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Estimation of Biochemical changes in wheat (*Triticum spp*.) due to Spot Blotch Diseases (*Bipolaris sorokiniana* Sacc.) by using different Treatments

Vishwa Vijay Raghuvanshi [1]*, Subhash Chandra [1], Shyam Narayan Patel [1], Suraj Kumar Patel [2], Shubham Patel [1], Abhishek Singh [1] and Prabha Siddharth [3]

[1] Department of Plant Pathology, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya (Uttar Pradesh), India.

[2] Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (Uttar Pradesh), India.

[3] Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur (Uttar Pradesh), India.

(Corresponding author: Vishwa Vijay Raghuvanshi)*

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ABSTRACT: *Bipolaris sorokiniana* (Sacc.) Shoemaker, a hemi biotrophic, phytopathogenic fungus, is the cause behind spot blotch disease. Warmer and more humid regions around the world are more prone to the spot blotch disease of wheat. Spot blotch (*Bipolaris sorokiniana*) symptoms start out as little, brown lesions that spread throughout the leaf and get larger as the disease progresses. Total phenolic contents and Phenylalanine ammonia-lyase that have been well-documented to play a significant role in disease resistance in a number of pathosystems, inhibiting the colonization of plant tissue. Different intervals of hours after inoculation (hai) have an impact on the total phenolic content (TPCs) and phenylalanine ammonia-lyase (PAL) in wheat leaves. Inoculation at various intervals (00 hai, 24 hai, 48 hai, and 72 hai) affects the amount of TPCs and PAL in wheat leaves. Maximum phenolic contents (134 mg/g, 156 mg/g, 221 mg/g, and 82 mg/g at 00 hai, 24 hai, 48 hai, and 72 hai, respectively) were recorded in T4 (seed treatment with *Pseudomonas fluorescens* @ 10g/kg of seed + 2 foliar spray garlic clove extract), while lowest phenolic contents were observed in T9 (untreated). The T4 treatment plot (seed treatment with *Pseudomonas fluorescens* @ 10g/kg of seed + 2 foliar spray garlic clove extract) had the highest phenylalanine ammonia-lyase concentrations (0.191, 0.214, 0.374, and 0.042 at 00 hai, 24 hai, 48 hai, and 72 hai, respectively), whereas the T9 control plot (untreated) had the lowest concentrations.

Keywords: Total phenolic contents, Phenylalanine ammonia-lyase, Spot blotch, *Bipolaris sorokiniana* and Hours after inoculation.

INTRODUCTION

Wheat (*Triticum* spp.) is the most important food grain crop in India after rice as well as recognized as a world's major cereal crop and staple food of many regions. Wheat belongs to family Poaceae (Yadawad *et al*., 2015). Wheat is C3 plant and as such it thrives in cool and moist environments. The optimum temperature is required for the cultivation of wheat is about 25ºC, with minimum and maximum growth temperatures 3-4ºC and 30-32ºC, respectively (Briggle, 1980). Wheat crop is affected by a number of diseases caused by fungi, bacteria, viruses and nematodes. Wheat crop is mostly affected by fungal diseases such as black stem rust [*Puccinia graminis tritici* (Pers.) Eriks and Henn.], leaf rust (*Puccinia recondita* Rob. Ex. Desm), yellow rust (*Puccinia striformis* West), loose smut [*Ustilago segetum tritici* (Jens) Schaf], karnal bunt [*Neovossia indica* (Mitra) Mundkur], foliar blight (*Bipolaris sorokiniana*) and powdery mildew (*Erysiphe graminis tritici* D, C,) etc. Among the foliar blight disease, spot blotch of wheat is emerged as a major threat in hot and humid wheat growing regions (Van Ginkel and Rajaram 1998).

Among the fungal diseases of wheat, spot blotch disease is caused by *Bipolaris sorokiniana* (Sacc.) Shoemaker, is a hemi biotrophic, phytopathogenic fungus. Spot blotch disease of wheat is prevalent in warmer and humid regions of the world. Symptoms of *Bipolaris sorokiniana* usually induce on the leaf, sheath and stem (Chand and Joshi 2004). In case of severe infection, symptoms may also appear on the spikes, resulting in low weight shrivelled grains (Kiesling, 1985) with black point at the embryonal end of kernels (Chand and Joshi 2004). The symptoms of spot blotch (*Bipolaris sorokiniana*) are appeared as small, brown lesions which are outspread throughout the leaf and enlarge in size with stage advancement. In later, these lesions are change in to large spot (oval to obolong and measuring 0.6 to 10 mm long and 3 to 5 mm wide) after one weak of infection. Spot blotch (*Bipolaris sorokiniana*) becomes more severe especially during

grain filling stage of crop (Joshi and Chand 2002) and causes huge yield losses (Sharma *et al*., 1997).

