



Impact of Humic Acid and Some Nanomaterials on Alternaria Leaf Spot Disease and Sugar Beet Productivity

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ABSTRACT

The effect of humic acid (HA) and three Nano materials (NPs) on alternaria leaf spot (ALS) disease caused by *Alternaria tenuis* (Nees) and sugar beet (*Beta vulgaris* var. *saccharifera* L.) productivity were evaluated. Field experiments were conducted over two seasons in the Experimental Farm of Sakha Agricultural Research Station (SARS) (latitude of 31°10' N and longitude 30° 93' E, at an elevation of 14 m above sea level), Kafr El-Sheikh governorate. Three HA rates along with 100g/L of Boron (B), Manganese (Mn) and Iron (Fe) NPs were used. Generally, the obtained results showed that, all studied traits recorded variability in reaction and the final impact was depending on the significance of material combinations (HA rates x NPs). ALS severity was generally reduced due to the tested materials and it was significantly decreased using B NPs with 3kg HA/fed (2.93%) compared with the control (19.07%). Therefore, the same treatment also showed the highest content of T-phenol (254.13 meq/100 g FW) and the lowest value of alpha amino nitrogen (1.82 %). Moreover, sugar beet plants treated with B NPs alone had the highest values of proline (5.16 mg/g⁻¹ FW), catalase enzyme (6.38 mM H₂O₂ g⁻¹ FW min⁻¹), sucrose (15.68%), extractable sugar (13.09%) and sugar quality (83.50%) as well as the lowest value (1.97%) of sugar lose to molasses% and Na⁺ content (1.41%). Furthermore, treatment of B NPs followed by the highest rate of HA (5kg /fed.) each one alone were recorded highest contents of Chlorophyll A (5.75 mg/g⁻¹ FW), Chlorophyll B (2.99 mg/g⁻¹ FW) and carotenoid (2.47 mg/g⁻¹ FW). Additionally, Fe NPs alone or combined with 3kg HA/fed. resulted in the lowest value of K⁺ (5.63%) and the highest contents of peroxidase (0.607 mM H₂O₂ g⁻¹ FW min⁻¹) respectively. Meanwhile, the highest values of root yield (30.41 ton/fed.) and sugar yield (4.41ton/fed.) were obtained using Mn NPs and 5kg of HA /fed separately.

Keywords: Alternaria leaf spot disease, *Beta vulgaris*, disease management, Sugar crops.

1. Introduction

Sugar beet (*Beta vulgaris* var. *saccharifera* L.) occupies an important place in the Egyptian crop rotation as a winter crop for sugar production not only on fertile soils, but also on poor, alkaline and calcareous soils. It can be grown economically in newly reclaimed soils such as the northern parts of Egypt as one of the most tolerant crops for a wide range of climates. The cultivated area reached about 637,000 Feddan and produces about 1.5 million ton of sugar (Gouda *et al.*, 2022). The total amount of sugar produced is not sufficient for our consumption. Therefore, increasing the cultivated area and sugar production per unit area is considered one of the important national goals to reduce the gap between sugar consumption and production. Sugar beet is an important crop for sugar extraction in many countries around the world (Smigocki *et al.*, 2009).

The production and yield of sugar beet in the world is affected by a number of diseases and they can be characterized as foliar, root and post-harvest diseases (Franc, 2009). One of the diseases that can affect sugar beet is alternaria leaf spot (ALS) (Franc, 2009) ALS is one of the important foliar diseases

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that can impact sugar beet. ALS has been reported as a pathogen not only in sugar beet, but also in a number of other crops (Jayawardana, 2022). ALS in sugar beet has a wide geographic distribution. It has been reported that ALS occurs in all regions where sugar beets are grown (Dunning and Byford, 1982 and Franc, 2009), but the level of severity varies in different regions (Dunning and Byford, 1982 and Franc, 2009). Recently ALS has caused increasing issues and potential significant yield losses in sugar beet fields in different country in the world (Rosenzweig *et al.*, 2017 and Rosenzweig *et al.*, 2019). Due to its relative lack of importance little research has been done on ALS in sugar beet (Franc, 2009). With the potential increasing issues from this disease, it is important to learn about this host-pathogen system and examine potential management strategies for this disease.

Nanotechnology is one of the promising scientific fields that have the potential to revolutionize agricultural production (Kumar *et al.*, 2019 and Mitter and Hussey 2019). Biofortification of micronutrients is the process of enriching the nutrient content of crops to provide a long-term solution to nutrient deficiencies in agricultural products, mainly Fe and Zn deficiency (Kumar *et al.*, 2019). Micronutrients, especially Fe, play an essential role in various physiological, metabolic, and cellular processes in plants due to their ability to promote oxidation and reduction reactions (Rout and Sahoo, 2015). Nanoparticles have been shown to affect microscopic soil properties such as humic acid content, bacterial communities, plant growth and development (Rajput *et al.*, 2018.). The nanoscale metals and metal oxide may damage or improve the germination, plant growth, development, photosynthesis and yield parameters (Rajput *et al.*, 2018.). Many studies reported that the application of iron oxide nanoparticles increased the germination, improved photosynthesis, reduced drought and metal stress and increased nutrients absorption, phytohormones production, and crops yield in maize, cotton, wheat, rice, tomato, and cereal crops (Du *et al.*, 2011.; Raja *et al.*, 2019 and Subbaiah *et al.*, 2016).

Nanoparticles (NPs) have demonstrated activity in suppressing plant diseases are metalloids, metallic oxides, nonmetals, and carbon nanomaterials. NPs have been integrated into disease management strategies as bactericides/fungicides and as nanofertilizers to enhance plant health. Although there are reports of over 18 different NPs of single element and carbon nanomaterials affecting disease and/or plant pathogens, only Ag, Cu and Zn have received much attention thus far. Some NPs act directly as antimicrobial agent while others function more in altering the nutritional status of the host and thus activate defense mechanisms. For example, NP of Ag and Cu can be directly toxic to microorganisms. Other NP of B, Cu, Mn, Si and Zn appear to function in host defense as fertilizers. As demand for food production increases against a warming climate, nanoparticles will play a role in mitigating the new challenges in disease management resulting in a reduction in active metals and other chemical inputs (Elmer *et al.*, 2018).

