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Impact of Mycoflora on Protein Content of Artificial Infested Roots of Medicinal Plant "*Asparagus Racemosus*".(L.)

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ABSTRACT: *Asparagus racemosus* (L.) is a vital drug plant in the pharmaceutical science. In the present investigation studies were carried out to understand the qualitative and quantitative pathogenic and nonpathogenic fungi on *Asparagus* plant during their developmental stages in field and also during storage and transport of drug plant to market. The findings are mainly on isolation of fungi from roots in field and under storage condition. Studied regarding biodeterioration of roots due to artificial infestation of fungi separately under different storage period were carried out and it was found that most of fungi were highly aggressive for causing loss protein content. The degree of deterioration of roots is variable. This clearly indicate that in nature there are number of micro-organisms capable to destroy drug plant parts under storage. Considering this situation the present studies on effect of mycoflora on stored drug plant parts used in ayurvedic medicines were started as a first step to fill up this major lacuna in the field of ayurvedic therapy.

KEYWORDS: Fungi, protein content, *Asparagus racemosus* roots

I. INTRODUCTION

Asparagus racemosus L. is a perennial drug plant grown in a wide range of soils and varied climatic conditions. *Asparagus* roots are largely used in ayurvedic medicines. Preservation of crude drugs needs sound knowledge of their physical and chemical properties. Quality of the drugs can be maintained or preserved properly. All the drugs should not be preserved in well closed containers. A number of drugs absorb moisture during their storage and become susceptible to the microbial growth. The environmental conditions like temperature, relative humidity, moisture and storage conditions have been reported to affect establishment of drug mycoflora, their role on biodeterioration and mycotoxin contamination. During survey, it was observed that phytopathogenic fungi cause severe harm to the drug plants. The plant part samples collected from field or forests are stored in warehouses where they are usually packed in gunny bags or spread on the ground. They have to endure fluctuating environment and diverse range of microorganism. Transport of drug plant parts to the market may involve major damage which may result into pathogenic infections. It is clear from the literature that damage to drug plants in field and during storage has been found mainly due to fungi. Drug plant parts undergo drastic chemical changes from field to factory due to microbial action. This may result in the qualitative and quantitative loss of drug plants and plant parts

During storage, the fungal organisms thrive in drug plant parts such as roots. where as loss in medicinally active ingredients from the drug plant parts have been reported by few workers, Proteins are the important constituents of the drug plant parts. The protein content of oats seeds were significantly decreased by seed borne pathogenic fungi during the storage period. The total of 10 fungal genera isolated from B2 and B7 seeds and stored for 360 days. The results showed that the content of protein of B2 and B7 seeds were significantly decreased by seed borne pathogenic fungi during the storage period [9]. Loss in protein content of seed due to associated mycoflora has also been reported in case of different oil seeds as in Pulse [3] Groundnut[7] and Green gram Black gram, Chickpea, Pigeon pea [1], Mustard [5],[2], observed leaves, where as decrease in protein content in infected leaves of sesame is due to the utilization of protein by the pathogen.

II. MATERIAL AND METHODS



Healthy Roots of *Asparagus racemosus* Infected Roots of *Asparagus racemosus*

Collection of healthy & infected roots of *Asparagus racemosus*

Healthy & infected root samples of *Asparagus racemosus* collected at regular intervals from fields, store houses and ayurvedic shops of various localities of Maharashtra. The collected root samples were dried at shade and kept separately in pre-sterilized polyethene bags and brought into the laboratory.

Detection of mycoflora from Roots of *Asparagus racemosus*

The mycoflora of medicinal plant such as roots of *Asparagus racemosus* were isolated by using Standard Blotter Method (SBM) and Agar Plate Methods (APM) as recommended by International seed Testing Association [4,6].

Identification of fungi

The fungi occurring on root pieces in the plates were identified preliminary on the basis of sporulation characters like asexual or sexual spores and fruiting structures with the help of stereoscopic binocular microscope. The identification and further confirmation of the fungi was made by preparing slides of the fungal growth and observing them under compound microscope. Pure cultures of different fungi prepared and maintained on Potato Dextrose Agar slants.