Bipolaris sorokiniana (Sacc.) Shoemaker is a seed and soil borne fungal pathogen, which causes foliar blight/spot blotch, seedling blight, head blight, common root rot and black point of wheat, barley and other small cereal grain crops (Wiese, 1998). Symptoms usually develop in the form of dark brown necrotic lesions (boat / eye shaped) appear on the leaves, coleoptiles, stem, crown and roots with or without yellow halo. The main symptoms caused by the *Bipolaris sorokiniana* is spot blotch, which is nothing but the disease of leaf. The initial spots on the leaves are 1 to 2 mm long, small and dark brown in colour. In later stage of crop the small lesions are extends very rapidly and change in to a large spot. The disease severity of spot blotch is affects more than 10 million hectare of wheat crop in Indo-Gangetic plains (Nagarajan and Kumar 1998). Spot blotch reduce the grain yield upto 25 percent in affected areas (Saari, 1998). Spot blotch is considered as a major constraint to wheat yield in South Asia due to loss of test weight and grain yield (Singh *et al.*, 2007).

It is generally known that phenolic chemicals, such as phytoalexins or phytoanticipins, or physical barriers, such as lignins, can play a role in a number of pathosystems. A crucial part in disease resistance, blocking the colonisation of plant tissue (Nicholson, Hammerschmidt, 1992). Rapid phenol buildup at the infection site inhibits pathogen growth and enables the activation of phytoalexins or other stress-related compounds (Matern and Kneusel 1988). According to a study, resistant varieties of *Alternaria triticina* were shown to have significantly greater total phenol contents (TPCs) than susceptible ones (Mishra *et al*., 2011). Being the first step in the phenylpropanoid biosynthesis pathway, phenylalanine ammonialyase (PAL) also plays a significant role in the biosynthesis of the various families of phenolics (coumarins, flavonoids, and lignins), phenolic derivatives, and its activity is correlated with the level of synthesis of the phenolic compound.

In several systems, fungal infection has been shown to induce PAL activity before a rise in the phenolic content (Mazeyrat *et al*., 1999; Pereira *et al*., 1999). According to Nicholson and Hammerschmidt (1992), PAL and phenolics play a direct impact in how distinct crop species display resistance. The goal should be to discover the biochemical elements responsible for resistance so that appropriate management measures can be developed or they can be used as molecular markers in plant breeding projects. TPC, PAL, and lignin deposition variations have been utilised as markers for early selection of various plant species that are resistant to certain diseases. These enzymes have been linked to pathogen defence mechanisms in a number of plant species (Thilagavathi *et al*., 2007). Peltonen & Karjalainen (1985) noted that after 24-32 and 40 hours following *Bipolaris sorokiniana* inoculation, PAL activity increased in the leaves of scarcely resistant cultivars. Numerous plant diseases have been shown to have their cell walls strengthened through the development of lignin and the accumulation of cell wall-bound phenolic chemicals (Niemann *et al*., 1991). Therefore, it is anticipated that investigating the mechanisms at the cellular and molecular levels would help us understand how wheat resists *Bipolaris sorokinana*. The purpose of the current study is to determine how PAL, TPC, and lignin contribute to the development of gradual blighting of spot blotch in wheat recombinant inbred lines (RILs). This study investigates if these elements could be combined with phenotypic criteria to effectively select wheat genotypes that are resistant to spot blotch. PAL accumulates more quickly and achieves higher levels of activity during resistive reactions in incompatible interactions than in compatible ones, and buildup of PAL is frequently linked to cell necrosis, a hypersensitive reaction of the invading tissue (Ralton *et al*. 1988). Due to their significance in determining a plant's susceptibility or resistance to disease, biochemical parameters in plants have attracted a lot of interest (Nyadanu *et al*. 2013). The rate of synthesis and/or the plants' pre-infection levels may affect the phenol content of infected plants. According to Bazzalo *et al*. (1985), certain phenols that accumulated in lesions exhibit fungitoxic effects on the mycelial development in vitro, suggesting that phenol levels may be correlated with lesion size. Secondary metabolic factors should be taken into account while choosing resistant cultivars, according to one suggestion (Wenzel 1985).

