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The Effects of Applications Humic Acids on Macronutrient, Micronutrient, Heavy Metal and Soil Properties

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Abstract: This study was conducted on the YYU campus area as a field survey, according to the randomized block experimental design carried out in three replicated. The yellow and orange marigold species with humic acids (HA0;0 kg HA da^{-1}, HA1;10 kg HA da^{-1}, HA2;20 kg HA da^{-1}, HA3;40 kg HA da^{-1}) were used. At the end of the experiment, the soil samples taken from the field of study were analyzed organic matter, soil reactions, total salt content, lime content, nitrogen, phosphorus, potassium, calcium, magnesium, natrium, iron, manganese, zinc, copper, cadmium, nickel and lead contents. The increased humic acid applications have influenced pH ($P<0.01$), lime ($P<0.01$), organic matter ($P<0.05$) with phosphorus ($P<0.01$), iron ($P<0.05$), zinc ($P<0.05$) and nickel ($P<0.01$) contents. The effect variety (V) has been determined on the available of soil iron ($P<0.01$), zinc ($P<0.01$), cadmium ($P<0.01$), nickel ($P<0.01$), and lead ($P<0.01$) contents. The interaction of only affected the nickel ($P<0.01$) and zinc ($P<0.01$) contents. As a result, humic acid applications have an important and positive effect on many properties of soil.

Humik Asit Uygulamalarının Toprak Özellikleri ile Makro Besin Elementi, Mikro Besin Elementi ve Ağır Metal İçerikleri Üzerine Etkisi

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Anahtar kelimeler

Ağır metal,
Humik asit,
Kadife çiçeği,
Toprak.

Öz: Bu çalışma YYU kampüsü deneme alanında tesadüf blokları deneme desenine göre üç tekerrürlü olarak kurulmuştur. Denemede test bitkisi olarak kadife çiçeği bitkisinin sarı ve turuncu çeşitleri ile humik asit (HA0;0 kg HA da^{-1}, HA1;10 kg HA da^{-1}, HA2;20 kg HA da^{-1}, HA3;40 kg HA da^{-1}) kullanılmıştır. Denemenin sonunda alınan toprak örneklerinde organik madde, pH, toplam tuz, kireç, azot, fosfor, potasyum, kalsiyum, magnezyum, sodyum, demir, mangan, çinko, bakır, kadmiyum, nikel ve kurşun elementlerinin analizleri yapılmıştır. Artan humik asit uygulamaları pH ($P<0.01$), kireç ($P<0.01$), organik madde ($P<0.05$) ile fosfor ($P<0.01$), demir ($P<0.05$), çinko ($P<0.05$) ve nikel ($P<0.01$) içeriklerine önemli düzeyde etki etmiştir. Çeşit uygulaması toprakların yarayışlı demir ($P<0.01$), çinko ($P<0.01$), kadmiyum ($P<0.01$), nikel ($P<0.01$) ve kurşun ($P<0.01$) içerikleri üzerine önemli etkide bulunmuştur. İnteraksiyon yalnızca nikel ($P<0.01$) ve çinko ($P<0.01$) üzerine etki etmiştir. Sonuç olarak humik asit uygulamaları toprağın birçok özelliği üzerine olumlu ve önemli etkiye sahip olduğu görülmüştür.

