Plant Pathol. J. 39(2) : 159-170 (2023)
https://doi.org/10.5423/PPJ.OA.06.2022.0080
eISSN 2093-9280

Research Article Open Access

The Plant Pathology Journal

Defense Inducer Compounds Up-regulated the Peroxidase, Polyphenol Oxidase, and Total Phenol Activities against Spot Blotch Disease of Wheat

Puja Kumari[1], Chandrashekhar Azad[1], Ravi Ranjan Kumar[2], Jyoti Kumari[1], Kumar Aditya[1], and Amarendra Kumar [1]*

[1]*Department of Plant Pathology, Bihar Agricultural University, Sabour Bhagalpur 813210, India*
[2]*Department of Molecular Biology and Genetic Engineering, Bihar Agricultural University, Sabour Bhagalpur 813210, India*

(Received on June 14, 2022; Revised on November 1, 2022; Accepted on November 24, 2022)

Spot blotch disease of wheat caused by *Bipolaris sorokiniana* (Sacc.) Shoem is considered as an economically important disease which affects all the growing stages of wheat crop. Therefore, it is important to search some effective management strategies against the spot blotch pathogen. Some synthetic elicitor compounds (salicylic acid, isonicotinic acid, and chitosan) and nano-particles (silver and aluminum) were tested against the pathogen to observe the change in biochemical activity and defense action of wheat plant against spot blotch disease. All the tested elicitor compounds and nano-particles showed a significant increase in activity of peroxidase, polyphenol oxidase (PPO), and total phenol over control. The highest increase in activity of peroxidase was recorded at 72 h from chitosan at 2 mM and 96 h from silver nano-particle at 100 ppm. Maximum PPO and total phenol activity were recorded from chitosan at 2 mM and silver nano-particle at 100 ppm as compared to pathogen-treated and healthy control. The lowest percent disease index, lowest no. of spots/leaf, and no. of infected leaves/plant were found in silver nano-particle at 100 ppm and chitosan at 2 mM, respectively. The use of defense inducer compounds results in significantly up-regulated enzymatic activity and reduced spot blotch disease. Therefore, chitosan and silver nano-particle could be used as alternative methods for the management of spot blotch disease.

Keywords **:** *Bipolaris sorokiniana*, elicitors, nano-particles, spot blotch disease, wheat

*Corresponding author.
Phone) +91-7903677928, FAX) +91-641-2452614
E-mail) kumaramar05@gmail.com
ORCID
Amarendra Kumar
https://orcid.org/0000-0003-0041-3270

Handling Editor : Chang-Jin Park

Wheat (*Triticum* spp.) is considered an important cereal crop and staple food for the South Asian population. In countries like India, Pakistan, Bangladesh, and Nepal, green revolution played a very important role in securing food security (Joshi et al., 2007). Wheat crop shows the greatest adoption to different weather condition in the world and provides 75% of carbohydrates required for human nutrition along with maize and rice. It is also a good source of dietary fiber, fat, riboflavin, protein, calcium, iron, magnesium, phosphorus, potassium, zinc, and manganese (Bodner-Montville et al., 2006). The research conducted on the yield trials by breeding centers of different countries has sown that wheat production is hampered by several different biotic and abiotic stresses (Duveiller, 2004; Van Ginkel and Rajaram, 1993). It is estimated that around 30% of wheat production all around the world is affected by different pests and diseases (Bockus et al., 2010). Some of the economically important diseases of wheat are caused by fungi, such as root rotting, rust, smut, and leaf blight. Particularly in the warmer part of the world, spot blotch disease is very common and spot blotch pathogen is aggressive when the relative humidity is high and the temperature is around 20ºC (Zillinsky, 1983). Spot blotch

disease of wheat caused by *Bipolaris sorokiniana* (Sacc.) Shoem (teleomorph, *Cochliobolus sativusis*) and this fungus is well known as a causal agent for many different diseases like common root rot, spot blotch or foliar blight, head blight, black point, and seedling blight disease of wheat (Wiese, 1998). Especially in the rice-wheat cropping system following areas of India are severely affected by spot blotch disease of wheat, because rice crop and rice stubbles act as a reservoir for the secondary inoculums of *B. sorokiniana* (Saari, 1998). Indian sub-continent has total 10 million ha of affected area by this disease, out of which India alone has 9 million ha and most of which is in the rice-wheat cropping system (Kumar and Nagarajan, 1998). This pathogen is both seed and soil-borne and secondary transmission is common through the air. The characteristics symptom of this disease appears on the leaves as small round to oval spot of 1-2 mm in size and dark brown in color with no sign of chlorotic margin but with the advancement in the disease the lesion size increases up to several centimeters and chlorotic margin can also be observed.

For the management of this pathogen, the use of fungicides at the commercial level is a common practice in agriculture sector. Seed treatment with chemical (synthetic fungicides) is widely practiced method for the management of this soil and seed-borne fungal pathogens. However intensive use of fungicides has two major consequences: first, overuse of fungicides threatens the environment and raise questions about human health and second, this practice encourages the emergence of fungicide-resistant pathogens in the environment (Lamichhane et al., 2017). Because the teleomorphic stage of this pathogen (*C. sativus*is) is rare in nature (Raemaekers, 1991), the development of resistance variety is a tough task for the researchers. The management of plant pathogens by enhancing the natural defense mechanism of plant is emerging as effective alternative method. Systemic resistance can be generated through many elicitors and synthetic chemical compounds which ultimately lead to the activation of various defense-related enzymes and also affects the production of phenolic compounds in plants (Thakur and Sohal, 2013). Defense inducer chemicals are not only effective in controlling disease but have a less residual effect on food (Gao et al., 2014). Along with inducer compounds, the application of nano-particles has been considered as an alternate approach for controlling different plant pathogens, if used properly by taking into consideration of all important aspects and recommended dose (Chandra et al., 2015).

These inducer compounds (elicitors and nano-particles) are effective in the induction of systemic resistance in plants against a broad range of pathogens i.e., race non-specific pathogens. It is already known that synthetic elicitors are small molecules that can induce plant immune responses and are structurally distinct from natural plant defense inducers, such as general or race-specific elicitors or endogenous plant defense signaling molecules. Synthetic elicitors may trigger defense reactions by mimicking interactions of natural elicitors or defense signaling molecules with their respective cognate plant receptors or by interfering with other defense signaling components. Often the term “plant activators” is used for molecules that can protect plants from diseases by inducing immune responses (Bektas and Eulgem, 2015). The alteration in metabolic activity of host plant is observed due to the infection of plant pathogen and this leads to the change in activity of biochemical defense enzymes and oxidative enzymes like peroxidase (POX), polyphenol oxidase (PPO), total phenol (Percival, 2001; Song and Goodman, 2001).

The main objective of present investigation is to observe the effect of elicitor compounds (salicylic acid, isonicotinic acid, and chitosan) and nano-particles (silver and aluminum) on the enzymatic activity of wheat plant and its effect on the severity of spot blotch disease. Isonicotinic acid is characterized as a functional analog of salicylic acid. It doesn’t trigger any changes in salicylic acid content so, acts downstream of salicylic acid. Similar to salicylic acid (Vernooij et al., 1995), isonicotinic acid has the ability to increase reactive oxygen species (ROS) production by inhibiting the activity of the enzymes catalase and ascorbate POX (Durner and Klessig, 1995). Up to now, only limited research work has been conducted regarding the effect of biochemicals like POX, PPO, and total phenol in wheat plant against *B. sorokiniana*. The experiments were conducted to determine the effect of all these biochemical enzymes on developing disease resistance and occurrence of spot blotch disease in wheat.

