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Evaluation of Chitosan Nanoparticles and Harpin Loaded Chitosan Nanoparticles on Elicitation of Defense Markers in Gerbera

Induced Defense Markers in Gerbera using Nanoparticles

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Abstract:- ***Gerbera jamesonii*** **Bolus ex Hooker F., an important ornamental plant which has global demand is facing serious consequences due to protected cultivation as repeated fertigation combined with biotic stress is greatly impacting its productivity. However, little to no work was initiated so far to overcome salt sensitive issues and developing biotic resistance in Gerbera cultivars. In the current study, we have developed chitosan nanoparticles and harpin loaded chitosan nanoparticles to improve the tolerance levels of *Gerbera* (yellow cultivar) towards various biotic stress conditions. With the topical application of CSNPs and CSHNPs on cut-flowers of Gerbera, a significant increase in elicitation of defense markers such as polyphenol oxidase (PPO), peroxidase (POD), and phenolic compounds was observed. With the increased levels of defense markers, Gerbera (yellow cultivar) cut-flowers have shown increased shelf life. The outcome of this study has not only benefits Gerbera, but can also be extended to several other ornamental plants which are facing fertigation issues and biotic stress due to closed environment in polyhouse grown plants.**

Keywords**:-** *Chitosan, Chitosan Nanoparticles, Harpin, Harpin Loaded Chitosan Nanoparticles, Gerbera, Defense Markers.*

I. INTRODUCTION

Plants cells respond to various biotic chemical signals in their environment including non-self factors such as cell wall fragments on the surface of a pathogen, self determinants such as cell wall fragments that are released by a plant in response to an invading pathogen or compounds that are secreted by plant pathogens [1]. Pathogens may produce toxins in various forms that promote disease development and many of which kill plant cells [2]. Alternatively, pathogens may produce compounds such as proteins, small peptides, glycoproteins/peptides, or oligosaccharides that activate mechanisms important in plant defense and are collectively known as elicitors [3]. Although the exact mechanisms of action of chitosan in reducing plant disease are currently not fully understood, there is growing evidence showing that chitosan regulates the immune system of the plant and induces the gene expression of pathogen related proteins, thereby improving resistance towards pathogenic attack [4]. Chitosan is known to induce hypersensitive reactions locally and systemically that involve signaling cascades, and the activation and accumulation of defenses-related antimicrobial compounds and proteins [5].

Chitosan nanoparticles (CSNPs) have proved to be potential delivery systems for introducing elicitor molecules onto plant for developing biotic and abiotic stress tolerance [6]. Till date, several methods of nanoparticle preparation are available but the polysaccharide based nanoparticle preparation is one of the best method employed so far to get uniform and well dispersed nanoparticles [4], The Covalent crosslinking, ionic crosslinking and polyelectrolyte complexation are the common methods by which nanoparticle preparations are prepared so far. Compared with covalent cross-linking, ionic cross-linking has more advantages which include mild preparation conditions and simple procedures. For charged polysaccharides like polyanion chitosan, low molecular weight polycations could act as ionic crosslinkers. To date, the most widely used polyanion crosslinker is tripolyphosphate (TPP) which is non-toxic and has multivalent anions. It can form a gel by ionic interaction between positively charged amino groups of chitosan and negatively charged counterions of TPP [7]. Taking the advantage of ionic-cross linking procedure, we have already developed harpin loaded chitosan nanoparticles (CSHNPs) for improving biotic stress resistance in plants [8]. However, till date no studies were reported on efficacy of CSNPs or CSHNPs in ornamental plants which face serious issue with pathogens such as microorganisms and insect pests. One of the important ornamental plant which is commercially grown in green houses across the globe is *Gerbera jamesonii* commonly known as Transvaal Daisy, Barberton Daisy or African daisy [9,10]. Our preliminary studies have shown that, few of the cultivars are highly susceptible (yellow flowered cultivar of Gerbera) to thrips (western flower thrip, WFT) whereas the white flowered cutivars are resistant and pink flowered cultivars are moderately resistant to WFT (Ebenezer et al., unpublished data). In the current study, we have focused on evaluation of CSNPs and CSHNPs on elicitation of defense markers, including polyphenol oxidase (PPO), peroxidase (POD), and phenolic compounds in yellow colored gerbera cut-flowers.

