Impact of three distinct inducers on potato plant (Solanum tuberosum) infected with Potato Virus Y (Potyvirus)

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ABSTRACT:

Potato virus Y (PVY) potyvirus leads to considerable potato production losses in Egypt and around the world. The PVY isolate confirmed by DAS-ELISA, (*Solanum tuberosum*) mechanically sap inoculated with PVY isolate under greenhouse condition, Thus Salicylic Acid (SA), Spermine and zinc sulphate (ZnSO4) were verified as foliar treatment for induce the systemic acquired resistance in *S. tuberosum* L. Bellini plants against PVY. In accordance with this study, when compared to virus-free plants, PVY infection dramatically reduced photopigments and biochemical components (total soluble proteins (TSP) and total soluble carbohydrates (TSC)) but boosted bioactive components (total polyphenol content (TPC) and proline (Pro) and antioxidant enzymes (POX, PPO, and SOD). In contrast, compared to the control plants, tested SA, spermine, and ZnSO4 shown a significant rise in all validated parameters. So, it can determine three inducers *S. tuberosum* L. Bellini resistance against PVY. The treatment with ZnSO4 was superior to other.

Keywords: PVY; Salicylic Acid (SA); ZnSO4; total soluble proteins (TSP); total soluble carbohydrates (TSC); total polyphenol content (TPC); proline (Pro)); antioxidant enzymes.

INTRODUCTION

One of the most commercially significant members of the family Solanaceae on a universal scale is the potato. The FAO lists potatoes (*S. tuberosum* L.) as the fourth-largest edible crop in the world. In many countries around the world, potatoes are one of the most significant crops of vegetables. Potato is the fourth most important crop in words of economic importance, after rice, wheat, and maize. It is a vital source of vitamins, antioxidants, and carbs. It is also used as a starting point for the biosynthesis of starch. (FAO, 2022).

However, a variety of viral and fungal diseases, comprising the family *Potyviridae*, genus *Potyvirus*, potato virus Y (PVY), harm potatoes under production conditions. (Lacomme et al., 2017). Furthermore, plants are subjected to harmful abiotic influences either simultaneously or successively, which can have a substantial impact on how protective responses to stressors are formed. (Valkonen et al., 2017; Pandey et al., 2017; Hamooh et al., 2021)

It's been demonstrated that SA directly affects microorganisms in bulk soil without the need for plant signalling. All of these findings point to the possibility that SA may influence the growth of community members directly, through interactions with other hormones, or through conventional signalling mechanisms. (Eichmann et al., 2021)

Furthermore, PAs-based such as spermine defensive mechanisms against viruses such as Citrus tristeza virus (CTV) might be incorporated with the detoxification of reactive oxygen species (ROS) (Doria et al., 2015). The CTV infection caused the infected cells to generate ROS, This then changed the way antioxidant enzymes functioned and reconfigured both the primary and secondary metabolic pathways. For instance, there was a rise in superoxide dismutase (SOD) and ascorbate peroxidase (APX) transcripts and the activity of enzymes. (Cheng et al., 2016; Hancevic et al., 2018).

Zinc (Zn) is a divalent cation (Zn^{2+}) that is present in plants and is a necessary element for healthy development and growth as well as protein function (Andreini et al., 2006; Bouain et al., 2017). Zn acting a central function in the triangle formed by a nutrient, a plant, and a pathogen. The metal defence theory suggests that multiple accumulations of Zn and various metals act as a defence mechanism versus pathogens (Helfenstein et al., 2015). Zn plays a crucial role in how plants react to illness. It might, however, differ based on how well Znrelated responses work to prevent pathogen attacks and reduce the pathogenicity of invaders by thwarting plant defence mechanisms while also taking the environment into account. Zn proteins help plants fight off intruders or help them avoid being attacked by pathogens, but not all plants will benefit from Zn proteins because certain plants may become more susceptible to them (Cabot et al.,

2019). Numerous research point to the enhancement of plant defence against diseases as a function of Zn nutrition (Malandrakis et al., 2019).

