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Studies on Biotic Stress Induced Biochemicals Activity in Root Knot Nematode *Meloidogyne incognita* Infested Mulberry Plants

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Abstract

Living organisms create biotic stress in plants, particularly microorganisms, insects and weeds. They directly robbing the host of its essential nutrients which leading to bring down drastically diminish plant vitality and health. Ultimately, which leads to host plant death. In agriculture field, substantial challenges arise from biotic stress, presenting a significant hurdle in the realm of plant health and growth eventually crop yield losses. Phyto-parasitic nematodes (PPNs) as a critical agricultural issue, precipitating significant harm to crops and causing marked yield and financial setbacks for farmers, particularly. Root-knot nematodes (RKNs) stand as a paramount threat, wielding a destructive impact of profound proportions in mulberry and other crops. In the face of biotic stress, plants mobilize intricate defense mechanisms, arrange a strategic response to safeguard for their well-being to scavenge this stress create free radicals. The primary aim of this study was to investigate stress-induced enzymes Phenylalanine ammonia lyase (PAL), Polyphenol oxidase (PPO) and Superoxide dismutase (SOD) specific activity in root knot nematode *Meloidogyne incognita* infested mulberry plants. Enhanced activities of defence enzymes have been noticed in nematode infested mulberry plants compared to control plants.

***Key words*:** *Biotic stress, Meloidogyne incognita,* PAL, PPO, *Root knot nematode,* SOD

Plants fight with different biotic stresses caused agents like protozoa, fungi, virus, bacteria, nematodes, insects etc. These microorganisms primarily grow on or inside plant tissues and bring various type of symptoms like chlorosis, stunting, rotting, or local lesions formation [1]. Different pathogens can lead to various diseases, infections, and harm to crop plants, ultimately impacting crop productivity [2-3]. Plants encounter a range of biotic stresses and challenging environmental conditions, prompting them to activate numerous morphological, biochemical, and molecular mechanisms in response [4]. Biotic agents significantly impact on agriculture, resulting in substantial yield losses. Wang *et al*. [5] reported that biotic stresses lead to yield losses of about 28.2%, 37.4%, 31.2%, 40.3%, 26.3%, and 28.8% in wheat, rice, maize, potatoes, soybeans, and cotton crops, respectively.

Plant defense mechanisms, such as morphological barriers, structural defenses, chemical compounds, proteins, and enzymes, play a pivotal role in conferring tolerance or resistance to biotic stresses by enhancing strength and rigidity. Following pathogen infestation, plants experience an increase in reactive oxygen species (ROS), prompting the activation of defense systems to eliminate these free radicals [6-8]. In response to pathogen attacks, plants increase cell lignification, a mechanism that obstructs parasite invasion and diminishes host susceptibility. Plant-parasitic nematodes pose a significant threat to agriculture, causing substantial crop damage and economic losses for farmers [9]. They rank among the most destructive biotic strains affecting a wide array of major crops, resulting in considerable yield and economic losses. Annual yield losses attributable to Plant parasitic nematodes are estimated to range between 8.8% to 14.6% of gross crop production [10]. Among these, Root-knot nematodes (RKNs) are particularly destructive, infecting various plants like eggplant, *Arabidopsis*, carrot, and tomato [11-13]. These nematodes primarily cause soil-borne diseases, attacking the plant's root system, extracting contents from plant cells and feeding on all parts of the plant. Their activity induces symptoms resembling nutrient deficiency, such as wilting or stunting. Mulberry (*Morus alba* L), a perennial woody plant, has *extraordinary* economic value in nutritional, medicinal and the leaves are important food for silkworms. Therefore, the primary aim of this study was to examine the stress induced enzymes vig- Phenylalanine ammonia lyase (PAL), Polyphenol oxidase (PPO) and Superoxide dismutase (SOD) specific activity in root knot nematode *Meloidogyne incognita* infested mulberry plants.

MATERIALS AND METHODS

This experiment was carried out in Sri Padmavathi Mahila Viswavidyayam, Tirupati, Andhra Pradesh. In this experiment, seventy-day-old V1 mulberry saplings were planted using a randomized block design with a spacing of 3' × 3'. Following a three-month establishment period, 1000 nematode juveniles were inoculated per plant, while maintaining control plants. Sixty days post-inoculation, the

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specific activity of stress-induced enzymes, namely Phenylalanine ammonia lyase (PAL), Polyphenol oxidase (PPO), and Superoxide dismutase (SOD) was assessed in both nematode-infested and control mulberry plants.