Alharby and Ali (2022) examined the combined effect of Fe nanoparticles (Fe NPs) and a chromium-resistant bacterium *Staphylococcus aureus*, on rice plants grown on chromium saturated medium and found that Fe NPs significantly improved plant growth, biomass, yield and photosynthetic activity by enhancing the chlorophyll contents and alleviating oxidative damage. Application of Fe NPs also reduced the uptake and accumulation of Cr in the plants by increasing the bioavailability of micronutrients to the plant. The Fe NPs decreased oxidative damage and enhanced the enzymatic and non-enzymatic activity in the plant to withstand Cr stress compared to the plants without Fe NPs treatments. Ahamad *et al.* (2023) studied the effect of Mgo NPs against *Alternaria dauci* infection on carrot (*Daucus carota* L.) under greenhouse conditions. foliar application of MgONPs at 50 and 100 mg L⁻¹ resulted in significant improvement of plant growth, photosynthetic pigments, phenol and proline contents, and defense enzymes activity of carrots with and without *A. dauci* infection. Spraying of MgONPs at 100 mg L⁻¹ had more plant length, shoot dry weight, plant fresh weight, and root dry weight in carrots when challenged with *A. dauci* over inoculated control. The leaf blight indices and percent disease severity were also reduced in *A. dauci* inoculated plants when sprayed with MgONPs. Humic acid was used widely by agriculturists, due to their benefits in disease suppression, frost damage and heat stress by promoting antioxidant activity, increasing root and shoot growth, fresh and dry weight and stimulating plant enzymes and hormones (Eyheraguibel *et al.*, 2008; El-Bassiouny *et al.*, 2014; Seydabadi and Armin, 2014). Humic acid treatment led to increases in defense compounds such as phenolic (phenylpropanoid), modifying antioxidant, phenols, and enzymes (Ertani *et al.*, 2011). Phenols have a direct effect against plant pathogens (Olivares *et al.*, 2015; Liu *et al.*, 2019) and microbial physical protection (Kaiser *et al.*, 2019). The main enzymes involved in the antioxidative defense and lignin biosynthesis, i.e. catalase, ascorbate, phenylalanine ammonia-lyase (PAL) and peroxidases were

monitored by humic acid (Kesba and El-Beltagi, 2012). Additionally, humic acid molecules may have a variety of physiological effects in plants, such as raising photosynthesis and respiration rates, as well as improving protein synthesis and plant hormone-like activity at the cell wall and membrane level or in the cytoplasm (Zaky *et al.*, 2006; Habashy *et al.*, 2008; Fathy *et al.*, 2009).

Sugar beet production can be improved through the use of optimum rates of humic acid as additions to the soil as well as the use of foliar spraying nano elements and its relationship to alternaria spot disease to achieve the highest yield per unit area. This crop is vulnerable to attack by foliar pathogens such as cercospora and/or alternaria that cause leaf spot diseases affecting the quantity and quality of final yield (El-Kholi *et al.*, 1994 and Fatouh *et al.*, 2011). Compared with other foliar diseases, little attention is focused on Alternaria leaf spot (ALS) in Egypt although it's globally importance (Hudec and Rohačik, 2002; Franc, 2009 and Lastochkina *et al.*, 2018). It has frequently been documented on cultivated sugar beets in India (Misra *et al.*, 2021), Pakistan (Abbas *et al.*, 2014), Russia (Gannibal, 2018) and United States (Rosenzweig *et al.*, 2019 and Khan *et al.*, 2020) in varied severities. Furthermore, nutrient deficiency and other stress conditions such as viral infection may increase the incidence of ALS in sugar beet fields (Franc, 2009).

Although, it has frequently been mentioned in Egypt as a minor disease, nowadays ALS seems to increase and must be controlled (Fatouh *et al.*, 2011). However, appropriate crop rotation including sugar beet resistant varieties followed by good cultural practices could play a crucial role in the management of sugar beet leaf spot diseases (Kiniec *et al.*, 2020). Meanwhile, the integrated nutrient management (INM) that enhances crop productivity (Bhatt *et al.*, 2018 and Joshi *et al.*, 2018) may improve plant resistance to biotic diseases as well (Yigit and Dikilitas, 2008). Therefore, the use of organic conditioners such as Humic acid (HA), may capable to improve physio-chemical and biological properties of the soil (Ampong *et al.*, 2022). In addition, HA reported to enhance crop productivity (Bhatt and Singh, 2022) and improve sugar beet yield traits (Rassam *et al.*, 2015; Abu-Ellail *et al.*, 2020 and Rahimi *et al.*, 2020).

Likewise, Nano-materials found to enhance productivity and yield technological quality of sugar beets (Dewdar *et al.*, 2018 and Hassnein *et al.*, 2019). Moreover, both of HA and nano-materials proved to be involved in plant disease control and some of them was effective in sugar beet disease management (El-Shoraky *et al.*, 2018; Ghazy *et al.*, 2021 and Abou-Salem *et al.*, 2022). Therefore, the present work aimed to evaluate the effect of Humic acid and some Nanomaterials on chlorophyll a, b, carotenoids, peroxidase, catalase, α -amino-N, Na^+ and K^+ , sucrose, sugar beet growth, yield and ALS severity to increase sugar beet productivity in Egypt.

2. Materials and Methods

2.1. Field Experiments

This study was carried out at the Experimental Farm of Sakha Agricultural Research Station (latitude of 31°10' N and longitude 30° 93' E, at an elevation of 14 m above sea level), Kafr El-Sheikh governorate, Egypt. For the two seasons, soils were clay in texture. Their physical and chemical analyses were estimated according to the standard methods of Page *et al.* (1982) and Klute, (1986) and the corresponding data are presented in Table(1).

