ECOFRIENDLY TREATMENTS AND DROUGHT CONDITIONS AS A TOOL FOR THE INDUCTION OF MEDICINALLY ACTIVE COMPOUNDS ON POTATOES.

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ABSTRACT

This experiment aimed to improve the qualitative traits and stimulate the accumulation of antioxidants in potato plants under environmentally sustainable agricultural practices. A field experiment was conducted during the spring season of 2023 at the fields of the College of Agricultural Engineering Sciences, University of Baghdad, using a Randomized Complete Block Design (RCBD) within split-plot arrangement with two factors and three replications (2 × 6 × 3). The first factor involved the application of titanium dioxide nanoparticles (TiO_2-NPs) at concentrations of 0 and 10 ml·L^{-1}, denoted as (T_0) and (T_1), respectively. The second factor included six treatments as follows: natural irrigation (I), prolonged irrigation (D), biofertilizers under prolonged irrigation (D_B), biofertilizers under prolonged irrigation with mannitol (D_{BM}), biofertilizers under prolonged irrigation with xanthan gum (D_{BZ}), and biofertilizers under prolonged irrigation with both mannitol and xanthan gum (D_{BMZ}). The results showed that foliar application of TiO_2-NPs (T_1) led to significant improvements in most of the studied traits. Moreover, the interaction treatment (T_1D_{BMZ}) recorded the highest qualitative and antioxidant traits compared to the prolonged irrigation treatment without any additions (T_0D).

Keywords: secondary metabolites, Biofertilizers, sugar alcohol, TiO_2 NPs, Xanthan gum, food safety.

مجلة العلوم الزراعية العراقية- 2025: 56 (2):648-656 الموسوي وآخرون

المعاملات الصديقة للبيئة والاجهاد المائي وسيلة لحث المركبات الفعالة طبياً في البطاطا

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المستخلص

هدفت التجربة الى تحسين الصفات النوعية وتحفيز تراكم مضادات الاكسدة في نبات البطاطا، بتطبيق معاملات زراعية مستدامة بيئيًا. نُفذت تجربة حقلية اثناء الموسم الربيعي لعام 2023 في حقول كلية علوم الهندسة الزراعية / جامعة بغداد، باستعمال تصميم القطاعات الكاملة المعشاة (RCBD) وفق ترتيب القطع المنشقة، وبعاملين وثلاث مكررات (3 × 6 × 2)، مثّل العامل الأول اضافة (TiO_2–NPs) بتراكيز (0 و10 مل.لتر$^{-1}$)، ورُمز لهما بـ (T_0) و (T_1)على التتابع. أما العامل الثاني فقد شمل ست معاملات كالاتي (ري طبيعي I، ري بمدة متباعدة D، اسمدة احيائية تحت ظرف الري المتباعد D_B، اسمدة احيائية تحت ظرف الري المتباعد+ مانيتول DBM، اسمدة احيائية تحت ظرف الري المتباعد+ صمغ الزنثان DBZ، اسمدة احيائية تحت ظرف الري المتباعد+ مانيتول+ صمغ الزنثان D_{BMZ}) . أظهرت النتائج أن الرش ثنائي اوكسيد التيتانيوم أدى إلى تفوق معنوي في معظم المؤشرات المقاسة. كما تميزت معاملة التداخل (T_1D_{BMZ}) بتسجيلها أعلى القيم في المؤشرات النوعية ومضادات الأكسدة، مقارنةً بمعاملة الري المتباعد بدون إضافة.(T_0D).

الكلمات المفتاحية : المركبات الثانوية ، الاسمدة الاحيائية ،سكر كحولي ،ثنائي اوكسيد التيتانيوم النانوي ،صمغ الزانثان، سلامة الغذاء.

