

DETECTION OF ACTIVE CHEMICALS IN ZEA MAYS WASTE AND SEPARATION ALLELOPATHIC SUBSTANCES UTILIZING HPLC TECHNOLOGY

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ABSTRACT

Some active compounds were detected in Zea mays plant waste, with the separation and estimation of the percentages of some of them. Allelopathic compounds were also isolated and diagnosed using a device. High-performance liquid chromatography (HPLC), as the results of the initial qualitative detections showed the presence of most secondary metabolite compounds, which include glycosides and alkaloids and tannins, flavonoids, terpenes, saponins, and resins in yellow corn waste. The results of analysis by high-performance liquid chromatography (HPLC) showed the presence of a number of phenolic compounds in corn waste compounds (Vanillin, Rutin, Resorcinol, Hydroquinone, Caffeic acid).

KEYWORDS: Allelopathic compounds, Secondary metabolic compounds, *Zea mays*.

1. INTRODUCTION

Allelochemicals are secondary metabolic compounds that are built during the metabolism of carbohydrates, fats, and amino acids and are transformed into the acetate acid pathway. Allelopathies are secondary metabolites that are built within the plant during its growth period. These compounds include alkaloids, phenols, and flavonoids and terpenes, after being released into the environment through washing and volatilization, root exudates, and decomposition of plant residues. Decomposition initiates allelopathic compounds. Its effect on other organisms within the biological system that includes plants, insects, fungi, as well as microorganisms.^[1] The allelopathic effects are caused by the liberation of Allelopathic compounds, so allelopathic research is incomplete without studying, isolating, diagnosing and estimating these compounds.^[2] Rice (1984) divided the allelopathic compounds into 14 groups, while^[3] Tang and Putnam (1986) divided them into 11 groups. In both divisions, the most common chemical compounds are: alkaloids, steroids, tannins, coumarins, and others. Then there was a succession of studies on these compounds and knowledge of their nature and mechanism of effect^[4] found that the presence of

allelopathic compounds in the environment and in certain concentrations is sufficient to affect plants growing with it or following it in agriculture. These compounds also have an acidic property.^[5]

It can change the pH of the soil surrounding it or affected by it, and the cause of the allelopathic effects is the compounds released from the plant that are in the form of phenolic compounds are released into the soil and cause a reduction in the growth of other plants. Phenolic compounds are chemical compounds that contain, the hydroxyl group, OH, is attached to an aromatic hydrocarbon group, which may be the plant's natural defenses against herbivores.^[6]

Allelopathic compounds are divided according to their different composition and properties into several types according to^[7] water-soluble organic acids, straight-chain alcohols, aldehydes and ketones, simple unsaturated complexes (complex quinine, anthroquinine, polyacetylenes), fatty acids, coumarins, flavonoids, tannins, cinnamic acid, phenols, benzoquinine steroids and terpenes. Phenolic compounds are among the most important and common allelopathic compounds in plants and ecosystems.

The presence of phenolic acids in allelopathic diseases is weak, but they contain a group of compounds that include aromatic phenols. Simple hydroxybenzoic acid, aldehyde, cinnamic acid derivatives, coumarins, tannins and a few flavonoids.

In addition to the role of phenolic compounds in the allelopathic phenomenon, which is protective against diseases such as heart disease, stroke, and cancer, due to the lack of knowledge about the effect of allelopathic compounds; Therefore, more attention must be given to vehicles being the basis for allelopathic effects in agricultural settings, crops use allelochemicals Gallandt and Srrantonio (8), Therefore, the research aims to detect some effective compounds in plant residues of Zea mays plants and extracted using appropriate chemical reagents, and the proportions of some of them were separated and estimated, in addition to the isolation and diagnosis of allelopathic compounds using a high-performance liquid chromatographic separation device.

2. MATERIALS AND METHODS

Research experiments were conducted in laboratories affiliated with the Ministry of Science and Technology, Department of Environment and Water, to reveal and estimate the proportions of some active compounds and to diagnose some phenolic compounds in plant residues of yellow corn plants. The research included the following steps.