Material and Methods

Experimental site. The experiment was conducted at Bihar Agricultural University, Department of Plant Pathology, Sabour which is situated at an altitude of 45 m above mean sea level, and lies between 24°30" and 25°30" at North latitude and 86°3" and 87°3" East longitude.

Plant material. Pot experiment was conducted under controlled conditions where, 24 ± 2ºC (day/night) temperature and relative humidity 85-90% were maintained inside the greenhouse. ‘Agra local’ cultivar of wheat, which is highly susceptible to spot blotch of wheat (Choudhary et al., 2018) was selected for the present investigation. For pot filling,

the soil was collected from the upper layer of 0-15 cm, from Bihar Agricultural University Farm, Bhagalpur. 1.5 kg of soil was packed in each plastic bag and autoclaved at 20 lb psi at 121.6ºC for 1 h on three consecutive days after that each pot was filled with 50% of sterilized soil and 50% of compost. Wheat seeds of uniform size were selected carefully, and surface sterilization was carried out with 70% ethanol followed by several times washing with sterilized distilled water. The seeds were then soaked in water for 8 h, after that the seeds were dried for 30 min. Seven seeds were counted and sown in each pot. Pots were watered regularly according to the moisture requirement and optimum condition was maintained.

Preparation of mycelial mat and conidial suspension. The pathogen *B. sorokiniana* (accession no. NFCCl3982) was grown *in-vitro* under lab condition on potato dextrose agar (200 g peeled potato, 1,000 ml distilled water, 20 g dextrose, and 20 g agar-agar) media and allowed to grow for 4-5 days, grey color colony with cottony growth was obtained (Fig. 1A), during the microscopy (Thermo Fisher Scientific, Bengaluru, India) study at 40× magnification it was observed that the conidia is of elliptical shape with 6-7 cells (Fig. 1B) and the size of conidia is approximately 15-20 µm. The active mycelium from the edge of the colony was cut into 3-4 bits of 5 mm with the help of sterilized cork borer and transferred to the 250 ml conical flasks containing potato dextrose broth (PDB) (200 g peeled potato, 1,000 ml distilled water, 20 g dextrose). The inoculated broth was kept in biochemical oxygen demand incubator at 26 ± 2°C for 7 days. After 7 days of incubation, a mycelia mat was developed on the PDB. The fully grown mycelia mat was harvested using blotter paper adjusted on a funnel and dried for 30 min. After drying the mycelia were crushed properly and diluted in distilled water and finally, blotter paper was used to filter the solution. The conidial solution was adjusted at 1×10^6 conidia/ml for spraying.

Preparation of spraying solution of elicitor compounds and nano-particles. Two elicitor compounds, salicylic acid, and isonicotinic acid (HiMedia Laboratories Pvt. Ltd., Thane, India), were taken in the required concentration of 1, 1.5, and 2 mM separately and dissolved in lukewarm distilled water (~25°C) and left undisturbed for 24 h. Chitosan (HiMedia Laboratories Pvt. Ltd.) solution was prepared by dissolving 1, 1.5, and 2 mM of chitosan separately in 100 ml of distilled water containing 0.5 ml (v/v) glacial acetic acid (Sisco Research Laboratories Pvt. Ltd., Kolkata, India), the solution was heated and agitated constantly and left undisturbed for 24 h. Fifty, 100, and 200 ppm of silver (Vedayukt India Pvt. Ltd.) and aluminum (Al_2O_3) nano-particles (Vedayukt India Pvt. Ltd., Jamshedpur, India) were dissolved in acetone respectively, and then left undisturbed for 24 h.

Spraying of pathogen and defense inducer compounds. Thirty days old wheat plants were used for the experiment i.e., for the analysis of peroxidase, polyphenol oxidase, and total phenol. Plants were inoculated once by spraying with the spore suspension of 1×10^6 spores/ml of *B. sorokiniana* by sterilized atomizer. Twenty-four hours after challenge inoculation (next day at 4 p.m.) with the pathogen, plants were sprayed singly with three elicitor compounds (salicylic acid, isonicotinic acid, and chitosan) and two nano com-

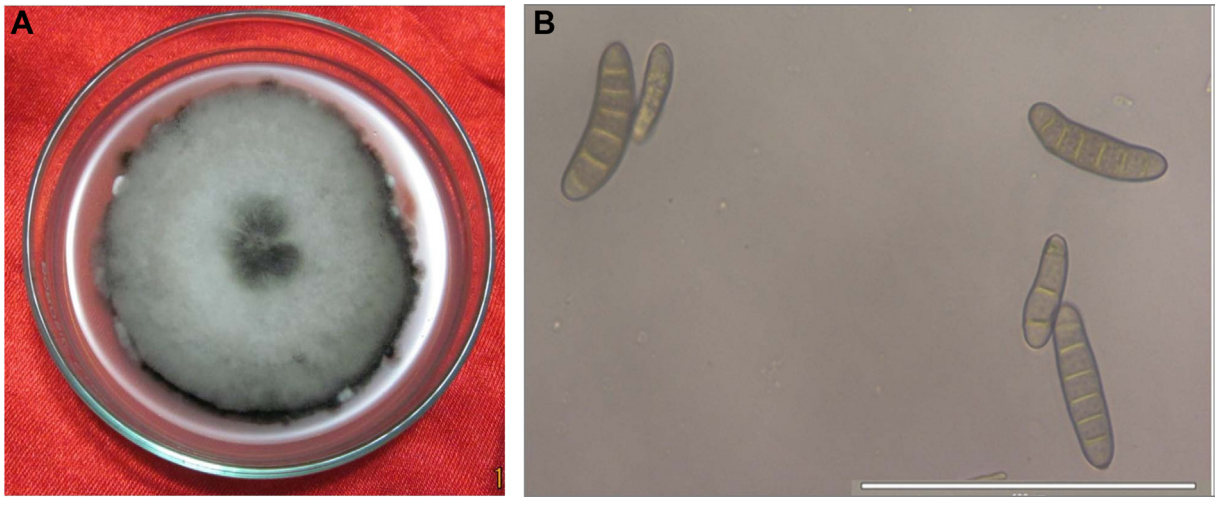

Fig. 1. Detail morphological study of *Bipolaris sorokiniana*. (A) Grey color colony with cottony growth. (B) Visualization of conidia at 40× magnification (scale bar = 400 µm) under microscope.

pounds (silver nano-particle and aluminum nano-particle). All the chemicals were tested at three different concentration of 1 mM, 1.5 mM, and 2 mM for elicitors compounds and for nano-particles 50 ppm, 100 ppm, and 200 ppm respectively. The elicitor compounds and nano-particles were sprayed on the plants homogeneously through an atomizer to the point of runoff except for the untreated control (pathogen treated + no inducer) and healthy control (no pretreatment + nothing).

Collection of leaf samples. The first leaf sample was collected at 0 h i.e., just after the spraying of inducer compounds and then the collection of samples was carried out at 24, 48, 72, 96, and 120 h. Every time the top leaves of the plant were cut down with the help of sterilized scissors, then carefully wrapped in sterilized blotter paper and again wrapped in aluminum foil and kept in a zip-lock plastic bag. All zip-lock ploy bags were stored at –80°C for further estimation of biochemical activity (POX, PPO, and total phenol).

