

Response of Ashwagandha (*Withania somnifera* L.) to sowing dates and organic liquid fertilizer (Vit-ORG)

Essam H. A. Al-Doghachi, Abdul-Razzak O. Hassan and Nada N. Faris

Department of Horticulture and land scape, University of Basra, Iraq

ABSTRACT

The experiment was conducted to study the response of Ashwagandha to sowing dates and organic liquid fertilizer (Vit-ORG) on chemical components of Ashwagandha sown at Medicinal and Aromatic Plants fields of the College of Agriculture , Basra University ,Iraq, during the growing season of 2013/2014 and 2014/2015. The experiment was laid out in Randomized Complete Block Design in a factorial experiment having three replications. The experiment involved two factors: sowing dates at three levels of 1 or 20 September and 10 October and organic liquid fertilizer (Vit-ORG) (0 and 2) ml.l⁻¹. The results showed that the nutritional contents were significantly affected by sowing dates and organic liquid fertilizer (Vit-ORG). Percentage of fixed oil and total alkaloid were not significantly affected by sowing dates during both growing seasons. The organic liquid fertilizer (Vit-ORG) caused increase the percentage of fixed oil in leaves and roots. The interactions between sowing dates and organic liquid fertilizer (Vit-ORG) gave a significant increase in most studied parameters during both seasons.

Key words: Ashwagandha (*Withania somnifera* L.), sowing dates ,Organic liquid fertilizer (Vit-ORG).

INTRODUCTION

Medicinal plants are wonder gifts of nature which are used as medicine, cosmetics, hygiene and food supplements in improving the quality of human life (1). *Withania somnifera* (Solanaceae), is an annual to perennial herb, with multiple medicinal properties. known in India as Ashwagandha or winter cherry, is one of the most valuable plants of the traditional Indian systems of medicines, is used in more than 100 formulations of *Ayurveda* (2) .All parts of Ashwagandha have medicinal properties and are used in preparation of various drugs, however, roots are mainly used for preparation of vital tonics. It is tonic, general tonic in arthritis and debility from old age. It is used in anxiety, depression, phobias, alcoholic paranoia, schizophrenia etc. The active ingredient that attributed to the medicinal property is the alkaloids and steroidal lactones.(3,4,5,6).

The environmental factors explicitly influence growth and development and yield of medicinal herbs. Sowing date is a very important parameter in crop production. The optimum sowing date paves the way for better use of time, light, temperature, precipitation and other factors (7).

Some substances affected on plant growth and its physiological activities, Including organic liquid fertilizer (Vit-ORG) which promote plant growth and induce soil microorganisms like bacteria and fungi and provide carbon as a source for the organisms organic liquid fertilizer (Vit-ORG) as well acting as chelating good martial, and reason the lack of mineral nutrient and losing them by leaching and also make many nutrient available in soil such as phosphate, calcium and trace elements and finally organic liquid fertilizer(Vit-ORG) possesses high capability in controlling soil pH against changes which might occurs from the use of chemical fertilizer (8). Hussein *et al.*(9) found that organic liquid fertilizer(Vit-ORG) caused increase physiological activities ,growth and chemical structure to pumpkin *Cucurbita pepo* L. and this was agreed with the results of Hussein and Majid(10)on melon plant *Cucumis melo* and Majid (11) to potato plants *Solanum tuberosum* L. and Abboud (12) on *Pimpinella anisum* L.

MATERIALS AND METHODS

Experimental Design

The study on response of *W. somnifera*(L.) to sowing date and organic liquid fertilizer (Vit-ORG) was conducted at Medicinal and Aromatic Plants fields of the College of Agriculture, Basra University, Iraq, during the growing season of 2013/2014 and 2014/2015.

The experiment was laid out in RCBD (Factorial) design with three replications. There were six treatment combinations comprised of three dates of sowing (Sep.1st, Sep. 20th and Oct.10th) and two levels of organic liquid fertilizer (Vit-ORG) (0 and 2) ml.l⁻¹. The seeds were sown in small pots with(15×12×12)cm and then transplanted in large pots with (30×30×30) cm when seedlings aged 5 month. The plants were treated with organic liquid fertilizer (Vit-ORG) (produced by Green, Italia)after 14 days of transplanting. Treatments were repeated three times. Each treatment was about 20 days after the other treatment took place. Tables (1 & 2) explains the chemical, physical, soil mixture, organic liquid fertilizer (Vit-ORG)and irrigation water used in this experiment .

