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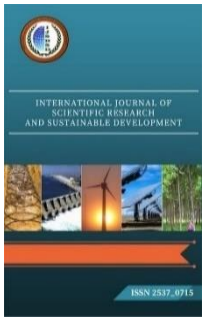

Impact of different materials in the normal and nanoform on the enzyme activities of infected potato plants with pathogenic bacteria *Pectobacterium spp.*

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Abstract

The potato (*Solanum tuberosum*) is the fourth most widely grown crop around the globe. It is used to make a variety of food products all over the world, including chips, crisps, potato flour, vegetable salad, canned food, and livestock supply. Unfortunately, a variety of abiotic and biotic stresses, such as soft rot pathogens, affect potato tuber yield and quality (Pectobacterium and Dickeya). Soft rot is most common throughout field production and storage. Our research study was aiming to analyse peroxidase and polyphenol oxidase, catalase, and phenol production in the infected potato Sponta and Mondial cultivars with *Pectobacterium spp* in response to 5 mM of glycyrrhizic acid ammonium salt, salicylic acid, and composites in the normal and nanoform treatments.

The obtained results showed that Peroxidasee activities in potato leaves increased to the highest level of 3.9 U.ml^{-1} in Mondial cultivar in response to 5 mM SA NPs compared to 0.73 U.ml^{-1} in the positive control and 0.47 U.ml^{-1} in the negative control. While Sponta cultivar scored 3.53 U.ml^{-1} of peroxidase activities in response to 5 mM SA NPs in comparison with 0.57 U.ml^{-1} and 0.33 U.ml^{-1} in the positive and negative control respectively. Also, Peroxidase activities in potato tubers boosted to the highest level of 3.9 U.ml-1 in Mondial cultivar in response to 5 mM SA NPs and 5mM nanocomposites (Ch NPs+ SA NPs + GSA NPs) compared to 0.6 U.ml-1 in the positive control and 0.5 U.ml-1 in the negative control. Treated Mondial cultivar showed the highest polyphenol oxidase activities in the potato leaves at 0.95 U.ml^{-1} in response to 5 mM SA NPs followed by 0.923 U.ml^{-1} and 0.813 in response to 5 mM nanocomposites (Ch NPs+ SA NPs + GSA NPs) and 5 mM GSA NPs treatments respectively, compared to 0.15 U.ml^{-1} in the positive control and 0.343 U.ml^{-1} in the negative control.

However, Sponta cultivar showed polyphenol oxidase activities in potato leaves 0.937 U.ml^{-1}, 0.86 U.ml^{-1}, 0.757 U.ml^{-1} and 0.72 U.ml^{-1} in response to 5 mM SA NPs, 5 mM nanocomposites (Ch NPs+ SA NPs + GSA NPs), SA and GSA NPs treatments respectively in comparison with 0.137 U.ml^{-1} in the positive control and 0.333 U.ml^{-1} in the negative control. Treated Mondial and Sponta cultivars with 5 mM SA NPs scored the highest polyphenol oxidase activities in potato tubers 0.777 U.ml^{-1} and 0.72 U.ml^{-1} respectively compared to all other treatments. Catalase activities in Mondial cultivar boosted to the highest level 2.173 U.ml^{-1} in response to SA NPS treatment.

The activities of catalase enzyme in potato tubers of Sponta cultivar 4.267 U.ml^{-1} in response to GSA compared to 1.267 U.ml^{-1} in response to GSA NPs. Catalase activities profile of potato tubers in the Mondial cultivar increased in response to all treatments in comparison with the Sponta cultivar except for GSA treatment. Phenol contents in the potato leaves of the Mondial cultivar boosted to 11.19 mg/gm, 10.98 mg/gm and 10.52 mg/gm in response to 5 mM SA NPs, 5 mM GSA NPs and 5 mM Ch NPs respectively. While potato leaves of the Sponta cultivar scored 10.48 mg/gm, 10.43 mg/gm and 9.71 mg/gm in response to 5 mM SA NPs, 5 mM GSA NPs and 5 mM Ch NPs respectively. The phenol contents in the potato tubers of both cultivars produced the same amount of 7.4 mg/gm in response to 5 mM SA treatment.

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While phenol contents in the potato tuber of the Sponta cultivar scored 7.38 mg/gm compared to 7.29 mg/gm in the Mondial cultivar in response to 5 mM SA NPs. In conclusion, different treatments with glycyrrhizic acid ammonium salt (GSA), Salicylic acid (SA), Chitosan (Ch), and Composites in the nano form increased the activities of peroxidase activities, polyphenol oxidase, catalase, and phenol contents in the Mondial cultivar in comparison with Sponta cultivar.

Keywords: Potato, *Pectobacterium spp*, Enzyme activities, Peroxidase, polyphenol oxidase, Catalase, Phenol production, Nanomaterials.

Introduction

The potato (*Solanum tuberosum*) is the fourth most widely grown crop around the globe (Dutt *et al.*, 2017; Rahaman and Shehab, 2019) .It is used to make a variety of food products all over the world, including chips, crisps, potato flour, vegetable salad, canned food, and livestock supply (Manzira, 2010). Unfortunately, a variety of abiotic and biotic stresses, such as soft rot pathogens, affect potato tuber yield and quality (Pectobacterium and Dickeya). Soft rot is common in the field and during storage (Pérombelon, 2002; Czajkowski *et al.*, 2011). The potential of these bacteria to produce vast quantities of extracellular plant cell wall-degrading enzymes (PCWDE), including pectin lyases, proteases, pectinase, and cellulases, caused enormous tissue maceration, rot, and resulting death of the plant (Barras *et al.*, 1994; Toth *et al.*, 2003).

They cause severe tissue damage, pervasiveness, dissemination, and colonisation, as well as the obliteration of plant cell walls. They also break down plant materials into smaller molecules that bacteria can simply take and use for growth and energy. The cumulative operation of pectinases (particularly pectate lyases), which also damage pectin, the plant cell wall's bonding substance, influences the symptoms of soft rot. Numerous physical and chemical methods were used to limit potato soft rot, including certified seed production, robust examinations, seed testing systems, sanitation facilities throughout harvest, tuber sorting and classifying, and mandating farmers to use cultivar resistance seed, however, these processes are complex and expensive, time-consuming, and therefore do not completely remove passageways or alternative routes through which disease becomes developed (Mauch-Mani *et al.*, 2017; Silva *et al.*, 2018).

Bacterial pathogens are also controlled using chemical treatment. Because of their detrimental consequences on humans and the environment, as well as the probability of selecting multidrug-resistant bacterial strains, synthesised bactericides are not the favoured method of managing plant diseases (Abd El-Kahir, 2004; Jess *et al.*, 2014). Plant hormones including salicylic acid (SA), which stimulates natural plant resistance against pathogenic bacteria, could be recognized as a possible option for the use of synthetic bactericides (Koo *et al.*, 2020). They act as chemokines in higher plants, controlling cellular activities (Kazan, 2015; Aymen, 2018).

Salicylic acid is a chemical substance created by plants that contain a hydroxyl or derivative group (Yousif, 2018). Plant phenolic compounds are customised metabolic byproducts that play significant roles in biosynthetic pathways of lignin and allelopathic substances that regulate adaptation mechanisms to living stimuli (Kubalt, 2016), thermoregulation (Klessig *et al.*, 2018), and defensive scheme signalling pathways function (Kubalt, 2016). This also provokes overall innate defence morphological, physiological, and biochemical pathways (Wang and Li, 2006; Vlot *et al.*, 2009). SA regulates ion utilisation and production of reactive oxygen species in plants as well as disease tolerance (Jayakannan *et al.*, 2015). SA has attracted a lot of attention because of its ability to contribute to plant protection response under biotic and abiotic stresses.