Disease severity. The effect of elicitor compounds and nano-particles on disease severity of spot blotch disease of wheat was observed; 0-9 rating scale proposed by Directorate of Wheat Research (2001): score 0 = no blight, 1 = up to 10% leaf area blighted, 2 = 11-20% leaf area blighted, 3 = 21-30% leaf area blighted, 4 = 31-40% leaf area blighted, 5 = 41-50% leaf area blighted, 6 = 51-60% leaf area blighted, 7 = 61-70% leaf area blighted, 8 = 71-80% leaf area blighted, and 9 = >80% leaf area blighted, was used for the estimation of the Percent Disease Index (PDI). PDI was recorded at 15, 30, 45, and 60 days after inoculation.

PDI was calculated as described by McKinney (1923) from the formula given below:

$$\text{PDI} = \frac{\text{Sum of all disease rating}}{\text{Total No. of plants observed} \times \text{Maximum rating value}} \times 100$$

No. of spots/leaf and no. of infected leaves/plant (seven plants per pot) were calculated, 15 days after inoculation, in three replications for each treatment.

Protein estimation. Protein estimation was done as per Lowry et al. (1951) and absorbance was recorded at 660 nm on UV-Vis spectrophotometer (Labman Scientific Instruments Pvt. Ltd., Chennai, India). Unknown protein concentration was estimated by comparing the absorbance on the standard curve of bovine serum albumin.

Enzymatic analysis. The assay of POX activity was carried out as per the procedure described by Putter (1974). Enzymes extract was prepared by homogenizing 250 mg of leaf samples in 0.1 M phosphate buffer (pH 7.0). It was centrifuged at 10,000 rpm for 20 min at 4°C and supernatant was collected and used as enzyme source. The reaction mixture of 3 ml consists of 1.5 ml of 1 M phosphate buffer of pH 7.0, freshly prepared 1 ml of 10 mM guaiacol (Sisco Research Laboratories Pvt. Ltd.), 0.1 ml of enzyme extract, and 0.1 ml of 12.3 mM hydrogen peroxide (H_2O_2) (Sisco Research Laboratories Pvt. Ltd.). The reaction mixture without enzyme extract serves as a blank. Initially, absorbance was taken at 436 nm and after that increase in the absorbance activity was noted at the interval of 30 s up to 3 min on the UV-Vis spectrophotometer. The molar extinction coefficient of POX is 25.2 and the enzyme activity will be expressed as µmol/min/mg protein. Enzyme activity was calculated as follows:

$$\text{Unit activity (units/min/gm FW)} = \frac{\text{Change in absorbance/min} \times \text{Total volume (ml)}}{\text{Extinction coefficient} \times \text{Volume of the sample taken (ml)}}$$

$$\text{Specific activity (µmol/min/mg protein)} = \frac{\text{Unit activity (units/min/g FW)}}{\text{Protein content (mg/g FW)}}$$

g/FW = 1 g fresh leaf tissue has been taken; Molar extinction coefficient = 25.2/mM/cm

PPO activity was determined as per the procedure given by Jang et al. (2002). One g of leaf tissue was homogenized in 20 ml of potassium phosphate buffer (0.05 M, pH 7.0). The potassium phosphate buffer containing 1% (w/v) polyvinyl pyrrolidone (Sisco Research Laboratories Pvt. Ltd.). The extract was centrifuged at 13,000 rpm for 20 min at 4°C; obtained supernatant was collected and used for the estimation of PPO. One point two ml of enzyme extract was added to 2.8 ml of catechol (Sisco Research Laboratories Pvt. Ltd.) solution of concentration 20 mM that was prepared in 0.01 M of potassium phosphate buffer (pH 6.8). An increase in absorbance was recorded at 410 nm for 3 min by using a UV-vis spectrophotometer. One unit of enzyme activity is defined as the amount of enzyme catalyzing the conversion of 1 µmol of substrate per min under standard assay condition (Harris and Keshwani, 2009). The enzyme activity was expressed as unit per milligram protein. Enzymatic activity was calculated as follows:

$$\text{Unit activity (units/min/g FW)} = \frac{\text{Change in absorbance/min} \times \text{Total volume (ml)}}{\text{Extinction coefficient} \times \text{Volume of the sample taken (ml)}}$$

$$\text{Specific activity (µmol/min/mg protein)} = \frac{\text{Unit activity (units/min/g FW)}}{\text{Protein content (mg/g FW)}}$$

g/FW = 1 g fresh leaf tissue has been taken; Molar extinction coefficient = 1,197/M/cm

Total phenol was determined as per the procedure given by Singleton and Rossi (1965) with some modification. One-gram of leaves was homogenized in 10 ml of 80% methanol (Sisco Research Laboratories Pvt. Ltd.). Then it was centrifuged at 15,000 rpm for 20 min at 24°C. The supernatant was collected and used for the enzymatic assay. Two hundred µl of methanolic extract were added to 2.8 ml of distilled water and 200 µl of Folin-Ciocalteu reagent (Sisco Research Laboratories Pvt. Ltd.), after this the solution was kept at 25°C for 3 min. After 3 min, 2 ml of a saturated solution (20%) of Na_2CO_3 was added and incubated for 30 min. Then the absorbance was taken at 760 nm. A 'blank' was prepared with distilled water and reagents were added to adjust the absorbance to zero.

After that, the sample concentration was calculated from the catechol standard curve equation and the result was expressed as mg catechol equivalents per gram of fresh weight sample. The total phenol content of extracts was calculated using the formula mentioned below. The analysis was done in three replications.

$$\text{Total phenol} = \frac{c \times v}{m}$$

Where, c is the sample concentration from the calibration curve (mg/ml), v is the volume (ml) of the solvent used for the extraction and m represents the weight (g) of the fresh sample used.

Statistical analysis. The statistical design used for this experiment was Randomized Complete Block Design (Factorial RBD), with three replications. The mean value (three replications) of concentration of POX, PPO, and total phenol at each hour (0, 24, 48, 72, 96, and 120) were used

Table 1. Effect of host defense inducing elicitors and nano-particles on the activity of peroxidase against spot blotch disease of wheat