Table 1: Physical and chemical properties of the soils under investigation during 2021/2022 and 2022/2023 seasons

Properties Seasons	Physical properties								
	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Soil texture	CaCO_3 (%)	Water table (cm)	Field capacity (%)	Real density (g/cm^3)
1 st season	3.5	13.8	35.8	46.9	Clay	4.66	97	40.6	2.67
2 nd season	4.2	14.9	34.4	46.5	Clay	4.74	100	39.9	2.65
Properties Seasons	Chemical properties						Available nutrients (mg kg^{-1})		
	pH*	EC** dS m^{-1}	Organic matter (%)		N	P	K		
1 st season	7.8	4.2	1.77		44.4	8.1	232		
2 nd season	7.9	4.1	1.86		46.3	8.9	223		

- Soil pH was determined in soil suspension (1: 2.5).

- Soil Electrical Conductivity (EC) was determined in saturated soil paste extract.

1-Evaluation of sugar beet cultivars to alternaria leaf spot disease

Nine sugar beet cultivars (KTARA, FARAIIDA, Bets 9830, Bets 2860, JAMPOL, DEL 1135, COLLINS, ASEEL and Raspoly) were obtained from Sugar Crops Res. Inst., Agric. Res. Center, Giza, Egypt and sown in the 2nd week of September and evaluated under natural infection during two growing season 2019/2020 and 2020/2021. Complete randomized design was used with three replicates. Each cultivar was planted in five rows with 5.0 m long and 0.60 m width.

2- Effect of humic acid and nanoparticles on alternaria leaf spot disease:

Humic acid and nanoparticles nutrients were used to study their effects against ALS in the field under natural infection. Highly susceptible sugar beet, *cv.* Raspoly to ALS was used. It sown in the 2nd week of September over two seasons (2021/2022 and 2022/2023). A split-plot design with three replicates was conducted. Main plot were three rates of humic acid (purchased from Lobal Chemie, Co. India), containing humic acid (dry basis) 90%; fulvic acid (dry basis) 20%; organic matter 92% and K (dry basis) 8%, was used at concentrations of 15 and 20g/l.) ie, 0, 3, and 5 kg/fed and applied at the first irrigation (35 days from sowing). Each one contain 24 rows, 5.0 m long and 0.60 m width with area 72 m². Sub-plot were Boron (B NPs), Manganese (Mn NPs) and Iron (Fe NPs) nanoparticles (purchased from Al-Gomhoria Co. for Chemicals, Cairo, Egypt), each one including 8 rows of 5.0 m long and 0.60 m width and area 24 m². Each one separately was sprayed twice at a rate of 100 mg/L, at 60 and 90 days after planting, and a non-sprayed treatment was used as a control. Cultivation was done using planter. Plants were thinned at the 4-6-leaf stage to one plant per hill. All recommended cultural practices such as irrigation and fertilization were done as recommended by Sugar Crops Research Institute, Agriculture Research Center, Ministry of Agriculture and Land Reclamation, Egypt. Humic acid made in Spanish, (humic acid 85%; fulvic acid 0.8%; K₂O 4%; N 0.7%; P₂O₅ 0.06%; Ca 0.89%; Mg 0.29%; Fe 0.89%; Mn 0.043%; Zn 0.013; Cu 0.056%; B 0.048 and soluble matter 5%) was used at concentrations of 15 and 20 g/L. Nanofertilizers were prepared at the Lazier Institute of Cairo University, Egypt. They were exposed to the high-power laser ray and applied as foliar application. Nanomaterial solutions were adjusted to obtain the desired concentrations at a final volume of 2 liter and tween 80 (0.1 ml/l) was added at the final solution. The treatments were applied at 90 days from sowing (when the disease appeared) with three sprays, 30 day intervals using a hand atomizer. The control treatment was sprayed with water only. Harvesting was done at 180 days after sowing in both seasons.

2.2. Evaluation of disease severity and growth parameters

Disease severity per sub plot was estimated after 15 days after the third spray and rated from 0 to 9 using the modified scale of Stewart (2020/2023) as follow:

Table 2: Modified scale for estimating *Alternaria* leaf spot disease infection on sugar beet plants

Rating scale	Infection intensity
0	No spots
1	Very few spots
2	Noticeable spotting (sparsely scattered and some leaves have no spots)
3	Leaves have many spots (100 or more), but spots are not merging together to form larger dead areas
4	Spots begin merging together to form desiccated, about 10% of leaf area affected
5	Leaves coping and bending down and regrowth begins and approximately of 25% of the leaf area is desiccated as flagging
6-8	Leaf damage in stages 6-8 and get progressively worse (from 50-90% leaf desiccated and
9	The entire leaf is dead

2.3. Determination of Prolein

Free proline in leaf tissue was estimated or determined according to the protocol of Bates *et al.* (1973).

2.4 Determination of total phenols

Total phenolic content (TPC) was determined as mg/g of fresh weight using the method described by Liyana-Pathirana and Shahidi (2007).

2.5. Photosynthetic pigments and enzymes activity assays.

Calorimetric method described by Wettstein (1957) was used to determine and calculate chlorophyll a, b, and carotenoids as mg/g⁻¹ leaf fresh weight.

2.6. Leaf area index = (leaf area / plant) / (soil area / plant). (Watson 1952).

2.7. Catalase enzyme activity:

Catalase activity was measured according to Aebi (1984).

2.8. Peroxidase enzyme activity:

Peroxidase activity was measured spectrophotometrically by following the change in absorption at 470 nm due to guaiacol oxidation. Peroxidase activity was measured according to Polle *et al.* (1994).

2.9. Productivity and quality traits.

The beets of 6 inner rows were harvested to calculate root yield per sub-plot and then converted into yield (ton/fed). After that ten plants were randomly collected per sub-plot at harvest time (195 day of sowing date) to estimate root weight in kg. Chemical and quality traits were assayed in the Quality Control Laboratory at Alexandria Sugar Factory, Alexandria, Egypt. Sucrose % was measured using polarimeter as described in A.O.A.C. (2005) and the method of Carruthers and Oldfield (1962) was used to determine the percent of purity.

