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Tomato Root Knot Nematode Control Through Biocontrol Agent *Pseudomonas Fluorescens*

Samaraj S. Thiyagarajan
New Product and Manufacturing Department, Biogen, 409,
Opp Carmel Garden School, Coimbatore, Tamil Nadu, India
Email: atsamraj@gmail.com

K. Hari
Sugarcane Breeding Institute, Sugar Cane Institute Rd,
Seeranaickenpalayam, Coimbatore 641007, India.
Email: sbi_hari@yahoo.com

Abstract **– Root knot Nematodes are prevalent pests for many commercial crops. Their impact is felt in the loss of agricultural production and acute shortage of agricultural produce at a regional level. One of the most important vegetable crops in India that is most impacted by this pest is the tomato (*Lycopersicum esculentus*). The primary nematode species infesting tomato plants is the root knot nematode *Meloidogyne incognita*. In this study the tomato variety CO-1 developed by Tamil Nadu Agriculture University was used as a host crop. Biological control agent *Pseudomonas fluorescens* was used for its antagonistic attributes in controlling root knot nematode in tomato plants. In summary, the study concludes that *Pseudomonas fluorescens* was comparatively more effective in sustained control of nematodes than chemical treatments.**

Keywords **– *Pseudomonas Fluorescens*, Root Knot Nematode, *Lycopersicum Esculentus*, Carbofuran.**

I. INTRODUCTION

Tomato crops are now grown worldwide for their edible fruits, with thousands of cultivars having been selected with varying fruit types and for optimum growth in various growing conditions [3]. Tomatoes are used in making salads, soups, ketchups, and for cooking curry and other dishes. It is grown as a warm climate crop. The tomato (Lycopersicum esculentus) belongs to the genus *Lycopersicum* under the Solanaceae family. Because of its importance as food, the tomato has been bred to improve the productivity and economic prosperity of the farming community in India. An attractive aspect of tomato cultivation is that it oftentimes returns a high value per square foot of cultivation. Poor environmental conditions, pests, and diseases majorly impact the yield and quality of tomato crops.

Among several adverse factors affecting tomato cultivation is the root knot nematode, a major pest that creates aggravated damage. Tomato root knot nematode is from the family Meloidogyne species. These are endoparasitic roundworms that create significant damage to crops around the world [16]. Nematodes penetrate the roots of tomato plant species through secretion of digestive enzymes [18], which dissolve the hard tissues and migrate to the vascular cylinder. They initiate a series of changes in the root resulting in the formation of galls otherwise called root knot. They develop specialized feeding cells called "giant cells [7] in their hosts. These biochemical and physiological changes affect nutrient and water uptake by the host plant.

Tomato crops infested with this nematode lose their vegetative growth including their flowering potential, which result in a lower yield [5]. Several chemical treatments are available with limited effectiveness. It is globally understood that alternative methods need to be identified or developed including a systematic, biological or integrated approach. Pseudomonas fluorescens has been identified as a viable biocontrol agent for controlling many plant diseases [19].

In recent years, introduction of bacterial and fungal biocontrol agents for managing soil borne pathogens are recognized by the agriculture community [15]. The main criteria that led to this approach are the inefficiency of synthetic pesticides and fumigants [26]. Pseudomonas fluorescens had been identified to be effective in controlling many soil diseases including Fusarium wilt, bacterial sheath blight and other phytopathological fungi [30]. In addition to controlling the diseases caused by microorganisms, Pseudomonas fluorescens controls ill effects of non-microorganisms [8] [10]. Tomato seedlings treated with the biocontrol agent Pseudomonas fluorescens were clear of nematode galls or eggs. The objective of the study is to assess the efficiency of Pseudomonas fluorescens in controlling root knot nematode Meloidogyne incognita. The efficiency was compared with a chemical treatment.

II. MATERIALS AND METHODS

A. Pseudomonas fluorescens Culture Preparation

The Pseudomonas fluorescens culture was developed internally from the strain PF1 (agar slants) obtained from Tamil Nadu Agriculture University. The strain was mass multiplied using King's B broth media [44]. The cultures were grown using PPE bottles. Cultures were incubated at room temperature for four days. Concentration of harvested broth was at 106 cfu/ml. The harvested culture was used without further modification.