ZnSO₄ considerably lessened the Cotton Leaf Curl Virus (CLCuV) severity and produced unfavourable conditions for the whitefly, which is the virus' vector, according to Kalsoom et al., (2019). By boosting resistance to viral diseases due to an increased level of phenolic compounds, which are crucial for the advance of defence mechanisms anti viruses, foliar spray of ZnSO4 was talented to reduce the indications and prevalence of potato viruses as PVY and PLRV (Ibrahim et al., 2016). Nevertheless, ZnSO₄ (0.5%) by itself did not significantly lessen the severity of papaya ringspot infection, according to findings from Deepika et al., 2021. But using ZnSO4 (0.5%) along with urea (1%) and humic acid (0.2%)reduced the severity of the condition significantly. Proteins and carbohydrates require Zn to be created. According to Dutta et al. (2017), Low Zn plants frequently store reducing sugars and amino acids in their tissues. A plant's accumulation of amino acids prepares it for sucking insects' feeding and reproduction. According to Dutta et al. (2017), greater feeding intensity and reproduction may also contribute to the development of diseases by increasing viral transmission. This is from the perspective of the vector.

Thus, the goal of this research is to examine the efficiency of SA, Spermine and ZnSO₄ in the defence of potato plants in contradiction of PVY infection and their effects on TSP, proline (Pro), total polyphenol (TPP), the protective antioxidant enzymes.

MATERIALS AND METHODS

Plant Materials:

Tuber of *S. tuberosum L. Bellini.* were acquired from the Ministry of Agriculture, Giza, Egypt (Agriculture Research Centre), and sprouted in perlite-filled seed plates. Seedlings were grown in natural lighting with a mean relative humidity of 60% and day/night temperatures of about 20/22°C. One-week-old seedlings were placed in soil and cultivated in the same environment.

Potato virus Y (PVY) inoculation

From the Virology Laboratory at the Fac. of Science at Al-Azhar Univ. in Cairo, Egypt, a PVY isolate was acquired. The Agriculture Research Centre in Giza, Egypt, graciously offered ELISA kits, and a double-antibody sandwich enzyme-linked immunoassay test (DAS-ELISA) was used to confirm the viral isolate serologically.

Techniques for Planting, Treating, and Sampling.

In the Bot. and Micro. Dep.'s greenhouse at Al-Azhar Univ.'s Fac. of Science in Cairo, Egypt, a pot experimentation were assessed as foliar treatment for induction systemic acquired resistance in S. tuberosum L. Bellini plants against PVY. Using a randomised complete block design. The pots were kept in a greenhouse that is lit normally, 20 to 22°C throughout the day, and 60% mean relative humidity. Following a growth period of two weeks, the two true-leaf seedlings of the plants treated with the (tap water, SA, Spermine and ZnSO₄), were challenged inoculated mechanically with PVY inoculum.

H= Healthy plants sprayed with tape water

H+X = Healthy plants sprayed with SA 1mM

H+Y = Healthy plants sprayed with spermine 50 ppm

H+Z = Healthy plants sprayed with (ZnSO₄) 50 ppm

I= Infected plants sprayed with tape water

I+X= Infected plants sprayed with SA 1mM

I+Y= Infected plants sprayed with spermine 50 ppm

I+Z= Infected plants sprayed with (ZnSO₄)50 ppm

Three to five weeks after the inoculation, the external symptoms began to appear. DAS-ELISA was used to confirm that the plants were infected.

Physiological factors were verified in healthy and challenged plants as following:

Photopigments concentration: The procedure utilized to quantify the amount of chlorophyll was (Vernon and Selly, 1966). The spectrophotometer with two wave lengths (649 and 665 nm) was used to measure the optical density of the plant extract. These are the points on the spectrum when chlorophyll (a) (Chl a) and chlorophyll (b) (Chl b) concentrations are at their highest. The formulae presented by Vernon and Selly (1966) were used to calculate the concentrations of (Chl a), (Chl b), and total chlorophyll in leaf tissue.

g tissue /mg Chl a =11.63(A665) -2.39(A649).

g tissue /mg Chl b =20.11(A649) -5.18(A665).

6.45 (A665) + 17.72 (A649) is the mass of Chl (a+b) per gram of tissue.

The concentration of carotenoids (Car) was determined using the equation of (Lichtentahler, 1987): Carx+c =1000×OD470-1.82Ca-85.02Cb/198 = mg/g fresh weight. A= the spectrophotometer's measuring of the optical density (nm).

