



## Effect of Humic Acid and Powders of Some Plants Producing Allelopathic Compounds on Soil Properties and Productivity of Faba Bean

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### Authors' contributions

This work was carried out in collaboration among all authors. Author AFA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AAF and AEMA EK managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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### ABSTRACT

This investigation was conducted at Shandaweel Agricultural Research Station Sohag Governorate during two successive growing seasons 2015/16 and 2016/17 to study the effect of using humic acid (200 mg kg<sup>-1</sup> soil), powder of some plants for producing allelopathic compounds (Powder at rate 10 gm kg<sup>-1</sup> soil of *Datura stramonium*, *Euphorbia geniculata*, *Trifolium*, *alexandrinum*) on soil properties and some physiological aspects in faba bean plants in addition to faba bean yield and broomrape retarding. The results demonstrated that, for all used treatments, a decrease in the bulk density and increase in total porosity, soil infiltration rate and hydraulic conductivity due to the increases in total porosity of the soil. Water holding capacity, field capacity and wilting point also increased, and this improved the hydro-physical properties of the soil. Chemical properties of the soil represented in pH were slightly lowered, while noticeably increased in cation exchange capacity, organic matter and available macronutrients content in the treated soils. Faba bean yield represented in number of pods, weight of pods, seeds weight (g) all / plant and 100-seed weight and seed yield (ardeb /fed) were increased compared to control. Photosynthetic pigments, proteins and carbohydrates contents in shoots and roots were higher in plants grown in the treated soil compared with untreated, while, total free amino acids and proline contents in shoots and roots. The analysis of allelopathic plants powder, indicated that these plants contains some acids and phenolic compounds in there shoots such as Vanillic, ferulic, syringic, p-coumaric, p-hydroxybenzoic, caffeic and protocateic acids.

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## 1. INTRODUCTION

Humic acids (HAs) comprise a mixture of weak aliphatic and aromatic organic acids which are soluble in water under alkaline conditions but are not soluble in water under acidic conditions. HAs consist of fraction of humic substances that are precipitated from aqueous solution when the pH is decreased below 2. Because of its molecular structure, it provides numerous benefits to crop production. Kononova [1] and Fortun et al. [2] had shown that the humic fractions (HA, fulvic acid and humin) of the soil organic matter are responsible for the generic improvement of soil fertility and improved productivity. HA also improve soil structure and change physical, chemical and biological properties of soil, promote the chelation of many elements and make these available to plants, enhancement of photosynthesis density and plant root respiration has resulted in greater plant growth with humate application, Smidova [3]; Chen and Avid [4]; Chen et al. [5]; Nardi et al. [6]. When HA and fulvic acids are applied to the soil, enhancement of root initiation and increased root growth was observed, Pettit [7]. The stimulatory effects of humic substances have been directly correlated with enhanced uptake of macronutrients, such as N, P and S as well as micronutrients, that is, Fe, Zn, Cu and Mn, Chen et al. [8]. Li ZR et al. [9]. Another study investigated that *Veronica persica* (Lour) Merr. had effective herbicidal activity; thus, a safer herbicide from allelochemicals can be developed. Humic substances may possibly enhance the uptake of minerals through the stimulation of microbiological activity, Mayhew [10]. Still very little work has been done in the direction of utilization of HA in crops and vegetables production and its effect on the plants which cultivated in soil infected by herbs especially the parasitic type as broomrape.

Allelopathy will become an important component in the development of future integrated weed management strategies. Kocacaliskan et al. [11] stated that the chemical interactions that occur among living organisms including plants, insects and microorganisms are called allelopathy, and the organic compounds involved in allelopathy are called allelochemicals. Incorporating allelopathy into agriculture management may reduce the use of herbicides, which cause less pollution and diminish autotoxin hazards. In practice, the allelopathic properties of plants

might be exploited by growing specific crop varieties, mixing certain crop residues with the soil and by using isolated allelochemicals as natural herbicides to reduce the impact of synthetic chemicals up on the environment, Alam et al. [12]. In future, worldwide there will be an increase in demand for better quality food and in large quantity due to the increasing of human population. Therefore, for sustainability of agriculture, we need to minimize the use of present pesticides (weedicides, insecticides, nematocides, fungicides), to control pests (weeds, insects, nematodes, diseases) in field crops, through use of allelopathic strategies for pests management. Rice [13] revealed that production of allelochemicals varies with environment and associated environmental stresses. It can occur in any plant organ, but roots, seeds and leaves are the most common sources. Leaves are the major source in most species. The location of the source is much less important than the total amount released. Olefosdotter et al. [14] mentioned that allelopathic rice causes severe root growth inhibition of *Echinochloa crus-galli* and, in strongly allelopathic cultivars, pruning of the root tip of the weed. Zeng et al. [15] indicated that there is no remarkable change in the soluble proteins of plants that are treated with secalonic acid F (major allelochemical produced by *Aspergillus japonicus*). Blum [16] reported that caffeic, ferulic, p-coumaric, protocatechuic, sinapic, caryogenic and vanillic are phenolic acids that had a primary allelopathic effects on plant process they reduce rates of photosynthesis, carbon allocation to root. Said-Al Ahl et al. [17] showed that foliar spray of humic acid enhanced plant growth, yield, and quality in several of plant species due to its action on different physiological and metabolic processes. Ali [18] stated that *Cyperus rotundus* tuber and foliage contained different flavonoids and phenolic compounds such as rutin, kaempferol, quercetin, myricetin, p-hydroxybenzoic, chlorogenic acid and ferulic acids. Uludag et al. [19] found that allelopathic compounds producing from tobacco, sunflower, chickpea, lentil, wheat, maize and cotton decreased the number of *Orobancha ramosa* tubercles up to 68% on tomato plants experiments. Khanh et al. [20] found that numerous phytotoxins such as cytokinins, diterpenoids, fatty acids, flavones, glucopyranosides, indoles, momilactones (A and B), oryzalexins, phenols, phenolic acids, resorcinols and stigmastanols have been

identified and determined as growth inhibitors in rice.

