JKAU: Met., Env & Arid Land Agric., Sci ***Vol. 33, No. 2****, pp: 11-36 (2024 A.D).*

DOI: 10.4197 /Met. 33-2.2

Effect of Salinity Stress, Humic Acid and Nanochitosan on plant

Ibtisam A. Alghabban[1.2], Hassan S. Al-Zahrani[1], Sameera A. Alghamdi[1]

[1.]*Department of Biological Sciences, Faculty of Science, KAU.*

[2.]*Department of Biology, Duba University College, UT.*

e.algaban@ut.edu.sa

Abstract. Salinity reduces the agricultural productivity of most plants. Humic acid (HA) and chitosan nanoparticles (CNPs) offer promising benefits for sustainable agricultural production due to their natural origins and potential to be used as a treatment for the effects of salt stress, it improves plant growth and productivity, which avoids or reduces the harmful resulting from the use of salty irrigation water on plants, as stated in previous literature. The present review represents details about salt stress and the damages and negative affects it causes on plant growth, agricultural production, and metabolites within the plant. It also sheds light on the definition of humic acid and its composition, effective and positive role in plant growth and increasing resistance to salinity. It also clarified the importance played by nanocomposites (nano chitosan) and their role as treatments for salinity.

Keywords: salinity stress, humic acid, nnochitosan, chitosan, nanoparticles.

1. Introduction

In various arid and semiarid agriculture regions, the water stress in land due to salinity results in significant yield reduction of crops which is considered a challenging issue exerting substantial variations in the metabolism and growth of plant (Aly et al., 2003). These variations as a result of salinity are primarily depends on the species and cultivars of plants as well as on salinity level and growth period (Turner and Kramer, 1980). Salt tolerances of plants are mainly associated with the regular uptake of salts for osmotic regulation (Lovato et al., 1999; Saad-Allah et al., 2022). The salinity of sodium chloride (NaCl) restrains the growth and development of plant by minimizing the water potential and escalating the toxic ions (sodium ion and chloride ion) which as results, leads to water discrepancy and disparity of plant nutrition (Ebrahim, 2005). Due to water deficiency and imbalance and/or lower nutrient uptake plants are directly or indirectly damaged and also physiological attributes of plants viz., plant germination, growth and development, respiration, photosynthesis and accumulation of metabolite are affected (Aly et al., 2003).

ROS are oxygen molecules having high reactivity and are generated as natural byproducts of various cellular processes, such as photosynthesis and respiration. In small amount, ROS play an important role in the cell signaling and defense against various biotic and abiotic stresses. Whereas the plants naturally have various mechanisms to regulate the ROS level and prevent their harmful effect (Dai et al., 1997). Molecular oxygen i.e reactive oxygen species (ROS) being used by plants as lethal oxidants within the cellular environment develops a continual oxidative risk to the structure and ongoing process in the cell (El-Shintinawy et al., 2004).

Humic substances (HS) are the complex organic molecules resulting from the decomposition and degradation of dead organic matter by soil microbiota and chemical processes. These are the fundamental components of soil organic matter which are classified into three main fractions based on their solubility in acid and alkaline solutions: humic acid, fulvic acid, and humin (Stevenson, 1994; Asli and Neumann, 2010). Humic acid (HA) significantly improves the growth and development of plant by improving the soil properties. HA sustain the bioactivity of plants by releasing bioactive compounds i.e phytohormones and derivatives which attach to the plant cell receptors that stimulate the plant growth and stress response. The effects of HS on plant growth may vary depending on the factors like the origin of HA, its concentration, the application method, and the stage of plant development. HA can stimulate the plant growth by impacting various physiological processes, including cell respiration, photosynthesis, protein synthesis, water and nutrient uptake, and enzyme activities. Its application as a soil amendment, either alone or in combination with other salts compounds, has shown significant increase in the plant growth and crop yield, particularly in sandy soil. This improvement is attributed to enhance the hydro-physical properties and the availability of nutrients in the soil (Senesi et al., 1996; Jindo et al., 2020). Further, Humic chemicals, when applied in saline soil, can contribute to improve the plant development and production which help to alleviate the adverse effects of moderate soil salinity by improving the availability of nutrients in soil. This suggested that HA can play a crucial role in enabling the plants to thrive in challenging saline environment (Osman and Ewers, 2008; Rady, 2011; Selim and Mosa, 2012). Overall, the use of humic acid and their bioactive compounds can offer substantial benefits to the plants for their growth and development, particularly in soil or environmental stress conditions and ultimately enhance the development (Vaughan and Linehan, 1976; Masciandaro et al., 2002).

The use of Chitosan nanoparticles (CNPs) is an emerging approach to mitigate the salt stress in plants and have distinct physicochemical properties than macro-particles (Alabdallah and Alzahrani, 2020; Cele, 2020). The benefits of CNPs in terms of surface effects and their nano size increase their efficacy than conventional chitosan “CS” (Divya and Jisha, 2018). Further, due to promising positive biological properties, it has widely been applied in various agricultural fields due to its strong potential to resolve a variety of ecological issues plant production and protection. It is an excellent candidate that has been extensively utilized to alleviate various abiotic stresses due to its potential to trigger the defense mechanism of plants against diverse abiotic stresses including salinity stress (Zou et al., 2015). Free hydroxyl radicals, hydrogen peroxide, superoxide anion radicals are neutralized by Chitosan which exhibits strong antioxidant properties. Foliar application of CNPs improved the antioxidant enzymes viz., Ascorbate peroxidase (APX) glutathione reductase (GR) and catalase (CAT) activity (Hassan et al., 2021). By reducing the level of superoxide radicals, H2O2, malondialdehyde and enhancing CAT activity, this polymer showed considerable reduction in the pessimistic effect of salt stress (Iber et al., 2022). In stressed plants, the treatment with CS significantly improves the antioxidant enzyme, opening and closing of stomata, photosynthesis rate, organic acid production, amino acids, sugars, and other metabolic compounds essential for osmotic regulation, metabolic energy and stress signals (Hidangmayum et al., 2019). Also, under salt stress conditions, the application of chitosan prolonged the shoots and roots of plants and ultimately improved the fresh and dry weight along with the increase in water content in plants (Mahdavi and Rahimi, 2013). Removal of acetyl group (−COCH3) and its conversion into amino acid (R-CH(NH2)-COOH), chitosan may be made from chitin (Sugiyama et al., 2001). It is a natural cationic polysaccharide (poly β-(1,4)-N-acetyl-D-glucosamine) with strong bio-stimulant potential to improve the growth and production of various horticultural crops (Pichyangkura and Chadchawan, 2015). In naturally occurring polymers, Chitosan is the second most significant with lower cost and least perilous effects which widely exist due to eco-friendly nature (Pirbalouti et al., 2017). As a result, it was a suitable fit for the study.

This review presents the most important studies conducted by researchers on the possibility of treating salty lands and mitigating the negative effects on plants growing in these lands using humic acid or chitosan in the form of nanoparticles. Highlighting the role of each of them as an effective healer in resisting the effects of salinity on plants.

2. Salinity stress

Various biotic and abiotic factors, whether are a serious issue in agriculture since they can significantly lower plant production and yield. These variables could include anything from temperature to chemicals to radiation to plant drought to salinity to microorganisms and more. Especially in various arid and semi-arid regions in drought season, among abiotic stress factor salt stress results in large economic losses and threatens global food security. Approximately 20 % of irrigated fields are also being impacted by it, though. A total of 7 % of agriculture areas are affected by salinity throughout the world, which is steadily rising due to climate change (Hernandez, 2019).

It's critical to distinguish between mild and severe stress when thinking about how salinity affects plants. Strong stress can seriously hurt a plant's metabolism and growth, whereas mild stress may have little to no impact on it. Persistent and severe stress, especially in plants that are sensitive to salinity, can induce wilting or even death in plants. This result varies according to the origin and cultivar of the plant (Ebrahim, 2005).

Agricultural soil and plants are adversely affected by various salt stresses (Lungoci et al. 2023). This stress can be brought on by several things, including overwatering that causes salt to build up on the soil's surface, inadequate irrigation water with high salt content, immediacy to sea level, and excessive evaporation where the salt augmented from underground water.

The naturally existing salts in soil are one of the main sources of salinity in groundwater due to various natural processes, including evaporation, weathering of rock, and sea salt precipitation due to rain and wind. On the other hand, human activities can lead to secondary salinization by upsetting the normal hydrologic balance of the soil by methods including employing irrigation water that is high in salt, over-extracting groundwater, and insufficient drainage. Water table in soil is affected and altered by these processes which ultimately enhance the concentration of salts to higher level and eventually causes the agricultural land to become more saline which influence the growth and production of crops (Rengasamy, 2006; Payen et al., 2016).

Based on the electrical conductivity (EC) of irrigation water the soil salinity is divided into three categories: 1. None/low- at this level the soil salinity is low ($EC < 0.75$ mmhos cm-1) and has no concern for the growth and development of plant. 2. Moderate ($0.75 \leq EC \leq 3.0$ mmhos cm-1) at this level of soil salinity, the irrigation water has a higher concentration of dissolved salts which may not severely affect the growth and development of the plants but could leads to some limitations and yield reduction particularly for salt sensitive plants. 3. Severe ($EC > 3.0$ mmhos cm-1)-at this level the soil salinity is high and can have significant negative effects on the plant growth. More, at this level the irrigation water contains higher concentration of dissolved salts, which can lead to osmotic stress and toxicity for plants and as a result, can reduce the plant growth, caused the damage to roots, and restrict the water uptake, leading to decreased crop yield (Ebrahim and Abu-Grab 1997; Aly et al. 2003).

Salt tolerance mechanisms are mainly associated with salt uptake and osmotic adjustment (Lovato et al. 1999; and Saad-Allah et al. 2022). The salinity of Sodium chloride (NaCl) enhances the concentration of detrimental ions of sodium and chloride by reducing the potential of soil water which restrict the growth and development of plants, and cause water scarcity which leads to disparity of available nutrients. More, due to this effect, the plants may lose their capability of uptake the nutrients, water, and other essential physiological components.

High salinity results from hyperosmotic and hypertonic solutions being created by the soil's high content of Na+ and Cl, which inhibit plants from absorbing water and nutrients (Aly et al., 2003). Ionic and/or osmotic stress results from the disruption of cellular, physiological, and biochemical processes of whole plant due to salt stress (Lungoci et al., 2023). The rhizosphere and exposed plant components may influence immediately, while long term impacts are mainly depending on the intensity, length, and type of stress (Aly et al., 2003; Lungoci et al., 2023). Salinity stress harms membranes, impairs photosynthesis, produces oxygen reactive species (ROS), affects metabolism within the cell, and may even lead to programmed cell senescence or death (Ebrahim and Saleem, 2017). Visible results may not appear till the plant level.

There are three ways in which the plants are stressed under saline conditions (Munns, 2002; Abdelhamid et al., 2010): (1) lower/reduced ability of plant for potential water uptake from roots; (2) discrepancy and reduction in the nutrients uptake and their transportation to shoots; (3) injuries caused by lethal ions of sodium and chloride. These problems are mainly caused by Na+ and K+ which compete for binding sites in plant cells (Marschner, 1995; Zhang et al., 2005). It is anticipated that the increasing salinity problems in agriculture could reached up to 40 % (Zahran, 1999; Payakapong et al., 2006) in future if proper management strategies would not be adopted.

Although there is a lot of knowledge and study on how plants respond to salinity, little is known about the mechanisms that underlie this process, especially when it comes to the utilization of stress-relieving agents. The effect of chitosan nanoparticles (CNPs) and humic acid (HA) on the susceptibility of plants to saline has not been fully documented. To close this gap, present study on Coriandrum was conducted to determine whether K-humate and nano-chitosan are capable to improve the tolerance of coriandrum plant to salt stress.

1.1. Effect of salt stress on the growth and yield

Proper respiration and photosynthesis are the most important factors to sustain the growth and development of plants. In yielding formation, both rate and duration are important parameters. Consequently, plant growth must be considered in plant productivity analyses. A plant's growth is usually accompanied by the formation of new organs, which include cell division, cell differentiation, and cell elongation.