History and Distribution. In India, the disease was first recorded in 1914 by Mohy from Pusa, Bihar (Joshi *et al*. 1986) and thorough research were later conducted by McRae (1924), who also reported a *Bipolaris sorokiniana* outbreak in the Pusa area in 1930. The spot blotch pathogen *Bipolaris sorokiniana* has been identified as the causal agent of wheat leaf blight in India's NEPZ (Joshi and Chand, 2002; Joshi *et al*., 2004). It is a serious disease in that mega-environment, which is characterized by high humidity levels both before and after the heading stage (Duveiller *et al*. 1998).

Symptomatology. Under favourable conditions the whole ear including awns are severely diseased and seeds are heavily infected and shrivelled, grain size largely reduced and grain yield adversely affected. When the fungus attacks spike, the grains are also affected causing light brown to blackish discolouration around the germination point of the seed which is called black point. The market value of grains also reduces due to shriveled and black pointed grains (Mahto and Bimb (1996). Leaf blight occurs 5-8 weeks after wheat sowing and the symptoms advance after heading and spread very fast. The dark brown necrotic spots (boat shaped) occur on the coleoptiles, leaves, crowns, stems, and roots with or without yellow halo around these. Darkening of the sub-crown internode is a characteristic symptom.

MATERIALS AND METHODS

Plant material and field trial: The present research entitled “Estimation of biochemical changes in wheat

(*Triticum spp*.) due to spot blotch diseases (*Bipolaris sorokiniana* Sacc.) by using different treatments" was carried out at main experimental station, wheat pathological laboratory, Plant Pathology laboratory and Agricultural Bio-chemistry laboratory of Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, U.P. India. This University is located in the Indo-Gangetic plains of Eastern Uttar Pradesh at latitude 26.47˚N, longitude 82.12˚S and at an altitude of 113 meter above the sea level. The experiments were conducted during *Rabi* season 2021-22.

For the estimation of biochemical changes in wheat due to spot blotch disease (*Bipolaris sorokiniana),* we conducted a field trial using different treatments such as two bio-agents, three botanicals, one bioenhancer, and two fungicides. Among these, **T_1** = Seed treatment with *Trichoderma viride* @ 4gm/kg of seed, **T_2** = Seed treatment with *Pseudomonas fluorescens* 10gm/kg of seed, **T_3** = Seed treatment with *Trichoderma viride* @ 4gm/kg of seed + 2 foliar spray of neem leaf extract @ 10%, **T_4** = Seed treatment with *Pseudomonas fluorescens* @ 10gm/kg of seed + 2 foliar sprays of garlic clove extract @ 10%, **T_5** = Seed treatment with *Pseudomonas fluorescens* @ 10gm/kg of seed + 2 foliar sprays of Tulsi leaf extract @ 10 %, **T_6** = Seed treatment with *Pseudomonas fluorescens* @ 10gm/kg of seed + 2 foliar sprays of Nativo (Tebuconazole 50% + Trifloxystrobin 25%) @ 0.4% / lit., **T_7** = Seed treatment with *Trichoderma viride* @ 4gm/kg of seed + 2 foliar sprays of Hexaconazole @ 0.1 % / lit., **T_8** = Seed treatment with Jeevamrit @ 5% + 2 foliar sprays of Hexaconazole @ 0.1 % / lit. and **T_9** = Control (untreated).

Field trial details:

Variety: NW - 1014
Design: RBD
No. of treatments: 09
No. of replications: 03

Inoculation of the pathogen: Artificial inoculation was produced using a pure culture of *Bipolaris sorokiniana*. The pathogen was grown on sorghum grain, and a spore suspension was uniformly sprayed at GS 50 in the late afternoon using a hemocytometer to adjust the concentration to 10-4 spore ml^{-1} of water.