Ninhydrin hydrindantin method (Cooke and Scott, 2006) was used to determine α -amino-N content (meq/100 g beet). Flame photometry method (Lilliand, 1964) was followed to determine Na⁺ and K⁺ in the lead acetate extract of fresh macerated root tissue. Subsequently, sugars lose to molasses % (SLM %) was calculated according to the equation of Devillers (1988) as follow:

$$[\text{SLM}\% = 0.14 (\text{Na} + \text{K}) + 0.25 (\alpha\text{-amino N}) + 0.50].$$

Root yield and sugar yield (ton/fed), sugar loss %, purity as well as sugar quality were recorded.

2.10. Data Analysis

All collected data were subjected to statistical analysis using WASP 2.0 (Web Based Agricultural Statistics Software Package, version 2.0). The least significant difference LSD ($P \leq 0.05$) was used to identify differences and compare means.

3. Results

Data in Table (3) showed that Raspoly variety recorded the highest values of infection with alternaria leaf spot disease under natural conditions in all tested sowing dates in both seasons while DEL 1135 sugar beet cultivar recoded the lowest disease severity of Altenaria leaf spot disease comparing with other tested varieties. It reflected that it is the most susceptible variety among them was Raspoly, so it used in this study.

Table 3: Disease severity% of Alternaria leaf spot disease under naturally infested sugar beet field

Sugar beet	1 st growing season (2019/2020)		2 nd growing season (2020/2021)	
Cultivars	1 st date DS%	2 nd date DS%	1 st date DS%	2 nd date DS%
KTARA	30.10	31.76	12.43	0.62
FARAIDA	26.76	17.43	14.11	1.11
Bets 9830	45.12	36.76	14.43	0.63
Bets 2860	48.43	38.43	41.76	1.43
JAMPOL	9.10	12.43	8.43	0.51
DEL 1135	7.43	8.43	8.43	0.27
COLLINS	30.10	25.11	21.76	0.31
ASEEL	31.76	38.43	31.77	1.03
Raspoly	55.10	45.18	48.43	2.11

Data in Table (4) showed that there were significant differences among humic acid levels in ALS%, T-phenol and proline in both seasons with exception of proline in the 2nd season. The highest values of ALS% were recorded by beets grown without humic acid in both seasons and proline in the 1st season only. On the other hand the highest T-phenol content was scored by 5 and 3 kg HA/fed in the 1st and 2nd seasons respectively. Data stated also, foliar application by nano materials resulted in significant differences in alternaria leaf spot %, and proline. In contrast, it did not significantly different in total phenols in both seasons. The control treatment recorded the highest disease severity by alternaria leaf spot compared to other treatments in both seasons.

Table 4: Effect of humic acid and selected nano materials on Alternaria leaf spot (ALS %) as well as total phenol ($\mu\text{g/g}$ fresh weight) and proline (mg/g^{-1} fresh weight) accumulation in sugar beet

Nano-Materials	Traits	2021/2022 season				2022/2023 season			
		Humic acid (HA) Rates (kg/fed)				Humic acid (HA) Rates (kg/fed)			
		0	3	5	Mean	0	3	5	Mean
Control	ALS%	19.07	17.60	12.33	16.33a	19.27	18.00	12.93	16.73a
Fe NPs		11.47	8.93	7.40	09.27b	8.60	8.13	6.73	07.82b
Mn NPs		4.60	4.60	3.79	04.33c	12.93	6.00	6.13	08.36b
B NPs		7.73	6.40	2.93	05.69c	12.60	8.07	6.27	08.98b
Mean		10.71a	9.53a	6.61b		13.35a	10.05b	8.02b	
LSD (5%) of factor (A x B)					4.227	4.813			
Control	T-Phenol	167.23	196.20	234.60	199.34a	199.87	124.17	191.97	172.00a
Fe NPs		146.87	153.90	223.50	174.75a	175.43	226.93	167.63	190.00a
Mn NPs		161.30	222.27	238.60	207.39a	146.73	221.93	158.47	175.71a
B NPs		178.43	194.90	254.13	209.15a	140.80	227.83	127.00	165.21a
Mean		163.45b	191.81b	237.70a		165.71b	200.22a	161.27b	
LSD (5%) of factor (A x B)					59.780	95.027			
Control	Proline	1.78	2.06	1.77	1.87ab	5.24	4.87	3.23	4.45ab
Fe NPs		3.30	1.99	1.70	2.33a	4.74	3.20	3.82	3.92b
Mn NPs		1.97	1.92	1.63	1.84ab	5.03	4.60	3.41	4.35ab
B NPs		1.88	1.85	1.56	1.76b	5.05	5.16	5.12	5.11a
Mean		2.23a	1.95ab	1.66b		4.30a	4.16a	4.91a	
LSD (5%) of factor (A x B)					0.797	1.873			

The highest proline content was recorded by iron nanoparticles treatment with no significant difference of control followed by manganese nanoparticles in the 1st season, also the same results was recorded by boron and manganese nanoparticles in the 2nd season. in the 1st and 2nd season respectively

using B NPs combined with 3kg HA/fed (Table 4).

Analysis of variance (ANOVA) revealed the existence of significant interaction between HA rates (Factor A) and used NPs (Factor B) as a source of variation in 17 and 3 tested traits in the 1st and 2nd seasons respectively. These traits are ALS%, T-phenol, proline, chlorophyll A, chlorophyll B, carotenoids, LAI, catalase, peroxidase, sucrose %, extractable sugar %, K⁺, Na⁺, SY and SQ in the 1st season as well as SLM%, N and RY in the 2nd season traits. Meanwhile, factors A and B were significant sources of variation in SLM%, while factor A was only a significant source of variation in SLM%, RY and N in the 1st season. The significance of the interaction (A x B) indicates that the effect of tested NPs is depending on the applied rate of HA and the vice versa. Accordingly, obtained result could be statistically explained as follows.

As a result, effect of Fe NPs and Mn NPs on ALS% was insignificantly different at the highest rate of HA (5 kg/fed), although it was significantly different at the 2 lower rates in the 1st season. The lowest ALS% (2.93%) compared with the control (19.07%) was obtained in the 1st season when B NPs with 5kg HA /fed were used. Meanwhile, the effect of Fe NPs and Mn NPs on the T-phenol and proline was significantly different at the lowest HA rate but it was insignificantly different at the two higher rates in the same season. In the 2nd season, the effect of Fe NPs and B NPs on the proline was significantly different only at HA rate of 3kg/fed although it was insignificantly different at the higher and lower rates. The highest content of T-phenol (254.13) and proline (5.16) were obtained.

