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STUDIES ON THE PRESERVATION OF HERBAL MEDICINES BY TREATMENT WITH HYDROGEN PHOSPHIDE

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Preservation of dried herbal medicines was studied by treatment of the material with hydrogen phosphide. Samples of *Ziziphus vulgaris* (Unnab), *Viola odorata* (Banafshah) and *Cordia latifolia* (Sapistan) were treated in a fumigation chamber under an atmosphere of $5x10^{-3}$ g/l of hydrogen phosphide for 24 hours. The treatment effectively killed all the insects and diminished the microbial counts. The general appearance of the samples (i.e. colour and lustre) was also maintained. Treated samples remained insect-free for a considerable time (more than four months) when stored under proper conditions and away from infested samples.

Studies were also carried out to evaluate the possible toxic effects of hydrogen phosphide by fumigating rat diet under identical conditions and feeding the animals with this diet. Pseudo-choline esterase, glutamate pyruvatetransaminase, alkaline phosphatase and bilirubin were determined in the serum. No significant difference was found in the values of these parameters when the control and experimental groups were compared.

These results indicate the suitability of hydrogen phosphide for the preservation of dried herbs from insect-invasion and safety of the material for subsequent use, when applied with proper precautions.

Key words: Medicinal herbs, Preservation, Hydrogen phosphide.

INTRODUCTION

Preservation of stored products from insect invasion is one of the major problems faced by almost all countries. Food and Agriculture Organization (FAO) of the United Nations estimated that the amount of food damaged or destroyed by insect pests each year may be sufficient to feed several hundred million people [1].

In view of the enormity of the problem, a lot of work has been carried out in the world to tackle this situation effectively and a large number of contact insecticides, aerosols and smokes etc. have been developed and used for this purpose. However, these products are active only on the surface and therefore do not completely exterminate the insects [2]. Moreover, they usually remain on the products and present residual toxic effects [3]. Fumigants developed later opened a new avenue for pest control. They are more suitable for the treatment of stored products because they reach otherwise in accessible habitats of the insects. They are also active against all developmental stages of the insects viz. egg, larva, pupa and adult [4]. The fumigants generally used are hydrocyanic acid [5], ethylene oxide [6], methyl formate [7], acrylonitrile [8], methyl bromide [9] and hydrogen phosphide [10]. Among these, hydrogen phosphide has many advantages. It has less residual toxicity than many other fumigants and is nearly inert. It does not react chemically with the foodstuffs. It binds temporarily to the fumigated commodity and leaves it unchanged chemically and physically when absolutely pure [11].

In the present studies, we tested the preservation of some medicinal herbs by hydrogen phosphide treatment and also evaluated the possible residual toxic effects by feeding fumigated diet to rats and estimating serum enzymes likely to be deranged as a result of neuro-and hepatotoxicity.

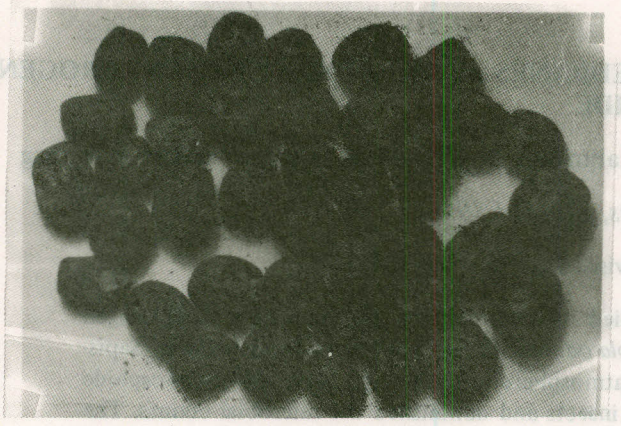
MATERIALS AND METHODS

Following samples were obtained from a local herbal drug factory for studies on their preservation.

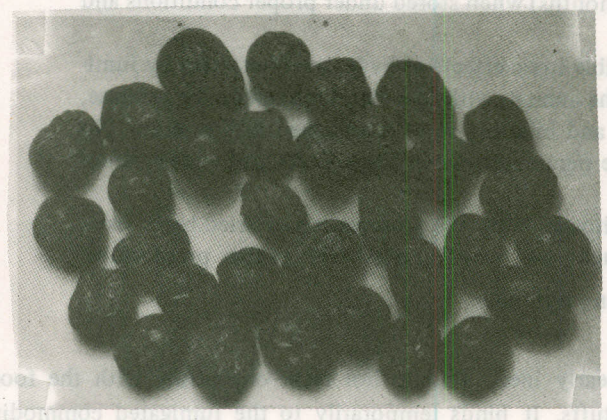
Zizyphus vulgaris (Unnab)
Cordia latifolia (Sapistan)
Viola odorata Linn (Banafshah)

These were infested with a number of insects, fungi and bacteria.