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INTRODUCTION

The practice, known as "antioxidant agriculture," refers to agronomic, chemical, genetic, and microbiological methods to boost plants' natural antioxidants, resulting in more resilient and fruitful crops (39). In actuality, vegetables with more antioxidant content offer more health advantages by scavenging the human body's dangerous free radicals, which lowers the risk of chronic illnesses like heart disease, stroke, and some types of cancer (2, 16, 29 33, 48).With the growing awareness of health and nutrition, the global functional food market is projected to experience substantial growth (9, 21). Potato (*Solanum tuberosum* L.) is considered one of the key global crops, alongside wheat, rice, and maize (17). It is a rich source of carbohydrates and energy, containing minerals such as potassium, as well as vitamins A, B, C, and E, in addition to antioxidants. Potatoes contribute to food security due to their ease of cultivation and high demand, particularly in developing countries (37, 38, 44). This underscores the importance of continued scientific research to employ sustainable practices on the mentioned crop, including the use of Biofertilizers. Which refers to growing living microorganisms that assist in enhancing plant growth, increasing productivity, and improving quality traits. These microorganisms naturally inhabit the soil or plant roots and aid plants in absorbing essential nutrients. Biofertilizers offer a natural alternative to chemical fertilizers, making them more sustainable and environmentally friendly (47). Biofertilizers represent one of the most promising solutions in sustainable agriculture. Numerous studies conducted in Iraq have demonstrated their effectiveness in enhancing plant growth and increasing yield, in addition to their role in improving soil properties and enhancing its biological efficiency over the long term (11, 18). Biofertilizers contribute significantly to enhance the qualitative traits and bioactive compounds of plants, with numerous studies confirming their effectiveness in promoting plant growth and productivity in Iraq (3, 4, 6, 42, 43) . Khan et al. (31) reported that the application of biofertilizer enriched with *Trichoderma* (BioF/compost) improved soil fertility, stimulated tomato plant growth, and increased yield by 12.9% compared to other treatments, including the recommended doses of NPK fertilizers. Furthermore, there was an elevated content of antioxidant compounds, including ascorbic acid, β-carotene, and lycopene. Similarly, Cordeiro et al. (13) demonstrated that biofertilizer application enhanced the qualitative attributes of potatoes and boosted their productivity by stimulating amino acid metabolism. In line with these findings, Tian et al. (40) showed that bio-inoculation with biofertilizers improved potato growth and quality traits by enhancing photosynthetic efficiency, sugar metabolism, and the biosynthesis of phenylpropanoid compounds. Mannitol, a hexose sugar alcohol, plays a vital role in enhancing plant growth and productivity, as well as improving the efficacy of biofertilizers under harsh environmental conditions such as drought and salinity (45). Through various physiological and biochemical mechanisms, mannitol functions as an Osmoprotectant, supporting the ability of plants and soil microorganisms to cope with environmental stress by improving water retention and nutrients uptake. This leads to increased biomass and yield (50). Mannitol also plays a key role in regulating the symbiotic relationship between plants and beneficial microorganisms by neutralizing reactive oxygen species (ROS), which are produced by plants as part of their immune response. By reducing ROS levels, mannitol facilitates fungal colonization of plant roots without triggering plant defense mechanisms. Conversely, plants metabolize fungal-derived mannitol through the enzyme mannitol dehydrogenase to maintain redox balance and sustain the symbiotic association (28). Additionally, mannitol serves as a valuable carbon source for soil microorganisms, thereby enhancing microbial activity and promoting key biological processes such as nitrogen fixation and root colonization (32). Plants treated with mannitol exhibit significant improvements in growth and productivity due to enhancing stress tolerance and more efficient resource utilization. These findings highlight the critical role of mannitol in supporting the effectiveness of biofertilizers, particularly in marginal agricultural environments and under suboptimal growing conditions (32). Titanium dioxide nanoparticles (TiO_2 NPs) are considered an effective tool in agriculture for enhancing plant growth, productivity, and the stimulation of bioactive compounds, including antioxidants under unfavorable conditions. the study of (22) focused on TiO_2 NPs effects on Vitex plant, revealing an increase in total chlorophyll content and soluble sugars at a concentration of 200 ppm. As the effects of climate change intensify; researchers have adopted techniques to preserve soil moisture, most notably the use of polymers, which reduce irrigation water consumption by 30–50% by absorbing, storing, and gradually releasing water to plant roots, thereby minimizing losses from evaporation and surface runoff (20, 41). In fact, Long-term studies have shown that conventional synthetic polymers are non-biodegradable and tend to accumulate in soil as end products that pose risks to both environmental and human health. Consequently, research has shifted toward the development of safer and more environmentally friendly alternatives, namely biopolymers. These are naturally derived compounds with complex structures, characterized by their ability to absorb and retain irrigation water, then gradually release it during periods of drought (27). This enhances water use efficiency without causing harmful effects on soil or plants. Xanthan gum is considered one of the most prominent examples of such biopolymers (46). Xanthan gum (XG) is a high molecular weight biomacromolecule considered an ideal raw material for the synthesis of superabsorbent hydrogels, due to its excellent properties such as biocompatibility, biodegradability, high water retention capacity, and renewability (34). Das et al (15). developed a slow-release fertilizer hydrogel using XG and gelatin as primary components, with glutaraldehyde as a crosslinking agent. Accordingly, this study aims to improve the qualitative traits of potato and enhance antioxidant production under drought stress, using environmentally friendly and sustainable agricultural techniques.