Superoxide dismutase (SOD), catalase (CAT), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), and peroxidase (POD) are enzymatic scavengers that are either participating in scavenging ROS under pathogen-loaded circumstances (Rahman et al., 2016). External treatments significantly (SA) induce signalling cascades which can improve plant growth and yield under a variety of environmental

stressful conditions by optimising and scavenging antioxidant activities ROS (Khan *et al.*, 2015). Salicylic acid is among the phenolics constituted by plants with a hydroxyl or derivative group; it functions as a ubiquitous biostimulant that can regulate various metabolic and physiological procedures and plays an essential part in the defensive measure against toxic effects (Cetinkaya and Kulak, 2019). (Zhang *et al.*, 2015). Glycyrrhizic acid (GA) is a vital biologically active material in liquorice derived from Glycyrrhiza glabra rhizomes and roots. Because of its antiviral (Pompei *et al.*, 1979), antimicrobial activity (Wang *et al.*, 2015; Oyama *et al.*, 2016), fungicidal (Alonso 2004), antioxidant (Fu *et al.*, 2013), antitumor (Kim *et al.*, 2013; Amirghofran 2010), antiulcer (Krausse *et al.*, 2004), and anti-inflammatory properties.

Chitosan is a homopolymer of -(1,4)-linked N-acetyl-glucosamine units and an abundant linear biopolymer gained by alkaline deacetylation of chitin (Islam *et al.*, 2017). A few types of research have proven that chitosan's action against microbial pathogens is dependent on the ability to stop microbial activity, negatively impacting sporulation, sporulation, and survivability. Chitosan's activity may be in the form of pathogen cell disruption or as an activator of host plant defence responses by provoking and constraining different biochemical operations during the plant-pathogen interaction (Bhaskar et al., 1999; Bhuvaneshwari *et al.*, 2013; Vickers *et al.*, 2017; Hassan and Chang, 2017). Because of modifications in physicochemical characteristics including size and shape, surface area, cationic nature, effective operational groups, and significantly larger encapsulation competency, chitosan nanoparticles have increased their features and functions in bioactivities (Saharan *et al.*, 2013). Irrespectively of their agricultural production applications, few studies have been conducted on the use of chitosan-NPs in phytopathogens, especially against fungal pathogens.

Throughout regular plant development, phenols are synthesized and compartmentalized in a weaker form in the vacuoles (Beckman, 2000). Enzymes oxidise phenols, causing them to polymerize with other host components such as carbohydrates to form lignin. They play a significant role in developing resistance to the pathogen (Im *et al.*, 2008).

Products of phenol oxidation provide tolerance by imposing restrictions on disease replication, stopping pathogen enzymes from degrading plant cell walls, or acting as precursors in the formation of structural components like lignin (Lyon *et al.*, 1992). Polyphenol oxidase (PPO), peroxidase, and peroxidase are the three main enzymes in the phenylpropanoid pathway (POD). PPO is an essential enzyme in primary infection because it catalysis the oxidation of phenols to quinones (Okey *et al.*, 1997). Peroxidase is required in the final stage of lignin biosynthetic pathways (Zhang *et al.*, 2008). Its catalysis is the oxidative polymerization of hydroxycinnamic alcohols to produce lignin and the cross-linking of isoditryosine structures in the cell wall (Ngadze *et al.*, 2012).

Nanotechnology has tremendous potential for enhancing crop productivity (Narrod and Abbott, 2011), plant protection (Pérez-de-Luque and Hermosn, 2013), and plant disease monitoring/detection (Frewer *et al.*, 2011). The high surface-to-volume ratio of nanoparticles (NPs) increases their excitability and prospective physiological activity (Dubchak *et al.*, 2010). In addition, nano-based materials are being used to improve the efficacy of fungicides and pesticides, enabling the use of low doses.

Our research study was aiming to analyse peroxidase and polyphenol oxidase, catalase, and phenol production in the infected potato Sponta and Mondial cultivars with *Pectobacterium spp* in response to 5 mM of glycyrrhizic acid ammonium salt, salicylic acid, and composites in the normal and nanoform treatments.

Material and Methods

Host plants

Potato seeds were germinated at Benha University's Faculty of Agriculture between November 2020 and October 2021. The Ministry of Agriculture provided certificated Sponta and Mondial seed. Sponta and Mondial were the cultivars used. As a base fertiliser, forty grammes of (7:28:7 NPK) were applied. Watering was applied as needed to keep the soils at field capacity. The experiment used a Randomized Complete Block design with a 5*6 treatment formation that was replicated three times.

Bacterial culture

Bacterial suspensions of *Pectobacterium spp.* in sterile distilled water.

Chemical Synthesis of Nanomaterials

Glycyrrhizic acid ammonium salt (CAS number: 53956-04-0) and salicylic acid (CAS number: 20283-92-5) were provided by Sigma-Aldrich (St. Louis, MO, USA). 10 mg from every material was prepared by dissolving 10 mg of absolute ethanol and sonicated for an hour at room temperature (20-25 C) at a frequency of 50 kHz (XUBA3Analogue Ul-ta-sonic Bath, Grant Company, Saint Joseph, MO, USA) (Ahmed *et al.*, 2022).

Chitosan nanoparticles have been created by gelating ionotropic chitosan with TPP anions. Chitosan (0.2%) was combined with Ascorbic acid (1%) and agitated at room temperature for 1 hour (1000 rpm). To make a TPP stock solution, dissolve 0.03 gm of TPP in 11 ml of water. When one ml of TPP stock solution was added dropwise to chitosan solution while stirring (1000 rpm, 1 hour) at ambient temperature, chitosan nanoparticles were mainly constituted. After that, the Chitosan nanoparticles were sonicated for an hour to ensure their small size.

Transmission Electron microscopy (TEM)

The form of Glycyrrhizic acid ammonium salt, Salicylic acid, and Chitosan nanoparticles was defined using electron microscopy. A drop of nanoparticle solution was sonicated before it was applied to carbon-coated copper grids (CCG), which were then completely dehydrated at room temperature. Electron micrographs were taken at Al-Azhar University's Regional Center for Mycology and Biotechnology (RCMB) using a JEOL GEM-1010 transmission electron microscope set to 70 kV. (2019, Amin and El-Sharkawy)

Treatments

The experiment utilised plants that were six weeks old. The plants were injected by inoculating 10 µl of the inoculum through into stems, about 5 cm from the ground. To avoid disease from other airborne contaminants, the wound was then covered with petroleum jelly. The injury was created by slashing off the plant's apical bud. The plants that were tested at 0 h received no treatment. On each occasion, leaves were collected through deleterious sample selection. Four leaves from the central core of the plant have been excised, with the mid-ribs eliminated, at 0, 12, 24, 48, 84, and 1 week after treatment, and samples have been stored in a freezer for later use in the enzyme assays. In each treatment group, five plants were used. A UV-VIS spectrophotometer (Model ST-UV 752N) was used for spectrophotometric analysis.

