

Storage Specificity for *Boerhavia diffusa* Linn as a Crude Drug

Shadab Ali¹, Amar Singh Kashyap^{2*}, Anuradha Singh¹

¹Department of Biochemistry, School of Basic & Applied Sciences, Galgotias University, Greater Noida, Uttar Pradesh, India

²Department of Botany, Multanimal Modi College, Modinagar, Uttar Pradesh, India

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Abstract The drug plants are inevitably consistent in view of other compositions and the standardization. They are influenced by many factors, such as surrounding environment, harvesting, storage and processing. The packaging also plays an important role in maintaining the quality and efficacy of the herbal drugs. The proper packaging and storage maintain the quality of herbal crude drugs. The storage specificity was studied on *Boerhavia diffusa* Linn. And the effect of fungal infection on stored plant material in different packaging was found. In the study, four different types of packaging were taken to observe the quality and efficacy *i.e.*, polythene, cloths, paper and gunny bag. One unpacked sample was used as a control set. It was a two-year study observed from December 2018 to December 2020. The total number of mycoflora was found on *Boerhavia diffusa* Linn material in variable number in different seasons. In 2019, there were 15 species in April, 24 species in August and 4 species in December. In 2020, there were 3 species in April, 7 species in August and 0 in December. The total number of 33 different fungal species belonging to 15 genera was recorded on all packaging. 12 species belong to *Aspergillus*, 01 *Alternaria*, 01 *Botrytis*, 01 *Botryodiplodia*, 01 to *Curvularia*, 01 to *Colletotrichum*, 03 to *Fusarium*, 02 to *Gilmaniella*, 01 to *Moniliella*, 01 to *Mucor*, 03 to *Penicillium*, 01 to *Paecilomyces*, 03 *Rhizopus*, 01 to *Thelavia* and 01 to *Zygosporium species*. The polythene packaging and cold winter climate were the best for the least microbial attack and minimizing the loss of quality of studied crude drugs.

Keywords Storage, Packaging, *Boerhavia diffusa*

Linn., Crude Drugs, Mycoflora, GMP

1. Introduction

The plants play a pivotal role in the human welfare as they are the source of food, clothing, shelter and medicine. Herbs have been used in all cultures throughout the history. For health care, the medicinal plants have been of a great interest since the beginning of civilization. WHO estimated that 80% world population relies on herbal medicines for the primary health care [7]. It is a major component in all indigenous people's traditional health care systems. Herbs have a higher position in Ayurvedic, Homeopathic, Siddha and other ancient cultures health care systems. Even in the modern systems they have a major role.

There are approximately 250,000 known species of higher plants on our planet and out of these about 35,000 species have been used by people for medicinal purposes. It is estimated that nearly 20,000 species are awaiting discovery which are to extinction. [9, 33]. Most of the crude drugs are obtained from the wild resources. Only a few are cultivated under control conditions. They are normally available in a certain season, location and amount but they can be procured as a raw material throughout the year from store houses and vendors [10]. The changed environmental conditions may bring about drastic alterations in the quality and efficacy of the medicinal plant [26, 27, 34]. Plant habitat is controlled by different environmental factors which may induce a potentially harmful to plant life secondary metabolites. These factors

may cause the microbial attacks. Contamination may cause retardation in growth, defoliation, die back of stems, poor flowering and less fruiting, etc. [11, 16].

Just after harvesting, the medicinal plants have to pass through many steps from collection to formulation e.g., harvesting, drying, transportation, packaging, storage and commerce. During these steps, the plant material has a fair chance to get contaminated and many have deterioration due to pollutants and microorganisms. These conditions may be major threats to decline the quality of crude drug plants [20].

Therefore, the safety measures should be taken in this respect which draws our attention. The drug material has been harvested and collected from the wild as well as cultivated resources by untrained people. They don't have proper training for the job and they don't know how to handle the drugs so that drug materials get contaminated [30]. There should be trained people to collect the crude drugs who could know the quality, maturity of herbs and handling methods. By the proper handling of the medicinal plants during harvesting and post harvesting stages the contamination and infection may be avoided [36].

