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RESEARCH ARTICLE

X-Ray Characterisation of Various Aluminium Phases in the Medicinal Herb *Bacopa Monnieri* Affected by Simulated Acid Rain

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ABSTRACT

In the present investigation various aluminium-based new phases formed due to substitution of sulphur *via* simulated acid rain in *Bacopa monnieri* have been analyzed using X-ray diffraction (XRD) technique. So far there is no report on the effects of acid rain on the *B. monnieri* herb and its vital properties like memory-boosting mechanism. Therefore, in the present study, an attempt has been made to analyze the various aluminium phase (salt) formations due to the substitution of sulphur *via* simulated acid rain (SiAR) in *B. Monnieri* because of its toxicological importance. The new phases like $AlH(SO_4)_2$ and Al_2S_3 along with usual Al_2O_3:H_2O, MgO, $FeAl_2(PO_4)_2(OH)_2$:$8H_2O$, ($K_2Ca(SO_4)_2$:H_2O), have been observed in *B. monnieri* when treated with sulphuric-simulated acid rain (S-SiAR) of two different pH (3.39 and 5.45) for 20 weeks. These Al-based new salts formed in the above medicinal herb due to the induction of S-SiAR may cause Alzheimer's disease and induce other abnormities.

KEYWORDS: Sulphuric-simulated acid rain, X-ray diffraction, *Bacopa monnieri*, phases, pH.

INTRODUCTION

Acid rain is a serious environmental problem that has adverse impact on plants, agriculture, forestry, medicinal herbs, animals, structured materials and human health. All living things whether living on land or in the water (aquatic) are affected directly or indirectly by acid rain (AR). AR has an adverse impact on medicinal herbs like other plants. It is because AR induces changes in the cellular biochemistry and physiology of the whole plant. Biological effects of acid deposition on plants [1-3] are numerous and complex, and these include visible symptoms of injury (chlorosis and/or necrosis), invisible effects such as reduced photosynthesis, nutrient loss from leaves, altered water balance, and variation of several enzyme activities. Acid rain is caused largely by emissions of sulphur dioxide (SO_2) and oxides of nitrogen (NO_x). These pollutants originate from human activities, interact with reactants present in the atmosphere and result into acid deposition. Natural rainfall has a pH around 6.0. This is because of the effect of CO_2 in the air which combines with water to form carbonic acid. The effect of this, however, is negligible, as it is neutralised in the soil by alkaline materials like limestone. Rain water is considered to be acidic when its pH is less than 5.6 or when it is lower than that expected for non-polluted rainfall. In this case, the soil cannot neutralise the acidity of the rain water, which varies from place to place. So, acid rain has a wide range of pH values that depend on the location and its environmental conditions. In some places the acidification is so severe that the pH drops to around 4.0. Rare cases have been reported of acid rain having a pH of around 2-2.5. Till date, the lowest pH recorded is 1.7 in the USA [4].

HERB MATERIAL:

Bacopa monnieri (Family: *Scrophulariaceae*; Genus: *Bacopa*; Species: *B. monnieri*; Common name: *Brahmi* (India)) is a medicinal herb. It commonly grows in wet, damp and marshy areas found throughout India, Nepal, Sri Lanka, China, Taiwan, Vietnam, Florida and other southern states of the USA. It has been used in traditional Indian system of medicine, the *Ayurveda*, for the treatment of anxiety, and in improving intellect and memory for several centuries. In addition to memory boosting activity, *B. monnieri* is also used in the indigenous system of medicine for the treatment of cardiac, nervous, respiratory and neuropharmacological disorders like insomnia, insanity, depression, psychosis, epilepsy and stress [5]. It has also been reported to possess anti-inflammatory, analgesic, antipyretic, sedative, free radical scavenging and anti-lipid peroxidative activities. It exhibits potent antioxidant and free radical scavenging properties. Besides, it also possesses anticancer, hepatoprotective, antiulcer, calcium antagonistic, bronchovasodilatory, smooth muscle relaxant and mast cell stabilizing properties. Within this tiny herb are numerous brain-boosting compounds, but the most active ones are bacoside A and bacoside B5. The pharmacological properties of *B. monnieri* were studied extensively and the activities were attributed mainly to the presence of characteristic saponins called "bacosides" [6, 7]. It is also used in rebirthing therapy to accelerate trauma

