preparation).

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Table: Protease activity of common and dominant vegetable fungi grown on liquid **Bhindi (*Abelmoschus esculentus L.*)** fruit powder nitrate (BN) medium (After ten days of incubation, by cup plate method)

Sr. No.	Common and dominant vegetable fungi	Protease activity zone (mm)
		Glucose-gelatin agar (GGA) Medium
1.	*Alternaria tenuis* Auct.	18
2.	*Aspergillus flavus* Link ex, Fr.	16
3.	*Aspergillus niger* van Tiegh	15
4.	*Curvularia lunata* (Wakker) Boedijn	14
5.	*Drechslera tetramera* Subram. & Jain	20
6.	*Fusarium moniliforme* Sheldon	21

The results in the Table clearly show that all the common and dominant vegetable fungi showed protease activity in different quantities. The fungus *Fusarium moniliforme* Sheldon showed maximum activity among all vegetable fungi (21 mm activity zone), followed by *Drechslera tetramera* Subram. & Jain. Minimum protease activity was shown by *Curvularia lunata* (Wakker) Boedijin (14 mm activity zone).Similar finding were recorded by Khandare (2008) stated that protease activity in variable degree.

III. CONCLUSION

Total thirteen fungi were found to be associated with the fruits and seeds of Bhindi. All the common and dominant vegetable fungi showed protease activity in more or less degree. The fungus *Fusarium moniliforme* Sheldon showed maximum proteolytic activity (21 mm activity zone). Minimum activity was recorded by *Curvularia lunata* (Wakker) Boedijin (14 mm activity zone).

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