Data in Table (5) illustrated that the application of beets with humic acid resulted in significant differences in photosynthetic pigments compared to control treatment in both seasons. The application of beets with 5 kg/fed humic acid gave the top values of chlorophyll a and b in both seasons however it did not significantly different from 3 kg HA/fed in chlorophyll b in the 2nd season. On the other hand the highest carotene content was found as a result of control treatment in both seasons.

Table 5: Effect of humic acid and selected nano materials on photosynthetic pigments (mg/g⁻¹ fresh weight) of sugar beet.

Nano-Materials	Traits	2021/2022 season				2022/2023 season			
		Humic acid (HA) Rates (kg/fed)				Humic acid (HA) Rates (kg/fed)			
		0	3	5	Mean	0	3	5	Mean
Control	Chlor. A	2.33	4.50	4.86	3.90a	2.42	5.01	5.12	4.19a
Fe NPs		2.23	3.18	5.06	3.49a	2.40	4.50	4.87	3.92a
Mn NPs		2.43	3.46	5.50	3.80a	2.33	4.60	5.06	3.99a
B NPs		2.42	2.96	5.75	3.71a	2.43	5.50	5.53	4.48a
Mean		2.35c	3.52b	5.29a		2.39c	4.90b	5.14a	
LSD (5%) of factor (A x B)					2.122	2.080			
Control	Chlor. B	0.91	1.23	2.39	1.51a	1.83	2.83	1.63	2.09a
Fe NPs		1.02	1.35	2.63	1.67a	1.82	0.96	2.22	1.67a
Mn NPs		1.57	2.16	2.22	1.98a	2.08	1.99	1.67	1.92a
B NPs		1.23	1.67	2.99	1.97a	2.41	2.39	2.14	2.31a
Mean		1.18b	1.60b	2.55a		2.03a	2.05a	1.91a	
LSD (5%) of factor (A x B)					1.079	1.502			
Control	Carotenoid	2.06	0.70	0.60	1.12a	2.29	0.76	0.72	1.26b
Fe NPs		2.35	1.14	0.61	1.36a	2.22	1.48	0.68	1.46ab
Mn NPs		2.34	1.61	0.48	1.48a	2.24	1.69	0.82	1.58ab
B NPs		2.36	0.71	0.59	1.22a	2.47	2.01	0.67	1.72a
Mean		2.27a	1.04b	0.57c		2.31a	1.48b	0.72c	
LSD (5%) of factor (A x B)					0.795	0.741			

Data in Table (5) cleared that the application of beets with nanoparticles had significant effect in carotene content in the 2nd season, however it had no significant in chlorophyll a and b in both seasons

and carotene in the 1st season only. The highest values of carotene were scored by boron with no significant different from manganese or iron nanoparticles.

In respect of photosynthetic pigments the interaction (A x B) was also significant so, the effect of Fe NPs and B NPs on chlorophyll B was insignificantly different at the highest and lowest rates of HA but it was significantly different at the 3kg/fed in both 2 season. Meanwhile, the effect of Fe NPs and B NPs on the carotenoids was insignificantly different at the lowest HA rate but it was significantly different at the 2 higher rates in the 1st season. In the 2nd season, their effect was insignificantly differed from each other at the highest rate of HA although it was significantly different at the 2 lower rates. The highest contents of Chlorophyll A (5.75 mg/g) and B (2.99 mg/g) were obtained using B NPs with 3kg/fed of HA. The highest carotenoid content (2.47 mg/g) was obtained using B NPs with 5kg/fed of HA (Table, 5).

Data in Table (6) stated that the application of beets with humic acid levels cased significant differences in LAI and peroxidase activity in both seasons but in catalase throw the 1st season only. The highest LAI and peroxidase activity in both seasons as well as catalase I the 1st seasons were resulted as a result of the application of beets with 5 kg HA/fed.

Data reflected that the foliar application of sugar beet plants with nano materials cased significant differences in LAI and peroxidase activity in both seasons however it was insignificant in catalase in the 2nd season. The top values of LAI were recorded by Mn nanoparticles with no significant different from iron nanoparticles and control in the 1st season and by boron nanoparticles in the 2nd season. Thus the top catalase activity was scored by the application of beets with nano materials with no significant differences among them in the 1st season. Consequently, the top peroxidase activity was reported by the application of beets with control treatment with no significant difference from iron nanoparticles in both seasons.

Table 6: Effect of humic acid and selected nano materials on leaf area index (LAI cm²) as well as catalase (CAT) and peroxidase (POX) enzymes (mM H₂O₂ g⁻¹ FW min⁻¹)

Nano-Materials	Traits	2021/2022 season				2022/2023 season			
		Humic acid (HA) Rates (kg/fed)				Humic acid (HA) Rates (kg/fed)			
		0	3	5	Mean	0	3	5	Mean
Control	LAI	49.67	52.49	54.29	52.15ab	28.313	39.18	40.02	35.84c
Fe NPs		35.38	52.57	67.51	51.82ab	27.690	49.90	51.33	42.98bc
Mn NPs		49.78	58.29	60.79	56.29a	41.297	35.17	58.59	45.02b
B NPs		38.23	38.55	56.62	44.47b	46.843	58.18	61.59	55.54a
Mean		43.26b	50.47b	59.80a		36.036b	45.61ab	52.88a	
LSD (5%) of factor (A x B)					17.834	15.988			
Control	CAT	0.84	2.43	5.50	2.92b	3.82	4.59	5.42	4.61a
Fe NPs		2.78	2.42	5.75	3.65ab	5.03	3.04	5.42	4.50a
Mn NPs		4.58	4.50	4.86	4.65a	5.12	6.30	3.14	4.85a
B NPs		6.38	5.06	3.18	4.87a	4.87	5.44	3.56	4.62a
Mean		3.65b	3.60b	4.82a		4.71a	4.84a	4.39a	
LSD (5%) of factor (A x B)					2.045	2.226			
Control	POX	0.090	0.600	0.703	0.464a	0.203	0.570	0.703	0.49a
Fe NPs		0.157	0.270	0.607	0.344abc	0.507	0.560	0.607	0.56a
Mn NPs		0.150	0.167	0.477	0.264c	0.150	0.123	0.477	0.25b
B NPs		0.217	0.210	0.593	0.340bc	0.217	0.173	0.593	0.33b
Mean		0.153c	0.312b	0.595a		0.269c	0.357b	0.595a	
LSD (5%) of factor (A x B)					0.214	0.232			