During the storage period, the crude drugs/ medicinal plants may deteriorate due to fungal attack so the proper storage is the main factor. The proper management of crude drugs in post harvesting conditions requires extra attention. Packaging is an unavoidable step to maintain the quality and efficacy of the drug material [17] during storage condition. In the present study of the effect of mycoflora was estimated on different packaging materials for *Boerhavia diffusa* Linn. The different packaging materials were taken viz. polythene bags, cloth bags, paper bags, gunny bags and open conditions were used for studied crude drugs [12, 14]. It is essential to set the parameters for GMP (Good Manufacturing Practice) and in ISM (Indian System of Medicine) for worldwide acceptance of AYUSH.

Ethnobotanical importance:

Boerhavia diffusa Linn. (Raktapunarnava) is an annual and perennial herbaceous, procumbent and highly diffused creeping plant (Fig 3.a). It shows polycarpic condition.

Ethno-botanically, all parts of this plant are useful as medicine. It has been administered for diverse disease since long time. It is bitter and pungent in taste. According to Indian Ayurveda system it is prescribed in sitaviryam (cold), pittam (caugh), raktam (blood purifier) and in laxative purpose [14].

The leaves are used in loss of digestive power, gulman (ball like movements in the intestine), in enlargement of the spleen. Generally, flower and leaves extracts are used as strong diuretic and in scrotal swelling and body swelling disease due to punarnavine and potassium salt which are present in the plant (300 mg/kg). It helps in the treatment of inflammatory renal diseases and common clinical problems like nephritic syndrome and in various eye

ailments too. The shoot of *Boerhavia diffusa* Linn. has antibacterial, antiviral and antiseptic properties against especially *Staphylococcus aureus* [15].

It is also helpful in bronchitis, cough, abdominal pain and dropsy. It is helpful in emetic, strangury, anti-gastric, Liver problems, jaundice, anti-biliary. It is effective in cases of oedema and ascites resulting from early cirrhosis or liver and chronic peritonitis and in abdominal tumors, piles and cancer [14,15].

It is also useful in anemia, rheumatism, in leucorrhea, in blood increasing and in increasing heart-beat and heart diseases. This is also used in diaphoretic in swelling, aphrodisiac, in scabies and leprosy. It may be useful in snake bite poison, in insect bite and in certain nervous diseases [14].

Objective of the Study

The fungal contamination of medicinal plants during storage may be hazardous to the consumer's health. The product will lose the good quality. The main objective of the present study is to store the medicinal plant in proper packaging. Its temperature, humidity and plants quality should be according to the materials safety. manner which could be free from any type of Microflora contamination. The stored material should be safe and it is available for further procurements.

2. Materials and Methods

The evaluation of the impact of fungal contaminants during storage condition is an important character for herbal based drugs. The samples of *Boerhavia diffusa* plant were subjected to the following steps:

2.1. Collection of Materials

The medicinal plants were collected/harvested at the time of flowering and fruiting during July, 2018. The plant material was collected from the wild area of Modinagar [longitude-77°34'38.76"E (77.577434), latitude-28°50'9.61"N (28.836001)]. About 70kg fresh biomass of harvested plant material was collected from the wild area of Modinagar [longitude-77°34'38.76"E (77.577434), latitude-28°50'9.61"N (28.836001)]. It was carried out to the Laboratory where it was separated from other foreign matter and adulterants (fig 3.a). They were washed with tap water and the material was cut into the small convenient pieces. Whole of the collected material was kept together for further processing. The clean and washed material let be dry for 25 to 30 days at 35°C to 45°C in shade and air. It was kept in sunlight for last 5 days just before packaging so that the Mycofloral infection could be avoided. (fig. 3.b). After sun dried, 1 kg material was filled in each bag and sealed. Before the packaging all the material was uniformly exposed to the open environmental

conditions. Each packaging had consisted constant dried weight of *Boerhavia diffusa* Linn. material *i.e.*, 1 kg.

2.2. Packaging and Storage

After being dried the well processed good quality material was packed in the different packaging manners *i.e.*, in Cloth bags, polythene bags, Paper bags and gunny bags. In each packaging 1 kg plant material was packed. Every type of packaging having seven packets. Each packet contained 1 kg plant material. Thus 7 packets of each packaging were kept uniformly as 7 CP, 7 PLP, 7 PPP and 7 GBP. An amount of 7 kg unpacked material was kept in open condition for control studies. All the packed and unpacked material was kept in the laboratory's store house. The stock material was used at every periodical experimental observation (Dec 2018, April 2019, August 2019, December 2019, April 2020, August 2020 and December 2020).

