ORIGINAL ARTICLE

The Effect of Spraying Haltite and Bokashi and the Extraction Method on the Medicinally Active Substances of the Aloe Vera Plant

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ABSTRACT

Introduction: This research was conducted in one of the private nurseries to study the effect of spraying several concentrations of haltite and Bokashi and the method of extraction in the medically effective compounds in Aloevera. The factors were (control and add a Bokashi), 4 concentrations of haltite (0, 25, 50 and 75mg.$^{L-1}$), while the third factor was the use of two extraction methods (aqueous and alcoholic). **Methods:** The plants were sprayed after one month of cultivation with four times, (R.B.D.C.) with four replicates was used. Each experimental unit contained three pots with one plant per pot. **Results:** The results showed that the factors caused significant effect, and the interaction of Bokashi with haltite at 50 mg.L^{-1} for the alcohol extraction method gave the highest rate of leaf content Anthronol and Sinapic acid reached to 121.06 and 97.42 mcg^{-1} respectively. Whereas the interaction of Bokashi with haltite at 75 mg.L^{-1} for the alcohol extraction achieved the highest rate of leaf content of Aloin acid reached to (250.6 mcg.g^{-1}), while the interaction of Bokashi with haltite at 50 mg.L^{-1} for the method of water extraction achieved the highest rate of leaf content of Cinnamic acid reached to (130.19 mcg.g^{-1}). **Conclusion:** The present study showed that the spraying the plants with several concentrations of halite and Bokashi were significantly superior compared to non-addition treatment and recorded the highest rate.

Keywords: Bokashi, Haltite, Extraction method.

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INTRODUCTION

Aloevera, is a succulent plant belonging to the Asphodelaceae family with seated leaves lubricated, with a pointed or thorny tip, white or greenish reddish flowers interspersed with yellow or orange, grouped in inflorescences carried on the stem. The plant was propagated by seeds and off shoots [1, 2]. It is one of the desert plants, and in its inner leaf tissue a substance in the form of viscous gum, which enables it to live in the desert dry areas [3]. The medically used portions of Aloevera are thick, greasy leaves to extract gel containing Anthraquinons [4]. Biological fertilizers are defined as the biomass resulting from the multiplication of microorganisms added to the soil in order to exploit its vital activity in supplying plants with a portion of their different nutritional needs that increasing plant production [5]. Several studies also showed that the use of plant extracts had a similar effect of growth regulators in improving plants growth and increasing the concentration of medically active substances by their ability to increase the formation of such compounds [6]. The Ferula of haltite (Fwrula assafoetida) herb powder has an important role in providing plants with many important food compounds and essential mineral elements that are mainly composed of carbohydrates and proteins that are present in a high rate, as well as it contains a percentage of vitamins such as carotene, riboflavin, niacin and a group of food minerals such as Calcium, magnesium, potassium, and sodium [7]. Due to the lack of studies related to the effect of this type of extracts and the organic bio-mixture with the method of extraction, the research was done to study the possibility of increasing growth indicators and medically effective compounds for the Aloevera.

MATERIALS AND METHODS

The research was conducted in one of the private nurseries from 12/9/2018 to 4/25/2019 to studying the effect of spraying with powder in several levels and adding organic biological fertilization represented by the Bokashi and the method of extraction in the medicinally effective compounds of the Aloevera plant. 6 month old plants obtained from a farmer were planted in plastic pots, 10kg filled with mixture soil. After that, the plants were sprayed with four concentrations of haltite powder (0, 25, 50 and 75ml.L^{-1}) after one month of planting. Four sprayings was done (two in autumn and the other two during spray during the early morning) with a 3 liter dorsal sprinkler. Bokashi was prepared in four steps (collecting the components of the mixture, mixing and stirring and adding the biological fertilizer, fermentation and incubation) about six months before carrying out the experiment. Homogeneous mixture on a thick piece of fabric, then add a mixture of distilled water and bio-formula EM1 with a quantity of molasses (brought from a local sugar factory) in a volumetric ratio (1:100:1) gradually added with stirring, continuous spraying and homogeneous mixing for the components of the mixture until reaching a humidity of 30-40%. The mixture became dough at that time with the addition of (150g) of the antibiotic to treat cases of rot that may occur during the fermentation process, then sterilization was done by exposing it to direct fire. Then it was placed in black polyethylene bags, closed tightly and placed in a dark and warm place for the purpose of increasing the speed of decomposition for about two months, with stirring the mixture every 10days. Then putting in tightly closed drum and when it starts to notice white growths on the surface of the mixture, this means that the mixture is ready for use [8].

