

Effect Addition method of Organic Fertilizer(Humic Acid) on Growth ,Yield and Active Ingredients of *Allium sativum L.*

Dr.Ayyub J.Abdl-Rahmaan Abayaty

Department of Biology,College of Women Education,Tikrit University

Abstract

A field experiment was conducted in College ducation for Women / Tikrit University during 2017 – 2018 season to study the effect of two addition method sprying the plant and sprying the soil of organic fertilizer humic acid with four concentration (1ml/l,2ml/l,3ml/l,4ml/l in addition of control(without treatment) on the growth , yield and active ingredients of *Allium sativum* , The experiment was factorial according to split plot design, addition method take the main plots while the concentration take the secondary plots with three replication ,results showed sprying the soil with humic acid led to significant increase on vegetative growth and yield characteristics and high value gained by spring the soil with concentration 4ml/l on compar with plant spray treatment.Analysis by using HPLC teqnique appeared existence many active ingredients on the leaves and cloves of plant as: S-allyl cysteine, Y- glutomy l cysteine, Allicin, Vinyl- {4H} 1.2dithlin (agoene), di-allyldisulfide and Diallyltrisulfide and higher percentage from allicin compound were at soil spray method with 3ml/l humic acid reached 54.59%.

1. Introduction

In the last years focus was on adoption on agricultural practices in particular fertilization with modern techniques and correct environmentally thus ensuring high productivity and quality products and reduce negative impact on environment [1],chemical fertilizer has great harm both on human health throw big increase on elements concentration and some compounds in the plant which adversely affects on human health,also throw increasing chemical fertilizer had adwers effect on soil useful organism and degradation of soil properties and resulting economic losses,all this causes urge specialists in agriculture to rely on organic fertilizers [2],the modern studies proved particularly the resulting danger of the remaining trace of nitrate which is considered from more dangerous compounds on human health [3],the chemical fertilizer dangerous comes from it doesn't metabolism on the body and its fixation on alkloidal soil and the higher its price [4] therfor global attention focus on organic fertilizers for many crops and vegetables [5],and organic agriculture concedred from the most important environmental regulations which restore for the environment its balance and provides health production wanted for human nutrition [6],verily using humidifiers and its acids as humic acid concider one of organic nutrition kind [7].Humic acid fertilizer is one of the decomposing materials produced from organic materials [8],assist to improve absorption of nutrients, create a good soil environment,improve plant ability on water retention and stimulate growth and activity soil microorganism particularly the responsible of ferment organic matter [9].

The chemical nature of humates is complex and depend to the source which consists of [10],and it origin from physical and biochemical transformation of living molecules and it has many types of salts as calisium hu mates,sodium humates,amonium humates and potassium humates [11] it improve soil quality and productivity on addition improving soil physical properties , And retention of soiland increasing interchangeable capacity which is important for soil fertility [12].

Allium sativum consider second important vegetative crops of Alliaceae family after onion crop [13] ,and its important winter vegetative crops on Iraq and the world for its high nutrition value , the frequent uses and its many benefits [14].the lobes contain 31% carbohydrates,6.29% proteins and its rich with elements as phosphorus, iron,potassium,magnesium, vitamins as riboflavin,niasin and ascorbic acid and it contain the most important compound Allicin which inhibitor growth of many numbers of bacteria,fungi

and viruses [Ankar and mirelman [15], [16] studies refer to superior organic fertilizer on growth properties of Califlower [17] and treatment with humic acid gave high yield from *Allium sativum* reached 3.38 t/do. And increasing its content from fatty acids [18].