1. Introduction

One of the important problems encountered in the agricultural activities is the decrease of the organic matter content due to time and the loss of yield in the soil. To prevent this, it is necessary to use farm manure, herbal waste farming (stubble sowing), green fertilization, compost or similar organic materials. As a result of the use of such organic fertilizers, microbial activity occurs in soil and many organic compounds emerge and humic and fulvic acids form their structure (Stevenson, 1982). Since Leonardite is an oxidized state found in the upper layers of lignite, all lignite deposits in our country are also a potential source of leonardite. An important source of humic and fulvic acid, leonardite is another source of organic matter used as a soil regulator in agricultural production (Engin and Cöcen, 2013). Humic acids are widely marketed in solid and liquid form. These products in many regions of studies of the wheat yield and protein content, sugar beet yield and sugar content of sugar, corn in the silage yield, lettuce, tomatoes, lentils, walnuts, pistachios, plum and many more products up to 80% increase in the product is reported (Gezgin, 2019). The presence of very high ion-exchange capacities and hydrolysis of humic and fulvic acids lead to a large amount of amino acids and organic acids. Humic and fulvic acid have colloidal properties. It improves the aggregate structure of soils with these properties (Kütük et al., 2000). Humic acid in addition to recovering the soils physical and chemical properties (Boyle et al., 1989; Schnitzer, 1992). By applying humic acid on its own or as a combination with chemical fertilizers to a plant nutrient medium, allows an ideal growth medium to be produced and reduces the environmental damage caused by chemical fertilizers (Sara et al., 2010; Ali et al., 2014). In a study by Yılmaz and Alagöz (2005), it was determined that the soil had a significant effect on aggregate formation and stability by applying humic acid to soils having different textures. Baran et al. (2002) observed that the applied humic acids significantly reduced potassium fixation in soils with different clay types. It has been reported that studies with humic acid applications increase the pH and salt content of the soil (Imbufe et al., 2004; Alagöz et al., 2006; Gümüş and Şeker, 2015).
Cacco and Agnolla (1984) reported that humic acid contained large functional groups, like carboxyl, phenolic hydroxyl, alcohol hydroxyl, and ketones. Humic acids prevent the retrieval of plants, mercury, cadmium and other harmful and heavy metals that are present in soil (Bozkurt, 2005). Piccolo (1988), in the study of the effect of humic substances on the usefulness of heavy metals in the soil in the study of the addition of humic matter in the soil, all metals in soluble and exchangeable form has been found to effectively immobilize the further propagation of mineral soils. According to Sönmez and Bozkurt (2006) with their work with humic acid applications of heavy metals from waste bioavailability can be limited. Naik and Das (2007) reported the highest increase in DTPA-Zn content with 0.2% humic acid.

In this study, the effects of increased humic acid applications on the soil physical properties, nutrient element content and heavy metal content of two different marigold (*Tagetes erecta* L.) grown soil were investigated.

2. Materials and Methods

This study was conducted on the YYU campus area as a field survey, according to the randomized block experimental design carried out in three replicated. The yellow and orange tagetes species were used. In the experiment were used of humic acids four doses (HA_0;0 kg HA da^{-1}, HA_1;10 kg HA da^{-1}, HA_2;20 kg HA da^{-1}, HA_3;40 kg HA da^{-1}) in the commercial name Agrolig (%85 HA). Parcel dimensions were designed to have dimensions 1.6×2: 3.2 m^2. Once the tagetes seedlings that were to be grown commercially were obtained, they were planted into row spacing and row tops so that they were in parcels of dimensions 40×40 cm. The experiment was carried out between 10.05.2014 and 21.10.2014.

At the end of the experiment, the soil samples taken from the field of study were tested organic matter by a modified Walkey Black method (Walkey, 1947) for texture (Bouyoucous, 1951) soil reaction (Jackson, 1958) total salt content (Richards 1954), lime content (Hızalan and Ünal, 1966); while, N content was determined according to the Kjeldahl method (Kacar, 1994) and available P content was determined by the Sodium bicarbonate (Olsen et al., 1954) method. According to Thomas (1982), extractable K, Ca, and Na were shaken with 1 N Ammonium acetate and, as also indicated by Lindsay and Norvel (1978). The available Fe, Mn, Zn, Cu, Ni, Pb and Cd were agitated with 0.05M DTPA with a pH of 7.3 (Kacar, 1994). The element content readings of the filtrates were done by using the ICP-OES tool.

The properties of humic acid used in the experiment were pH 3.75, organic matter content 86.0%, nitrogen, 1.05%, phosphorus, potassium, calcium, magnesium, iron and manganese contents respectively 4.7 mg kg^{-1}, 97 mg kg^{-1}, 3.04%, 0.59% 7800 mg kg^{-1} and 180 mg kg^{-1}.
At the end of the experiment, the obtained values were analyzed by using the SAS (1988) statistical program according to the randomized block design. The significant differences between the averages were subjected to Fisher's Least Significant Difference (LSD) test.