It has been documented that soil salinity adversely influences the biochemical and physiological processes and reduce the biomass accumulation produced by plants (Aly et al., 2003; Ebrahim and Saleem, 2017). This negative effect is present during all stages (seedling, germination, vegetative, reproductive) of plant growth and development (Nawaz et al., 2010). Additionally, the salinity stress influences all processes including growth and yield, synthesis of proteins, photosynthesis, and metabolic processes of lipid and energy production (Ramoliya et al., 2004;

Amarin et al., 2020; Gholamnia et al., 2021; Denaxa et al., 2022; Lungoci et al., 2023; Tahjib-Ul-Arif et al., 2023). Growth inhibition, rapid senescence development, and death following extended exposure are the general signs of salt stress damage (Levitt, 1980). As a result of higher soil salinity, growth of plant inhibited and ultimately programmed cell death occurs which is the prominent damage of plants along with other symptoms. In response to saline stress, the resulting Abscisic acid which is supplied to epidermal cells sealed the stomata (Munns and Tester 2008; Amarin et al., 2020) and closure of stomata decreases the photosynthesis as well as photo inhibition, oxidative stress, and photo inhibition. Immediately, cell development either directly or indirectly by abscisic acid is inhibited by osmotic stress (Jouyban, 2012).

The capacity of plants to absorb water is decreased by salinity stress, which inhibits the growth of plants. This is how salt stress manifests as an osmotic or water-deficit effect. Drought frequently results in osmotic stress from salt, which affects both cellular and metabolic functions. However, under drought stress condition, the ability of soil to uptake water negatively affects the required rate essential for the development of leaves. As the salt enters xylem, may rapidly fit inside by elongating the cells, expanding vacuoles and could not accumulate in newly developed tissues to hinder the growth. Consequently, the amount of salt absorbed by plants could not prevent the development of new leaf (Munns, 2005; Tejera et al., 2006).

Irrigation water salt levels dramatically inhibited seed germination (Othman et al. 2006). It may be preferable to use another supply of fresh water at this point to prevent delaying germination when saline water is used experimentally. This result agreed with those from El-Dardiry (2007), who discovered that salinity in the water has a more significant impact on germination than salinity in the soil. In calcareous and sandy soils with variable salt content, lower germination rate was recorded (Ragab et al. 2008). According to the researcher, calcareous soil had a 16% loss in grain production whereas sandy soil saw a 23% reduction when water salinity was increased up to 4.85 ds m-1.

Moreover, the crop yield is mainly associated with the salinity level of irrigation water and at higher salinity level (8.86 dSm-1) considerable yield reduction was recorded. Furthermore, negative impact on grain quality, weight and straw yield was also observed at higher salinity of irrigation water. Increase in the salinity of irrigation water had a detrimental impact on the grain, straw, height, spike length, and the weight of wheat grain (Zein et al. 2003). Further, the length, fresh and dry weight of roots is significantly increased by increasing the salinity whereas, biomass accumulation, growth, shoot, and yield is reduced (Gholamnia et al. 2021). Other researchers documented the identical findings for the plant growth and yield of Zea maize (Aly et al., 2003), Salvadora persica (Ramoliya et al., 2004), Dianthus caryophyllus (Amarin et al., 2020), Mentha piperita (Gholamnia et al., 2021), Strawberry (Denaxa et al., 2022) and Nepata racemosa (Lungoci et al., 2023).

1.2. Effect of salinity on chemical constituents

The amount of solar energy produced during the photosynthesis, is widely engrossed by various biological processes which occurs in plant leaves demonstrated as:

$$6CO_2 + 12H_2O \longrightarrow C_6H_{12}O_6 + 6H_2O + 6O_2\uparrow$$

In isoprenoid plant lipids, chlorophylls, photosynthetic pigment molecules, and carotenoids are usually referred as “prenyl lipids”. In algae and higher plants, the chlorophyll A is primary pigment while carotenoids and chlorophyll (Chl) B are accessory pigments which are the true constituents of photosynthetic membrane and occurred as 3-1 (a/b) which are affected and/or modified by various environmental factors and growth conditions (Lichtenthaler, 1977).

Nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP) produced from the light (photochemical) reaction by plant leaves are then used in CO2 assimilation during the dark (biochemical) reaction. The main part of the assimilated carbon during this process is carbohydrates (Gordon, 1986). Sugars synthesized in leaves are partly utilized in respiration and partly in growth. The remainder moves from leaves (lower concentration) to all other plant organs, especially stem (higher concentration); i.e., against the concentration gradient (Hartt, 1936). Since light is required for photosynthesis, carbohydrate formation by green leaves occurs mainly during the day. In contrast, the translocation of sugars to the stem proceeds day and night (Dillewijn. 1952). Because the rate of sugar formation during the daytime exceeds the transport capacity of the phloem, some sugars have to be stored during the daytime in the leaf blade. As starch is a water-insoluble compound and the translocated sugars must be in solution, starch cannot be moved without first being digested to soluble sugars.

Carbohydrates are anabolized in the leaf blade during two distinct phases of particular importance. The first consists of a process, in which Carbon dioxide (CO2) is reduced during the existence of NADPH and ATP which results the triose-phosphates (light assimilation, photochemical reaction) formation. During the second phase, which requires neither chlorophyll nor light (dark assimilation, biochemical reaction), triose-phosphates are converted into oligosaccharides, polysaccharides. However, 80% of triose-phosphate is used in starch and sucrose synthesis and 20 % for the biosynthesis of organic acid and amino acid (Gordon, 1986). The assimilation of CO2 by photosynthesis results in significant (90%) dry matter accumulation of higher plants (Zelitch, 1988). In leaf blades, the pathway of starch and sucrose synthesis is mutually dependent identical pool of triose-P produced by Calvin cycle (Huber and Israel, 1982). Therefore, when sucrose synthesis is diminished, more photosynthates become available for starch formation and vice versa.

However, Britz (1990) attributed the presence of higher amounts of sucrose and starch compared to hexose concentrations in the leaf to the breakdown of hexoses at night for growth and respiration. Whereas, a positive correlation in the synthesis of sucrose-phosphate and sucrose-starch ratio was observed in tomato leaves (Galtier et al. 1993).

For herbaceous plants, extensive information on the metabolism of carbohydrate has been reported rather than the age of leaves. However, considerable decrease in sugar contents from younger to older leaves of spinach was found (Heilos and Deitz 1990) and decrease in the fixation capacity of CO2 due to the leaf senescence of plant was reported (Sobrado, 1994). After the transfer of soluble sugars from plant leaves to the stem through phloem tissues, they are taken up by meristematic and storage tissues. This process is one of several parameters that influence plant yield and productivity. Extensive studies on photosynthetic response to stress conditions, inducing damage to the photosynthetic apparatus, showed that chlorophyll contents are the sensitive indicators of salt stresses. Under various long lasting stress conditions, the amount of chlorophyll contents (per leaf area) are relatively lower than the normal green leaves and this decrease in the accumulation of chlorophyll is mainly associated with the stress (Lichtenthaler and Rinderle 1988).

The efficacy of salinity stress on biodegradation and biosynthesis of chlorophyll is mainly dependent on the factors involved in plant origin, its adaptation, age of exposed tissue and the duration of exposure. The seawater application (40%) on two spinach cultivars showed considerable reduction in chlorophyll (A and B) contents (Sun et al. 2010). Whereas, significant reduction in chlorophyll (A and B; ratio a/b) contents was recorded in Zea mays under salt stress (Aly et al. 2003). Furthermore, various researcher documented the identical findings on various plants viz., faba beans (Dawood et al., 2014), tomato (Ebrahim and Saleem, 2017), Dianthus caryophyllus (Amarin et al., 2020), Mentha piperita (Gholamnia et al., 2021) and Nepata racemose (Lungoci et al., 2023) under salt stress conditions.

Various studies reported the significant effects of salt stress on antioxidant enzymes, protein, mono and disaccharides sugar, amino acids (proline), and carbohydrates depending upon the duration and the level of salinity as well as the stage of growth and development of plants. Increase in the concentration of sodium chloride salt significantly augmented the protein contents in Vigna mungo L (Kapoor and Srivastava 2010) and in Hordeum vulgare L (Tort and Turkyilmaz 2004) while decrease in soluble protein contents was observed (Chen et al. 2007).

2. Humic acid biostimulants

2.1. Humic substances

Humic substances (HS) refer to naturally occurring material found in or obtained from sediments, soil, and ordinary waters (Mac-Carthy, 2001). These are naturally occurring diverse organic polymers with higher molecular mass characterized with yellow to black color (Aiken et al., 1985) which results from putrefaction of animal and plant residues and classified into three types viz., humin, fulvic acid, and humic acid.

2.3.1.1. Humin

The fraction of humic substances that is not soluble in water at any pH is referred as insoluble humic substances. Naturally, the humin has black color and it has been widely reported that humic substances of black color are mainly considered as the part of system but has not been fully identified (Gersende et al., 2008). Fulvic acids and humic acids are mainly differentiated in the variation of polymerization level, molecular weight and number of functional group (phenolic OH and carboxyl COOH). A significant relationship among the molecular weight has been reported as acidity, oxygen and carbon content, and polymerization systematically changes with the increase in the molecular weight.

2.2. Fulvic acid

Fulvic acid is a natural compound and the part of humus which is found in soil and produced by the decomposition of organic material by soil microbes and the fraction of HS is soluble in water at all pH level and subsequently exists in the solution even after removing the humic acid through acidification. Fulvic acid (FA) has low molecular size and weight (2000 dalton) with light yellow to yellow brown color having higher oxygen (45-48 %) and low nitrogen (< 4 %). Due to low molecular weight, the surface charge (-ive) peptize macromolecule in slight alkaline or neutral conditions which results the portability in soil (Fiorentino et al., 2006).

2.3. Humic acid

Humic acid (HA) is a complex mixture of organic compounds found in soil, peat, coal, and other organic material and characterized by dark brown to black color and capable to chelate to metal ions and incapable to dissolve in water with low pH (< 2) and acidic conditions but become soluble at high pH.

HA is a major component of HS of soil that can be extracted and has average molecular size with higher molecular weight (5000-100000 dalton) as reported (Fiorentino et al., 2006) along with sufficient oxygen (33-36%) and low nitrogen (4%). Due to average size and sufficient negative charge on surface, macromolecules occurs only in highly alkaline medium (pH > 8) which limits the mobility in soil under acidic and/or alkaline as well as neutral conditions. Additionally, it regulates the fertility of soil by affecting water holding capacity (Sutton and Sposito, 2005; Ali et al., 2019).

Constant fraction of C is constituted by HA which improve the soil properties by improving the pH buffering, water holding capacity, as well as thermal lagging (Izhar et al., 2020) and also increase the nitrogen use efficacy and ultimately stimulate the growth of root and shoot of plant (Lodhi et al., 2013; Leite et al., 2020). More, HA also assist the fertility and stability of soil by improving the chemical, physical and biological properties of soil which leads to astonishing growth of plant and their capacity to uptake the essential nutrients (Ata-Ul- Karim et al., 2020). Further, HA as a biostimulants, drastically influence the growth and development of plant and ultimately, the crop yield is increased. In the present study, humic acid was applied as potassium humate (K-humate). As HA is dark in color, spraying pants with it may hinder the process of photosynthesis, therefore it was used granular k-humate which was firstly dissolved in the plant irrigation water.

2.3.1. Structure and composition of HA

Initially, the structure of humic acid was presented by Berzelius (Mulder, 1840) and empirical formula (C40H30O15) which prevailed for long time and accounted even for years was also published (Mulder, 1840) along with the model of HA (Stevenson, 1982; Steelink, 1999). Steelink structure used for computer modeling and its slight modification about the chemical structure and composition of elements obtained from the liquid state nuclear magnetic resonance (NMR) and circular dichroism measurement of sample humic acid extracted sample from a living plant Pilayella litoralis. More, the existing structure was considered as a structural block of HA. More, accurate and recent humic acid model showing the complexity of system was also presented (Simpson et al. 2001).