Treatment	Peroxidase (µmol/min/mg protein)					
	0 h	24 h	48 h	72 h	96 h	120 h
Salicylic acid 1 mM	8.60 ± 0.05	10.27 ± 0.03	10.32 ± 0.02	14.19 ± 0.01	7.41 ± 0.02	5.68 ± 0.02
Salicylic acid 1.5 mM	9.30 ± 0.01	10.67 ± 0.02	13.87 ± 0.01	17.95 ± 0.02	12.44 ± 0.02	8.80 ± 0.04
Salicylic acid 2 mM	7.14 ± 0.04	9.12 ± 0.02	9.17 ± 0.02	10.32 ± 0.02	6.89 ± 0.02	4.98 ± 0.02
Isonicotinic acid 1 mM	6.09 ± 0.06	7.89 ± 0.02	9.23 ± 0.04	10.46 ± 0.02	9.62 ± 0.05	6.92 ± 0.03
Isonicotinic acid 1.5 mM	6.99 ± 0.01	8.33 ± 0.03	9.82 ± 0.03	12.25 ± 0.01	10.41 ± 0.02	7.46 ± 0.02
Isonicotinic acid 2 mM	5.76 ± 0.03	7.40 ± 0.01	8.02 ± 0.03	9.08 ± 0.03	7.72 ± 0.02	6.33 ± 0.02
Chitosan 1 mM	7.83 ± 0.03	8.93 ± 0.03	11.03 ± 0.03	13.98 ± 0.01	11.34 ± 0.02	7.30 ± 0.04
Chitosan 1.5 mM	8.08 ± 0.03	10.01 ± 0.02	12.24 ± 0.02	15.64 ± 0.03	8.17 ± 0.02	8.15 ± 0.03
Chitosan 2 mM	9.50 ± 0.02	10.87 ± 0.02	13.98 ± 0.02	29.08 ± 0.01	19.28 ± 0.03	11.84 ± 0.02
Silver nano-particle 50 ppm	11.26 ± 0.02	12.37 ± 0.02	13.72 ± 0.03	14.86 ± 0.01	20.68 ± 0.02	17.55 ± 0.03
Silver nano-particle 100 ppm	13.21 ± 0.04	14.64 ± 0.02	23.28 ± 0.01	24.95 ± 0.01	31.64 ± 0.02	28.40 ± 0.02
Silver nano-particle 200 ppm	5.59 ± 0.01	12.09 ± 0.02	12.40 ± 0.04	13.56 ± 0.02	18.92 ± 0.02	16.50 ± 0.01
Aluminum nano-particle 50 ppm	11.28 ± 0.03	13.67 ± 0.02	15.83 ± 0.04	16.50 ± 0.04	26.17 ± 0.02	19.62 ± 0.02
Aluminum nano-particle 100 ppm	12.09 ± 0.02	14.44 ± 0.02	17.37 ± 0.02	19.41 ± 0.02	29.37 ± 0.02	20.23 ± 0.02
Aluminum nano-particle 200 ppm	10.30 ± 0.03	10.45 ± 0.03	12.92 ± 0.02	13.53 ± 0.03	14.09 ± 0.05	13.63 ± 0.03
Control (pathogen treated + no inducer)	8.00 ± 0.02	12.44 ± 0.02	10.42 ± 0.02	10.16 ± 0.01	7.11 ± 0.01	1.68 ± 0.02
Healthy control (no pretreatment + nothing)	6.00 ± 0.01	6.02 ± 0.02	6.03 ± 0.01	6.03 ± 0.01	6.03 ± 0.01	6.04 ± 0.02
CD ($P \leq 0.05$)	A = 0.009, B = 0.007, C = 0.010, A × B = 0.016, B × C = 0.018, C × A = 0.023					
CV %	0.196					

A = Treatment, B = Concentration, C = Hour; Mean ± standard deviation value of 3 replications.
CD, critical difference; CV, coefficient of variation.

statistical analysis was performed using three-way analysis of variance (three-way ANOVA). Indostat software 9.1 version was used for the analysis of experimental data. The correlation analysis between different indicators (POX, PPO, and total phenol) and their comparison with PDI in the form of heat map were calculated by using SPSS software.

Results

Effect of elicitor compounds and nano-particles on POX, PPO, and total phenol activity in wheat. It was revealed that a significant increase in enzymatic activity after a particular period. POX (Table 1), PPO (Table 2), and total phenol (Table 3) activity were increased significantly in the case of the treated plant (pathogen + inducers) as compared to the control (pathogen treated + no inducers) and healthy control (no pretreatment + nothing). An increase in POX, PPO, and total phenol activity were recorded at 24 h and reached its highest value at 72 h but after 72 h, there had been a decrease in activity in all the elicitor compounds and in case of nano-particles the regular increase in POX and PPO. The total phenol activity were recorded up to 96 h and at 120 h which displayed decrease in the activity but that was higher than the activity recorded at 0 h. Among all elicitor compounds, the maximum rise in POX, PPO, and total phenol activity were recorded in plant treated with chitosan at 2 mM (pathogen + inducer) at 72 h (29.08 µmol/min/mg protein, 3.44 µmol/min/mg protein and 1.026 mg/g of fresh leaf, respectively), which was highest as compared to treatment with all other elicitor compounds followed by salicylic acid at 1.5 mM as compared to pathogen treated control and healthy control. Among nano-particles maximum activity of POX, PPO, and total phenol were recorded from silver nano-particle at a concentration of 100 ppm at 96 h (31.64 µmol/min/mg protein, 3.49 µmol/min/mg protein and 1.077 mg/g of fresh leaf, respectively) followed by aluminum nano-particle (100 ppm) as compared to control. Enzymatic activity regularly increased up to 96 h; however, after 96 h, a decline in activity was observed at 120 h.

Table 2. Effect of host defense inducing elicitors and nano-particles on the activity of polyphenol oxidase against spot blotch disease of wheat

Treatment	Polyphenol oxidase (µmol/min/mg protein)					
	0 h	24 h	48 h	72 h	96 h	120 h
Salicylic acid 1 mM	1.34 ± 0.01	1.72 ± 0.02	1.81 ± 0.02	2.14 ± 0.02	1.98 ± 0.02	1.87 ± 0.02
Salicylic acid 1.5 mM	1.39 ± 0.01	1.78 ± 0.01	1.82 ± 0.02	3.03 ± 0.03	2.62 ± 0.02	2.26 ± 0.02
Salicylic acid 2 mM	1.23 ± 0.01	1.32 ± 0.02	1.48 ± 0.03	1.83 ± 0.02	1.58 ± 0.02	1.42 ± 0.02
Isonicotinic acid 1 mM	0.58 ± 0.01	1.09 ± 0.02	1.27 ± 0.02	1.81 ± 0.02	1.48 ± 0.01	1.23 ± 0.03
Isonicotinic acid 1.5 mM	1.07 ± 0.02	1.14 ± 0.01	1.72 ± 0.02	2.07 ± 0.02	1.48 ± 0.02	1.41 ± 0.03
Isonicotinic acid 2 mM	0.42 ± 0.03	0.74 ± 0.01	1.24 ± 0.05	1.68 ± 0.02	1.22 ± 0.01	0.86 ± 0.02
Chitosan 1 mM	1.23 ± 0.02	1.44 ± 0.05	1.46 ± 0.04	2.08 ± 0.04	1.94 ± 0.02	1.56 ± 0.02
Chitosan 1.5 mM	1.24 ± 0.02	1.66 ± 0.03	1.75 ± 0.02	2.37 ± 0.04	2.32 ± 0.03	2.07 ± 0.03
Chitosan 2 mM	1.92 ± 0.02	1.99 ± 0.01	2.45 ± 0.04	3.44 ± 0.02	2.46 ± 0.02	2.20 ± 0.02
Silver nano-particle 50 ppm	1.15 ± 0.03	1.67 ± 0.02	1.75 ± 0.02	2.04 ± 0.01	2.95 ± 0.02	2.09 ± 0.02
Silver nano-particle 100 ppm	2.06 ± 0.02	2.42 ± 0.01	3.24 ± 0.01	3.31 ± 0.01	3.49 ± 0.01	3.35 ± 0.04
Silver nano-particle 200 ppm	1.09 ± 0.01	1.12 ± 0.03	1.42 ± 0.02	1.57 ± 0.03	2.87 ± 0.01	1.91 ± 0.02
Aluminum nano-particle 50 ppm	1.72 ± 0.03	2.03 ± 0.03	2.04 ± 0.01	2.47 ± 0.02	3.07 ± 0.01	2.97 ± 0.01
Aluminum nano-particle 100 ppm	1.98 ± 0.01	2.31 ± 0.02	2.58 ± 0.02	2.63 ± 0.03	3.19 ± 0.01	3.06 ± 0.02
Aluminum nano-particle 200 ppm	1.15 ± 0.01	1.27 ± 0.02	1.30 ± 0.01	1.41 ± 0.01	2.13 ± 0.03	1.74 ± 0.01
Control (pathogen treated + no inducer)	0.66 ± 0.01	0.82 ± 0.02	0.63 ± 0.03	0.50 ± 0.01	0.40 ± 0.02	0.35 ± 0.02
Healthy control (no pretreatment + nothing)	0.39 ± 0.02	0.40 ± 0.01	0.40 ± 0.01	0.41 ± 0.02	0.41 ± 0.01	0.41 ± 0.01
CD ($P \leq 0.05$)	A = 0.008, B = 0.006, C = 0.009, A × B = 0.014, B × C = 0.015, C × A = 0.019					
CV %	1.124					

A = Treatment, B = Concentration, C = Hour; Mean ± standard deviation value of 3 replications.
CD, critical difference; CV, coefficient of variation.