MATERIALS AND METHODS

This experiment was conducted at Research Station (A) of the College of Agricultural Engineering Sciences, University of Baghdad (Al-Jadriyah), during the spring season of 2023. The chemical and physical characteristics of the soil are presented in Table (1). Potato tubers of the Santana hybrid (Elite) were planted in a single row at the center of the furrows on January 28, 2023. The field was equipped with a drip irrigation system. The spacing between plants was 0.25 meters within rows and 0.75 meters between rows, resulting in a planting density of 53,333 plants per hectare. All experimental plots were harvested 120 days after planting.

Experimental Design:

The experiment was conducted using a Randomized Complete Block Design (RCBD) in a split-plot arrangement within three replications (2 × 6 × 3). Titanium dioxide (TiO_2) served as the main plot factor, applied at two concentrations (0 and 10 $mg \cdot L^{-1}$), denoted as T_0 and T_1, respectively. The sub-plot factor comprised six drought mitigation strategies (DMS), as follows:

-I : Regular irrigation every 4 days (control),

-D : Prolonged irrigation every 8 days, based on the recommendations of (5)

-DB : Prolonged irrigation combined with fungal biofertilizers,

-DBM : Prolonged irrigation with fungal biofertilizers and mannitol,

-DBZ : Prolonged irrigation with fungal biofertilizers and xanthan gum,

-DBMZ : Prolonged irrigation with fungal biofertilizers, mannitol, and xanthan gum.

Table 1. Physical and chemical properties of the soil .

Character	Value
pH	6.16
$EC_{1:1}$ ($ds.m^{-1}$)	390.8
Total N ($mg\ kg^{-1}$)	53.8
P ($mg\ kg^{-1}$)	12.5
K ($mg\ kg^{-1}$)	168.8
Ca ($mg\ kg^{-1}$)	185.8
Mg ($mg\ kg^{-1}$)	168.8
Fe ($mg\ kg^{-1}$)	1.4
Na ($Meq\ L^{-1}$)	59.8
Cl^- ($Meq\ L^{-1}$)	49.8
SO_4^{-2} ($Meq\ L^{-1}$)	205.8
HCO_3^- ($Meq\ L^{-1}$)	475.8
O.M. (%)	9.1
Gypsum (%)	318.8
Sand (%)	10.8
Silt (%)	38.8
Clay (%)	46.8
Texture	Clay loam

Nano-anatase titanium dioxide was applied via foliar spraying three times during key phenological stages of potato growth: the vegetative growth stage, the tuber initiation stage, and the tuber filling stage. Fungal biofertilizers (*Glomus intraradices* and *Trichoderma asperellum*) were mixed with corn cob residues at a concentration of 50 $spores \cdot g^{-1}$ and placed as a pad beneath each tuber at sowing, at a rate of 20 g per tuber. Xanthan gum was incorporated into the soil at 1% (w/w) prior to planting. Mannitol was applied three times by injection into the rhizosphere at a concentration of 30 mM·L⁻¹, in synchronization with the same growth stages during which titanium dioxide was applied.

Study traits: The following traits were measured: T.S.S. percent (%) , protein percent (%) (14), tubers Specific Density ($kg.cm^3$),tubers Firmness ($kg.cm^2$). The measured antioxidant traits included tuber ascorbic Acid percent (%) (26), lutein concentration ($mg.g^{-1}$ f.w.) (25) ,β-carotene concentration ($mg.g^{-1}$. f.w.) (35), total carotenoids concentration ($mg.g^{-1}$ f.w.) (24). The quality and antioxidant indicators were measured at the Plant Laboratory, Agricultural Research Department, Ministry of Science and Technology. Data were analyzed using analysis of variance (ANOVA), and means were compared using the Least Significant Difference (L.S.D) test at a 5% probability level.

