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Impacts of Wheat Enzymatic Defense Mechanisms Response to Cereal Cyst Nematode (*Heterodera avenae*) Infection

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Biochemical trial was conducted to find out the variation in enzymic activities (PO, PPO, and PAL) infested with *Heteroderaavenaeon* wheat under cage house conditions. Results exhibited highest enzymatic activities (PO, PPO, and PAL) with application of chemical treatment (fluopyram 34.48% SC at 5 ml/kg seed). The chemical treatment significantly reduced nematode reproduction, as indicated by number of females per 5g root (9.10), cysts per 200 cc soil (12.45) and final larvae population per 200 cc soil (212.70).

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Keywords: Wheat; cereal; cyst; nematode; infection.

1. INTRODUCTION

Wheat (*Triticum spp.*) is among the most essential cereal crops globally providing sustenance to a significant portion of the population and occupying a prominent role in food security and agricultural economies. Wheat is rich in carbohydrates and proteins, and it is an important source of essential micronutrients, such as B vitamins, iron, and zinc (Gupta & Singh, 2020). Major wheat-producing countries including China, India, United States and Russia contribute to global wheat trade and food supply helping stabilize food prices and availability (FAO, 2021). However, wheat production faces multiple biotic and abiotic challenges such as climate variability and pest infestations which impact yield and quality (Langridge et al., 2022).

Among biotic stresses, cereal cyst nematode (*Heterodera avenae*) is a destructive pest that damages wheat roots reducing nutrient uptake and compromising yield (Meagher et al., 1978). *H. avenae* infection interferes with wheat's physiological processes prompting research into breeding for resistance and understanding the biochemical pathways that facilitate this resistance (Sharma et al., 2022). Integrated pest management (IPM) strategies combining chemical, biological and varietal approaches have proven effective for nematode control with a growing focus on wheat cultivars exhibiting natural resistance to nematodes and other pathogens (Ali et al., 2020).

2. METHODOLOGY

Anexperimentwasconductedtofindoutbiochemical variationofPeroxidase (PO), Polyphenol oxidase (PPO) and Phenylalanine ammonia lyase (PAL) in wheat caused by cereal cystnematode *H. avenae*. The trial was conductedin Department of Nematology, RCA, MPUAT, Udaipur under cage house conditions in earthen clay pots filled with naturally infested soil with test nematode. Susceptible wheat variety RAJ-4037 was sown in each pot and three treatments *viz*., cyst nematode alone, soil application with fluopyram 34.48 % SC at 5ml/kg seed and absolute control with ten replication. The pots were watered regularly and rotated in order to avoid effect of sun and shade. Utmost care was taken for better plant growth. After 120 days of sowing, the plants were uprooted and following observations *viz:* number of females/5g root, number of cysts/200 cc soil, final larvae population/200cc soil were recorded. Enzyme analysis of Peroxidase (PO), Polyphenol oxidase (PPO) and Phenylalanine ammonia lyase (PAL) were determined after completion of experiment. Data were analyzed statistically for interpretation of experimental findings.

3. RESULTS AND DISCUSSION

3.1 Biochemical Variation

3.1.1 Specific activity of peroxidase (PO) (changes in absorbance min^{-1} μg^{-1}protein)

Data showed that peroxidase activity was significantly increased in wheat as compared to untreated check. The peroxidise activity was recorded maximum in fluopyram at 5ml/kg seed (0.12 µg)followed by absolute control (0.08 µg) and nematode alone (0.064 µg).

3.1.2 Specific activity of polyphenol oxidase (PPO) (changes in absorbance min^{-1} μg^{-1}protein)

Results of pool analysis (2022-23 and 2023-24) revealed thatpolyphenol oxidase activity was observed maximum with fluopyram at 5ml/kg seed (0.09 µg) followed by absolute control (0.05 µg). and nematode alone (0.03 µg). These treatments were significantly differ from each other.

