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Biologocal control of root-knot nematode, *Meloidogyne incognita* in chilli (*Capsicum annum* L.)

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Abstract
Pests are enormously dangerous to the agriculture industry especially the root-knot nematodes. Furthermore, as the global population is expected to reach 9 billion people in 2050; the need to advocate for efficiency in farming as influenced by climate change; the necessity for diseases control on crops has been brought about. Many of these pests have in the past been controlled by chemical fertilizers and pesticides but due to their high prices, their negative impacts on the environment and their prohibitive charges other natural methods have had to be considered. Microorganism and particularly plant growth-promoting rhizobacteria (PGPR) especially *P. fluorescens* has been established as one of the solution to the biologic al control. Some of the benefits of these microorganisms include facilitating of growth and yield of the plant, suppression of nematode diseases, as well as enhancement of the plants ability to fight disease through enzymatic and metabolically. Some bacteria found to improve the nematodes regulation as well as yield include PGPRs such as *Bacillus*, *Trichoderma*, and *Pseudomonas* species. Further, PGPRs can lead to systemic resistance in plants to make the plant immune to pest and diseases in the longer run. This review mainly focuses on enlightening about biocontrol and how PGPRs can replace the toxic chemical with explosive advantages to agriculture.

Keywords: Nematodes, biological control, plant growth-promoting rhizobacteria (PGPR), *Pseudomonas fluorescens*, *Bacillus*, *Trichoderma*, disease resistance, sustainable agriculture, crop yield.

Introduction

Overall, pests, and diseases are many, but nematodes are one of the most crucial pests. The effect of this pest has been observed to lead to great loss to the growers hence the name "world's costliest pest". In recent past, population growth and climate change have been the region's most severe constraint to both farmers in the last two decades and researchers. Presently, according to the United Nation estimates, global population will reach about 9 billion by 2050, which will require 60% more food than current requirement and this pressure forces farmers to produce food and for researchers to improve their yields by making them disease free crops. The farmers in the present world rely a lot with chemical fertilizers and pesticide to boost food production and control diseases. Chemical control of root-knot nematodes has been very efficient but this method is also very costly (Thiyagarajan *et al*., 2014) [41].

This study shows that bacteria in the rhizosphere creates a great impact for bio-fertilizer and psycho-stimulant, disease resistance and heavy metal clean up but it is in definite relation with colony formation in the rhizosphere. Plant growth promoting microorganisms are expressing in rhizosphere can enhance plant growth through the formation of specific plant growth factors, and also protect the plants against nematode contagion and growth (Basu *et al*. 2021) [8].

The first group includes *Azotobacter sp*., *Bacillus subtilis*, fluorescent *Pseudomonads* and *Rhizobium sp*., which has been used in the biocontrol against different phytopathogen in the last few years (Tuzun 2001). Owing to their versatility in catabolism, pathogen controlling ability and ability to produce assortment of enzymes and metabolites that confers stress tolerance to the plant the fluorescent *Pseudomonads* have become the largest and potentially the most preferred candidates for bio-control of plant diseases among these rhizosphere competent bacteria, and ability to produce a variety of enzymes and metabolites that help the plant withstand various biotic and abiotic stresses.

1. Biological management of plant diseases:

It is clear from various literature relating to the use of BCAs that control of disease achieved after the application of a BCA to a crop is equally effective like that achieved after using a fungicide as has been stated by O'Brien *et al*. (2017) [29]. When a fungicide was used in *Phytophthora cactorum* infected apple, the disease was completely wiped out while, various BCAs applied individually disease suppression were between 79 and 98% depending on the BCA used.

Parveen *et al*., (2020) stated that a biocontrol potential of rhizobia either solo or in consort with *Pseudomonas aeruginosa* against root rot and root-knot diseases in chilies have been assessed. Thus, of the seven rhizobia isolates in the *vitro* study NFB-30 (*Sinorhizobium sahelens*) affected all four test fungi *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium oxysporum* and *F. solani* had zone of inhibition of 10, 2, 2 and 11mm respectively. Comparable experiment was performed using seven strains of *Pseudomonas aeruginosa*, PGPR-4, PGPR-6 and PGPR-11 Successfully the growth of the four tested fungi was suppressed by them all. Chili screen house experiments also demonstrated that PGPR-6, PGPR-8, PGPR-11 and PGPR-37 had significant reduction in root rot fungi infection. However, the present study found that NFB- 28, NFB- 30, NFB- 31 & NFB- 32 rhizobia isolates were effective.

During field experiment we observed that compared to the individual treatment of the strains of PGPR when co-inoculated with rhizobia it was much more effective in suppressing root rotting fungi and root knot nematode in chili.

Among the 19 bacterial strains isolated from the rhizosphere soils and plant tissues from Yunnan province, the highest antagonistic activity against the Tomato root-knot nematode *Meloidogyne incognita* was recorded Rp2-2 *Bacillus methylotrophicus* and the *Lysobacter antibioticus* strain 13-6 as noted by Zhou *et al*., (2016) [46]. When used as soil drenches or seed treatments in green house experiments, both the strains checked root-knot disease intensity and incidence on tomato than no bacteria controls. Field tests of either strain in multi-year trials with tomato plants showed a mitigation of root-knot disease and improved yields of the plants (The strain composition and infectivity of the J2s or eggs of nematode for assessment of *Meloidogyne incognita* in tomato field experiment alley cropping systems). The strains' pest control and yield improvement was more effective than that of the chemical treatments abamectin and carbofuran. It is the first time that *B. methylotrophicus* has been used as a bio-control against a plant parasitic nematode and the first time that the combination of *B. methylotrophicus* and *L. antibioticus* has been shown to reduce root-knot nematode disease in the field.

