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RR Jadhav

Research centre of Botany,
M.B.S.K. Kanya
Mahavidyalaya, Kadegaon,
Dist.- Sangli – 415304 (M.S.)
India.

Effect of mycoflora on alkaloid content of artificially infested roots of *Rauvolfia serpentina*. (L.)Benth.ex.Kurz

RR Jadhav

Abstract

Rauvolfia serpentina (L.)Benth.ex.Kurz.is an important medicinal plant in the pharmaceutical world due to presence of it's immense therapeutic properties. The plant is known for curing various disorders because of the presence of alkaloids. The plant part root have been used in ayurvedic medicines. Studies regarding biodeterioration of plant parts due to infestation of fungi were carried out and it was found that fungal pathogens were highly aggressive for causing degradation of alkaloid contents of the roots at different developmental stages. Plant parts undergo drastic chemical changes from field to factory due to microbial action. During 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.

Keywords: Mycoflora, alkaloid contents, infestation, *Rauvolfia serpentina* roots.

1. Introduction

Medicinal plants provide medicines for the disease of human beings. Man started using drug plants for curing diseases, protecting their lives and sustaining for a longer period as noted in Vedas. The wealth of India is stored in the enormous natural flora of medicinal plants which has been gifted to our nation. It is necessary that more and more medicinal crops be commercially cultivated as field crops. At present, bulk of the raw material is obtained from wild sources, whereas only a few are under systematic cultivation of drug plants in India. The medicinal plants have great value in the medicinal science. The leaves, roots, stems, fruits and seeds yield good quality of phytochemicals Thus it is necessary to study the nature of the important diseases of these important medicinal plants to protect them. During a survey of phytopathogenic fungi cause severe attack to different medicinal plants. The different medicinal plants and plant parts reported to be affected by different microbial origin. The plant samples collected from field or forests are stored in traditional warehouses where they are usually packed in gunny bags or spread as such as ground and have to face fluctuating environment and diverse range of microbes. During transport of medicinal plant parts to the market may involve various types of damages which may result into infections. This may result in the qualitative and quantitative loss of medicinal 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 be come hazardous rather than curative, It is clear from the literature that damage to medicinal plants in field and during storage has been found mainly due to fungi. Medicinal plant parts undergo drastic chemical changes from field to factory due to microbial action. During transport of crude drugs to the market may involve various types of damages which may result into qualitative and quantitative loss of the samples.

Preservation of crude drugs needs sound knowledge of their physical and chemical properties. A good 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. *Rauvolfia serpentina* L. Benth. Ex Kurz. is an evergreen, woody, glabrous and perennial shrub

Correspondence

RR Jadhav

Research centre of Botany,
M.B.S.K. Kanya
Mahavidyalaya, Kadegaon,
Dist.- Sangli – 415304 (M.S.)
India.

with maximum height up to 60 cm. The plant possess tuberous root with pale brown cork and elliptic to lanceolate or obovate leaves in whorls of three.⁸ The plant belongs to the family Apocynaceae and occurs in habitats of tropical and subtropical regions. The family includes 50 species, distributed worldwide in the region of the Himalayas, Indian peninsula, Burma, Indonesia and Sri Lanka and is indigenous to India, Bangladesh and other regions of Asia. The plant is commonly known as Sarpagandha.

Studies regarding biodeterioration of roots of *Rauvolfia serpentina* due to infestation of fungi were carried out and it was found that fungal pathogens were highly aggressive for causing degradation of alkaloid content of the roots of *Rauvolfia serpentina* at different developmental stages. The species of *Aspergillus* were highly harmful for deterioration of plant parts. Roy *et al* (1988)^[7] isolated about seven fungi *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus candidus*, *Aspergillus luchuensis*, *Aspergillus ocharaceus*, *Fusarium moniliforme* and *Penicillium sp.* from infected roots of *Rauvolfia serpentina*.

2. Material and Methods

2.1 Collection of infected root samples

Infected roots samples of *Rauvolfia serpentina* (L.)Benth.ex.Kurz. 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 roots of *Rauvolfia serpentina*

The mycoflora of medicinal plant such as roots of *Rauvolfia serpentina* (L.)Benth.ex.Kurz. were isolated by using Standard Blotter Method (SBM) and Agar Plate Methods (APM) as recommended by International seed Testing Association (ISTA, 1966)^[3] and Neergard (1973)^[5].

2.3 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 these fungi prepared and maintained on potato dextrose agar slants.

2.4 Biodeterioration

The roots of *Rauvolfia serpentina* (L.)Benth.ex.Kurz. 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 °C 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 alkaloid

10 gms of finely powdered root sample was soaked with 28% ammonium hydroxide (NH₄OH) solution for a few hours and then little dried up. This was latter soxhleted with the mixture of chloroform and ethanol (3:1) for eight hours. After that, 100 ml of solvent extract shaken with 25 ml N/2 sulphuric acid (H₂SO₄) and collected acidic extract. This process was repeated thrice for the total extraction of alkaloids. Collected acidic extract was then made alkaline with ammonia hydroxide solution, followed by chloroform extraction. Chloroform extraction was made twice with 20 ml and 15 ml chloroform respectively. Extract obtained was distilled on water bath up to dryness and weighted on balance.

3. Results

Table1: Changes in alkaloid content of artificially infested roots of *Rauvolfia serpentina*-

Fungi	Storage period in months			
	1	3	6	12
<i>Aspergillus flavus</i>	0.12	0.09	0.06	0.04
<i>Aspergillus niger</i>	0.13	0.08	0.06	0.03
<i>Aspergillus terreus</i>	0.12	0.09	0.07	0.05
<i>Alternaria alternata</i>	0.14	0.11	0.08	0.06
<i>Curvularia lunata</i>	0.11	0.08	0.05	0.04
<i>Fusarium moniliforme</i>	0.12	0.09	0.07	0.05
<i>Rhizoctonia solani</i>	0.13	0.09	0.08	0.06
<i>Rhizopus stolonifer</i>	0.15	0.12	0.09	0.07
Control		0.15		

Values in gm/10 gm.

4. Discussion

In order to study alkaloid content of roots of *Rauvolfia serpentina* (L.)Benth.ex.Kurz. due to artificial infestation, the alkaloid 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 significant decrease in alkaloid 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 alkaloid content, their concentration was significantly reduced under infestation. It is clear from result that there was no decrease in alkaloid 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 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 of medicinally active ingredients like alkaloid 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 ingredients, number of medicinal plants have been worked out in a detailed manner by Dutta and Roy (1987)^[1], Dutta (1988)^[2], Roy A. K. (1989)^[8], Kumar and Roy (1996)^[4], Roy A. K. (2003)^[9].

Regarding the effect of mycoflora on alkaloid content of roots of *Rauvolfia serpentina*, it was seen that, After 6 to 12 month storage period all fungi caused maximum loss in alkaloid content, their concentration was significantly reduced under infestation. Similar results regarding decrease in alkaloid

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. et al 1987) [6], *Aspergillus flavus* (Kumar and Roy, 1996) [4].

5. Conclusion

Among all fungi *Aspergillus* species caused maximum loss in alkaloid content. The concentration of alkaloid was significantly reduced under artificially infestation with increase in storage period.

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