2-Materials and methods:

A field experiment conducted using plastic pots 30cm diameter and 30cm high during the season 2017-2018 using sandy silty soil treated with compound fertilizer N,P,K (20,20,20) the planting date were 15/10/2017 each treatment contain 5 plant to study effect of two method addition spraying plant and spraying soil with organic fertilizer (humic acid) in growth, yield components and the contents of active constituents of garlic plant, using factorial experiment in CRD design. The first factor were spraying the plant with by concentration (1m/L, 2m/L, 3m/L, 4m/L) in addition to control treatment, the second factor were spraying the soil with same concentration, the number of treatment were 10 with three replication. Harvesting of crop were 10/5/2018 and the data taken on the harvest date as range three plants, to study growth properties: plant high (cm), leaves number, plant-1, stem diameter (cm), Total chlorophyll and yield properties: Head diameter (cm), lobes number, head-1, lob weight (gm), head weight (gm) and active constituent of the plant.

2.1. Chlorophyll determination: wet weight (0.5) gm from leaves put on dark glass bottle then (20) ml acetone by 80% concentration added and left for 24 hours the process replicate more than once to extract chlorophyll completely, the size of extract reach (50) ml and chlorophyll estimated according [19] through reading the extract absorption on the wave length (645-663) nm by spectrophotometer apparatus.

2.2. Determination of active ingredients of plant: Active ingredients on lobes of plant determined by using High Performance Liquid Chromatography (HPLC) technique. The plant dried at the shade and milled then 10 gm from the sample put in 50 ml boiled water (90-100°C) for 3 hours then extracted with Whatman papers no. 1 the extraction collected and put in closed glass tube [Weerachang & Dung 1998] [20] in order measuring the concentration of active ingredients by (HPLC) apparatus at condition show in (table 1) and the data recorded by calculator which drawed the peak area and retention time. A standard solution of *Allium sativum* plant used and separated by HPLC apparatus and identification the peak area and retention time of standard solution and comparing it with the peak area and retention time of studied plant sample at the same condition [21].

Concentration of compounds in the sample calculated by the equation:

$$\text{Conc. Compound in the plant} = \frac{\text{peak area of compound}}{\text{peak area of standard pattern}} \times \text{standard pattern conc.} \times \text{delution factor}$$

Table 1. Chromatographic separate condition.

Colum	Mobile phase	Flowing rate	Type of detector	Temperature	Fast of recorder paper	Size of injected sample
Reverse phase column (50×4.6 mm I.D)	Distill water : Ethanol 70% 2: 80v/v	10 ml/min.	Ultra violate ray 250 nm	25C ⁰	10 mm/min	20µl

The means compared according Duncan test at significant level 5% using program [22].

3. Results and discussion:

The results of table (2) significant effect of treatment with humic acid on the growth properties in compar with control treatment also their were difference between method of uses and the used concentration,all the vegetative parameters increased with increasing humic acid concentration when spryed the plant and sprying the soil,the highest values when sprayed the soil with 4m/l concentration reached 77.26,14.58,1.70 and 24.10 for parametrs plant high (cm),number of leaves.plant-1,stem diameter(cm) and total chlorophyll (mg/g) respectively,while the least values were on control treatment when plant spry method reached 30.42,6.0 ,0.80 and 20.11 for plant high (cm),number of leaves.plant-1,stem diameter (cm) and total chlorophyll(mg/g) respectively.

table (3) appear the significant effect of method of treatment and the effect of the difference between the concentration on the yield parameters.The highest values were in the sprying soil method by 4m/l concentration reached 3.81,12.0,5.30 and 55.70 for the parameters head diameter (cm),number of lobs.plant-1,lob weight (gm) and head weight (gm) respectively.,while the least values on the the same method were on control treatment reached 2.80,7.10,1.88 and 18.0 for the parameters head diameter (cm),number of lobs.plant-1,lob weight (gm) and head weight (gm) respectively. The reason of the increasing on vegetative and yield parameters may due to to the role of organic fertilization(humic acid) on imoroving soil bio ,chemical and physical properties and increasing its ability to absorption water and increasing its content from minor nutrient elements specially nitrogen,phosphorus and potassium[23],and the increasing on nitrogen concentration led increase on formation chlorophyll pigments and this increase photosynthesis process[24],also humic acid affect on exchange capacity which led to increase the nutrient element and improve soil texture and this easing root growth inside the soil which led to absorption more of water and elements and this affect on vegetative group as plant hight and other vegetative and yield paramete[25] [26].the analysis of humic acid gave carbonic acid which is weak acid analysis to ion(H⁺) and (HCO₃⁻) and this contribute on change soil (pH)assisting on desolving some macronutrients mineral elements as P,K,Mg,Ca and available some micronutrients and this absorbed by the plant and increasing plant physiology action which reflect possitvly on both growth and yield parameters.[27]

