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Evaluation the effectiveness of Nano-chitosan with wax to control the growth of water hyacinth (*Eichhornia crassipes*)

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Abstract

Water hyacinth is an aggressive invasive plant. The infestation can double in size every 5 days under ideal conditions, quickly covering the surface of the water and taking over an entire stream. This plant's ability to tolerate a wide range of temperatures, nutrients, and pH levels gives it a superior advantage over other native freshwater plants whose growth is generally slower. In the current research, the growth of the water hyacinth plant was treated in an environmentally friendly manner. This is done by spraying a thick layer of the nano-chitosan mixture with wax repeatedly for 10 consecutive days. The result was yellowing and gradual wilting of the leaves. From the first 24 hours of spraying the nano-mixture until 10 days of treatment have passed. During the treatment period, stiffness, blackening, and tearing of the leaf edges were observed, which led to a decrease in the wet weight from (109 - 75) grams. The number of dead leaves increased from (1 - 29) and the length of the plant decreased, reaching (40 - 26) cm compared to the control plant. Decreased chlorophyll reaching (28.3 – 9.8) cm compared to the control plant The water evaporation rate was measured, reaching (23-20), and the laboratory temperature was measured continuously during the plant's acclimatization period and after the treatment period.

The current study aims to identify the effect of spraying a mixture of chitosan nanoparticles with wax on the growth and production water hyacinth plants, evaluate the amount of water lost in evaporation and transpiration, and the possibility of developing an effective and successful biological control strategy in the biological control of invasive plants.

Keywords: Biological Control; Chitosan Nanoparticles; Water Hyacinth Plant; Iraq

1. Introduction

1.1. Water hyacinth, Eichhornia crassipes (Mart.) Solms

Water hyacinth, Eichhornia crassipes (Mart.) Solms. (Fig. 1) is a South American aquatic weed that floats freely. It has been regarded as invasive across Africa and Asia since the 1990s [1]. The ideal conditions for water hyacinth growth are 28 to 30 degrees Celsius, high levels of nitrogen, phosphorus, and potassium. When conditions are right, water hyacinth grows incredibly quickly in freshwater environments. In ideal circumstances, A water hyacinth mat can spread up to 60 meters each month, and its seeds can survive for up to 20 years on the bottom of the infected body of water [2] Water hyacinth can overrun marshes and lakes, outcompete native aquatic species, deplete fish oxygen levels, and provide an ideal environment for disease-carrying mosquitoes. Large infestations of water hyacinths can jam dams, restrict fishing, and impede transit along rivers [3]. Dangerous floods can result from obstructions in rivers and canals. On the other side, increased water hyacinth evapotranspiration could have detrimental effects on areas where there is still not enough water. The other issue related to water hyacinth is the loss of biodiversity.

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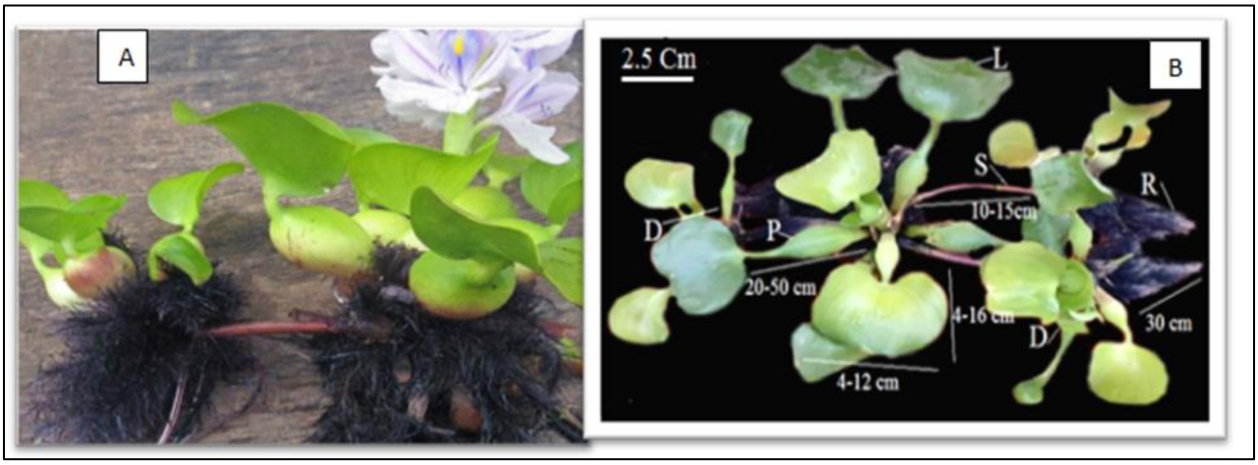

