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Research Article

Changes in the Hormones Content of Common Bean (*Phaseolus vulgaris* L.) cv. Valentino Plant to Treatment with Industrial Ceramic-Waste Water Sludge

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ABSTRACT

This study aimed to highlight the phytohormones aspects impressed by different levels of ceramic waste water sludge on common bean (*Phaseolus vulgaris* L.) cv. Valentino cultivated in sandy soils. The studies concerning the effects on phytohormones at relatively low concentrations (0.5%, 1% and 2%) for 30 days. Within the different levels used of CWWS, especially at 1%, Endogenous phytohormones Indoleacetic Acid (IAA), Indolebutyric Acid (IBA) and Abscisic Acid (ABA) showed enhanced levels and a reverse trend was obtained with Gibberellic Acid (GA3) level in plants exposed to different levels of ceramic sludge.

Keywords: Ceramic-wastewater sludge; Common bean; Heavy metals; Phytohormones

INTRODUCTION

As a by-product of treating industrial or municipal wastewater, semi-solid sewage sludge is a sink for a variety of chemicals, some of which are harmful to living things. It is the end result of the techniques used to treat wastewater. It is made up of various raw wastewater constituents as well as anthropogenic activity by products such pesticides, heavy metals and medication residues that end up in a sewerage system catchment region [1]. Wastewater from homes and businesses is included in municipal sewage. High concentrations of important organic materials and nutrients, including phosphate and nitrogen, can be found in sewage sludge. Sewage sludge is an effective soil enhancer because of these ingredients. It enhances the physical, chemical and biological characteristics of the soil, such as bulk density, water mobility, aggregate stability and high crop yields can be achieved by increasing the amount of nutrients in the soil and reducing the demand for artificial fertilizers through the use of sewage sludge in agriculture. Due to the bio-solid matter's low cost and fertilizer qualities, solid sewage sludge has gained popularity in metropolitan areas where agriculture is practised. According to Kacprzak et al. one waste management tactic used in agriculture is the exploitation of sewage sludge. It also emphasizes the use of sewage sludge in farming as a means of resolving issues related to sewage sludge disposal. Additionally, there are materials in sewage sludge that are not good for the soil. Sewage sludge contains pathogens like bacteria and viruses as well as inorganic substances like heavy metals.

Pathogens, such as bacteria and viruses. Studies have indicated that contaminants from sludge may seep into crops. Obasi, et al. found that plants cultivated on dumpsite soil absorbed heavy metals and Fei-Baffoe, et al. found that plants grown on soil treated with sewage sludge accumulated heavy metals as well. Vegetables cultivated in sludge-treated soil showed a variety of heavy metals and other pollutants, according to Nunes et al. The application of sludge to agricultural soil that contains pollutants such as pesticides, surfactants and polycyclic aromatic hydrocarbons poses a risk to human health and the environment. Therefore, it is of concern that heavy metals leaking from sewage sludge could contaminate vegetables. Considering the world over; people are encouraged to consume a lot of vegetables to improve health as vegetables are associated [2].

In spite of five classical plant hormones, *i.e.*, Gibberellins (GAs), Cytokinins (CKs), auxins, Abscisic Acid (ABA) and ethylene, Jasmonate (JA), Brassinosteroids (BR) and Salicylic Acid (SA) are also well known for regulating many physiological processes and

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heavy metal stress tolerance. Heavy metal-mediated alteration in hormonal balance correlates with their toxicities in plants. Treatment of chickpea seeds with increasing concentrations of either Pb or Zn generally decreased the Gibberellic Acid (GA3) content. Several studies revealed that GA alleviates various abiotic stresses including heavy metal toxicity [3]. In *A. thaliana*, GA (5 μM) is reported to ameliorate Cd toxicity by reducing Cd uptake and lipid peroxidation. These studies clearly indicate that GA plays an important role in protecting plant metabolism against various stresses.

MATERIALS AND METHODS

Pure lots of seeds of common bean (*Phaseolus vulgaris* L. cv. Valentino) were obtained from the department of vegetable crops production and technology, horticulture research institute, agriculture research centre, Giza, Egypt. Ceramic-wastewater sludge was collected from a drainage outlet of a ceramic industrial factory in Abou-Zaabal, Egypt. Virgin sandy soil was obtained from Belbeis desert, Sharquia Governorate Northern East Cairo.

Methods

All the experiments have been carried out in the green house at the botanic garden, faculty of science, Ain Shams university, where the different analyses were done in the laboratory of plant physiology, department of botany, faculty of science, Ain Shams university, Cairo, Egypt [4].

Analyses of some physical and chemical properties of the soil used in this study were done as described by Cottenie et al. Sodium, potassium, calcium, magnesium, chloride, sulphates, carbonates as well as bicarbonates were determined by atomic absorption spectrometer GBC (Savantaa) [5]. Organic matter was determined using the method of Walkley (Table 1).

Table 1: Physical and chemical analysis of sandy soil.

Properties	Value
pH	8
EC (dS m^{-1})	0.28
Soluble anions (%)	
Na^+	2.17
HCO_3	3.4
Cl^-	6.5
SO_4	12.9
Soluble cations (%)	
Ca^{++}	15.2
Mg^{++}	4.6
K^+	0.89
Organic carbon (g/100g soil)	0.03
Physical analysis	
Coarse sand (%)	64.1
Fine sand (%)	24.4
Silt (%)	8.8
Clay (%)	2.7
Texture	Sandy soil

Ceramic wastewater sludge was analyzed in central laboratory, faculty of science, Ain Shams university by atomic absorption apparatus and the data was recorded in the Table 2 [6].