Estimation of Total Phenolic Contents (TPCs): At 0, 24, 48, and 72 hours after inoculation (hai), 1 gramme of leaf from each of the three replications was collected, mixed with 20 ml of 80% alcohol, and the mixture was then centrifuged at 1000 g for 15 minutes before being filtered through Whatman filter paper. 1 ml each of distilled water, standard gallic acid solution, and filtrate were transferred into three test tubes. When the volume reaches 5 ml, 1 ml of sodium carbonate and 1 ml of phenol reagent were added. All test tubes were held at room temperature for an hour before the spectronic 20 was used to record the colour intensity at 750 nm.

Reagents for TPCs:

I. Folin-ciocalteu reagent (FCR).
II. 80% Ethanol.
III. 20% Sodium carbonate.

$$\text{TPC}\left(\frac{\text{mg.}}{\text{100g}}.\right) = \frac{\text{Amount of gallic acid from standered}}{\text{O. D. of known gallic acid solution}} \times \frac{\text{sample O. D.}}{\text{sample weight}} \times \frac{\text{vol. made up}}{\text{vol. of aliquot}}$$

Estimation of phenylalanine ammonia-lyase (PAL): Flag leaf was collected from all the three replications in the field after inoculation. PAL activity was analysed as the rate of conversion of L- phenylalanine into trans-cinnnamic acid at 270nm UV-spectrophotometer. Sample was prepared by crossing 100mg plant tissue in a 50µl buffer (100mM sodium borate buffer, 1.4mM β-mercaptoethanol and 1 per cent PVPP). After homogenization samples were centrifuged at 10,000 rpm for 5 minutes and supernatant was stored at -20° C for further analysis. This supernatant was treated as crude enzyme extract. 50µl of enzyme extract were treated with 0.5ml of 0.1M trisodium borate buffer (pH 8.5) and 0.5ml of 12mM L-phenylalanine in same buffer. The volume of reaction mixture was made up to 3ml with deionized H_2O and immediately mixed by inversion and recorded the increase in absorbance at 270nm for approximately 5 minutes. The samples were prepared in duplicate for each analysis and the mean value of the ΔA270nm/minute was obtained using the maximum linear rate for both the test and blank. Units/ml= (ΔA_{270}nm/minute Test - ΔA_{270}nm/minute blank) (df)/ (19.73) volume of enzyme. Where ΔA is the change in absorbance, (dfl), 19.73 is the milli molar extinction coefficient of trans-cinnamate at 270nm.

RESULTS AND DISCUSSION

1. Estimation of total phenolic contents (mg/g): Total phenolic content (TPCs) in leaves of wheat is influenced by different intervals of hours after inoculation (hai) have been clearly presented in Table 1 and in Fig. 1. TPCs content in wheat leaves is influenced by inoculation at different intervals (00 hai, 24 hai, 48 hai and 72 hai).

Maximum phenolic contents (134 mg/g, 156 mg/g, 221 mg/g and 82 mg/g at 00 hai, 24 hai, 48 hai and 72 hai respectively) was recorded in T_4 (seed treatment with *Pseudomonas fluorescens* @ 10g/kg of seed + 2 foliar spray garlic clove extract) and lowest phenolic content was recorded in T_9 (untreated). Total phenolic content increased significantly with increasing time from 24 to 48 hai and drastically decreased at 72 hai. Chand *et al.* (2013) and Malik *et al.* (2017) reported the total phenolic content (TPC) at different intervals of hours after inoculation (hai) and it is increased significantly with increased time from 24 to 48 hours after inoculation and reduce at 72 hours after inoculation.

Table 1: Estimation of total phenolic contents (mg/g).

Total Phenolic Contents (mg/g)					
Sr. No.	**Treatments**	**0 hai**	**24 hai**	**48 hai**	**72 hai**
T_1	Seed treatment with *Trichoderma viride* @ 4g/kg of seed	130	149	210	74
T_2	Seed treatment with *Pseudomonas fluorescens* 10g/kg of seed	127	147	206	72
T_3	Seed treatment with *Trichoderma viride* @ 4g/kg of seed + 2 foliar spray of neem leaf extract @ 10%	132	152	214	78
T_4	Seed treatment with *Pseudomonas fluorescens* @ 10g/kg of seed + 2 foliar sprays of garlic clove extract @ 10%	134	156	221	82
T_5	Seed treatment with *Pseudomonas fluorescens* @ 10g/kg of seed + 2 foliar sprays of tulsi leaf extract @ 10 %	130	143	196	66
T_6	Seed treatment with *Pseudomonas fluorescens* @ 10g/kg of seed + 2 foliar sprays of nativo (tebuconazole 50% + trifloxystrobin 25%) @ o.4% / lit.	122	137	176	64
T_7	Seed treatment with *Trichoderma viride* @ 4g/kg of seed + 2 foliar sprays of hexaconazole @ 0.1 % / lit.	121	134	172	62
T_8	Seed treatment with jeevamrit @ 5% + 2 foliar sprays of hexaconazole @ 0.1 % / lit.	119	131	170	59
T_9	Control (untreated)	112	124	176	43
SE(m)		**1.09**	**1.10**	**1.14**	**0.59**
CD (1%)		**3.22**	**3.25**	**3.36**	**1.73**