Extraction methods and preparation
First/ alcoholic extract

Alcoholic extract prepared by accidentally cutting plant leaves into small pieces with removing the peel from the leaves and taking the gel and then drying it by grinding the dried gel by the ceramic mortar. 10g of crushed dry gel was taken and placing it in thimble , then adding 250ml of ethanol in a circular glass beaker, then placed in the sexolate for 6 hours at 80 °C, then passed the model using the Watman filter paper (0.45µm), then filtered by (0.22µm) Milipore Syringe , then focused by the Rotary evaporator until a thick extract was obtained quite then the form is moved to dry the freezer to turn it into powder for the purpose of measuring laboratory tests [9].

Second/ aqueous extract: The extraction method was conducted according to the method of [10]. 100 ml of distilled water was added to about 20g of leaf powder in a glass jar with continuous stirring and left for 7days after which it was filtered with filter papers and the filtrate was concentrated by rotary evaporator until obtaining very thick extract by evaporating as much of the solvent as possible. Then preparing the final extract by dissolving 0.4gm in 1ml of sterile distilled water, placing it in opaque glass vials, then leaving in the refrigerator until use. At the end of the research experiment, the leaf content of medically active substances was studied.

Estimating medically effective compounds

Medicinal active compounds were measured in the material analysis laboratories of the Ministry of Science and Technology, as 0.01gm of the standard material was taken for high-purity measured compounds individually and dissolved with high-purity methanol separately and completed the volume for each substance measured in a volume vial to 250 ml (40mg.L-1). Then take 1ml of the standard solution prepared for each substance and putting in a 10ml volume vial and complete the volume up to the mark using the ultrapure methanol so the new concentration became 4mg.L-1 and then transferred to the HPLC device [11]. As shown in the following Fig(1) that state the standard solution for HPLC detecting medicinally effective Compounds for Aloe Vera plant measured in research experience.

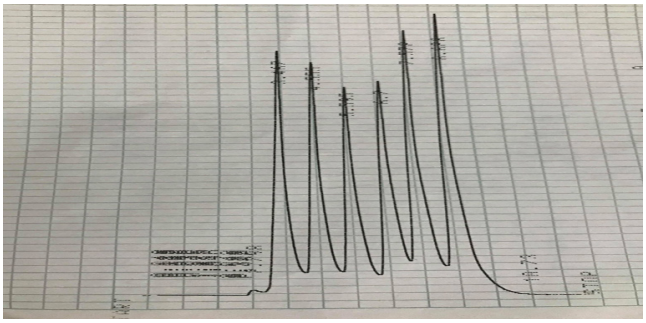

Fig 1: The standard solution for HPLC detecting medicinally effective Compounds for Aloe Vera plant measured in research experience

High-performance liquid chromatography (HPLC) method for detecting clinically effective compounds: The studied models were loaded using a high-performance liquid chromatography device (HPLC model SYKNM German origin with a pump model 1122S and an automatic injector 5200S as the vehicles were detected using the S3210 UV/VIS UV detector. The following vehicles were estimated: (Cinnamic acid, Aloin, Anthronol and Sinapic acid).

RESULT

The leaf content of Anthronol, Aloin, Sinapic, Cinnamic: Table I showed that the factors caused significant effect on the content Anthronol compound in the Aloevera leaves. Several concentrations of herbaceous herb caused a significant increase and the concentration of 50 and 75 were superior compared to control and 25mcg.g^{-1} and achieved the highest rate of 111.73 and 110.72mcg.g^{-1}, respectively. While the control treatment recorded the lowest rate of 99.59.