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Characterize	Season1	Season2
(E.C)	5.03	5.04
(pH)	7.32	7.30
Total nitrogen(ml.l ⁻¹)	0.85	0.90
Phosphorus(ml.l ⁻¹)	17.76	20.85
Potassium(ml.l ⁻¹)	22.18	30.04
Organic matter%	0.38	0.40
Separates of soil		
Sand%	46.90	46.51
Silt%	18.00	20.04
Clay%	14.00	13.11
Soil texture	Sandy silt	
Irrigation water		
(E.C)	40.2	40.3
(pH)	7.90	7.92

Density	1.25gm/cm ³
(pH)	7.0
(E.C)	400meq/100g
Particle size	<100 Microns
Solubility in water	100%
Organic Nitrogen(N) soluble in water	3 %
Potassium oxide (K ₂ O) soluble in water	6 %
Organic Carbon (C)	18 %
Organic matter	31 %

Table (3) maximum and minimum temperature grades and relative humidity for two seasons

Month	2013/2014		
	Temperature		Humidity%
	Max.	Min.	
September	42.23	24.79	19.26
October	33.41	17.33	26.84
2014/2015			
September	41.48	27.45	19.87
October	30.88	18.86	22.47

Measurements

Immediately after harvest, three plants in each replication were used for measurement of factors. Measured factors consisted of :-

1. Chemical Compositions

A. Total nitrogen percentage (N %) by micro-Kjeldahl according to Page *et al.*(13).

B. Phosphorus content (P %) according to Olsen and Dean (14).

C. Potassium (K) and calcium (CA) content was measured by using Flame photometer according to Page *et al.*(13).

2. Fixed Oil percentage

extracted fixed oil from leaves and roots by Soxhlet according to Stahl (15) using organic solvent ether petrolatum Spirit (Distillation range 40-60C °). percentage of fixed oil by applying the following formula :-

$$\text{Fixed Oil percentage (\%)} = \frac{\text{Fixed oil weight (g)}}{\text{Samples weight (g)}} \times 100$$

3. Total alkaloid percentage

extracted fixed oil from leaves and roots by according to Harborn (16). Percentage of total alkaloid applying the following formula :-

$$\text{Total alkaloid percentage (\%)} = \frac{\text{Total alkaloid (g)}}{\text{Samples weight (g)}} \times 100$$

Statistical procedure

The data recorded from three plants were subjected to statistical analysis, using analysis of variance (ANOVA) using Gen Stat Release 10 statistical software. The differences among various treatments were analyzed through Least Significant Difference test at probability of 0.05.(17).

RESULTS AND DISCUSSION

A) Nutritional contents

The nutritional contents of Ashwagandha affected by different sowing dates and organic liquid fertilizer (Vit-ORG) and their interactions are shown in tables (4,5,6). Data presented in table (4) revealed that sowing dates was significant effect of macro nutrients (N, P, K and Ca) in leaves and roots, except for the nitrogen and potassium of roots and phosphorus of leaves during both seasons. The maximum percentage of nitrogen in leaves and phosphorus in roots during first season were obtained by plant sown on 1st Sep. followed by other sowing date. Also the highest percentage of phosphorus and calcium in roots were obtained by plant sown on 20th Sep. during second season only. Also, plant sown on 10th Oct. during second season gave maximum percentage of calcium in leaves.

Treatment with organic liquid fertilizer (Vit-ORG) caused increase of percentage of nitrogen in roots during first growing season only, and increase percentage of phosphor in leaves during both growing season and potassium and calcium in roots during second seasons, followed by untreated plants. (Table 5).