2.3.1.1. Action of HA

A negative charge provided by the oxidized site with a complete molecule enables them to absorb the micronutrient and HA directly improve the clay characterization while growth and development of plant by indirectly.

a. Clay disaggregation

Clay disaggregation is a process of breaking the clay aggregate particles into individual particles. These are fine and small particles which clumps jointly due to the existence of water and consistent character. The rich amount of clay contents causes the compactness and opaque of soil which resist the roots of plant. This is mainly due to the presence of rich salt in soil which carries negative charge and causes the repulsion of clay particles and also, due to higher (%) clay particles bearing positive charge on the border of clay particles which attract the negative charges on the horizontal surface and results a three-dimensional structure (Bergaya and Lagaly 2013).

b. Water penetration enabled

Application of HA holds the clay particles which allow frequent water penetration. This phenomenon takes place in two ways. At first, it separates the salts by removing them from the facade of clay particles results in appearance of negative charge which allow the repletion of clay particles and cause the lost of soil structures while other phenomenon involves the attachment of carboxyl group (attached to humic acid molecule) which contain positively charged clay particles that causes the break-down of positive and negative charged forces on the surface of particles (Piccolo 1997).

2.3.2. Effect of humic acid on growth and yield

Humic acid as biostimulant, regulate the growth and development of plant by increasing their length, biomass accumulation, and uptake of essential nutrient required for plant growth. Further, along with an increase in the seed germination, HA also increases the biosynthesis of protein, phenomenon of respiration, enzyme activity and photosynthesis (Senesi et al., 2009).

Various studies documented that the foliar application HA results in significant increase in the stem diameter, plant height, root length, and biomass of plants (Farahat et al., 2012). More, on cowpea plant, the foliar application (50 M) of HA along with nitrogen (45 kg/ha) showed significant increase in plant height and the yield related parameters (Azarpour et al. 2011). A study documented the positive impact on the plant height, branches and leaves of Chemlali olive tree when treated with HA, macro element, amino acid, trace element as a combination (Aml et al. 2011) and also increased the leaf area and the diameter of plant. Additionally, these treatments showed considerable increase in weight and length of roots.

Another positive impact of the foliar application of HA on olive tree (Aggizi) was recorded and significant increase in the fruit quality, and yield was observed (Hagagg et al., 2013). More, the application of HA applied at full bloom stage considerably increased the size, and weight of berries (Ferrara and Brunetti 2010) which confers the positive effect of HA that increased the uptake of essential nutrients and minerals along with plant hormone i.e cytokinin, auxin, and gibberellins. Furthermore, various studies reported the positive effects of HA which increased the growth and biomass accumulation of apricot (Fathi et al. 2002; Fawzia, 2003; Shaddad et al. 2005; Fathy et al. 2010). Various concentrations of HA continuously applied (7 days) on maize seedlings increased the surface area and the root length (Canellas et al., 2022) and the density of root hairs of Arabidopsis thaliana, treated with humic substances considerably increased which may induce the nutrient acquirement response that supported the nutrients uptake by increasing the assimilative surface area (Schmidt et al. 2007).

In order to confer the response of HA on auxin production, the application of HA in the presence of water extractable humic substances (WEHS) was studied on the roots of Arabidopsis (Schmidt et al., 2007) and significantly modify the morphology of roots by increasing the density and hair length of roots, formed ectopic hairs and increased the proliferation of cells in the ground tissues of roots. The genes involved in the action of epidermal cells were considerably affected in the presence of WEHS which modify the differentiation of root cells at early stage which confers the reshaping and morphology of roots and increased the root surface.

To investigate the morphological modification due to HA in the cells and leaves in tissue culture numerous studies were conducted which provided an economical and rapid approach for the screening of compounds than the experiments performed for whole plant to study various aspects (Ehsanpour and Fatahian, 2003; El-Hak et al., 2012) and the results showed that the foliar application with HA and/or antioxidant compound promoted the yield, growth, seeds and biomass accumulation. Thus, these studies suggested that under identical growth condition and the foliar application with HA (1g/L) and salicylic acids (200 ppm) provided higher and quality yield.

The combined application of neutral and organic fertilizer with biofertilizer (NPK) and HA improved the production and quality of crimson grapevine (Shaheen et al. 2012). In another study, the foliar application of HA significantly improve the yield and growth of cowpea and ultimately increase the leaf, height, flowers, fruits and yield of cucumber (Magdi et al. 2011). Additionally, the length of shoots and roots of jowar and wheat seedlings increased on the foliar application of potassium humate (Patil, 2010) and the application of HA showed significant effect on soybean growth rate (Peymaninia et al., 2012) and also increased the growth related parameters of wheat i.e number of leaves, height as well as fresh and dry weight (El-Bassiouny et al., 2014).

Application of HA to auxin results in the enlargement of roots and shoots and positively affected the morphological attributes of plants. Prolongation of roots and shoots in the presences of HA is mainly associated with the elongation of cells (Gawlik et al., 2014). The application of HA showed significant increase in plant growth, organic matter and yield production in various vegetable crops (Kaya et al., 2005; Selim et al., 2010). Significant increase in grain yield, biomass accumulation, and seed production (per plant) was recorded upon the foliar application and/or soil drenching inoculation of HA (Khan et al. 2018) and an increase in the growth of roots was also reported (Nikbakht et al., 2008). Further, application of HA promote the growth and development of plants through hormone like action which stimulate the division of cells (Dobbss et al., 2007). The solution of HA (dissolved in water) regulates the root hair and ultimately the epidermal cell and corticosteroide (Zandonadi et al., 2010).

2.3.3. Effect of HA on the chemical constituents

Various studies reported the Direct and indirect effect of HA on plants including the increase in metabolic potential in plants along with the increase in biological and physio-properties of soil (Mallikaarjuna Rao et al. 1987; Tejada et al. 2006). Apparently, significant increase in the chlorophyll contents in the plants treated with K-humate was observed. The application of HA enters into plant cells, the functional groups of FA and HA as complementary basis of respiratory catalyst (polyphenol) and reduction and/or oxidation regulator (quinine) (Vaughan & Ord, 1991; Irfan et a1., 2005; Sritharan and Mallika 2005). More, the HS positively influence the physiological attributes of plants (Turkmen et al., 2004; Atefe and Ali, 2012) and showed an increase in the chlorophyll contents which ultimately improved the photosynthesis of plants and increase the yield (Zeng, 2002).

The foliar application of K-humate (1%) significantly increased the total phenol, proteins, Beta-carotene, chlorophyll contents and reducing sugar in wheat (Patil et al., 2013) whereas another study reported that the application of HA (4%) for metabolism and growth initiation of plants showed considerable increase in NPK ratio, pigmentation, and soluble sugar of roots and leaves (Zancani et al. 2009) and reduced the Na and proline contents. Thus, the application of HA to plants regulates the metabolic process due to the abundant production of protein, amino acid, antioxidant enzymes and metabolic compounds (Nardi et al., 1996; Ryosuke et al., 2006) as significant increase in the soluble sugar in the shoots of Thuja orierntalis was recorded when the plants were sprayed with K-humate (Zaghloul et al. 2009). Various studies documented the momentous increase in the degradation of chlorophyll and/or chlorophyll synthesis in leaves treated with HA (Vaughan et al. 1985; Nardi et al. 1996) and foliar application of HA (20 ppm) showed an increase in the chlorophyll contents in garden strawberries (Ameri and Tehranifar, 2012) and increases the chlorophyll synthesis and /or delayed the degradation of chlorophyll in the leaves of grapevine (Ferrara and Brunetti 2008). Whereas, in wheat leaves, application of HA demonstrated significant increased in carotenoids, proline, chlorophyll a and b, as well as the pigments (El-Bassiouny et al. 2014).

3. Nanoparticles (NPs)

In the era of 21st century, Nanotechnology is a emerging and promising approach in science, leading towards new revolution in multiple scientific domains (Sabir et al., 2014; Ditta et al., 2015) which mainly deals with the use of nanoparticles (NPs) which are the atomic cumulatives identified by two dimensional structure with various particle size (1-100 nm) (Sabir et al., 2014; Rico et al., 2015). Nanoparticles (NPs) are the diverse form of basic element resulting from the alteration in the molecular properties of element (Kato, 2011). Due to their small size and large surface area (relative to volume), NPs often exhibit unique properties compared to their bulk counterparts which makes NPs as useful in a wide range of applications across various fields, such as materials science, medicine, electronics, and environmental science (Sabir et al., 2014; Saxena et al., 2016). Due to their nano size NPs can be thoroughly dissolved in gaseous and liquid media (Buzea et al., 2007) while, the biological and physio-chemical characteristics makes them unique. The application of NPs in agriculture, open new up new horizon to control various plants diseases, protect the plants, and ultimately improve the crop yield (Ghormade et al., 2011; Duran et al., 2017).

The application of NPs to plants may have both negative and positive effects (Tripathi et al., 2015; Kasim et al., 2017) which positively influence the structure of cells, growth and development of plants, and physiological attributes (Siddiqui el al., 2015; Rico et al., 2015). However, the efficacy of NPs the metabolic process, growth and development of plants may varies among the crops (Duran et al., 2017). Various studies have reported the negative (toxic) as well as positive (beneficial) effects of NPs on plants (Siddiqui el al., 2015).

3.1. Chitosan nanoparticles (CNPs)

Recently, the bio-stimulators like chitosan and humic acid are gaining attention for their potential benefits in agriculture. Chitosan (CS) and humic acid are the best examples of bio-stimulants that have been studied for their ability to enhance plant growth, improve stress tolerance, and mitigate the negative effects of salinity in agriculture. Both chitosan and humic acid offer promising benefits for sustainable agricultural production due to their natural origins and potential to improve plant resilience and productivity. However, the effectiveness of these bio-stimulants can vary depending on factors such as soil type, plant species, application methods, and environmental conditions. It is encouraging to witness the growing interest for the use of these bio-stimulators as an alternative of chemical fungicides, especially in the context of addressing salinity-related challenges in agriculture (Salachna and Zawadzinska, 2014; Byczyńska, 2018). It has been widely reported that during low stress the application of CS positively influenced the ornamental plants by increasing the flowering and growth attributes, photosynthesis, chlorophyll contents, as well as the up-taking potential essential nutrients and minerals (Dzung et al., 2011; Salachna et al., 2015).

Whereas the application of CS in salt stress conditions significantly alleviated the negative effect salts (Jabeen and Ahmad, 2013; Krupa-Malkiewicz and Smolik, 2019). Furthermore, it has widely been reported that the damage on plants caused by various salt stresses can be mitigated with CS application by the modification of the concentration of intracellular ions and by promoting the capability of antioxidant enzymes (Safikhan et al., 2018). The application of CS positively stimulates the opening and closing of stomata and photosynthesis, increase the activity of antioxidants (H2O2 signaling pathways), regulate the production of sugar, organic acid and amino acid production, and metabolic compounds that are essential for stress signaling, osmotic adjustment, metabolic energy production under salt stress conditions (Hidangmayum et al., 2019).

Nano-chitosan is a derivative of chitosan, which is a natural polymer, derived from chitin and has gained attention due to its unique physio-chemical characteristics, which make it suitable for various applications. Chitosan particles have reduced to nanoscale dimensions, extremely small sizes, ranging from 1-100 nm in diameter. This reduced size can lead to increase the surface area and their unique properties compared to bulk chitosan. The arrangement of atoms in its molecular structure may contribute to its unique properties and exhibit impressive tensile strength and may have electrical conductivity characteristics which enhance the elasticity compared to bulk chitosan (Jackson et al., 2013). The application of nano-chitosan with lower concentration positively affected the yield, growth and development, and water transportation, water absorption, uptake of nutrients in French bean plants (Hasaneen and Omer 2016) while higher concentration negatively affected the multiplication of reactive oxygen species (ROS) (Mondal et al., 2011).