Figure 1 (A) Morphology parts of Water hyacinth (B) L: Leave or (lamina), S: Stolon, R: Root, P: Petiole, D: Daughter plant

2. Chitosan

2.1. Chitosan nanoparticles (CHNPS): An adaptable growth enhancer in contemporary farming practices.

Because they impart superior characteristics than chitosan, such as an increase in bioactivity, chitosan nanoparticles have been used extensively in textile applications recently. The world's most common natural polymer is chitin. Two marine crustaceans—shrimp, crabs, lobster, and crawfish—are the main sources that are used; these species account for 20–30% of total dry weight and are adequate to support a commercial chitin/chitosan industry [4] Chitin is a linear poly-β-(1,4)-N-acetyl-D glucosamine that resembles cellulose [5]. In nature, chitin takes the form of arranged crystalline microfibrils. Three polymorphic variants are identified for it: α-chitin, β-chitin, and γ-chitin. Deacetylated chitin, or chitosan, is a polycationic polysaccharide that occurs naturally and is obtained from partial deacetylation of chitin (Figure 2). On a dry basis, estimates of the yearly output of shell wastes from clams and oysters, crab, lobster, shrimp, and krill range up to 1.44 million metric tons worldwide. These characteristics were shown to be influenced by the molecular weight of chitosan and the degree of deacetylation, which is expressed as the molar fraction of deacetylated units or percentage of deacetylation. Its special qualities make it useful in biological and medicinal processes like as water treatment, materials for healing wounds, drug delivery, and scaffolding for tissue engineering. Chitosan nanoparticles (CHNPS) have the ability to function as powerful antibacterial agents against bacteria and harmful fungi as well as growth enhancers on their own in the agricultural sector [6]. They are also known as chitosan-based agronanochemicals since they have the ability to serve as nano carriers for currently used agrochemicals [7], [8]. With or without the combination of macronutrients, chitosan could function as a substituted sustained powerful biocide agent against agricultural pathogens such bacteria, fungus, and viruses in plant pathogen control and disease management [9]. Chitosan is a naturally occurring, biocompatible, biodegradable, and non-hazardous biopolymer with a wide range of uses. When applied to plants, chitosan increases the rate of photosynthetic reaction, closes stomata, and activates antioxidant enzymes through nitric oxide and hydrogen peroxide signaling pathways. Additionally, it promotes the production of sugars, amino acids, organic acids, and other metabolites, all of which are necessary for energy metabolism and stress tolerance [10].

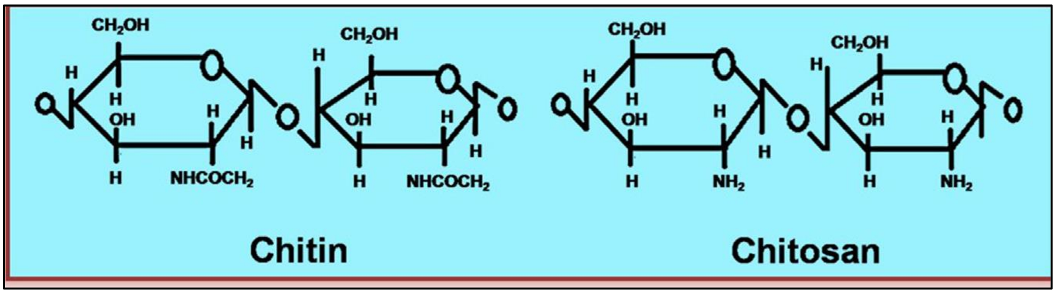

Figure 2 Structure of chitin and chitosan

3. Water hyacinth management

Four strategies—mechanical, chemical, environmental, and biological—have been put forth to limit and regulate the growth and spread of water hyacinth [11].

