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Effects of Plant Density and Vermicompost on the Rate of two Active Substances, Aloenin and Aloe emodin of *Aloe Vera* (*Aloe barbadensis* mill.)

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Abstract

For many years, the gel and distillate of *Aloe vera* as a medicinal plant have been used in medical and cosmetic industries. *Aloe vera* gel is used in medical and cosmetic industries. *Aloe vera*'s gel has various biologic and physiologic features such as curing the burning and skin ulcers, anti dirt, stopping the growth of bacterium and parasites, having resistance impact against chemical synthesis due to Antraquinone compounds and stopping the growth of HIV activity. In order to examine the influence of planting density and vermicompost on the rate of active substances Aloenin and Aloe emodin in *AloeVera*, an experiment was performed in Ahwaz experimental farm, Khozestan province, 2009. The experiment was done as a factorial on the basis of randomized complete blocks design in three replications. Vermicompost and density treatments were used in three levels involving 5, 10 and 15 ton/hec and levels of 2, 4 and 6 plants/m^2, respectively. After extracting the gel from the leaves of *Aloe vera*, the rate of active substances of Aloenin and Aloe emodin has been evaluated using high performance liquid chromatography (HPLC). Regarding the obtained results, Vermicompost and planting density treatments had a significant effect on the rate of active substances and the highest amounts of Aloenin and Aloe emodin were measured as 1193.3 µg/g and 536.3 µg/g in the fertilizer treatment of 15 ton/hec and density of 4 plants/m^2.

Key words: *Aloe vera L., Vermicompost, Plant density, Aloenin , Aloe emodin*

Introduction

Nowadays, clarifying the side complications of chemical medicines, the use of medicinal plants is increasing. Since many researchers have not been done on the increase of medicinal plant production, introducing appropriate methods for medicinal plant cultivation is of importance so as to increase the quality and quantity of medicinal plants (Farooqi *et al*., 2001). *Aloe vera* has 300 species and the most important species called *Aloe barbadensis* is used in pharmaceutical, food, cosmetic and health industries (Yogee Swaran *et al*., 2005). *Aloe vera* is classified as the perennial meat plant from the family of *liliaceae* and has a variety of eatures (Hsanuzzaman *et al*., 2008). It is used as the gel for curing the burning, wound, insect bite, rash, spot and freshness, dandruff, blotch and dermatitis and as an oral medicine to improve the body immune system against the diseases such as AIDS, leukemia and diabetes (Talmadge *et al.,* 2004). The leaves of this plant are also widely- used because they contain the required gel, which is applied along with the distillate in pharmaceutical, food, cosmetic and health industries (Davis et *al.,* 2003). The most primary ingredients of Aloe Vera are found in the class of Antraquinone and Glycoside (Josias *et al.,* 2008). Among these compounds, we can refer to Aloenin and Aloe emodin which are formal medicines in the U.S.A pharmacopoeias (Joshi, 1998).

Vermicompost is one of suitable fertilizers for the organic agriculture. Vermicompost is considered as a rich source of macro/micro elements, vitamins, enzymes and growth stimulating hormones which accelerate the growth of medicinal plants. It also increases the access to nitrogen and phosphours by increasing the Nitrogen fixation and dissolving phosphorus (Prabha *et al.,* 2007).

Most nutrients in vermicompost are nitrates, phosphates, exchangeable calcium and soluble potassium (Edwards, 1998). Due to large

particulate surface area, Vermicompost has many microsites for microbial activity and strong retention of nutrients. Plant growth regulators and other materials which are affecting the growth are available in vermicompost(Atyieh et al., 2002). Large amounts of humic substances are found in vermicompost which have some plant growth effects similar to the impacts of soil applied plant growth regulators (Muscolo et al., 1999).