In the store house all 4 types packed and 1 unpacked material was kept. The store house had the constant environmental conditions. The material was kept at the room temperature (20°C), humidity between 50% to 60% and an absolute pressure of 1 atm (14.696 psi, 101.325 kPa).

2.3. Sample Preparation

The drug samples were aseptically pulverized and 1 gm of each sample was transferred to a dry sterilized measuring cylinder (25 ml capacity). All the samples were taken from each storage conditions. The volume was made up to 25 ml with sterilized phosphate buffer pH 7.2 after which the cylinder was covered with a sterilized glass stopper and manually shaken for about 20 minutes to liberate active, inactive mycelia's spores and propagules present in the samples.

2.4. Preparation of Culture Media Plates

Culture Media- Sabouraud agar, potato dextrose agar and Rose Bengal agar medium used to culture the mycoflora. 20 ml of the prepared sterile culture media was aseptically poured into the three sterilized Petri dishes. The Petri dishes were covered and media were allowed to solidify at room temperature.

2.5. Inoculation & Incubation

1 ml of the prepared media suspension was poured on the surface of the sterilized media. The Petri dishes were shaken gently to disperse the suspension throughout the culture media uniformly, 3 replicates were used for isolation of fungi for each drug sample. Two plates of media were used without drug sample for control. All the plates were incubated in BOD incubator regulate at 20°C to 22°C ($\pm 2^\circ\text{C}$) for 172 hours for incubation.

2.6. Estimation of Mycoflora

During course of time, the samples were analyzed for mycofloral population each after four months' period. A total of 33 fungal species were recorded on all packaging and control, which were belonged to 15 genera (Table 1).

2.7. Observation

The periodical changes in mycofloral population on the drugs samples which were collected from fields and kept in the different storage packaging. They were observed under compound microscope and studied the fungal mycelium and spores in the stored material packaging. We saw the significant effect of the different packaging. The similar studies of fungal load on some medicinal plants were carried out by Roy [24].

Observation took place under microscope at 100X, 400X, and 1000X magnification by using compound light microscope (Olympus CH20i). The colonies which were developed on the glass plates, counted carefully and took photography. The photographs were tally with the literature to determine the species of the infected fungi. The identifications were confirmed by macroscopic as well microscopic analysis by using fungal taxonomic guidelines. The standard procedures and other relevant literature available on fungal systematics [5, 8, 35]. The microphotography of different genera and species were identified during investigation under the microscope (Figure 4). A total of 33 fungal species were recorded on all packaging and control, which were belonged to 15 genera, *i.e.*, *Aspergillus*, *Alternaria*, *Botrytis*, *Botryodiplodia*, *Curvularia*, *Colletotrichum*, *Fusarium*, *Gilmaniella*, *Moniliella*, *Mucor*, *Penicillium*, *Paecilomyces*, *Rhizopus*, *Thelavia* and *Zygosporium* (Table 1).

Table 1. Mycoflora during storage period in all packaging's and periods founds on *Boerhavia diffusa* Linn.