3.1. Physical and mechanical control

Mechanical control is the process of controlling weeds with tools. Some methods of mechanical control of weeds with tools include: Water weed cutters (A), netting, (B), barriers, (C), chaining, and (D). In the event of a large or deeper water body, mechanical control entails manually removing the plants and using boats, rakes, and booms to assist in gathering and transferring the plants to the land.

3.2. Chemical Regulation

When chemical herbicides are the quick fix for the water hyacinth issue, they should be used as the primary method of control [12]. Among herbicides, glyphosate and [2,4-D (2,4-dichlorophenoxy) acetic acid] are most frequently employed. Herbicides are thought to be a safe and efficient way to control water hyacinth. It takes a few days to a few weeks for the treated plants to die and decay. Herbicides are effective, but their primary drawbacks are that they are non-selective and can have a major negative impact on the ecosystem if used improperly [14] .

3.3. Biological Control

Biological control is acknowledged as a practical, long-lasting, and eco-friendly alternative to chemical control when the latter is not environmentally benign or economically possible [12]. The use of an organism's natural enemies to restrain its uncontrollably rapid growth is known as biological control. Can, if used improperly, result in serious environmental issues [13]

4. Material and Method

4.1. Collection of Water Hyacinth

In March 2023, samples of young, healthy water hyacinths were randomly gathered from three distinct locations along the Diyala River throughout their growth cycle. Samples were transported straight to the Ministry of Science and Technology's laboratory in Baghdad using plastic containers filled with five liters of river water. Plants were then repeatedly washed with water to remove any attached organic and inorganic materials, which, if used improperly, could have a major negative impact on the environment [14].

and periphyton or macroinvertebrates in the roots and leaves with distilled water, after which the plant was blotted dry. One water hyacinth daughter plant was placed in plastic pots with measurements of 45 cm by 35 cm and 20 cm of depth in order to acclimate the plant and monitor its progress [15]. Each container was labeled, filled with dirt from the same location, weighed one kilogramme after 13 liters of water was added, and oxygen compressors were supplied for the duration of the study figure (3). A straightforward test system was employed for three replicates in a plant that is homogeneous in weight and size, with three replicates for each plant and a total of 12 per site, in accordance with the Complete Randomized Design (CRD). Weighed in grams, the plant's total length in centimeters, the number of leaves in each centimeter, the number of daughters per plant, and size balance were among the characteristics recorded. The air temperature was also regularly monitored using a digital thermometer with a range of (0-100 digital thermometer).

Figure 3 Plant acclimatization stage in the laboratory

4.2. Water Evapotranspiration Experiment

In order to compare how much water the water hyacinth used by transpiration or water evaporation (evapotranspiration, or ERT), three plastic containers—one without the plant—filled with 13 liters of water and equipped with oxygen compressors were used for the duration of the study. The same container is planted on plants that are homogeneous in weight and size, with three replicates for each plant and a rate of 12 per site. A numerical ruler was attached to the water meter to measure the water drop in the container and compensate the lost water to maintain the same level of water. The temperature and relative humidity are continuously measured every day in this manner.

4.3. Preparation of chitosan nanoparticles

The following modified approach from [16] was used to prepare the nano chitosan: In distilled water with 1% acetic acid, chitosan (2 weight percent) was suspended using a mechanical blender running at 500 rpm for 20 minutes. After that, the solution was run through an ultrasonicate for 30 minutes at 35 watts. It was then utilized for other procedures.

4.4. Preparation of wax

100g of honey and beeswax were weighed using a modified version of [17] methodology. Using a weigh balance, 300 mL of acidified methanol with hydrologic acid was added to a two-liter conical flask and shaken ferociously. It was sealed, labeled, and let to stand for 48 hours in order for it to dissolve. After that, it was put in a reaction boiler meant for certain heating purposes (called Reflux), where the temperature was maintained between 60 and 80 degrees Celsius for an hour. It was cooled before being filtered using Whatman number one filter paper and a 150-micron laboratory sieve before being placed into a conical flask. With minor adjustments, an established basic oil/water emulsion method [18] was used to create honey wax encapsulated microparticles (WEM). The methanolic honey wax extract was combined with 400 ml of 2% w/v sodium alginate solution to create the organic solution (oil phase), which was then stirred with a mechanical stirrer set at 4000 rpm for five hours at room temperature.