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release and make continuous breathing easier. *B. monnieri* is a well-known nootropic plant reported for its tranquilizing, sedative, cognitive-enhancing, hepatoprotective and antioxidant action. Again, Kumar *et al.* [8] have reported the presence of various elements like Al, Br, Ca, Cl, Fe, K, Mg, P, etc. in the *B. monnieri* herb. Our interest is focused on Al, whose concentration in this herb is about 1.88 ± 0.86 mg/g [8] and can induce toxicity beyond threshold value. About 70% of the acid in rain is due to the contribution of H_2SO_4 [9, 10]. Therefore, considering the induction of acid rain (sulphuric acid based) on *B. monnieri*, the above Al may be converted to Al salts.
The probable chemical reactions are as follows:

$$2\,Al + 2\,dil.H_2SO_4 \rightarrow 2AlH(SO_4)_2 + H_2\uparrow,$$

$$2\,Al + 3\,dil.H_2SO_4 \rightarrow Al_2(SO_4)_3 + 3H_2\uparrow,$$

$$2\,Al + 3\,dil.H_2SO_4 \rightarrow Al_2S_3 + 6\left(OH\right) + 3O_2\uparrow.$$

Acid rain causes toxic metals to break loose from their natural chemical compounds. The released toxic metals might be absorbed by the drinking water, crops, medicinal herbs, and animals that humans consume. These foods that are consumed could cause nerve damage to children or severe brain damage or even death. Scientists believe that the metal aluminium is suspected to be related to Alzheimer's disease. Perl [11] has shown the "relationship of aluminium to Alzheimer's disease". The Alzheimer's disease is a progressive degenerative brain disease of unknown etiology, characterized by the development of a large number of neurofibrillary tangles and senile plaques in the brain. Although some studies prior to his work had reported [12] the increased amounts of aluminium in the brains of Alzheimer's disease victims, these bulk analysis studies have been difficult to replicate and remain controversial. Finally, in the same paper, Perl discussed the implications of changes in the geochemistry and ecosystem associated with acid rain, and their potential implications to altered risks for aluminum neurotoxicity.

Therefore, in the present study, an attempt has been made to analyze the various aluminium phase (salt) formations due to the substitution of sulphur *via* simulated acid rain (SiAR) in *B. Monnieri* using X-ray diffraction (XRD) technique.

MATERIALS AND METHOD:

MATERIALS AND SAMPLING:

B. monnieri herbs were grown in three identical pots to evaluate the effect of acid on the growth, appearance, and the formation of various crystallographic phases due to sulphuric-simulated acid rain (S-SiAR). Initially, 10 plantlets of around 50 mm length and having 2/3 buds were planted in each of the three pots and herbs were waterred regularly using normal water for 2 weeks. After that, the total planted pots were divided into three groups, viz-one for the treatment with normal water (pH = 6.29-6.85) and the other two with acidic (H_2SO_4) solution of two different pH value (3.39 ± 0.02 and 5.45± 0.01) in controlled amount (400 ml) for 20 weeks. Stems with leaves of the above herb samples were collected for investigation. Because of the medicinal-importance of the plant materials as such, no chemical path or physical process was followed for the processing of plant materials. So, the herb materials were sun dried and crushed into powder. Then, they were thoroughly grinded by using agate mortar and pressed into pellets of 10 mm diameter in a KBr press for X-ray diffraction studies.

INSTRUMENTATION:

X-ray diffraction patterns were recorded using a high-resolution X-ray powder diffractometer (Bragg-Brentano geometry, X'Pert-MPD, PANalytical, The Netherlands) with a step size of 0.01° on a 5°-90° range with a scanning rate of 2°/min. A line focus and collimated CuK_α-radiation from an X-ray tube operated at 40 kV and 30 mA was passed through a fixed divergence slit of 1° and a mask (10 mm) before getting it diffracted from the plant sample. Then, the diffracted beam from the sample was well collimated by passing it through a programmable anti-scattering slit of 1°, receiving slit of 2 mm and Soller slit before getting it reflected by the graphite monochromator. Experimental data such as different crystallographic phases, *d* spacing, and intensity were calculated using X'Pert Graphics & Identify software (1999) [13].