The analysis of standar patterns of the *Allium sativum* plant by HPLC apparatus appeared verily the plant contain several compounds: S-allyl cysteine, Y-glutomyl cysteine, Allicin, Vinyl-{4H}1.2dithlin (agoene), di-allyldisulfide and Diallyltrisulfide (Figure 1 and table 5), while analysis studied plant by HPLC teqnique showe higher contain the plant from allicin compound reached 54.59% when soil spraying method with concentration 3ml/l from humic while.

Table 2.Effect of treatment method with humic acid on vegetative properties of *Allium sativum* L. Properties

properties treatment		plant high(cm)	Number leaves.plant-1	Stem diameter (cm)	Total Chlorophyll
soil spry	0	42.41e	5.26d	0.80c	20.0c
	1ml/l	53.85d	7.24c	1.13b	21.39bc
	2ml/l	67.58c	12.04b	1.90a	22.60b
	3ml/l	72.30b	12.90b	1.90a	22.60b
	4m/l	77.26a	14.58a	1.70a	24.10a
plan spry	0	30.42d	6.0cd	0.70cd	20.11b

	1m/l	50.48d	7.01c	0.80c	21.12b
	2m/l	65.49c	11.01b	1.00b	21.40b
	3m/l	70.11ab	11.10b	1.08b	23.0a
	4m/l	73.13a	13.0a	1.20a	23.90a

* Same later on one column mean no significantly difference at the level 5%.

Table 3. Effect of treatment method with humic acid on growth components of *Allium sativum L.*

properties		Head diameter(cm)	Number lobes.head-1	Lob weight(gm)	Head weight(gm)
treatment					
soil spry	0	2.81bcd	7.01cd	1.91dc	17.17de
	1m/l	3.01cd	8.15bc	2.00bc	19.45d
	2m/l	3.11abc	8.21bc	3.13b	32.30c
	3m/l	3.32ab	9.0b	3.60b	40.04b
	4m/l	3.81a	12.0a	5.30a	55.70a
Soil spry	0	2.80bcd	7.10bcd	1.88de	18.0e
	1m/l	3.10cd	8.25bc	2.02cd	20.45de
	2m/l	3.21abc	8.61bc	3.33bc	35.31c
	3m/l	3.44ab	10.30abc	4.12ab	45.60b
	4m/l	3.55	11.51	4.83	50.12

* Same later on one column mean no significantly difference at the level 5%.

analysis by HPLC apparatus proved its activity in fast on diagnosis this compounds through its ability on calculating the curve with its high and determine active ingredients in one operation [35] It is also has the advantage in compare with other methods such as GC by ability on the dealing with non volatail materials including inorganic ions and thermally stable materials [36], results of study agree with [24][37] whom refers To contain A. sativum to Allicin compound and agree with [38] whom confirmed contain this plant to sulfide compounds.