This study aiming at the impact assessment of HA alone or with some powders of plants application on soil physical and chemical properties especially nutrient availability and uptake by faba bean. Moreover, this study was undertaken to choose the right type of these powders and quantifying some of the benefits that could be realised from using some powders of plants for producing allelopathy on control broomrape in faba bean.

## 2. MATERIALS AND METHODS

Two pot experiments were carried out at Shandaweel Agricultural Research Station Sohag Governorate during winter seasons at 2015/16 and 2016/17 to study the effect of HA and powder of some plants for producing allelopathic compounds on soil physical and chemical properties, yield of faba bean and control broomrape. In plastic pots, HA was added at a ratio of soil weight by 200 mg kg<sup>-1</sup> soil, powder of plants (*Trifolium alexandrinum*, *Datura stramonium* and *Euphorbia geniculata*) at rate 10 g kg<sup>-1</sup> soil, and untreated soil as control. Powder was taken from the full of plants with inhibitory effect of *Orobancha* (broomrape) growth and added mixing with the soil cultivated with beans.

Faba bean Fabaceae (*Vicia faba* cv Giza 843) was employed as test plants during all experiments conducted in this study. Faba bean were planted in 23<sup>th</sup> and 28<sup>th</sup> of November in both seasons, respectively. The used pots were 40 cm in depth 30 cm in diameter and filled with 10 kg of the used soil then planted by 5 plants of faba bean using of complete randomized design with six replications.

### 2.1 Analysis of Soil

The main analytical data of the soil are presented in Table 1. Soil samples were taken before planting faba bean and at the end of the experiment (after 160 days from plantation) and analysed for physical and chemical characteristics as follows:

### 2.2 Mechanical Analysis

For soil mechanical analysis, the pipette method described by Richards [21] was followed.

### 2.3 Chemical Analysis

**Organic matter:** OM was determined by the method of Walkley and Black [22].

**Soil pH:** Soil reaction was in a 1:2.5 soil-water suspension using a pH meter apparatus. **NPK content:** Total nitrogen content of treated and untreated soils was determined after digestion with H<sub>2</sub>SO<sub>4</sub>-HClO<sub>4</sub> mixture (Jackson) [23].

**Total soluble salts:** Total soluble salts were determined in a 1:2.5 soil-water extract by measuring the EC of solution (Jackson) [24].

**Total calcium carbonate:** Total calcium carbonate was determined according to Page et al. [25].

**Available potassium** was extracted using 1 M neutral CH<sub>3</sub>COONH<sub>4</sub> and measured with Atomic Absorption Spectrophotometer (Perkin-Elmer, 3300). **Available P** was extracted with NaHCO<sub>3</sub> and measured as described by Olsen and Sommers [26].

**Pore spaces:** The analysis of pore spaces was done according to Deleenher and Boodt [28]. In soil samples, the pore spaces were classified to quickly drainable, slowly drainable, volume drainable water holding and fine capillary pores.

**Hydraulic conductivity:** Hydraulic conductivity was determined by using soil samples according to Richard [21] Values of hydraulic conductivity were calculated by using Darcys low:

$$Q/AT=K \times \Delta H/L.$$

Where Q = the volume (cm<sup>3</sup>) of water passing through the soil column at time t, and A is the cross-sectional area of the soil column.

L=the length of soil column.

H/L=hydraulic gradient.

K=the hydraulic conductivity (in terms of length/time).

**Total porosity:** Total porosity was calculated as percentage from the obtained values of bulk densities (Richard) [21].

**Broomrape:** The number and dry weight of broomrape/pot as well as number of days for broomrape emergence above soil surface were determined before faba bean harvesting.

**Table 1a. Mechanical analysis of soil**

Sand	Silt	Clay	Soil texture
Fine 20-200 $\mu\%$ 30.36	2-20 $\mu\%$ 31.51	<2 $\mu\%$ 38.13	Clay loam

**Table 1b. Chemical analysis of soil**

pH 1:2.5	EC 1:5 (dS/m)	CaCO <sub>3</sub> (%)	CEC (c mol/kg)	OM (%)	Available macro-nutrients (ppm)					Cation Na	Anions		
					N	P	K	Mg	Ca		Cl	HCO <sub>3</sub>	SO <sub>4</sub>
7.82	1.42	8.6	7.82	1.57	49.60	25.80	75.14	9.60	10.11	3.79	1.90	1.23	8.5

CEC: Cation exchange capacity; OM: Organic matter

**Table 1c. Hydrophysical analysis of soil**

Bulk density (%)	Total porosity (%)	Water-holding Capacity (%)	Field Capacity (%)	Wilting percentage	Hydraulic Conductivity (m/day)	Mean diameter of soil pores ( $\mu$ )
1.414	25.53	31.29	19.35	7.11	7.4	6.9

WHC: WHC was determined using the standard methods outlined by **Dewis and Freitas** [27]

Bulk density: Bulk soil density values were determined by using the soil column according to **Richard** [21]

**Faba bean yield and its components:** At harvest, five plants were taken to study the following criteria: plant height (cm), number of pods/plant, weight of pods/plant (g), weight of seeds/plant (g), 100-seed weight/plant (g) and seed yield (ardab/fed) was calculated as well as, to carry out the chemical analysis as following criteria:

**Photosynthetic pigments:** (chlorophyll a, b and carotenoids) of the plants were determined using the spectrophotometric method recommended by Metzner et al. [29].

**Carbohydrates:** Soluble, insoluble and total carbohydrates were determined by the anthrone sulphuric acid method described by Fales [30].

**Proteins:** The soluble, insoluble and total proteins were determined according to Lowry et al. [31] using bovine serum albumin as standard.