3. Results

At the end of the study, the f values from the sample soil analysis were represented in Tables 1, while average Duncan results were shown in Tables 2, 3, and 4. As can be observed in the tables showing F values, humic acid has 0.1% effect on pH, lime, phosphor, zinc, and nickel content, 5% effect on organic matter content. It was observed that lead and nickel had a significant effect of 0.1%, while zinc and iron had a significant effect of 1% as sources of species variation (Table 1).
As can be observed in Table 2, with increasing humic acid applications, lime content of soil in the study area decreased. While lime content was 15.99% in the control group, when 40 kg da^{-1} humic acid was added, this content decreased to 13.00%. Similar results were observed for soil pH. While the control group had an 8.48 pH, after 40 kg da^{-1} humic acid was applied, the pH dropped to 8.21. The organic material content of the parcels increased with the application of increasing amounts of humic acid. While the control group had 0.84% organic matter, after the addition of 40 kg da^{-1} humic acid, this increased to 1.29%.

Table 1. F values of the variance analyses physical and chemical the growth media

Variation Source	D.F.	pH		Salinity		Lime		O.M.	
		MS	F value	MS	F value	MS	F value	MS	F value
Varieties (V)	1	0.0009	0.011 ns	3432.0	1.97 ns	0.0267	1.48 ns	0.0210	0.33 ns
Humic acids (HA)	3	0.0964	11.24**	1279.5	0.73 ns	9.4806	6.47**	0.2138	3.40*
V×HA	3	0.0011	0.13 ns	5272.6	2.83 ns	0.4811	1.45 ns	0.0335	0.53 ns
Error	7	0.0419		3298.3		4.2731		0.1089	

Variation Source	D.F.	Phosphorus		Potassium		Calcium		Natrium	
		MS	F value	MS	F value	MS	F value	MS	F value
Varieties (V)	1	1.601	1.50 ns	176.04	0.60 ns	2380	0.24 ns	2.667	0.02 ns
Humic acids (HA)	3	20.540	19.28**	594.26	2.03 ns	20694	2.07 ns	34.778	0.23 ns
V×HA	3	0.387	0.31 ns	356.48	1.22 ns	15377	1.54 ns	14.333	0.09 ns
Error	7	9.198		432.61		9992		21.428	

Variation Source	D.F.	Iron		Manganese		Zinc		Cupper	
		MS	F value	MS	F value	MS	F value	MS	F value
Varieties (V)	1	0.8626	11.39**	3.010	3.47 ns	0.0400	6.61**	0.0150	3.22 ns
Humic acids (HA)	3	0.2658	3.51*	0.948	1.09 ns	0.0420	6.29*	0.0003	0.05 ns
V×HA	3	0.1082	0.11 ns	1.349	1.56 ns	0.0278	4.37*	0.0007	0.16 ns
Error	7	0.2835		1.415		0.0356		0.0026	

Variation Source	D.F.	Cadmium		Nickel		Lead	
		MS	F value	MS	F value	MS	F value
Varieties (V)	1	5.97×10^{-6}	7.73**	0.0008	16.34**	0.0023	14.99**
Humic acids (HA)	3	6.34×10^{-7}	0.82 ns	0.0027	55.44**	0.0005	3.32 ns
V×HA	3	5.02×10^{-7}	0.65 ns	0.0007	14.20**	0.0001	0.30 ns
Error	7	1.34×10^{-7}		0.0015		0.0006	

*, **: %5, %1; ns: non significant

Table 2. Effects of humic acid treatments on pH, salinity, lime and organic matter of the of the growth media.

Treatments	pH	Salinity	Lime	Organic Matter
		µS cm^{-1}	%	
Humic acids (kg da^{-1})				
Control (HA0)	8.48 a	317	15.9 a	0.84 b
10 (HA1)	8.35 b	346	14.9 a	0.99 ab
20 (HA2)	8.23 bc	316	13.7 b	1.02 ab
40 (HA3)	8.21 c	315	13.0 b	1.29 a
LSD*	0.11*	52	1.2	0.32
Varieties				
Yellow	8.32	336	14.34	1.06
Orange	8.31	312	14.41	1.01
LSD	0.08	36	0.88	0.24

*: LSD values (P<0.05); a, b, c: Means followed by the same letter within the same column are not statistically different (Duncan's test, P<0.05).