Determination of enzymes activity activities:

Polyphenol oxidase (PPO)

The tests were performed exactly as described by Zhang et al (2008). Liquid nitrogen was used to grind the leaves or tubers. In a mortar and pestle, one gramme of the leaves was homogenised and mixed with 5 ml of 0.05 M phosphate buffer solution (pH 5.8) containing 5% (w/v) polyvinylpyrrolidone (PVP). The tissues were homogenized and then filtered through a cheesecloth, and the extract was filtered and then centrifuged at room temperature for 5 minutes at 10 000 r/min. 3.9 ml of 0.05 M phosphate buffer solution (pH 5.5), 1.0 ml of 0.1 M catechol, and 0.5 ml enzymatic extract comprised the final reaction mixture. As a control, the same reaction mixture without the enzymatic extract was used. At room temperature, the reaction was spectrophotometrically monitored for 2 minutes at 20-second intervals. At 525 nm, absorbance was measured, and the results were expressed as a change in absorbance Δ 525 min/g of fresh mass.

Peroxidase (POD)

Liquid nitrogen was used to grind the leaf or tuber sample. In a mortar and pestle, one gramme of leaves was homogenised in 0.05 M phosphate buffer solution (pH 5.8) containing 5% (w/v) PVP. The homogenate was then filtered through muslin cloth, and the filtrates were centrifuged at room temperature for 5 minutes at 10 000 r/min. 2.9 ml 0.05 M phosphate buffer solution (pH 5.5), 1.0 ml 0.05 M guaicol, 1.0 ml 2 percent hydrogen peroxide (v/v), and 0.1 ml enzymatic extract were added to the reaction mixture.

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As a control, the same reaction mixture without the enzymatic extract was used. At room temperature, guaiacol oxidation was monitored spectrophotometrically for 2 minutes at 20-second intervals. Absorbance was read at 470 nm and results were expressed as Δ470 min/g fresh mass.

Catalase (CAT) activity

Catalase activity was assayed using a hydrogen peroxide test based on the formation of a stable complex with ammonium molybdate. 0.2 ml of plant extract was incubated for 4 minutes at 25°C in 1 ml of a reaction mixture containing 65mM hydrogen peroxide in 60mM sodium-potassium phosphate buffer, pH 7.4. The enzymatic process was stopped with 1 ml of 32.4mM ammonium molybdate, and the concentration of the yellow molybdate and hydrogen peroxide complex was detected at 405 nm (Luhová *et al.*, 2003).

Determination of total phenols content:

In a pestle and mortar, half a gramme of fresh plant tissue was mixed with 10 mL of 80% ethanol, then filtered and centrifuged at 10,000 rpm for 20 minutes. The supernatant was dried completely by evaporating it. By dissolving the residue in 5 mL of 80% ethanol, the extract was created. Ten drops of strong hydrochloric acid were added to 0.2 ml of the prepared sample in a test tube before it was rapidly heated to boiling point over a free flame with room for condensation. After that, the tubes were immersed in a 100°C water bath for 10 minutes. After chilling, 1 mL of the reagent and 2.5 mL of 20% Na_2CO_3 were added to each tube. With distilled water, the liquid was diluted to 50 mL and then tested. (BRAY & THORPE, 1954).

Statistical analysis

The experiment was repeated twice in a completely randomised design with a 5*6 factorial treatment structure replicated twice. ANOVA was performed on data obtained for the enzyme activity of different cultivars at different time intervals using Genstat 14th Edition. Where $P< 0.05$, all significant means were separated using Fischer's protected Least Significant Difference.

Results

Transmission Electron microscopy (TEM)

TEM characterization of salicylic acid nanoparticles applied in this study ranging from 5.48-20 nm. The results showed that the biggest size for SA NPs was 48.3 nm, and the smallest size of SA NPs was 17.3 nm. Also, Glycyrrhizic acid ammonium salt nanoparticles (GAS NPS) showed that the range sizes between 20-48 nm), and chitosan nanoparticles ranged (from 78.1 -176 nm).

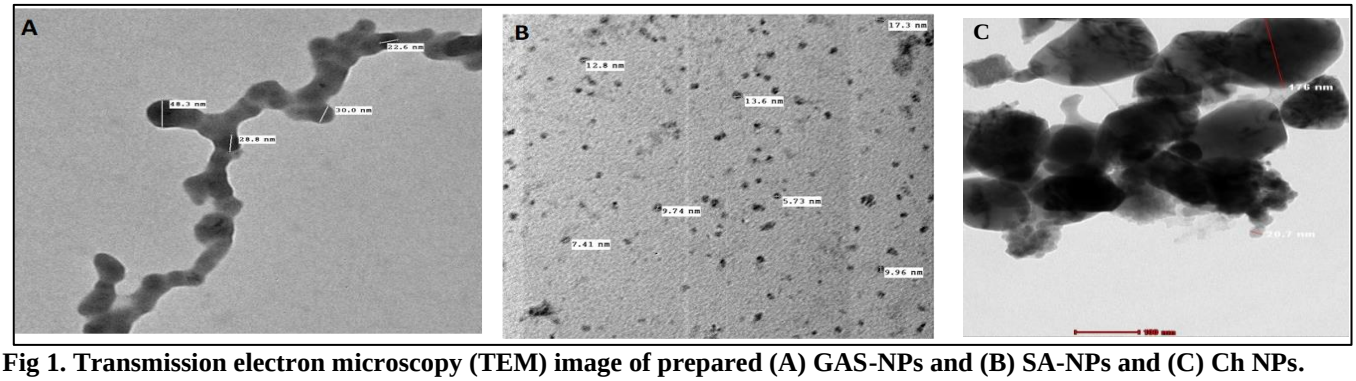

Fig 1. Transmission electron microscopy (TEM) image of prepared (A) GAS-NPs and (B) SA-NPs and (C) Ch NPs.

Peroxidase activities in Potato Sponta and Mondial cultivars in response to different treatments

Figure (2) showed that peroxidase activities in potato leaves increased to the highest level of 3.9 $U.ml^{-1}$ in Mondial cultivar in response to 5 mM SA NPs compared to 0.73 $U.ml^{-1}$ in the positive control and 0.47 $U.ml^{-1}$ in the negative control. While Sponta cultivar scored 3.53 $U.ml^{-1}$ of peroxidase activities in response to 5 mM SA NPs in comparison with 0.57 $U.ml^{-1}$ and 0.33 $U.ml^{-1}$ in the positive and negative control

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respectively. Potato leaves in Sponta cultivar scored 3.87 U.ml^{-1} of peroxidase activities compared to 3.57 U.ml^{-1} in response to 5 mM of nanocomposites (Ch NPs+ SA NPs + GSA NPs). However, treated potato leaves with 5 mM GSA NPs scored 3.7 U.ml^{-1} in the Mondial cultivar and 3.53 U.ml^{-1} in the Sponta cultivar of peroxidase activities respectively. Potato leaves of Sponta cultivar scored 2.5, 2.7, 1.9, 1.7, 1.93, 1.77 U.ml^{-1} in response to 5 mM of GSA, SA, Ch, Ch+GSA, Ch+SA and Ch+GSA+SA respectively. While treated Mondial leaves with 5 mM of GSA, SA, Ch, Ch+GSA, Ch+SA and Ch+GSA+SA scored 2.9, 3.03, 2.2, 2 and 2,57 U.ml^{-1} of peroxidase activities respectively in comparison with 0.73 U.ml^{-1} in the positive control and 0.47 U.ml^{-1} in the negative control (Figure 2).