RESULTS AND DISCUSSION:

The main composition of the *B. Monnieri* herb (Fig.1) material is cellulose $(C_6H_{10}O_5)_n$. The X-ray diffraction pattern showing the superimposed halo indicates that the fibrous (cellulose) material is semi-crystalline in nature. Usually, cellulose possesses monoclinic crystal structure. The crystalline peaks of the herb cellulose are 101, $10\bar{1}$ and 002 as shown in Fig. 2(a). Again, the two-phase morphology of crystalline (ordered) and amorphous (disordered) regions is highly influenced by the chemical activity of fibers [14]. The broad halo at about $2\theta \approx 35^0$, just after these crystalline peaks, indicates that a part of the fibrous material is amorphous (having very small crystallites size) in nature. There are only few reports on the crystallographic phase analysis of pharmaceuticals and medicinal plants [15-17]. The X-ray powder diffraction study of plant materials shows various crystallographic phases, which are formed due to the presence of multiple-elements in it. However, it is practically difficult to observe these crystallographic phases in the fibrous materials because of : (i) the contents of the multiple-elements are low (mg/g), so they possess low-intense X-ray diffraction peak, and (ii) the fibrous materials usually possess high background intensity that creates problems to observe these low intense peaks. So, with a special care and using high resolution X-ray diffraction system, it is possible to observe these low intense diffraction peaks. X-ray powder diffraction study of the above plant materials shows various crystallographic phases, which are formed due to the presence of multiple-elements in *B. monnieri*. The smoothening diffraction profile of the In *vivo*-grown *B. monnieri* (outdoor plant of same species) is shown in Fig. 2(a). In the present study, phase identification was carried out with PCPDFWIN, V 1.3 (1999) package, using PDF2 reference patterns database [18]. The crystallographic phases observed from the *B. monnieri* herb grown in normal habitat are Paravauxite ($FeAl_2(PO_4)_2(OH)_2{:}8H_2O$) (JCPDS-ICDD, 14-0247), Syngenite, syn ($K_2Ca(SO_4)_2{:}H_2O$) (JCPDS-ICDD, 28-0739), Bohmite ($Al_2O_3{:}H_2O$) (JCPDS-ICDD, 01-1283), Periclase, syn (MgO) (JCPDS-ICDD, 43-1022), etc. The various peak parameters like interplaner spacing d (Å), relative intensity I_r (%), peak intensity I_p in counts/second (cps), and background intensity I_b (cps), with the statistical significance α of the smoothen profile are tabulated in Table 1. The herbs grown in S-SiAR environment possess a few new phases along with the above phases (Fig. 2(a)) and are shown in Fig. 2(b) and Fig. 2(c) respectively. Since our interest is on the aluminum phases formed due the effect of acid rain, as discussed earlier, the present paper has concentrated on the Al + S phases only. The herbs grown in high-acidic environment (pH = 3.39) show more distinct new phases, *viz* a aluminum hydrogen sulphate ($AlH(SO_4)_2$) (JCPDS-ICDD, 37-0697), aluminum sulphate ($Al_2(SO_4)_3$) (JCPDS-ICDD, 42-1410) and aluminum sulphiide (Al_2S_3) (JCPDS-ICDD, 37-0697) as shown in Fig. 2 (b). The number of diffraction peaks of the above phases has decreased in case of herbs grown in the less acidic medium (pH = 5.45). The Al + S phases observed in case of the *B. Monnieri* (grown in less acidic medium) become less prominent except for the MgO phase as shown in Fig. 2(c). In this case, a more prominent MgO phase observed, which indicates that more crystallization takes place in the less acidic medium. This may be due to the easy detachment of Mg from the chlorophyll in a low acidic medium and synthesis of more MgO molecules because of low electron affinity of the Mg (<0). The peak intensity, I_p (subtracting background) of this MgO phase degrades (1/6) part in highly acidic medium (pH = 3.39) and is given in Table 2. Again, the peak of $AlH(SO_4)_2$ (2θ=35.42°, d = 2.5304 Å) and MgO (2θ = 42.28°, d = 2.1358Å) of S-SiAR-affected *B. Monnieri* (grown in relatively large acidic medium) splits into two peaks showing diphase behavior. Other peaks like $Al_2O_3{:}H_2O$ phase vanish due to acidic effect. The corrected peak (background correction) intensity I_p, slightly increases in the herb grown in AR of lower pH (more acidic) value. In XRD profile, the 100% relative intensity (I_r) peaks play an important role in understanding the structure of the materials. The plot, Fig. 3(a), of pH vs. $(I_p)_{avg}$ of most prominent and highly resolved phase of $K_2Ca(SO_4)_2{:}H_2O$ shows a slight increase of its peak intensity with acidic concentration. Also, it is observed that the background intensities (I_b) increase with doping concentration of H_2SO_4 *via* simulated acid rain. Again, plot of pH vs. $(I_b)_{avg}$, as in Fig. 3(b), shows that the background intensity of the pattern increases in the herb sample treated with high-acidic AR. As reported by Makinson *et al.* [19], the experimental background intensities (I_b) increase with high doping concentration. This increase in I_b is related to the concentration of vacancies as predicted by the Laue formula [20], $I_b = C_a C_v (F_a - F_v)$, where C and F are the concentrations and atomic scattering factors, respectively, of the atoms (a) and vacancies (v). Since the scattering factor of the vacancies is zero, the above relation can be written as, $I_b = C_a C_v F_a$. This simplified relation shows that the background intensity is directly proportional to concentration of the additive atom. The X-ray diffraction patterns (Fig. 2a) show that the I_b increases with the doping concentration of S ion (in H_2SO_4). This increase in I_b is because of the breaking of metal complexes due to acidic effect of the S-SiAR. It may be suspected that the above aluminium salts formed due to S-SiAR can cause protein misfolding in the brains, hence cause Alzheimer's disease