B. Seedling treatment

Three different treatments were done. Tomato variety CO-1 developed by Tamil Nadu Agriculture University was raised in the nursery. The seedlings were harvested at the 35th day. The seedlings were treated as following:

Treatment 1: 35 days old tomato seedlings roots were dipped in the Pseudomonas fluorescens broth for 30 minutes.

Treatment 2: 35 days old tomato seedlings roots were dipped in 1% Carbofuran – Furadan (Rallis) (chemical treated) for 30 minutes.

Treatment 3: 35 days old tomato seedlings were dipped in PBS (Phosphate buffered Saline) for 30 minutes. This served as control.

C. Experimental designs

The field trials were conducted at Pertholuvzhu, Coimbatore district and Kangeyam, Tk, subsequently. The land was ploughed and leveled and the experiments were laid out in a split plot design. Each plot was 2.5 m wide x 20.0 m long with two rows of 20 plants each. Tomato seedlings drenched in the respective treatment were transplanted with a spacing of 60 cm between plants. Weed control was carried out manually.

D. Nematode extraction and counting

Soil sample was collected and nematode presences in the soil per 10 inch depth were calculated using the decanting and sieving method where the soil samples were mixed and sieved through coarse sieve to remove rocks and other debris. Soil samples were then mixed with water and stirred few times before changing sieves. The samples were then sieved with 60 mesh and again sieved in 325 mesh after stirring. Remains in the 325 mesh sieve were further washed and counted after staining and observing under a microscope [23].

Five plants from each plot were harvested at 60 days and 90 days from the day of nursery development. The following parameters were evaluated: plant height (including shoot height and root length), root nodule presence through visually checking and average number of galls per plant root system (each root was counted separately and average was taken).

E. Statistical analysis

The data was analyzed using the JMP program version 10.0. (SAS). Data was subjected to analysis of variance (ANOVA) at significant levels ($P < 0.05$) and means were compared by each pair Student's t test.

III. RESULTS

Nematode counting resulted in 43 per sq inch of the soil. The characteristics of the nematode symptoms in the tomato plant include root knot formation called galls. The root galls caused by the nematode are a distinctive bulging of the roots, the symptoms above the ground are stunted growth, yellowing leaves, thinning plants, damage in patches and premature wilting. Uprooted plants were examined for these symptoms.

A. Nematode control at 60 days

At 60th day, plants were visually checked for their physical characteristics to see if those plants exhibited any of the symptoms mentioned earlier. Control showed stunted growth and yellowing of leaves, indicating slight to moderate wilting appearance. Root examination consistently showed root galls present in all the roots. All these symptoms clearly indicated the nematode infestation of control plants. Chemically treated plants were healthy and devoid of any symptoms above the ground. However, there were a few root galls observed in these plants. Overall, chemically treated plants showed considerable growth as indicated in figures 1 & 2. Plants treated with *Pseudomonas fluorescens* did not exhibit any nematode attack symptoms except for a few galls in the roots. These tomato plants appeared healthy with considerable vegetative growth and flowering.Comparing total plant height (both shoot and root) indicated plants treated with *Pseudomonas fluorescens* had a light increase in height. However, comparing both chemically treated and *Pseudomonas fluorescens* treated plants were not statistically different. Comparing both the chemical treatment and *Pseudomonas fluorescens* to control plants, indicated that both plants were significantly different with control having lower plant height (Figure 1). Root gall count indicated control plant had higher average galls per plant root system compared to chemical, and *Pseudomonas fluorescens* treated plants. *Pseudomonas fluorescens* treated plants had fewer root galls per root. Statistical analysis showed all three treatments were significantly different from one another at the 60th day from the nursery development (Figure 2).

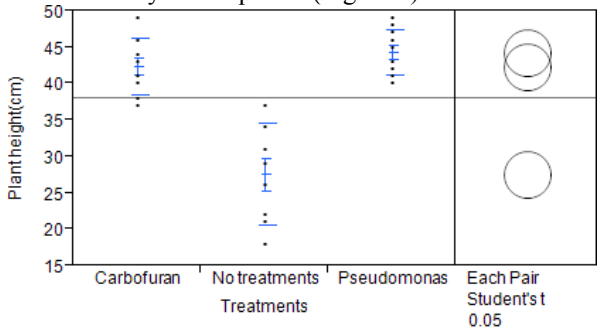

Fig.1. Influence of treatments on plant height at the 60th day from nursery development. Plant heights in cm are measured at the 60th day from the nursery development. Data was analyzed using ANOVA and Student t test. Circles that do not overlap indicate that the means are significantly different for p value ($p< 0.05$).