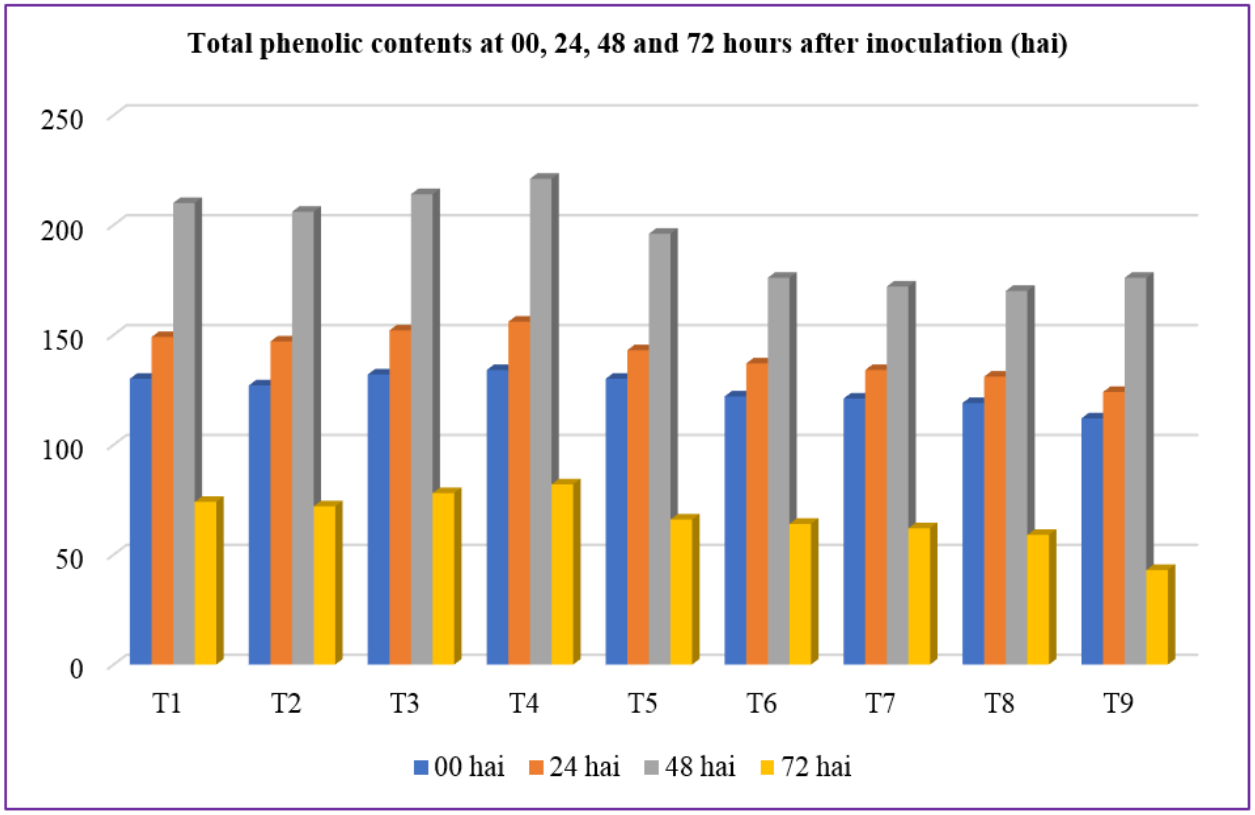

Fig. 1. Total phenolic contents (TPCs) at 00, 24, 48 and 72 hai in different treatments.