**Proline:** It was extracted according to the method of Bates et al. [32].

**Free amino acids:** They were extracted from the plant tissues and determined according to the method of Moore and Stein [33].

**Phenolic acids:** Vanillic, ferulic, syringic, p-coumaric, p-hydroxybenzoic, caffeic and protocateic. Phenolic acids were separated by Shimaduz (Kyoto, Japan) HPLC apparatus (model, LC-4A) equipped with visible / UV detector (model, SPD-2AS) at 280 nm and stainless steel column (25.0 cm × 4.6 mm i.d.) (Phenomenex Co., USA) coated with ODS, (RP-18). An aliquot of the sample suspended in MeOH was diluted with 10 mM phosphoric acid buffer (PH3.5) to the same concentration as initial mobile phase (15% MeOH). Samples were next filtered through a 0.2 µm poly (tetrafluoroethylene) (PTFE) filter prior to injection. The two solvent systems consisted of MeOH (A) and 10 mM phosphoric acid buffer, PH 3.5 (B), operated at follow rate of 1.5 ml/min. The phosphoric acid buffer consisted of 10 mM  $\text{NH}_4\text{H}_2\text{PO}_4$  adjusted to pH 3.5 with 10 mM  $\text{H}_3\text{PO}_4$ .

**Statistical analysis:** Obtained results were subjected to the proper statistical analysis according to Snedcor and Cochran [34] and the treatments were compared by L.S.D. at 0.05 level of probability.

### 3. RESULTS AND DISCUSSION

#### 3.1 Soil Hydrophysical and Chemical Properties

Nowadays, the use of HA and powder of some plants as soil conditioners is still limited research. Humic acids are an important soil component that can improve nutrient availability and impact on other important chemical, biological, and physical properties of soils (Khaled and Fawy) [35], so that, the using of HA as conditioners is a way to solve soil problems mainly nutrients availability and water limitation. With regard to the use of allelopathic plants powder is a way to solve problems of broomrape infection. In this study, HA and powder of some plants were mixed with soils and investigated carefully for their effect on soil physical and chemical properties. The analytical data of the used soil was estimated before plantation and the data were presented in Table 1. These results show that the soil used in this experiment suffers from a lack of nutrients, in addition to that this soil contains a small proportion of organic matter and these soils tend to alkalinity. In the same way regarding the hydrophysical properties of these soil, the results showed that the soil is poor in these characteristics.

#### 3.2 Soil Hydrophysical Properties

The enhancement effect of HA and powder of plants on some hydrophysical properties of tested soil was estimated and the data were presented in Table 2. It is believed that addition of HA and powder of plants induced substantial changes in the physical properties of the soil.

##### 3.2.1 Soil bulk density

Organic matter reduces soil bulk density through increasing aggregation. Data in Table 2 show that, bulk density of the soil was influenced by treating the used soil with HA and powder of plants. The values of soil bulk density of used soil treated by HA or powder of plants or both of them together were relatively lower than those of control, and the maximum decrease exists in case of HA mixed with *Datura stramonium* compared to other treatments and control. These results are in agreement with the results of Amlinger et al. [36] who observed that compost application influences soil structure in a beneficial way by lowering soil density as a result for the admixture of low density organic matter

into the mineral soil fraction. In addition, the organic fraction is much lighter in weight than the mineral fraction in soils. Accordingly, the increase in the organic fraction decreased the total weight and bulk density of the soil, (Brown and Cottone) [37]. This positive effect has been detected in most cases and it is typically associated with an increase in porosity because of the interactions between organic and inorganic fractions.

### 3.2.2 Total soil porosity

Total soil porosity is a special formula which explains the relationship between both the soil real and bulk densities. On the other hand, it is an index of the relative volume of pores in soil. Data in Table 2 indicated that the values of total soil porosity increased in soil treated with HA and powder of plants at any treatment compared to control, where the highest value was found in the treatment of HA mixed with *Trifolium alexandrinum*. These results agree with those of Vengadaramana et al. [38]. Similar results were obtained by Oo et al. [39] who reported that the use of organic amendments resulted in substantial flocculation and the formation of many soil aggregates. As a consequence aggregate stability, soil porosity, water infiltration, and water-holding capacity of soil are improved, which result in minimizing the impact of drought.

### 3.2.3 Soil hydraulic conductivity and infiltration rate

Hydraulic conductivity refers to the rate at which water flows through soil. For instance, soils with well-defined structure contain a large number of macropores which allow for relatively rapid flow of water through the soil. In our results the values of hydraulic conductivity and infiltration rate were increased by adding HA and powder of plants (Table 2). The highest values of hydraulic conductivity and infiltration rate were observed by applying HA combined with powder of plants together compared to control treatment or using each of them without the other. Patrick [40] mentioned that soil hydraulic conductivity in saturated soil matrix depends mainly on the soil structure, which can be described in terms of spatial distribution of pore spaces. On the other hand, Tayel and Abdel Hady [41] reported that soil EC and pH had a higher direct effect on hydraulic conductivity value through negative relationship and described on the base of soil alkalinity.

### 3.2.4 Soil moisture constants

The amount of water available to plant depends on two factors: the quantity of water that is able to infiltrate into the soil and the quantity of water that the soil is able to hold onto. Field capacity and available water holding capacity are influenced by the particle size, structure and content of OM. However, clay soils, due to its higher matric potential and smaller pore size will generally hold significantly more water by weight than sandy soils (reference). In this respect, data in Table 2 indicate that the addition of HA and powder of plants to the used soil increased the water holding capacity, field capacity, wilting point and available moisture contents compared with the control. The highest values of field capacity and available water were observed at the treatment of HA mixed with *Trifolium alexandrinum* compared to other treatments and control treatment. Brown and Cottone [38] indicated that, texture is the primary factor affecting water holding capacity and increasing organic carbon is a significant factor in improving soil water holding capacity. They also confirmed that compost application had the greatest effect on soil water holding capacity on coarser textured soils with smaller to no change in water holding capacity on finer textured soils.