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II. MATERIALS AND METHODS

The CSNPs and CSHNPs used in the current experiment are prepared as per Kongala et al., 2021 [6].

Effect of CSNPs & CSHNP on Post Harvest Gerbera Cut-Flowers: Freshly harvested Gerbera Cut-Flowers of same age (raised in polyhouse of Department of Biotechnology, Telangana University) with no surface injuries or infections were used in the current experiments.

Chemicals: Chitosan , TPP, Harpin stock, Deionised water, Catechol, Guaiacol , H_2O_2 , HCl , Methanol, Sodium Phosphate buffer, Polyvinyl Poly Pyrrolidone stocks were prepared as per Kongala et al 2021 [6].

Test Regimen: Chitosan solution (2.1 mg/ml), Harpin (3.2 mg/ml), TPP (0.7 mg/ml), CSNP, CSHNP, Deionised water and unwounded cut-flower condition taken as test regimen.

Testing on Gerbera flowers: Gerbera cut-flowers were injured (3 mm by 3 mm wound) by sterile scalpel blade. 15 µl of each seven regimens was injected in the wound space and the cut-flowers were stored at 25^0C. Tissue samples were taken from the surrounding area of the wound starting from day "0" (6 hours of post wounding followed by sample treatment) and subsequently followed by day 1, day 2 and day 3. Enzymatic (POD and PPO) and total phenolic content assays were performed as described below.

PPO And POD Activity Assay Extract Prepration: PPO and POD were extracted as per the method previously developed by Mayer *et al.*, 1965 [11] and Hammerschmidt et al., 1982 [12] with little modifications. Isolated tissue (2 gm) from around the wound were homogenized in 10 ml of 100 mM sodium phosphate buffer (pH 6.4) containing 0.2 g of polyvinyl polypyrrolidone (PVPP) and temperature maintained at 4ºC. Subsequently the homogenate was centrifuged at 15,000xg for 30 min at 4ºC. Supernatant from these extracts were used for POD and PPO assay.

The PPO activity was determined by adding 0.1 ml of enzyme extract to 3.0 ml of Catechol (500 mM , in 100 mM Sodium Phosphate buffer, (pH 6.4) and increase in absorbance measured immediately at 398 nm. POD activity was determined using Guaiacol as substrate using standard method. An 0.1 ml of crude extract was mixed with 2 ml of Guaiacol (8 mM, in 100 mM Sodium Phosphate buffer, (pH 6.4) and incubated for 30 min at 30ºC followed by addition of 1 ml of H_2O_2 (24 mM). Increase in absorbance was measured immediately at 460 nm.

Extract preparation and Quantification of total phenolic compounds: 1 gm of cut-flower was homogenized with ice cold 1% HCl-methanol solution and centrifuged at 15,000xg for 15 min at 4ºC following method of Swain and Hills 1959 [13]. The collected supernatant was measured at 280 nm to estimate changes in total phenolic compounds.

***Statistical analysis*:** The data present in the given study are the mean values (±SE) of replicates conducted on different days and it was analyzed statistically by one way ANOVA (Holm-Sidak method) using SigmaPlot version 12.0.

III. RESULTS AND DISCUSSION

Gerbera cut-flowers (yellow) treated with Chitosan have shown a significant increase in PPO levels and it was maintained same until 24 hours of treatment. However from the day 2, there was sudden fall in PPO levels. CSNPs and CSHNPs induced high PPO activity and the induction of the PPO activity was higher than the Chitosan and other treatment (TPP / Harpin/ Water) throughout the 3 days tested (Figure 1).

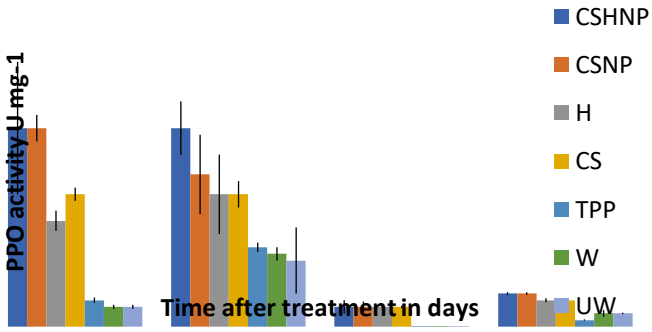

Fig 1:- Total change of PPO activity in the *Gerbera flower* (yellow genotype) after treatment with chitosan loaded haprin nanoparticles, chitosan nanoparticles, harpin, chitosan, TPP and water. Flower buds wounded and treated with water and unwounded served as controls.