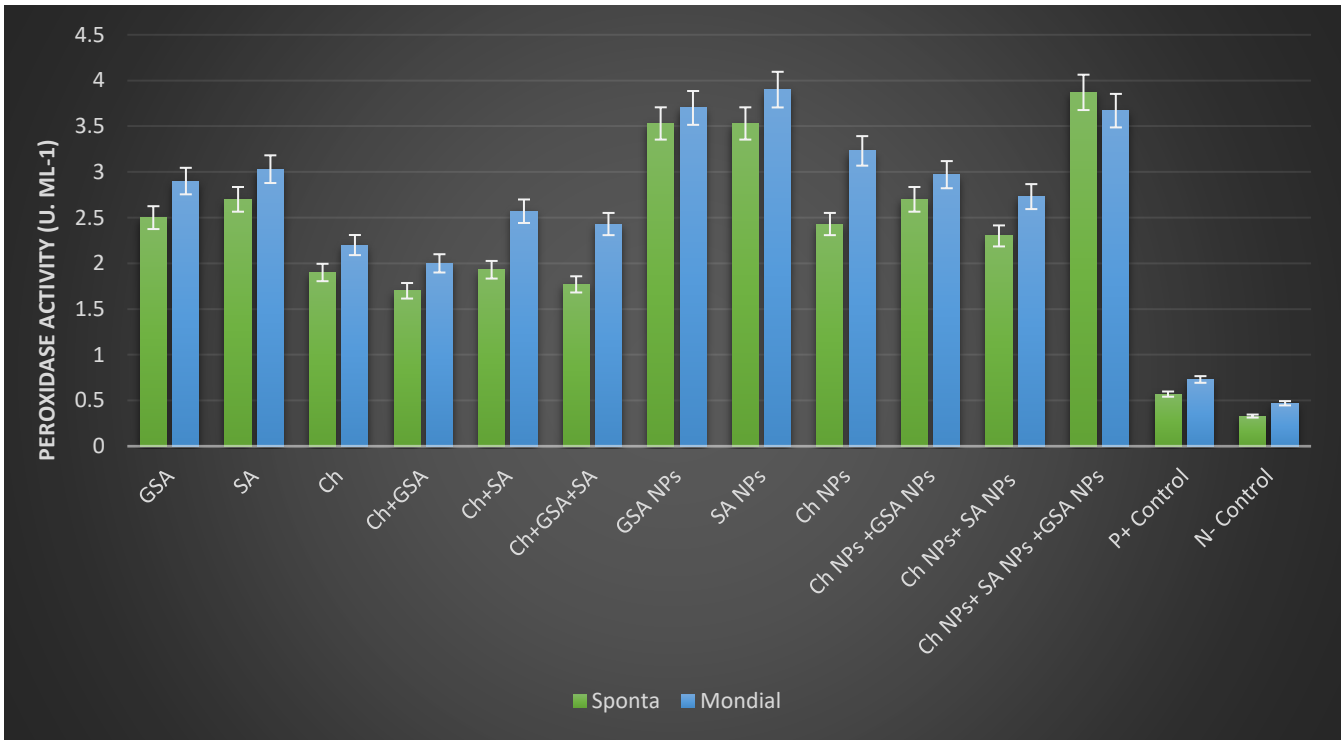

Figure 2. Effect of 5 mM glycyrrhizic acid ammonium salt (GSA), Salicylic acid (SA), Chitosan (Ch), and Composites in the normal and nanoform on peroxidase activities of infected potato cultivars (Sponta and Mondial) leaves with *Pectobacterium spp* in comparison with positive and negative control. Data are presented as mean ± SE.

Figure (3) presented that peroxidase activities in potato tubers boosted to the highest level of 3.9 U.ml^{-1} in Mondial cultivar in response to 5 mM SA NPs and 5mM nanocomposites (Ch NPs+ SA NPs + GSA NPs) compared to 0.6 U.ml^{-1} in the positive control and 0.5 U.ml^{-1} in the negative control. Whereas Sponta cultivar scored 3.53 U.ml^{-1}, 3.6 U.ml^{-1} and 3.47 U.ml^{-1} of peroxidase activities in response to 5mM nanocomposites (Ch NPs+ SA NPs + GSA NPs), 5 mM SA NPs and 5mM GSA NPs respectively in comparison with 0.5 U.ml^{-1} in the positive control and 0.3 U.ml^{-1} in the negative control. Potato tubers in the Mondial cultivar scored 3.07 U.ml^{-1}, 3.03 U.ml^{-1} and 2.83 U.ml^{-1} of peroxidase activities in response to SA, Ch NPs and GSA treatments respectively. Generally, peroxidase activities profile in Mondial cultivar were higher than in Sponta cultivar in all treatments (Figure 3).

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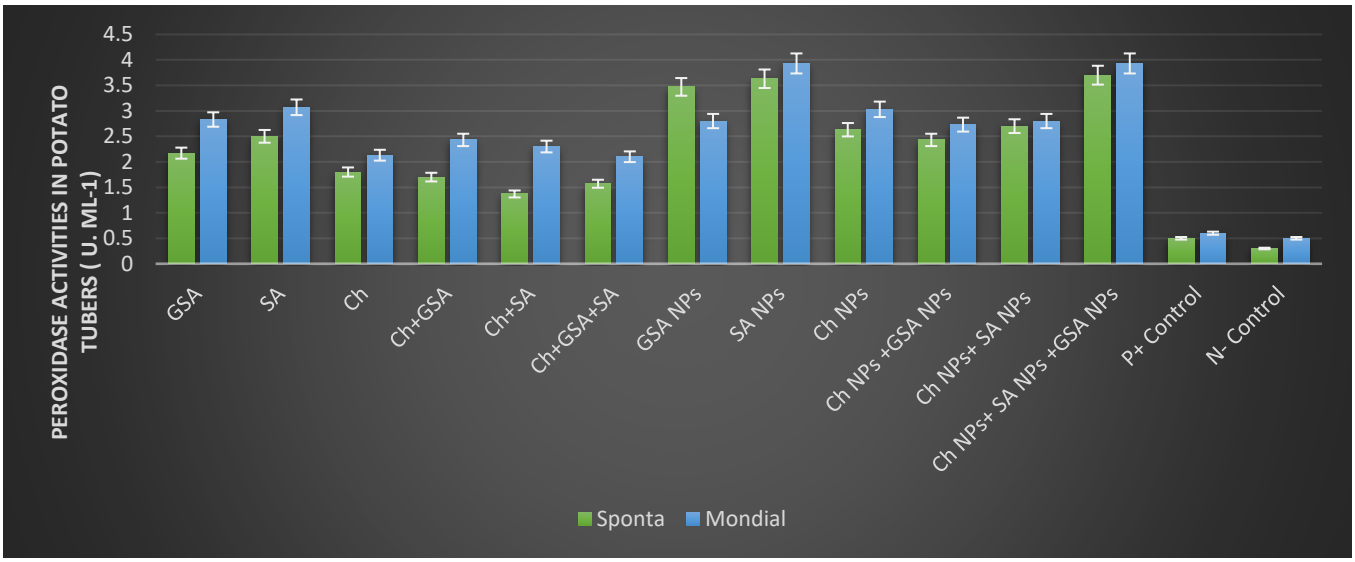

Figure 3. Effect of 5 mM glycyrrhizic acid ammonium salt (GSA), Salicylic acid (SA), Chitosan (Ch), and Composites in the normal and nanoform on peroxidase activities of infected potato cultivars (Sponta and Mondial) tubers with *Pectobacterium spp* in comparison with positive and negative control. Data are presented as mean ± SE.