Generally, our results were in agreement with Mohamed [42] and Hoda, et al. [43] whom reported that HA treatments improve soil aggregation, structure, water permeability, air conditioning, fertility, and moisture holding capacity, and other soil physical properties of the tested soil are improved.

## 3.3 Soil Chemical Properties

### 3.3.1 Soil pH

Soil pH has a considerable impact on soil chemical properties. Data in Table 3 show the changes existing in the tested soil chemical properties in response to the application of HA and powder of some plants. Data showed that the soil pH of soil treated with HA and powder of some plants was lower than that of the untreated soil (Control). The decreases in soil pH was higher in the case of soil treated with HA in addition to powder of some plants together than that of using each of them unless the other. These finding are in agreement with those of El-Sherief et al. [44]. A decrease in pH values could be attributed to various acids or acid is forming

**Table 2. Effect of HA (2 g / pot) and powder of plants at rate of (100 g / pot) on some hydro-physical properties of the used soil in average of 2015/16 and 2016/17 seasons**

Treatments	Bulk density kg m <sup>-3</sup>	Total porosity (%)	W.H.C.	F.C. (%)	W.P. (%)	Available moisture%	Infiltration rate (mm/h)	Hydraulic conductivity (cm h <sup>-1</sup> )
Untreated (control)	1.482	25.36	31.52	25.65	13.12	12.53	28.12	0.085
HA only	1.391	27.19	33.61	31.32	16.02	15.30	31.65	0.099
<i>Trifolium alexandrinum</i>	1.362	31.25	36.52	33.98	16.33	17.65	32.87	0.101
<i>Datura stramonium</i>	1.313	30.78	35.46	34.97	16.54	18.43	31.66	0.095
<i>Euphorbia geniculata</i>	1.319	33.16	34.89	34.11	17.86	17.25	32.25	0.11
HA + <i>Trifolium alexandrinum</i>	1.345	34.27	37.52	36.53	16.13	20.40	36.08	0.123
HA + <i>Datura stramonium</i>	1.286	31.98	36.2	35.02	16.45	19.57	34.34	0.118
HA + <i>Euphorbia geniculata</i>	1.305	33.26	35.98	34.87	16.91	18.96	35.01	0.126
LSD 0.05%	<b>0.13</b>	<b>0.87</b>	<b>1.84</b>	<b>2.07</b>	<b>0.86</b>	<b>1.32</b>	<b>2.18</b>	<b>0.005</b>

W.H.C.: Water-holding capacity; F.C.: Field Capacity; W.P.: Water potential

**Table 3. Effect of HA (2 g/pot) and powder of plants at rate of (100 g/pot) on some chemical properties of the used soil in average of 2015/16 and 2016/17 seasons**

Treatments	pH 1:2.5 H <sub>2</sub> O	OM %	CEC C mol. kg <sup>-1</sup>	Available macro-nutrients (ppm)				
				N	P	K	Mg	Ca
Untreated (control)	7.83	1.51	7.82	49.60	25.80	85.62	10.35	12.17
HA only	7.56	1.69	10.56	75.87	43.81	130	24.65	28.36
<i>Trifolium alexandrinum</i>	7.61	1.85	8.59	69.23	35.56	115	18.87	18.54
<i>Datura stramonium</i>	7.59	1.79	9.32	68.85	46.2	123	21.68	20.15
<i>Euphorbia geniculata</i>	5.64	1.95	8.89	79.36	39.3	108	16.95	21.38
HA + <i>Trifolium alexandrinum</i>	7.52	2.02	11.24	88.88	48.79	136	28.10	31.24
HA + <i>Datura stramonium</i>	7.56	1.99	12.21	92.31	66.33	155	35.56	25.61
HA + <i>Euphorbia geniculata</i>	7.58	1.86	10.87	81.42	58.65	148	31.71	29.64
LSD 0.05%	<b>0.20</b>	<b>0.09</b>	<b>1.73</b>	<b>8.56</b>	<b>0.86</b>	<b>10.18</b>	<b>11.25</b>	<b>9.53</b>

OM: Organic matter; CEC: cation exchange capacity; N: Nitrogen; P: potassium; K: phosphor

compounds that were released from the added organic acids Abdel Fattah [45]. Brady [46] concluded that, the applying of organic matter to clay soils had no significant change in soil pH because of it is higher buffering capacity.

### 3.3.2 Soil organic matter and cation exchange capacity

Organic amendments are very important since they contain both major and minor elements necessary for plant growth and help in improving physical and chemical properties of the soil. Results show that all applied treatments increased OM content as compared to the untreated soil (control). The soil amended with HA mixed with *Trifolium alexandrinum* has a highest content of OM compared to the other soil treatments where, in this treatment OM increased by 33% compared to control. In this respect, the results agree with results reported by Gulser et al. [47] and Ouni et al. [48].

Cation Exchange Capacity is one of the most important indicators for evaluating soil fertility, more specifically for nutrient retention and thus it prevents cations from leaching. The cation exchange capacity of the soil under different treatments take the same trend of organic matter where the treatment of the soil with HA recorded more increases in CEC than that of powder of some plants. More increases in CEC were recorded with supplement of soil with HA combined with powder of some plants. According to Amlinger et al. [49], soil organic matter contributes about 20 – 70% of the CEC for many soils. In absolute terms, CEC of organic matter varies from 300 to 1,400 c mol kg<sup>-1</sup> soil being much higher than CEC of any inorganic material.

Similar results were obtained from Dadhich et al. [50] who stated that application of farmyard manure significantly increased the organic carbon and CEC of the soil. These results are in agreement with those of Agegnehu et al. [51]; Abdel-Rahman [52] and Mohammad et al. [53] whom explained that compost amendment resulted in an increase of CEC due to input of stabilized OM being rich in functional groups into soil.