It might be due to controlled release of Chitosan as well as harpin from the nanoparticles at the site. Non wounded cut-flower showed relatively lesser PPO activity among all the samples tested. Similar observations were found with regard to POD activity and total phenolics compounds, where there was a gradient fall in POD levels from Day 0 to Day 2 (Figure 2 & 3). There was a cumulative increase in POD activity across all samples on Day 3. CSHNPs took the peak on all sampling days with regards to POD. As per expected harpin loaded Chitosan nanoparticles exhibited highest level of both enzymatic activity as well as total phenolics compounds in the treated Gerbera cut-flowers followed by CSNP, harpin and Chitosan. Peaks at each day starting from day 0 (6 hr) is observed to be taken by CSHNP despite the fact that each of the enzymes i.e. POD and PPO as well as Phenolics compounds follow a definite characteristic pattern.

Elevated concentrations of defense-related proteins such as PPO, POD, lipoxygenase, and protease inhibitors have frequently been well documented in several plants in response to wounding or feeding by arthropod herbivores [14,15]. Similarly, Chitosan also has the potential for inducing defense related enzymes [16] and phenolics in plants [17]. Our laboratory studies on CSNPs and CSHNPs have reported that PPO, POD and the level of phenolics

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compounds in Chitosan treated samples were increased significantly (Chaturvedi unpublished data). In the current experiment we have found similar observations, however there was significant increase in PPO and POD levels initially and slowly the levels of enzyme and total phenolics compounds showed a decrease with regard to their activity.

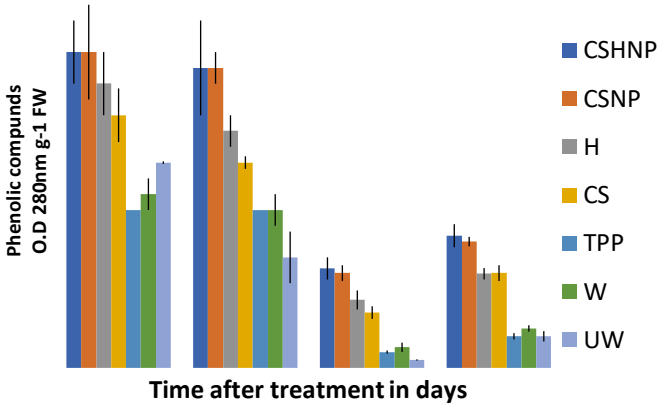

Fig 2:- Total change of phenolics compounds in *Gerbera flower* (yellow genotype) after treatment with chitosan loaded haprin nanoparticles, chitosan nanoparticles, harpin, chitosan, TPP and water. Flower buds wounded and treated with water and unwounded served as controls.

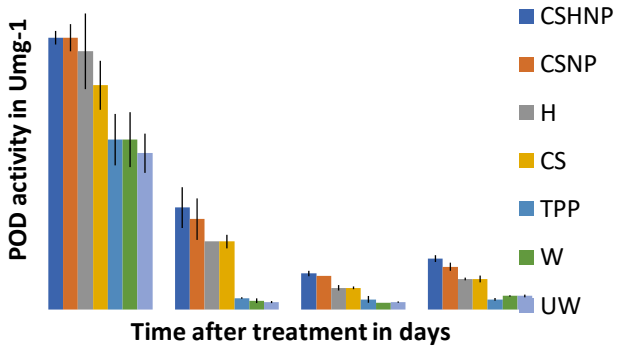

Fig 3:- Total change of POD activity in the Gerbera flower (yellow genotype) after treatment with chitosan loaded haprin nanoparticles, chitosan nanoparticles, harpin, chitosan, TPP and water. Flower bus wounded and treated with water and unwounded served as controls.

IV. CONCLUSION

The synthesized CSNPs and CSHNPs has shown a significant increase in PPO and POD activities along with the total phenolics content which may better help in developing resistance towards both the biotic and abiotic stress in Gerbera. Such studies may be extended to other ornamental plants which are reported to have issues of repeated fertigation and biotic stress in polyhouse conditions.

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