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Biological control of root knot nematodes in chillies through *Pseudomonas fluorescens's* antagonistic mechanism

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Abstract: Among many pests and diseases, nematodes are one of the most important pests. Managing nematode chemically is very expensive. The damage caused by this pest results in considerable economical loss for the agriculture community. The important factor for increased pest management expenses, and decreased return on investment is due to inefficiency of chemical nematicides to provide a prolonged pest resistance. Chilli (*Capsicum annum*) is an important crop in India and is cultivated throughout India. Nematodes are an important pest for chillies. Primary nematode species infesting chili plants are the Root knot nematode *Meloidogyne Incognita*. In this study, susceptible variety PKM-1 developed by Tamil Nadu Agriculture University was used. Nematode control through biopesticides *Pseudomonas fluorescence* was assessed. *Pseudomonas fluorescences's* antagonistic activity was studied to understand the efficiency and duration of its antagonistic activity. *Pseudomonas fluorescens'*s nematode resistance was compared with chemical and untreated plants. In summary, the study concludes that *Pseudomonas fluorescens* comparatively was more effective in sustained control of nematodes than chemical treatments.

Keywords: *Pseudomonas Fluorescens*, Root Knot Nematode, Carbofuran. Chilli Capsicum Annum

1. Introduction

Root-Knot caused by nematode in vegetable crop is a serious problem in India and all over the world. Crops are affected both quantitatively and qualitatively. Among the pests that infest commercial crops, Root-knot nematode *Meloidogyne* sp is the most prevalent pest. Eleven species of this nematode have been reported from different states of India. (Sitaramaiah,1984). Diverse effects of the pesticide approach and inefficiency of the chemicals to control nematode lead to search for alternative methods. Identifying and developing integrated pest and disease management had given the growers a relief to reduce the nematode population in crop fields. (Nusbaum and Ferris 1973).

Chille (*Capsicum annum*) is an important commercial crop and is grown worldwide for its edible fruits; it is a rich source of protein, and essential minerals. This crop is crucial for the industrial economy as it is used in food processing and other commercial industries. United Nations (FAO, 2003). Dried chillies are often ground into powders; they are also made into paste. Coloring industry uses it as raw material. Hence it has a more commercial interest for both farmers and industrial stakeholders. Any disruptions to its cultivation wills have a ripple effect on the upstream markets.

Root knot nematode causes damage for several crops like chilli, tomato, potato. Meloidogyne species are the most prominent family affecting vegetable crops. (Williamson VM and Hussey RS et al, 1996). They initiate a series of changes in the root, resulting in the formation of galls, commonly called as root knots. Nematodes are often identified as sedentary endo-parasites, but they need to be mobile to reach the root system (Bird AF, 1961). Once the nematodes are at the root it, then pierces the hard tissues by using a biochemical process to gain entry into the vascular system (Giebel J, 1974).

Chilli crops infested with this nematode appears to be stunted in growth, low flowering, and low yield. Chemical

control proved to be ineffective and expensive. Integrated pest management approach, including biological approach and nutrient enrichment will provide a long term solution. (Sosamma VK, and Koshy PK, 1997). *Pseudomonas fluorescens* had been identified as a viable Biocontrol agent for controlling many plant diseases (Schroth MN and Hancock, 1982, Samaraj ST and Hari K, 2014). Farmers are increasingly using the bacterial agent to control soil borne pathogens (Duffy BK, and Defago G, 1997). Several studies in other crops have been proven to be effective. In this research, the biocontrol effect on nematode control in the chilli crop was studied.