2. Estimation of phenylalanine ammonia-lyase (unites/g): Phenylalanine ammonia-lyase (PAL) in wheat leaves is influenced by different intervals of hours after inoculation (hai) have been presented in Table 2 and in Fig. 2.

Highest phenylalanine ammonia-lyase (0.191, 0.214, 0.374 and 0.042 at 00 hai, 24 hai, 48 hai and 72 hai respectively) was noted in T_4 (seed treatment with *Pseudomonas fluorescens* @ 10g/kg of seed + 2 foliar spray garlic clove extract) and lowest was found in T_9 control plot (untreated). Phenylalanine ammonia-lyase increased significantly with increasing time from 24 to 48 hai and drastically decreased at 72 hai. Malik *et al.* (2017) reported induction of PAL activities were recorded in resistant genotype NIDW 295 (356 %, 184.3%) and PDW 314 (253.6 %, 156.6%) whereas minimum response was recorded in susceptible genotypes.

Table 2: Estimation of phenylalanine ammonia lyase (unites/g).

Phenylalanine ammonia lyase (unites/g)					
Sr. No.	**Treatments**	**0 hai**	**24 hai**	**48 hai**	**72 hai**
T_1	Seed treatment with *Trichoderma viride* @ 4g/kg of seed	0.182	0.201	0.361	0.029
T_2	Seed treatment with *Pseudomonas fluorescens* 10g/kg of seed	0.177	0.197	0.357	0.025
T_3	Seed treatment with *Trichoderma viride* @ 4g/kg of seed + 2 foliar spray of neem leaf extract @ 10%	0.181	0.204	0.36	0.032
T_4	Seed treatment with *Pseudomonas fluorescens* @ 10g/kg of seed + 2 foliar sprays of garlic clove extract @ 10%	0.191	0.214	0.374	0.042
T_5	Seed treatment with *Pseudomonas fluorescens* @ 10g/kg of seed + 2 foliar sprays of tulsi leaf extract @ 10 %	0.174	0.195	0.351	0.025
T_6	Seed treatment with *Pseudomonas fluorescens* @ 10g/kg of seed + 2 foliar sprays of nativo (tebuconazole 50% + trifloxystrobin 25%) @ 0.4% / lit.	0.171	0.191	0.347	0.021
T_7	Seed treatment with *Trichoderma viride* @ 4g/kg of seed + 2 foliar sprays of hexaconazole @ 0.1 % / lit.	0.167	0.187	0.341	0.018
T_8	Seed treatment with jeevamrit @ 5% + 2 foliar sprays of hexaconazole @ 0.1 % / lit.	0.165	0.184	0.338	0.016
T_9	Control (untreated)	0.142	0.179	0.249	0.014
SE(m)		**0.001**	**0.002**	**0.003**	**00.00**
CD (1%)		**0.004**	**0.006**	**0.01**	**0.001**