3.1.1. Effect of CNPs on growth and yield

Various studies have reported the effective response of nanoparticles including nanochitosan on plant growth, and relatively fewer studies documented the response of chitosan nanoparticles and comprehensive understanding for underlying the mechanisms. Most of the studies indicated that even lower concentrations of NPs significantly improve the growth and development of plant, increase the yield, and tolerance of plants to various biotic and abiotic stresses. Under drought stress conditions, the foliar spray of various concentrations (0 ppm, 30 ppm, 60 ppm and 90 ppm) of nanochitosan significantly increased the yield and growth Russian sage (Dowom et al., 2022) while a concentration 50 mgl-1 in the combination with a plant growth promoting rhizobacteria (PGPR) improved the germination of seeds, height of root and shoot, leaves, and biomass of root and shoot of maize in field experiments (Chaudhary et al., 2021). Furthermore, this concentration (50 mgl-1) of nanochitosan increased the length of cob, as well as plant and grain weight of Zea mays. In another experiment, the foliar application of chitosan at various potassium and nitrogen levels to potato plants not only increased the vegetative traits but also significantly increased the yield and size of potato (Harfoush et al. 2017). Various studies documented that the foliar application of nanochitosan (4 ml-1) on coriander plants increased the plant height, number of branches, dry weight, growth and components of yield including fruit yield and no. of inflorescence (El-Shayeb et al. 2021) and various studies reported the identical findings on various plants including radish (Farouk et al., 2011) okra (Mansour and El Mesairy, 2015), Pea (Khan et al., 2018), Tomato (Reyes-Perez et al., 2020), Wheat (Zein El-Abdeen and Farroh, 2019), Vitex trifolia (Ashour et al., 2021), and Vitis (Aazami et al., 2023).

3.1.2. Effect of CNPs on chemical constituents

For the eco-friendly management of resources in arid and semiarid lands, the utilization of organic NPs helps to combat the water and stress related challenges. A study focused to investigate the stress alleviatory effect of chitosan NPs (0, 10 and 20 mM) on coriander with respect to humic acid and salinity stress. A study on the application of chitosan to soil considerably increased the yield and growth of reddish by improving the physiological traits but showed immaterial effect to combat the salt stress

(Farouk et al. 2011). Furthermore, application of chitosan significantly increased the soluble sugars and chlorophyll contents in plants with various stresses. However, under saline conditions, the application of chitosan (200 ppm) increased the availability of mineral (NPK) in okra plant (Mansour and El Mesairy, 2015). A study by (Harfoush et al. 2017) showed substantial increase in chlorophyll contents of potato plants whereas, the foliar application of nanochitosan (4 ml-1) results in the higher level of carbohydrate, NPK and chlorophyll contents of coriander plant (EL-Shayeb et al., 2021). In a field experiment, the foliar spray inoculation of nanochitosan (50 mgl-1) in the combination with a PGPR not only increased (1.5-2fold) the amount of carotenoids, chlorophyll contents, proteins and sugar contents in Zea mays plants but also increased the defence related enzymes and antioxidants (Chaudhary et al. 2021). In drought stress conditions, Dowom et al. (2022) found that the application of chitosan not only showed significant increase in leaf chemical constituents' viz., chlorophyll contents, carotenoids, proteins, proline contents, soluble sugar, total phenols, and flavonoids of Russian sage but also enhanced the antioxidant enzyme activity to minimize the effect of water discrepancy. A study by Aazami et al. (2023) showed that the application of chitosan salicylic acid as nanocomposite in salinity stress conditions significantly increased the minerals, essential nutrients, chlorophyll contents, H2O2, malondialdehyde, and antioxidant enzymes of Vitis plant.

4. Combined effects of salinity, humic acid and/ or chitosan nanoparticles

In various abiotic stresses, salinity is the most challenging factor which reduces the crop productivity throughout the world particularly in arid and semiarid regions. Various physio-chemical and adoptability to environment can help to mitigate the salinity stress of plant. The use of bio stimulants viz., humic acid (HA) and chitosan nanoparticles (CNPs) could be an eco-friendly substitute for the improvement of various crops under convenient stress conditions.

4.1. Combined effects of salinity and humic acid on plant growth

Salinity management involves improving the physical, chemical, and biological properties of soil to make it suitable for plant growth and sustainable agricultural practices. Humic acid (HA) which is derived from the degradation of microbial activities, animal and plant remnants (Gulser et al., 2010) positively influence the growth and yield of plants in saline soil (Turkmen et al., 2004; Paksoy et al., 2010; Pizzeghello et al., 2013). In moderate salinity, spray inoculation to various plants regulates the growth of roots and shoots, biomass accumulation, length of hypocotyls and cotyledon (Pizzeghello et al., 2013) and as growth regulator, it control the hormones by enhancing the tolerance against stress (Çimrin et al., 2010). Plants have evolved various mechanisms to adapt to adverse soil conditions, such as high salinity. When soil becomes saline, the concentration of salt ions increases, making it challenging for plants to take up water and nutrients. In response to this stress, plants can activate specific physiological and biochemical mechanisms that help them cope with these conditions (Romheld and Neumann, 2006). A affirmative correlation between the yield of wheat and the level of HA was reported (Brunetti et al. 2007) and application of HA under saline conditions positively affected the seedlings of pepper by increasing the germination (Turkmen et al. 2004) and also in the combination with putrescine (Put) showed significant increase in growth of cotton plants (Hanafy et al., 2010).

Under moderate salinity stress, the application of HA in the combination with phosphorus increased the fresh and dry biomass of plants (Cimrin et al., 2010). Studies showed that in extreme saline stress, the biochemical and vegetative growth both were strongly restricted in caraway (Hassan 2019) and wheat plant (Abbas et al. 2022) and the saltwater irrigation to Carum carvi plants reduce the vegetative and physiological traits while the inoculation with HA significantly improved the physiological traits. Likewise, salinity reduced shoot biomass of wheat plants, whereas humic acid application enhanced the plant shoot biomass.

4.2. Effect of humic acid and salinity on the chemical components

Chemical constituents are important components of plants responsible for the activation of defense system in plants. Foliar application of HA significantly increases the carotenoids and chlorophyll contents under slat stress conditions (Hanafy et al., 2010). Increase in the level of salinity significantly increased the availability of amino acids and phenol contents in wheat On Myrtle plants (Verma and Mishra 2005) and under higher salt stress conditions, the foliar application of HA (20 gl-1) increased the amino acids and total phenol contents in snap bean (Hanafy et al. 2010). Under slat stress conditions, significant increase in the overproduction of hydrogen peroxide, and malondialdehyde along with the leakage of membrane in wheat was recorded which considerably reduced upon the foliar application of HA.

4.3. Combined effect of salinity stress and nanoparticles on plant growth

In arid and semiarid regions, the excessive amount of salts on soil surface hinders the plant growth and ultimately causes the significant reduction of productivity. Thus, among abiotic stress factors, salinity has become a challenging factor which drastically affects the physio-chemical process of plants. Using nanoparticles in salt affected regions can reduce the destructive salt effects. In this regard, studies have reported the utilization of Chitosan in agriculture and observed positive effect on maize plants under salt stress and significant increase in the plant growth and development was recorded (Al-Tawaha et al. 2018). More, negative impact of salinity the root and shoot development, height and weight of maize plants was observed. Further, foliar application chitosan considerably increased the growth and development of plants. Various studies documented that the foliar application of chitosan nanoparticles (CNPs) and Zinc nanoparticles (Zn-NPs) applied under salt stressed conditions, significantly improved the biomass accumulation, physiological traits and antioxidants in various plants (Balusamy et al. 2022) including sunflower (Torabian et al. 2016). More various researchers reported the positive effects of NPs on various plants including Borage (Sah et al., 2011), Strawberries (Luksiene et al., 2020), and maize (Shinde et al., 2020). Further, the application of zinc nanoparticles diminishes the pessimistic effect of various abiotic stresses (Sturikova et al., 2018). In plants, various environmental factors influence the amalgamation of NPs in leaves depending on the leaf type and level and duration of stress (Fernandez et al., 2013). Additionally, the application of Zn-NPs stimulates the biosynthesis of Indole acetic acid (IAA) resulting the division and elongation of cells, potential for the uptake of essential nutrients and augmented the growth of plants (Cakmak, 2000; Cakmak, 2008).

4.4. Effect of salt and nanoparticles on the plant chemical components

Chitosan nanoparticles as bio stimulants have gained significant attention due to their potential to alleviate the salt stress, efficiently increase the yield chlorophyll (A & B) contents, amino acids, antioxidant enzymes and carotenoids but caused the reduction ROS by increasing the species of revealed the role of chitosan nanoparticles under salt stress to improve pigments such signaling of jasmonic acid (Balusamy et al. 2022). In another study, under various level of salt stress (NaCl), significant increase in essential nutrients, carotenoids, chlorophyll contents, H2O2, carbohydrates, antioxidant enzymes, malondialdehyde, and proline contents of Vitis leaves treated with nanocomposite of salicylic acid ad chitosan was recorded (Aazami et al. 2023).

In the response of plants to various abiotic stresses, photosynthetic pigment is the most perceptive indicators and Zn-NPs positively influenced these pigments even under salt stress. As the application of Zn-NPs noticeably increased the carotenoids, protein and chlorophyll (a,b) contents in cotton (Venkatachalam et al. 2017), tomato (Singh et al. 2016), common beans (Salama et al., 2019), Muskmelon (Wang et al., 2019) proline contents in potato (Jaarsma et al., 2013) and Beta vulgaris (Ebrahim, 2005) by increasing the salinity. Significant increase in the accumulation of proline contents during salinity stress sturdily associated with the tolerance of stress (Levitt, 1980) because Proline as osmo-regulator allows the absorption of water even in stress conditions. The proline contents are relatively higher in stressed plants as compared to the sensitive ones (Aly et al., 2003) and saline stress results in the accretion of N compound that have tolerance against salt compounds (Ebrahim, 2005).

Furthermore, soluble sugar and salinity stress (along with NPs) are closely linked to each other as various studies documented that substantial increase in soluble sugar was recorded with the increase in salt and NPs in maize (Aly et al., 2003), wheat (Sadak, 2019), and carrot (Elizabath et al., 2017). Production of ROS (product of metabolic pathway) (Ahmad et al., 2010), during salt stress is the most important response by plant containing O2−, H2O2, O2 and OH− that are highly toxic to the cells of plant (El-Shintinawy et al., 2004). However, ROS has diverse nature from molecular oxygen due to their capability to oxidize the cellular component which leads to the oxidative burst of cell (Meloni et al., 2003).

Higher salt stress conditions cause the disparity of cellular ions which results the osmotic stress and the toxicity of ions and ultimately ROS are produced which caused the impairment of organic molecule in plants (Ebrahim and Saleem, 2017). More, continuous production of ROS in mitochondria, cytosol, apoplastic and chloroplast (Dai et al., 1997) causes the rummage of antioxidant enzymes such as SOD and CAT which protect the plant cell from oxidative stress of hydroxyl radical (Biswal and Mohanty, 1976). To improve the salt stress tolerance, maximizing the antioxidants metabolism and production of antioxidants enzyme is a fundamental method to protect the plants from abiotic stresses (Dai et al., 1997) because higher SOD and CAT activity even under salt stress provide the protection to plants against oxidative burst (Meloni et al., 2003; El-Shintinawy et al., 2004; Ebrahim and Saleem, 2017).