Biodeterioration

The roots of *Asparagus racemosus* were surface sterilized separately with 0.1% mercuric chloride solution and washed twice with sterile distilled water. Excess water was discarded, the plant part were distributed into sterilized conical flasks (25 g/flasks) and were inoculated separately with 2 ml spore suspension of different fungi of drug plant. The flasks were incubated at room temperature at 1, 3, 6 and 12 months respectively and were harvested for recording chemical changes in the drug plant part due to fungi. For which the plant part were thoroughly washed under running tap water in order to remove mycelial growth from their surface. Subsequently the drug plant part were dried at 60°C for 48 hours and crushed into fine powder for the estimation of protein content. For the control, plant part were incubated in a similar manner but without inoculating the spore suspension.

Estimation of protein :-

Estimation of crude protein was made by Microkjeldahl method (A.O.A.C., 1965). 300 mg root powder was placed in 50 ml Microkjeldahl flask to which 60 mg catalyst and 7.5 ml of H₂SO₄ were added. The flasks were heated for 6-8 hours (digestion) till colourless digest is obtained. On cooling, the digest was diluted to 50 ml in a volumetric flask. 5 ml of the aliquot was introduced in a Markham's distillation unit through the side tube funnel to which glass stopper was fitted. 10 ml of 10% NaOH solution was added into the funnel of side tube and was allowed it into the digest. The quantity of ammonia liberated was collected in 50 ml conical flask containing 5 ml of 2 % boric acid as an indicator. The distillate was titrated against 0.035 N HCl till the end point (faint pink) was achieved.



1ml of 0.035 N HCl = 0.5 mg nitrogen. The amount of nitrogen obtained in aliquot and subsequently in total volume of digest per 300 mg root powder was calculated and the value was expressed as percent nitrogen. The crude protein was calculated by using the formula.

Percent crude protein = $6.25 \times$ percent nitrogen.

III. RESULTS AND DISCUSSION

Table:1 Changes in protein content of artificially infested roots of *Asparagus racemosus*:

Fungi	Storage period in months			
	1	3	6	12
<i>Aspergillusflavus</i>	6.17	4.80	3.70	2.81
<i>Aspergillusniger</i>	6.12	4.93	3.61	2.58
<i>Aspergillusterreus</i>	6.05	5.08	3.90	2.42
<i>Alternariaalternata</i>	6.18	5.60	4.10	3.23
<i>Curvularialumata</i>	6.20	5.18	3.88	2.94
<i>Fusariummoniliforme</i>	6.29	5.09	3.91	2.87
<i>Rhizoctoniasolani</i>	6.20	4.98	4.06	3.10
<i>Rhizopusstolonifer</i>	6.23	5.19	4.11	3.14
Control		6.60		

Values in mg.

In order to study protein content of artificial infestation of roots of *Asparagus racemosus*, the protein content was estimated after one, three, six and twelve months respectively and results are summarized in Table 1. It is clear from the results that, the loss in protein content of the roots was found to be variable with the different fungi. All the species of *Aspergillus* showed deterioration of protein content of roots on one month storage. Rate of degradation at the end of six and twelve month storage periods was found to be more in all the fungi.

Regarding protein content of roots of *Asparagus racemosus* maximum reduction was recorded due to *Aspergillus*. It is interesting to note that rate of degradation at the end of six and twelve month storage period was found to be more in all the fungi. Similar results regarding decrease in protein content was recorded due to *Aspergillusflavus* caused maximum loss in protein content of *Strychnosnux-vomica*, *Strychnospotatorum*, *Datura metal* and *Piper longum* seeds [8]. Protein content of infected leaves of sesame is decreased due to the pathogen [2]. This clearly indicate that in nature there are number of micro-organisms capable to destroy drug plant parts under storage.

IV. CONCLUSION

Changes in the protein content of roots of *Asparagus racemosus*, among all fungi *Aspergillus species* utilized maximum protein content of root, *Fusariummoniliforme* and *Rhizoctoniasolani* caused maximum protein degradation of the roots. The loss in protein content of the roots of *Asparagus racemosus* was found to be variable with different fungi

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