Total carbohydrate extraction:

Total soluble carbohydrates (TSC) were obtained corresponding to (Said *et al.*, 1964)

(*TSC*):

Anthrone methods were employed to determine the TSC in compliance with (Umbriet et al., 1969). TSC (equals in expressions of sucrose). At 620 nm, it was detected using a spectrophotometer (Unico 2000).

Total polyphenol (TPP):

TPP compounds in the leaves were investigated using the methodology outlined via (Daniel and George, 1972) using a 725 nm wavelength by spectrophotometer (Unico 2000).

Proline (Pro) content:

Using a UV spectrophotometer (Unico 2000), the free proline content was determined in accordance with the protocol specified by (Bates et al., 1973) at 520 nm.

Total soluble proteins (TSP):

Corresponding to the procedure of (Lowery *et al.,* 1951) utilizing a (Unico2000) spectrophotometer set at 750 nm in order to find out the TSP of leaves using casein as the standard protein.

Antioxidant enzymes:

Ten milliliters of phosphate buffer (pH 6.8; 0.1 M) was used to smooth two grams of the terminal buds, and the mixture was centrifuged at 2C for 20 minutes at 20,000 rpm in a frozen centrifuge. as the plant materials used for the enzyme estimate. The enzyme-containing clear out supernatant was identified as the primary source of enzymes (MuKherjee and Choudhuri, 1983).

SOD enzyme activity:

SOD activity was ascertained by measurement of the suppression of the autooxidation of pyrogallol by means of a procedure designated by (Marklund and Marklund, 1974) applying UV spectrophotometer (Labomed, inc.23) at 325 nm.

POX enzyme activity:

POX activity was assessed applying the frequency of rise in intensity in optical density as pyrogallol was regulated (Bergmeyer, 1974) using (Labomed, inc.23) UV spectrophotometerat 470 nm.

PPO enzyme activity:

The polyphenol oxidase enzyme's PPO activity was established using the methodology used by Matta and Dimond (1963). Using a UV spectrophotometer, the optical density was determined at 495 nm (Labomed, inc. 23).

Statistical analysis:

Two-way ANOVA (SigmaPlot 12.0) was used to statistically analyse all plant chemical analysis data at the 0.05 probability level. The values listed in the biochemical investigation's values are typically the means of six replicates.

RESULTS AND DISCUSSION

Confirmation of PVY

By using DAS-ELISA, specific polyclonal antibodies against PVY get serologically confirmed the infectious ness of the PVY.

One of the primary physiological processes essential to plant growth is photosynthesis. PVY effect on the process of photosynthesis in various potato treatments, different treatments of potato also showed different responses because of PVY isolate infection by assessing the contented of both chlorophylls and carotenoids in the leaves of potato plants.

Records demonstrated clearly in fig.1 (A, B, C, D) shows the effect of SA, Spermine and ZnSO₄ on (Chl_s) and (Car) values in the potato plants leaves under PVY infection. Chl a, b, total a+b and carotenoids values were significantly declined in potato influenced with PVY as compared to healthy control plants. Likewise, vs the control plants that were infected, foliar treatment with salicylic acid, spermine, and ZnSO₄ significantly increased the amounts of Chl a, b, total a+b, and Car. The most effective inducer was ZnSO₄ that caused a significant improvement in Chl a (12.1%, 33.4%), Chl b (17.1%, 10.3%), total a+b (14.5%, 22.4%) and Car (56.1%, 186.2%)

amounts in healthy and infected plants, in contrast to the values of the H and I controls, respectively. Concurring to Rahoutei et al. (2000) and Akladious and Mohamed (2017), photosynthesis is one of the physiological processes crucial to plant growth, the plantvirus relationship either inhibit photosystem II action or has an impact on pigment generation in the chloroplast. Our research's conclusions concurred with Sofv et al. (2021), who found that ToMV infection decreased tomato plant leaves' photosynthetic qualities in comparison to uninfected plants. As plants respond to biotic stress through transcription and translation, Zn supports the healthy operation of several critical plant processes, including growth, apoptosis, cell division, and differentiation (Chen et al., 2021).