It has been reported that antioxidant enzymes play a significant role in the scavenging of ROS which enhance capability of plants to combat the salinity stress (El-Shintinawy et al., 2004). Superoxide Dismutase (SOD) is a widespread antioxidant enzyme found in various cell compartments, including the cytoplasm, mitochondria, and extracellular spaces and protects the cells from the harmful effects of reactive oxygen species (ROS) and is the first defense line against oxidative stress in aerobic organisms. Additionally, ROS are highly reactive molecules containing oxygen, generated as natural byproducts of cellular metabolism and play an important role in various cellular processes. Excessive accumulation of ROS can cause oxidative damage to cellular components (proteins, lipids, and DNA). One of the primary functions of SOD is the detoxification of superoxide radical (O2−). More, SOD catalyzes the dismutation (conversion) of O2− into H2O2 and O2 because O2− leads to the formation of more harmful ROS. Hydrogen peroxide (H2O2) is another ROS that can be harmful to cells if it accumulates but H2O2 is less reactive than O2− but can cause significant damage. Other enzymes such as catalase and glutathione peroxidase present in the cell breakdown H2O2 into oxygen and water (Dai et al., 1997). More, SOD helps the breakdown of O2− into O2 and H2O2. By regulating the levels of superoxide radicals and other reactive oxygen species, SOD contributes to maintaining the balance of oxidative stress in cells (Meloni et al., 2003; Ebrahim and Saleem, 2017). The SOD and CAT are considered as the most important and proficient antioxidants which reduce the cell damage and detoxification of ROS (El-Shintinawy et al., 2004). Under salt stress conditions, the application of NPs significantly improves the CAT and SOD activity and optimizes CAT activity to increase the scavenging of H2O2 (Ahmad et al., 2017).

4.5. Combined effects of humic acid and chitosan nanoparticles on plants

The presences of acidic groups in HA that are the small organic polymers play an important role in bioavailability, transportation, and assist the heavy metal to dissolve. Chitosan is an organic polymer (organic plant growth stimulant) obtained from de-acetylation of chitin (Khan et al., 2018). Foliar spray inoculation of HA, chitosan and/or both demonstrated positive effects and exhibited considerable increased in the physiological traits, growth and development of plants as well as yield by mitigating the detrimental effect of salt. The foliar application of HA and/or chitosan as soil drenching (100-200 mg/kg) not only increases the biomass accumulation, number of branches and leaves as well as the height and length of plant but also considerably enhanced the availability of NPK, protein, sugar contents, amino acids, chlorophyll contents, water contents, and soluble proteins in plant tissue (Farouk et al. 2011). Whereas the foliar application of chitosan (200 mg/kg) showed significant effects as compared to HA at various concentrations in mitigating the salinity stress on radish whereas, significant increase in the physiological traits and yield of potato was recorded (Harfoush et al. 2017). The combined application (chitosan + humic acid) significantly increased the photochemical and antioxidant compounds of potato and physiological traits of pea plants (Khan et al. 2018) as higher pods, length was observed.

5.6. Combined effects of salinity, humic acid, and chitosan nanoparticles on plants

Various studies reported the positive effects of HA, chitosan and nanochitosan on the reaction of plants to salt stress conditions and fewer studies has been conducted to observe the combined effects of HA, and salinity stress. So far, the literary references indicated no hint for investigating the plant response to the combined effects of salinity x humic acid x chitosan nanoparticles. All studies were made to investigate the plant response only to either one or two factors. The combined application of HA and chitosan on okra plant was studied at various concentrations of HA as K-humate (0 kg, 2 kg, 4 kg and 6 kg) and chitosan (0 ppm, 100 ppm, 150 ppm and 200 ppm) and results in the significant increase in the growth and development of plant as well as yield of okra (Ramadan and El Mesairy 2015). Further, the higher concentration of HA, chitosan and NPs increased the leaves, height, biomass, availability of essential minerals fruits and yield along with protein contents. Under salt stress conditions, the yield and production of okra was relatively higher at higher concentration of HA in the combination with the higher concentration (200 ppm) of chitosan. Furthermore, the saline water irrigation of Vitex trifolia and foliar application of HA and chitosan potentially mitigate the salinity stress (Ashour et al. 2021) and significantly increased the vegetative growth and antioxidant enzymes along with the increase in carbohydrates and chlorophyll (A, B) contents while the ratio of sodium and potassium was decreased. The foliar application of chitosan, NPs, and HA to mitigate the salinity stress, significantly increased the phenolic compounds, proline contents, proteins and antioxidant enzymes. Further, the foliar spay of HA and chitosan increases the vegetative growth and chemical constituents and reduced the accumulation of phenols, the toxicity of calcium and sodium ions in leaves while HA showed more efficacy than CH. Thus, the application of HA and chitosan with higher concentration, can be effective in mitigating the salinity effects in various plants and considerably increase the vegetative growth of plants.

6. Conclusions

Many previous researchers have proven the negative effect of salt stress on plants. Previous studies also showed that both humic and nano-chitosan have proven effective in improving the plant's ability to withstand salt stress, as both (HA) and (CNPs) enhance growth characteristics and biochemical factors in plants grown either without or under salinity stress. Both had a significant impact on improving the morphological characteristics and metabolic products of plants.

References

Aazami MA, Maleki M, Rasouli F, Gohar G. 2023. Protective effects of chitosan based salicylic acid nanocomposite (CS-SA NCs) in grape (Vitis vinifera cv. 'Sultana') under salinity stress. Scientific Reports 13: 883-897.

Abbas G, Rehman S, Siddiqui MH, Ali HM, Farooq MA, Chen Y. 2022. Potassium and Humic Acid Synergistically Increase Salt Tolerance and Nutrient Uptake in Contrasting Wheat Genotypes through Ionic Homeostasis and Activation of Antioxidant Enzymes. Plants 11: 263.

Abdelhamid MT, Shokr M, Bekheta MA. 2010. Growth, root characteristics, and leaf nutrients accumulation of four faba bean (Vicia faba L.) cultivars differing in their broomrape tolerance and the soil properties in relation to salinity. Communications in Soil Science and Plant Analysis 41(22): 2713-2728.

Ahmad P, Ahanger MA, Alyemeni MN, Wijaya L, Egamberdieva D, Bhardwaj R, Ashraf M. 2017. Zinc application mitigates the adverse effects of NaCl stress on mustard [Brassica juncea (L.) Czern and Coss] through modulating compatible organic solutes, antioxidant enzymes, and flavonoid content. Journal of Plant interactions 12(1): 429-437.

Ahmad P, Jaleel CA, Salem MA, Nabi G, Sharma S. 2010. Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress. Critical reviews in biotechnology, 30 (3): 161-175.

Aiken GR, McKnight DM, Wershaw RL, MacCarthy P. 1985. An introduction to humic substances in soil, sediment and water 1-9.

Al-Tawaha AR, Turk MA, Al-Tawaha ARM, Alu'datt MH, Wedyan ME, Al-Ramamneh M, Hoang AT. 2018. Using chitosan to improve growth of maize cultivars under salinity conditions. Bulgarian Journal of Agricultural Science 24 (3):437-442.

Alabdallah N M, Alzahrani HS. 2020. The potential mitigation effect of ZnO nanoparticles on [Abelmoschus esculentus L. Moench] metabolism under salt stress conditions. Saudi Journal of Biological Sciences 27 (11): 3132-3137.

Ali I, Khan AA, Imran I, Khan A, Asim M, Iqbal B. 2019. Humic acid and nitrogen levels optimizing productivity of green gram (Vigna radiate L.). Russian Agricultural Sciences 45: 43-47

Aly MM, El-Sabbagh SM, El-Shouny WA, Ebrahim MKH. 2003. Physiological response of Zea mays to NaCl stress with respect to Azotobacter chroococcum and Streptomyces niveus. Pakistan J. Biological Sciences 6 (24): 2073-2080.

Amarin R, Kafawin O, Ayad J, Al-Zyoud F, Ghidan A. 2020. Effect of Saline Water Irrigation and Growing Media on Growth, Physiological and Mineral Parameters of Clove Pink Dianthus caryophyllus. Jordan Journal of Agricultural Sciences 16(3): 55-69.

Ameri A, Tehranifar A. 2012. Effect of Humic Acid on Nutrient Uptake and Physiological Characteristic Fragaria x Ananassa Var: Camarosa. Journal of Biological Environment Science 6: 77-79.

Aml RM, Yousef EAM, Mostafa AM, Saleh MMS 2011. Olive seedlings growth as affected by humic and amino acids, macro and trace elements applications. Agriculture and Biology Journal of North America 2(7): 1101-1107.

Ashour HA, Esmail EA, Kotb SM. (2021). Alleviative effects of chitosan or humic acid on Vitex trifolia 'Purpurea' grown under salinity stress. Ornam. Hortic 27(1): 88-102.

Asli S, Neumann PM. 2010. Rhizosphere humic acid interacts with root cell walls to reduce hydraulic conductivity and plant development. Plant Soil 336: 313–322.

Ata-Ul-Karim ST, Cang L, Wang Y, Zhou D. 2020. Effects of soil properties, nitrogen application, plant phenology, and their interactions on plant uptake of cadmium in wheat. Journal of hazardous materials 384: 121452.

Atefe A, Ali T. 2012. Effect of humic acid on nutrient uptake and physiological characteristic Fragaria ananassa var: Camarosa. Journal of Biodiversity and Environmental Sciences 6(16): 77-79.

Azarpour E, Danesh RK, Mohammadi S. 2011. Effects of nitrogen fertilizer under foliar spraying of humic acid on yield and yield components of cowpea (Vigna unguiculata). World Applied Sciences Journal 13: 1445-1449.

Balusamy SR, Rahimi S, Sukweenadhi J, Sunderraj S, Shanmugam R, Thangavelu L, Mijakovic I, Perumalsamy H. 2022. Chitosan, chitosan nanoparticles and modified chitosan biomaterials, a potential tool to combat salinity stress in plants. Carbohydrate Polymers 284: 15.

Bergaya F, Lagaly G. 2013. Handbook of clay science. Newnes.

Biswal UC, Mohanty P. 1976. Aging induced changes in photosynthetic electron transport of detached barley leaves. Plant Cell Physiol 17: 323-331.

Britz SI. 1990. Regulation of photosynthetic partitioning into starch in soybean leaves. Response to natural daylight. Plant Physiol 94 :350 -356.

Brunetti G, Senesi N, Plaza C. 2007. Effects of amendment with treated and untreated olive oil mill wastewaters on soil properties, soil humic substances and wheat yield. Geoderma 138: 144-152.

Buzea C, Pacheco II, Robbie K. 2007. Nanomaterials and nanoparticles: sources and toxicity. Biointerphases 2(4): MR17-MR71.

Byczyńska A. 2018. Chitosan improves growth and bulb yield of pineapple lily (Eucomis bicolor Baker) an ornamental and medicinal plant. World Scientific News (110): 159-171.

Cakmak I. 2000. Tansley Review No. 111: possible roles of zinc in protecting plant cells from damage by reactive oxygen species. New Phytologist 146(2): 185-205.

Cakmak I. 2008. Enrichment of cereal grains with zinc: agronomic or genetic biofortification? Plant and soil 302(1-2): 1-17.

Canellas LP, Olivares FL, Canellas NOA, Jindo K, Rosa RCC, Piccolo A. 2022. Challenge of transition: the history of a case study involving tropical fruits polyculture stimulated by humic acids and plant-growth promoting bacteria. Chem Biol Technol Agric 9:76.

Cele T. 2020. Preparation of Nanoparticles. In Silver Nanoparticles-Health and Safety. Intech Open.

Chaudhary BU, Lingayat S, Banerjee AN, Kale RD. 2021. Development of multifunctional food packaging films based on waste Garlic peel extract and Chitosan. International Journal of Biological Macromolecules 192: 479-490.

Chen H,Gonzales-Vigil E, Wilkerson CG, Howe GA. 2007. Stability of plant defense proteins in the gut of insect herbivores. Plant physiology 143(4): 1954-1967.

Çimrin KM, Türkmen Ö, Turan M, Tuncer B. 2010. Phosphorus and humic acid application alleviate salinity stress of pepper seedling. African Journal of Biotechnology 9(36): 5845-5851.

Dai Q, Yan B, Huang S, Liu X, Peng S, Miranda MLL, Chavez AQ, Vergara BS, Olszyk DM. 1997. Response of oxidative stress defense systems in rice (Oryza sativa) leaves to supplemental UV-B radiation. Physiol. Plant 101: 301-308.