### 3.3.3 Available macro-nutrients in soil

In regarding to the effect of applied HA and powder of some plants on soil available N, P and K, data are showing in Table 3. The data indicated that available N, P and K in the used soil increased considerably due to the application of HA and powder of some plants compared to the untreated soil. Soil application of HA was associated with significant increases in available N, P and K. The combined application of HA and powder of some plants recorded higher values in respect to N, P and K as compared to the treatments received HA or powder of some plants each of them only and the highest values of available N, P and K (92.31, 66.33 and 155 mg/kg soil, respectively) were obtained under the treatment of HA along with powder of *Euphorbia geniculata*. Such increase in N, P and K as a result of HA addition may be attributed to the improving in soil nutrients retention and enhancing soil microbial activity which works to convert the organic form of nutrients to mineral form, Stevenson [54]. Similar observations were also obtained by Zaky et al. [55] who mentioned that treated soil with HA through the irrigation water caused marked increases in available N, P and K in soil.

In regard to available Mg and Ca, the same behavior of N, P and K were obtained, where HA and powder of some plants increased the available Mg and Ca of the soil compared to control, the data are showing in Table 3. The combined application of HA and powder of some plants recorded higher values in respect to Mg and Ca as compared to the treatments received HA or powder of some plants each of them only and the highest values of available Mg and Ca (35.56 and 31.24 mg/kg soil, respectively) were obtained under the treatment of HA along with powder of *Euphorbia geniculata* in the case of Ca and HA along with powder of *Trifolium alexandrinum*.

Generally, in respect to the effect of HA on soil chemical and physical properties in previously, Boyle et al. [56] and Schnitzer [57] reported that HA affects physical and chemical properties of soils. Also, Nisar and Mir [58] reported that humic substances can be used as soil conditioner as fertilizer or plant growth promoter. Similarly, Ibrahim and Goh [59] reported that humic substance applications increased pH, cation exchange capacity and organic carbon content of the soil. In this regard, Chen and Aviad [4], Ayuso et al. [60], Sharif et al. [61] explained that humic substances can improve soil properties such as aggregation, aeration, permeability, water holding capacity, hormonal activity, microbial growth, organic matter (OM) mineralization, and solubilization and availability of microelements (e.g., Fe, Zn, and Mn) and some macro elements (e.g., K, Ca, and P).

**2-Broomrape:** Data in Table 4 show that the effect of HA and powder of plants were significantly inhibited broomrape growth in both seasons. Using of powder of *Datura stramonium*, *Trifolium alexandrinum* and *Euphorbia geniculata* significantly decreased the numbers of spikes/pot, weight of spikes (g/pot) and late the date of emergence of spikes compared with the untreated (control) in both seasons. This inhibition in broomrape growth was more effective when we used HA at the same time with using powder of *Datura stramonium*, *Trifolium alexandrinum* and *Euphorbia geniculata*. For example using of HA and powder of *Datura stramonium* decreased the numbers of spikes (pot) and weight of spikes (g/pot) by (73.21, 75.0%) and (70.53 and 67.60%) in both seasons, respectively compared with untreated (control).

### 3.3.4 Yield and its components as growth parameters

Data in Table 5 show the effect of HA and powder of plants on yield and its components of faba bean plants in both seasons. The obtained data show that plant height (cm), weight of pods/plant, seed weight/plant (g), weight of 100-seeds (g) and seed yield (ardab/fed) of faba bean plants were significantly increased when these plants treated with HA or powder of plants each of them without the other compared with untreated (control) in 2015/16 and 2016/17 seasons. Furthermore, this increase was more significant with adding HA and powder of plants together to the soil in which faba bean plants

**Table 4. Effect of HA (2 g / pot) and powder of plants at rate of (100 g / pot) on broomrape growth in 2015/16 and 2016/17 seasons**

Treatments	Broomrape growth					
	No. of spikes /pot		Weight of spikes /pot		Date of emergence of spikes (days)	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
Untreated (control)	13.7	12.00	56.0	46.60	78.00	83.33
HA only	7.67	3.00	32.6	18.67	120.00	125.67
<i>Trifolium alexandrinum</i>	6.00	4.67	24.8	21.50	122.00	126.33
<i>Datura stramonium</i>	4.00	3.00	16.8	14.10	108.67	114.67
<i>Euphorbia geniculata</i>	4.00	2.67	17.7	12.83	124.00	137.33
HA + <i>Trifolium alexandrinum</i>	3.00	2.00	13.2	9.93	137.67	140.33
HA + <i>Datura stramonium</i>	3.67	3.00	16.5	15.10	131.33	127.00
HA + <i>Euphorbia geniculata</i>	3.33	2.80	15.1	10.73	114.33	118.00
LSD 0.05%	<b>4.39</b>	<b>2.46</b>	<b>17.3</b>	<b>12.67</b>	<b>6.88</b>	<b>9.41</b>

**Table 5. Effect of HA (2 g / pot) and powder of plants at rate of (100 g / pot) on yield and yield components of faba bean in 2015/16 and 2016/17 seasons**