Analysis of variance showed that interactions between sowing dates and organic liquid fertilizer (Vit-ORG) was significantly affected of nutritional contents of Ashwagandha (Table 6). Maximum percentage of nitrogen in leaves (9.18 and 8.81)% during both seasons and percentage of calcium in leaves (3.35)% during first season, were obtained with treatment 1st Sep. with organic liquid fertilizer (Vit-ORG) followed by other treatments. Testament 10th Oct. with organic liquid fertilizer (Vit-ORG) produced the highest percentage of phosphors in leaves (3.55)% followed by other treatment. The maximum increment for percentage of phosphors in roots (5.05) during first growing season only was recorded for 20th Sep. without organic liquid fertilizer (Vit-ORG) over the other treatment. However plant sown on 20th Sep., with organic liquid fertilizer (Vit-ORG) for second season produced maximum percentage of potassium in leaves (4.63)% and phosphors in roots (5.27)% and calcium in leaves and roots (4.08 and 3.30)% followed by other treatment.

The exceed of early sown plants in the nutritional contents, may be due the plants growth under favorable weather conditions led to the length of the vegetative growth period and abundance represented by increasing the number of branches and vegetative number of leaves and leaf area which led to increase the efficiency of photosynthesis, which reflected significant increase in processed food in stock accumulation and delayed transmission of those manufactured materials to the centers polarization (flowers and fruits) which provided carbonate needed to build the amino acid structures led to increase the nutritional contents (18). This results agree with Abd *et al.* (19) to sweet basil *Ocimum basilicum* var. *basilicum* L.

The high percentage of nitrogen, phosphorus, potassium, calcium, may be caused by the effect of organic acids humic and fulvic in the organic liquid fertilizer to increase metabolic processes activity such as construction photosynthesis and respiration and carbohydrates, in addition to increasing the permeability of cell membranes and facilitate the movement of nutrients process, especially nitrogen and phosphorus, potassium, calcium, resulting in increased plant efficiency of the uptake and accumulation of these elements in the leaves and roots, this result agree with Farhan (20) to potato plants.

Generally, the organic fertilizers changed the proportion of organic matter in the soil, which improved the characteristics by increasing the readiness of nutrients and increase microbiology activity and the quantity and effectiveness of the enzymes analyzed in the soil which increases the readiness of the elements and the absorption of the plant (21).

The organic fertilizers are prepared initially from either animal or plant residues. All organic manures improve the behaviors of several elements in soils through that active group (fulvic and humic acids) which have the ability to retain the elements in complex. These materials release the elements over a period of time and are broken down slowly by soil microorganisms. The extent of availability of such nutrients depends on the type of organic materials and microorganisms (22).

Table 4: Effect of sowing dates on nutritional contents of Ashwagandha during both seasons

Sowing dates	Season (2013/2014)							
	%N		%P		%K		%Ca	
	Leaves	roots	Leaves	roots	Leaves	roots	Leaves	roots
D1	8.81	5.14	3.25	4.95	3.42	2.80	2.73	1.31
D2	7.58	4.36	3.25	4.10	4.32	2.56	2.79	1.41
D3	4.89	3.91	3.16	4.64	4.40	2.30	2.78	1.37
L.S.D.	2.20	N.S	N.S	0.33	0.90	N.S	N.S	N.S
Season (2014/2015)								
D1	7.90	5.08	2.70	4.79	3.40	3.17	2.43	1.73
D2	7.47	4.41	2.91	5.20	4.16	3.05	3.09	2.55
D3	6.01	5.55	2.75	4.53	4.26	2.72	3.37	1.99
L.S.D.	N.S	N.S	N.S	0.60	N.S	N.S	0.79	0.63

* D1:-1 September, D2 :- 20 September, D3 :-10 October.

Table 5: Effect of organic liquid fertilizer (Vit-ORG)on nutritional contents of Ashwagandha during both seasons

Organic liquid fertilizer (Vit-ORG)	Season (2013/2014)							
	%N		%P		%K		%Ca	
	Leaves	roots	Leaves	roots	Leaves	roots	Leaves	roots
V0	6.95	4.58	3.02	4.91	4.05	2.43	2.54	1.20
V1	7.25	4.36	3.42	4.81	4.05	2.68	3.00	1.53
L.S.D.	N.S	N.S	0.31	N.S	N.S	N.S	N.S	N.S
Season (2014/2015)								
V0	6.46	4.52	2.49	4.73	3.56	2.59	2.69	1.52
V1	7.80	5.51	3.08	4.95	4.32	3.37	3.24	2.66
L.S.D.	N.S	1.26	0.58	N.S	N.S	0.74	N.S	0.51

* V0 :- without Vit-ORG , V1:- with Vit-ORG (2 ml.l⁻¹).