Table 3. Effect of host defense inducing elicitors and nano-particles on the activity of total phenol against spot blotch disease of wheat

Treatment	Total phenol (mg/gm of fresh leaf)					
	0 h	24 h	48 h	72 h	96 h	120 h
Salicylic acid 1 mM	0.519 ± 0.003	0.549 ± 0.008	0.594 ± 0.004	0.827 ± 0.004	0.822 ± 0.003	0.742 ± 0.003
Salicylic acid 1.5 mM	0.538 ± 0.001	0.592 ± 0.004	0.602 ± 0.004	0.859 ± 0.004	0.825 ± 0.002	0.772 ± 0.007
Salicylic acid 2 mM	0.507 ± 0.004	0.526 ± 0.003	0.581 ± 0.006	0.768 ± 0.008	0.647 ± 0.004	0.599 ± 0.008
Isonicotinic acid 1 mM	0.352 ± 0.004	0.503 ± 0.005	0.531 ± 0.005	0.769 ± 0.002	0.650 ± 0.009	0.540 ± 0.002
Isonicotinic acid 1.5 mM	0.483 ± 0.006	0.520 ± 0.003	0.538 ± 0.008	0.785 ± 0.001	0.718 ± 0.014	0.604 ± 0.006
Isonicotinic acid 2 mM	0.372 ± 0.002	0.493 ± 0.002	0.523 ± 0.002	0.671 ± 0.005	0.664 ± 0.002	0.533 ± 0.007
Chitosan 1 mM	0.486 ± 0.001	0.531 ± 0.002	0.588 ± 0.003	0.803 ± 0.005	0.770 ± 0.002	0.615 ± 0.010
Chitosan 1.5 mM	0.489 ± 0.004	0.537 ± 0.002	0.601 ± 0.003	0.834 ± 0.002	0.812 ± 0.006	0.683 ± 0.008
Chitosan 2 mM	0.556 ± 0.008	0.592 ± 0.004	0.735 ± 0.007	1.026 ± 0.002	0.971 ± 0.004	0.815 ± 0.008
Silver nano-particle 50 ppm	0.460 ± 0.009	0.486 ± 0.005	0.579 ± 0.003	0.609 ± 0.006	0.729 ± 0.012	0.555 ± 0.005
Silver nano-particle 100 ppm	0.552 ± 0.001	0.639 ± 0.024	0.836 ± 0.004	0.887 ± 0.005	1.077 ± 0.189	0.965 ± 0.003
Silver nano-particle 200 ppm	0.348 ± 0.003	0.439 ± 0.004	0.461 ± 0.002	0.486 ± 0.004	0.614 ± 0.001	0.513 ± 0.031
Aluminum nano-particle 50 ppm	0.546 ± 0.014	0.553 ± 0.003	0.663 ± 0.004	0.679 ± 0.006	0.926 ± 0.005	0.655 ± 0.002
Aluminum nano-particle 100 ppm	0.550 ± 0.005	0.554 ± 0.009	0.695 ± 0.013	0.797 ± 0.011	0.957 ± 0.029	0.674 ± 0.001
Aluminum nano-particle 200 ppm	0.447 ± 0.011	0.462 ± 0.002	0.488 ± 0.014	0.547 ± 0.028	0.625 ± 0.010	0.588 ± 0.003
Control (pathogen treated + no inducer)	0.486 ± 0.007	0.333 ± 0.017	0.269 ± 0.005	0.250 ± 0.003	0.235 ± 0.003	0.210 ± 0.001
Healthy control (no pretreatment + nothing)	0.400 ± 0.001	0.402 ± 0.002	0.402 ± 0.003	0.403 ± 0.001	0.403 ± 0.003	0.405 ± 0.001
CD ($P \leq 0.05$)	A = 0.013, B = 0.010, C = 0.014, A × B = 0.022, B × C = 0.024, C × A = 0.031					
CV %	5.334					

A = Treatment, B = Concentration, C = Hour; Mean ± standard deviation value of 3 replications.
CD, critical difference; CV, coefficient of variation.

Effect of elicitor compounds and nano-particles on PDI, number of spots/leaf and number of infected leaves/plant of spot blotch disease of wheat. The increase and decrease in the activity of POX, PPO, and total phenol has shown a relation with PDI, number of spots/leaf, and number of infected leaves/plant. The results of correlation analysis showed that there was a significant decrease in the severity with the increase in POX, PPO, and total phenol. The result of the experiment presented in Table 4 affirmed that all the elicitors and nano-particles showed a reduction in the PDI as compared to control after 15 days of inoculation. At 60 days after inoculation, in case of plants sprayed with elicitor at 2 mM concentration, PDI was minimum (13.00) in chitosan (Fig. 2A) followed by salicylic acid at 1.5 mM concentration. Whereas in case of nano-particles, 60 days after inoculation a similar pattern was observed at a concentration of 100 ppm where the disease severity was minimum (9.00) in silver nano-particles (Fig. 2B) followed by aluminum nano-particles as compared to control (pathogen treated + no inducer) (Fig. 2C). No. of spots/leaf and

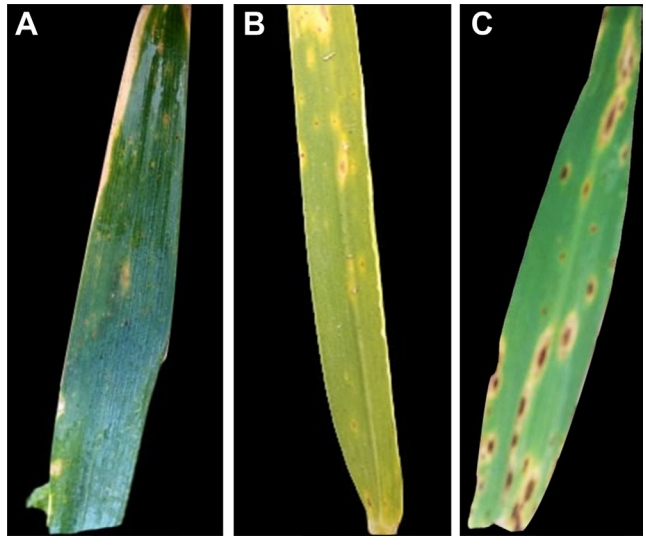

Fig. 2. Spot blotch disease symptom. (A) Symptoms appeared in plant treated with 2 mM of chitosan. (B) Symptoms appeared in plant treated with 100 ppm of silver nano-particle. (C) Symptoms appeared in plant inoculated by pathogen.