3.1.3 Specific activity of phenylalanine ammonia lyase (PAL) (nmol trans-cinnamic acid min^{-1} μg^{-1}protien)

Pooled analysis showed that phenylalanine ammonia lyaseactivity was significantly increased in wheat as compared to untreated check. PAL activity was noticed highest influopyram at 5ml/kg seed (0.07 µg) followed by absolute control (0.06 µg)and nematode alone (0.03 µg).

3.2 Nematode Reproduction Parameters

Seed treatment with fluopyram 34.48% SC at 5ml/kg challenged with *H. avenae* in wheat crop at the time of harvesting was effective in suppressing the incidence of *H. avenae*. Further, it reduces nematode reproduction *viz*., number of females/5g root (9.10), cysts/200 cc soil (12.45) and larvae population/200 cc soil (212.70) were observed. However, nematode reproduction

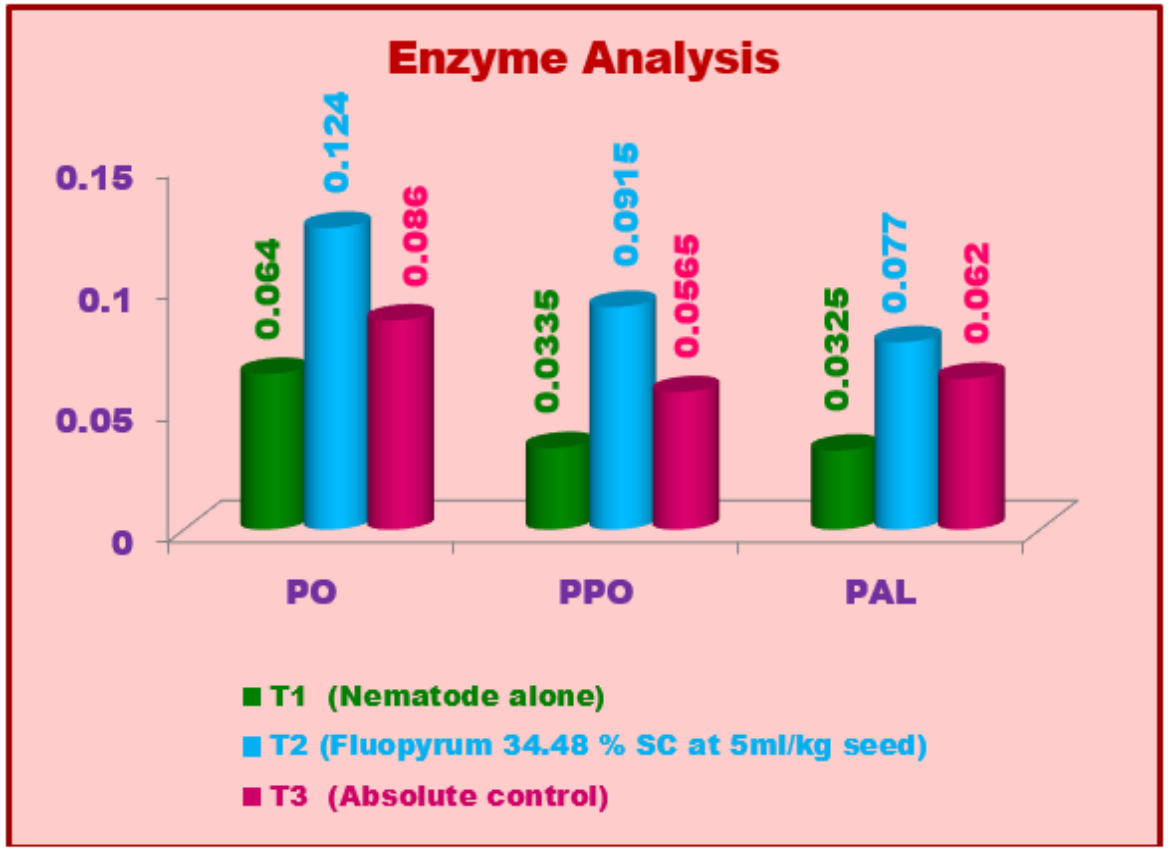

Fig. 1. Effect of chemical on enzymatic activity on wheat infected with cereal cyst nematode

