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SCAN ME

Research Paper

IMPACT OF PATHOGENIC FUNGI ON GLYCOSIDE CONTENT OF ARTIFICIAL INFESTED RHIZOME OF *Glycyrrhiza glabra* (L.)

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Abstract

Glycyrrhiza glabra (L.) is an vital drug plant in the pharmaceutical science. The plant is known for curing various disorders due to the presence of phytochemical glycoside. The plant part rhizome have been used in ayurvedic medicines. Various studies regarding biodeterioration of plant parts due to infestation of fungi suggest that fungal pathogens are highly aggressive for causing degradation of glycoside contents of the rhizome at different developmental stages. Microbial action causes plant parts to undergo drastic chemical changes from field to factory. Transport of crude drugs to the market may involve various types of damages which may result into qualitative and quantitative loss of the samples. 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.

Key words: Fungi, Glycoside content, infestation, *Glycyrrhiza glabra*, rhizome.

INTRODUCTION

Drug plants are crucial in the pharmaceutical science. Nature is a source of drug plants. A large number of modern drugs have been isolated from natural sources., many based on their uses in ayurved medicine. The raw material of drug plants are obtained from wild sources, but few are under systematic cultivation in India. The drug plant parts like leaves, roots, stems, fruits and seeds yield good quality of phytochemicals. This necessites the study of the important diseases of medicinal plants especially to protect them.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. This may result in the qualitative and quantitative loss of drug plants and plant parts.If such infected or contaminated herbal parts are used for preparation of medicines, the quality of the resulting medicine is likely to be adversely affected and the medicine may become hazardous rather than curative. 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.

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 relative humidity, temperature, moisture and storage conditions have been reported to affect establishment of drug mycoflora,their role on biodeterioration and mycotoxin contamination.

Studies regarding biodeterioration of rhizomes of *Glycyrrhiza glabra* due to infestation of fungi were carried out and it was found that fungal pathogens were highly aggressive for causing degradation of glycoside content of the rhizomes of *Glycyrrhiza glabra* at different developmental stages.

2. MATERIALS AND METHODS

Fig.1 : Healthy rhizome

Fig.2: Infected rhizome

2.1 Collection of healthy & infected rhizome samples

The healthy & infected rhizome samples of *Glycyrrhiza glabra* at different developmental stages were collected at regular intervals from fields, store houses and ayurvedic shops of various localities of Maharashtra. The collected samples were dried

at shade and kept separately in pre-sterilized polyethene bags and brought into the laboratory.

2.2 Detection of mycoflora from rhizomes of *Glycyrrhiza glabra*

The mycoflora of medicinal plant such as rhizomes of *Glycyrrhiza glabra* were isolated by using Standard Blotter Method (SBM) and Agar Plate Methods (APM) as recommended by International Seed Testing Association (ISTA, 1966) and Neergard (1973).

2.3 Identification of fungi

The fungi occurring on rhizome 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 these fungi prepared and maintained on Potato Dextrose Agar slants.

2.4 Biodeterioration

The rhizomes of *Glycyrrhiza glabra* 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 plants. The flasks were incubated at room temperature 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 60^0C for 48 hours and crushed into fine powder for the estimation of alkaloid contents. For the control, plant part were incubated in a similar manner but without inoculating the spore suspension.

2.5 Estimation of glycoside

10 gm of coarsely powdered rhizome and macerate with 50 ml of 70% alcohol for 1 hour. Filter and retain the filtrate, Then slowly add strong solution of lead subacetate until precipitation is complete, centrifuge and retain the supernatant. Then slowly add 6.3% sodium sulphate solution to precipitate excess lead, and centrifuge or filter as before. The aqueous supernatant contains the glycosides. Extract with successive 25 ml portions of chloroform and reduce the volume of chloroform to 5 ml by distillation. The

glycosides are in the chloroform layer and can be crystallized out with evaporate the chloroform layer and weighed on balance.

