RESEARCH ARTICLE

STUDIES ON ENZYME PROTEASE ACTIVITY PRODUCTION BY COMMON AND DOMINANT MYCOFLORA OF RADISH *(RAPHANUS SATIVUS L.)*

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Abstract:

Radish *(Raphanus sativus L.*) is one of the most popular root vegetable crops which is cultivated for its enlarged edible roots. Radish is not only a versatile and delicious root vegetable they are also health and nutritional benefits. It contains smaller amount of Calcium, Riboflavin, Niacin, Iron, Folate etc. The health benefit including Digestive health, Potential Anticancer properties, Diabetes management, Liver function, Heart Health, Radish are a favourite vegetable all over the planet they remove bilirubin from the Liver preventing Jaundice and respiratory problem such Asthma or Bronchitis. The vegetables and their seeds carry large number of fungi both in field and during the storage most of the fungi cause decay and rots. The biodeterioration of vegetable and their seeds directly related to the toxin and enzyme production by the associated fungi. Determination of protease activity was done with the help of cup plate method adopted by Hislop and Rajamoni. Protease production by *Alternaria tenuis* Auct., *Aspergillus flavus* Link ex. Fr., Aspergillus niger van Tiegh., *Curvularia lunata (*Wakker) Boedijn., *Drechslera tetramera* Subram. & Jain. and *Fusarium moniliforme* Sheldon. was studied by growing them on liquid Radish powder nitrate medium (RN medium) separately. This study present Radish protease activity of enzyme. The proteases essay by complete method and result are presented table. The common and dominant seed born of fungi producer protease enzyme in variable quantity which help the fungi degrade the seeds and ultimately affect seed quality yield. These common and dominant seed born fungi produced protease enzyme in variable quantity which help the fungi degrade the seeds and ultimately affected seed quality yield.

Keywords: Isolation, Radish, Mycoflora, Protease Activity, Post Harvest Fungi.

Introduction:

Radish (*Raphanus sativus* L.) is vegetable belongs to the Cruciferous family there are several types of Radishes in colour size and shape. Radish nutritive value health benefits come from fibre vitamins and minerals proteins carbohydrate low in calories. Medicinal benefits for human body in digestion health antioxidant properties and antimicrobial properties and support liver health. Radish total twelve fungi found on roots and seeds. Most of the fungi cause Decay and rots (Kunte and Yawalkar, 1991). The biodeterioration of vegetables and their seeds show many changes in their contents (Verma *et al.,* 1991). Chamraj *et al.* (2011). Bio-deterioration of Radish and their seeds directly related with the toxin and enzyme production by the associated fungi. The Chary and Reddy (1982) reported studied amylase production by seed borne fungi of green gram and its adverse effects on seed content. Prasad (1979) studied seed borne fungi and found proteolytic activity of coriander seed borne fungi responsible for seed biodeterioration. Protein is an important constituent of pulses its degradation due to seed borne fungi has been reported by Sinha and Prasad (1977), Shesha (2017) twelve fungi pathogens were detected. Experiment entitled with study on seed health status of Radish fungi where recorded common and dominant fungi *Alternaria tenuis* Auct., *Aspergillus flavus* Link ex. Fr., *Aspergillus niger* van Tiegh*., Curvularia lunata* (Wakker) Boedijn., *Drechslera tetramera* Subram. & Jain, *Fusarium monaliforme* Sheldon among them most dominant in seed mycoflora of Radish present investigation study in paper economical approach for the bio conservation of vegetable waste for production of proteins and activity of enzyme protease activity of common and dominant vegetable fungi studied. For this the vegetable fungi were grown on liquid Radish root powder nitrate (RN) medium over a period of ten days. After incubation period, culture filtrate (crude enzyme preparation) was collected. The protease activity was assayed in agar solidified basal Glucose gelatine agar (GGA) medium by cup plate method. The results are presented in Table.

Materials and Methods:

1. Preparation of Spore Suspension:

Spore suspension of common and dominant vegetable mycoflora was prepared separately by adding 10 ml of sterile distilled water into the sporulating pure cultures Radish 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. Composition of media reagents and indicators used during present study

Radish root powder Nitrate (RN) Medium

Radish root powder-10 gm; KNO_3-2.5 gm; KH_2PO_4- 1gm

$MgSO_4.7H_2O$-0.5 gm; Distilled water1000 ml.