Detection of some active compounds in plant wastes (flavones, saponins, glycosides, alkaloids, tannins, terpenes, and resins), along with estimating the percentages of some active compounds in plant wastes, such as the percentage of glycosides in pure, crystalline solid form. Estimating the percentage of alkaloids based on dry weight and estimating the percentage of tannins based on dry weight according to the following equation:

$$\frac{\text{The weight of the remaining residue before burning} - \text{the weight of the remaining residue after burning}}{100} \times 100 \text{ x \% for tannins}$$

Estimating the percentage of flavonoids based on dry weight and identifying phenolic compounds in plant wastes. High performance liquid chromatography (HPLC) technology was followed, as alcoholic extracts of plant samples were prepared from the preparation of twelve standard compounds by dissolving 100 mg/30 ml of standard compounds were made by injecting 5 microliters of previously prepared standard compounds into the separation device. 15cm× 4.6 5mm and its dimensions are SUPELCOSIL™ Column LC using a separation column 18 LC 2010 HT type to obtain the complete retention time of the standard compounds that were prepared. Retention time of prepared standard compounds include (1.99 Hydroquinone, 3.25 Quercetin, 2.32 Rutin, 2.36 Caffeic acid, 2.44 P-Hydroxybenzoic acid, 2.49 Vanillin, 2.66 Salicylic acid 2.67 Catechol, 2.67 Gallic acid, 2.68 Benzoic acid, 2.75 Resorcinol).

3. RESULTS AND DISCUSSION

3.1. Preliminary detection of secondary chemical compounds in Zea mays plant waste and estimation of the proportions

The initial detections of the chemical compounds studied in the plant wastes included glycosides, alkaloids, flavonoids, terpenes, tannins, resins, and saponins. The preliminary detections indicated the investigation of the presence of active compounds in the plant wastes, as the results appear positive detection of active chemical compounds in all tested replicates of plant wastes that were released into the environment as a result of the washing process, or by the influence of microorganism's decomposers.

To reach the effective concentration to show its effect on the physiological and biochemical characteristics necessary for the germination process to occur, such as water absorption of the seeds, stimulating the embryo, and the effectiveness of enzymes, as well as its effect on osmotic potential and mitochondrial activity. These compounds also affect seed germination through their effect on membrane permeability and plant division and differentiation, as^[9] mentioned the variation in the allelopathic effects of soil extracts containing on sunflower waste with an addition rate of (0.5, 1.5, 2.5%) in the percentage of germination for two types of corn and sunflower. Studies have shown that alkaloids affect cell division and flavonoids affect plant hormones. As for the saponins, which the results showed are present in plant waste, they have an inhibitory effect in Seed germination and growth, as it affects chromosome division.^[10]

As for estimating the proportions of some secondary chemical compounds in the studied plant wastes, Tannins recorded the highest amount of 2.24% in waste, while the highest amount of glycosides was 0.78%, while the alkaloids reached their highest level of 1.04%. The highest amount of flavonoids was recorded at 2.89%, and thus the waste was distinguished by containing percentages, higher than most of the secondary chemical compounds studied.

The results showed the highest concentration of flavonoids among these compounds. Studies have indicated that allelopathic compounds found in plant residues and added to the soil are released into the environment through washing (soluble in water) and that these compounds may suffer from chemical transformations as they may It suffers from decomposition under the influence of microorganisms in the soil, such as oxidation, decomposition, and polymerization, which changes its nature and concentration, and some of the compounds can bind with clay or soil elements.^[7,11]

Humus may increase soil fertility, and some compounds, such as phenolic acids, can accumulate in the soil to reach the level of toxicity (inhibitory effect). Also, allelopathic compounds released into the soil may suffer biochemical transformations due to microorganisms, which leads to their transformation into other simpler compounds or more complex, which reflects their allelopathic influence on germination, growth, and physiological processes associated with the plant. The preliminary detection results also showed that plant wastes contain high concentrations of glycosides and flavonoids, and that these compounds have a clear effect on the physiological processes of the plant, as well as affecting germination and growth.^[12]

Glycosides have a role in physiological changes in the germination stage. They are also considered among the substances necessary for the metabolism process, and alkaloids have an effect on cell division, while flavonoids affect plant hormones.