2. Materials and Methods

2.1. Pseudomonas Fluorescens Culture Preparation

Pseudomonas fluorescens culture 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 Nandakumar R *et al*, (2001). The cultures were grown using PPE bottles. Cultures were incubated at room temperature for 72 hours. Cultures were harvested and their concentration was at 10^6 cfu/ml. The broth was added to the talc for making the talc based formulation. (Vidhyasekaran and Muthamilan, 1995). 10 kg of talc powder processed with adjusting pH to neutral by adding CaCO3 at the rate of 10 g/kg. CMC was added to talc and mixed well. The mixture was autoclaved for 30 min each, twice on two consecutive days. 600 ml of bacterial suspension containing 6×10^9 cfu/ml was mixed with carrier-cellulose mixture under aseptic conditions. The powder mixture was then dried overnight. Once its moisture was approximately 35%, it was stored in an in polypropylene room temperature. Prior to application, the bacterial count was at 4×10^6 cfu/g of talc powder.

2.2. Experimental Designs

The field trials were conducted at Pertholuvzhu, Coimbatore district (*Field A*). The second trial was conducted at the Sukkanputhur, Erode district (*Field B*). PKM 1 variety released by Tamil Nadu Agriculture University was used. The nursery was raised and the seedlings were transplanted after the field was ploughed and leveled. Plots were separated into three different blocks. Each plot was at 30m X 20m in dimensions. Ridges and furrows were formed at a spacing of 60 cm. 40 days old seedlings were transplanted. Seedlings were transplanted at 60 x 45cm spacing. Weed control was done through hand weeding.

2.3. Nematode Extraction and Counting

Soil sample was collected, and nematode presences in the soil per 10 cubic inches were calculated using the decanting and sieving method. Soil samples were mixed, sieved through coarse sieve to remove rocks and other debris. Soil samples were then mixed with water and stirred a few times before changing sieves. The samples were then sieved with 60 mesh, and again sieved in 325 mesh after stirring. Soil remaining in the 325 mesh sieve was further washed and counted after staining and observing under microscope (Barker KR, 1985). The count resulted in 63 per sq inch of the soil.

The *Pseudomonas florescence* talc formulation was mixed at the rate of 50gm/kg of soil and the mixture was applied around the transplanted plants. The plot was designated as biocontrol treated. In the second plot, chemical Carbofuran was added at the rate of 10gm per plant. The plot was designated as chemical treated plot. Third plot was designated as control and was not treated with anything.

Ten plants from each plot were harvested after 100 days and 160 days. Following parameters were evaluated: Each plant was measured for the Plant shoot height, root height, both these measurements were combined into one single measurement as a total plant height. Each root from a single plant was cut and root galls were counted. The average of the galls per root system was recorded.

2.4. Statistical Analysis

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

3. Results

Typical symptoms of nematode attack are root knot formation called galls, a distinctive bulging of the roots, stunted growth, leaves losing chlorophyll, thinning plants, damage in patches, and premature wilting. (lsgouhi Kaloshian et al, 1995). The uprooted plants were examined for these symptoms.

3.1. Nematode Control at 100th Day

At 100th day, control plot plants had stunted growth and leaves appeared premature yellowing. Root examination showed root galls presence in all the roots. Chemically treated plants appeared with lesser symptoms compared to the control, but it had moderate galling in the roots. However, the shoots were less stunted and leaves showed little yellowing. Comparing the control, the chemically treated plants were better as indicated in the table 1; Figure 1&2. The plot that had *Pseudomonas fluorescens* application did not exhibit any nematode attack symptoms, except for few galls on the roots. The plants were healthier and had good vegetative growth and flowering. For all treatments, shoot height and root height were measured and recorded as total plant height. Nodules were counted for each root in the root bunch. Average nodules per plant root system were recorded. In comparison with control and chemical treated plots, *Pseudomonas fluorescens* treated plant showed significant increase in total plant height (table 1). Comparing chemically treated plants with control indicated chemically treated plants were less affected by the nematodes. *Pseudomonas*

fluorescens treated plants were showing they were statistically different in all parameters from the other two treatments. (Table 1, Figure 1). Root gall count indicated control plant had higher average galls per plant root system compared to chemically treated plants, and *Pseudomonas fluorescens* treated plants (table 1). *Pseudomonas fluorescens* treated plants had lower root galls per root. Statistical analysis showed all three treatments were significantly different from one another at 100th day from the nursery development (Table 1, Figure 2).