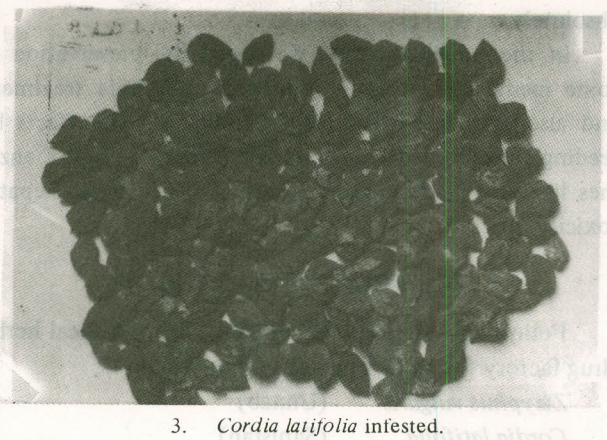
Fumigation treatment. The samples were placed in a wooden fumigation chamber (120 x 68 x 108cm) fitted with an exhaust pump. The herbs were placed on trays and aluminium phosphide tablets were placed in the chamber near the trays. The aluminium phosphide slowly decomposes to release gaseous hydrogen phosphide that diffuses



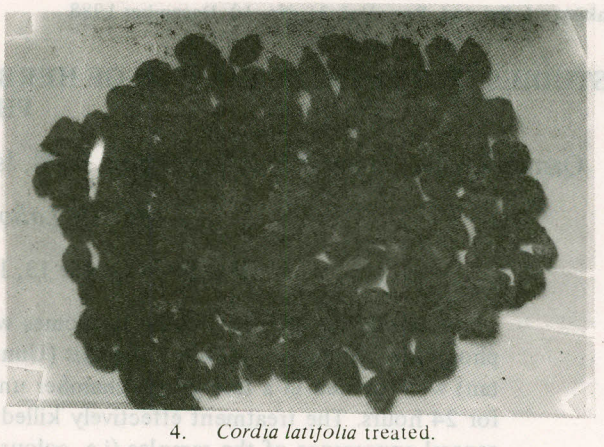
1. *Zizyphus vulgaris* infested.



2. *Zizyphus vulgaris* treated.

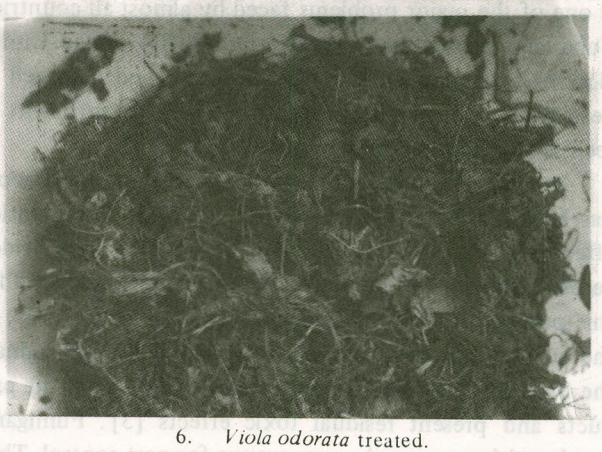


3. *Cordia latifolia* infested.



4. *Cordia latifolia* treated.

5. *Viola odorata* infested.



6. *Viola odorata* treated.

Photographs 1-6. showing comparison between infested and treated samples.

and distributes evenly in the chamber. Each tablet weighed 3g and is reported to release 1gm of gaseous hydrogen phosphide. Five tablets were used for each treatment.

1.5 kg of material was fumigated under an atmosphere of 5×10^{-3} g/litre of hydrogen phosphide gas with an exposure time of 24 hours after which time the gas was completely evacuated by running the exhaust pump for about four hours.

The treated samples were divided into two parts. One set was sealed and stored in a drawer. The other part was stored in open conditions, in close proximity to infested samples. Weekly observations of the samples were made.