RESULTS AND DISCUSSION

The foliar application of titanium dioxide nanoparticles (TiO_2-NPs) showed a significant superiority in the evaluated traits, recording the highest total soluble solids (T.S.S) (6.278%), Tuber firmness (12.83 $kg·cm^{-2}$), protein percent (6.512%), and specific density (1.086 $kg·cm^{-3}$), compared to control, which exhibited the lowest across all measured traits. According to the data presented in Table (2.), plants treated with biofertilizers, biopolymer, and mannitol under drought stress conditions demonstrated a clear enhancement, achieving the highest TSS percent (6.367%), tuber firmness (13.16 $kg·cm^{-2}$), protein percent (6.733%), and^{-3} specific density (1.088 $kg·cm^{-1}$) In contrast, the drought stress treatment without any additives (I) recorded the lowest: (5.633%, 11.00 $kg·cm^{-2}$, 6.267%, and 1.080 $kg·cm^{-3}$) consequently. Regarding the interaction treatments, the combination treatment (T_1D_{BMX}) involving TiO_2-NPs foliar application and drought stress mitigation practices show a significant superiority in both T.S.S (6.633%) and protein percent (6.933%) when compared to the (T_0I) interaction treatment, which recorded the lowest (5.433% T.S.S and 6.167% protein). However, the interaction effects were not statistically significant for tuber firmness and specific density.

Table 2. Effect of titanium dioxide (T), and drought mitigation strategies (DMS): biofertilizers (B), mannitol (M), and xanthan gum (X) on T.S.S.(%) , T. Firmness ($Kg.cm^{-2}$) , Protein(%) and Specific-Density($kg.cm^{-3}$) of potato plant under drought conditions

traits / treatments	T.S.S. (%)	T. Firmness ($Kg.cm^{-2}$)	Protein (%)	Specific-Density ($kg.cm^{-3}$)
		T		
$T_{0\ (control)}$	5.644	11.66	6.292	1.085
T_1	6.278	12.83	6.512	1.086
LSD($_{0.05}$)	0.248	0.828	0.135	0.0006
		DMS		
I [irrigated (control)]	5.633	11.00	6.267	1.080
D [drought]	5.867	11.83	6.010	1.085
D_B	5.867	12.16	6.367	1.086
D_{BM}	5.900	12.50	6.517	1.087
D_{BX}	6.133	12.83	6.517	1.088
D_{BMX}	6.367	13.16	6.733	1.088
LSD($_{0.05}$)	0.167	0.659	0.092	0.0014
		TXDMS		
T_0I (control)	5.433	10.66	6.167	1.080
T_0D	5.633	11.33	6.017	1.084
T_0D_B	5.433	11.66	6.300	1.086
T_0D_{BM}	5.467	11.66	6.367	1.087
T_0D_{BX}	5.800	12.00	6.367	1.087
T_0D_{BMX}	6.100	12.66	6.533	1.087
T_1I	5.833	11.33	6.367	1.081
T_1D	6.100	12.33	6.003	1.086
T_1D_B	6.300	12.66	6.433	1.087
T_1D_{BM}	6.333	13.33	6.667	1.088
T_1D_{BX}	6.467	13.66	6.667	1.089
T_1D_{BMX}	6.633	13.66	6.933	1.089
LSD($_{0.05}$)	0.249	N.S	0.137	N.S

The results presented in Table (3.) demonstrated that foliar application of (TiO_2-NPs) had a significant effect compared to the control. This treatment led to a notable increase in the accumulation of total carotenoids (0.132 $mg\cdot g^{-1}$), β-carotene (0.067 $mg\cdot g^{-1}$), and lutein (0.040 $mg\cdot g^{-1}$), in addition to an increase in ascorbic acid percent, which reached 3.442%.In a related context, plants treated with biofertilizers, biopolymer, and mannitol under drought stress conditions exhibited superior performance, as shown in the same Table. These treatments recorded the highest concentrations of the studied biochemical compounds, including total carotenoids (0.155 $mg\cdot g^{-1}$), β-carotene (0.078 $mg\cdot g^{-1}$), lutein (0.047 $mg\cdot g^{-1}$), and ascorbic acid (3.650%), compared to the regularly irrigated plants (I), which showed the lowest (0.063 $mg\cdot g^{-1}$, 0.033 $mg\cdot g^{-1}$, 0.020 $mg\cdot g^{-1}$, and 3.055%) respectively. Furthermore, the interaction between foliar application of TiO_2-NPs and drought conditions revealed that the combined treatment (T_1D_{BMX}) significantly outperformed all other treatments in all measured traits except for ascorbic acid percentage. This treatment resulted in the highest accumulation of antioxidants in potato tubers, (0.214 $mg\cdot g^{-1}$, 0.108 $mg\cdot g^{-1}$, 0.064 $mg\cdot g^{-1}$, compared to the regularly irrigated treatment (T_0I), which recorded the lowest for all studied indicators (0.060 $mg.g^{-1}$, 0.032 $mg.g^{-1}$, 0.019 $mg.g^{-1}$) consequently.