Polyphenol oxidase activities in Potato Sponta and Mondial cultivars in response to different treatments:

Treated Mondial cultivar showed the highest polyphenol oxidase activities in the potato leaves at 0.95 U.ml^{-1} in response to 5 mM SA NPs followed by 0.923 U.ml^{-1} and 0.813 in response to 5 mM nanocomposites (Ch NPs+ SA NPs + GSA NPs) and 5 mM GSA NPs treatments respectively, compared to 0.15 U.ml^{-1} in the positive control and 0.343 U.ml^{-1} in the negative control. However, Sponta cultivar showed polyphenol oxidase activities in potato leaves 0.937 U.ml^{-1}, 0.86 U.ml^{-1}, 0.757 U.ml^{-1} and 0.72 U.ml^{-1} in response to 5 mM SA NPs, 5 mM nanocomposites (Ch NPs+ SA NPs + GSA NPs), SA and GSA NPs treatments respectively in comparison with 0.137 U.ml^{-1} in the positive control and 0.333 U.ml^{-1} in the negative control (Figure 4).

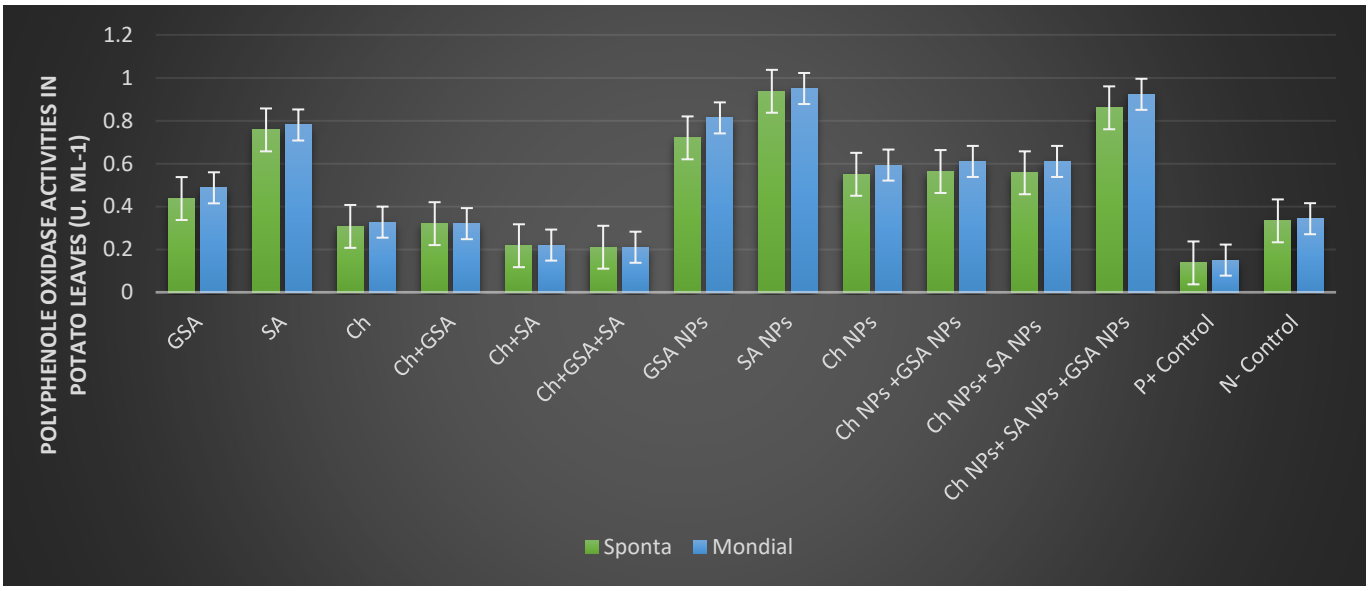

Figure 4. Effect of 5 mM glycyrrhizic acid ammonium salt (GSA), Salicylic acid (SA), Chitosan (Ch), and Composites in the normal and nanoform on Polyphenol oxidase activities of infected potato cultivars (Sponta and Mondial) leaves with *Pectobacterium spp* in comparison with positive and negative control. Data are presented as mean ± SE.

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Figure 5 showed that treated Mondial and Sponta cultivars with 5 mM SA NPs scored the highest polyphenol oxidase activities in potato tubers 0.777 U.ml^{-1} and 0.72 U.ml^{-1} respectively compared to all other treatments. Mondial cultivar scored 0.713 U.ml^{-1}, 0.677 U.ml^{-1} and 0.643 U.ml^{-1} of polyphenol oxidase activities in response to 5 mM GSA NPs, 5 mM nanocomposites (Ch NPs+ SA NPs + GSA NPs) and SA respectively. While Sponeta cultivar scored 0.647 U.ml^{-1}, 0.65 U.ml^{-1} and 0.6 U.ml^{-1} of polyphenol oxidase activities in response to 5 mM GSA NPs, 5 mM nanocomposites (Ch NPs+ SA NPs + GSA NPs) and SA respectively. Commonly, treated potato cultivars with different nanomaterials showed an increase in the polyphenol oxidase activities in the tubers profile compared to the normal materials (Figure 5).