Table 4. Effect of host defense including elicitors and nano-particles on percent disease index (PDI) of spot blotch disease of wheat

Treatment	PDI at 15 DAI	PDI at 30 DAI	PDI at 45 DAI	PDI at 60 DAI
Salicylic acid 1 mM	9.00 ± 0.01	14.33 ± 0.03	18.33 ± 0.06	22.33 ± 0.01
Salicylic acid 1.5 mM	5.00 ± 0.02	9.00 ± 0.03	13.00 ± 0.08	17.00 ± 0.02
Salicylic acid 2 mM	10.33 ± 0.01	18.33 ± 0.02	22.33 ± 0.05	30.33 ± 0.01
Isonicotinic acid 1 mM	9.00 ± 0.12	13.00 ± 0.03	21.00 ± 0.10	29.00 ± 0.02
Isonicotinic acid 1.5 mM	9.00 ± 0.06	13.00 ± 0.12	17.00 ± 0.09	25.00 ± 0.01
Isonicotinic acid 2 mM	10.33 ± 0.01	18.33 ± 0.19	26.33 ± 0.04	34.33 ± 0.01
Chitosan 1 mM	9.00 ± 0.03	13.00 ± 0.15	17.00 ± 0.06	25.00 ± 0.01
Chitosan 1.5 mM	6.33 ± 0.03	10.33 ± 0.07	14.33 ± 0.03	18.33 ± 0.01
Chitosan 2 mM	5.00 ± 0.08	5.00 ± 0.15	9.00 ± 0.06	13.00 ± 0.02
Silver nano-particle 50 ppm	7.67 ± 0.04	9.00 ± 0.03	13.00 ± 0.08	17.00 ± 0.01
Silver nano-particle 100 ppm	1.00 ± 0.03	1.00 ± 0.07	5.00± 0.10	9.00 ± 0.01
Silver nano-particle 200 ppm	5.00 ± 0.02	9.00 ± 0.03	13.00 ± 0.08	21.00 ± 0.57
Aluminum nano-particle 50 ppm	6.33 ± 0.03	7.33 ± 0.02	9.00 ± 0.06	13.00 ± 0.10
Aluminum nano-particle 100 ppm	5.00 ± 0.06	5.00 ± 0.04	9.00 ± 0.04	13.00 ± 0.06
Aluminum nano-particle 200 ppm	10.33 ± 0.05	14.33 ± 0.02	18.33 ± 0.02	22.33 ± 0.02
Control (pathogen treated + no inducer)	21.00 ± 0.01	29.00 ± 0.06	38.33 ± 0.01	49.00 ± 0.03
Healthy control (no pretreatment + nothing)	-	-	-	-
CD ($P \leq 0.05$)	A = 1.43, B = 0.94 A × B = 2.49	A = 1.43, B = 0.94 A × B = 2.49	A = 1.17, B = 0.76 A × B = 2.03	A = 1.17, B = 0.76 A × B = 2.03
CV %	14.70	10.18	5.89	4.10

A = Treatment, B = Concentration; Mean ± standard deviation value of 3 replications.
DAI, days after inoculation; CD, critical difference; CV, coefficient of variation.

Table 5. Effect of host defense inducing elicitors and nano-particles on leaf infection of spot blotch disease of wheat

Treatment	No. of spots/leaf	No. of infected leaves/plant
Salicylic acid 1 mM	6.56 ± 0.03	4.67 ± 0.01
Salicylic acid 1.5 mM	5.33 ± 0.08	3.89 ± 0.01
Salicylic acid 2 mM	8.00 ± 0.10	5.67 ± 0.07
Isonicotinic acid 1 mM	7.33 ± 0.15	5.22 ± 0.02
Isonicotinic acid 1.5 mM	7.33 ± 0.07	5.00 ± 0.03
Isonicotinic acid 2 mM	9.89 ± 0.09	6.89 ± 0.02
Chitosan 1 mM	6.67 ± 0.02	4.78 ± 0.01
Chitosan 1.5 mM	6.33 ± 0.03	4.56 ± 0.06
Chitosan 2 mM	4.33 ± 0.10	3.22 ± 0.08
Silver nano-particle 50 ppm	5.89 ± 0.09	4.00 ± 0.06
Silver nano-particle 100 ppm	4.11 ± 0.11	3.00 ± 0.18
Silver nano-particle 200 ppm	6.56 ± 0.06	4.56 ± 0.01
Aluminum nano-particle 50 ppm	5.33 ± 0.03	3.89 ± 0.03
Aluminum nano-particle 100 ppm	5.11 ± 0.07	3.56 ± 0.02
Aluminum nano-particle 200 ppm	6.67 ± 0.03	4.67 ± 0.02
Control (pathogen treated + no inducer)	12.78 ± 0.02	8.89 ± 0.03
Healthy control (no pretreatment + nothing)	-	-
CD ($P \leq 0.05$)	A = 0.58, B = 0.41, A × B = 1.01	A = 0.41, B = 0.29, A × B = 0.71
CV %	12.15	12.98

A = Treatment, B = Concentration; Mean ± standard deviation value of 3 replications.
CD, critical difference; CV, coefficient of variation.

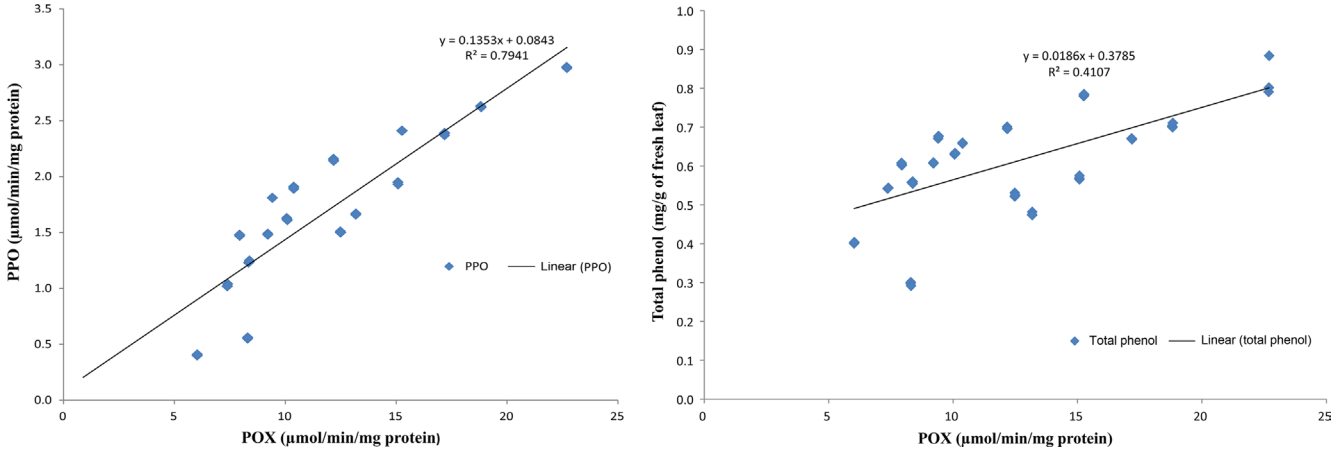

Fig. 3. (A) The positive correlation between peroxidase (POX) and polyphenol oxidase (PPO). (B) The positive correlation between POX and total phenol.

no. of infected leaves/plant showed the same trend as PDI, the data was collected 15 days after inoculation. The results have been presented in Table 5. Minimum no. of spots/leaf and no. of infected leaves/plant were recorded from the treatment with chitosan at the concentration of 2 mM (4.33 and 3.22 respectively) whereas in case of nano-particles, silver nano-particle at the concentration of 100 ppm has given best result with minimum no. of spots/leaf (4.11) and no. of infected leaves/plant (3.00) respectively. The trend showed similarity with the increase in POX, PPO, and total phenol activity, at higher concentration of enzyme at a particular period provided resistance against *B. sorokininan*.