Effect of SA, Spermine and ZnSO₄ on Biochemical and bioactive components under PVY infection

In addition, potato plants foliar sprayed with SA, Spermine and ZnSO₄ showed a significant increase in biochemical components (TSC and TSP) and bioactive compound (Pro and TPP) contents as compared to the healthy and infected controls as in fig.2 (A, B, C, D).

The data graphic illustrations in fig. 2 (A, B, C, D) clearly demonstrate how salicylic acid, spermine, and ZnSO₄ affect the amounts of protein, and carbs in the leaves of potato plants infected with PVY. When comparing potato plants infected with PVY to absolute control plants, there was a significant drop in the levels of both TSP and TSC. In addition, when foliar treated with ZnSO4, salicylic acid, and spermine, the levels of Salicylic Acid and carbohydrates increased significantly in comparison to control-infected plants. ZnSO4 was the most effective inducer, resulting in a considerable improvement in the levels of TSP (56.5%, 47.7%) and TSC (25.16%, 31.46%) in both uninfected and challenged plants. respectively in contrast to H and I control values, in that order. These results are in harmony with reports that sugar buildup in virus-infected plants was seen. The virus modifications to fructose-1,6causes diphosphate production, which impacts the metabolism of carbohydrates and lowers the plant's energy stores. This decrease in metabolic activity would promote the formation of fructose-1,6-diphosphate, which would hinder the infected leaves' ability to synthesise sucrose. (Stitt and Hurry, 2002). However, other investigations indicated that phloem necrosis, which may inhibit sugar transfer, may be the cause of the increased

reduction in sugars in infected plants. (Ahmed et al., 1986). Lastra and Gil (1981) also noted a reduction in sugar translocation in virusinfected plants as a result of damaged phloem. In contrast to the research conducted by Khalil et al. (2014). Zinc (Zn) is a divalent cation (Zn²⁺) that is present in plants and is a necessary element for healthy development and growth as well as protein function (Andreini et al., 2006; Bouain et al., 2017). Zn proteins help plants fight off intruders or help them avoid being attacked by pathogens, but not all plants will benefit from Zn proteins because certain plants may become more susceptible to them (Cabot et al., 2019). Sarma et al. (1995) suggested that a virus infection raised the amounts of whole protein in the leaves. Variations in the overall quantity of protein throughout viral replication exhibit a multifaceted pattern and are contingent on the leaf's developmental stage at the time of infection. According to additional findings, the TSP content of a healthy leaf increases during its expansion and decreases with age. (Fraser, 1987). Mature leaves that are infected stop the overall loss of whole protein as they age. This could be clarified by the accumulation of TMV coatprotein in addition to the net loss of host proteins. (Fraser, 1987). Conversely, Sofy et al. (2017) worked with TYLCV, whereas Taiwo and Akinjogunla (2006) dealt with Mung bean (Phaseolus aureus L.) infection caused by MYMV. The diseased carrot (Daucus carota L.) plant showed a reduction in TSP (Afreen 2011). PVY infected potato plant leaves (I) demonstrated a significant raise in the content of Pro content, and TPP content by 129.9 % and 60.8%, respectively compared to H values. Additionally, potato plants foliar sprayed with different treatments of SA, Spermine and ZnSO₄ showed a significant enhancement in proline and phenols content as contrasted to the H and I control (Figure 2). The maximum increase in Pro (28.3%) and in TPP (45.7%) content was find out in infected plants spray applied with ZnSO₄ as competed to (I) control.

ROS generation is the one of typical feature of relations between viruses and plant cells (Hernández et al., 2016 and Király et al., 2021). During host-pathogen interactions, oxidative damage takes place by the rapid generation of ROS. ROS are mostly produced via the reaction of Mehler in chloroplasts, photorespiration in peroxisomes, and electron transport in mitochondria (Zhao et al., 2016). Oxidative stress, a rapid build-up of ROS, and oxidative damage to biological components could result from a pace of ROS generation that exceeds the capacity of cells to scavenge

them (Hernández et al., 2016; Gomaa and Dawood, 2021). As а non-enzymatic antioxidant, proline can assist stabilise subcellular elements including proteins and cell membranes. It can also scavenge free radicals under stressful circumstances and buffer redox potential (El-Beltagi et al., 2019; El-Sheshtawy et al., 2021). Plants are only protected by Pro as a suitable solute from single oxygen and radical damage caused by surplus of ROS. (Szabados and Savoure, 2010; Dawood et al., 2021). As a non-enzymatic antioxidant, proline may assist stabilise subcellular components like proteins, buffer redox potential, and scavenge free radicals and cell membranes in stressed conditions. (Maksoud et al., 2022). Proline is the only appropriate solute that provides protection to plants against the damage that single oxygen and radicals produced by excessive ROS can inflict.