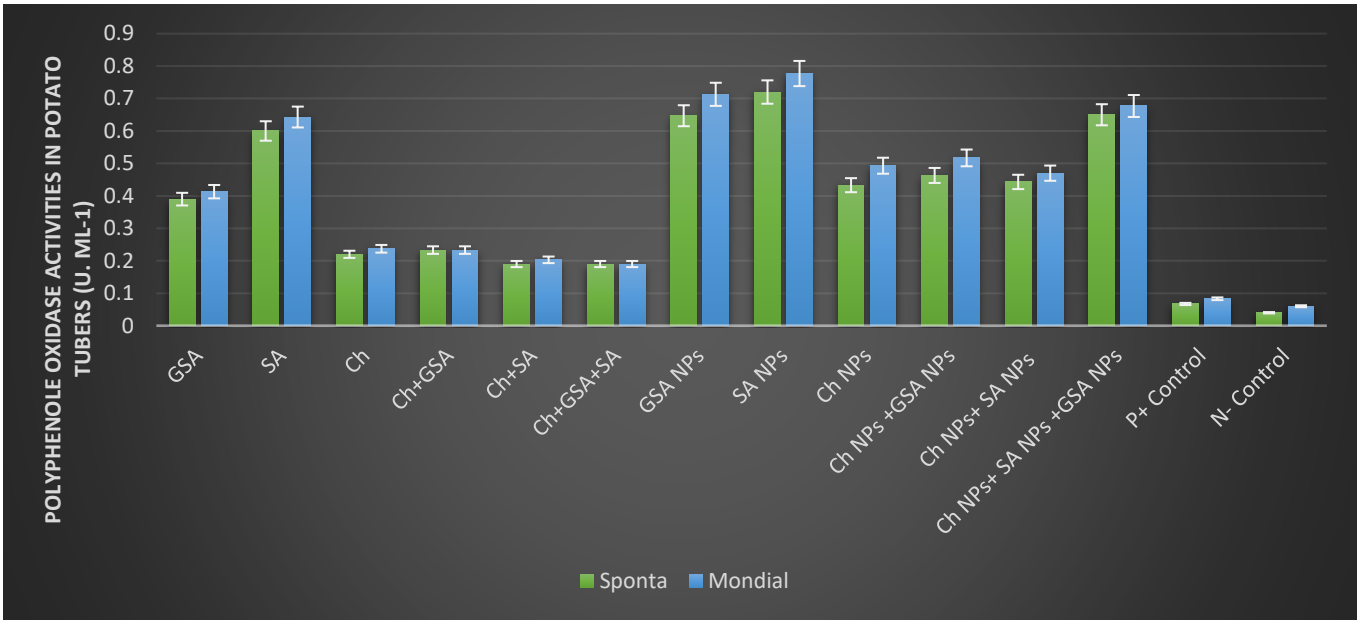

Figure 5. Effect of 5 mM glycyrrhizic acid ammonium salt (GSA), Salicylic acid (SA), Chitosan (Ch), and Composites in the normal and nanoform on Polyphenol oxidase activities of infected potato cultivars (Sponta and Mondial)tubers with *Pectobacterium spp* in comparison with positive and negative control. Data are presented as mean ± SE.

Data presented in figure (6) showed that catalase activities in Mondial cultivar boosted to the highest level of 2.173 U.ml^{-1} in response to SA NPS treatment. Additionally, catalase activities in potato leaves (Mondial cultivar) increased in response to all treatments and scored 1.976 U.ml^{-1}, 1.976 U.ml^{-1}, 1.867 U.ml^{-1}, 1.733 U.ml^{-1}, 1.633 U.ml^{-1} and 1.6 U.ml^{-1} in response to 5 mM GSA NPs, 5 mM nanocomposites (Ch NPs+ SA NPs+ GSA NPs), SA, CH NPs + SA NPs and Ch NPs respectively. Interestingly, potato leaves in the Mondial cultivar showed the same catalase activities of 1.976 U.ml^{-1} in response to 5 mM GSA NPs and 5 mM nanocomposites (Ch NNPs+ SA NPs+ GSA NPs). Remarkably, catalase activities in the potato leaves of the Mondial cultivar increase in response to all treatments except positive and negative control. However, catalase activities in the potato leaves of Sponta cultivar increased to 2.1 U.ml^{-1},1.4 U.ml^{-1},1.267 U.ml^{-1}, in response to 5 mM SA NPs, GSA NPs and 5 mM nanocomposites (Ch NNPs+ SA NPs+ GSA NPs) respectively (Figure 6).

Data presented in figure (7) showed that significant increase in the activities of catalase enzyme in potato tubers of Sponta cultivar 4.267 U.ml^{-1} in response to GSA compared to 1.267 U.ml^{-1} in response to GSA NPs. Catalase activities profile of potato tubers in the Mondial cultivar increased in response to all treatments in comparison with the Sponta cultivar except for GSA treatment (Figure 6).

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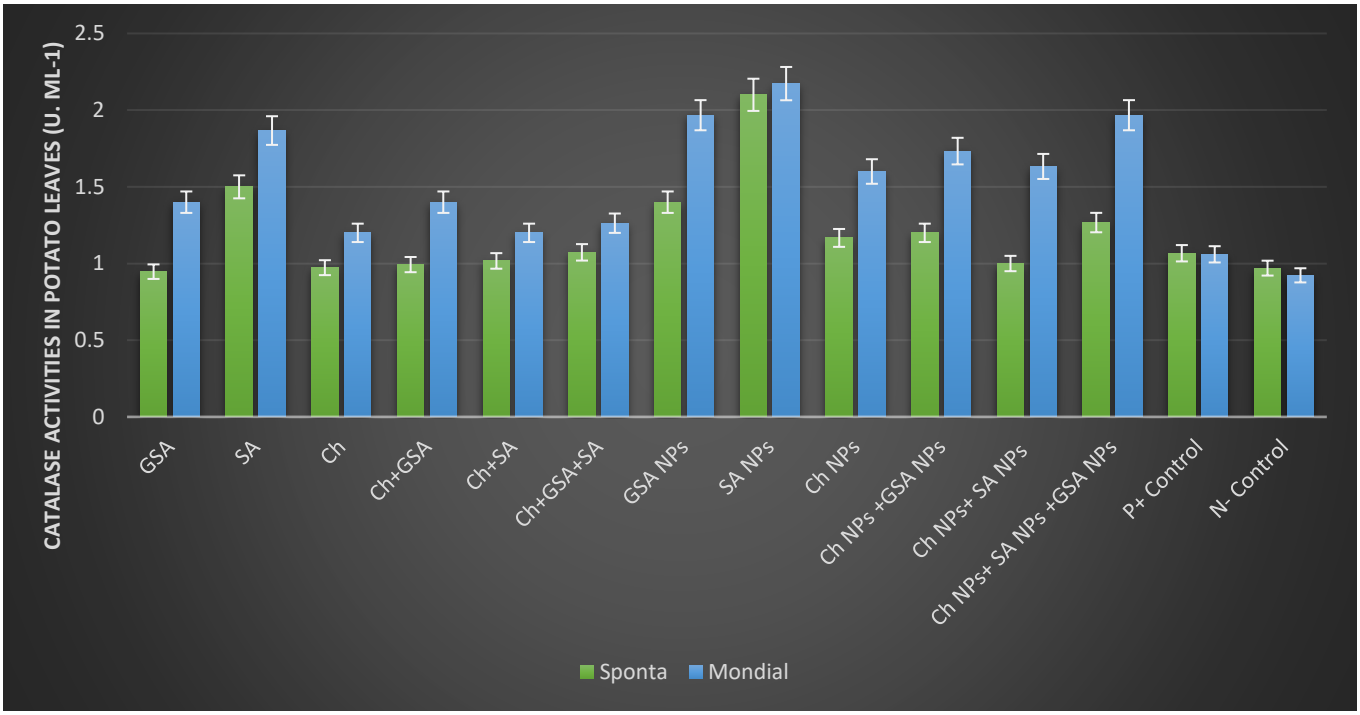

Figure 6. Effect of 5 mM glycyrrhizic acid ammonium salt (GSA), Salicylic acid (SA), Chitosan (Ch), and Composites in the normal and nanoform on Catalase activities of infected potato cultivars (Sponta and Mondial) leaves with *Pectobacterium spp* in comparison with positive and negative control. Data are presented as mean ± SE.