Regarding the ALI and enzymatic activity, the interaction (A x B) was also significant. So, the effect of Fe NPs and Mn NPs on LAI was insignificantly different at the lowest or highest HA rate but

it was significantly different at HA rate of 3kg/fed in the 1st season. In the 2nd season, the effect of Fe NPs and B NPs on LAI was significantly different at the lowest HA rate but it was insignificantly different at the 2 higher rates. Meanwhile, the effect of Fe NPs and Mn NPs on catalase was insignificantly different at the lowest rate of HA but it was significantly different at the 2 higher rates in the 2nd season. Meanwhile, the effect of Fe NPs and Mn NPs on the peroxides was insignificantly different at the highest HA rate but it was significantly different at the 2 lower rates in the 2nd season. The highest Catalase (6.38 mg/g) content was obtained using B NPs alone. The highest peroxidase content (0.607 mg/g) content was obtained using Fe NPs with 3kg/fed of HA (Table 6).

Data in Table (7) illustrated that the application of sugar beet plants with humic acid as a soil amendment resulted in significant differences in extractable sugar % and SLM% in both seasons as well as sucrose% in the 1st season. The maximum value of sucrose% in the 1st season and extractable sugar % in both season were scored by the application of beets with humic acid level (5 kg/fed) with no significant different from (3 kg/fed) level except in extractable sugar % in the 1st season. But the top value of sugar lose to molasses % was reported from beets treated without humic acid with no significant different from that treated with humic acid (3 kg/fed) in both seasons.

Table 7: Effect of humic acid and selected nano materials on sucrose (S %), extractable sugar (Ext. S %) and sugar lose to molasses (SLM %) of sugar beet.

Nano-Materials	Traits	2021/2022 season				2022/2023 season			
		Humic acid (HA) Rates (kg/fed)				Humic acid (HA) Rates (kg/fed)			
		0	3	5	Mean	0	3	5	Mean
Control	S %	10.77	11.83	11.93	12.75a	13.82	14.98	14.03	14.28a
Fe NPs		11.02	12.87	14.77	12.89a	14.72	15.01	12.90	14.21a
Mn NPs		11.62	14.46	13.87	12.67a	14.90	14.98	14.20	14.69a
B NPs		12.55	13.42	13.69	12.63a	15.68	14.19	14.44	14.77a
Mean		11.4b	13.58a	13.39a		14.78a	14.79a	13.89a	
LSD (5%) of factor (A x B)					1.793	2.121			
Control	Ext. S%	8.87	9.68	10.66	9.73a	11.14	11.06	12.39	11.53a
Fe NPs		9.89	8.16	11.64	9.89a	10.11	11.77	11.98	11.29a
Mn NPs		8.97	8.86	11.45	9.76a	11.51	11.84	12.14	11.83a
B NPs		7.91	10.64	10.70	9.75a	11.65	11.51	13.09	12.08a
Mean		8.91b	9.33b	11.11a		11.10b	11.54ab	12.40a	
LSD (5%) of factor (A x B)					1.628	2.085			
Control	SLM%	2.58	2.35	2.29	2.41a	1.97	2.29	2.16	2.14b
Fe NPs		2.55	2.37	2.24	2.39a	2.63	2.19	2.13	2.32a
Mn NPs		2.35	2.42	2.16	2.31a	2.55	2.12	2.15	2.27a
B NPs		2.44	2.13	2.25	2.27a	2.08	2.17	1.97	2.07b
Mean		2.48a	2.32ab	2.24b		2.31a	2.19ab	2.10b	
LSD (5%) of (A x B)					0.393	0.212			

Data in Table (7) stated that the application of beets with nano materials did not significantly affect sucrose S % and extractable sugar % in both seasons as well as sugar lose to molasses % in the 1st season. But it was significant in sugar lose to molasses % in the 2nd season where the top values of that were recorded by the application of beets with iron or manganese nanoparticles with no significant differences between them.

The significance of the interaction (A x B) shows that effect of Fe NPs and Mn NPs on sucrose % and extractable sugar % was significantly different at 0 rate of HA but it was insignificantly different at the higher rates of HA in the 1st season. Their effect was insignificantly different in the 2nd season. Significance of A and B factors over (A x B) in respect of SLM% in the 1st season indicates that the effect of NPs and HA are independent. Significant difference was only found herein between 1st and 2nd

rates of HA. However, in the 2nd season, the significance of (A x B) shows that effect of Fe NPs and B NPs on SLM % was insignificantly different at the lowest or the highest rate of HA although it was significantly different only at 3kg/fed of HA. The highest values of Sucrose % (15.68%) and extractable sugar (13.09%) as well as the lowest value of SLM% (1.97%) were obtained using B NPs alone (Table, 7).

Data in Table (8) showed that the application of beets with humic acid caused significant differences in K⁺ and Na⁺ meq/100 gm beet roots in both seasons. The top values of them were scored in the 1st season by control treatment with no significant differences from humic acid level (5 kg/fed), however it was recorded in the 2nd season by 3kg/fed humic acid level with no significant different from 5kg/fed level. On the other hand the application of beets with humic acid had insignificant effect in α -amino N contents meq/100 gm in sugar beet roots in both seasons.