Phenolics build-up at infection sites has been connected to resistance of mango to infection of *C. fimbriata* (Araujo et al., 2014). Additionally, applying potassium phosphite to mango plants suppressed mango wilt and created systemic resistance. This could be because of the increased phenolics content, the development of an antifungal partition, and the quick tylose deposit. (Mourad et al., 2021).

Effect of SA, Spermine and ZnSO₄ on enzymatic antioxidants under PVY infection

Figure 3 (A, B, and C) illustrates the impact of viral infection and the administration of SA, spermine, and ZnSO₄ on antioxidant enzyme activity, including that of peroxidase (POX), polyphenoloxidase (PPO), and superoxide dismutase (SOD).

The potato leave challenged by PVY (Infected control), the activity of POX (36.2%), PPO (53.8%) and SOD (21.2%) was significantly increased in comparison to healthy control plants. Additionally, SA, Spermine and ZnSO4 promotes systemic resistance by activating POX, PPO, SOD that act as defense-related enzymes.

ZnSO₄ was the most successful inducer, resulting in a considerable improvement in the levels of POX (131.5%, 87.3%), PPO (184.6%, 105%), SOD (75.3%, 61.2%), and in both infected and uninfected plants, respectively, as compared to the values of healthy and infected control plants.

Plant resistance to viral infections depends critically on these pathogenesis-related enzymes. (Tamandegani et al., 2021). When

proteins, lipids, and DNA are reacted with the ROS created upon viral infection, normal plant cell processes are disrupted. (Vitti et al., 2015). Plants therefore create both enzymatic and non-enzymatic which reduce the generation of reactive oxygen species (ROS) and oxidative destruction throughout infectivity (Lehmann et al., 2015). SOD, the most important defence system enzyme, dismutates superoxide into O₂ • and H_2O_2 . (Abu-Shahba et al., 2022). Because ROS are lethal and unstable, CAT converts them into components that are less toxic and more stable, such as H₂O and O₂. (Gill and Tuteja, 2010). The current work shown a generally substantial rise in the activity of POX, PPO, and SOD in PVY infested plants as compared to the identical uninfected one at all verified potato plant treatments across PVY isolates. This work addressed the impact of PVY isolate on activity of antioxidant enzyme in the leaves. These findings are in accordance utilizing the research conducted by El-Dougdoug et al., (2014); Sofy et al., (2017). They noticed that certain stresses had an improve in the activity of antioxidant enzyme in leaves. Plants have evolved into complex metabolism repair antioxidant to the destruction initiated by ROS. Numerous oxidative enzymes found in plants, including POX and PPO, can catalyse lignin and other oxidative phenolics synthesis that support cell wall throughout pathogen attack. (Deng et al., 2015).

According to Radwan et al. (2010), The first enzyme to reveal alterations in activity when under stress was POX and was thought to be one of the enzymes that combat free radicals implicated when a plant defends itself against a pathogen attack. Growing insights into plant defence systems against pathogens are shedding light on an increasingly complex process.

The existing study noticed that adding SA, Spermine and ZnSO₄ to potato plants heightened activity of antioxidant, which aided to lower the harmful effects of virus infection. In zinc-proteins and enzymes, Zn ions mostly play catalytic, co-catalytic, or structural roles. When numerous metal ions are linked closely to one another, a situation known as co-catalysis occurs. In this circumstance, one metal ion acts as a catalyst while the other metal ions boost the site's catalytic activity. The Zn ions clearly show up as a catalytic component in the formation or disruption of the bond. In stabilizing the tertiary structure of enzymes, the zinc ion has a structural role akin to that of disulfide bonds.

(Cabot et al., 2019). According to Mohamed and Abd-El Hameed (2014), SOD shields tissues from oxidative stress brought on by pathogen and pest infestation. Mango plants showed elevated metabolite concentrations linked to oxidative stress responses and enhanced enzyme activity following C. fimbriata inoculation, corresponding to Araujo et al. (2014).