Increasing humic acid applications, when compared to the control parcel, resulted in an increase in useful phosphor and available iron and nickel content. The most increase observed in these contents were when 40 kg da^{-1} humic acid was added for phosphor, 10 kg da^{-1} humic acid was added for iron, and 20 kg da^{-1} humic acid added for nickel, and the values were 12.43 mg kg^{-1}, 2.23 mg kg^{1}, and 0.0913 mg kg^{-1} respectively. The calcium content in the study parcels, with increasing humic acid applications, firstly increased a little then decreased when compared to the calcium content in the control group. While the calcium content in the control group was 2287 mg kg^{-1}, in the group with 10 kg da^{-1} addition of humic acid, this value increased to 2345 mg kg^{-1}, and the calcium content in the group with 40 mg da^{-1} humic acid added decreased to 2211 mg kg^{-1}. However, zinc content decreased with increasing humic acid applications. While the zinc content in the control group was 0.64 mg kg^{-1}, this amount decreased to 0.46 mg kg^{-1} when 20 and 40 kg da^{-1} humic acid were added. With regards to other elements, increases and decreases in their content were observed, however these were deemed insignificant changes (Table 3).

Table 3. Effects of humic acid treatments on contents phosphorus, potassium, calcium and natrium of the growth media.

Treatments	Phosphorus	Potassium	Calcium	Natrium
	mg kg^{-1}			
Humic acids (kg da^{-1})				
Control (HA0)	8.07 c	214	2287 ab	56.5
10 (HA1)	10.18 b	230	2345 a	58.2
20 (HA2)	11.22 ab	224	2241 ab	53.3
40 (HA3)	12.43 a	208	2211 b	53.3
LSD*	1.28*	21	123	15.8
Varieties				
Yellow	10.73	222	2281	55.0
Orange	10.22	216	2261	55.7
LSD	0.90	15	88	9.1

*:LSD values (P<0.05); a, b: Means followed by the same letter within the same column are not statistically different (Duncan's test, P<0.05).

The soil of the tested areas, where the different varieties of the tagetes were grown, showed significant changes in only usable iron, cadmium, and lead. The samples with the highest concentrations of useable iron, cadmium, and lead were found in the soils where yellow tagetes species were grown of which the concentrations were as follows: 2.20 mg kg^{-1}, 0.00778 mg kg^{-1}, and 0.1602 mg kg^{-1} respectively. The study areas where orange tagetes species were grown showed usable iron, cadmium, and lead concentrations, when compared to the control group, significant ratios of 14.9%, 22.2%, and 13.9% respectively. Other elements did not show statistically significant changes in their concentrations (Table 4).

Table 4. Effects of humic acid treatments on contents iron, manganese, zinc, copper, cadmium, nickel, and lead of the growth media.

Treatments	Iron	Manganese	Zinc	Copper	Cadmium	Nickel	Lead
	mg kg^{-1}						
Humic acids (kg da^{-1})							
Control (HA0)	1.75 b	12.28	0.64 a	0.49	0.00756	0.0443 c	0.1589 a
10 (HA1)	1.97 ab	13.25	0.51 b	0.51	0.00754	0.0846 a	0.1576 a
20 (HA2)	2.25 a	12.82	0.46 b	0.49	0.00693	0.0913 a	0.1415 b
40 (HA3)	2.06 ab	12.70	0.46 b	0.50	0.00706	0.0660 b	0.1434 ab
LSD*	0.44*	1.20	0.11	0.09	0.00113	0.0121	0.0180
Varieties							
Yellow	2.20 a	13.12	0.56 a	0.53	0.00778 a	0.0658 b	0.1602 a
Orange	1.82 b	12.41	0.48 b	0.48	0.00677 b	0.0773 a	0.1406 b
LSD	0.31	0.82	0.10	0.05	0.00072	0.0190	0.0125

*:LSD values (P<0.05); a, b, c: Means followed by the same letter within the same column are not statistically different (Duncan's test, P<0.05).

The nickel content of experimental area has increased significantly with increased humic acid applications. Nickel content of 0.0443 mg kg^{-1} in the control parcel increased to 0.0913 mg kg^{-1} in 20 kg HA application. Soil available lead and cadmium contents of yellow and orange tagetes were significantly different from each other. Yellow tagetes cultivated soil lead and cadmium contents of the soil can be taken in the orange tagetes variety of lead and cadmium contents of the soil was observed to be more. The soil of cadmium and lead contents of the yellow tagetes species were 0.00778 mg kg^{-1} and 0.1602 mg kg^{-1}, respectively. Orange tagetes were 0.00677 mg kg^{-1} and 0.1406 mg kg^{-1}. The nickel content of the soil in which the orange tagetes were grown was determined to be higher than the nickel content of the soil in which the yellow tagetes were grown (Table 4).