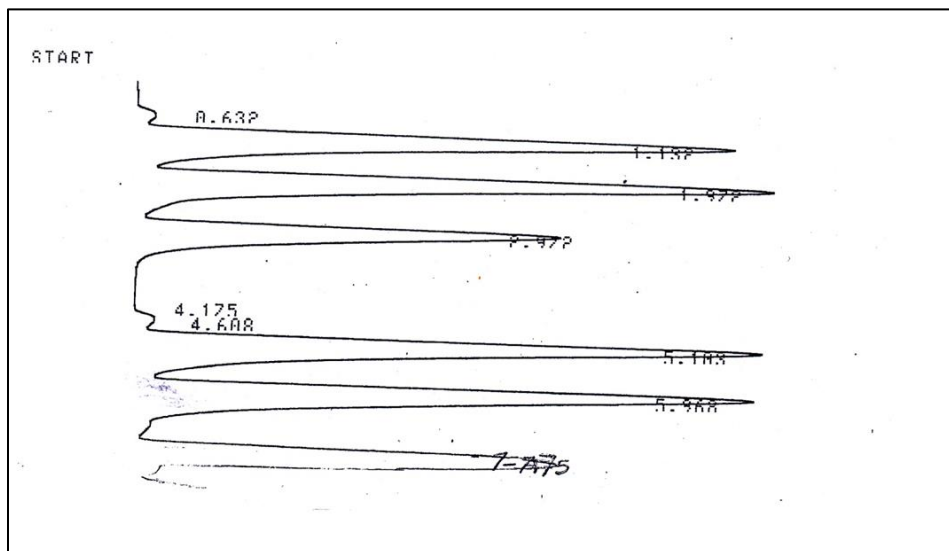


Figure 1.Retention time and Peak area of *Allium sativum* plant standard pattern.

Table 4.Compounds ,Pick area and the concentration of *Allium sativum* plant standard solution

Compounds	Retention time	Peak Area	Concentration(mg/ml)
S-Allyl cystine	1.22	30583	25
Y-Glutomyl cysteine	1.95	32646	25
Allicin	2.99	24533	25
Vinyl-4H-1,2 dithilin(Agoene	5.20	35445	25
Diallyl disulfide	5.88	34993	25
Diallyl trisulfide	8.07	21989	25

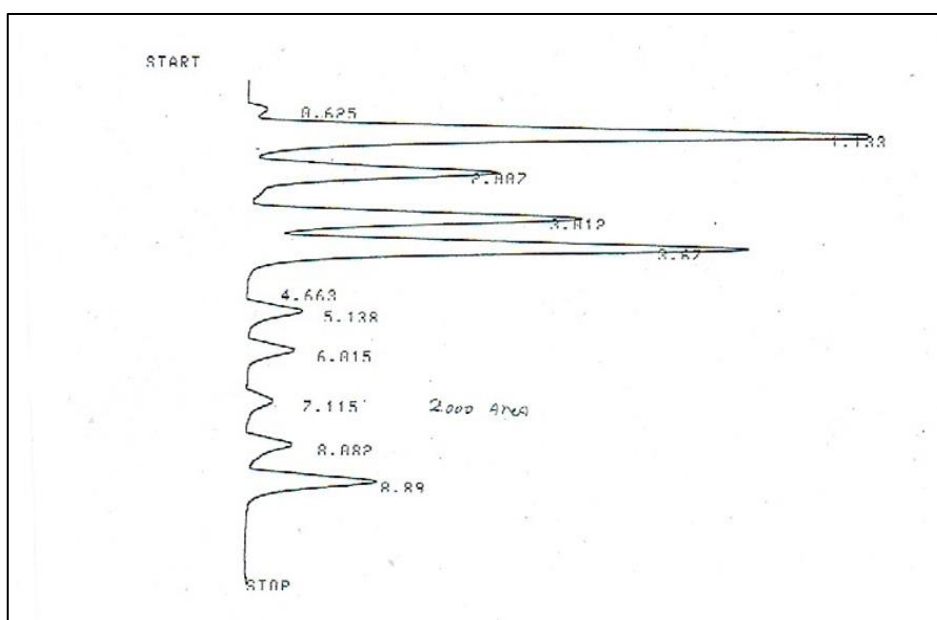


Figure 2.Retention time and Peak area of *Allium sativum* studied plant.