Name of the fungal species	Periodical changes in the mycoflora of the drug <i>Boerhavia diffusa</i> Linn.												
	Unpacked							Cloth Packed					
	2018	2019			2020			2019			2020		
	Dec	Apr	Aug	Dec	Apr	Aug	Dec	Apr	Aug	Dec	Apr	Aug	Dec
<i>Aspergillus amstelodami</i>	-	+	+	-	-	-	-	+	+	+	+	+	-
<i>Aspergillus bruneouniseriatus</i>	-	+	+	-	-	+	-	+	+	+	+	-	-
<i>Aspergillus candidus</i>	-	+	+	-	-	-	-	+	+	+	-	-	-
<i>Aspergillus flavus</i>	-	+	+	+	+	+	-	+	+	+	-	-	-
<i>Aspergillus fonsecaceus</i>	-	-	-	-	-	-	-	+	+	-	-	-	-
<i>Aspergillus fumigatus</i>	+	+	+	-	-	+	-	+	+	+	-	+	-
<i>Aspergillus funiculosus</i>	+	+	+	-	-	-	-	+	+	+	-	-	-
<i>Aspergillus nidulans</i>	-	-	+	-	-	-	-	-	+	+	-	-	-
<i>Aspergillus niger</i>	+	+	+	+	+	+	-	+	+	-	+	+	-
<i>Aspergillus sulphureus</i>	+	-	+	-	-	-	-	+	+	-	-	-	-
<i>Aspergillus sydowii</i>	-	-	+	-	-	-	-	+	+	+	-	-	-
<i>Aspergillus terricola</i>	+	-	+	-	-	+	-	-	+	+	-	+	-
<i>Alternaria alternata</i>	-	+	+	+	-	-	-	+	+	+	+	+	-
<i>Botryodiplodia theobromae</i>	+	+	-	-	-	-	-	+	+	+	+	+	-
<i>Botrytis cinerea</i>	-	-	-	-	-	-	-	-	+	+	-	-	-
<i>Curvularia lunata</i>	-	-	-	-	-	-	-	-	+	+	+	+	-
<i>Colletotrichum capsica</i>	-	-	+	-	-	-	-	-	+	-	-	+	-
<i>Fusarium nivale</i>	-	+	+	-	-	-	-	+	-	-	-	+	-
<i>Fusarium oxysporum</i>	-	-	+	-	-	-	-	-	-	-	-	+	-
<i>Fusarium sp.</i>	-	-	+	-	-	-	-	-	+	+	-	-	-
<i>Gilmaniella humicola</i>	-	-	+	-	-	-	-	-	+	+	-	-	-
<i>Gilmaniella penicillioides</i>	-	-	-	-	-	-	-	-	+	-	-	-	-
<i>Moniliella sp.</i>	-	-	+	-	-	-	-	+	+	+	+	+	-
<i>Mucor sp.</i>	-	+	+	+	+	+	-	+	+	-	+	+	-
<i>Penicillium purpurogenum</i>	-	-	+	-	-	-	-	+	+	-	-	-	-
<i>Penicillium brefeldianum</i>	-	-	+	-	-	-	-	-	+	-	-	+	-
<i>Penicillium citrinum</i>	-	+	-	-	-	-	-	-	-	-	-	-	-
<i>Paecilomyces variotii</i>	-	-	+	-	-	-	-	-	+	-	-	-	-
<i>Rhizopus nigricans</i>	-	+	-	-	-	+	-	-	+	-	-	+	-
<i>Rhizopus oryzae</i>	-	+	-	-	-	-	-	-	+	-	-	-	-
<i>Rhizopus stolonifera</i>	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Thelevia sp.</i>	-	-	+	-	-	-	-	-	-	-	-	-	-
<i>Zygosporium sp.</i>	-	+	+	-	-	-	-	+	-	+	-	-	-
Total number of mycoflora	6	15	24	4	3	7	0	17	27	17	8	14	0

Table 1. Continued

Periodical changes in the mycoflora of the drug <i>Boerhavia diffusa</i> Linn.																	
Polythene Packed						Paper Packed						Gunny Bag Packed					
2019			2020			2019			2020			2019			2020		
Apr	Aug	Dec	Apr	Aug	Dec	Apr	Aug	Dec	Apr	Aug	Dec	Apr	Aug	Dec	Apr	Aug	Dec
-	-	-	-	-	-	+	+	+	-	-	-	+	+	+	+	+	-
-	+	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-
-	+	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	-
-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-
-	+	-	-	-	-	-	+	+	-	+	-	+	+	+	+	+	-
-	+	-	-	-	-	+	+	-	-	-	-	+	+	+	-	+	-
-	+	-	-	-	-	+	-	-	-	+	-	+	+	+	-	+	-
-	+	-	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-
+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
-	+	-	-	-	-	+	+	-	-	+	-	-	+	+	+	+	-
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-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	+	+	-
-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-
-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-
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-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-
-	-	-	-	-	-	+	+	-	-	-	-	+	+	+	+	+	+
-	+	-	-	-	-	+	+	-	-	-	-	+	+	+	+	+	+
-	+	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-
+	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-
-	-	-	-	-	-	+	+	-	-	-	-	+	+	+	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
2	11	0	0	0	0	18	31	16	4	8	1	21	33	24	10	17	3

Table 2. Number of mycoflora during storage period CP–Clothes Packed, PL–Polythene Packed, PP–Paper Packed, GP–Gunny bag Packed, UP–Unpacked/open (Controlled)

Sr. No.	Packaging	Periods					
		2019			2020		
		April	August	December	April	August	December
	UP (Control)	15	24	04	03	07	00
	CP	17	27	17	08	14	00
	PL	02	11	00	00	00	00
	PP	18	31	16	04	08	01
	GP	21	33	24	10	17	03

