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Effect of relative humidity on the growth of fungal species over *Adhatoda vasica* L. and its quality deterioration during storage

Riti Kumari & B. K. Dayal*
University Department of Botany, B.N. Mandal University, Madhepura, Bihar, India

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Abstract- Procured sample of *Adhatoda vasica* L. was kept to the laboratory in small pockets of muslin clothes and weighed properly. The samples contained in pockets were stored under 60, 70, 80 and 90%. Relative humidity for different incubation 15, 30, 45 and 60 days. The control was also stored for 60 days. On interval of every 15 days the sample was washed with distilled water thrice and filtered. The filtrates were agar plated on Czapex Dox agar medium. The frequencies of different fungal species in the form of (%) per cent incidence was calculated. The quality of the drug sample was determined in terms of quantity deterioration of different chemical constituents which was estimated by Spectrophotometry method using calibration curve. All together 13 species of fungi were observed. Lowest frequency (% incidence) was formed after 15 days at 70% Relative humidity for *Aspergillus ustus* as 1.2 while at 90% the percent incidence found was 8.1 for this species. The maximum percent incidence for *Aspergillus elagans* was observed at 90% Relative humidity after 60 days of incubation as 9.8. Quantitative estimation of sugar, protein, alkaloid, saponin were carried out. The result obtained showed a sharp reduction in the Total sugar, Reducing sugar, Protein and Alkaloid etc. Maximum deterioration was found at 90% R.H. after 60 days of incubation which corresponded to 48% for sugar deterioration. For protein maximum deterioration was found at 90% R.H. after 60 days over control which corresponded to 29%. In the same way maximum deterioration for total alkaloid content showed 34%, while it was 31% for saponin content.

Key words: Relative humidity, Incubation, Fungal incidence, Quality deterioration

INTRODUCTION

Adhatoda vasica L. is a perennial shrub native to Asia and it belongs to family Acanthaceae. Leaves of the plant are medicinally important and contain a very important active chemical compound Vasicine. Besides, a number of other chemical constituents- Alkaloids, Saponin, Flavenoids, Phenols, tannines etc. are also present in leaves of the plant. The plant and plant parts has a no. of traditional uses in Siddha, Ayurveda and Unani system of medicine. The leaves of this plant are used in the treatment of cough, cold and bronchitis. Due to presence of active compound Vasicine, the leaves of the plant serve as raw material for manufacturing of cough syrup in pharmaceutical industry. So, storage of this plant parts specially leaves is a matter of concern.

The various factors responsible for fungal growth over the surface of plant material include relative humidity, moisture content of the material, temperature of the environment, incubation period and nutrients available for proper growth of the fungal mycelium over the surface of

*Corresponding author :
Phone : 9431890157
E-mail : binodkumardayal@gmail.com

plant material but the most important factors are relative humidity and temperature which play significant role in the fungal growth.

The quality of herbal drugs or plant parts under storage depends upon Relative humidity (RH) and temperature. Due to absorbing tendency of the storage samples, moisture content presents in the store house plays an important role in the fungal invasion. During pre and post harvesting, transporting, handling and processing these medical plants parts get infected.

Because of the unfit infrastructure of store houses which are not built scientifically damage of store material may occur. The quality as well as the quantity of the stored material may decrease.[1,2]

The present paper focused on the effect of relative humidity on the growth of fungal species over leaves of *Adhatoda vasica* L. and its quality deteriotation during storage.

MATERIALS & METHODS

The leaves of *Adhatoda vasica* L. were procured from local jaributti shop of Sigheshwarsthan, Madhepura and brought to the laboratory in a polythene bag to avoid further contamination. The sample material was weighed properly and stored in small muslin cloth bags under various R.H. -60%, 70%, 80% and 90% at room temperature (27°C±30°C) for 2 months. The Relative humidity levels were maintained in sterilized glass desiccators using pure glycerol diluted properly to get various R.H. % levels adopting Braun & Braun 1958 method. On interval of 15 days samples were taken out and thoroughly washed with 100 ml sterilized glass distilled water to remove fungal mycelium. The fungal suspension so obtained was washed and centrifused to get residue. The same was diluted by adding 2 ml of sterilized distilled water and incubated on agar plate. Growth of different fungal species was observed on agar plate. In each case percentage (%) incidence of fungal growth was recorded using the following formula-