Table 5. effect of treatment method with humic acid on active ingredients concentration (%) of studied *Allium sativum* L. plant. Compounds

properties		Head diameter(cm)	Number lobes.head-1	Lob weight(gm)	Head weight(gm)
treatment					
soil spray	0	2.81bcd	7.01cd	1.91dc	17.17de
	1ml/l	3.01cd	8.15bc	2.00bc	19.45d
	2ml/l	3.11abc	8.21bc	3.13b	32.30c
	3ml/l	3.32ab	9.0b	3.60b	40.04b
	4ml/l	3.81a	12.0a	5.30a	55.70a
Soil spray	0	2.80bcd	7.10bcd	1.88de	18.0e
	1ml/l	3.10cd	8.25bc	2.02cd	20.45de
	2ml/l	3.21abc	8.61bc	3.33bc	35.31c
	3ml/l	3.44ab	10.30abc	4.12ab	45.60b
	4ml/l	3.55	11.51	4.83	50.12

* Same letters on one column mean no significantly difference at the level 5%.

References:

- [1] Chen, Y. and Avid, T. (1990). Effect of humic substances on Plant growth. Pp.161-186. In: American Society of Agronomy and Soil Science Society of America (eds.), Humic substances in soil and crop science; selected Readings. American Society of Agronomy, Madison, WI.
- [2] Al-Zahawi, S.M.A. (2007). Effect of different organic fertilizer and mulching on growth, production and quality of *Solanum tuberosum* L. Ms. theses, college of Agriculture, Baghdad University, Iraq.
- [3] Othman, J.Y. (2007). Study effect using organic fertilizer on culturing and production of *Solanum tuberosum* L. as input on clean organic production. Ms. theses, college of Agriculture, Narain University, Syria.
- [4] Al-Tememe, J.Y. (2009). Effect of humic acid and seaweed extracts on growth and chemical properties of *Rosemarinus officinalis* L. sixth conference of biology dept. College of Education, Tikrit University, Iraq. 1-17.
- [5] Willer, H. and Yusessefi, M. (2006). The word of organic Agriculture Statistics and emerging trends 2006 International Federation of Organic Agriculture Movement (IFOAM), Bonn, Germany. And Research Institute of Agriculture (FIBL), Frick, Switzerland.