Table 3. Periodical assessment of total number of mycofloral species

Total Number of Species	Period (Timing)					
	2019			2020		
	April	August	December	April	August	December
	31	16	05	03	11	03

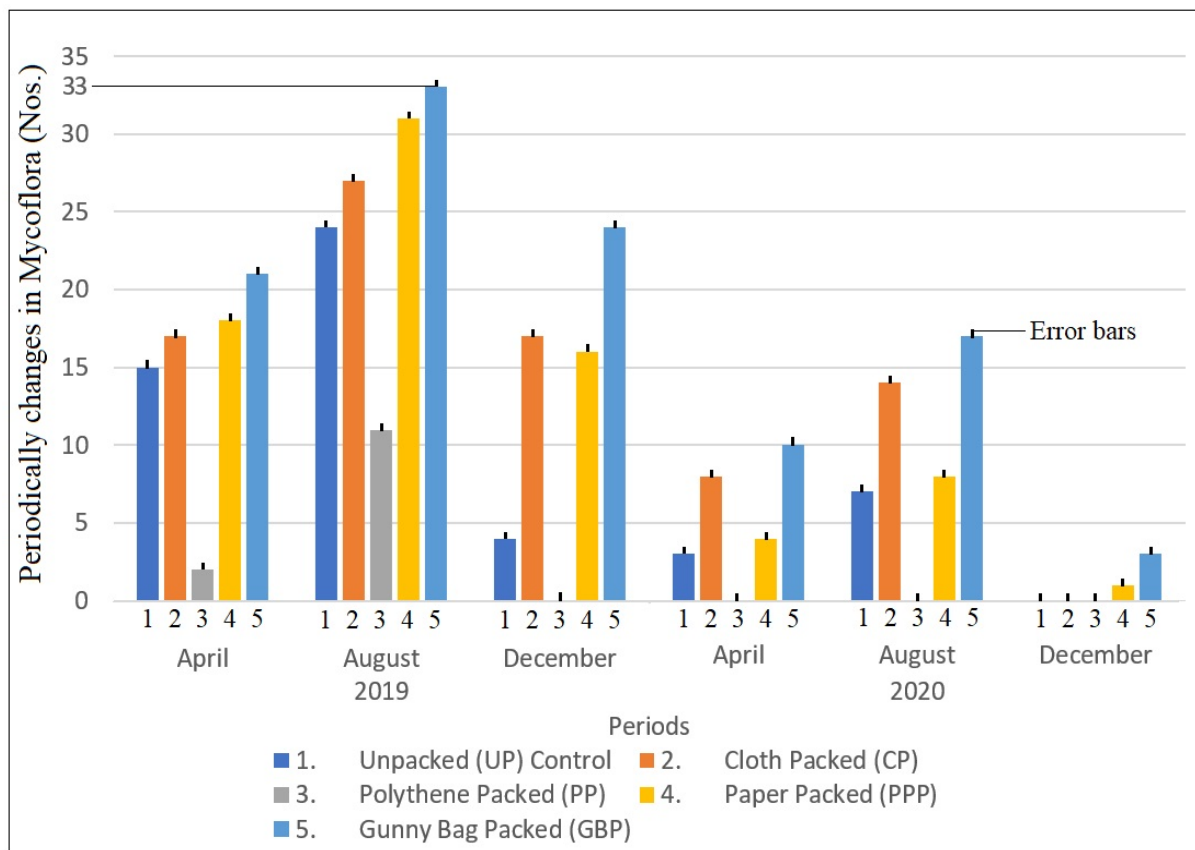


Figure 1. Mycoflora during storage period in all packaging and periods founds on *Boerhavia diffusa* Linn.