Dawood MG, Taie HAA, Nassar RMA, Abdelhamid MT, Schmidhalter U. 2014. The changes induced in the physiological, biochemical and anatomical characteristics of Vicia faba by the exogenous application of proline under seawater stress. South african journal of botany 93: 54-63.

Denaxa NK, Nomikou A, Malamos N, Liveri E, Roussos PA, Papasotiropoulos V. 2022. Salinity effect on plant growth parameters and fruit bioactive compounds of two strawberry cultivars, coupled with environmental conditions monitoring. Agronomy 12(10): 2279.

Dillewijn CV. 1952. Botany of sugarcane. The Chronica Botanica Company, Waitham, Mass U.S.A.

Ditta A, Arshad M, Ibrahim M. 2015. Nanoparticles in sustainable agricultural crop production: applications and perspectives. Nanotechnology and plant sciences: nanoparticles and their impact on plants 55-75.

Divya K, Jisha MS. 2018. Chitosan nanoparticles preparation and applications. Environ. Chem. Lett 16: 101-112.

Dobbss LB, Medici LO, Peres LEP, Pino-Nunes LE, Rumjanek VM, Façanha AR, Canellas LP. 2007. Changes in root development of Arabidopsis promoted by organic matter from oxisols. Annals of Applied Biology 151(2): 199-211.

Dowom AS, Karimian Z, Mostafaei DM, Samiei L. 2022. Chitosan nanoparticles improve physiological and biochemical responses of Salvia abrotanoides (Kar.) under drought stress. BMC Plant Biology 22(1):364.

Duran NM, Savassa SM, Lima RGD, de Almeida E, Linhares FS, van Gestel CA, Pereira de Carvalho HW. 2017. X-ray spectroscopy uncovering the effects of Cu based nanoparticle concentration and structure on Phaseolus vulgaris germination and seedling development. Journal of Agricultural and Food Chemistry 65(36): 7874-7884.

Dzung NA, Khanh VTP, Dung TT. 2011. Research on impact of chitosan oligomer on biophysical characteristics, growth, development and drought resistance of coffee. Carbohydr. Polym 84:751–755.

Ebrahim MKH, Abu-Grab OS. 1997. Effect of water quality and alar or cycocel on yield, chemical constituents, and water-use efficiency of onion bulb. J. Union Arab. Biol., Cairo, Egypt 4: 179-196.

Ebrahim MKH. 2005. Amelioration of sucrose-metabolism and yield changes, in storage roots of NaCl-stressed sugar beet, by ascorbic acid. Agrochimica 3-4: 93-103.

Ehsanpour AA, Fatahian N. 2003. Effects of salt and pro- line on Medicago sativa callus. Plant Cell, Tissue and Organ Culture 73(1): 53–56.

El Shayeb NS, Hassan RH, Mohaseb MI. 2021. Impact of nano-chitosan rate and glutamine acid concentration on growth, yield and volatile oil production of coriander plants. Journal of Bio-agriculture 15-24.

El-Bassiouny HS, Bakry BA, El-Monem AA, Abd Allah MM. 2014. Physiological Role of Humic Acid and Nicotinamide on Improving Plant Growth, Yield, and Mineral Nutrient of Wheat (Triticum durum) Grown under Newly Reclaimed Sandy Soil. Agric Sci 5:687-700.

El-Dardiry EI. 2007. Effect of soil and water salinity on barley grains germination under some amendments. World J. of Agricultural Sciences 3(3): 329-338.

El-Hak SG, Ahmed AM, Moustafa YMM. 2012. Effect of foliar application with two antioxidants and humic acid on growth, yield and yield components of peas (Pisum sativum L.). Journal of horticultural science & ornamental plants, 4(3): 318-328.

El-Shintinawy F, Ebrahim MKH, Sewelam N, El-Shourbagy MN. 2004. Activity of photosystem 2, lipid peroxidation, and the enzymatic antioxidant protective system in heat shocked barley seedlings. Photosynthetica 42 (1): 15-21.

Elizabath A, Bahadur V, Misra P, Prasad VM, Thomas T. 2017. Effect of different concentrations of iron oxide and zinc oxide nanoparticles on growth and yield of carrot (Daucus carota L.). Journal of pharmacognosy and phytochemistry 6(4), 1266-1269.

Farahat MM, Mazhar AA, Mahgoub MH. 2012. Response of Khaya senegalensis seedlings to irrigation intervals and foliar application of humic acid. Journal of Horticultural Science & Ornamental Plants 4(3): 292-298.

Farouk S, Mosa AA, Taha AA, Ibrahim M, EL-Gahmery AM. 2011. Protective Effect of Humic acid and Chitosan on Radish (Raphanus sativus L. var. sativus) Plants Subjected to Cadmium Stress. Journal of Stress Physiology & Biochemistry 7 (2): 99-116.

Fathi MA, Fawzia ME, Yahia MM. 2002. Improving growth, yield and fruit quality of ‘Desert Red’ peach and ‘Anna’ apple by using some biostimulants. Minia J. Agric. Res. & Develop 22(4): 519-534.

Fathy MA, Gabr MA, El-Shall SA. 2010. Effect of humic acid treatments on "Canino" apricot growth, yield and fruit quality. New York Sci. Journal 3(12): 109-115.

Fawzia ME. 2003. Use of some biostimulants in activation of soil microflora for yield and fruit quality improvement of ‘Canino’ apricot. J. Agric. Res. Tanta Univ 29(1): 175-194.

Fernandez MBM, Cosio LE, Montero DC, Garcia AC, Lopez DM. 2013. Potentiality of vermicompost humic acids in banana in vitro micropropagation clone: Enano Guantanamero. J Environ Sci Eng A 2:677-685

Ferrara G, Brunetti G. 2008. Foliar Applications of Humic Acids in Vitis vinifera L. cv Italia. J Int Sci Vigne Vin 42: 79-87.

Ferrara G, Brunetti G. 2010. Effects of the times of application of a soil humic acid on berry quality of table grape (Vitis vinifera L.) cv Italia. Spanish Journal of Agricultural Research 8(3): 817-822.

Fiorentino G, Spaccini R, Piccolo A. 2006. Separation of molecular constituents from a humic acid by solid-phase extraction following a transesterification reaction. Talanta 68(4): 1135-1142.

Galtier N, Foyer CH, Huber J, Voelker TA, Huber SC. 1993. Effects of elevated sucrose phosphate synthase activity on photosynthesis, assimilate partitioning and growth in tomato. Plant Physiol 101: 535-543.

Gawlik A, Kulpa D, Golkebiowska D, Bejger R. 2014. Influence of the auxin-like activity of humic acid on bio and microbiometric parameters of Pisum sativum L. by in vitro cultures of pea plants. J Food, Agric Environ 12: 209–212.

Gersende B, Dou S, Peakman T, Lichtfouse E. 2008. Identification of bound alcohols in soil humic acids by GC-MS. Euro. J Mass Spectrometry 6: 439-444.

Gholamnia A, Mosleh AA, Sodaeizadeh H, Tarkesh ES, Ghasemi S. 2021. The effects of salinity and heat stress on some physiological and vegetative characteristics of peppermint (Mentha piperita L.) at different time intervals. Iranian Journal of Plant Biology 13(2): 39-52.

Ghormade V, Deshpande MV, Paknikar KM. 2011. Perspectives for nano-biotechnology enabled protection and nutrition of plants. Biotechnology advances 29(6): 792-803.

Gordon AJ. 1986. Diurnal patterns of photosynthates allocation and partitioning among sinks. In: phloem transport (eds., J., Grondshaw, W. J. Lucas and R. T. Giaquinta) Alan R., Liss, New York 499-518.

Gulser F, Sonmez F, Boysan S. 2010. Effects of calcium nitrate and humic acid on pepper seedling growth under saline condition. J. Environ. Biol 31: 873-876.

Hagagg LF, Shahin MFM, Merwad MA, Fikria HK, El-Hady ES. 2013. Improving fruit quality and quantity of “Aggizi” olive trees by application of humic acid during full bloom and fruit set stages. Middle East j 2(2): 44-50.

Hanafy AH, Nesiem MR, Hewedyand AM, Sallam HES. 2010. Effect of some simulative compounds on growth, yield and chemical composition of snap bean plants grown under calcareous soil conditions. J. American Sci 6(10): 552-569.

Harfoush EA, Abdel-Razzek AH, El-Adgham FI, El-Sharkawy AM. 2017. Effects of Humic Acid and Chitosan under Different Levels of Nitrogen and Potassium fertilizers on Growth and Yield potential of Potato plants (Solanum tuberosum, L.). Alex. J. Agric. Sci 62, (1):135-148.

Hartt CE. 1936. The fluctuations of sugars in the leaf sheaths of the sugar cane plant during the day and the night. Hawaii Plant Rec 40:329-354.

Hasaneen MNA Omer AM. 2016. Nano chitosan-NPK fertilizer enhances the growth and productivity of wheat plants grown in sandy soil. Spanish J Agric Res 14:1-9

Hassan AA. 2019. Effects of irrigation water salinity and humic acid treatments on caraway plants. J. Plant Production 10(7): 523-528.

Hassan FAS, Ali E, Gaber A, Fetouh MI, Mazrou R. 2021. Chitosan nanoparticles effectively combat salinity stress by enhancing antioxidant activity and alkaloid biosynthesis in Catharanthus roseus (L.) G. Don. Plant Physiology and Biochemistry 162: 291-300.

Heilos L, Dietz KJ. 1990. Carbon metabolism in spinach leaves as affected by leaf age, phosphorus and sulfur nutrition. Plant physiol 93:1219-1225

Hernandez JA. 2019. Salinity Tolerance in Plants: Trends and Perspectives. Int. J. Mol. Sci 20: 2408.

Hidangmayum A, Dwivedi P, Katiyar D, Hemantaranjan A. 2019. Application of chitosan on plant responses with special reference to abiotic stress. Physiology and molecular biology of plants 25(2): 313-326.

Huber SC, lsrael DW. 1982. Biochemical basis for partitioning of photosynthetically fixed carbon between starch and sucrose in soybean leaves. Plant physiol 69:691-696.

Iber BT, Kasan NA, Torsabo D, Omuwa JW. 2022. A review of various sources of chitin and chitosan in nature. Journal of Renewable Materials 10(4): 1097.

Irfan A, Shahzad M, Iqbal A. 2005. The effects of seed soaking with plant growth regulators on seedling vigour of wheat under salinity stress. J. Stress Physiology and Biochemistry 1: 6-14.

Izhar SM, Adnan M, Fahad S, Wahid F, Khan A, Yue Z, Datta R. 2020. Application of single superphosphate with humic acid improves the growth, yield and phosphorus uptake of wheat (Triticum aestivum L.) in calcareous soil. Agronomy 10(9): 1224.

Jaarsma R, de Vries RS, de Boer AH, 2013. Effect of salt stress on growth, Na+ accumulation and proline metabolism in potato (Solanum tuberosum) cultivars. PLoS One 8(3): 60183.

Jabeen N, Ahmad R. 2013. The activity of antioxidant enzymes in response to salt stress in safflower (Carthamus tinctorius L.) and sunflower (Helianthus annuus L.) seedlings raised from seed treated with chitosan. J Sci Food Agric 93:1699–1705.

Jackson C, Patel J, Fernando N, Salehi E, Wong V, Kim J. 2013. Chitosan against cutaneous pathogens. AMB Express 3(1): 1-8.

Jindo K, Canellas LP, Albacete A, Santos LF, Rocha RLF, Baia DC, Canellas NOA, Goron TL, Olivares FL. 2020. Interaction between humic substances and plant hormones for phosphorous acquisition. Agronomy 10: 640-657.

Jouyban Z. 2012. The Effects of Salt stress on plant growth. TJEAS Journal 2(1): 7-10.

Kapoor K, Srivastava Al. 2010. Assessment of salinity tolerance of Vigna mungo var. Pu-19 using ex vitro and in vitro methods. Asian Journal of Biotechnology 2(2): 73-85.