The toxic effect of residual phosphine was studied by fumigating a standard rat diet. 12 kg of the diet was prepared. 10 kg out of this preparation was fumigated with a dose of 5×10^{-3} g/litre of hydrogen phosphide in the fumigation chamber. After 24 hours of contact time, the gas was evacuated and the diet was used for feeding the experimental rats. The control group was fed on non-fumigated diet. From the 1st to 3rd day of consumption of fumigated diet, six animals were killed each day and blood samples were taken for analysis. For chronic effect of toxicity, 6 animals were killed each day from the 15th to 17th day of consumption of fumigated diet and blood samples were collected for analysis of the following parameters:

- Pseudocholinesterase
- Glutamate-pyruvate-transaminase
- Alkaline phosphatase
- Bilirubin

These parameters were measured by using commercial diagnostic kits purchased from E. Merck, Darmstadt (West Germany).

Presence of insects and larvae in the samples was ascertained with the naked eye or with the help of a dissecting microscope. For the microbial counts, 1 gm each of the treated and untreated sample was taken in separate conical flasks containing 100 ml of sterile Nutrient broth (E. Merck) and shaken well. From each flask, 0.1 ml of the broth was removed and poured in separate sterile petri dishes, over which 20 ml sterile molten nutrient agar (E. Merck) was poured, and allowed to set. Triplicate sets were made in this manner for each sample and incubated at 37° alongwith the control. The colonies were counted after 24 and 48 hours.

Tests for fungi were similarly undertaken by pouring 20 ml of sterile Sabouraud Glucose 4 % agar (E. Merck) over each sample in petri dish. The plates were poured in triplicate and incubated at room temperature alongwith the control for a period of one week. The colonies were counted after completion of the incubation.

RESULTS AND DISCUSSION

Table 1 shows the type of infestation present in the samples before fumigation. A number of insects, fungi and bacteria were identified. Table 2 shows the counts of the micro-organisms per gram of sample. All three herbs investigated (*Zizyphus vulgaris, Viola odorata* and *Cordia latifolia*) had fairly high counts of micro-organisms. However visual inspection after fumigation showed that almost all the insects were dead and the count of the micro-organisms was also drastically reduced Table 3. This indicates that the treatment effectively killed the insects and

Table 1. Infestation found in the samples before fumigation.

Insects	Fungi	Bacteria
Triboleum confusum	*Aspergilus niger*	*Bacillus cereus*
Lasioderma serricorne	*Paccilomyces* sp.	*Aerobactor aerogenes*
Oryzaephilus mercator	*Trichoderma* sp.	*Aeromonas* sp.
Ephestia cantilla (Larvae)	*Fusarium* sp. (only spores were found)	
Plodia interpunctella (Larvae)		

Table 2. Levels of microbial infestation before fumigation (n = 3)

Herb examined	Bacteria	Count/gm	Fungi	Count/gm
Zizyphus vulgaris	*Bacillus cereus*	7×10^7	*Aspergillus* niger	3×10^3
	Aerobactor aerogenes	6×10^6	*Paccilomyces* sp.	4×10^3
	Aeromonas sp.	9×10^7	*Fusarium* sp.	4×10^3
Viola odorata	*Bacillus cereus*	5×10^7	*Paccilomyces* sp.	1×10^2
	Aerobactor aerogenes	7×10^7	*Trichoderma sp.*	4×10^2
	Aeromonas sp.	7×10^7	*Fusarium* sp.	4×10^3
Cordia latifolia	*Bacillus cereus*	3×10^6	*Aspergillus niger*	1×10^5
	Aerobactor aerogenes	4×10^6	*Paccilomyces* sp.	1×10^2
	Aeromonas sp.	4×10^6	*Trichoderma* sp.	2×10^3

Table 3. Treated but unsealed samples kept with infested samples (results after 2 months) (n = 3)

Sample	Insects	Bacteria	Count/gm	Fungi	Count/gm
Zizyphus vulgaris	*Lasioderma serricorne*	*Bacillus cereus*	5×10^2	*Aspergillus niger*	4
		Aeromonas sp.	5×10^3		
Viola odorata Linn.	*Tribolium confusum*	*Aerobactor aerogenes*	2×10^2	*Trichoderma* sp.	1
		Bacillus cereus	9×10		
Cordia latifolia	*Oryzaephilus mercator*	*Bacillus cereus*	7×10	*Fusarium* sp.	2
	Tribolium confusum	*Aerobactor aerogenes*	1×10^2		

diminished the microbial counts to a very low level. The unsealed samples remained pest free for a period of about two months. However, after two months, those samples which were kept in close proximity to the infested samples were reinfested with insects as well as having increased microbial counts (Table 4). It appears that the primary degradation of the samples was caused by insects which then make the herbs vulnerable to microbial attack. If insects are controlled, samples can be stored for much longer time. Thus samples packed in cellophane bags after fumigation, could be stored for more than four months without appearance of insects.