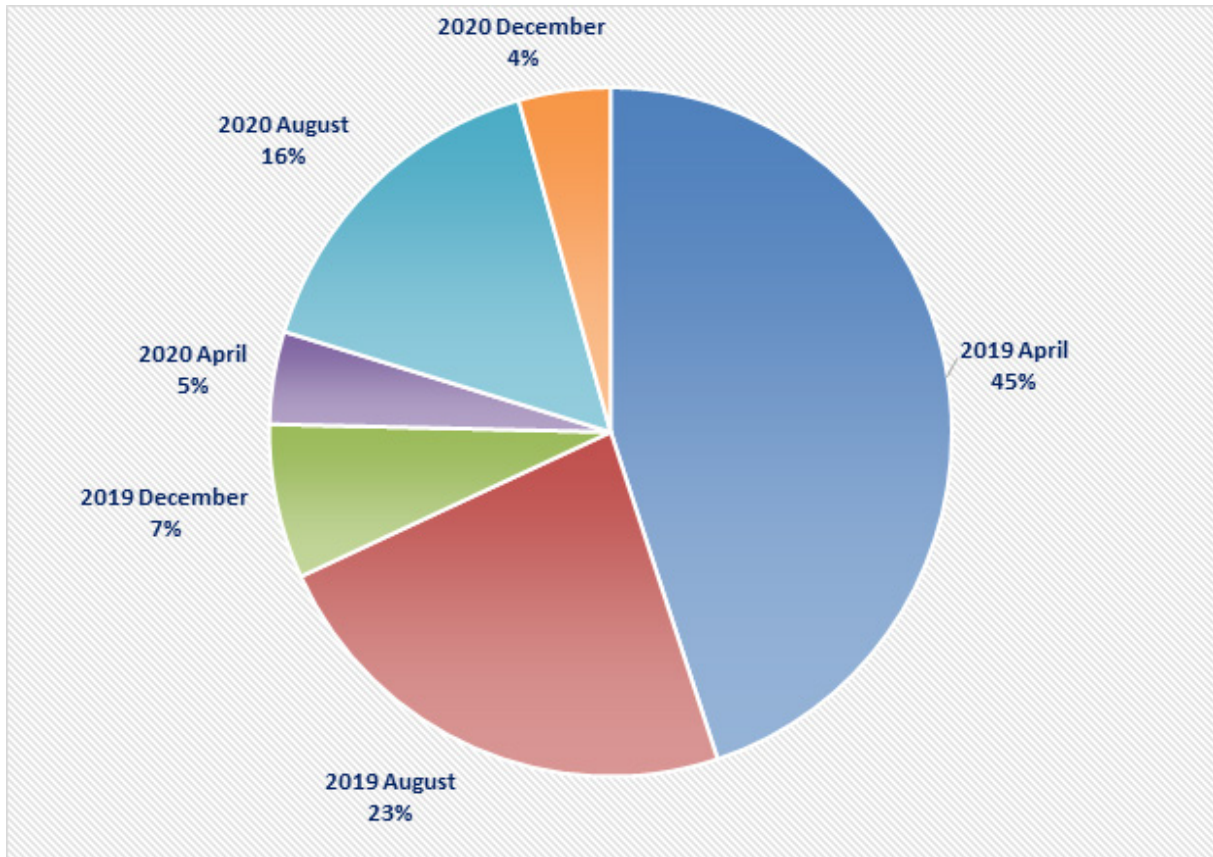


Figure 2. Periodical assessment of total number of mycofloral species



Figure 3. *Boerhavia diffusa* Linn. (a- Fresh Plant, b- Dried Plant)