Vermicompost has some beneficial influences on horticultural and agronomic crops (Goswami et al., 2001; Roy et al., 2010). According to previous studies, vermicompost has a positive effect on the growth and yield of medicinal plants such as garlic (Arguello et al., 2006), basil (Anwar et al., 2005), fennel (Darzi et al.,2008) and chamomile (Azizi *et al*., 2009). It has also been shown to increase the flower yield and essential oil of roman chamomile (Liuc and Pank, 2005). Furthermore, previous works have reported that using vermicompost enhances the essence rate and improves the quality of the medicinal plant(*Arthemis nobilis*) (Liuc & Pank, 2005).

Surveying the sources indicates that much researches have not been done on the agriculture management, especially the impact of climatic elements such as organic fertilizer and plant density on the growth, behavior and substances (Hsanuzzaman *et al.,* 2008; Cruz *et al.,* 2002 & Alagukannan *et al.,* 2008). Studying the effect of mulch on *AloeVera* has shown that mulch increases the bush weight (20.81 ton/ hectare), leaf number, leaf weight and its performance (Cruz *et al.,* 2002). In examining the performance and its components, the highest leaf length and highest performance were seen in the planting distance of 120 cm (two bushes) and 90 cm, respectively (Yogee swaran *et al.,* 2005). In another investigation done on the various ecotypes of *Aloe Vera*, it was determined that the nutrients have a positive influence on the rate of the active substance, Aloenin. As the gel weight increases, the rate of active substance is enhanced (Alagukannan *et al.,* 2008). Examining *Aloe Vera* indicated that plants being able to use the most of nutritive and optical sources had a better growth and the plants with shadowing produce more leaves and splits (Kawther *et al.* ,2002). In another investigation on the medicinal plant, namely *Aloe Vera*, it was observed that the nutrients such as Nitrogen increase the plant growth and rate of active substance (Alejandra *et al.,* 2000). This study aims to measure the influences of vermicompost and plant density on active substances in medicinal plant, *Aloe vera*

Material and Methods

I) Planting

This research aims to study the impact of the rate of the active ingredient from *Aloe Vera* that is considered as a medicinal plant, in various levels of vermicompost and plant density by using a factorial design based on randomized complete blocks in 3 replications. The experiment was performed in an area of 450m^2 in Ahwaz experimental farm, located in Khozestan province. Each furrow has 5 planting lines in the length of 4 m and the distance between two planting lines is 50 cm (the area, length and width of a furrow are 10 m^2, 4 and 2.5 m, respectively). The climatic features and soil of the studied area are presented in Tables 1 and 2. The experimental treatments involved the use of vermicompost in three levels consisting of 5, 10 and 15 tons per hectare. The main characteristics of the vermicompost used in this study are presented in Table 3. The plant density was 2, 4 and 6 plants/m^2; and the distance between two plants were 100, 50 and 33.3 cm for the densities of 2, 4 and 6 plants, respectively. Firstly, the farm was plowed, flattened and furrowed. The plant splits with the same size were cultivated in the farm in May, 2009. Then, Vermicompost fertilizer treatments were applied in the planting rows. The weeds were rooted out by hand several times. During the experiment, no pests and diseases were observed.

II) Collecting

Harvesting was done manually in December, 2010. The plants were cut below the crown and the leaves were cut from the lowest leaf near to the crown with a clean knife. Then, they were prepared for the required analyses after washing and cleaning.

III) Extracting

Some features including the rates of Aloenin and Aloe emodin were measured. The leaves were gathered from various furrows after washing with distilled water to extract the gel and perform the related experiments.

IV) Method to Measure the Active Substances, Aloenin and Aloe emodin using HPLC

The gel was broken and homogenized, and the mixture was then centrifuged at 4^oC for 30 minutes. The above solution was passed through a membrane Millex – GV filter with the thickness of 0.22 micrometer. It was purified and injected into the column of HPLC machine which was Inlersil Ops-2 type having 4.6 × 150 mm dimensions made in Glsciences, Japan. This column was washed using Acetonitrile solution with a concentration slope of 15 to 40 percents for 10 minutes. The transit velocity was computed as 1 millimeter per minute and the absorption measurement was performed in the wavelength of 293 nm.