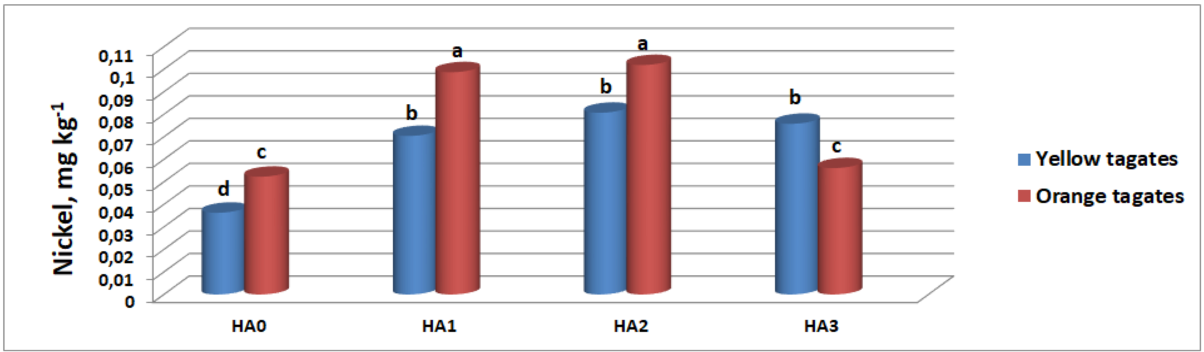

Figure 1. Effect of humic acid applications on the available nickel content of soil, HA0; 0 kg HA da^{-1}, HA1; 10 kg HA da^{-1} HA2; 20 kg HA da^{-1}, HA3; 40 kg HA da^{-1}

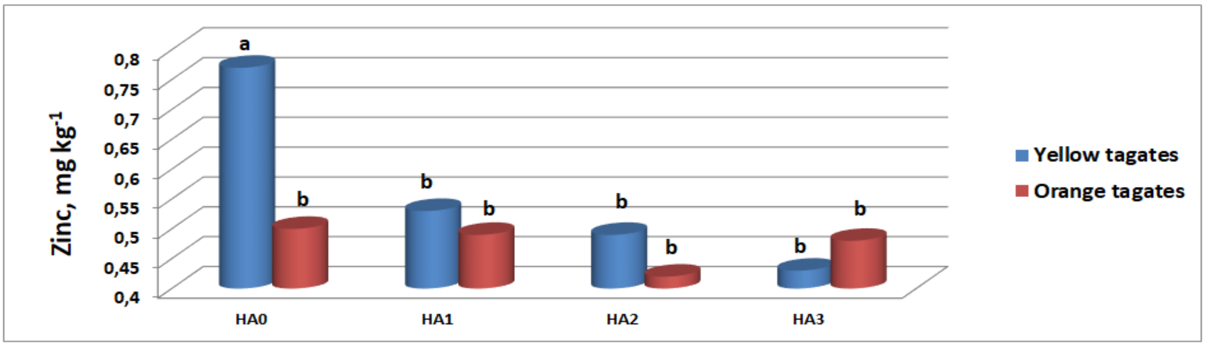

Figure 2. Effect of humic acid applications on the available zinc content of soil, HA0; 0 kg HA da^{-1}, HA1; 10 kg HA da^{-1} HA2; 20 kg HA da^{-1}, HA3; 40 kg HA da^{-1}

4. Discussion and Conclusion

In the field of study where the experiment was conducted, a statistically significant decrease was observed in pH. Between the control soil parcel and the parcel where 40 kg da^{-1} HA was applied, the observed difference was approximately 3.3%. This effect occurred due to the various functional groups present in humic acid that neutralizes the pH of the soil (Çelik, 2008). Therefore, lime content can result in a small decrease in the pH of high soil. Hence, with increasing humic acid applications, a decrease in soil lime content at a significant level was determined. This decrease occurred at 22.3%. This situation can be explained as the freeing of carbon dioxide (CO_2) from calcium carbonate by humic acid (Larcher, 2003) resulting in carbon dioxide mixing with the water in the soil solution to form carbonic acid; the carbonic acid then separates into its ions from which the hydrogen ions help lower the soil pH. With increasing humic acid, the organic matter content of the tested soil increased. This increase was 53.6 % for the 40 kg da^{-1} HA applied group when compared to the control group (Table 3).