Table (I) : Effect of spraying haltite and Bokashi and extraction method on leaf content of Anthronol (mcg.g^{-1})

Bokashi× Extraction	Haltite				Extraction way	Bokashi
	75	50	25	0.00		
99.45	101.01	100.83	100.82	93.45	aqueous	Non addition
110.97	115.31	116.23	109.25	103.11	alcoholic	
102.57	106.67	108.82	100.45	94.33	aqueous	addition
116.46	119.90	121.06	117.42	107.45	alcoholic	
3.337	L.S.D. 0.05				4.675	
Bokashi	Bokashi Ч Haltite					
105.00	108.16	108.53	105.03	98.28	Non addition	
109.51	113.28	114.94	108.93	100.89	addition	
1.653	L.S.D. 0.05				3.306	
Extraction	Haltite Ч Extraction way					
100.80	103.84	104.82	100.64	93.89	Non addition	
113.71	117.60	118.64	113.33	105.28	addition	
1.653	L.S.D. 0.05				3.306	
	110.72	111.73	106.98	99.59	Haltite	
	L.S.D. 0.05				2.337	

Table (II) : Effect of spraying haltite and Bokashi and the extraction method on the leaf content of Aloin acid (mcg.g^{-1})

Bokashi× Extraction	Haltite				Extraction way	Bokashi
	75	50	25	0.00		
156.3	164.6	166.8	156.4	137.6	aqueous	Non addition
184.1	184.5	201.1	182.4	168.4	alcoholic	
176.4	184.3	187.1	187.9	146.1	aqueous	addition
253.8	250.6	327.1	232.1	204.9	alcoholic	
16.97	L.S.D. 0.05				33.93	
Bokashi	Bokashi× Haltite					
170.2	174.5	184.0	169.4	153.0	Non addition	
215.1	217.4	257.1	210.3	175.5	addition	
12.00	L.S.D. 0.05				23.99	
Extraction	Haltite × Extraction way					
166.4	174.4	177.0	172.2	141.8	aqueous	
219.0	217.5	264.1	207.6	186.6	alcoholic	
12.00	L.S.D. 0.05				23.99	
	196.0	220.5	189.9	164.2	Haltite	
	L.S.D. 0.05				16.97	

The addition of the Bokashi was significantly superior compared to non-addition treatment, and recorded the highest rate of 109.51 compared to 105.00mcg.g^{-1}. The extraction method was differ significantly, as the alcohol extraction method produced a significant response and achieved the highest rate of 113.71 compared to water extraction method that recorded a rate of 100.80 mcg.g^{-1}. The table showed that the triple interference had a significant effect and the addition of the Bokashi and the alcohol extraction method with spraying haltite at a 50mg.L^{-1} achieved a significant superiority over most others by achieved the highest rate of 121.06 mcg.g^{-1} compared to not adding Bokashi and haltite with water extraction which recorded the lowest rate of 93.45 mcg.g^{-1}.

Table II showed that spraying the plants with several concentrations of haltite caused significant effect and 50 mg.L^{-1}. achieved the highest rate of 220.5, while the control treatment recorded the lowest rate of 164.2mcg.g^{-1}. The addition Bokashi achieved the highest rate of 215.1 mcg.g^{-1}, significantly compared to non-additive treatment that recorded a rate of 170.2 mcg.g^{-1}. The method of extracting were differ significantly and alcoholic extraction was superior compared to water-extracting treatment, by recording the highest rate of 219.0mcg.g^{-1} versus 166.4mcg.g^{-1}.The triple interaction between the factors caused a significant effect and the addition of Bokashi with haltite at 50ml.L^{-1} and alcohol extraction method recorded a significant superiority over all others by achieving the highest rate of 327.1mcg.g^{-1} versus 137.6mcg.g^{-1} from control.

Table (III) : Effect of spraying haltite and bokashi mixture and the extraction method on leaf content of Cinnamic acid (mcg.g^{-1})

Bokashi× Extraction	Haltite				Extraction way	Bokashi
	75	50	25	0.00		
88.65	88.15	114.14	81.09	71.24	aqueous	Non addition
55.33	57.72	62.06	54.38	47.15	alcoholic	
102.36	103.77	130.19	103.49	71.98	aqueous	addition
60.06	59.57	70.63	58.97	51.01	alcoholic	
3.148	L.S.D. 0.05			6.295		
Bokashi	Bokashi ×Haltite					
71.99	72.93	88.10	67.73	59.20	Non addition	
81.20	81.67	100.41	81.23	61.50	addition	
2.226	L.S.D. 0.05			4.451		
Extraction	Haltite × Extraction way					
95.51	95.96	122.16	92.29	71.61	aqueous	
57.69	58.65	66.35	56.67	49.08	alcoholic	
2.226	L.S.D. 0.05			4.451		
	77.30	94.26	74.48	60.35	Haltite	
	L.S.D. 0.05			3.148		

Table IV : Effect of spraying haltite and Bokashi and extraction method on leaf content of Sinapic acid (mcg.g^{-1})