3. RESULTS

Table1: Changes in glycoside content of artificially infested rhizomes of *Glycyrrhiza glabra:*

Fungi	Storage period in months			
	1	3	6	12
Aspergillus flavus	0.49	0.37	0.24	0.13
Aspergillus niger	0.51	0.35	0.20	0.11
Aspergillus terreus	0.48	0.31	0.17	0.10
Alternaria alternata	0.52	0.43	0.28	0.18
Curvularia lunata	0.50	0.39	0.27	0.21
Fusarium moniliforme	0.52	0.38	0.30	0.16
Rhizoctonia solani	0.49	0.33	0.24	0.12
Rhizopus stolonifer	0.48	0.34	0.22	0.14
Control		0.59		

Values in gm/ 10gm.

4. DISCUSSIONS

Studied regarding biodeteriortion of rhizome of *Glycyrrhiza glabra* 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 loss in medicinally active ingradients like glycoside content.The degree of deterioration of plant part caused by storage fungi was found to be variable. This clearly indicate that in nature there are number of micro-organisms capable to destroy drug plant parts under storage. Similar type of work regarding degradation of active ingradients, number of medicinal plants have been worked out in a detailed manner by Dutta and Roy (1987), Dutta (1988), A. K. Roy (1989), Kumar and Roy (1996), Roy A. K. (2003).

Regarding the effect of mycoflora on glycoside content of infested rhizome of *Glycyrrhiza glabra,* it was seen (Table 1) that, After 6 to 12 month storage period all fungi caused maximum loss in glycoside content. The concentration of glycoside was siginificantly reduced under infestation. Similar results regarding decrease in glycoside content was recorded due to *Aspergillus flavus, A.candidus, A. clavatus, A. luchuensis, A.niger, A. nidulans, A ochraceus* and *A. Sydowii* (Roy A. K. 1987), *Aspergillus flavus* (Kumar and Roy, 1996).

In order to study glycoside content of rhizome of *Glycyrrhiza glabra* due to artificial infestaton, the glycoside content was estimated after one, three, six and twelve months

respectively and results are summarised in table 1. It is clear from the results that, there was siginificant decrease in glycoside concentration with increase in storage period in case of all the fungi. After twelve month storage period *Aspergillus flavus, Aspergillus niger* and *Aspergillus terreus* caused maximum loss in glycoside content, their concentration was significantly reduced under infestation. It is clear from result that there was no decrease in glycoside concentration in control.

In the present investigation studies were carried out to understand the qualitative and quantitative pathogenic and non pathogenic fungi on different cultivated medicinal plants during their developmental stages in field and also during storage and transport of drug plants to market. The findings are mainly on isolation of fungi from roots in field and under storage condition. Studied regarding biodeterioration of rhizome 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 of medicinally active ingradients like glycoside 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.Similar type of work regarding degradation of active ingradients, number of medicinal plants have been worked out in a detailed manner by Dutta and Roy (1987), Dutta (1988), Roy A. K. (1989), Kumar and Roy (1996), Roy A. K. (2003).

5. CONCLUSION

The loss in glycoside content of the rhizomes of *Glycyrrhiza glabra* was found to be variable with different fungi. Maximum loss in glycoside content was due to *Aspergillus niger* and *Rhizoctonia solani* it's concentrations was reduced with increase in storage period. Among all fungi *Aspergillus* species caused maximum loss in glycoside content. The concentration of glycoside was significantly reduced under artificially infestation with increase in storage period. The loss in glycoside content of the rhizomes of *Glycyrrhiza glabra* was found to be variable with different fungi. Maximum loss in glycoside content was due to *Aspergillus niger* and *Rhizoctonia solani* it's concentrations was reduced with increase in storage period.The loss in glycoside content of the rhizomes of *Glycyrrhiza glabra* was found to be variable with different fungi. Maximum loss in glycoside content was due to *Aspergillus niger* and *Rhizoctonia solani* it's concentrations was reduced with increase in storage period.

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