Kasim WA, Abokassem EM, Ragab GA. 2017. Ameliorative effect of yeast extract, IAA and green-synthesized nano zinc oxide on the growth of Cu- stressed Vicia faba seedlings. Egyptian journal of Botony 57:1-15.

Kato H. 2011. Tracking nanoparticles inside cells. Nature Nanotechnology, 6(3): 139-140.

Kaya MU, Atak ME, Khawar KM, Çiftçi CY, Ozcan S. 2005. Effect of pre-sowing seed treatment with zinc and foliar spray of humic acids on yield of common bean (Phaseolus vulgaris L.). Int. J. Agric. Biol 7(6): 875-878.

Khan R, Manzoor N, Zia A, Ahmad I, Ullah A, Shah SM, Muhammad NM, Ali S, Khan IH, Zia D, Malik S. 2018. Exogenous application of chitosan and humic acid effects on plant growth and yield of pea (Pisum sativum). International Journal of Biosciences12(5): 43-50.

Krupa-Małkiewicz M, Smolik B. 2019. Alleviative effects of chitosan and ascorbic acid on Petunia atkinsiana D. Don under salinity. European Journal of Horticultural Science 84(6): 359-365.

Leite JM, Arachchige PSP, Ciampitti IA, Hettiarachchi GM, Maurmann L, Trivelin PC, Sunoj SJ. 2020. Co-addition of humic substances and humic acids with urea enhances foliar nitrogen use efficiency in sugarcane (Saccharum officinarum L.). Heliyon 6(10).

Levitt J. 1980. Responses of Plants to Environmental Stresses. Academic Press, New York, London 2:365-454.

Lichtenthaler HK, Rinderle U. 1988. The role of chlorophyll fluorescence in the detection of stress conditions in plants. CRC Critical Reviews in Analytical Chemistry 19(1): 29-85.

Lichtenthaler HK. 1977. Lipids and lipid polymers in higher plant. 231. Springer-Verlag. Berlin and New York.

Lodhi A, Tahir S, Iqbal Z, Mahmood A, Akhtar M, Qureshi TM, Naeem A. 2013. Characterization of commercial humic acid samples and their impact on growth of fungi and plants. Soil Environment 32: 63-70.

Lovato MB, De-Lemos Filho JP, Martins PS. 1999. Growth responses of Stylosanthes humilis (Fabaceae) populations to saline stress. Environ. Exp. Bot 41: 145-153.

Luksiene, Z.; Rasiukeviciute, N.; Zudyte, B. and Uselis, N. (2020). Innovative approach to sunlight activated biofungicides for strawberry crop protection: ZnO nanoparticles. Journal of photochemistry and photobiology B: Biology, 203: 111656.

Lungoci C, Motrescu I, Filipov F, Rimbu CM, Jitareanu CD, Ghitau CS, Puiu I, Robu T. 2023. Salinity stress influences the main biochemical parameters of nepeta racemosa lam. Plants 12(583):1-15.

Mac-Carthy P. 2001. The principles of humic substances. Soil Science 166(11): 738-751.

Magdi TA, Selim EM, El-Ghamry AM. 2011. Integrated effects effects of Bio and Mineral fertilizers and humic substances on Growtth, yield and nutrient contents of fertigated Cowpea (Vignaunguiculata L.) grown on sandy soils. Journal of Agronomy 10(1): 34-39.

Mahdavi B, Rahimi A. 2013. Seed priming with chitosan improves the germination and growth performance of ajowan (Carum copticum) under salt stress. EurAsian Journal of BioSciences 76:69-76.

Mallikaarjuna Rao MM, Govindasamy R, Chandrasekaran S. 1987. Effect of humic acid on Sorghum vulgare var. CSH-9. Current science 1273-1276.

Mansour R, El Mesairy M. 2015. Effect of humic acid and chitosan on growth and yield of okra (abelmoschus esculentus L.) Under saline conditions. Egyptian Journal of Desert Research 65(1): 47-60.

Marschner H. 1995. Mineral nutrition of higher plants. (Academic Press: London.).

Masciandaro G, Ceccanti B, Ronchi V, Benedicto S, Howard L. 2002. Humic substances to reduce salt effect on plant germination and growth. Comm. Soil Sci. Plant Anal 33: 365-378.

Meloni DA, Oliva MA, Martinez CA, Cambraia J. 2003. Photosynthesis and activity of superoxide dismutase, peroxidase, and glutathione reductase in tomato under salt stress. Environ. Exp. Bot 49: 69-76.

Mondal MMA, Rana MIK, Dafader NC, Haque ME. 2011. Effect of foliar application of chitosan on growth and yield in Indian spinach. J. Agrofor. Environ 5(1): 99-102.

Mulder GJ. 1840. Uber die Bestandteile der Ackercrde. J. prakt. Chem 21(203).

Munns R, Tester M. 2008. Mechanisms of salinity tolerance. Annual Review of Plant Biology 59: 651-681.

Munns R. 2002. Comparative physiology of salt and water stress. Plant Cell Environ 25: 239-250.

Munns R. 2005. Genes and salt tolerance: bringing them together. New Phytol 167: 645- 663.

Nardi S, Concheri G, Dell'Agnola G. 1996. Biological activity of humic substances. In: Piccolo, A., (Ed.), Humic Substances in Terrestrial Ecosystems, Elsevier, Amsterdam 361-406.

Nawaz K, Hussain K, Abdul Majeed KF, Afghan S, Ali K. 2010. Fatality of salt stress to plants: Morphological, physiological and biochemical aspects. African Journal of Biotechnology 9(34): 5475-5480.

Nikbakht A, Kafi M, Babalar M, Xia YP, Luo A, Etemadi NA. 2008. Effect of humic acid on plant growth, nutrient uptake, and postharvest life of gerbera. Journal of Plant Nutrition 31(12): 2155-2167.

Osman AS, Ewees MSA. 2008. The possible use of humic acid incorporated with drip irrigation system to alleviate the harmful effects of saline water on tomato plants. Fayoum Journal of Agricultural Research and Development 22: 52-70.

Othman Y, Al-Karaki G, Al-Tawaha AR, Al-Horani A. 2006. Variation in germination and ion uptake barley genotypes under salinity conditions. World Journal of Agricultural Sciences, 2: 11-15.

Paksoy M, Türkmen Ö, Dursun A. 2010. Effects of potassium and humic acid on emergence, growth and nutrient contents of okra (Abelmoschus esculentus L.) seedling under saline soil conditions. Afr. J. Biotechnol 9: 5343-5346.

Patil RB, Chavan SB, More AD, Shinde JB. 2013. Effect of potassium humate on biochemical aspects of wheat. Indian Journal of Fundamental and Applied Life Sciences 3(1): 89-91.

Patil, R. (2010). Effect of Potassium Humate and Deproteinised Juice (DPJ) on Seed Germination and Seedling Growth of Wheat and Jowar. Annals of Biological Research 1: 148-151.

Payakapong W, Tittabutr P, Teaumroong N, Boonkerd, N, Singleton P, Borthakur D. 2006. Identification of two clusters of genes involved in salt tolerance in Sinorhizobium sp. Strain BL3. Symbiosis 41: 47-53.

Payen S, Basset-Mens C, Núñez M, Follain S, Grünberger O, Marlet S, Perret S, Roux P. 2016. Salinisation impacts in life cycle assessment: a review of challenges and options towards their consistent integration. The international journal of life cycle assessment 21(4): 577-594.

Peymaninia Y, Valizadeh M, Shahryari R, Ahmadizadeh, M, Habibpour M. 2012. Relationship among morpho-physiological traits in bread wheat against drought stress at presence of a leonardite derived humic fertilizer under greenhouse condition. Int Res J Appl Basic Sci 3:822-830

Piccolo A, Pietramellara G, Mbagwu JSC. 1997. Use of humic substances as soil conditioners to increase aggregate stability. Geoderma 75(3-4): 267-277.

Pichyangkura R, Chadchawan S. 2015. Bio stimulant activity of chitosan in Horti- culture. Sci. Hortic 196: 49-65.

Pirbalouti AG, Malekpoor F, Salimi A, Golparvar A. 2017. Exogenous application of chitosan on biochemical and physiological characteristics, phenolic content, and antioxidant activity of two species of basil Ocimum ciliatum and Ocimum basilicum under reduced irrigation. Sci. Hortic 217: 114-122.

Pizzeghello D, Francioso O, Ertani A, Muscolo A, Nardi S. 2013. Isopentenyladenosine and cytokinin-like activity of different humic substances. J. Geochem. Ex 129: 70-75.

Rady MM. 2011. Effect of 24-epibrassinolide on growth, yield, antioxidant system and cadmium content of bean (Phaseolus vulgaris L.) plants under salinity and cadmium stress. Scientia Horticulturae 129: 232-237.

Ragab A, Hellal F, Abd El-Hady. 2008. Irrigation water salinity effects on some soil water constants and plant. Twelfth International Water Technology Conference, IWTC12 Alexandria Egypt :14.

Ramadan M, El Mesairy M. 2015. Effect of humic acid and chitosan on growth and yield of okra (Abelmoschus esculentus L.) under saline conditions. Egypt. J. Desert Res 65 (1): 47- 60.

Ramoliya PJ, Patel HM, Pandey AN. 2004. Effect of salinization of soil on growth and macro- and micro-nutrient accumulation in seedlings of Salvadora persica (Salvadoraceae). For Ecol Manag 202: 181-193.

Rengasamy P. 2006) World salinization with emphasis on Australia. Journal of experimental botany 57(5): 1017-1023.

Reyes-Perez JJ, Enríquez-Acosta EA, Ramírez-Arrebato MÁ, Zúñiga-Valenzuela E, Lara-Capistrán L, Hernández-Montiel LG. 2020. Effect of chitosan on variables of tomato growth, yield and nutritional content. Revista mexicana de ciencias agrícolas 11(3): 457-465.

Rico CM, Barrios AC, Tan W, Rubenecia R, Lee SC, Varela-Ramirez A, Gardea-Torresdey JL. 2015. Physiological and biochemical response of soil-grown barley (Hordeum vulgare L.) to cerium oxide nanoparticles. Environmental Science and Pollution Research, 22: 10551-10558.

Romheld V, Neumann G. 2006. The Rhizosphere: Contributions of the soil-Root Interface to Sustainable Soil Systems. In: Uphoff, N. et al. (ed.) Biological Approaches to Sustainable Soil Systems. CRC Press, Boca Raton, FL 91-107.

Ryosuke N, Eiji K, Akiyoshi S, Masayuki S. 2006. The novel hypoglycemic agent YM440 improves hepatic insulin resistance in obese Zucker fatty rats. Journal of pharmacological sciences 101(4): 311-317.

Saad-Allah KM, Sobhy SE, Hassan FAS, Al-Yasi H, Gad D. 2022. Assessment of selenium contribution to salt and water stress tolerance in hydroponically grown cotton (Gossypium barbadense L.). J Plant Nutr 45:2405-2421.

Sabir S, Arshad M, Chaudhari SK. 2014. Zinc oxide nanoparticles for revolutionizing agriculture: synthesis and applications. The Scientific World Journal. .

Sadak MS. 2019. Physiological role of trehalose on enhancing salinity tolerance of wheat plant. Bulletin of the national research centre 43(1): 53.

Safikhan S, Khoshbakht K, Chaichi MR, Amini A, Motesharezadeh B. 2018. Role of chitosan on the growth, physiological parameters and enzymatic activity of milk thistle (Silybum marianum (L.) Gaertn.) in a pot experiment. Journal of Applied Research on Medicinal and Aromatic Plants 10: 49-58.

Sah S, Sorooshzadeh A, Rezazadehs H, Naghdibadi HA. 2011. Effect of nano silver and silver nitrate on seed yield of borage. Journal of medicinal plants research 5(2):171-175.

Salachna P, Wilas J, Zawadzinska A. 2015. The effect of chitosan coating of bulbs on the growth and flowering of Ornithogalum saundersiae. In Proceedings of the XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014), Brisbane, Australia 1104: 115–118.