Bokashi× Extraction	Haltite				Extraction way	Bokashi
	75	50	25	0.00		
44.28	47.72	44.19	45.40	39.81	aqueous	Non addition
89.09	90.88	89.52	90.56	85.40	alcoholic	
50.85	52.50	53.33	51.83	45.74	aqueous	addition
91.16	93.11	97.42	91.07	83.06	alcoholic	
1.186	L.S.D. 0.05			2.371		
Bokashi	Bokashi×Haltite					
66.69	69.30	66.86	67.98	62.61	Non addition	
71.01	72.81	75.37	71.45	64.40	addition	
0.838	L.S.D. 0.05			1.667		
Extraction	Haltite × Extraction way					
47.57	50.11	48.76	48.61	42.78	aqueous	
90.13	92.00	93.47	90.81	84.23	alcoholic	
0.838	L.S.D. 0.05			1.667		
	71.05	71.11	69.71	63.50	Haltite	
	L.S.D. 0.05			1.186		

Table III shows that the factors caused significant effect on the leaf content of Cinnamic acid for Aloevera plants, and the haltite at 50mg.L^{-1} was superior significantly by giving the higher rate of 94.26mcg.g^{-1}, while control treatment gave the lowest rate of 60.35mcg.g^{-1}. The addition of the Bokashi caused significant increase in this trait and achieving the highest rate of 81.20mcg.g^{-1}, while the control treatment recorded the rate of 71.99mcg.g^{-1}. The extraction methods also differed significantly, and alcohol extraction giving the highest rate of 95.51mcg.g^{-1}, while water extraction achieved the lower rate of 57.69mcg.g^{-1}. The interaction of spraying Haletite at a concentration of 50mg.L^{-1} with the addition of Bokashi and the method of water extraction achieved the highest rate of 130.19mcg.g^{-1}, while without Bokashi and haltite with alcohol extraction method was recorded the lowest rate of 47.15mcg.g^{-1}.

Table IV showed that the factors had a significant effect on the leaf content of Sinapic acid, spraying haltite at 50mg.L^{-1} achieved the highest rate of 71.11mcg.g^{-1}, while the lowest rate was recorded from the control treatment 63.50mcg.g^{-1}. The addition of the Bokashi had a significant effect in this capacity, and achieved rate of 71.01mcg.g^{-1} compared to control. The method of extracting were differ significantly, and alcoholic extraction recorded the highest rate of 90.13 mcg.g^{-1}. The triple interaction had a significant effect, spraying of Haltite at 50mg.L^{-1} with the addition of Bokashi and the alcohol extraction method achieved the highest rate of this trait (97.42 mcg.g^{-1}, while no adding Bokashi without haltite and water extraction recorded 39.81mcg.g^{-1}.

DISCUSSION

The reason for the superiority achieved in all indicators of the studied characteristics of the medicinally effective substances can be attributed to the role that spraying played in different concentrations of the herbaceous herb by containing a high percentage of vitamins and a high group of essential nutrients, the most important of which are phosphorus, potassium, magnesium, calcium and sodium, which feed positively in The growth and development of the plant and this is reflected in the productivity of the plant from the active substances

it produces [12]. The fact that the herbaceous herb contains high levels of proteins, carbohydrates and many organic acids, including coumarin, feriolic and clucuronic acid, may cause an explicit reflection in the level of growth and production of active substances of medicinal value [13]. [14] stated that the herbaceous plant is one of the most important naturally-growing plants that are distinguished by its contain biologically active substances such as alkaloids, flavonoids and phenols that are of high importance in promoting growth as anti-microbial activities that affect the plant appear at the same time and its role is influential in increasing the strength of the plant, thus It helps in building its various materials. [15,16] also mentioned that the group of organisms contained in the Bokashi mixture contribute to the secretion of many substances such as amino acids and carbohydrates, which encourage plant growth and stimulate other species of other microorganisms, which improves the characteristics of the agricultural chemical and physical characteristics, including increasing its retention of moisture and increasing air pores that increase From plant aeration and improving plant growth standards. These neighborhoods also improve the indicators of the root system of the plant and increase its branches, which increases the plant's ability to absorb water and nutrients, thus increasing its growth and development even if the plant growth encounters various vital and inanimate stresses [16-18].

CONCLUSION

The present study showed that the spraying the plants with several concentrations of halite and Bokashi were significantly superior compared to non-addition treatment and recorded the highest rate.

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