Table 6: Effect of interactions between sowing dates and organic liquid fertilizer (Vit-ORG)on nutritional contents of Ashwagandha during both seasons

Sowing dates & Organic liquid fertilizer (Vit-ORG)	Season (2013/2014)							
	%N		%P		%K		%Ca	
	Leaves	roots	Leaves	roots	Leaves	roots	Leaves	roots
D1V0	8.45	4.69	3.06	4.97	3.24	2.75	2.11	1.03
D2V0	8.38	5.48	3.24	5.05	4.44	2.18	2.73	1.01
D3V0	4.03	3.58	2.77	4.70	4.46	2.36	2.77	1.56
D1V1	9.18	5.59	3.44	4.93	3.61	2.86	3.35	1.58
D2V1	6.79	3.23	3.27	4.92	4.19	2.94	2.86	1.81
D3V1	5.76	4.25	3.55	4.58	4.35	2.24	2.79	1.18
L.S.D.	3.11	N.S	0.53	0.46	N.S	N.S	1.16	0.75
Season (2014/2015)								
D1V0	6.99	4.43	2.33	4.69	3.61	2.86	2.42	0.95
D2V0	7.29	3.87	2.77	5.12	4.19	2.65	2.10	1.89
D3V0	5.10	5.25	2.37	4.38	4.35	2.27	3.56	1.83
D1V1	8.81	5.73	3.06	4.89	3.74	3.48	2.45	2.52
D2V1	7.65	4.95	3.05	5.27	4.63	3.45	4.08	3.30
D3V1	6.92	5.85	3.13	4.68	4.59	3.17	3.19	2.15
L.S.D.	2.80	N.S	N.S	0.85	1.38	N.S	1.11	0.89

* D1V0:-1stSeptember without Vit-ORG ; D2V0 :- 20th September Vit-ORG ; D3V0 :-10th October without Vit-ORG ; D1V1:-1stSeptember with Vit-ORG ; D2V1 :- 20th September with Vit-ORG ; D3V1 :-10th October with Vit-ORG**B) Percentage of fixed oil and total alkaloid**

Evident from the data related to percentage of fixed oil and total alkaloid presented in Table (7) the effect of sowing dates was not significant during both growing seasons.

On the other hand data presented in table (8) showed the organic liquid fertilizer (Vit-ORG)was significantly affected of fixed oil percentage. Plants treated with organic liquid fertilizer (Vit-ORG)produced maximum percentage of fixed oil in leaves (0.65)% during first growing season only, and in roots (0.52)% during second growing season only, compared with untreated plants. Regarding total alkaloid, it could be easily noticed that treated with organic liquid fertilizer (Vit-ORG), had no significant effect, in both growing seasons.

Analysis of variance showed that interactions between sowing dates and organic liquid fertilizer (Vit-ORG)was significantly affected of fixed oil and total alkaloid percentage of Ashwagandha (Table 9). The maximum percentage of fixed oil in leaves (0.71 and 0.59)% in both growing seasons ,and percentage of total alkaloid in leaves (11.51)% during first growing season, were obtained with treatment 10th Oct. with organic liquid fertilizer(Vit-ORG)followed by other treatments. Testament 20th Sep. with organic liquid fertilizer (Vit-

ORG)produced the highest percentage of fixed oil in roots (0.60 and 0.61)% in both growing seasons and total alkaloid in roots(9.94)%during first growing season ,followed by other treatments.

Alkaloids are secondary metabolism products so its production is effected by environmental conditions that effect the photosynthesis . The significant exceed of the plants sown in the 20th Sep. and 10th Oct. that treated with organic liquid fertilizer (Vit-ORG) in the percentage of the total alkaloids in leaves and roots ,which is due to the suitable weather conditions in these dates and increase the quality of the photosynthesis and increase the produced nutritional components which reflected in the strength of the plants growth represented in the increase branches of the plants, leaves number and leaf area to increase the dry matter percentage and carbohydrates and increase the alkaloids building and accumulation which connect directly with the dry matter percentage in plants.(22). this results agree with Kakaraaparthi *et al.*(23) to Ashwagandha plant.