Stress induced enzyme assays

Tests for three stress-induced enzymes were conducted on both nematode-infested and control mulberry plants, specifically evaluating the specific activities of i. Phenylalanine ammonia lyase (PAL), ii. Polyphenol oxidase (PPO), and iii. Superoxide dismutase (SOD). The specific activity of Phenylalanine ammonia lyase (PAL) in the samples was determined using the method described by Dickerson *et al*. [14]. Polyphenol oxidase (PPO) activity was assessed following the protocol outlined by Esterbaner *et al.* [15]. The enzyme Superoxide dismutase was assayed following Dhindsa *et al*. [16].

Statistical analysis

All the experimental data collected in three replicates has been subjected to statistical analysis (SPSS-2.0 Version). The analysis of variance has been done to find out the significant differences between the control and infested plants for the T-test and Two-way ANOVA test following the procedures laid down in Agricultural statistics [17].

RESULTS AND DISCUSSION

Plants possess innate secondary metabolic defense mechanisms that serve multiple biological functions and play a crucial role in safeguarding against various biotic and abiotic stresses. In this study, the stimulation of specific enzymes namely, polyphenol oxidase, phenylalanine ammonia lyase, and Superoxide Dismutase resulted in increased activity levels (Table 1, Fig 1), potentially mitigating the onset of biotic stress. Elevated activities of these defense enzymes are believed to directly or indirectly contribute to inducing systemic resistance in plants against pathogens [18].

i. *Phenylalanine ammonia lyase activity (min^{-1} mg^{-1} protein)*

There was a notable and significant increase in the specific activity of phenylalanine ammonia lyase observed in the leaves of *M. incognita* infested mulberry plants which was observed as 1.44 min^{-1} mg^{-1} protein and it was noted as 0.80 min^{-1} mg^{-1} protein in healthy plants. The percentage of increase was recorded as 80.00 over healthy plants. Phenylalanine ammonia-lyase is one of the most important enzymes of plant secondary metabolism. Phenylalanine ammonia lyase facilitates the de-amination process of phenylalanine amino acid, leading to the production of trans-cinnamic acid. This compound serves as a precursor for various phenolic compounds within the phenylpropanoid pathway, ultimately culminating in the production of lignin as the final product [19].

Phenylalanine ammonia lyase activity represents a typical reaction in plant cells triggered by both biotic and abiotic stresses. Moreover, due to their capability to trap free radicals, these enzymes might also serve as antioxidants [20]. This study observed that the pal activity significantly higher in leaves of root knot nematode infested mulberry than control plants. Similar studies were conducted by Sirohi and Dasgupta [21] who reported phenylalanine ammonia-lyase initiation and increased activity due to root knot nematode, *M. incognita* in cowpea cultivar during the early stages of infestation. Silva *et al*. [22] noted an elevation in phenylalanine ammonia lyase activity in coffee plants infested with the nematode *Meloidogyne exigua* compared to the control plants. Shannon *et al*. [23] documented increase in enzyme activity rapidly following infestation, likely stemming from either the de novo synthesis of enzymes or the activation of previously inactive enzyme forms.

Table 1 Effect of root knot nematode *Meloidogyne incognita* on stress induced enzyme activity of mulberry leaves

S. No	Parameters (min^{-1} mg^{-1} protein)	Control	Infested	% of increase over control	T- Test	Level of significance
1	Phenylalanine ammonia lyase	0.80	1.44	80.00	33.764	**
2	Polyphenol oxidase	0.34	0.66	94.11	24.787	**
3	Superoxide dismutase	0.03	0.05	66.66	4.243	*

p<0.01, **Highly significant at 0.01
p<0.05, *Significant at 0.05

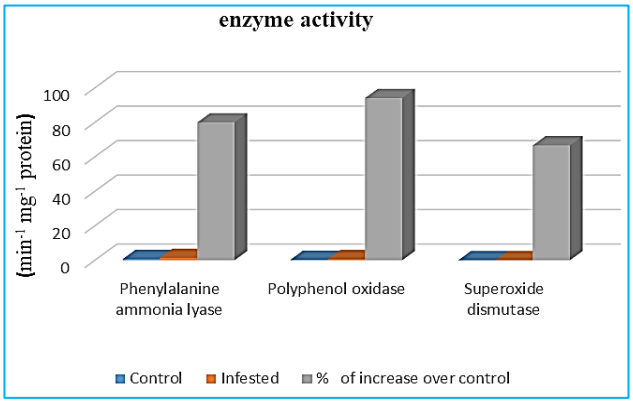

Fig 1 Effect of root knot nematode *Meloidogyne incognita* on stress induced enzyme activity of mulberry leaves