Table 4. Levels of microbial infestation after fumigation and storage in sealed containers (n = 3).Results after 4 months.

Herb examined	Bacteria	Count/gm	Fungi	Count/gm
Zizyphus vulgaris	*Bacillus cereus*	3 x 10	*Aspergillus niger*	–
	Aerobactor aerogenes	–	*Paccilomyces* sp.	–
	Aeromonas sp.	3	*Fusarium* sp.	–
Viola odorata Linn.	*Bacillus cereus*	2 x 10	*Paccilomyces* sp.	–
	Aerobactor aerogenes	–	*Trichoderma* sp.	–
	Aeromonas sp.	–	*Fusarium* sp.	–
Cordia latifolia	*Bacillus cereus*	–	*Aspergillus niger*	–
	Aerobactor aerogenes	7	*Paccilomyces* sp.	–
	Aeromonas sp.	–	*Trichoderma* sp.	–

Table 5 shows the effect of consumption of fumigated diet by rats on the serum enzymes: choline esterase, alkaline phosphatase, glutamate-pyrurate-transaminase and also on bilirubin. Choline esterase is deranged in cases of brain damage and an increase in alkaline phosphatase, glutamate-pyruvate-transaminase and bilirubin is indicative of liver damage. When compared with the control group, the P value for each parameter was found to be > 0.05. Hence the difference was non-significant, suggesting that feeding of rats on fumigated diet did not cause any change in the brain and liver function. Additionally, no change in the general health and activity of the animals was observed.

Cabrol Telle and co-workers [12] have also studied the effects of long-term feeding of phosphine-fumigated diet to rats and have concluded that it does not cause any marked modification of growth, feed intake, nitrogen balance, body composition, functional behaviour or incidence of tumours. On the basis of these observations it is concluded that treatment of herbs with hydrogen phosphide is safe and is not likely to lead to any toxic effects if proper precautions are taken and degasification is complete. It was also observed that fumigation with hydrogen phosphide did not lead to any change in the appearance of the treated samples in terms of colour, lustre etc. The appearance of the non-fumigated samples was greatly altered due to heavy infestation with insects and microbes.

In Pakistan the traditional system of medicine is still quite popular, particularly in the rural areas. Large amounts of herbs are collected for use within the country and also for export. The problem of harmful infestation of this

Table 5. Table showing the effect of consumption of fumigated diet on rats. (Results are given as mean values ± s.e.m.).

Test	Control group (Normal diet)	Experimental group (fed on fumigated diet)					
		1st day	2nd day	3rd day	15th day	16th day	17th day
Choline esterase (U/ml of serum)	2.43 ± 0.18 (6)	2.72 ± 0.39 (6)	2.77 ± 0.24 (6)	2.87 ± 0.34 (6)	2.72 ± 0.28 (6)	2.82 ± 0.42 (6)	2.45 ± 0.16 (6)
Alkaline phosphatase (U/L of serum)	40.0 ± 7.75 6)	42.0 ± 7.41 (6)	32.0 ± 5.81 (6)	44.2 ± 8.93 (6)	37.1 ± 7.41 (6)	37.7 ± 7.24 (6)	36.8 ± 6.59 (6)
Glutamate-pyrurate-transaminase (U/L of serum)	6.4 ± 1.80 (6)	8.2 ± 2.56 (6)	12.3 ± 2.88 (6)	11.42 ± 1.47 (6)	9.1 ± 1.86 (6)	7.1 ± 1.96 (6)	7.9 ± 2.00 (6)
Bilirubin (mg/dl of serum)	0.41 ± 0.12 (6)	0.55 ± 0.09 (6)	0.53 ± 0.13 (6)	0.34 ± 0.08 (6)	0.49 ± 0.13 (6)	0.68 ± 0.13 (6)	0.72 ± 0.16 (6)

All values are statistically non-significant when compared with the respective control values.

valuable crop can be solved by fumigation with hydrogen phosphide and storage in sealed containers.

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