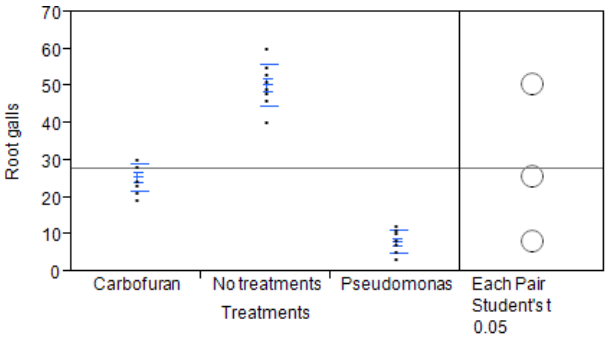

Fig.2. Influence of treatments on root gall formation at the 60th day from nursery development. Root galls are measured as an average of all roots in the plant. Data was analyzed using ANOVA and Student t test. Circles that do not overlap indicate the means are significantly different for p value ($p< 0.05$).

B. Nematode control at 90th day

Visual inspection for the symptoms mentioned earlier indicated control plants exhibited symptoms of wilting, stunted growth, and had excessive damage in terms of fruit production. Chemically treated plants showed considerable damage with stunted growth, yellowing of leaves and reduction in flowering. Whereas, *Pseudomonas fluorescens* treated plants maintained their growth vigor and flowering. For assessing the plant height, five plants from each plot were uprooted. Plants were measured for

their total plant height including the shoot and root length. *Pseudomonas fluorescens* treated plants had higher total plant height. Chemically treated plants had lower total plant height. Control plants had even lower total plant height. Comparing *Pseudomonas fluorescens* and chemically treated plants to the control plants, indicated *Pseudomonas fluorescens* had significant increase in total plant height whereas the chemically treated plants showed increases in total plant height but less than *Pseudomonas fluorescens*. All three treatments showed significant difference in their mean with p-value of less than 0.05 (Figure 3). Root gall count at the 90th day indicated control plants had higher than average galls per root system followed by chemically treated plants. *Pseudomonas fluorescens* treated plants showed no significant increase in the root galls compared to 60th day observations. When *Pseudomonas fluorescens* and chemically treated plants were compared to the control, *Pseudomonas fluorescens* had very low gall incidence compared to the chemically treated plants. Statistical analysis indicated that all three treatments were significantly different from one another at the 90th day from nursery development. P value of less than alpha of 0.05 for all treatments showed the mean root gall were different from one another (Figure 4).

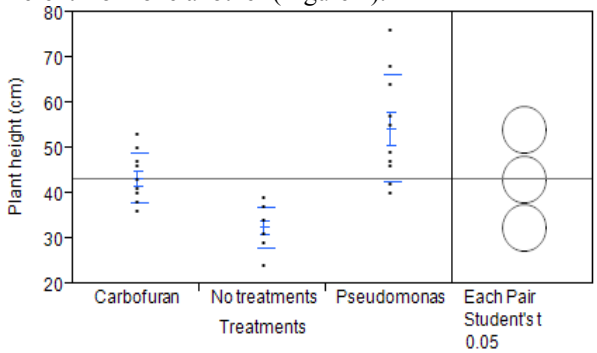

Fig.3. Influence of treatments on plant height at the 90th day from nursery development. Plant heights in cm are measured at the 90th day from the nursery development. Data was analyzed using ANOVA and Student t test. Circles that do not overlap indicate that the means are significantly different for p value ($p < 0.05$).

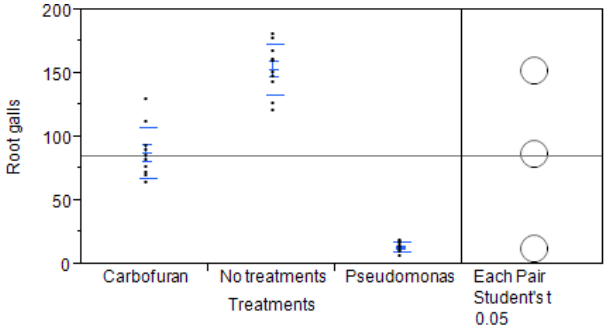

Fig.4. Influence of treatments on root gall formation at the 90th day from nursery development. Root galls are measured as an average of all roots in the plant. Data s analyzed using ANOVA and Student t test. Circles that do not overlap indicate that the means are significantly different for p value ($p < 0.05$).