Treatments	Yield and yield components of Faba bean									
	Plant height (cm)		Weight of pods / plant		Seed weight (g) / plant		Weight of 100 seeds		Seed yield ardab / fed	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
Untreated (control)	60.87	63.33	34.07	38.33	29.40	34.23	67.70	69.70	4.20	3.26
HA only	64.27	68.8	57.57	58.83	45.97	51.97	83.23	87.43	6.07	5.77
<i>Trifolium alexandrinum</i>	78.23	83.80	51.67	65.80	46.80	47.23	78.70	80.53	5.70	6.90
<i>Datura stramonium</i>	79.23	83.83	65.23	73.83	59.93	65.47	74.93	77.70	5.83	6.03
<i>Euphorbia geniculata</i>	72.27	75.57	61.47	67.13	57.60	63.90	84.07	87.27	5.75	5.96
HA + <i>Trifolium alexandrinum</i>	86.83	92.17	71.77	81.83	66.70	74.70	84.13	87.93	6.03	6.55
Ha + <i>Datura stramonium</i>	82.00	92.27	86.03	90.47	80.33	86.43	91.47	93.13	6.33	7.87
HA + <i>Euphorbia geniculata</i>	84.33	87.80	75.13	84.20	70.00	80.20	87.70	90.77	6.10	7.48
LSD 0.05%	<b>8.22</b>	<b>9.51</b>	<b>26.06</b>	<b>17.33</b>	<b>24.48</b>	<b>15.32</b>	<b>10.86</b>	<b>10.97</b>	<b>0.76</b>	<b>0.51</b>

were cultivated. Treatment of faba bean plants with HA and powder of *Datura stramonium* significantly increased seed yield (ardab/fed) by 38.81 in 2015/16 season and by 84.97% in 2016/17 season, respectively compared with untreated (control). The positive effects of HA and powder of plants on growth parameters may be due to the enhancement of nutrients supplied by these amendments or to the effects of the amendments on nutrient and water retention, pH, aeration, and other physical and chemical characteristics of the soil, in addition to the retardation of broomrape growth and lateness of its appearance. These results are in agreement with those reported by Said- Al Alh and Hussein [62] who found that the HA application led to an enhancement of growth parameters compared with control due to the effect of HA on solubilization and uptake of nutrients.

#### 4. PLANT TISSUE ANALYSIS

##### 4.1 Photosynthetic Pigments of Plants Tissue

The responses of tissue content of photosynthetic pigments fractions (*chlorophyll a*, *chlorophyll b* and *carotenoids*) in faba bean plants growing on soils amended with HA and powder of plants in both seasons are presented in Table 6. The content of photosynthetic pigments was increased in response to the

presence of HA. The enhancement in pigment content was more significant, when the cultivated plants treated with powder of *Trifolium alexandrinum*, where, the total pigment content of the leaves of faba bean plants was increased by 1.28 and 1.21- fold compared with controls, in the two seasons, respectively. Further effects were observed for using powder of plants only or with HA than that of using HA alone. In this respect, HA combined with powder of *Trifolium alexandrinum* or *Datura stramonium* significantly increased faba bean plants tissue content of Chl. a, Chl. b and carotenes by (58.17 and 52.4%), (33.61 and 30.25%) and (6.21 and 8.28%) in first season and by (26.81 and 24.68%), (31.09 and 28.79%) and (29.92 and 25.20%) in second season, respectively, compared to untreated plants.

##### 4.2 Proteins Content of Plants Tissue

In order to test the interactive effects of the application of HA and powder of plants to soil on shoot and root protein content, resulted are shown in Tables 7 and 8. The faba bean plant treated with HA, its shoot revealed a significant increase in the soluble, insoluble as well as total protein content, in respect to the control (Table 7). Also, the soluble and insoluble protein content of faba bean plant roots were increased with treatment of the plants with HA in the two tested season (Table 8). In the same way, treatment of

**Table 6. Effect of HA (2 g/pot) and powder of plants at rate of (100 g/pot) on photosynthetic pigments (mg /g Fresh weight) of faba bean in 2015/16 and 2016/17 seasons**

Treatments	Photosynthesis pigments (mg /g Fresh weight)							
	Chl. A		Chl. B		Carotene		Total chl.	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
Untreated (control)	2.08	2.35	1.19	1.32	1.45	1.27	4.72	4.94
HA only	2.35	2.57	1.28	1.43	1.51	1.43	5.14	5.43
<i>Trifolium alexandrinum</i>	3.03	2.82	1.50	1.66	1.53	1.49	6.06	5.97
<i>Datura stramonium</i>	2.53	2.57	1.34	1.49	1.50	1.40	5.37	5.46
<i>Euphorbia geniculata</i>	2.71	2.69	1.38	1.53	1.52	1.44	5.61	5.66
HA + <i>Trifolium alexandrinum</i>	3.29	2.98	1.59	1.77	1.54	1.65	6.42	6.40
HA + <i>Datura stramonium</i>	3.17	2.93	1.55	1.70	1.57	1.59	6.29	6.22
HA + <i>Euphorbia geniculata</i>	2.91	2.80	1.45	1.61	1.57	1.59	5.93	6.00
LSD 0.05%	<b>0.18</b>	<b>0.51</b>	<b>0.03</b>	<b>0.08</b>	<b>0.04</b>	<b>0.10</b>	<b>0.14</b>	<b>0.17</b>

Chl. A: chlorophyll A, Chl. B: chlorophyll B

**Table 7. Effect of HA (2 g / pot) and powder of plants at rate of (100 g / pot) on shoot protein contents (mg/g Dry weight) of faba bean in 2015/16 and 2016/17 seasons**

Treatments	Shoot protein contents (mg/g Dry weight)					
	Soluble protein in shoot		Insoluble protein in shoot		Total protein in shoot	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
Untreated (control)	81.46	88.47	110.80	141.2	192.26	229.67
HA only	103.73	106.20	137.80	165.47	241.53	271.67
<i>Trifolium alexandrinum</i>	99.46	110.23	184.87	174.77	284.33	285.00
<i>Datura stramonium</i>	104.40	120.27	163.07	171.73	267.47	292.00
<i>Euphorbia geniculata</i>	91.46	124.07	167.60	183.6	259.06	307.67
HA + <i>Trifolium alexandrinum</i>	122.46	130.87	200.40	181.47	322.86	312.33
HA + <i>Datura stramonium</i>	142.06	162.47	213.53	201.53	355.59	364.00
HA + <i>Euphorbia geniculata</i>	153.46	163.73	214.67	214.6	368.13	378.33
LSD 0.05%	<b>23.60</b>	<b>10.73</b>	<b>36.71</b>	<b>15.35</b>	<b>39.92</b>	<b>35.41</b>