HPLC system is of reverse type and its pump is lc – 10 AT. The output peaks recorded by the detector and standard peaks of Aloenin and Aloe emodin were evaluated and their concentrations were determined (Kuzuya *et al.,* 2001).

The study data were analyzed using the statistic software, MSTAT-C and the means comparison was performed by Duncan method.

Table 1.Climatic characteristics of the experimental farm

Longitude	Latitude	Mean precipitation	Mean humidity	Maximum temperature	Minimum temperature
Northern 48˚,40'	Northern 20˚,31'	335-420 mm	81%	34	15

Table 2 . Chemical and physical characteristics of the soil from the experimental farm (at the soil depth of 0 - 30 cm)

Soil texture	pH	EC (ds/m)	Water absorption %	Organic Carbon %	Total Nitrogen %	Absorptive Phosphorus % (p.p.m)	Absorptive Potassium % (p.p.m)	Clay %	Sand %	Silt %
Loamy-clay	7.2	1.03	57	4.24	0.12	19.80	290	34	39	27

Table 3. Chemical analysis of vermicompost used in the experiment

pH	EC (ds/m)	O.C %	Organic carbon	Total Nitrogen	Absorptive Phosphorus % (p.p.m)	Absorptive Potassium % (p.p.m)	Fe (p.p.m)	ZN (p.p.m)	Mn (p.p.m)
7.10	2.36	11.80	4.24	0.12	35	368	42	33	20

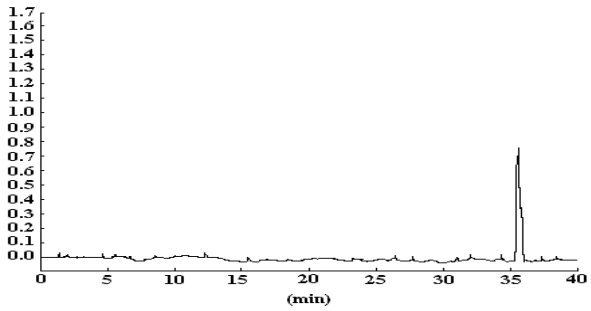

Fig 1. Standard curve of Aloenin with R7=17'/2''

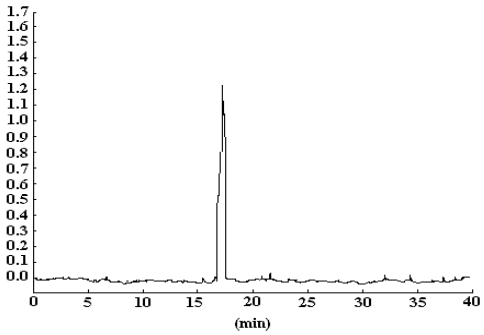

Fig 2.Standard curve of Aloe emodin with R7=36'/1''

Results

Plant Density

Variance analysis results showed that various levels of density have the significant level of 0.01 (Table 4). Also, comparing the means by Duncan method indicated that the highest rates of Aloenin computed as 983.2 and Aloe emodin as 426.5 µg/g were obtained in the density of 4 plants/m^2 (Table 5). Between the densities of D_1 and D_2, a significant difference was not seen for three studied characteristics and they were classified in the same statistical group.

Vermicompost

Variance analysis showed that Vermicompost levels were significant at 0.01 level (Table 4). The mean comparisons using Duncan test indicated that highest rates of active substances of Aloenin and Aloe emodin computed as 1112 and 472.7 µg/g were measured for the fertilizer V_3 treatment (Table 5).

''Interaction of Plant Density and Vermicompost''

The variance analysis results demonstrated that the interaction of planting density and Vermicompost has a significant level of 0.01 for all studied features. Comparing the means using Duncan test, it was clear that the highest rate was obtained in V_3D_2 treatment (Table 6).