Table 1. *Average plant height (shoot and root) of the tomato crop at 100th from the nursery development.*

Treatments							
		Control		Carbofuran		*Pseudomonas*	
No	Fields	Plant height	Avgerage no of galls/root	Plant height	Avgerage no of galls/root	Plant height	Avgerage no of galls/root
1	Field A	28	148	37	60	48	12
2	Field A	23	108	31	73	41	10
3	Field A	28	211	38	93	50	15
4	Field A	21	175	33	65	51	18
5	Field A	18	158	31	70	43	14
6	Field A	24	194	39	56	46	12
7	Field A	27	163	27	76	41	12
8	Field A	23	149	30	65	44	18
9	Field A	30	153	33	43	50	9
10	Field B	36	147	35	52	38	11
11	Field B	25	131	39	52	50	10
12	Field B	24	182	37	57	55	17
13	Field B	25	132	38	55	52	12
14	Field B	27	163	32	63	58	15
15	Field B	31	142	29	58	61	14
16	Field B	35	174	33	56	42	14
17	Field B	22	131	40	64	51	12
18	Field B	26	145	36	59	48	16
19	Field B	28	153	27	62	52	13
20	Field B	21	127	20	55	47	10

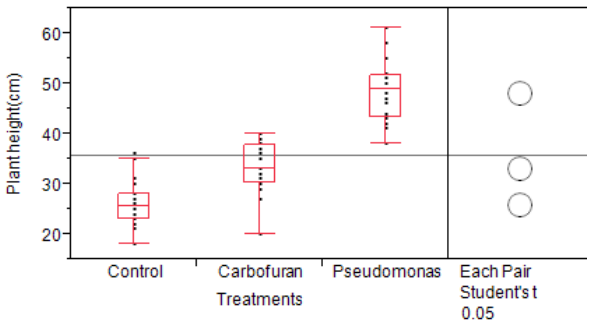

Figure 1. *Influence of treatments on plant height at 100th day from the nursery development. Plant heights in cm are measured at 100th day from the nursery development. Data was analyzed using ANOVA and Student t test. Circles that do not overlap indicate the means are significantly different for p value (p< 0.05)*

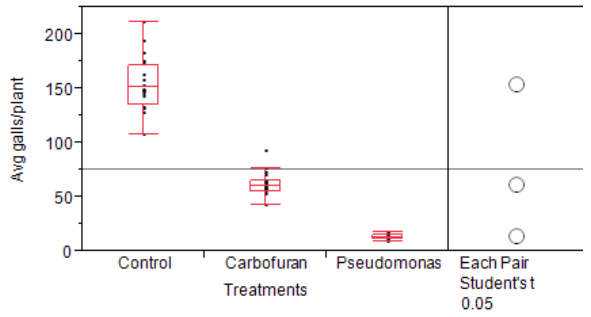

Figure 2. *Influence of treatments on root gall formation at 100th day from nursery development. Root galls are measured as an average of all roots in the plant. ANOVA and Student t test. Circles that do not overlap indicate the means are significantly different for p value (p< 0.05)*

3.2. Nematode Control at 160th Day

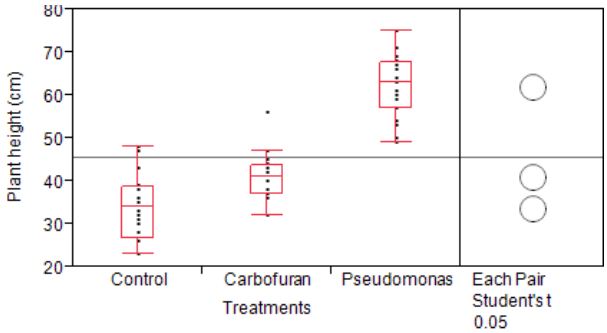

Figure 3. *Influence of treatments on plant height at 160th day from nursery development. Plant heights in cm are measured at 160th day from the nursery development. Data was analyzed using ANOVA and Student t test. Circles that do not overlap indicate the means are significantly different for p value (p< 0.05)*

Prior to uprooting the plants, they were visually inspected, and the symptoms were recorded. Both control and chemically treated plants exhibited severe symptoms of wilting and loss of growth. Plants in the control plot showed a complete damage by the nematode. They had little growth compared to the measurement made on the 100th day. The leaves showed wilting and no flowering. Chemically treated plants were moderately better than the control, but, still had significant loss of vegetative growth and flowering. Leaves exhibited strong wilting symptoms.