In case of elicitor compounds regular increase in POX, PPO, and total phenol activity was observed up to 72 h and

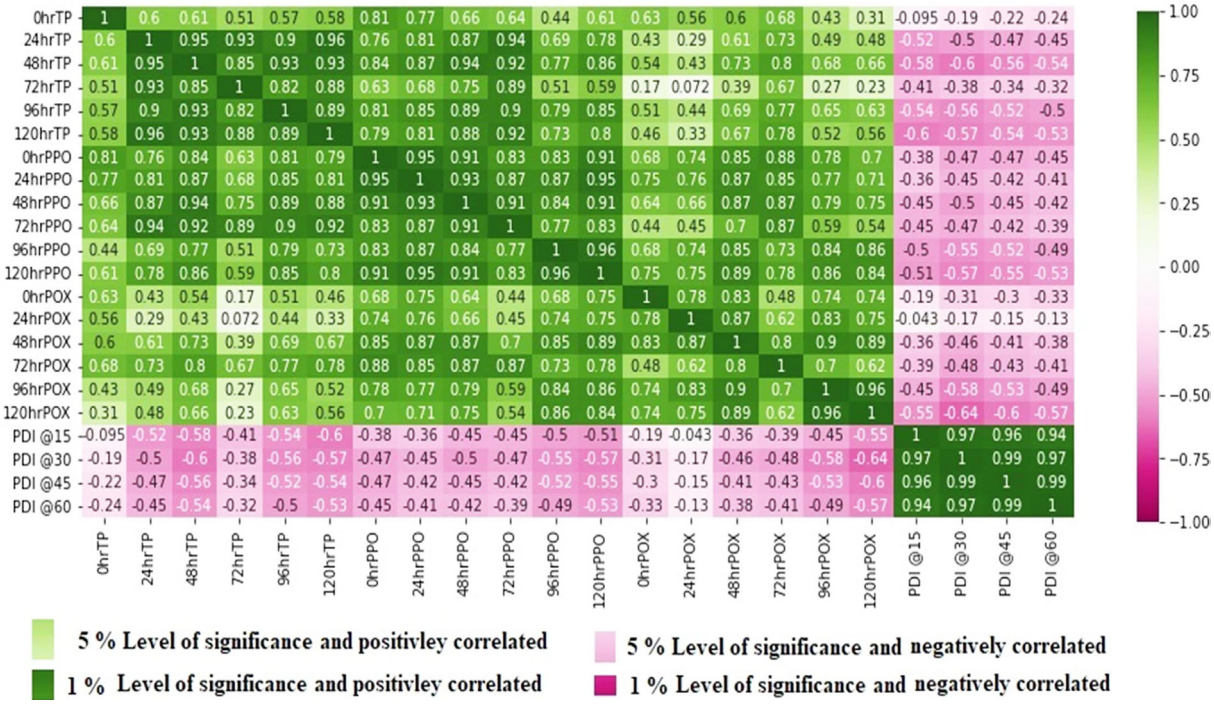

Fig. 4. Correlation heat map showing the negative correlation between the different concentration of enzymatic activity of different inducers and PDI at 15, 30, 45, and 60 DAI. The concentration of enzymatic activity of different inducers at different hrs is positively correlated with each other. TP, total phenol; PPO, polyphenol oxidase; POX, peroxidase; PDI 15, 30, 45, 60, Percent Disease Index at 15, 30, 45, 60 DAI.

whereas for nano-particles regular increase in POX, PPO, and total phenol activity was observed up to 96 h. Rise in the biochemical activity played a key role in the reduction of disease severity.

The correlations between POX and PPO and between POX and total phenol have been presented in Fig. 3A and B. The positive correlation has been observed between all indicators, with the increase in POX the PPO concentration increased by 79.40%, whereas total phenol concentration increased by 41%. The correlation between several inducers at different concentration and PDI has been presented in Fig. 4 in the form of correlation heat map. The negative correlation has been observed, with the increase in the concentration of POX, PPO, and total phenol the PDI gradually decreases.

Discussion

The signaling compounds have the ability to mimic the pathogen insight by plant following the activation of complex defense reaction which includes anti-microbial compound synthesis, hypersensitive response, lignin accumulation in the cell wall and production of ROS (Acharya et al., 2011; Chakarborty et al., 2016; Chandra et al., 2014). PPO and POX also act as a transducer of signals to neighboring unaffected cells (Lamb and Dixon, 1997). According to Schmitz-Hoerner and Weissenböck (2003), total phenol acts by increasing the production of ROS. It accumulates in the sub-epidermal layer of plant tissue and plays a crucial role in the development of disease resistance in plants.

The present study showed that the application of such synthetic compounds like salicylic acid, isonicotinic acid, chitosan, silver, and aluminum nano-particles at different concentrations played an essential and prominent role in inducing immune responses by increasing the enzymatic activity against spot blotch disease. The application of chitosan resulted into notable increase in POX, PPO, and total phenol activity which was acted to minimize disease infection. These results are in accordance with other studies on induced resistance against spot blotch disease in wheat plants where the leaves were treated with chitosan and vanillin-modified chitosan (1-15 mM), showed weaker disease symptoms and also decrease in the injured area on leaves. So, chitosan was considered to be an effective inducer that leads to resistant reaction in wheat against the pathogenic infection of *B. sorokiniana*, which was correlated with change in the leaf POX activity (Popova et al., 2016). In another study, it was found that chitosan salicylate (0.1%) reduced the root rot damage to wheat by 79% and yellow rust by 29.1% and increased the yield by 32.8%, and caused an increase in indices of wheat growth, development and productivity (Kolesnikov et al., 2022). Devi et al. (2019) conducted a field trial and they suggested that soaking wheat seeds in chemical compounds like salicylic acid (10^{-5} M) and $CuSO_4$ (10^{-4} M and 10^{-5} M) increases the accumulation of total phenol and POX and could be used in the management of spot blotch of wheat. The two nanoparticles used in the present experiment increased biochemical enzymatic activity thereby decreasing the disease infection. Hasanuzzaman and Fujita (2013) also reported that the application of silver nanoparticles on wheat plant against abiotic stress enhances the activity of different antioxidant enzymes like polyphenol oxidase, POX, and total phenol. Therefore, it can be concluded that the treatments with chitosan and silver nano-particle increased plant defense responses against the infection of *B. sorokiniana* by up-regulating the enzymatic activity in plant system. Such treatments may be used for wheat crop as a part of disease management strategy in order to avoid the use of fungicides.

Based on the result obtained from the experiment, it is concluded that the use of defense inducer compounds like chitosan and silver nano-particle, successfully restrict the spread and development of spot blotch disease in wheat. Furthermore, this will also enhance the defense activity which stimulates multiple aspects of the plant defense system and can provide resistance against spot blotch pathogens.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

The authors are highly grateful to the Department of Plant Pathology, Bihar Agricultural University, Sabour, Bhagalpur for providing experimental facilities to carry out this work. This research work was financially supported by Directorate of Research, Bihar Agricultural University, Sabour, Bhagalpur.

References

Acharya, K., Chandra, S., Chakraborty, N. and Acharya, R. 2011. Nitric oxide functions as a signal in induced systemic resistance. *Arch. Phytopathol. Plant Prot.* 44:1335-1342.

Bektas, Y. and Eulgem, T. 2015. Synthetic plant defense elicitors. *Front. Plant Sci.* 5:804.