ii. *Polyphenol oxidase activity (min^{-1} mg^{-1} protein)*

In the present investigation, the polyphenol oxidase specific activity was recorded as 0.66 min^{-1} mg^{-1} protein in the nematode infested mulberry leaves and was 0.34 min^{-1} mg^{-1} protein in healthy leaves. The percentage of increase was observed as 94.11. Polyphenol oxidase (PPO) plays a role in the defense mechanisms of plant cells by oxidizing phenolic compounds, transforming them into quinones, which exhibit toxicity against nematodes [24]. In this current study, there was an observed increase in the specific activity of polyphenol oxidase. The rise in polyphenol oxidase activity observed it might be contribution to increased phenol production which is responsible plant defence against pathogen. Vacheiskvili *et al*. [25] observed elevated activity levels of peroxidase and polyphenol oxidase in tomato plants infested with *M. incognita* compared to healthy ones. Similarly, Osman *et al.* [26] and Korayem *et al.* [27], investigated the impact of *M. incognita* on potatoes and sugar beet they noted increased polyphenol oxidase activity compared to the control. Additionally, Neena *et al*. [28] documented alterations in peroxidase and polyphenol oxidase activity in tomatoes due to *M. incognita* infestation. The activity of these enzymes increased after infestation with root knot nematode. Root knot nematode infestation led to heightened levels of peroxidase activity, phenol content, polyphenol oxidase activity, phenylalanine ammonia lyase, and tyrosine

ammonia lyase in the roots of cotton, coffee, chickpea, banana, and rice [29-32].

iii. *Superoxide dismutase (SOD) (min^{-1} mg^{-1} protein)*

In the present study, greater specific activity of Superoxide dismutase 0.05 min^{-1} mg^{-1} protein was observed in the leaves of nematode infested mulberry leaves as compared with the healthy plants which were 0.03 min^{-1} mg^{-1} protein with an increase of 66.66 percentages.

Catalase, which converts H_2O_2 to water and oxygen, may also be integral to the development of anti-nematode defence and superoxide dismutase. The enzyme catalyse the dismutation of O^{2-} into oxygen and hydrogen peroxide. They play an active role in scavenging reactive oxygen species (ROS), potentially enhancing plants capacity to tolerate ROS stress and delaying senescence [33]. Rajasekhar *et al*. [34] conducted investigations aiming to establish connections between the activities of catalase, peroxidase, and superoxide dismutase in tomato plants during interactions with the nematode *M. incognita*. They found that the susceptible cultivar exhibited the highest increase in enzyme activity following nematode inoculation. Kathiresan and Mehta [35] documented alterations in catalase activity in sugarcane attributed to *Pratylenchus zeae* and further they found maximum increase in the activity of catalase and superoxide dismutase in both the roots and leaves of susceptible sugarcane. Sahebani and Hadavi [36] conducted a study focusing on hydrogen peroxide generation and the activities of enzymes involved in its metabolism, namely superoxide dismutase (SOD), guaiacol peroxidase (GPOX), and catalase (CAT), within tomato roots infected with the root-knot nematode (*Meloidogyne javanica*). Their findings indicated a significant increase in the specific activities of SOD and GPOX in the nematode-infested plants. Korayem *et al*. [27] documented comparable findings in sugar beet genotypes affected by the root knot nematode *M. incognita*, noting substantial increases in the activities of peroxidase, catalase, and superoxide dismutase across multiple genotypes.

CONCLUSION

In conclusion, numerous studies on host-parasite interactions indicate that nematode infestations significantly impact the growth and development of host plants, causing substantial losses in yield and quality within agricultural settings. These infestations have the capacity to modify host metabolic processes, inducing various changes in the production of biochemical compounds during nematode pathogenesis. The present investigation mention that host plant defence system respond with pathogen activities continuously to avoid pathogen infestation. Hence, there is a need to have knowledge on the complexities or subtleties involved in the pathogenicity of root knot disease to develop appropriate management strategies to potential nematode control or prevention approaches in mulberry.

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