Table 4.Variance analysis results of plant density and Nitrogen effects on active substance characteristics of *Aloe vera*

S.O.V	df	Square Means	
		Aloenin	Aloe emodin
replication	2	68.8 ns	93.7 ns
density	2	195718.5**	62863.8**
Vermicompost	2	187464**	63957.8**
Density* Vermicompost	4	4932.8*	58130372**
Error	16	1029.7	113.2
C.V%		3.6	3.7

*Means and ** : Non significant ,and significant at the 5% and 1% levels of probability, respectively

Table 5.Mean comparisons of various density and Vermicompost levels on active substance characteristics of *Aloe vera*

Density (plant/m^2)	Measured Features	
	Aloenin	Aloe emodin
D_1	963.6a	436.2a
D_2	983.3a	426.5a
D_3	813.8b	285b
Vermicompost(ton/hec)		
V_1	843.6c	308.8c
V_2	982.3d	367.1d
V_3	1112a	472.7a

*Means with the same letters in each column are not significantly different.
*D_1: 2(Plant/m^2) , D_2: 4(Plant/m^2) , D_3: 6(Plant/m^2), V_1 = 5ton/hec, V_2 = 10 ton/hec and V_3 = 15 ton/hec

Table 6: Mean comparison of density and Vermicompost interaction for the features of active substances in *Aloe Vera*

Density Treatment and vermicompost	Measured Features	
	Aloenin	Aloe emodin
D_1V_1	896c	317.4e
D_2V_1	835.8c	354d
D_3V_1	711.4e	177.4g
D_1V_2	988b	432b
D_2V_2	998.8b	412.3c
D_3V_2	782.6d	196f
D_1V_3	1198a	539.3a
D_2V_3	1193.3a	536.3a
D_3V_3	872.6c	298.6de

Conclusion

By investigating the literature, it was shown that there are sufficient data on the medicinal and hygienic influences of *Aloe Vera* but a few studies deal with farming management of this plant (Hasnuzzaman *et al.,* 2008; Cruz et *al.,* 2002; Alagukannan *et al.,* 2008).

Environmental factors play important roles in the quality and quantity of active substances of medicinal plants.

Among these factors, we can mention the plant density and organic fertilizer. Active substance biosynthesis of *Aloe Vera* depends on the light treatments and plant respiration (Alagukannan *et al.,* 2008). Therefore, due to the less shadowing and receiving more light through the lower parts in D_1 and D_2 densities, the highest rate of active substance was obtained. In D_1 and D_2 densities, the plant receives higher levels of light and the increase in light has a positive effect on the active substance rate but due to the shadowing, Aloenin and Aloe emodin‘s active substances are decreased in D_3 density.

Investigating the growth of *Aloe Vera* and production of Aloenin demonstrated that the plants being able to consume the light and food sources optimally had grown better and their active substances were increased to 145.6 µg/g as compared to the shadowing plants. These observations are compatible with the study results. As the gel rate increases, the active substance rate is increased (Alagukannan *et al.,* 2008). While higher levels of Vermicompost are applied, investing the photosynthetic substances is increased in various parts of leaf and it leads to the increase in Alkaloid production in the leaves. This increase in turn enhances the active substance (Alagukannan *et al.,* 2008). The increase in the nutrients of *Aloe Vera* results in higher levels of sugar in the plant (Alagukannan *et al.,* 2008).

In other words, the plants which received more levels of Vermicompost produce active substances more than those receiving lower levels of Vermicompost because of lower growth of vegetative organs.

A study done on the vegetative growth and Aloenin rate in a variety of ecotypes showed that the increase in active substance rate depends on the environmental conditions such as nutrients Vermicompost and density and the plants having access to these factors have more vegetative growth and active substance (Alagukannan *et al.,* 2008).

In this study, the treatment having the desired density and highest rates of nutrients (V_3D_2) has acquired the highest levels of active substance. In D_3 density, the active substance rate is reduced because of shadowing, competing of bushes for light and water absorption and the increase in consuming the photosynthetic substances.

The research results indicated that in Ahwaz, Khozestan province, if there are the soil and nutrients similar to the experiment conditions and the weeds are rooted out, the maximum rate of active substance will be obtained using 15 ton/hec of vermicompost fertilizer and 4 plants/m^2.

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