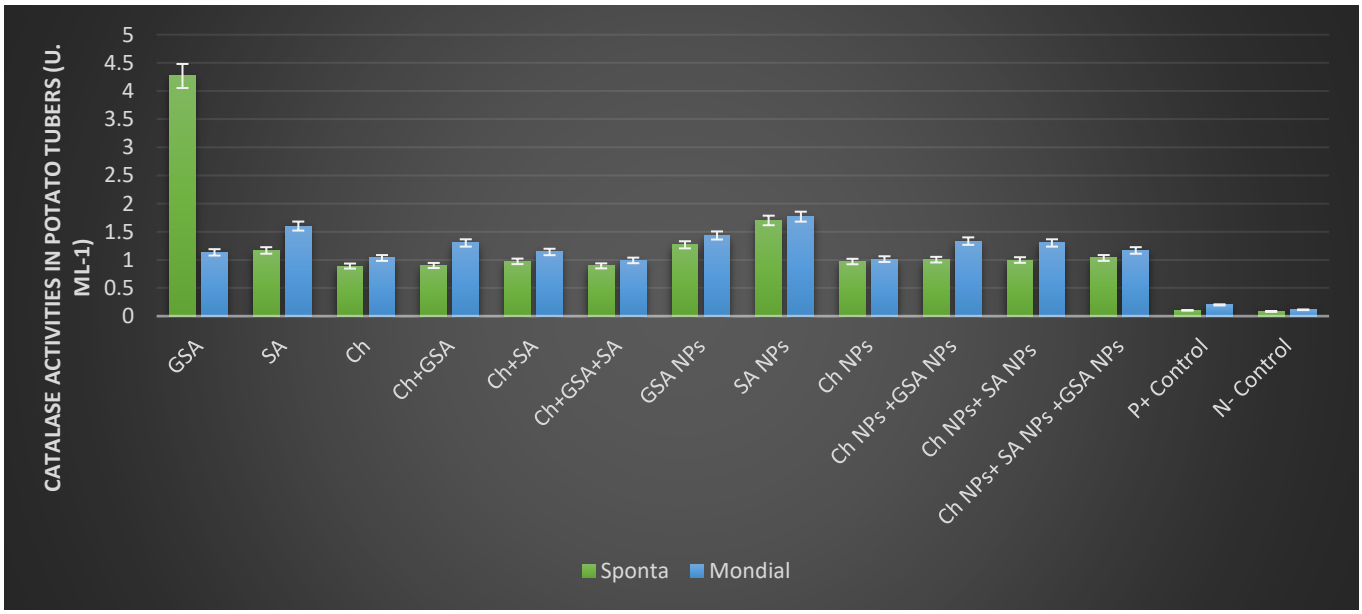

Figure 7. Effect of 5 mM glycyrrhizic acid ammonium salt (GSA), Salicylic acid (SA), Chitosan (Ch), and Composites in the normal and nanoform on Catalase activities of infected potato cultivars (Sponta and Mondial) tubers with *Pectobacterium spp* in comparison with positive and negative control. Data are presented as mean ± SE.

Figure (7) presented that phenol contents in the potato leaves of the Mondial cultivar boosted to 11.19 mg/gm, 10.98 mg/gm and 10.52 mg/gm in response to 5 mM SA NPs, 5 mM GSA NPs and 5 mM Ch NPs respectively. While potato leaves of the Sponta cultivar scored 10.48 mg/gm, 10.43 mg/gm and 9.71 mg/gm in response to 5 mM SA NPs, 5 mM GSA NPs and 5 mM Ch NPs respectively. Interestingly, potato leaves

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of both cultivars produced the same amount of phenol contents 9.07 mg/gm in response to SA, while the phenol contents increased to 9.04 mg/gm compared to 7.83 mg/gm in response to 5 mM GSA. Also, Phenol contents in potato leaves of the Mondial cultivar increased to 9.18 mg/gm compared to 8.79 mg/gm in sponta cultivar in response to 5 mM nanocomposites (Ch NPs+ SA NPs+ GSA NPs). Generally, phenol contents in the potato leaves of the Mondial cultivar increased in response to all treatments (Figure 7).

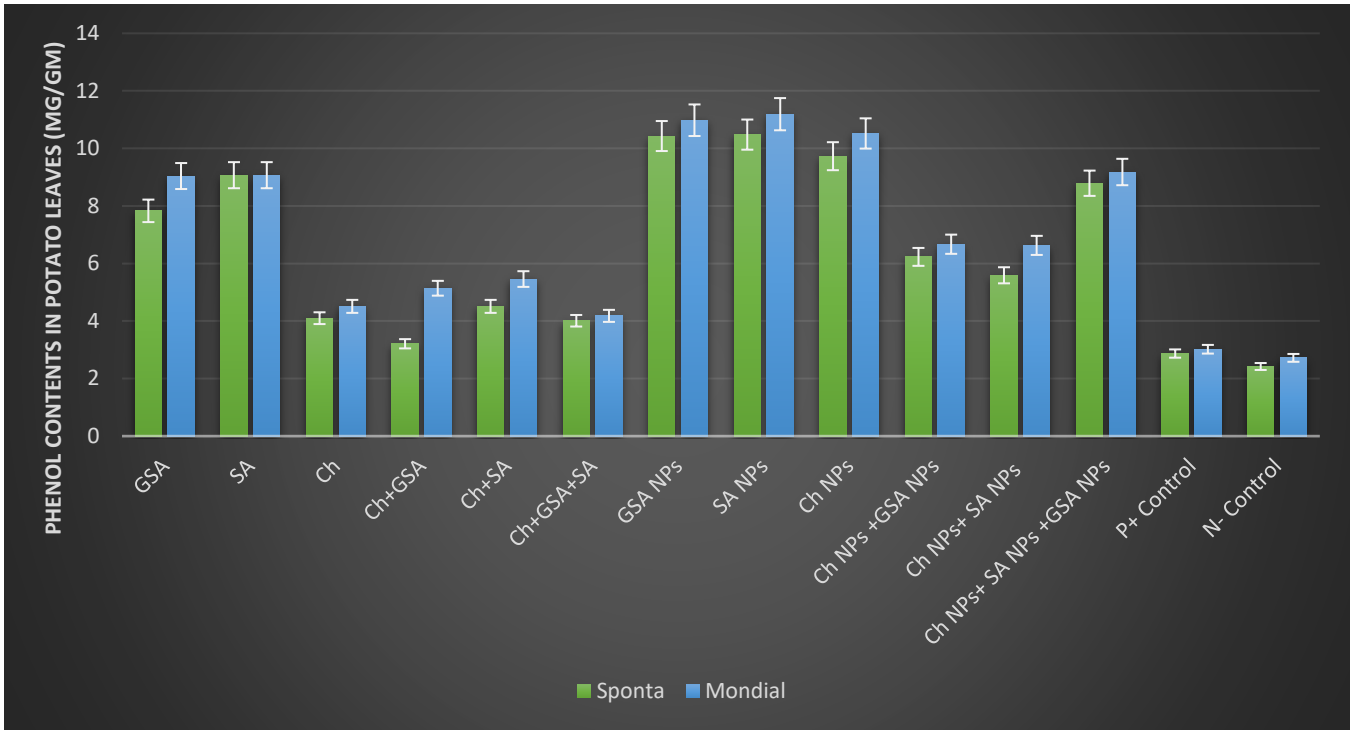

Figure 7. Effect of 5 mM glycyrrhizic acid ammonium salt (GSA), Salicylic acid (SA), Chitosan (Ch), and Composites in the normal and nanoform on Phenol contents of infected potato cultivars (Sponta and Mondial) leaves with *Pectobacterium spp* in comparison with positive and negative control. Data are presented as mean ± SE.