The increase of alkaloids percentage in leaves and roots is may be due to role of organic liquid fertilizer(Vit-ORG) in supporting the plants growth, and building a root system which is featured with high quality in absorption the nutritional contents this is reflected on cells divisions and growth, and increase the leaf area ,as well as increase the nutritional contents that are made in leaves such as carbohydrate and proteins that are important to building plants tissues which are reflected increasing the dry weight and plant growth and composing the effective components in different plant parts .(24). The organic liquid fertilizer (Vit-ORG) includes organic nitrogen (table 2) and it may be because of its effective role in activating the biological operations and especially photosynthesis which increase the amino acid which are part of preparing alkaloids and increase its percentage in the plant. This is similar to what was produced by Abdul Hussein *et al.*(25) on *Trigonell foenum-graecum*.

Table 7: Effect of sowing dates on percentage of fixed oil and total alkaloid of Ashwagandha during both seasons

Sowing date	fixed oil %				Total alkaloid %			
	Season (2013/2014)		Season (2014/2015)		Season (2013/2014)		Season (2014/2015)	
	Leaves	roots	Leaves	roots	Leaves	roots	Leaves	roots
D1	0.54	0.46	0.40	0.39	7.78	7.91	13.84	10.16
D2	0.57	0.54	0.47	0.47	6.66	8.78	14.63	13.39
D3	0.62	0.50	0.47	0.48	10.34	8.37	12.75	12.09
L.S.D.	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

* D1:-1 September, D2 :- 20 September, D3 :-10 October.

Table 8: Effect of organic liquid fertilizer (Vit-ORG)on percentage of fixed oil and total alkaloid of Ashwagandha during both seasons

Vit-ORG	fixed oil %				Total alkaloid %			
	Season (2013/2014)		Season (2014/2015)		Season (2013/2014)		Season (2014/2015)	
	Leaves	roots	Leaves	roots	Leaves	roots	Leaves	roots
V1	0.51	0.46	0.41	0.38	7.46	7.99	12.91	11.75
V2	0.65	0.54	0.48	0.52	9.06	8.71	14.57	12.01
L.S.D.	0.09	N.S	N.S	0.08	N.S	N.S	N.S	N.S

* V0 :- without Vit-ORG , V1:- with Vit-ORG (2 ml.l⁻¹).

Table 9: Effect of interactions between sowing dates and organic liquid fertilizer (Vit-ORG)on percentage of fixed oil and total alkaloid of Ashwagandha during both seasons

Sowing dates & Vit-ORG	fixed oil %				Total alkaloid %			
	Season (2013/2014)		Season (2014/2015)		Season (2013/2014)		Season (2014/2015)	
	Leaves	roots	Leaves	roots	Leaves	roots	Leaves	roots
D1V0	0.48	0.45	0.34	0.33	8.38	6.92	14.06	10.66
D2V0	0.50	0.49	0.44	0.34	4.83	7.62	13.25	13.40
D3V0	0.53	0.43	0.44	0.47	9.18	9.43	11.42	11.18
D1V1	0.59	0.46	0.50	0.46	7.18	8.89	13.62	9.66
D2V1	0.64	0.60	0.54	0.61	8.49	9.94	16.00	13.37
D3V1	0.71	0.58	0.59	0.50	11.51	7.31	14.08	13.00
L.S.D.	0.15	0.17	0.17	0.14	3.19	2.63	N.S	N.S

* D1V0:-1stSeptember without Vit-ORG ; D2V0 :- 20th September Vit-ORG ; D3V0 :-10th October without Vit-ORG ; D1V1:-1stSeptember with Vit-ORG ; D2V1 :- 20th September with Vit-ORG ; D3V1 :-10th October with Vit-ORG

CONCLUSION

The results showed that early sowing had the optimum yield and the following traits revealed the largest quantities: nutritional contents and fixed oil and total alkaloid of Ashwagandha . And can be concluded that organic liquid fertilizer (Vit-ORG)had safe of agricultural treatments to hazard undesirable impact of mineral fertilizers and had a favorable effect on growth and availability of chemical composition nutritional contents and fixed oil and total alkaloid of *Withania somnifera* (L.) seedling.



Photo (10) the experiment fields

Photo (11) *Withania somnifera* (L.)