4.5. Biological Treatment

A. Effects of foliar wax and Nano-chitosan on water hyacinth growth: Two groups of plants were treated following a fifteen-day acclimatization period.

The first group served as the control, with merely distilled water sprayed on the whole water hyacinth plant. In order to cover the surface of the leaves and stem, a 500 ml extract of the wax combination with chitosan was sprayed liberally over the second group of water hyacinth plant beds. For five days in a row, the spraying procedure was carried out once per day. The first signs of the leaves' wilting emerged three days after the treatment. It was quantified. everyday temperature in the lab [19].

5. Results

The plant was acclimatized in the laboratory for 15 days. The fresh weight of the plant reached (109-130) grams at a laboratory temperature of 34 °C. At the same time, the weight of the plant reached 130 grams at a temperature of 45 °C after 5 weeks of acclimatization. The plant also reached a length of (40-47) cm. The length of the root reached (12-17) cm, and the number of leaves increased from (25-39 leaves) to laboratory temperature (34-42. °C) and the rat of chlorophyll (34.5-20 ccl). Table (1) Shows the Morphological characteristics changes of the plant during the acclimatization stage in the laboratory.

Table 1 Morphological characteristics of the water hyacinth after acclimatization (control)

	Weekes				
Characters plant	First week	second week	third week	fourth week	fifth week
Air temp.°C	34	36	38	40	42
Wet weight / gm	109	112	117	124	130
Plant height / cm	40	42	44	45	47
Root length / cm	12	13	13	15	17
Number of leaves	25	28	31	35	39
Chlorophyll /ccl	34.5	27.8	29.7	20.4	20

Table (2) shows the amount of water consumption by the plant in the amount of plants/liter before treatment with Nano-chitosan extract with wax. Water consumption by the plant was in laboratory conditions and temperature changes, as the amount of water decreased by evaporation through transpiration between (23 - 14) liters as of The first week to the fifth week in a same at a temperature that ranged between (34 - 42°C) compared to the control tank without plants in the same laboratory conditions of air temperature, where the decrease in the amount of water ranged between (23 - 17) liters.

Table 2 Measured of Evapotranspiration Rate of Water hyacinth in Vitro before treatment (control)

	Week				
Characters	First week	second week	third week	fourth week	fifth week
Air temp.°C	34	36	38	40	42
Wet weight / gm	109	112	117	120	124
Plant height / cm	40	42	44	45	47
Root length / cm	12	13	13	15	17
Number of leaves cm	25	28	31	35	39
Evaporation in container with plant / l. day	23	21	19	16	14
Evaporation in container without plant / l. day (Control)	23	22	20	18	17

The control: spraying with foliar wax and Nano-chitosan Spraying with distilled water only(comparison). Figure (4)

Figure 4 Control ponds (A) contain a water hyacinth plant without treatment to estimate the rate of transpiration (B) a pond without a plant contains only water to estimate the amount of evaporation

Table 3 shows the effect of spraying Nano-chitosan with wax on the rate of plant death and cessation of growth, as the number of yellowed leaves reached 17.

Table 3 Morphological characteristics of water hyacinth water evaporation rate after treatment in vitro

	Week				
Characters	First week	second week	third week	fourth week	fifth week
Air temp.°C	36	39	40	40	42
Wet weight / gm	109	90	85	82	75
Plant heigh / cm	40	40	38	29	26
Root length / cm	12	13	13	14	14
Number of damaged leaves	17	22	25	25	29
Evaporation in container with plant l/ day/ (Control without treatment)	23	21	19	16	14
Evaporation in container without plant l / day / (Control)	23	22	20	18	17
Chlorophyll /ccl	28.3	18.9	13.8	9.8	9.8

By following up on the results of bio-spraying nano-chitosan with wax on the leaves of the water hyacinth plant within 24 hours, it was observed that the color of the leaves changed from light green to dark green tending to brown. After 48 hours, wilting, curling, and cracking developed in the edge of the leaves, and after 72 hours, the loss of chlorophyll pigment was observed. Completely and after the first week of treatment by spraying Nano-chitosan with wax. figure (5) shows the stages of morphological changes in the leaves after treatment. It was also observed that the fresh weight of the plant decreased, the number of green leaves decreased, the total length of the plant decreased and stopped, and the chlorophyll pigment disappeared from (28.3 -9.8 ccl) table (3). As for the rate of evaporation, the plant retained a deficiency in the rate of transpiration is a result of damage to water-conducting tissues, as well as the plant stopping the absorption of water through the transpiration process. Table. (3) shows the amount of water consumed by evaporation. And damage in chlorophyll from (28.3- 9.8 ccl).