CONCLUSION

The current study focuses on Salicylic Acid (SA), Spermine and Zinc sulphate (ZnSO4) in suppressing PVY in potato plants. The findings suggest that potato plants exposed to ZnSO4 rates became more resistant to the PVY, most likely as a result of improved physiological performance. That favours the mounting of host defence responses by increasing secondary metabolites, defence antioxidant enzyme activity. As a result, developing management methods that favour increasing ZnSO4 supply could be a beneficial strategy for reducing PVY severity.

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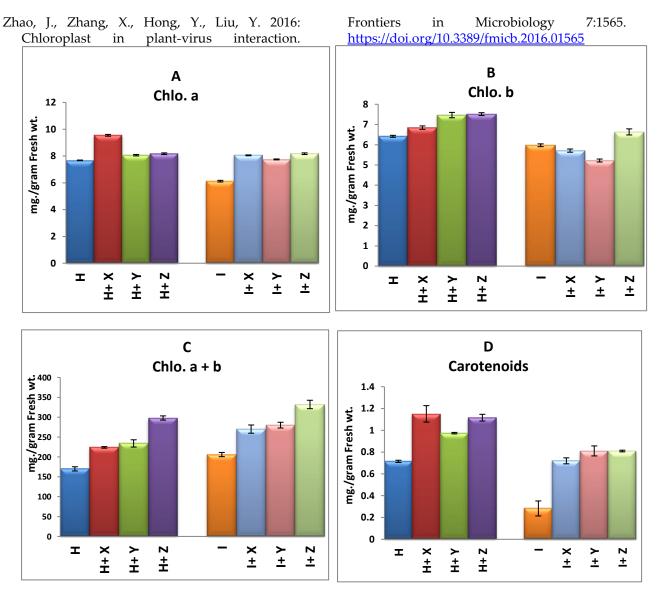


Figure 1 (A, B, C, D): Foliar spray's impact with SA, Spermine and ZnSO₄ on Chl (a), Chl (b), total (a+b) and Car of potato plants under PVY infection (mg/g Fresh wt.) via two-way analysis of variance (ANOVA). Every value is the average of six replicates \pm standard error of average, H=Healthy plants, H+X = Healthy plants sprayed with SA, H+Y = Healthy plants sprayed with spermine, H+Z = Healthy plants sprayed with ZnSO₄, I= Infected plants, I+X= Infected plants sprayed with SA, I+Y= Infected plants sprayed with SA, I+Y= Infected plants sprayed with Significancy at P<0.050

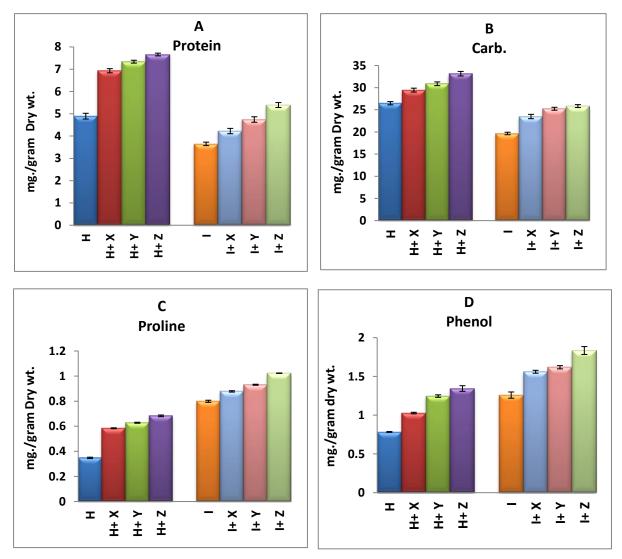


Figure 2 (A, B, C, D): Foliar spray's impact with SA, Spermine and ZnSO₄ on TSP, TSC, Pro and TPP of potato plants under PVY infection (mg/g dry wt.) via two-way analysis of variance (ANOVA). Every value is the average of six replicates \pm standard error of average, H= Healthy plants, H+X = Healthy plants sprayed with SA, H+Y = Healthy plants sprayed with spermine, H+Z = Healthy plants sprayed with ZnSO₄, I= Infected plants, I+X= Infected plants sprayed with SA, I+Y= Infected plants sprayed with SPA, I+Y= Infected plants sprayed with SA, I+Y= Infected plants sprayed with SO₄, I= Infected plants sprayed with ZnSO₄ with Significancy at P<0.050