CONCLUSION:

The purpose of this work is to determine the effect of sulphuric acid *via* simulated acid rain (SiAR) in *B. monnieri*. It has been confirmed from the X-ray diffraction analysis that various aluminium salts (phases) were formed in the different body parts of *B. monnieri* herb grown in the acid rainfall area. Because of acid rain, a medicinal herb like *B. monnieri* may lose its vital properties like memory-boosting mechanism. The pollutants of acid rain cause the formation of aluminium salts in the different parts of *B. monnieri* herb. Therefore, these Al-salts formed due to S-SiAR may lead to protein misfolding in the brain and cause Alzheimer's disease.

Figure No. 1 Naturally Occurring *Bacopa Monnieri* (*B. Monnieri*) Herb in the Designed Botanical Garden.

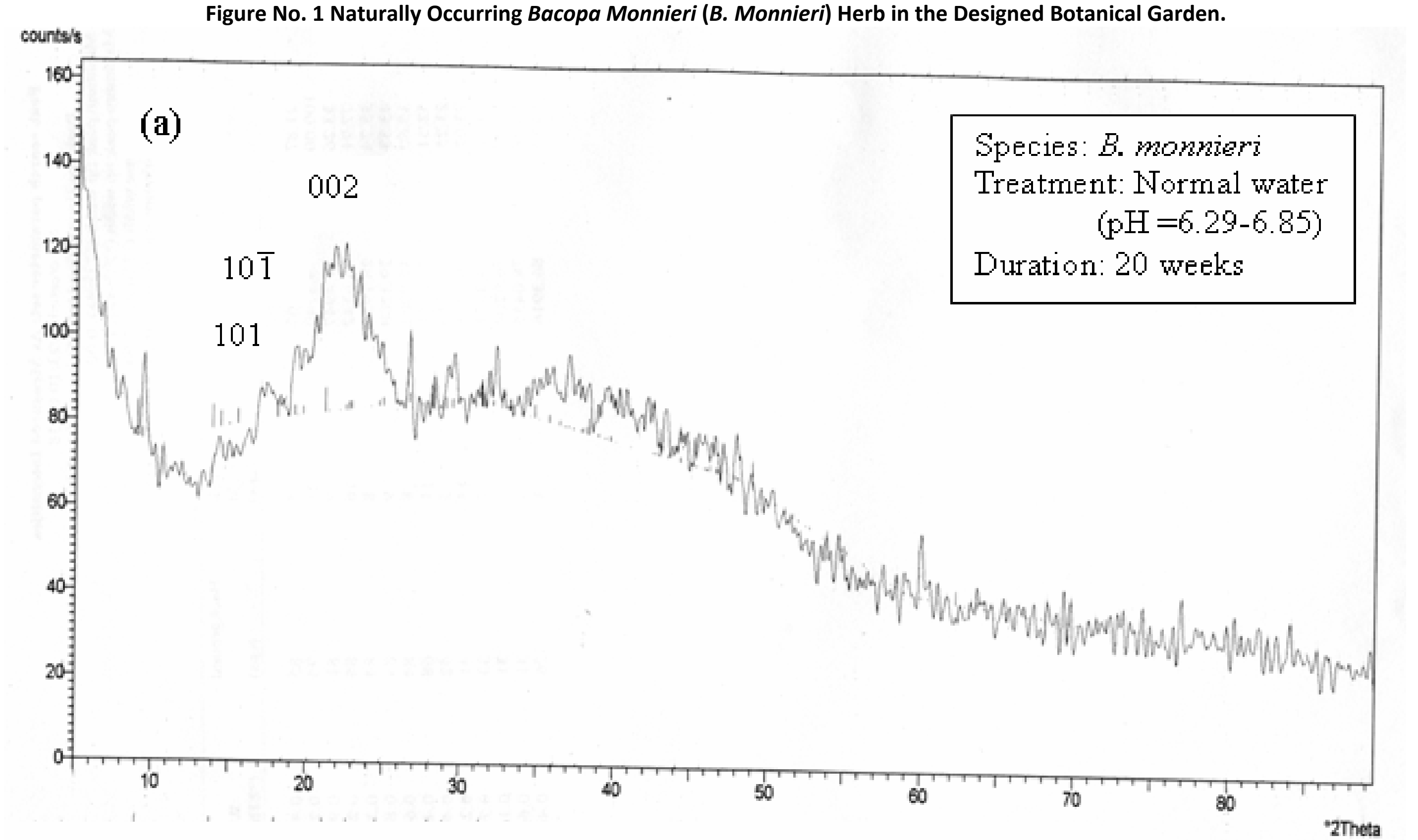

Figure No. 2 XRD spectrum of *Bacopa monnieri* : (a) the plant materials (stem and leaves) of *vivo*-grown *B. Monnieri* ; and sulphuric-simulated acid rain affected