Salachna P, Zawadzińska A. 2014. Effect of chitosan on plant growth, flowering and corms yield of potted freesia. Journal of ecological engineering 15(3).

Salama DM, Osman SA, Abd El-Aziz ME, Abd Elwahed MS, Shaaban EA. 2019. Effect of zinc oxide nanoparticles on the growth, genomic DNA, production and the quality of common dry bean (Phaseolus vulgaris). biocatalysis and agricultural biotechnology 18: 101083.

Saxena J, Sharma PK, Sharma MM, Singh A. 2016. Process optimization for green synthesis of silver nanoparticles by Sclerotinia sclerotiorum MTCC 8785 and evaluation of its antibacterial properties. SpringerPlus 5(1): 1-10.

Schmidt W, Santi S, Pinton R, Varanini Z. 2007. Water-extractable humic substances alter root development and epidermal cell pattern in Arabidopsis. Plant and Soil 300: 259-267.

Selim EM, Mosa AA, El-Ghamry AM. 2010. Evaluation of humic substances fertigation through surface and subsurface drip irrigation systems on potato grown under Egyptian sandy soil conditions. Agric Water Manag 96:1218-1222.

Selim EM, Mosa AA. 2012. Fertigation of humic substances improves the yield and quality of broccoli and nutrient retention in sandy soil. Journal Plant Nutr. Soil Science 175: 273-281.

Senesi N, Miano TM, Brunetti G. 1996. In “Humic-Like Substances in Organic Amendments and Effects on Native Soil Humic Substances”. Piccolo, A. (ed.). Humic Substances in Terrestrial Ecosystems. USA, New York: Elsevier 531-593.

Senesi N, Plaza C, Brunetti G, Polo A. 2009. Effects of composted and thermally dried sewage sludges on soil and soil humic acid properties. Pedosphere 19(3): 281-291.

Shaddad G, Khalil A, Fathi MA. 2005. Improving growth, yield and fruit quality of "Canino" apricot by using bio, mineral and humate fertilizers. Minufiya, J. Agric. Res 30(1): 317-328.

Shaheen GMA, Abdel-Wahab MS, Hassan EA, Abdel Aziz AMR. 2012. Effect of some soil conditioners and organic fertilizers on vegetative growth and quality of crimson seedless grapevines. Journal of Horticultural Science & Ornamental Plants 4: 260-266.

Shinde S, Paralikar P, Ingle AP, Rai M. 2020. Promotion of seed germination and seedling growth of Zea mays by magnesium hydroxide nanoparticles synthesized by the filtrate from Aspergillus niger. Arabian journal of chemistry 13(1):3172-3182.

Siddiqui MH, Al-Whaibi MH, Firoz M, Al-Khaishany MY. 2015. Nanotechnology and plant sciences; role of nanoparticles in plants. Springer International Publishing Switzerland 19-35.

Simpson AJ, Burdon J, Graham CL, Hayes MHB, Spencer N, Kingery WL. 2001. Interpretation of heteronuclear and multidimensional NMR spectroscopy of humic substances. European Journal of Soil Science 52(3): 495-509.

Singh A, Singh NB, Hussain I, Singh H, Yadav V, Singh SC. 2016. Green synthesis of nano zinc oxide and evaluation of its impact on germination and metabolic activity of Solanum lycopersicum. Journal of biotechnology 233: 84-94.

Sobrado MA. 1994. Leaf age effects on photosynthetic rate, transpiration rate and nitrogen content in a tropical dry forest. Physiol. Plant 90: 210 – 215.

Sritharan AA, Mallika V. 2005. Effect of foliar spray of nutrients and plant growth regulators (PGRS) for yield maximization in blackgram Madras Agric. J 4(6): 301-307.

Steelink C. 1999. Understanding humic substances: advanced methods, properties and applications. In E. A. Ghabbour, & G. Davies (Eds.), Cambridge: The Royal Society of Chemistry.

Stevenson FJ, 1982. Humus chemistry: Genesis, composition, reactions. Wiley-Interscience, New York.

Stevenson FJ. 1994. Humus Chemistry: Genesis, Composition, Reactions; John Wiley & Sons: Hoboken, NJ USA.

Sturikova H, Krystofova O, Huska D, Adam V. 2018. Zinc, zinc nanoparticles and plants. Journal of hazardous materials 349: 101-110.

Sugiyama H, Hisamichi K, Sakai K, Usui T, Ishiyama JI, Kudo H, Senda Y. 2001. The conformational study of chitin and chitosan oligomers in solution. Bioorganic & medicinal chemistry 9(2): 211-216.

Sun J, Jia YX, Guo SR, Li J, Shu S. 2010. Resistance of spinach plants to seawater stress is correlated with higher activity of xanthophyll cycle and better maintenance of chlorophyll metabolism. Photosynthetica 48(4): 567-579.

Sutton R, Sposito G. 2005. Molecular structure in soil humic substances: the new view. Environmental science & technology 39(23): 9009-9015.

Tahjib-Ul-Arif M, Hasan MT, Rahman MA, Nuruzzaman M, Rahman AS, Hasanuzzaman M, Brestic M. 2023. Plant response to combined salinity and waterlogging stress: current research progress and future prospects. Plant Stress 100-137.

Tejada M, Gonzalez JL. 2006. The relationships between erodibility and erosion in a soil treated with two organic amendments. Soil and Tillage Research 91(1-2): 186-198.

Tejera N, Ortega E, Rodes R, Lluch C. 2006. Nitrogen compounds in the apoplastic sap of sugarcane stem: Some implications in the association with endophytes. J. Plant Physiol 163: 80-85

Torabian S, Zahedi M, Khoshgoftarmanesh A. 2016. Effect of foliar spray of zinc oxide on some antioxidant enzymes activity of sunflower under salt stress. Journal of agricultural science and technology 18(4): 1013-1025.

Tort N, Turkyilmaz B. 2004. A physiological investigation on the mechanisms of salinity tolerance in some barley culture forms. Jfs 27: 1-16.

Tripathi DK, Singh VP, Prasad SM, Chauhan DK, Dubey NK. 2015. Silicon nanoparticles (SiNp) alleviate chromium (VI) phytotoxicity in Pisum sativum (L.) seedlings. Plant Physiology and Biochemistry 96: 189-198.

Turkmen O, Bozkurt MA, Yildiz M, Çimrin KM. 2004. Effects of nitrogen and humic acid applications on the head weight, nutrient and nitrate contents in lettuce. Advances in food sciences 26(2): 59-63.

Turner, N. C. and Kramer, P. J. (1980). Adaptation of plants to water and high-temperature stress. Proceedings of a seminar, Stanford, Calif. (USA), 6-10 Nov 1978.

Vaughan D, Linehan DJ. 1976. The growth of wheat plants in humic acid solutions under axenic conditions. Plant and Soil 44(2): 445-449.

Vaughan D, Malcom RE, Ord BG. 1985. Influence of humic substances on biochemical processes in plants. In: Vaughan, D, Malcom, R.E. (Eds.), Soil Organic Matter and Biological Activit, Martinus Nijhoff/Junk W, Dordrecht, The Netherlands 77-108.

Vaughan D, Ord BG. 1991. Influence of natural and synthetic humic substances on the activity of urease. Journal of Soil Science 42(1): 17-23.

Venkatachalam P, Priyanka N, Manikandan K, Ganeshbabu I, Indiraarulselvi P, Geetha N, Muralikrishna K, Bhattacharya R, Tiwari M, Sharma NJP, Sahi SV. 2017. Enhanced plant growth promoting role of phycomolecules coated zinc oxide nanoparticles with P supplementation in cotton (Gossypium hirsutum L.). Plant physiology and biochemistry 110:118-127.

Verma S, Mishra SN. 2005. Putrescinealleviation of growth in salt stressed Brassicajuncea by inducing antioxidative defence system. J. Plant Physiol 162: 669- 677.

Wang Y, Wang S, Xu M, Xiao L, Dai Z, Li J. 2019. The impacts of γ-Fe2O3 and Fe3O4 nanoparticles on the physiology and fruit quality of muskmelon (Cucumis melo) plants. Environmental pollution 249: 1011-1018.

Zaghloul SM, El-Quesni FEM, Mazhar AAM. 2009. Influence of Potassium Humate on Growth and Chemical constituents of Thuja Orientalis L seedlings. Ozean Journal of Applied Sciences 2(1):73-78.

Zahran HH. 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiology and Molecular Biology Reviews 63: 968-989.

Zancani M, Petrussa E, Krajňáková J, Casolo V, Spaccini R, Piccolo A, Macrì F, Vianello A. 2009. Effect of humic acids on phosphate level and energetic metabolism of tobacco BY-2 suspension cell cultures. Environmental and Experimental Botany 65(2-3): 287-295.

Zandonadi DB, Santos MP, Dobbss LB, Olivares FL, Canellas LP, Binzel ML, Okorokova-Façanha AL, Façanha AR. 2010. Nitric oxide mediates humic acids-induced root development and plasma membrane H+-ATPase activation. Planta 231: 1025-1036.

Zein El-Abdeen A, Farroh Y. 2019. Preparation and characterization of nano organic soil conditioners and it's effected on sandy soil properties and wheat productivity. Nat. Sci 17: 115–128.

Zein FI, Abd El-Wahab SA, El-Abaseri MA, Moustafa ATA. 2003. Evaluation of some wheat varieties to salt tolerance. Egypt. J. Soil Sci 43(3): 319-327.

Zelitch I. 1988. The close relationship between net photosynthesis and crop yield. Biolog. Sci., 32:796-802.

Zeng K, Hwang HM, Yu H. 2002. Effect of Dissolved Humic Substances on the Photochemical Degradation Rate of 1-Aminopyrene Atrazine. International Journal of Molecular Sciences 3(10): 1048-1057.

Zhang ZJ, Mao BZ, Li HZ, Zhou WJ, Takeuchi Y, Yoneyama Y. 2005. Effect of salinity on physiological characteristics, yield and quality of microtubers In vitro in potato. Acta Physiol. Plant 27: 48-490.

Zou P, Li K, Liu S, Xing R, Qin Y, Yu H, Li P. 2015. Effect of Chito oligosaccharides with different degrees of acetylation on wheat seedlings under salt stress. Carbohydrate Polymer 126: 62-69.

تأثير الإجهاد الملحي، حمض الهيوميك، وجسيمات الشيتوزان متناهية الصغر على النبات

ابتسام أحمد الغبان[١-٢]، حسن سعيد الزهراني[١]، سميرة عبد الله الغامدي[١]

[١] *قسم علوم الاحياء، كلية العلوم، جامعة الملك عبد العزيز*

[٢] *قسم الأحياء، الكلية الجامعية بضباء، جامعة تبوك*

e.algaban@ut.edu.sa

المستخلص. تقلل الملوحة من الإنتاج الزراعي لمعظم النباتات. بينما يملك حمض الهيومك (HA) وجسيمات الشيتوزان النانوية (CNPs) فوائد واعدة للإنتاج الزراعي المستدام بسبب أصولها الطبيعية وإمكانية استخدامها كعلاج لآثار الإجهاد الملحي، حيث تقوم بتحسين نمو النبات وإنتاجيته مما يجنب أو يقلل النبات الاضرار الناتجة عن استخدام مياه الري المالحة في الزراعة كما جاء في الأدبيات السابقة. تقدم المراجعة الحالية دراسات مفصلة حول الإجهاد الملحي وأضراره وآثاره السلبية التي يسببها للنبات والإنتاج الزراعي وعلى نواتج عملية الايض ومضادات الاكسدة داخل النبات. كما يسلط الضوء على تركيب حمض الهيوميك ودوره الفعال والإيجابي في نمو النبات وزيادة مقاومته للملوحة. كما تم توضيح أهمية المركبات النانوية (الشيتوزان) ودورها كعلاجات للملوحة.

الكلمات المفتاحية: الإجهاد الملوحة، حمض الهيوميك، جسيمات الشيتوزان النانوية، الشيتوزان، الجسيمات النانوية.