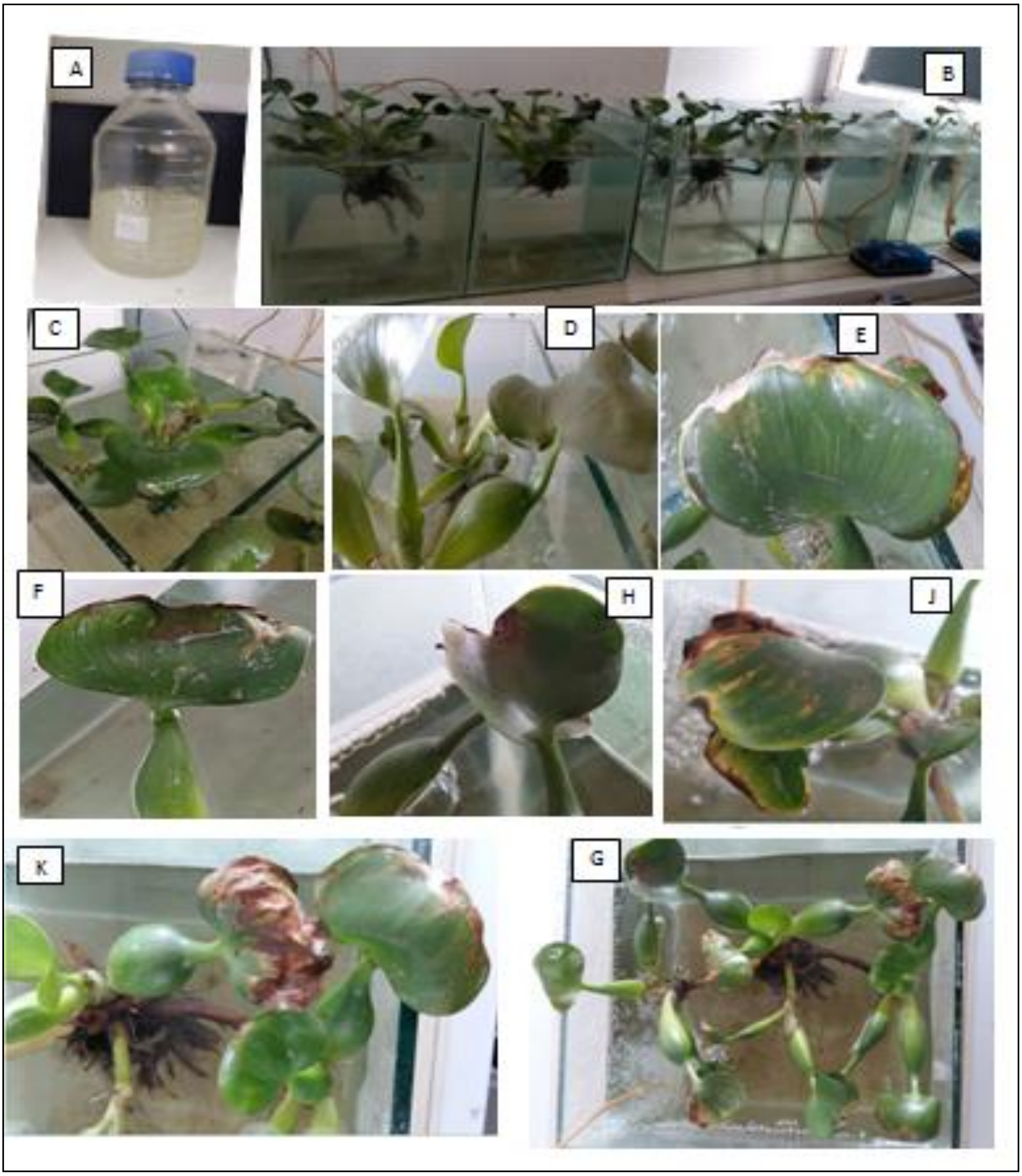

Figure 5 The stages of biotreatment by spraying with nanoparticles chitosan with wax of the water hyacinth plant physiological and morphological changes continue after ten days of, until the stage of complete death of all the leaves of the plant is reach