Table 2: Analysis of the ceramic wastewater sludge (%).

Mineral ion	Concentration (% W/W)
Iron (Fe)	1.77
Zinc (Zn)	0.65
Lead (Pb)	0.04
Calcium (Ca)	1.93
Magnesium (Mg)	0.25
Sodium (Na)	1.59
Potassium (K)	1.01
Aluminum (Al)	8.33
Silicon (Si)	29.47

Endogenous phytohormones were assayed following the method described by Shindy and Smith. 10 grams fresh plant leaves from different treatment were ground in cold 80% ethanol, the macerated tissues were transferred to a flask with fresh volume of ethanol and stirred for 30 minutes with magnetic stirrer, then filtered again, the procedure was repeated once more and the combined extracts were evaporated to the aqueous phase in the a rotatory evaporator.

To estimate the amounts of acidic hormones (fraction 1), the aqueous phase (10 mL-30 mL) was adjusted to pH 8.6 with 1% NaOH and partitioned three times with equal volumes of ethyl acetate. The combined ethyl acetate fraction was evaporated to dryness and held for further purification. The aqueous phase was adjusted to pH 2.8 with HCl and portioned three times with equal volume of ethyl acetate. The remaining aqueous phase was discarded [7]. The combined acidic ethyl acetate was evaporated and then dissolved in methyl alcohol (fraction 1) to be ready to High Performance Liquid Chromatography (HPLC) for determination of acidic hormones (IAA, ABA, IBA and GA3).

Injected of 10 µl into HPLC 510b was used for identification and determination of hormone using data model (Waters 746), detector (U.V Tumable Absorbance), pump (HPLC 510). The chromatography was fitted (equipped with 3.9 × 300 mm Bondapack C18 capillary column). The HPLC was operated under temp 25°C. The Retention Time (RT) and the area of peaks of different phytohormones of authentic standards were used for the identification and characterization of peaks of samples under investigation [8].

RESULTS

Changes in the hormones content

Changes in auxins content: The results presented in Table 3 reveal that the contents of Indoleacetic Acid (IAA) and Indolebutyric Acid (IBA) of common bean plants were significantly increased under different levels of ceramic wastewater sludge. The maximum increases were recorded at 1% level in case of IAA which reached about 173.9% of the control value and in case of IBA was 208.3% at 2% level.

Changes in gibberellin content: The ceramic wastewater sludge induced a significant decrease in the content of gibberellin (GA3) of *P. vulgaris* (cv. Valentino) plants. The percentage of decrease was elevated with the progressive increase of ceramic sludge from 0.5 to 1 and 2% (12.6%, 45.1% and 73.3%, respectively).

Changes in abscisic acid content: The increase in the content of Abscisic Acid (ABA) was manifested in the common bean plants grown under different levels of ceramic wastewater sludge [9]. The highest accumulation of ABA content was recorded at 2% level which was 260.8%, as compared with the control value.

GA3/IAA and GA3/ABA ratios: The GA3/IAA and GA3/ABA ratios were declined in the *P. vulgaris* (cv. Valentino) plants in response to the application of different wastewater sludge [10]. The percentage of decrease was increased with the increase of ceramic sludge level where the maximum decrease was noticeable at 2% level.

DISCUSSION

Heavy metals induced changes and disturbances in the balance of growth hormones, which led to some defense pathways for the synthesis of phytochelatins, metallothioneins and specific stress proteins [11]. The hazardous effect of heavy metals induced signaling pathways mainly associated with the accumulation of Abscisic Acid (ABA), as well as a decline in the levels of the growth promoting hormones as Indoleacetic Acid (IAA), Gibberellic Acid (GA3) and cytokinins. Plant hormones (ABA, IAA and GA3) are key players in regulating plant growth, development and morphogenesis processes and play an important role in plant stress tolerance and stress signal transduction.

ABA as a growth-inhibiting hormone and IAA and GA3 as growth-promoting hormones has been also reported by Yan and Chen [12].

In the present study, ceramic wastewater sludge at differentially levels caused pronounced increases in IAA, IBA and ABA levels in common bean cv. Valentino plants (Table 3).

Table 3: Effect of different levels (w/w) of Ceramic Waste Water Sludge (CWWS) on indoleacetic acid (IAA), Indolebuytaric Acid (IBA), Gibberellic Acid (GA3) and Abscisic Acid (ABA) contents as well as GA3/IAA, GA3/ABA ratios in *Phaseolus vulgaris* L. (cv. Valentino) plants (30-day-old) grown in sandy soil.

Parameters/ CWWS (%)	IAA	IBA	GA3	ABA	GA3/IAA	GA3/ABA
	(µg/gFW)					
0.0	2.3	0.24	12.02	0.23	5.22	50.2
0.5	4.2	0.35	10.5	0.35	2.5	30
1.0	6.3	0.4	6.6	0.47	1.04	14.04
2.0	3.5	0.74	3.2	0.83	0.91	3.85

CONCLUSION

The increase of levels of IAA and IBA in young *P. vulgaris* plants, as a result of amended soil with ceramic wastewater sludge (mostly polluted by heavy metals), might be attributed to a decrease in the activities of IAA oxidase and peroxidase leading to formation of IAA and/or due to increase in IAA biosynthesis. Abscisic acid is a generic stress hormone that has multiple functions, including induction of genes involved in stress protection and stomata closing. Furthermore, the noticeable decline in gibberellins (GA3) of common bean plants caused by the application of ceramic wastewater sludge might result from the conversion of free active gibberellins into bound inactive ones or due to inhibition of the biosynthesis of gibberellins.

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