**Table 8. Effect of HA (2 g / pot) and powder of plants at rate of (100 g / pot) on root protein contents (mg/g dry weight) of faba bean in 2015/16 and 2016/17 seasons**

Treatments	Root protein contents (mg/g dry weight)					
	Soluble protein in root		Insoluble protein in root		Total protein in root	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
Untreated (control)	63.46	65.2	67.73	76.8	131.19	142.00
HA only	75.46	77.07	78.13	125.27	153.59	202.33
<i>Trifolium alexandrinum</i>	68.93	84.17	81.60	165.5	150.53	249.67
<i>Datura stramonium</i>	93.53	137.43	60.33	170.9	153.86	308.33
<i>Euphorbia geniculata</i>	83.86	138.67	80.40	182	164.26	320.67
HA + <i>Trifolium alexandrinum</i>	95.13	141.73	98.33	204.27	193.46	346.00
HA + <i>Datura stramonium</i>	106.26	145.93	125.33	208.73	231.59	354.67
HA + <i>Euphorbia geniculata</i>	105.26	155.87	102.73	196.13	207.99	352.00
LSD 0.05%	<b>21.21</b>	<b>10.04</b>	<b>35.83</b>	<b>18.35</b>	<b>21.21</b>	<b>34.74</b>

the soil with powder of plants appeared positive effect on soluble, insoluble as well as total protein content of faba bean plants shoots and roots (Tables 7 and 8). The percent of the increases in protein content of faba bean tissues varied from shoots to roots and between the powders of plants producing allelopathy which used in this study, but in all conditions the results revealed increases in protein fractions as well as total protein contents of shoots and roots of faba bean plants, compared to control (Tables 7 and 8).

The interaction between HA and powder of plants addition in the soil revealed more increase in the soluble and insoluble as well as total protein contents of faba bean plants in respect to the controls or using one of them unless the other (Tables 7 and 8). In most cases, the increase in shoot and root total protein content ranged from 1.5 to 2-fold as a result of this combination between HA and powder of plants compared with the control. But it was noteworthy

that in both shoot and root, the total protein content of faba bean treated with *Datura stramonium* or *Euphorbia geniculata* was more than that *Trifolium alexandrinum*. Protein synthesis is essential for normal cell proliferation, differentiation and growth. A variety of environmental stress factors have been reported to influence the synthesis of plant proteins, William [63]. Cooper [64] reported that, incorporation of HAs into soils stimulated root growth as well as stimulated the branching and initiation of root hairs and could partially be attributed to enhanced nutrient uptake, Atiye [65]. Several studies showed that HA increase root length, root number and root branching. When HA and fulvic acid are applied on the soil, enhancement of root initiation and increased root growth may be observed, Pettit [7]. Humic substances have a very strong influence on the growth of plant roots. Stimulation of root growth is generally more apparent than shoot growth, Nardi et al. [6]. These enhancement in root and shoot growth of the test plants may be reflected

on their contents of soluble, insoluble as well as total protein.

#### 4.3 Carbohydrates Content of Plants Tissue

The amendment of the soil with HA and powder of plants effect on faba bean shoot and root carbohydrates content are shown in Tables 9 and 10. The faba bean plant treated with HA, its shoot revealed a significant increase in the soluble, insoluble as well as total carbohydrate content, in respect to the control (Table 9). Similarly, the soluble and insoluble carbohydrate content of faba bean plant roots increased with treatment of the plants with HA in the two tested season (Table 10). This increases were also

noticed, with soil treatment by powder of plants, where, these treatments appeared positive effect on soluble, insoluble as well as total carbohydrate content of faba bean plants shoots and roots (Tables 9 and 10). The percent of the increases in carbohydrate content of faba bean tissues varied from shoots to roots and between the powders of plants producing allelopathy which used in this study, but in all conditions the results revealed increases in protein fraction as well as total carbohydrate contents of shoots and roots of faba bean plants, compared to control. Total carbohydrate contents of shoots and roots of faba bean plants treated with *Datura stramonium* was more than that of *Trifolium alexandrinum* and *Euphorbia geniculata* treatments.

**Table 9. Effect of HA (2 g/pot) and powder of plants at rate of (100 g/pot) on shoot carbohydrate contents (mg/g dry weight) of faba bean in 2015/16 and 2016/17 seasons**

Treatments	Shoot carbohydrate contents (mg/g Dry weight)					
	Soluble carbohydrate in shoot		Insoluble carbohydrate in shoot		Total carbohydrate in shoot	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
Untreated (control)	35.6	38.47	94.07	101.4	129.67	139.87
HA only	41.43	46.20	126.23	135.49	167.66	181.69
<i>Trifolium alexandrinum</i>	40.1	41.23	115.9	128.76	156	169.99
<i>Datura stramonium</i>	44.3	48.27	127.37	152.11	171.67	200.38
<i>Euphorbia geniculata</i>	43.03	44.07	119.60	148.32	162.63	192.39
HA + <i>Trifolium alexandrinum</i>	60.13	63.73	163.87	173.64	224	227.37
HA + <i>Datura stramonium</i>	57.37	62.47	171.53	165.59	228.9	228.06
HA + <i>Euphorbia geniculata</i>	46.23	50.87	155.40	152.17	201.63	203.04
LSD 0.05%	<b>3.40</b>	<b>4.23</b>	<b>11.27</b>	<b>13.43</b>	<b>16.01</b>	<b>17.31</b>

**Table 10. Effect of HA (2 g/pot) and powder of plants at rate of (100 g/pot) on root carbohydrate contents (mg/g dry weight) of faba bean in 2015/16 and 2016/17 seasons**