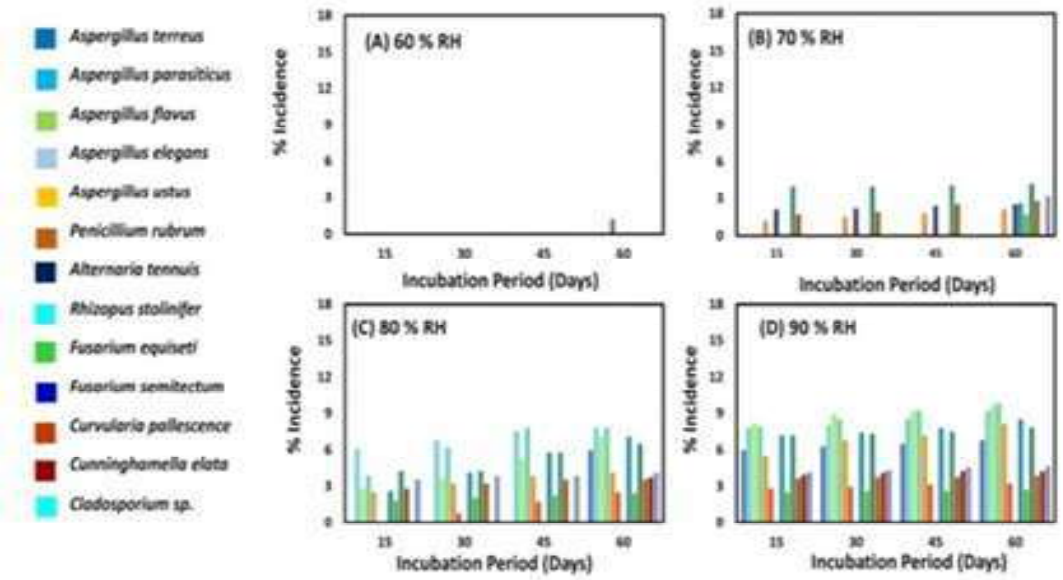
$$\% \text{ incidence of fungal growth} = \frac{\text{No. of colonies of a particular species}}{\text{Total no. of colonies of all the species}} x\ 100$$

OBSERVATION AND IDENTIFICATION OF FUNGAL SPECIES

Observation of different fungal species was carried out under stero binocularo microscope. Identification of different fungal species was done with the help of standard text.[3-6]

Table 1- Incidence of fungal organisms on *Adhatoda vasica* leaves stored under various R.H %

	60% R.H.					70% R.H.				80% R.H.				90% R.H.			
Mycoflora cont.	**Days**	15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60
Aspergillus terreus ------		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.0	6.0	6.3	6.5	6.8
Aspergillus parasitic ---		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.1	6.8	7.5	7.8	7.8	8.0	8.6	9.2
Aspergillus flavous -----		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.8	3.6	5.2	7.1	8.2	8.9	9.2	9.6
Aspergillus elegans -----		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.8	6.2	7.8	7.8	7.9	8.9	9.2	9.8
Aspergillus ustus -----		0.0	0.0	0.0	1.2	1.2	1.5	1.8	2.1	2.5	3.2	3.8	4.1	5.5	6.8	7.2	8.1
Penicillium rubru ----		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	1.7	2.5	2.8	2.9	3.1	3.2
Alternaria tenuis ----		0.0	0.0	0.0	0.0	2.1	2.2	2.4	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rhizopus stonlonife ----		0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6	2.6	4.1	5.8	7.1	7.2	7.4	7.8	8.5
Fusarium equiseti ----		0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	1.8	2.0	2.2	2.4	2.5	2.6	2.6	2.7
Fusarium semitectum --		0.0	0.0	0.0	0.0	2.5	4.0	4.1	4.2	4.2	4.2	5.8	6.5	7.2	7.3	7.5	7.8
Curvularia pallescense--		0.0	0.0	0.0	0.0	1.7	1.9	2.5	2.8	2.8	3.2	3.5	3.5	3.6	3.7	3.7	3.8
Cunninghamella elata --		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.7	3.9	4.1	4.2	4.2
Cladosporium sps. -----		0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.2	3.5	3.8	3.8	4.1	4.1	4.3	4.5	4.6
Total % Incidence		0.0	0.0	0.0	1.2	7.5	9.6	10.1	19.1	30.1	37.9	47.1	62.6	66.7	70.2	74.6	78.3



Graphical representation of the data given above in the table-1

Quality deterioration of the sample leaves was determined in terms of quantity loss during storage using spectrophotometry with calibration curve. Estimation of chemical constituent's like- total sugar was carried out by adopting Dubois *et al.* (1951)[7] method. For the estimation of reducing sugar DNS method was adopted. The total protine in the sample was estimated by Lowry's method (1951)[8]. Estimation of total alkaloids was done by adopting harborne method while, total saponin content was estimated by Ejkme *et al.* (2014)[9] and Obedoni & Ochuko method.