- [6] Mohmmad,R.S.(2002).Comparision organic agriculture with classic agriculture on production Cucmis sativus L.and soil fertilization. Ms.theses,college of Agriculture,Baghdad University.Iraq .
- [7] Al-Tememe,J.Y.and Bas ma,H.A.(2009).Effect of organic fertilizer and chelating iron on vegetative growth properties of Mentha piperita L.Tikritv J.Agric. Sci.2(14):181-187.
- [8] Leonard, A. G. (2008). Humic Acid: 100% Natural, Many Uses. Golden Harrest Organic., LLCTM.
- [9] Al-Gartany,A.A.,&Al-Saamray,A.A.(2008).Effect of biofertilizer with fungi Glomus mosseae and Trichoderma hazianum and organic fertilizer humic acid and their combination on balance N,P,K on Lycopersicon esculentum Mill. Tikritv J.Agric. Sci.2(8):256-280.
- [10] Eladia,M.,Pena,M.,Josef,h.,and Jiri,P.(2005).Humic substances application in Agriculture,industry,environment,and biomedicine J.apple.BIOMED.3:13.
- [11] Buckan, G. , Hooker , P. ,and Moulin , V. (2000). Versatile companents of plant soils and water In : Ghabbour E.A. and Davies G.(eds): Humic Substabces ,RSC,Cambridge.
- [12] Zhang,M.and He.Z.(2004).long-term Changes in organic carbon and nutrition of an Ultisol under rice cropping in southeast China Geoderma, 118:167-179.
- [13] Al-Shammary,A.M.&Al-Mfargi,O.Kh.(2015).Effect of organic and chemical fertilization and their combination on growth and yield for two garlic cultivars of Allium sativum L.Diala J.Agric. sci.2(7).
- [14] Hasan,A.A.(2007).Bioteqnlology and plant breeding.Adar Alarabia for publication and distribution.Egypt.
- [16] Al-Bayaty,A.J.;Abdulraheem.A.A&Altef.R.H.(2018). Determain kind and concentration of Heliotropium suaveolens,Plantago major and Silybum marianum plants ingredients and its effect on some plant pathogenic fungi IOP Conf. Series: Journal of Physics: Conf. Series 1003 (2018) 012008
- [17] Abbas,Kh.;Hadi,Y.;Hussen,J.&Kahtan,A.J.(2013).Effect of sewage Sludge,Humic acid and mineral sepray of some traits of Califlower.Furat J.Agric Sci.5(4):316-323.
- [18] Zahwan.T.A.;Nadhim,A.A.&Ziyad,Kh.S.(2013).Effect of humic acid and nutrient solution prosol in growth ,yield garlic Allium sativum L.and content from fatty acid.Diala J.Agric. Sci.5(1):159-169.
- [19] Bajracharya,D.(1999).Experiments in plant physiology.Narosa publishing Host.New Delhi,India.
- [20] Weerachai,P.and Duang,B.(1998).simple isolation and purification of glycyrrhizic acid. J.Sci.Fac.Cmu.25(2):87-91.
- [21] Nishizawa,H.;Okimura,S.and Abe,Y.(1991).Application liquid particle extraction to the purification of glycyrrhizin.Chemical pharm.Bull.39.969 - 971.
- [22] SAS.(2001).Users Guide,Statistics(Version 6.121)SAS.Inst.Cary,N.C.USA.
- [23] Al-Sahhaf,F.H.and Aaty,A.S.(2007).Effect of organic on growth and yield of tubes and its quality.Iraq,J.Agric sci48(4):65-82.
- [24] Al-Sahhaf,F.H.(1989).Natural plant nutrition.Baghdad Unuversity.Ministry of Higher Education and Scientific Research.Iraq.
- [25] Sarheed,M.M.(2012).Effect of addition organic fertilization to soil and spray with extract of sea herbbs Kelpak and Ultra kelpak 40 on growth and active ingredients of Apium graveolens L. Ms.theses,college of Agriculture,Tikrit University.Iraq .
- [26] Omar,T.J.and Salih,Sh.M.(2018).The effect of humic acid and sea algae extracts on the growth ,for Lepidium sativum and aloe vera plants . Tikritv J.Agric. Sci.18(3):22-32.
- [27] Al-Tememe ,J.Y.and Al-Dory,T.O.(2011).Effect spraying micronutrient on growth and chemical contents and active ingredients of Apium graveolens L. seventh conference,Bio.Dept. Colli.Education,Tkrit University.
- [28] Al-Hedwony, A.K.H. 2004. Effect Fertilization and foliar application of some nutrient elements on quantitative and qualitative of some active compounds in seed two kind of (Trigon olla foenum) Ph.D Theses. Col. Agric. Univ. Baghdad, Iraq.
- [29] Settle, F.A. 1997. Handbovk of instrumental techniques for analytic chemistry. N.J . Prentic Hall PTR:945
- [30] Tsao, S.M.; Yin, M.C. 2001. Invitro antimicrobial activity of four diallyl sulphides occurring naturally in garlic and chinese leek oils. J. Med. Microbial, 50:646-649.
- [31] Ankri, S. and Mirelman, D. 1999. Antimicrobial properties of allicin from garlic, Microbes and Infect, 2: 125-129.
- [32] Lawson, L. D.; Wood, S.G. and Hughes, B.G. 1991. HPLC analysis of allicin and other thiosulfates in garlic clove homogenates . Planta Med. 57(3): 263-70.