IV. DISCUSSION

Comparing all three treatments at 60th day from setting nursery, indicates both *Pseudomonas fluorescens* and chemical Carbofuran provided similar resistance to nematode attack. Seedlings transplanted into the experimental plot were able to initiate and sustain growth in all three treatments. In case of untreated plants, nematode attack to take place as soon as after transplantation. Plants treated chemically showed significant resistance to the attack. Nematode enters the root system and reaches the vascular system [6][2]. The mode of pathological action includes blocking the nutrients and water availability for plants [29]. Hence, root treatment with chemicals proved to be effective at least until a certain point in the tomato life cycle. In case of *Pseudomonas fluorescens,* the bacterium establishes itself with the rhizosphere and forms colonization in the tomato plant's rootswithin few days after transplanting [12][20]. However, during the first few days while *Pseudomonas fluorescens* establishes within the root system, Nematodes have a slight advantage to attack the transplanted seedling roots and have some wriggle room for entering into the root system. As the results indicated, there were few root nodules seen that confirm this. The results clearly indicate both *Pseudomonas fluorescens* and Carbofuran were successful in withholding the nematode progression.

On the other hand, analyzing the results for the same parameters at the 90th day showed varying results for each treatment. Chemically treated plants also indicated significant distress, similar to untreated plants. One reason could be the possibility of the chemical losing its toxicity for nematodes over a period of time. Whereas *Pseudomonas fluorescens* treated plants showed progressive growth and flowering. Also, most of the roots were clear of root nodules. *Pseudomonas fluorescens* was able to establish in the soil and supplement the rhizosphere [28]. A symbiotic interaction between bacteria and plants is often times proved to beneficial for partners. Plant root surface and the surrounding soil serve to provide a favorable environment where nutrients released by the plant in the form of root exudates are available to the rizhosphereic bacteria [17]. In return, rizhosphereic bacteria like *Pseudomonas* can improve plant growth and also act as protective agent against pathogens including nematodes. *Pseudomonas* colonizes within the roots of the tomato plant [9].

One of the main factors that acts as a growth factor for plants as well as an antagonistic factor to the root knot nematodes are the PGPR compounds including HCN, and other scavenging compounds[28][35]. The concept of biocontrol agents for disease control had already been well demonstrated in several crops. Microorganisms in the rhizosphere interact either symbiotically or antagonistically. A number of antagonistic actions have been identified and proven for controlling pathogens including nematodes [34]. Among the mechanisms that are more effective and have been studied extensively is the production of cell wall lytic enzymes [36], which induce systemic resistance [38]. The antagonistic properties were

explained as exuding certain polysaccharides like lipopolysaccharide or salicylic acid [4], which reduce the mobility of the nematodes [33][27]. As reported earlier, ISR induced by *Pseudomonas fluorescen*s will activate a series of plant protection mechanisms, which result in increased activity of PO, PPO, PAL and phenol [24][21]. This provides a series of events that lead to preventing nematode attacks [21]. As time progresses, conducive soil conditions enhance the population of *Pseudomonas fluorescens,* which are the best inducers of plant chitinase and peroxidase, which is crucial for ISR [35] against nematode attack [14]. This in turn stimulates the growth of tomato plants due to an increased presence of PGPR substance and other antagonistic toxins that provide a sustained plant protection mechanism. The study showed clear difference between the chemical and biocontrol treatment, with the later proving to be a more sustainable mechanism of plant protection.

V. Conclusion

In conclusion, the results indicate biocontrol was more effective in providing a prolonged nematode attack resistance for the tomato crop compared to the chemical Carbofuran, which was effective up to a certain point in the tomato crop life cycle. In the present study, evidence in terms of plant growth and few galls in the *Pseudomonas* treated plants proves a future path in nematode management for tomato crops. Bacterial biocontrol agent *Pseudomonas fluorescens* provided sustained plant protection from nematode attack in the tomato crops. In addition, it has dual benefits for promoting the growth of tomato crops.

Conflict of Interest: Authors declare there is no conflict of interest.

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