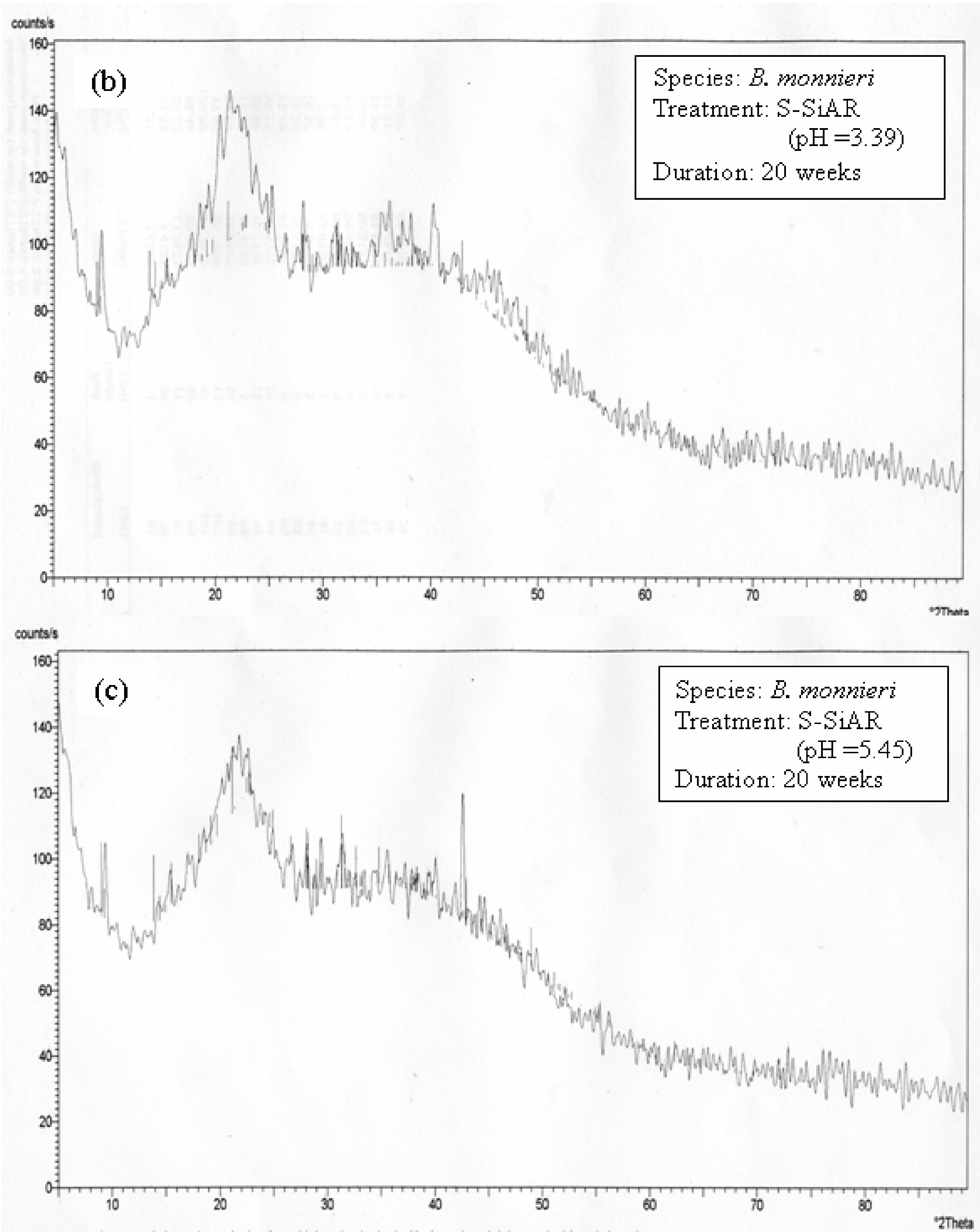

Figure No. 2 XRD spectrum of *Bacopa monnieri*: (b) *S*-SiAR of pH 3.39, and (c) S-SiAR of pH 5.45.

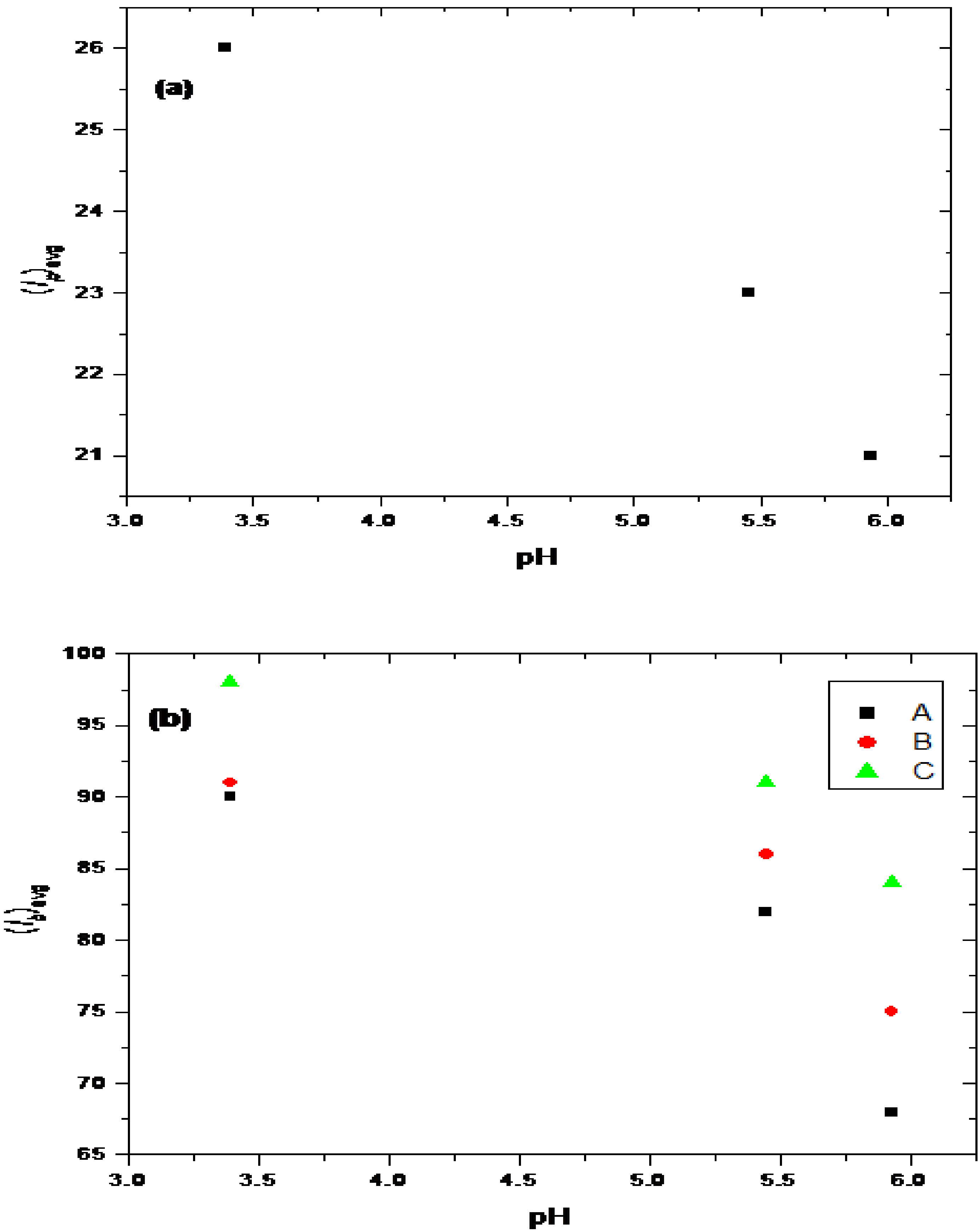

Figure No. 3: Effect on X-ray diffraction intensity of the pH of simulated acid rain: (A) pH vs. $(I_p)_{avg}$ of the $K_2Ca(SO_4)_2{:}H_2O$ phase, and (B) pH vs. $(I_b)_{avg}$ of MgO (A: square), $K_2Ca(SO_4)_2{:}H_2O$ (B: circle), $FeAl_2(PO_4)_2(OH)_2{:}8H_2O$ (C: Triangle).