Data presented in figure (9) showed that the phenol contents in the potato tubers of both cultivars produced the same amount of 7.4 mg/gm in response to 5 mM SA treatment. While phenol contents in the potato tuber of the Sponta cultivar scored 7.38 mg/gm compared to 7.29 mg/gm in the Mondial cultivar in response to 5 mM SA NPs. Interestingly, phenol contents of potato tubers increased to 7.18 mg/gm and 6.64 mg/gm in the Monial and sponta cultivars respectively, in response to 5 mM nanocomposites (Ch NPs+ SA NPs+ GSA NPs). While phenol contents in the potato tubers scored 3.45 mg/gm and 3.01 in the Mondial and Sponta cultivars respectively in response to 5 mM nanocomposites (Ch+ SA+ GSA) (Figure 9)

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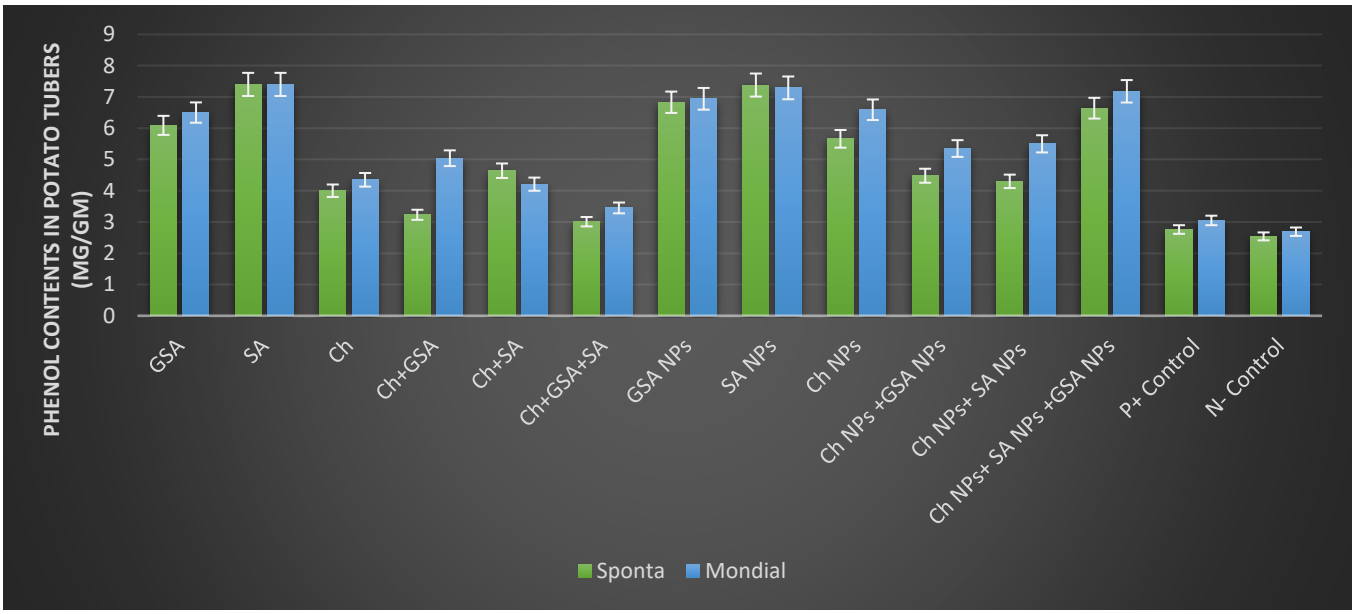

Figure 9. Effect of 5 mM glycyrrhizic acid ammonium salt (GSA), Salicylic acid (SA), Chitosan (Ch), and Composites in the normal and nanoform on Phenol contents of infected potato cultivars (Sponta and Mondial) tubers with *Pectobacterium spp* in comparison with positive and negative control. Data are presented as mean ± SE.

Discussion

Treatment with glycyrrhizic acid ammonium salt (GSA), salicylic acid (SA), chitosan (Ch), and nanocomposite enhanced peroxidase activities, polyphenol oxidase, catalase, and phenol contents in the Mondial cultivar compared to the Sponta cultivar in our study. It has been shown that SA plays a major role in the initiation of plant systemic acquired resistance (SAR) (Hayat *et al.*, 2010). Other previous studies showed that enzymatic and non-enzymatic antioxidants have risen throughout SA treatments on crop plants, which supports our findings (Sofy, 2015; Chen *et al.*, 2016; Razmi *et al.*, 2017; Moharramnejad *et al.*, 2019).

The correlating pathway was that SA might freely move among tissues and cells as a long-distance mediating role (Kumar, 2014), stimulating the operations of one or more antioxidative enzymes and thus increasing the tolerance of plants to oxidative stress caused by various abiotic and biotic stresses (Idrees *et al.*, 2011; Ibrahim *et al.*, 2017).

External SA utilisation regulates the expression of defence-related genes, which were systematically stimulated after infection with tobacco mosaic virus (TMV) (Nandi and Babu, 2013). SA is an intrinsic signal that activates definite plant systemic resistance, such as pathogenesis-related (PR) gene expression and stress resistance formation (Klessig *et al.*, 2018). A significant rise in gene expression following SA application under infection stress aided in the reversal of pathogen-induced ROS on growth and photosynthesis. Morris et al. (2000) revealed that the application of salicylic acid enhances gene expression all through leaf senescence, which is consistent with the finding.

Sprinkling nanoparticles on infected plants may promote kinase 3 genes leading to the activation of many signalling channels, including the oxidative inducible gene (oxi1 gene), ROS, and calcium signalling pathways cascades (Shoala *et al.*, 2021). In reaction to both biotic and abiotic stress factors, reactive oxygen species (ROS) are generated automatically within a few seconds; they may also act as protectants against both biotic and abiotic stresses (Rentel *et al.*, 2004). ROS are important in the activation of Mitogen-activated protein kinases (MAPKs), which are well-studied signalling families in higher plants.

Moreover, MAPKs control a wide variety of important cellular activities, including cellular division, stress responses, metabolic activity, and a variety of developmental changes (Shoala *et al.*, 2021). Oxidative

signal Inducible gene 1 (Oxi1) is a serine/threonine kinase required for plant tolerance to external cues and oxidative burst-mediated signalling in plant roots. Induction of Oxidative signal Inducible gene may augment resistance mechanisms and quickness the process of recovery, but overexpression may induce apoptosis as a rapid response to external stress (Shoala *et al.*, 2021).

Nanotechnology has the potential to elevate management techniques against various phytopathogens to new heights. Natural nanomaterials, such as (GAS-NPs), that can be prepared and extracted from plants, play an important role in increasing plant resistance to various stimuli while minimising environmental impact (Shoala 2018). The preparation of nanomaterials saves time and money. Nanomaterials, depending on the prepared material, could be stored at room temperature for a few months due to their stability. Nanotechnology may play a dual role in phytopathogen resistance in plants by interacting with phytopathogens and enhancing growth pathways, both of which have a positive impact on plant resistance.

The study's thorough biochemical and enzyme assays enable us to determine the pathophysiology by which SA NPs, GAS NPs, Ch NPs, and nanocomposites boost phenol production, peroxidase, polyphenol oxidase, and catalase activities in potato tuber and leaves. The SA route is part of the pathway. The mechanism includes (i) reduction in exoenzyme production, (ii) decrease in microbial fission, growth, and movement, and (iii) boost in antioxidant defence system and plant growth appearance.

According to the findings, foliar implementation of SA NPs, GAS NPs, Ch NPs, and nanocomposites (5 mM) to potato plants elevated activities of antioxidant enzymes and phenol production, thus also boosting the plant's defence stress. Based on our findings, soft rot disease in potato tubers can be managed using 5mM (SA NPs, GAS NPs, Ch NPs, and nanocomposites) before storage or planting. Extensive field trials are required to further optimise the use of SA, GAS, Ch, and composites in their normal and nano forms for soft rot control.

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