3. Basal Medium for Protease Assay

Glucose-10 gm; Agar-2 gm; Gelatin-1 gm; Distilled water-100 ml

4. Protease Production:

Protease production by some common and dominant vegetable mycoflora such as *Alternaria tenuis* Auct., *Aspergillus flavus* Link ex. Fr., *Aspergillus niger* van Tiegh., *Curvularia lunata* (Wakker) Boedijn., *Drechslera tetramera* Subram. & Jain. and *Fusarium moniliforme* Sheldon. was studied by growing them on liquid Radish powder nitrate (RN) medium 25 ml of the RN medium was poured in 100 ml conical flasks separately and autoclaved at 15 lbs pressure for 20 minutes. The flasks on cooling were inoculated separately with spore suspension of test vegetable fungi prepared from seven day old culture grown on PDA slants. These flasks were incubated 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 presterilized bottles and used as crude enzyme preparations for the protease assay by cup plate method and the results are presented in Table.

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) a Rajamani *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 cavities (cups) were made in the center 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% Hgcl2 in 7M HCl was added to the plates. 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 protease activity and non-appearance of clear zone considered absence of protease in the culture filtrate (crude enzyme preparation).

Table 1: Protease activity of common and dominant vegetable fungi grown on liquid Radish (*Raphanus sativus* L.) root powder nitrate (RN) 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.	19
2.	*Aspergillus flavus* Link ex, Fr.	12
3.	*Aspergillus niger* van Tiegh	14
4.	*Curvularia lunata* (Wakker) Boedijn	15
5.	*Drechslera tetramera* Subram. & Jain	24
6.	*Fusarium moniliforme* Sheldon	15

Result:

The results in Table reveal that, all common and dominant vegetable fungi exhibited protease activity in different quantities. The fungus *Drechslera tetramera* Subram. & Jain. showed maximum protease activity (24 mm activity zone) followed by *Alternaria tenuis* Auct. Minimum protease activity was shown by *Aspergillus flavus* Link ex. Fr. in (12 mm activity zone).

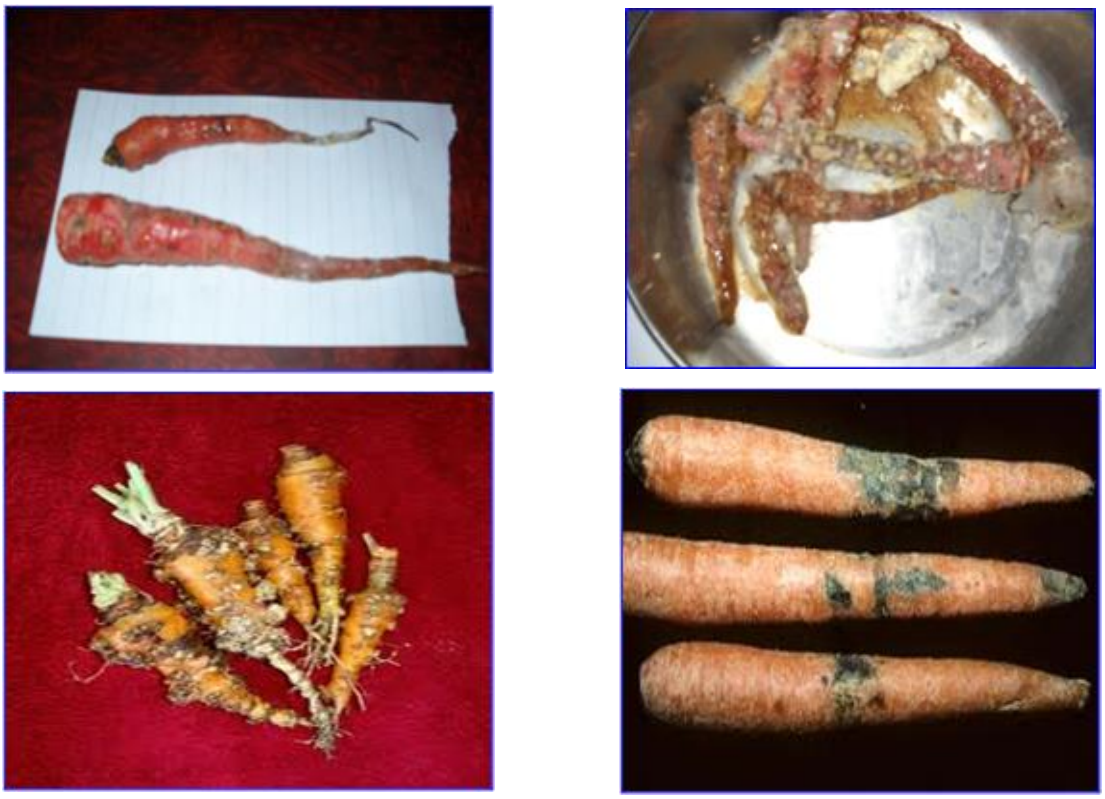

Plate 1: Incidence of mycoflora on the roots of Radish

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