Table 3. Effect of titanium dioxide (T), and drought mitigation strategies (DMS): biofertilizers (B), mannitol (M), and xanthan gum (X) on T. Carotenoids($mg.g^{-1}$) , β _Carotene($mg.g^{-1}$) ,lutein($mg.g^{-1}$) and Ascorbic acid(%) of potato plant under drought conditions.

Traits treatments	T. Carotenoids ($mg.g^{-1}$)	β _Carotene ($mg.g^{-1}$)	Lutein ($mg.g^{-1}$)	Ascorbic acid (%)
		T		
$T_{0\ (control)}$	0.089	0.046	0.027	3.293
T_1	0.132	0.067	0.040	3.442
LSD($_{0.05}$)	0.0248	0.0124	0.0074	0.0769
		DMS		
I $_{[irrigated\ (control)]}$	0.063	0.033	0.020	3.055
D $_{[drought]}$	0.090	0.047	0.028	3.250
D_B	0.106	0.055	0.033	3.400
D_{BM}	0.130	0.066	0.040	3.400
D_{BX}	0.122	0.062	0.037	3.450
D_{BMX}	0.155	0.078	0.047	3.650
LSD($_{0.05}$)	0.0071	0.0034	0.0021	0.0764
		TXDMS		
T_0I $_{(control)}$	0.060	0.032	0.019	0.023
T_0D	0.091	0.047	0.028	3.167
T_0D_B	0.094	0.049	0.029	3.333
T_0D_{BM}	0.098	0.050	0.030	3.333
T_0D_{BX}	0.098	0.050	0.030	3.367
T_0D_{BMX}	0.095	0.048	0.029	3.533
T_1I	0.066	0.035	0.021	3.087
T_1D	0.088	0.046	0.027	3.333
T_1D_B	0.117	0.060	0.036	3.467
T_1D_{BM}	0.162	0.083	0.049	3.467
T_1D_{BX}	0.146	0.074	0.044	3.533
T_1D_{BMX}	0.214	0.108	0.064	3.767
LSD($_{0.05}$)	0.0188	0.0094	0.0056	N.S

The foliar application of titanium dioxide nanoparticles led to a significant improvement in both the qualitative and medicinal traits of plants, due to their role in enhancing photosynthetic efficiency by promoting chloroplast development and increasing the plant's ability to cope with drought stress (23). Additionally, titanium dioxide nanoparticles acted as a protective barrier against ultraviolet radiation, which helped reduce leaf damage caused by sunburn (30). Furthermore, the nanoparticles stimulated photosynthetic activity by increasing light absorption by chloroplasts, enhancing the plant's photosynthetic capacity, and preventing chloroplast aging) (49). These effects also contributed to the activation of biologically active compounds, including antioxidants. Biofertilizers composed of mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) demonstrated high efficiency in improving the qualitative traits and antioxidant content of potato plants under drought conditions (Tables 2 and 3). This effect is attributed to their role in enhancing water and nutrient uptake and stimulating the plant's physiological processes. Microorganisms are a fundamental component of sustainable agriculture, contributing to improved soil fertility, increased nutrient availability, and enhanced plant resilience to environmental stress (10, 12). Both PGPR and AMF have proven effective in supporting plant growth and productivity by enhancing root interactions and stimulating the production of beneficial bioactive compounds (9, 19, 36). The effective impact of mannitol is attributed to its role as an osmotic agent that induces water deficiency, thereby simulating drought conditions. This triggers a series of physiological and biochemical responses within the plant, stimulating the accumulation of compatible solutes that help maintain cellular homeostasis and sustain vital functions under stress. Additionally, mannitol promotes the production of Osmoprotectant, which play a fundamental role in enhancing the plant's tolerance to water scarcity (1). The addition of xanthan gum to soils under stress enhances the cohesion between their fine particles, potentially leading to a significant increase in soil binding strength up to three times higher compared to untreated soils, particularly when moisture content drops to certain critical levels. This improvement is attributed to the gel-like nature of xanthan, which forms a network that integrates and binds soil particles together (7). In conclusion, ensuring safe food with high quality and exceptional nutritional value is of paramount importance. Additionally, adopting sustainable development practices contributes to mitigating the effects of climate change in Iraq, thereby enhancing the stability of ecosystems, and ensuring environmental sustainability for future generations.

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