The application of beets with nano materials had no significant effect in K⁺, Na⁺ and α -amino N contents meq/100 gm of sugar beet roots in both seasons except in K⁺ content meq/100 gm of sugar beet roots in the 2nd season. The top values of root K⁺ content meq/100 gm were recorded by the application of beets with manganese nanoparticles with no significant differences from iron nanoparticles or control treatments (Table, 8).

The interaction (A x B) was also significant source of variation in K and Na in both seasons as well as A-amino N in the 2nd. Factor A was a significant in respect of A-amino N in the 2nd. So, the effect of Fe NPs and B NPs on the K was insignificantly different at the lowest or the highest HA rate although it was significantly different at intermediate rate in the 2nd season. The effect of Fe NPs and Mn NPs on the Na was significantly different at the highest rate of HA although it was insignificantly different at lower 2 rates in the 1st season. The lowest values of K (5.63%) and Na (1.41%) were obtained using only Fe NPs and B NPs respectively. The lowest value of A-amino N (1.82%) was obtained with B NPs following 5kg HA /fed (Table, 8).

Table 8: Effect of humic acid and selected nano materials on K⁺, Na⁺ and α -amino N contents meq/100 gm of sugar beet roots

Nano-Materials	Traits	2021/2022 season				2022/2023 season			
		Humic acid (HA) Rates (kg/fed)				Humic acid (HA) Rates (kg/fed)			
		0	3	5	Mean	0	3	5	Mean
Control	K	7.52	7.05	8.30	7.62a	6.58	5.72	7.27	6.52ab
Fe NPs		8.35	6.99	7.67	7.67a	5.63	8.24	5.99	6.62ab
Mn NPs		7.33	6.70	6.72	6.92a	5.86	8.98	7.00	7.28a
B NPs		7.59	7.23	6.92	7.25a	6.24	6.39	6.72	6.45b
Mean		7.70a	6.99b	7.40ab		6.08b	7.33a	6.74ab	
LSD (5%) of factor (A x B)					1.506	1.174			
Control	Na	1.88	1.81	1.77ab	1.82	1.59	1.50	1.90	1.66a
Fe NPs		1.98	1.68	1.43b	1.69	1.56	1.85	1.79	1.73a
Mn NPs		1.71	1.61	2.02a	1.78	1.50	1.87	1.73	1.70a
B NPs		2.06	1.48	1.85a	1.80	1.41	1.70	1.39	1.50a
Mean		1.91a	1.65b	1.77ab		1.51b	1.73a	1.70ab	
LSD (5%) of (A x B)					0.549	0.409			
Control	A-amino N	2.48	2.23	2.21	2.31a	2.08	1.85	2.52	2.14a
Fe NPs		2.62	2.28	2.08	2.32a	2.03	2.43	2.31	2.26a
Mn NPs		2.19	2.21	2.38	2.26a	2.06	2.59	1.99	2.21a
B NPs		2.60	2.14	2.06	2.27a	1.97	1.89	1.82	1.90a
Mean		2.47a	2.22a	2.18a		2.04a	2.19a	2.16a	
LSD (5%) of (A x B)					0.805	0.711			

The application of beets with humic acid had significant effect in root yield (RY, ton/fed), sugar yield (SY, ton/fed) and sugar quality (SQ %) in both seasons except in root yield in the 1st season. The highest root and top yields were scored by the application of beets with 5 kg/fed of humic acid in the 2nd season, which scored the top sugar yield in the 1st season with no significant different from 3 kg/fed humic acid. Otherwise, the top sugar quality% values were recorded by beets untreated with humic acid in both seasons with no significant different differences from that treated with 5 and 3 kg/fed humic acid in the 1st and 2nd seasons respectively (Table, 9).

There were no significant effects on beets treated with nano materials in both seasons except in sugar yield and quality percentage in the 1st and 2nd seasons respectively. The top sugar yield was produced by beets treated by iron nanoparticles with no significant differences manganese nanoparticles and control treatments, while the highest quality% was scored from beets untreated by humic acid with no significant difference from that treated by 3 kg/fed humic acid (Table, 9).

Table 9: Effect of humic acid and selected nano materials on root yield (RY, ton/fed), sugar yield (SY, ton/fed) and sugar quality (SQ %) of sugar beet

Nano-Materials	Traits	2021/2022 season				2022/2023 season			
		Humic acid (HA) Rates (kg/fed)				Humic acid (HA) Rates (kg/fed)			
		0	3	5	Mean	0	3	5	Mean
Control	RY	25.65	27.54	27.96	27.05a	23.93	23.33	24.83	24.03a
Fe NPs		26.43	27.06	30.02	27.83a	23.43	24.03	24.91	24.12a
Mn NPs		26.29	25.62	27.08	26.33a	23.62	25.12	30.41	26.38a
B NPs		25.17	25.52	27.30	25.99a	24.25	24.43	28.61	25.76a
Mean		25.88a	26.43a	28.09a		23.81b	24.45b	27.19a	
LSD (0.5) of : (Ax B)					6.107	4.481			
Control	SY	2.61	2.81	2.89	3.16ab	3.38	3.48	3.50	3.45a
Fe NPs		3.09	3.03	4.18	3.39a	3.07	3.55	3.65	3.42a
Mn NPs		2.94	3.14	3.55	3.21ab	3.46	3.52	4.41	3.79a
B NPs		2.92	3.21	3.65	2.91b	3.40	3.56	4.16	3.71a
Mean		2.89b	3.04ab	3.65a		3.32b	3.52b	3.93a	
LSD (0.5) of : (Ax B)*					0.639	0.795			
Control	SQ	76.82	77.13	74.93	76.29a	79.75	82.70	79.38	80.61ab
Fe NPs		78.71	73.94	76.85	76.50a	81.14	78.37	78.30	79.27b
Mn NPs		75.14	76.16	79.10	76.80a	81.50	79.01	80.81	80.44ab
B NPs		77.77	73.41	79.48	76.89a	83.50	81.03	80.61	81.71a
Mean		77.11ab	75.16b	77.59a		81.47a	80.28ab	79.77b	
LSD (0.5) of : (Ax B)					3.130	3.131			

Data in Table (9) demonstrated that the application of sugar beet plants with naon materials resulted in insignificant differences in root, sugar yields and quality percentage in both seasons except in sugar yield and quality% in 1st and 2nd seasons respectively. The top sugar yield was recorded from the beets treated with iron nanoparticles with no significant difference from that treated with manganese nanoparticles or control treatments, while the highest sugar quality% was found in plants treated with boron nanoparticles with no significant difference from that treated with iron nanoparticle or control treatments.