Sr. No	2θ (deg.)	*d*-value (Å)	I_r (%)	I_p (cps)	I_b (cps)	phase	α
1	9.38	9.4258	100	21	75	$K_2Ca(SO_4)_2{:}H_2O$	0.16
2	14.30	6.1906	16	6	67	$(C_6H_{10}O_5)_n$	0.21
3	17.50	5.0651	33	7	81	$(C_6H_{10}O_5)_n$	0.19
4	22.57	3.9358	30	10	115	$(C_6H_{10}O_5)_n$	0.21
5	26.61	3.3466	78	16	85	$K_2Ca(SO_4)_2{:}H_2O$	0.16
6	28.19	3.1626	36	8	84	$FeAl_2(PO_4)_2(OH)_2{:}8H_2O$	0.17
7	29.33	3.0430	43	9	84	$K_2Ca(SO_4)_2{:}H_2O$	0.43
8	32.10	2.7862	36	8	84	$K_2Ca(SO_4)_2{:}H_2O$	0.21
9	42.57	2.1219	18	8	74	MgO	0.35
10	47.89	1.8981	53	11	68	$Al_2O_3{:}H_2O$	0.32
11	58.95	1.5654	23	5	42	$K_2Ca(SO_4)_2{:}H_2O$	0.28

Table No.1: X-ray crystallographic phases of in *vivo*-grown *B. Monnieri*

Symbols used: *d* is the interplaner spacing, I_r is the relative intensity in %, I_p is the peak intensity in counts/second (cps), I_b is the background intensity in cps, and α is the significance.

Sr.No	Phase	*pH=3.39*					*pH=5.45*				
		2θ (deg.)	*d* (Å)	I_p (cps)	I_b (cps)	α	2θ (deg.)	*d* (Å)	I_p (cps)	I_b (cps)	α
1	$FeAl_2(PO_4)_2(OH)_2{:}8H_2O$ (Paravauxite)	17.77	4.9885	11	93	0.19	17.15	5.1661	12	90	0.38
		24.50	3.6304	7	108	0.24	-	-	-	-	-
		28.25	3.1565	16	92	0.40	28.09	3.1741	14	91	0.24
2	$AlH(SO_4)_2$ (Aluminum Hydrogen Sulphate)	14.42	6.1374	11	78	0.32	14.30	6.1906	6	81	0.21
		18.85	4.7038	18	96	0.20	-	-	-	-	-
		20.84	4.2589	40	99	0.22	-	-	-	-	-
		23.72	3.7481	17	107	0.26	23.60	3.7667	16	102	0.41
		35.42	2.5304	20	96	0.28	35.56	2.5225	10	93	0.20
3	$Al_2(SO_4)_3$ (Aluminum sulphate)	15.23	5.8127	10	96	0.21	15.39	5.7513	11	86	0.18
		21.13	4.2011	45	101	0.20	-	-	-	-	-
		40.31	2.2357	15	94	0.22	40.06	2.2488	12	88	0.19
4	Al_2S_3 (Aluminum Sulphide)	25.58	3.4795	12	107	0.16	25.34	3.5119	8	102	0.32
		31.50	2.8378	11	93	0.19	31.37	2.8497	17	91	0.20

Table No: 2 Identified crystallographic phases of Aluminium in S-SiAR affected *B. Monnieri* using X-ray diffraction

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REFERENCES:

1. V. Velikova, I. Yordanov, and A. Edreva, *Oxidative stress and some antioxidant systems in acid rain-treated bean plants Protective role of exogenous polyamines*, Plant Sci. 151 (2000), pp. 59 - 66.

2. A. Wyrwicka, and M. Sklodowska, *Influence of repeated acid rain treatment on antioxidative enzyme activities and on lipid peroxidation in cucumber leaves*, Environ. Exp. Bot. 56 (2006), pp. 198 - 204.