Bodner-Montville, J., Ahuja, J. K. C., Ingwersen, L. A., Haggerty, E. S., Enns, C. W. and Perloff, B. P. 2006. USDA food and nutrient database for dietary studies: released on the web. *J. Food Compos. Anal.* 19:S100-S107.

Bockus, W. W., Bowden, R. L., Hunger, R. M., Morrill, W. L., Murray, T. D. and Smiley, R. W. 2010. Compendium of wheat diseases and pests. 3rd ed. APS Press, St. Paul, MN, USA. 171 pp.

Chakraborty, N., Ghosh, S., Chandra, S., Sengupta, S. and Acharya, K. 2016. Abiotic elicitors mediated elicitation of innate immunity in tomato: an *ex vivo* comparison. *Physiol. Mol. Biol. Plants* 22:307-320.

Chandra, S., Chakraborty, N., Chakraborty, A., Rai, R., Bera, B. and Acharya, K. 2014. Induction of defence response against blister blight by calcium chloride in tea. *Arch. Phytopathol. Plant Prot.* 47:2400-2409.

Chandra, S., Chakraborty, N., Dasgupta, A., Sarkar, J., Panda, K. and Acharya, K. 2015. Chitosan nano-particles: a positive modulator of innate immune responses in plants. *Sci. Rep.* 5:15195.

Choudhary, D. K., Aggarwal, R., Shanmugam, V., Bashyal, B. M. and Vinod. 2018. Image-based phenotyping of spot blotch disease symptoms in wheat varieties. *Int. J. Fauna Biol. Stud.* 5:11-13.

Devi, H. M., Mahapatra, S. and Das, S. 2019. Management of spot blotch of wheat using inducer chemicals under field conditions. *J. Cereal Res.* 11:152-158.

Directorate of Wheat Research. 2001. Annual report, 2000-2001. Directorate of Wheat Research, Karnal, Haryana, India. 98 pp.

Durner, J. and Klessig, D. F. 1995. Inhibition of ascorbate peroxidase by salicylic acid and 2,6-dichloroisonicotinic acid, two inducers of plant defense responses. *Proc. Natl. Acad. Sci. U. S. A.* 92:11312-11316.

Duveiller, E. 2004. Controlling foliar blights of wheat in the rice-wheat systems of Asia. *Plant Dis.* 88:552-556.

Gao, Q.-M., Kachroo, A. and Kachroo, P. 2014. Chemical inducers of systemic immunity in plants. *J. Exp. Bot.* 65:1849-1855.

Harris, T. K. and Keshwani, M. M. 2009. Measurement of enzyme activity. *Methods Enzymol*. 463:57-71.

Hasanuzzaman, M. and Fujita, M. 2013. Exogenous sodium nitroprusside alleviates arsenic-induced oxidative stress in wheat (*Triticum aestivum* L.) seedlings by enhancing antioxidant defense and glyoxalase system. *Ecotoxicology* 22:584-596.

Jang, M. S., Sanada, A., Ushio, H., Tanaka, M. and Ohshima, T. 2002. Inhibitory effects of. 'Enokitake' mushroom extracts on polyphenol oxidase and prevention of apple browning. *LWT Food Sci. Technol.* 35:697-702.

Joshi, A. K., Ortiz-Ferrara, G., Crossa, J., Singh, G., Alvarado, G., Bhatta, M. R., Duveiller, E., Sharma, R. C., Pandit, D. B., Siddique, A. B., Das, S. Y., Sharma, R. N. and Chand R. 2007. Associations of environments in South Asia based on spot blotch disease of wheat caused by *Cochliobolus sativus*. *Crop Sci.* 47:1071-1081.

Kolesnikov, L. E., Popova, E. V., Novikova, I. I., Kolesnikova, Y. R. and Balagurova, E. D. 2022. Application of chitosan to protect wheat from diseases and boost yields. *Appl. Biochem. Microbiol.* 58:329-335.

Kumar, J. and Nagarajan, S, 1998. Role of flag leaf and spike emergence stage on the incidence of Karnal bunt in wheat. *Plant Dis.* 82:1368-1370.

Lamb, C. and Dixon, R. A. 1997. The oxidative burst in plant disease resistance. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48:251-275.

Lamichhane, J. R., Dürr, C., Schwanck, A. A., Robin, M.-H., Sarthou, J.-P., Cellier, V., Messéan, A. and Aubertot, J.-N. 2017. Integrated management of damping-off diseases: a review. *Agron. Sustain. Dev.* 37:10.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265-275.

McKinney, H. H. 1923. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. *J. Agric. Res.* 26:195-218.

Percival, G. C. 2001. Induction of systemic acquired disease resistance in plants: potential implications for disease management in urban forestry. *J. Arboricult.* 27:181-192.

Popova, E. V., Domnina, N. S., Kovalenko, N. M., Sokornova, S. V. and Tyuterev, S. L. 2016. Effect of chitosan and vanillin-modified chitosan on wheat resistance to spot blotch. *Appl. Biochem. Microbiol.* 52:537-540.

Putter, J. 1974. Peroxidases. In: *Methods of enzymatic analysis*, ed. by H. U. Bergmeyer, pp. 685-690. Academic Press, New York, USA.

Raemaekers, R. H. 1991. First occurrence in nature of *Cochliobolus sativus*, the teleomorph of *Bipolaris sorokiniana*. In: *Contribution to the epidemiology of Bipolaris sorokiniana diseases and the development of rainfed wheat, a new crop in Zambia*, pp. 70-85. Katholieke Universiteit e Leuven, Leuven, Belgium.

Saari, E. E. 1998. Leaf blight disease and associated soilborne fungal pathogens of wheat in South and Southeast Asia. In: *Helminthosporium blights of wheat: spot blotch and tan spot,* eds. by E. Duveiller, H. J. Dubin, J. Reeves and A. McNab, pp. 37-51. CIMMYT, Mexico City, Mexico.

Schmitz-Hoerner, R. and Weissenböck, G. 2003. Contribution of phenolic compounds to the UV-B screening capacity of developing barley primary leaves in relation to DNA damage and repair under elevated UV-B levels. *Phytochemistry* 64:243-255.

Singleton, V. L. and Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* 16:144-158.

Song, F. and Goodman, R. M. 2001. Molecular biology of disease resistance in rice. *Physiol. Mol. Plant Pathol.* 59:1-11.

Thakur, M. and Sohal, B. S. 2013. Role of elicitors in inducing

resistance in plants against pathogen infection: a review. *Int. Sch. Res. Notices* 2013:762412.

Van Ginkel, M. and Rajaram, S. 1993. Breeding for durable resistance to diseases in wheat: an international perspective. In: *Durability of disease resistance*, eds. by T. Jacobs and J. E. Parlevliet, pp. 259-272. Springer, Dordrecht, Germany.

Vernooij, B., Friedrich, L., Goy, P. A., Staub, T., Kessmann, H. and Ryals, J. 1995. 2,6-Dicholoroisonicotinic acid-induced resistance to pathogens without the accumulation of salicylic acid. *Mol. Plant-Microbe Interact*. 8:228-234.

Wiese, M. V. 1998. Compendium of wheat diseases. In: *Helminthosporium blights of wheat: spot blotch and tan spot,* eds. by E. Duveiller, H. J. Dubin, J. Reeves and A. McNab, pp. 114-118. CIMMYT, El Batan, Mexico.

Zillinsky, F. J. 1983. Common diseases of small grain cereals: a guide to identification. CIMMYT, Mexico. 141 pp.