Table 2- Estimation of Total Sugar content (mg/gm)

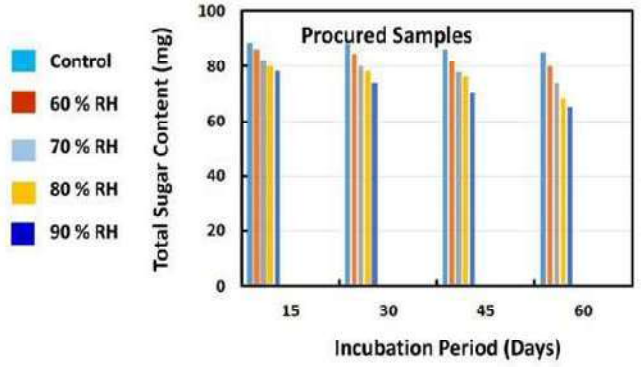
Days	15	30	45	60
RH (%)↓				
Control	88.56	88.21	86.23	85.23
60%	86.23	84.57	82.01	80.12
70%	82.12	80.12	78.17	74.23
80%	80.12	78.25	76.24	68.12
90%	78.25	74.21	70.12	65.27

Data given in the table are mean of three replicates

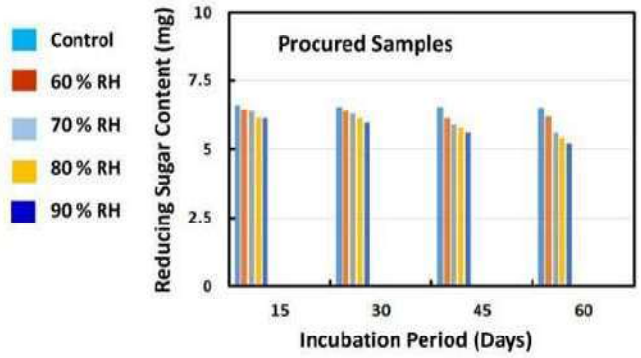
Table 3- Estimation of Reducing sugar content (mg/gm)

Days	15	30	45	60
RH (%)↓				
Control	6.58	6.52	6.52	6.51
60%	6.42	6.41	6.12	6.19
70%	6.38	6.31	5.92	5.61
80%	6.15	6.12	5.78	5.41
90%	6.12	5.98	5.62	5.22

Data given in the table are mean of three replicates



Graphical representation of the data given in the table- 2

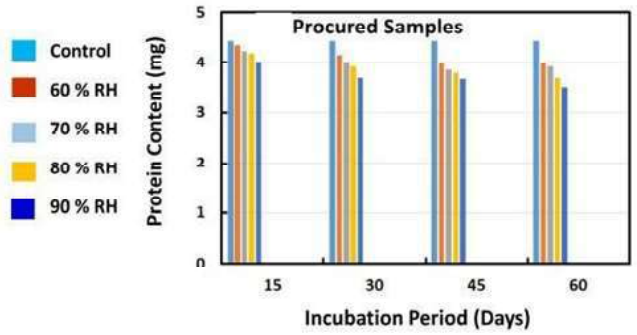


Graphical representation of the data given in the table- 3

Table 4- Estimation of Total Crude Protein Content (mg/gm)

Days	15	30	45	60
RH (%) ↓				
Control	4.42	4.42	4.42	4.42
60%	4.34	4.15	3.99	3.98
70%	4.23	4.01	3.85	3.92
80%	4.18	3.92	3.78	3.69
90%	4.01	3.68	3.67	3.51

Data given in the table are mean of three replicates

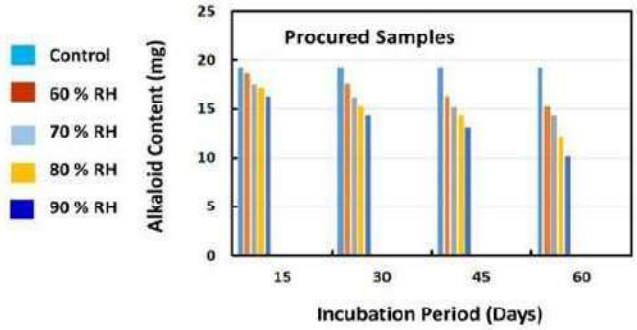


Graphical representation of the data given in table 4

Table 5- Estimation of Total Alkaloid Content (mg/gm)

Days	15	30	45	60
RH (%) ↓				
Control	19.25	19.23	19.23	19.23
60%	18.72	17.65	16.35	15.23
70%	17.52	16.24	15.21	14.38
80%	17.24	15.23	14.32	12.12
90%	16.32	14.32	13.12	10.23