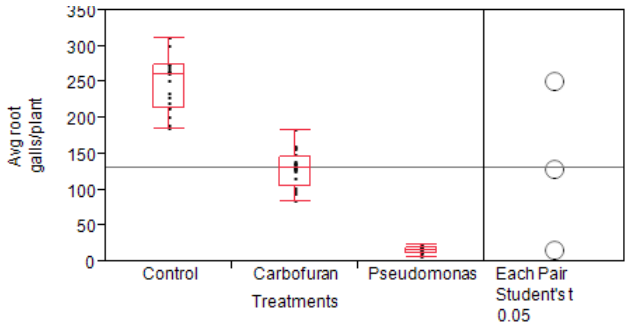

Figure 4. *Influence of treatments on root gall formation at 160th day from nursery development. Root galls are measured as an average of all roots in the plant. Data was analyzed using ANOVA and Student t test. Circles that do not overlap indicate the means are significantly different for p value (p< 0.05)*

However, *Pseudomonas fluorescens* treated plants maintained its growth vigor, and flowering. For assessing the plant height, 10 plants from each plot were uprooted. Shoot and root height were measured for all the plants and were recorded as total plant height. *Pseudomonas fluorescence* treated plants were significantly better in terms of the shoot and root height. (Table 2). All three treatments showed significant difference in the means with p-value of less than 0.05 (Table 2, Figure 3).

Comparing root galls incidence, *Pseudomonas* treated plants had lower average galls compared to control and chemical treatments. Root gall count at the 160th day indicated control plant had higher average galls per root system followed by chemically treated plants. *Pseudomonas fluorescens* treated plants showed no significant increase in the root galls compared to 100th day observation (Table 2) Statistical analysis indicated that all three treatments were significantly different from one another at 160th day. p value of less than alpha of 0.05 for all treatments showed the mean of the root galls were different from one another (Table 2, Figure 4).

Table 2. *Average root galls per plant root system of the tomato crop at 100th day from the nursery development*

Treatments							
No		**Control**		**Carbofuran**		***Pseudomonas***	
		Plant height	**Avgerage no of galls/root**	**Plant height**	**Avgerage no of galls/root**	**Plant height**	**Avgerage no of galls/root**
1	Field A	33	261	40	134	68	20
2	Field A	35	274	43	128	63	17
3	Field A	39	263	56	115	69	21
4	Field A	26	211	36	98	66	21
5	Field A	23	273	37	155	59	16
6	Field A	31	311	44	102	71	15
7	Field A	38	200	37	160	57	11
8	Field A	36	184	32	137	60	23
9	Field A	35	227	38	83	61	12
10	Field B	48	262	40	93	49	16
11	Field B	39	311	42	131	66	20
12	Field B	26	233	32	182	54	17
13	Field B	23	189	43	127	68	12
14	Field B	47	219	45	124	57	18
15	Field B	32	312	47	135	75	11
16	Field B	43	269	47	83	64	15
17	Field B	26	264	42	158	63	9
18	Field B	30	251	37	129	67	15
19	Field B	28	300	43	148	50	5
20	Field B	36	213	38	139	53	15