REFERENCES

- [1] Meena AK, Singh B, Yadav AK, Singh U, Kaur R, Sachan A, Gautam V and Pal B, *J. Pharm. Res.*, **2010**, 3(4):866-868.
- [2] Kaileh M, Berghe WV, Boone E, Essawi T and Haegeman G, *J. Ethnol. Pharmacol.*, **2007**, 113: 510-516.
- [3] Williamson EM, Major herbs of Ayurveda. *Churchill Livingstone; London, UK*, **2002**, pp. 322-323.
- [4] Umadevi M, Rajeswari R, Rahale CS, Selvavenkadesh S, Pushpa R, Sampath KKP, and Bhowmik D, *T.P.I. Journal*, **2012**, 1 (9) : 102-110.
- [5] Ven Murthy MR, Ranjekar P K, Ramassamy C, and Deshpande M, *Central Nervous System Agents in Medicinal Chemistry*, **2010** 10 (3): 238-246.
- [6] Ahmad M K, Mahdi AA, Shukla KK, Islam N, Rajender S, Madhukar D, Shankhwar S N, and Ahmad S, *Fertility and Sterility*, **2010**, 94 (3): 989- 996.
- [7] Vishwanath RD, Chandra R, and Kumar R, *Research Journal of Agriculture and Biological Sciences*, **2010**, 6(4): 548-551.
- [8] Saleh AL, Abd El- Kader AA, and Hegab SAM, *Egypt .J. Appl. Sci.*, **2003**, 18 (12): 707 – 716.
- [9] Hussein WA, Bayan HM, and Noora JJ, *Journal of Tikrit University for Agricultural sciences*, **2009**, 9(2) : 381-391.
- [10] Hussein WA, and Majid BH, *Diyala Agricultural Sciences Journal* ,**2009**, 37 : 229-239.
- [11] Majid BH, *The Iraqi Journal of Agricultural Sciences*, **2010**, 41(4):1-7.
- [12] Abboud HJK, Thesis ,*College of Agriculture, Basra University, Iraq*. **2015**.
- [13] Page AL, Miller RH, and Keeney DR, Methods of soil analysis. Part 2 , 2nd Ed. *Medison Son, Wisconsin, USA*, **1982**, 1159 .
- [14] Olsen SR, and Dean LA, Phosphorus methods of soil analysis. *Amer .Soc . Agron . Monogr.*, **1965**, 90:1059-1063.
- [15] Stahl R, Thin layer chromatography, A laboratory handbook, 2nd . *Translated by Ashworth M.R. Springer, Verlag, Berlin.*, **1969**.
- [16] Harborne JB, *Phytochemical methods a guide to modern techniques of plant analysis*-springer netherlands. *Chapman and Hall Publishing, New York*, **1984**: 282p.
- [17] AL-Rawy KM, Kalaf Allh A, *Design and analysis of agricultural experiments National Library for printing and publishing , Mosul , Iraq*, **1980**, 488 p.
- [18] Almohamadi OHM, Thesis, *College of Agriculture, Bagdad University, Iraq*, **2009**.
- [19] Abd MM, Sad AAA, Jamal AA, *Journal of Babylon University of Pure and Applied Sciences*, **2012**, 20(3)1087-1098.
- [20] Farhan HN, *Anbar Journal of Agriculture Sciences*, **2008**, 6(1), 144-163.
- [21] Appireddy G K, Saha S, Mina BL, Kundu S, Selvakumar G, and Gupta HS, *Arch. Agron. Soil Sci.*, **2008**, 24: 127- 137.
- [22] Saleh AL, Abd El- Kader AA, and Hegab SAM, *Egypt .J. Appl. Sci.*, **2003**, 18 (12): 707 – 716.

- [23]Sa'adEldenShMK, Adel YN, and Ali A,*Iraqi Journal of Agricultural Sciences*,**2005**,36(1):89-96.
- [24] Kakaraparathi PS, Rajput DK, Komaraiah K, Kumar N, and KumarRR, *Journal of Scientific Research & Reports*,**2013**, 2(1): 121-132.
- [25]Almously MAD,*Thesis, College of Agriculture, Mouosul University, Iraq*,**2005**.
- [26]Abdul Hussein TM, AljbouyRKh, and Sa'ad AA, *Euphrates journal of Agricultural ciences*,**2013**,5(4):104-112.