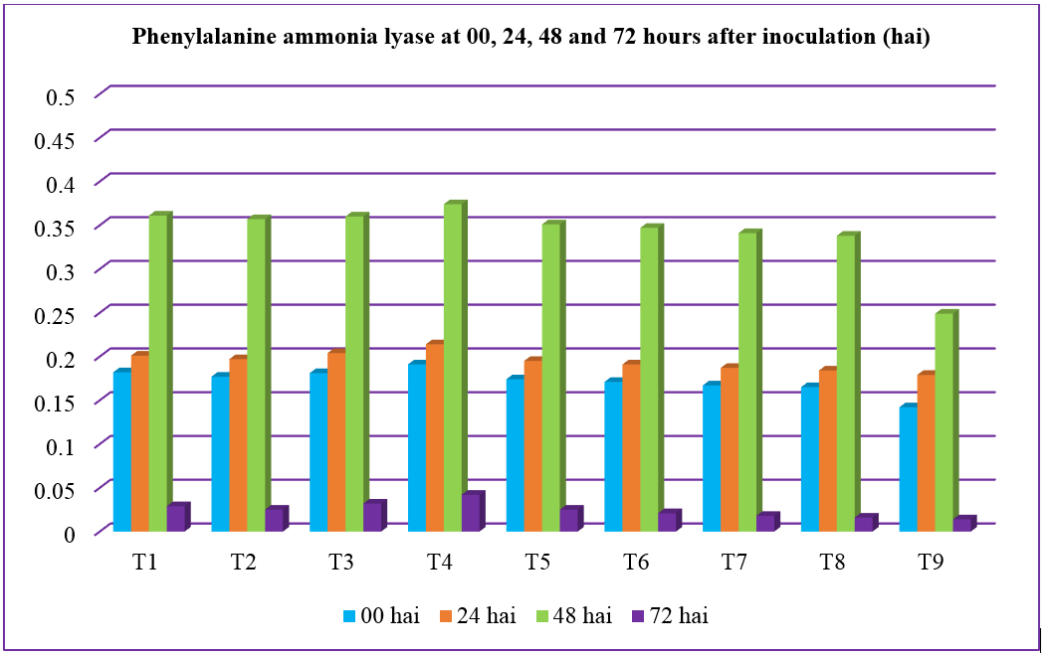

Fig. 2. Phenylalanine ammonia lyase (PAL) at 00, 24, 48 and 72 hai in different treatments.

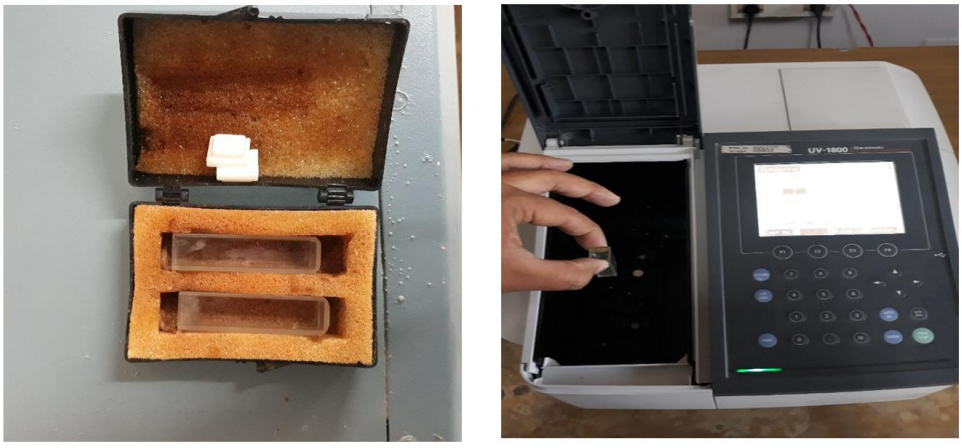

Fig. 3. Estimation of total phenolic contents and phenylalanine ammonia-lyase in Spectrophotometer.

CONCLUSION

1. In the present investigation, botanical and bio-agent treated plots recorded higher total phenolic contents (TPCs) and phenylalanine ammonia lyase (PAL) as compared to chemically treated and untreated plots.
2. Different time intervals after inoculation have an impact on the total phenolic content (TPCs) and phenylalanine ammonia-lyase (PAL) in wheat leaves.
3. Total phenolic Content (TPC) and Phenylalanine Ammonia-lyase (PAL) were measured at various time points after inoculation (hai). They considerably increased with passing time, from 24 to 48 hours after inoculation, and then decreased at 72 hours after inoculation.
4. Maximum phenolic contents (134, 156, 221 and 82 at 00 hai, 24 hai, 48 hai and 72 hai respectively) was recorded in T_4 (seed treatment with *Pseudomonas fluorescens* @ 10g/kg of seed + 2 foliar spray garlic clove extract) and lowest phenolic content was recorded in T_9 (untreated).
5. Highest phenylalanine ammonia-lyase (0.191, 0.214, 0.374 and 0.042 at 00 hai, 24 hai, 48 hai and 72 hai respectively) was noted in T_4 (seed treatment with *Pseudomonas fluorescens* @ 10g/kg of seed + 2 foliar spray garlic clove extract) and lowest was found in T_9 control plot (untreated).

FUTURE SCOPE

If the phenolic content and phenylalanine ammonia-lyase (PAL) present in plants are higher, then disease attack will decrease. Botanicals and bioagents help increase the total phenolic content and PAL in plants, which provide resistance against diseases. Neem leaf extract, garlic clove extract, tulsi leaf extract, *Trichoderma viride* and *Pseudomonas fluorescens* are more effective at increasing phenolic content.

Conflicts of Interest. Nil.

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