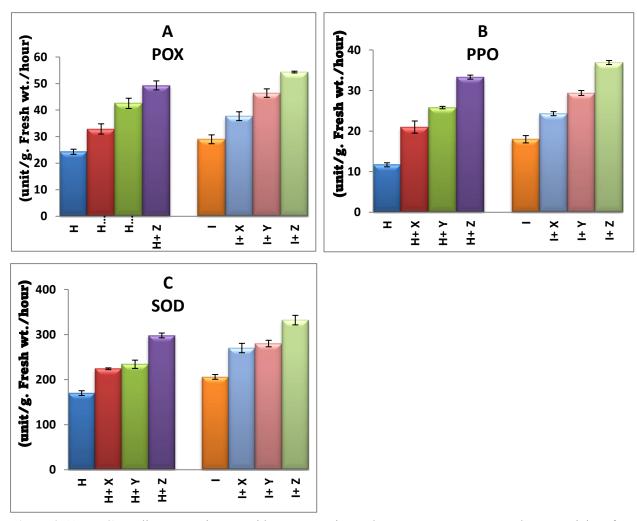


Figure 3 (A, B, C): Foliar spray's impact with SA, Spermine and $ZnSO_4$ on POX, PPO and SOD activity of potato plants under PVY infection (unit/g fresh wt./hour) via two-way analysis of variance (ANOVA. Every value is the average of six replicates \pm standard error of average, H= Healthy plants, H+X = Healthy plants sprayed with SA, H+Y = Healthy plants sprayed with spermine, H+Z = Healthy plants sprayed with ZnSO₄, I= Infected plants, I+X= Infected plants sprayed with SA, I+Y= Infected plants sprayed with SA, I+Y= Infected plants sprayed with SA, I+Z= Infected plants sprayed with SA, I+Y= Infected plants sprayed with SA, I+Z= Infected plants sprayed with SA, I+Z

تأثير ثلاثة محفزات متميزة على نباتات البطاطس المصابة بفيروس البطاطس Y

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الملخص العربي:

يسبب فيروس البطاطس (PVY) خسائر فادحة في إنتاج البطاطس سواء في مصر أوحول العالم. تم تأكيد عزل PVY سيرولوجيا باستخدام -DAS ELISA، تم اختبار حامض السلسيليك ,والاسبرمين وكبريتات الزنك لاستحثاث المقاومة الجهازية. خلصت الدراسة إلى أن عدوى PVY تسببت في انخفاض كبير في كل من الأصباغ الضوئية والمكونات الكيميائية الحيوية (الكربوهيدرات والبروتينات) بينما تمت زيادة المكونات النشطة بيولوجيًا (الفينول والبرولين) والإنزيمات المضادات للأكسدة (بيروكسيديز وبولى فينول أوكسيديز وسوبرأوكسيد ديسميوتيز) بشكل كبير مقارنة بالنباتات السليمة. على العكس من ذلك، أظهر حامض السالسيليك والاسبرمين وكبريتات الزنك التي تم اختبارها زيادة كبيرة في جميع المعلمات الختبرة عند مقارنتها باستهة. على العكس من ذلك، أظهر حامض السالسيليك والاسبرمين وكبريتات الزنك التي تم اختبارها زيادة كبيرة في جميع المعلمات الختبرة عند السليمة (المجموعة الضابطة). ومن ثم، يمكن القول إن نبات البطاطس صنف بيليني يصبح مقاوماً للالاث بفعل ثلاثة عوامل محفزة: حامض سالسيليك والإسبرمين وكبريتات الزنك. وقد فاقت العاملة بكبريتات الزنك التي تم اختبارها زيادة كبيرة في حريا بفعل بعد الساليمة.

الكليات الاسترشادية: فيروس البطاطس Y, حامض السلسيليك, كبريتات الزنك, البروتينات الكلية, السكريات الكلية, الفينولات الكلية, البرولين , إنزيمات الأكسدة.