3. Y. Lee, J. Park, K. Im, K. Kim, J. Lee, K. Lee, J. A. Park, T. K. Lee, D. S. Park, J. S. Yang, D. Kim, and S. Lee, *Plant, Arabidopsis leaf necrosis caused by simulated acid rain is related to the salicylic acid signaling pathway*, Plant Physiol. Biochem. 44 (2006), pp. 38 - 42.

4. L. Taiz, and E. Zeiger, *Plant Physiology,* Sinauer Associates, Inc., Sunderland, MA,1998.

5. K. Anbarasi, K. E. Sabitha, and C. S. S. Devi, *Lactate dehydrogenase isoenzyme patterns upon chronic exposure* *to cigarette smoke: Protective effect of bacoside A. Environ. Toxicol. Pharmacol*, 20 (2005), pp. 345 - 350.

6. O. Prakash, G. N. Singh, R. M. Singh, S. C. Mathur, M. Bajpai, S. Yadav, *Determination of bacoside A by HPTLC in Bacopa monnieri extract*, Int. J. Green Pharma. 2 (2008), pp. 173-175.

7. M. Deepak, and A. Amit, *The need for establishing the identities of bacoside A and B, the putative major bioactive saponins of Indian medicinal plant Bacopa monnieri*, Phytomedicine 11 (2004), pp. 264 -268.

8. A. Kumar, A. G. C. Nayr, A. V. R. Reddy, and A. N. Garg, *Analysis of essential elements in Pragya-peya-a herbal drink and its constituents by neutron activation*, J. Pharm. Biomed. Anal. 37 (2005), pp. 631-638.

9. T. Shvetsova, J. Mwesigwa, A. Labady, S. Kelly, D'Jahna Thomas, K. Lewis, and A. G. Volkov, *Soybean electrophysiology: effects of acid rain,* Plant. Sci. 162 (2002), pp. 723-731.

10. A. G. Volkov, and J. Mwesigwa, *Interfacial electrical phenomena in green plants: Action potentials*, A. G. Volkov, ed., Marcel Dekker, NewYork, 2001, pp. 649-681.

11. D. P. Perl, *Relationship of Aluminum to Alzheimer's Disease*, Environ. Health Perspect. 63 (1985), pp. 149-153.

12. R. D. Terry, and C .Penâ, *Experimental production of neuro-fibrillary degeneration*, *2(Electron microscopy, phosphatase histochemistry and electron probe analysis),* J. Neuropath. Exptl. Neurol. 24 (1965), pp. 200-210.

13. *Graphics & Identify Software*, PANalytical X-ray, The Netherlands, 1999.

14. D. Ciolacu, *On the supramolecular structure of cellulose allomorphs after enzymatic degradation*, J. Optoelectron. Adv. M. 9(2007), pp. 1033-1037.

15. X.-h Gao, L-h Guo, and H. Li, *XRD Fingerprint and Digital Characteristics of Rhizoma gastrodia*, Nat. Prod. Res. Dev. 17 (2005), pp. 42 - 46.

16. B. Mahitha, B. D. P. Raju, G. R. Dillip, C. M. Reddy, K. Mallikarjuna, L. Manoj, S. Priyanka, K. J. Rao, and N. J. Sushma, *Biosynthesis, Characterisation and antimicrobial studies of AgNPs extract from Bacopa monniera whole plants*, Digest. J. Nano. Biostr. 6 (2011), pp. 135-142.

17. N. V. Y. Scarlett, I. C. Madsen, L. M. D. Cranswick, T. Lwin, E. Groleau, G. Stephenson, M. Aylmore, and N. Agron-Olshina, *Outcomes of the International Union of Crystallography Commission on Powder Diffraction Round Robin on Quantitative Phase Analysis: samples 2, 3, 4, synthetic bauxite, natural granodiorite and pharmaceuticals*, J. Appl. Cryst. 35 (2002), pp. 383-400.

18. *CPDFWIN, V 1.3*, JCPDS-ICDD Powder Diffraction Files, International Center for Diffraction Data, Newtown Square, PA, USA 1997.

19. J. D. Makinson, J. S. Lee, S. H. Magner, R. J. De Angelis, W. N. Weins, and A. S. Hieronymus, *Advances in X-ray Analysis*, JCPDS-ICDD, vol. 42 , 2000.

[20] A. Guinier, *X-ray Diffraction,* Freeman and Company, San Francisco, 1963.

20. S. C. Bondy, and A. Truong, *Potentiation of beta-folding of β-amyloid peptide 25-35 by aluminum salts*, Neurosci. Lett. 267 (1999), pp. 25-28.