The useable phosphor content in the area of study increased with increasing humic acid applications. The 40 kg da^{-1} HA applied group showed a 54.0 % increase in useable phosphor content when compared to the control group. This case could not only be the result of humic acid applications and the resulting decrease in soil pH plus the decrease in lime content, but also be the result of phosphor-humic compound formations from humic acid and phosphor (Kacar and Katkat, 2008). The phosphorus in these compounds can, in time, become unbound thus increases the useable phosphor content in the soil. Likewise, this case could also result from the phosphor present in humic acid. Similarly, Erdal et al. (2000) and Wang et al. (1995) reported in their study that with increasing humic acid applications useable phosphorus content in the soil also increased.

At the end of the study, according to the analysis of the results, the extractable calcium contents in the studied soil showed a certain amount of increase but then decreased to a significant level with increasing applications of humic acid. While the calcium content in the control was 2287 mg kg^{-1}, this content increased to 2345 mg kg^{-1} when 10 kg da^{-1} HA was applied, after which it decreased to 2211 mg kg^{-1} when 40 kg da^{-1} HA was added. While the decrease was 3.4% compared to the control group, the increase in calcium content compared to the 10 kg da^{-1} HA applied group was 6.1%. This situation most probably resulted from the washing away of calcium in the soil that, due to the addition of humic acid to increase the solubility of calcium was, therefore, easily carried away from the root areas. The decrease in calcium content is in accordance with the decrease in lime content. Kloster and Avena (2015) reported that the addition of a small dose of humic acid resulted in a little increase in calcium content but that adding a large dose of humic acid decreased the calcium content.

With the application of humic acid, significant changes were observed in soil Fe, Zn, and Ni content. With increasing HA applications, useable Fe content in the soil increased when compared to the control group. While the Fe content was 1.75 mg kg^{-1} in the control group, this level increased to 2.25 mg kg^{-1} when 20 kg da^{-1} HA was added and this increase was at 28.9%. A similar case was observed for useable Ni content in the soil. While the nickel content in the control soil was 0.0443 mg kg^{-1}, this amount increased by 106.9% and reached 0.0913 mg kg^{-1} in the group where 20 kg da^{-1} HA was applied. A significant decrease in nickel content, from the 20 kg da^{-1} group, was observed in the group where 40 kg da^{-1} HA was applied. This decrease was of 38.3%. This may be due to the heavy metal content of the commercially available humic acids. Pekcan et al. (2018) reported that the heavy metal content of leonardit-derived organic materials sold in the market was above the permissible limits. This may be due to the fact that the yellow tagetes variety can take lead and cadmium elements more than the soil according to the orange tagetes variety (Sönmez et al., 2018).

The available zinc content in the soil decreased by 39.1% with increasing HA dose applications (Table 4). This phenomenon may have occurred due to the functional groups present in humic acid. These functional groups form complexes with metals (Livens, 1991). Thus, while some metals become easier for the plant to take-up, some become more difficult (Stevenson, 1994; Kerndorff and Schnitzer, 1980). In their study, Clement and Bernal (2006) reported that while Cu and Fe content in soil increased when humic acid was added, Zn and Pb content decreased. Pilanali and Kaplan (2002) reported that they work with the soil nutrient content of liquid humic acid applications between the important relationships.

The pH of the medium has a significant effect on the formation of metal-organic comple×compounds between humic acid and metals. With an increase in soil pH, the strength of bond formation between humic acid and metals also changes (Liu and Gonzales, 1999). This phenomenon was explained by the changes that occur in humic acid structure when the pH of the soil alters (Engebretson and Von Wandruszka, 1998).

As a result, humic acid applications of soil, pH, lime, organic matter, phosphorus, calcium, iron, zinc, and nickel contents have a positive and significant effect. It has been observed that there are differentiated effects on soil properties in different varieties of the same plant.

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