Treatments	Root carbohydrate contents (mg/g dry weight)					
	Soluble carbohydrate in root		Insoluble carbohydrate in root		Total carbohydrate in root	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
Untreated (control)	20.33	24.93	85.12	95.9	105.45	120.83
HA only	26.13	28.47	117.41	140.12	143.54	168.59
<i>Trifolium alexandrinum</i>	23.25	29.63	110.19	113.83	133.44	143.46
<i>Datura stramonium</i>	24.16	22.53	119.25	136.17	143.41	158.7
<i>Euphorbia geniculata</i>	27.24	31.20	111.93	131.61	139.17	162.81
HA + <i>Trifolium alexandrinum</i>	28.53	34.27	164.64	149.29	193.17	183.56
HA + <i>Datura stramonium</i>	35.13	36.83	168.39	145.36	203.52	182.19
HA + <i>Euphorbia geniculata</i>	32.14	31.50	155.98	141.97	188.12	173.47
LSD 0.05%	<b>1.11</b>	<b>1.42</b>	<b>6.94</b>	<b>13.43</b>	<b>7.43</b>	<b>8.52</b>

**Table 11. Effect of HA (2 g / pot) and powder of plants at rate of (100 g / pot) on total free amino acid and proline contents (mg/g dry weight) of faba bean in 2015/16 and 2016/17 seasons**

Treatments	Total free amino acid and proline contents (mg/g dry weight)							
	Amino acid-shoot		Amino acid-root		Proline-Shoot		Proline-Root	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
Un treated (control)	17.24	16.23	12.99	13.13	7.99	9.20	8.770	9.70
HA only	10.72	12.03	7.84	8.93	5.84	6.47	5.840	3.30
<i>Trifolium alexandrinum</i>	11.26	13.47	9.48	9.47	4.48	7.20	6.60	8.23
<i>Datura stramonium</i>	9.75	10.50	9.47	7.93	4.47	7.60	6.08	8.67
<i>Euphorbia geniculata</i>	10.92	11.67	8.24	10.40	6.24	5.30	7.66	6.17
HA + <i>Trifolium alexandrinum</i>	7.76	8.93	7.64	6.60	3.64	3.77	4.53	3.80
HA + <i>Datura stramonium</i>	6.92	4.90	4.23	3.03	2.23	1.17	1.32	1.43
HA + <i>Euphorbia geniculata</i>	8.95	9.80	12.99	5.90	3.86	3.63	4.74	2.73
LSD 0.05%	<b>2.56</b>	<b>2.37</b>	<b>1.64</b>	<b>3.27</b>	<b>1.42</b>	<b>1.41</b>	<b>0.62</b>	<b>1.94</b>

**Table 12. Phenolic acids contents of some powder of plants which producing allelopathic compounds (concentration µg/100 mg)**

Plant name	HPLC analysis :Phenolic acids (concentration µg /100 mg)						
	Vanillic	ferulic	syrungic	p-coumaric	p-hydroxybenzoic	caffic	protocatoic
<i>Trifolium alexandrinum</i>	<b>81.14</b>	<b>284.41</b>	<b>169.95</b>	<b>18.54</b>	<b>26.96</b>	<b>14.16</b>	<b>36.25</b>
<i>Datura stramonium</i>	88.36	271.14	143.36	8.24	22.84	21.16	30.14
<i>Euphorbia geniculata</i>	89.71	253.33	178.42	18.47	49.58	24.47	25.58

The combined effect of HA and powder of plants addition in the soil revealed more increase in the soluble and insoluble as well as total carbohydrate contents of faba bean plants in respect to the controls or using one of them individually (Tables 9 and 10). In most cases, the increase in shoot and root total carbohydrate content was nearest 1.5-fold as a result of this combination between HA and powder of plants compared with the control. But it was noteworthy that in both shoot and root, the total protein content of faba bean treated with *Trifolium alexandrinum* and *Datura stramonium* was more than that *Euphorbia geniculata*.

#### 4.4 Amino Acids Content of Plants Tissue

Data in Table 11 show the effect of HA and powder of plants on total free amino acids and proline content of faba bean plants shoots and roots in both seasons. All treatments, HA only, powder of plants or HA combined with powder of plants revealed significantly decrease in total free amino acids in shoot and root, compared with un-treated (control) in 2015/16 and 2016/17 seasons. Similarly, proline content of faba bean plants shoots and roots showed a decrease with all the treatments compared with un-treated (control) in 2015/16 and 2016/17 seasons.

Combination effect between HA and *Datura stramonium* showed the highest decrease in total free amino acids in shoot and root, as well as proline in shoot and root compared with all other treatments or un-treated (control). These decreases in total free amino acid and proline in the branches and roots of faba bean plants cultivated in soil treated with HA and powders of some plants producing of allelopathy indicate to the lower injury of faba bean plants by broomrape and its combat. This would counter broomrape by blocking growth broomrape penetrate to the inter roots of faba bean, so that, the biotic stress of broomrape and abiotic stress of soil properties were lowered.

#### 5. ANALYSIS OF POWDER OF PLANTS FOR ITS PHENOLIC ACIDS CONTENT

Analysis of powder of plants for its Phenolic acids content is showing in Table 12. The data revealed that powder of plants producing allelopathic compounds contain many of phenolic acids which extracted and then determined by HPLC analysis. These phenolic acids are vanillic, ferulic, syringic, *p*-coumaric, *p*-hydroxybenzoic, caffeic and protocateic acids. Powder of the tested

plants contents of phenolic acid are varied from plant to other and between the different types of the determined phenolic acid.

#### 6. CONCLUSION

In results of this study, it can be concluded that the use of plants producing of allelopathy and HA enhanced the soil physical and chemical properties and hampered germination and growth of broomrape in faba bean. We can take advantage of HA and these plants which have ability to Allelopathy to use them in the future as biological control of weeds and soil improving within agricultural systems. The use of vehicles allelochemicals (Allelopathy) structural models for new pesticides and herbicides material environmentally safe. If we can extract those materials we have been able to control broomrape of faba bean at high rates up to more than 70% and to increase the crop by about 4-5 (ardeb /fed).

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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