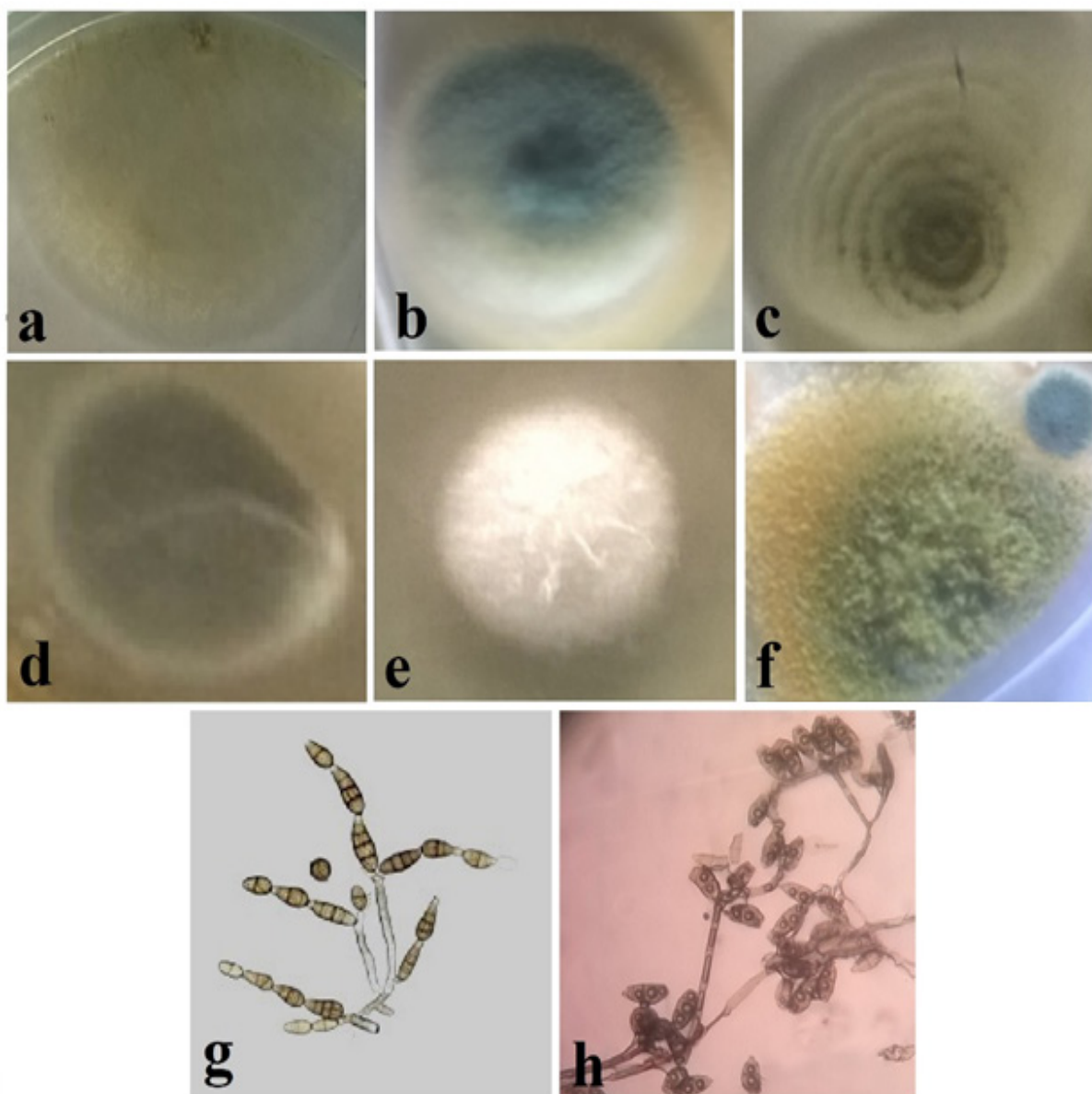


Figure 4. Certain fungi isolated on culture media showed in figures a, b, c, d, e & f, while g and h showing microscopic images of *Alternaria alternata* and *Curvularia lunata* respectively.

3. Result & Discussion

The result of the present study clearly showed that the crude drugs in raw form were attacked by different species of fungi when they were stored in various packaging. Studies on storage specificity of *Boerhavia diffusa* Linn. were taken during December 2018 to 2020 and found out the attacking genera and species of mycoflora (Table 1). Analysis of the drugs and their formulation was made at every four months. The samples of the studied plants material were collected from fields and cut into small pieces, dried and then packed in the different types of packaging viz. clothes, paper, polythene, gunny bags and unpacked (Table 2). After certain period, the stored material in different packaging was seen to have the effect

of mycoflora population. After every four months from unstressed sites. The periodical changes in mycofloral population on the drugs samples which were collected from fields and kept in the different storage packagings (Table 3). The microbial load was observed under microscope and studied quality of storage materials. We saw the significant effect of the different packaging [24]. During course of time, the samples were analyzed for mycofloral population each after four months' period (Figure 1, 2).