4. Discussion

Among all three treatments, *Pseudomonas fluorescens* had better resistance to nematode attack. In all three plots, seedlings were able to establish themselves. Once nematodes sensed the root exudates, they migrate to the plant rhizosphere. The signals stimulate egg hatch, attraction to target tissues (Zhao X et al, 2000). Previous studies have identified root exudates even from a single root could alter the behavior of the nematodes (Griffin D M et al, 1972). As the results indicate, nematode attack took place within a few days after transplantation in the control plots. Nematode infestation is based on biochemical substance present within the root surface and the rhizosphere (Pierson L S, 2000). Since control plants have the natural biochemical signal that favors the nematode mobility and infestation to its root

system. *M. incognita* undergoes a reversible change in the motility that helps in its migration, and the existing favorable conditions help nematodes to enter the root system and reach the vascular system (Bird A F, 1959). On entering the root system, the mode of pathological action includes blocking the nutrient, and water availability for plants (Sikora RA, 1988). In chemically treated plots, soil rhizosphere undergoes changes, which makes nematode immobile. A general concept of the chemical action on the nematode is that acetyl cholinesterase enzyme is inhibited, causing the nematode to undergo physiological and behavioral effects that incapacitates the nematodes PerryD J *et al,* (1990). Although these changes are reversible, and nonlethal as indicated in previous research that had shown the effects of toxicology of the carbamate reduced and were reversible in the nematodes. This study reiterates previous findings. Assessing the plant symptoms at 100^{th} day and 160^{th} day clearly shows the progression of the symptoms after 100^{th} day. Chemically treated plants were similar to untreated plants at the 160^{th} day. They had extensive symptoms, including loss of growth, leaves and almost no flowering.

Analyzing the results for the *Pseudomonas fluorescens* treated plots showed that the bacteria were able to establish and increase the population in the rhizosphere. Bacteria then colonize the chilli roots Yeole R D *et al*, (2001) within few days after transplanting. Fewer root galls were seen in the *Pseudomonas fluorescens* treated plants. However, comparing 100^{th} day and 160^{th} day, there was no progression of the root galls as seen in the chemically treated plants. This clearly indicates the efficacy of *Pseudomonas fluorescens* in preventing the nematode entry into the root system. Also, these plants exhibited progressive growth and flowering. Examining the roots, showed fewer root nodules. Bacteria were able to establish a symbiotic relationship with the plant root system. A favorable environment exists, where nutrients released by the plant in the form of root exudates are available to the *Pseudomonas* to establish and increase its population. The root colonization creates a protective layer against nematodes (Bull CT, 1991).

Plant Growth Promoting Substance (PGPR) compounds, including Hydrogen Cyanide (HCN), and other scavenging compounds (Kloeppe J W, and Rodriguez-Kabana R, 1999) was identified as factor for antagonistic activity. Microorganisms in the rhizosphere interact either symbiotically or antagonistically. Earlier studies had identified and enumerated the mechanisms of controlling pathogens, including nematodes (Nandakumar R et al, 2001). Among the mechanisms that are more effective and studied extensively are productions of cell wall lytic enzymes, exuding certain polysaccharides like lipopolysaccharide or salicylic acid (Singh P P *et al,* 1999, Van Peer R and Schippers B, 1992.), which reduce the mobility of the nematodes (Robin Duponnois and Amadou M, 1998). Other well characterized factors are ISR induced by *Pseudomonas fluorescen*s, which resulted in increased activity of PO, PPO, PAL and phenol. This provides universal events that lead to preventing nematode attack. Kandan A *et al,* (2002). Pf1 are the best inducers of plant chitinase and peroxidase, which is crucial for ISR Nandakumar R et al, (2000) against nematode attack (Anita E and Rajendran G, 2002). The study as reported showed the efficiency of the biocontrol in controlling the nematode infestation in the chile crop.

5. Conclusion

The study concludes *Pseudomonas fluorescens* as an efficient biocontrol for nematode management in the chilli crops and also, it clearly indicates that biocontrol were more effective in providing a prolonged nematode attack resistance to the chilli crops compared to the chemical Carbofuran. With the reversible nature of the chemicals, crop protection was not sustainable as compared to biological control. Developing and further characterizing more beneficial microorganisms will in future provide a comprehensive crop protection.

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