3.2. Diagnosis and quantitative determination of phenolic compounds in plant wastes

The detection process resulted using high-performance liquid chromatography technology on the separation of phenolic compounds by drawing curves of the absorption peaks for each compound coupled with chromatography (HPLC) approximate to what this study used.^[13] Specific to it, the retention time values of the standard compounds that were prepared in the laboratory were adopted to match them with the RT values of the retention time of the compounds separated from plant waste extracts. It is evident from the compounds, the Rt values of the HPLC curves are the presence of several compounds in each curve, which were diagnosed using the HPLC technology.^[14,15]

Diagnostics in plant extracts and alcoholic extracts of crops using HPLC using Rt technology include (Hydroquinone 2.4, Coffee acid 2.55, Rutin 2.7, Quercetin 3.65, P-Hydroxybenzoic acid N, Vanillin 2.9, Salicylic acid N, Catechol N, Resorcinol 3.22).

The results showed the presence of several phenolic compounds known for their high allelopathic ability, which were diagnosed with this technology. It seems clear that there is a high percentage of the number of diagnosed phenolic compounds, and the compounds (Hydroquinone and Caffeic acid were diagnosed Quercetin, Resorcinol and Rutin), this agree with.^[16]

Normal cell growth requires regulating the level of these two hormones. Allelopathic compounds also affect the balance of some hormones, such as auxin, as mentioned in studies have also shown that disruption of the level of this compound affects the growth of the plant's secondary roots, and the content of the peroxidase enzyme Phenylalanine ammonia-lyase reduces root growth and reduces the effectiveness of the Caffeic acid enzyme. Which increases the rigidity of the cell wall, prevents root growth, and increases the number of lignin units in roots exposed to contamination, this agree with.^[16-18]

3.3 Quantitative determination of phenolic compounds isolated from plant wastes

The concentration of phenolic compounds was calculated and estimated quantitatively by comparing the area under the curve of the HPLC material based on the analysis technique with the area under the curve of plant waste samples, as the results indicated that there were important differences in the concentration of chemical compounds isolated from plant waste. We find that the highest concentration is 296 µg/g. The

reason for the effectiveness of plant waste and aqueous extracts of these wastes is due to Allelopathic compounds liberated from it due to the process of decomposition of plant residues and the washing process. This has been confirmed more precisely in our current study through the detection, diagnosis and measurement of some chemical compounds (secondary metabolite compounds) from yellow corn residues according to HPLC ethanol extract and according to the estimation of the amount of phenolic acids ($\mu\text{g/g}$) in diagnostic for plant waste.

4. CONCLUSION

The chemical compounds in the plant perform the function of protecting the plant from external damage. Many of these compounds were extracted and used for medical purposes. These compounds are concentrated in the secretory structures of the plant. These structures were diagnosed in the leaves of the corn plant and the nature of the chemical compounds in them was identified through the use of Chemical reagents, as fats, fatty acids, neutral fats, essential oils, resins, phenols, lignin, pectic substances, starch, alkaloids and proteins were detected, and most of the secretory structures and chemical compounds of the methanolic extract of the leaves of the plants under study appeared through HPLC technology. It has been shown that there are many active compounds that are important to the plant, and that the cause of the allelopathic effects is the compounds released from the plant, which are in the form of phenolic compounds that are released into the soil, and cause a reduction in the growth of other plants due to the types of pollution affected by this plant or other plants, and that this plant contains phenolic compounds it binds to an aromatic or free hydrocarbon group that may be a natural defense of the plant against herbivores.

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