Data given in the table are mean of three replicates

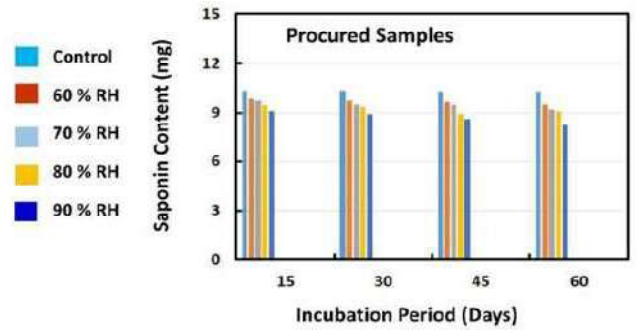


Graphical representation of the data given in table 5

Table 6- Estimation of Total Saponin Content (mg/gm)

Days	15	30	45	60
RH (%) ↓				
Control	10.31	10.31	10.3	10.3
60%	9.84	9.74	9.62	9.52
70%	9.72	9.52	9.51	9.21
80%	9.45	9.34	8.89	9.1
90%	9.12	8.92	8.62	8.21

Data given in the table are mean of three replicates



Graphical representation of the data given in table 6

RESULTS & DISCUSSION

In *Adhatoda vasica*, a total of 13 species of fungi were observed and identified leaves stored at 70% R.H. for 15 days incubation period showed only four fungal sps. i.e. *Aspergillus ustus* with 1.2% incidence, *Alternaria tenuis* with 2.1% incidence, *Fusarium sps*. with 4.0% incidence and *curvularia* sps. with 1.7% incidence.

For 15 days incubation period at 80% R.H. nine fungal sps. were observed i.e. *Aspergillus parasiticus* with 6.1% incidence, *Aspergillus flavus* with 2.8%, *Aspergillus ustus* with 2.5%, *Aspergillus elegans* with 3.8%, *Rhizopus stolonifer* with 2.6%, *Fusarium semitectum* with 4.2%, *Fusarium equiseti* with 1.8%, *Curvularia pallescens* with 2.8% and *Cladosporium sps.* with 3.5% of incidence.

Maximum of incidence of above fungal sps. was observed under 90% R.H. After 60 days of incubation period at 90% R.H. % incidence of different fungi shows increasing tendencies. The maximum % incidence at this stage was recorded for *Aspergillus elegans* as 9.8%.

Next to this was 9.6% for *Aspergillus flavus* and for *Aspergillus parasiticus* 9.2%. While *Aspergillus ustus* shows 8.1% and *Rhizopus stolonifer* with 8.5% of incidence.

For quality deterioration various chemical constituence like total sugar, reducing sugar, total crude protiene, total alkaloid and saponin content were estimated. The result in case of total sugar showd that after 15 days

of incubation the control showed a total sugar content 88.56mg. The sample stored at 60% RH after 15 days of incubation showed 86.23mg sugar content which decreases to 80.12mg after 15 days of incubation. Maximum deterioration of total sugar content after 60 days of incubation at 90% RH occurred. At this RH level total sugar decreases to 65.27mg corresponded to 26% deterioration.

While the maximum loss in reducing sugar content was found under the same storage condition which corresponded to 20%.

For protein content the control showed 3.55mg of total protein after 15 days of incubation. The stored sample at 60% R.H. after 15 days of incubation showed 3.34mg protein content which decreased to 2.94 after 60 days of incubation. At 70% R.H. and 80% R.H. after 15 days there appeared a slight change in the protein content which were 3.23 and 3.18. Maximum deterioration was found at 90% R.H. after 60 days of incubation which showed 2.5 mg corresponded to 29% deterioration over control.

In case of alkaloid content, after 15 days of incubation the control showed totak alkaloid content 132.55mg, which slowly decreased to 127.32mg after 60 days of incubation. At 60% R.H. the sample contained total alkaloid content 131.09mg which redused to 122.38mg after 60 days of incubation. Maximum deterioration was found at 90 % R.H. after 60 days of incubation which showed total alkaloid content 86.37mg corresponded to 34 Deterioration.

For Saponin content the control showed a total amount of 7.61mg Saponin content after 15 days of incubation, which slightly changed to 7.57mg after 60 days of incubation. The stored sample at 60 % R.H. showed 6.84 Saponin content after 15 days of incubation which reduced to 6.52mg after 60 days of complete storage. Maximum deterioration in this case occurred after 60 days at 90% R.H. which showed a total amount of Saponin 5.29mg corresponded to 31% deterioration over control.

CONCLUSION

Conclusively, 90% R.H. and 60 days of incubation period play a significant role in fungal growth thus directly effect the quality of *Adhatoda vasica*, (leaves). This is evident from the quantitative estimation of various chemical constituents at various R.H. levels .The highest amount of deterioration in the chemical constituents occurred at 90% R.H. after 60 days of incubation thus showed straight relationship between % incidence of fungal growth over the sample material and its quality deterioration.

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