A total of 33 fungal species were recorded on all packaging and control, which were belongs to 15 genera, i.e., *Aspergillus*, *Alternaria*, *Botrytis*, *Botryodiplodia*, *Curvularia*, *Colletotrichum*, *Fusarium*, *Gilmaniella*, *Moniliella*, *Mucor*, *Penicillium*, *Paecilomyces*, *Rhizopus*,

Thelavia and *Zygosporium* (Table 1). The total number of mycoflora in 2019 on *Boerhavia diffusa* Linn was found 15 species in April, 24 species in August, 04 species in December while in 2020 there were 03 species in April, 07 species in August and no growth found in December (Table 2). A total number of 33 fungal species were recorded on all packaging and control set which were belonging to 15 genera. Twelve species belong to *Aspergillus*, 01 *Alternaria*, 01 *Botrytis*, 01 *Botryodiplodia*, 01 to *Curvularia*, 01 to *Colletotrichum*, 03 to *Fusarium*, 02 to *Gilmaniella*, 01 to *Moniliella*, 01 to *Mucor*, 03 to *Penicillium*, 01 to *Paecilomyces*, 03 *Rhizopus*, 01 to *Thelavia* and 01 to *Zygosporium species* (Table 1, Figure 4). The polythene packaging and cold winter climate were the best calibration to check the microbial attack to minimizing the least loss of quality of crude drugs (Table 2, 3).

The different storage materials were affected by the pathogens because the packaging material was also effected. The surrounding environment loaded the spores on the open material and perforated packaging. At the room temperature the storage material gets contamination with the spores. The material was contaminated in the available humidity due to packaging materials facilitation. Polythene material was air tight and not allowed any type of spore to entry into it. Therefore, the polythene packaging was more ideal to safe the material from the spores [14].

The effect of Mycoflora during storage was studied, at room temperature on the seeds of *Zinnia by Shrivastava and Gupta* [29]. They found that during the storage period, the fungal colonies increased rapidly at room temperature storage but they decreased at low temperature.

Prashad and Das [23] also reported that two medicinal plants viz. *Terminalia bellirica* and *Terminalia chebula* were infested artificially with three dominant fungi viz. *Aspergillus flavus*, *Curvularia lunata* and *Fusarium moniliforme*.

Roy [24] studied to the medicinal plants and drugs which were threatened by fungi [24]. Roy (1989) has been reported that the efficacy of the medicinal plants depends on active constituents which are altered due to fungi. The fungi play the most vital role in deteriorating drug plant at various stages.

According to Agarwal and Chauhan [1], some food plants were also rotted from microbial attack and showed the effect on hypolipidemic effect. In a study Halder and Gupta [10, 21] found that after a long period of storage of *Sunflower seeds at high and very low relative humidity in solute leaching the essential biochemical*. Due to the deterioration of qualities, it was useless for utility.

Singh *et al.*, [31] found that *Aspergillus* was the most dominant genus causing the infection to raw materials of plant samples and about the 13 species of *Aspergillus* isolated from the raw materials had toxic effects on the plants. Similarly, fungal strains were isolated during the storage of plant material and showed the fungal infection on the medicinal plant material *Embellica officinalis*

Gaertn. by Tajeja in 1982 [32].

The fungal infection was observed on the Solanum leaves during storage period on the Medicinal plants in the different types of packaging by Joshi *et al.*, 1982 [12] and by Khulbe and Bist in 2001 on different Medicinal plants [17]. The decay of bamboo (*Dendrocalamus strictus*) during outside storage degradation of cellulose of the plant was also studied by Chandra and Guha in 1979 [6].

Mishra and Kanaujia in 1973 isolated more diverse microflora from cultivated soil and they found that the *Aspergillus flavus* and *Aspergillus niger* were the most dominant fungi due to cold climate and acidic nature of the soils [19]. The effect of Mycoflora on the storage conditions and viability of *Zinnia* seeds had bad effect [29].

Sharma in 2002 gave the different aspects of collection, processing and storage of different crude drugs so that the GMP in ISM could be maintained [28].

The studies showed that medicinal plants and plant drugs are threatened by fungi. In the present study *Aspergillus sp.* dominantly found in relatively high temperatures, whereas found in relatively high temperatures, whereas species of *Penicillium* dominated at low temperatures. Polythene packaging is more reliable packaging which showed the least number of fungal infection whereas open and cloth pack is unsafe for storage. Rainy season is the high more susceptible for fungal attack on storage medicinal plants. The gunny bag, cloth pack and paper pack are unsafe for storage.

4. Conclusions

Polythene packaging is recommended for safe packaging during storage. All the environmental conditions and post harvesting practices should be ideal.

Declaration

The work was performed in the Department of Botany, M. M. H. (P. G.) College, Ghaziabad later on it was repeated for the correct results in the Department of Botany, M.M. (P. G.) College, Modinagar for updating the work and reality check for storage.

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