Table 1. Effect of chemical on PO, PPO, PAL activity and nematode reproduction on wheat infected with H. avenae

Treatments	Peroxidase			Polyphenol oxidase			Phenylalanine ammonia lyase			No. of females/5g root			No. of cysts/200 cc soil			Final larvae population/200 cc soil		
	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled
T_1(Nematode alone)	0.06	0.06	0.06	0.03	0.03	0.03	0.03	0.03	0.03	13.10 (0.00)	16.10 (0.00)	14.60 (0.00)	17.60 (0.00)	21.80 (0.00)	19.70 (0.00)	313.00 (0.00)	351.00 (0.00)	332.00 (0.00)
T_2(Fluopyrum 34.48 % SC)	0.11	0.13	0.12	0.09	0.08	0.09	0.08	0.07	0.07	10.00 (23.66)	8.20 (49.06)	9.10 (36.36)	13.60 (22.72)	11.30 (48.16)	12.45 (35.44)	228.40 (27.02)	197.00 (43.87)	212.70 (35.93)
T_3(Absolute control)	0.08	0.09	0.08	0.05	0.05	0.05	0.07	0.05	0.06	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)
SEM±	0.0011	0.0007	0.0007	0.0001	0.0001	0.0000	0.0001	0.0001	0.0001	0.261	0.183	0.159	0.254	0.173	0.154	5.230	3.583	3.170
CD at 5 %	0.0032	0.0021	0.0019	0.0002	0.0002	0.0001	0.0003	0.0002	0.0002	0.758	0.532	0.452	0.737	0.502	0.435	15.175	10.397	8.987

***Figures in parentheses are percent decrease over untreated check*

parameters*viz*., females/5g root (14.60), cysts/200 cc soil (19.70) and larvae population/200 cc soil (332.00) were found maximum in nematode alone (untreated check with nematode).

Klotz et al., (1998) observed the increase in the PO, PPO, PAL and LOX activity in susceptible wheat roots could be correlated with resistance responses following the application of the three elicitors at their highest concentration. It is known that peroxidase is involved in creating chemical and physical barriers against invading pathogens via cell wall lignification and protein cross-linking, generation of cytotoxic compounds, and/or oxidizing compounds that are important for pathogen metabolism. Andrés et al. (2001) also reported the cyst nematode infection enhances plant class III peroxidases (PRX), esterase, and superoxide dismutase activity in wheat roots carrying Cre2, Cre5 or Cre7 resistance genes.

Pokhareet al., (2012) showed the greater activity of PO, PPO, PAL and LOX following the foliar application of these elicitors could be correlated with the reduced nematode penetration, final nematode population and fecundity of *H. avenae* considering all the plant variables and nematode development during earlier studies BABA at 8000 µg/ml was the best elicitor, followed by JA at 200 µg/ml and salicylic acid at 200 µg/ml, in protecting wheat plants from the cereal cyst nematode. Wildermuth et al. (2001) suggested that the phenylpropanoid pathway is responsible for the rapid production of salicylic acid associated with local cell death and in turn, levels of PAL increase with increase in salicylic acid concentration in plants. The increase in PAL activity has frequently been reported as a defence reaction of plants to pathogen attack, showing significant increases after infection by pathogens or wounding. Abhi et al. (2024) showed that *T. harzianum* not only showed the highest biochemical activity of peroxidase (PO) and PAL content but also showed increase in the biometric parameters of tomato and decrease in root-knot nematode reproduction.

4. CONCLUSION

The findings exhibited that reactive oxygen species (ROS) degrade protein lipids and DNA as they contain extra electron. PO, PPO and PAL are antioxidant enzymes. These enzyme degrade the ROS and antioxidant enzymes to protect plant from reactive oxygen species. If plant has higher activity of antioxidant enzymes, plant become healthy. Hence, the study proved that the tested new nematicide (fluopyrum) has ability in decrease of nematode reproduction in soil and plant by increase activity of releasing of defense enzymes like., PO, PPO and PAL content in wheat infected with *H.avenae*.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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