Significance of the interaction (A x B) was also proved in cases of RY in the 2nd season as well as SY and SQ in both 2 seasons (Table 7). Thus, effect of Fe NPs and Mn NPs on the RY was significantly different at the highest HA rate although it was insignificantly different at the 2 lower rates in the 2nd season. Meanwhile the effect of Fe NPs and Bo NPs on sugar yield was significantly different at 0 HA but it was insignificantly different at the higher rates of HA in the 1st season. It was significantly different at the highest rate but it was insignificantly different at the lower 2 rates of HA in the 2nd

season.

Regarding the SQ%, significance of the treatments (A x B) shows that effect of Fe NPs and Mn NPs on SQ% was significantly different at 0 HA but it was insignificantly different at the higher rates of HA in the 1st season. Their effect was insignificantly different in the 2nd season. The lowest value of SLM% (1.97%) and the highest value of sugar quality (83.50%) were obtained with B NPs alone. The highest values of RY (30.41 ton/fed) and Sugar yield (4.41 ton/fed) were obtained using Mn NPs following 5kg HA /fed (Table 9).

4. Discussion

More than 55% ALS disease severity was recorded in the field observation including 9 sugar beet cultivars grown in Sakha experimental farm, Kafr El-Sheikh district over 2 seasons (2019-2021). This finding led to imply the probable increase of ALS in case of susceptible cultivars and favorable conditions exist. However, ALS, caused by several *Alternaria* species, is most common, widespread, and economically important in many countries (Khan *et al.*, 2020, Haque and Parvin, 2021 and Misra *et al.*, 2021). So, to avoid the probable increase in ALS severity and to improve the quantitative as well as qualitative traits of sugar beet, HA and NPs were suggested to employ in this respect. Tested materials in the present work were generally effective against ALS disease compared with the control. Similarly, the use of NPs in the control of sugar beet disease was previously reported by Ghazy *et al.*, (2021) and Abou-Salem *et al.*, (2022). Free radicals produced by nanoparticles and their ions can damage microbial cell membrane, DNA, and mitochondria resulting in death of microbes (Rajput *et al.*, 2017). On the other hand, HA was also reported to be used in sugar beet disease control (Farahat, *et al.*, 2022). Furthermore, nano materials reported to enhance sugar beet productivity, yield and technological quality (Mamyandi *et al.*, 2012 and Barlog, *et al.*, 2016). Thus the lowest ALS severity compared with the control was achieved in the current study using B NPs (100g/L) spray following HA (3kg/fed) application. This combination also resulted in the highest content of T-phenol and the lowest value of alpha amino nitrogen. Increase of HA rate (5kg /fed) in the previous combination resulted in the highest contents of the photosynthetic pigments. On the other hand, B NPs alone resulted in moderate content of T-phenol, the lowest value of sugar lost to molasses % and Na content as well as the highest values of proline, catalase, sucrose %, extractable sugar % and sugar quality %. These findings reflect the important and effective role of B NPs alone or combined with HA in the growth, development as well as quantity and quality of the final yield. However, boron consider one of the essential trace elements required for optimal plant growth, phenolic metabolism and various physiological function of the higher plants (Marschner 2012 and Shireen *et al.*, 2018). It is essentially located in the leaf cell walls (95%-98% B) and be responsible for the integrity of the cell membrane due to its involvement in its protein and enzymatic function (Hu and Brown, 1994; Brown *et al.*, 2002 and Goldbach and Wimmer, 2007). Likewise, Fe NPs alone or combined with 3kg HA/fed resulted in the least value of K⁺ and the highest contents of peroxidase, respectively. Iron is known to enhance the chlorophyll contents, photosynthetic activity, enzymatic activity, plant growth and yield as well as decrease the oxidative damage (Alharby and Ali, 2022). Furthermore, the highest values of root yield and sugar yield were obtained using Mn NPs with 5kg HA /fed. Mn also is an essential element for photosynthesis, respiration, enzymatic activity, hormone signaling, plant growth development and productivity as well as pathogen defense. Its bio-availability can decrease in soils containing high amounts of organic matter. So, plants have specific mechanisms to regulate Mn requirements (Alejandro *et al.*, 2020). In addition to above mentioned roles of HA and NPs, positive increase in the plant growth and productivity may attributed to hormone-like activity of HA and its impact on photosynthesis, protein synthesis, cell respiration, and various enzymatic reactions (Vaughan, 1974; Muscolo *et al.*, 1993; Zhang and Schmidt, 1999 and Türkmen *et al.*, 2004). Thus, refined sugar beet yield might be increased up to 20%-25% compared to the control due to HA application (Feckova *et al.*, 2005 and Sadeghi-Shoae *et al.*, 2013).

5. Conclusion

There are many factors that influence the ability of plants to fight disease. Nutrient management has been a key factor in controlling the spread of plant pathogens. Severity of most diseases may be reduced by good nutrient management in crops. when crops are undernourished, nutrient management leads to a greater reduction in disease severity (Gupta *et al.*, 2017). Nutrients have a significant role in

disease resistance and plant growth and development (Datnoff *et al.*, 2007). Certain nutrients have a higher influence on plant diseases; nevertheless, various diseases and circumstances may have opposing effects on the same nutrient (Agrios 2005). The NPs and HA rates combinations could be variably affect ALS severity as well as the quality and quantity of sugar beet yield in the current study. Even if the ALS consider a minor foliar disorder for some times, its increase probably will takes place in favorable conditions where susceptible cultivars are grown. Since more than 1.5 million ton of annual Egyptian sugar production are extract from sugar beet, suppression of attacking pathogens is needed. So, further studies must be contacted to reach the maximal impact of our tested and/or new materials on the sugar beet health and final yield particularly with the existence of climate change.

6. References

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