6. Discussion

Age of the leaves, position at the canopy, and climatic conditions all affect stomatal conductance. The application of nano-chitosan to wax-coated leaves reduces their absorption of light for photosynthesis, hence influencing their stomatal conductance, plant biomass, and photosynthesis rate. Additionally, the increased absorption of solar radiation by leaves coated in nano-chitosan and wax raises the temperature of the leaves. Furthermore, fine nano-chitosan mixed with wax may restrict stomatal openings, which would negatively impact photosynthesis and leaf temperature.

Foliar spray of engineered nanoparticles (NPs) such as nano fertilizers, nano pesticides, nano sensors, and nanocarriers is increasingly being employed in the agricultural industry. Foliar spraying of NPs improves the effectiveness of plant protection technologies compared to traditional soil–root application. Foliar-sprayed NPs mainly enter the leaves through stomata and are transported to different plant parts via apo plastic and simplistic pathways. Foliar NPs enhance defenses and resistance against pests and diseases and improve the yield and quality of crops. However, Moreover, important factors limiting foliage uptake of NPs such as wax deposits on leaf surfaces, environmental factors (e.g., light, temperature, and humidity), and physical and chemical properties of NPs should be investigated to further improve this technology [19]

Out of a wide range of biopolymers, chitosan's use in agriculture is becoming more popular because of its biodegradability, biocompatibility, nontoxicity, ability to control plant growth, antimicrobial activity, and ability to inhibit plant stress [20]. In addition, it is thought to be a cost-effective biopolymer because it is easily extracted from fungi, insects, and marine trash. These characteristics of nanoparticles based on chitosan have made them attractive for usage in a variety of agricultural applications as NPs, microbeads, and microfibers [21]. The results of the present research agree with the results of previous research, for example, [22] Study the effect of chitosan nanoparticles (NPs) on Catharanthus roseus through foliar treatment during salt stress alleviation. After application of chitosan NPs, a significant increase in the activity and expression of antioxidant enzymes was detected. Additionally, after treatment with chitosan NPs during exposure, the implicated genes were increased. [23]. It has also been discovered that chitosan NPs (400 mg/L) are applied. It works well to lessen the effects of cold stress on bananas. Banana plants that were both control and chitosan-treated were exposed to cold stress in a study. outcomes It demonstrated that the treated plant was able to develop more antioxidant enzymes, including POD and SOD, and that it was generally healthy. leading to a larger decrease in reduced MDA and ROS levels [24]. In another study, it was discovered that chitosan NPs at a concentration of 90 ppm were effective in reducing the negative effects of drought stress in wheat [25]. The samples treated with chitosan NPs exhibited a noteworthy rise in the accumulation of SOD and CAT, as demonstrated by the enzymatic assay. Using a foliar spray, investigated how wheat responded to salicylic acid-chitosan NPs (0.01%e0.08%; w/v), which increased the accumulation of polyphenol oxidase, SOD, CAT, and POX. It was previously thought that the antioxidant enzymes were crucial in helping plants fight off oxidative stress caused by unfavorable conditions. This suggests that chitosan nanoparticles (NPs) can be extremely effective in reducing plant stress by stimulating the molecules indicated above.

7. Conclusion

From a plant point of view, chitosan-based nanoparticles have a wide range of functional properties that spread easily into many application fields. Many elements directly influence the formulation in order to produce the desired features.

In the current study, the ability of chitosan nanoparticles mixed with wax was achieved, which is considered a factor that helps the adhesion of chitosan nanoparticles to the surface of the plant, which has the ability to penetrate the openings (stomata) and directly affect the vital activities of the plant by impeding the process of photosynthesis by spraying a thick layer covering Plant surface. the paper. And the rest of the plant parts, which leads to blocking light and reducing vital processes such as transpiration, which in turn leads to reducing water consumption through transpiration. As the plant temperature rose, the dense layer of wax and nano-chitosan spray caused the inactivation and loss of the chlorophyll pigment as a result of damage to the work of chloroplasts and damage to the tissue membrane of the plant.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed

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