Concurring with Fan *et al*., (2020) [12], we isolated a total of 890 fungal isolates from the rhizosphere soil associated with the various crops and screened for nematicidal property. The isolate Snef1910 was very virulent against the second stage juveniles of *M. incognita* and was identified morphologically and bio molecularly as *Trichoderma citrinoviride*. Besides, an egg development inhibition was also recorded using *T. citrinoviride* Snef1910 whereby hatching inhibition percentages of 90.27%, 77.50 % and 67.06 % were incurred at 48, 72 and 96 h after treatment respectively. According to pot experiment the metabolites *of T. citrinoviride* Snef1910 reduced the number of root galls, J2s, and egg-masses and J2 population density in soil and enhanced the growth of tomato plants. Field experiment revealed that biocontrol application of *T. citrinoviride* Snef1910 has a control efficacy of more than 50% against root-knot nematode. Meanwhile, *T. citrinoviride* Snef1910 increased the tomato plant biomass.

More recently, Radwan *et al*., (2012) [32] documented a glasshouse experiment on the effectiveness of four commercial bioproducts containing the bioagents *Bacillus megaterium, Trichoderma album, Trichoderma harzianum* and *Ascophyllum nodosum* against the root-knot nematode, *Meloidogyne incognita* infecting tomato. Their activity was compared to ortho-para directing directing group such as oxamyl or carbofuran. The treatments were further on to evaluate the extent that these treatments affected the growth parameters of the tomato plant. Present findings revealed that all treated samples were effectual in reducing the root galls and J2 of the nematode in the soil compared to the untreated check except *T. harzianum* at 10 and 25 ml/kg soil J2 were statistically comparable to control. Among bioproducts *B. megaterium* at 10 g/kg soil showed the significant reduction in root galling index 89.20% followed by T. album 87.77%, *A. nodosum* 86.96% and *T. harzianum* 69.79%. The highest rate of the tested bio-products resulted in the lowest number of galls and hence more effective than oxamyl or carbofuran.

The various impacts of biological control of root-knot nematode (*Meloidogyne javanica*) by *Pseudomonas fluorescens* on plant accession described by Norabadi *et al*., (2014) mentioned some results on greenhouse and laboratory tests. *P. fluorescens* at 109 (CFU/ml) lowered the infection with nematode and others by presenting cut-off values as compared with the control group. *P. fluorescens* lowered the hatching rate of nematode eggs and the disintegration of the matrix of egg mass of nematode. Nonetheless, biochemical activities linked with resistance to phytotoxins are induced in plants inoculated with *P. fluorescens*; however, specific induction was noted for POX and PAL enzymes only. The highest level of POX and PAL was observed at the 5th day after inoculation of the plants in both control and treatment. These findings indicated that the deploy essential mortality factors and the suppression of plant defence sign which lead to systemic resistance are two critical suppression modes used by P.

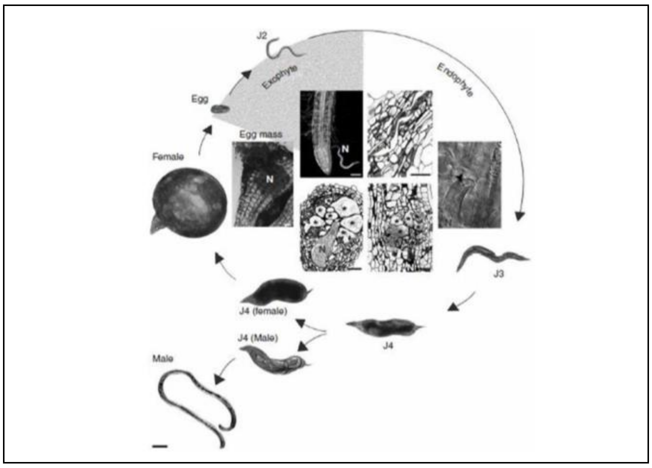

Fig 1: Parasitic life cycle of *Meloidogyne incognita* (Source: Abad *et al*., 2008)

Role of *Pseudomonas fluorescens* in management of plant disease

As mentioned by Thiyagarajan *et al*., 2014 [41] the antagonistic activity of *Pseudomonas fluorescens* was investigated to determine it's efficiency and the period of antagonistic activity. This enhancement prompted a comparative study of *Pseudomonas fluorescens'*s nematode resistance with chemical and untreated plants. In this study, susceptible only variety PKM-1 of blackgram developed by Tamil Nadu Agriculture University has been selected. Coimbatore district was used as the Field A for the field trials to be carried out. The second trial was done at the Sukkanputhur, Erode district which is referred to in this study as Field B. *Pseudomonas florescens* tale formulation was prepared at the concentration of fifty gm/ kg of soil and the formulation was applied at the around the transplanted plants. The plot was assigned to the biocontrol treated category. In the second plot chemical Carbofuran was applied at the rate of 10gm/ plant. The plot was chosen and used for chemicals treatment plot. The third plot was labeled control and was given nothing. In this experiment, ten plants from the plot were collected after-growing period of 100 days and 160 days. The parameters were as follows; each plant was scored for the PCSH, RL, and then an overall height – the total shoot height was obtained by adding the RL to the shoot height of each plant. A single plant was harvested and the roots cut to determine the number of excised root-galls. The frequency of galls per root system was determined and their average noted. Out of all three treatments, *Pseudomonas fluorescens* had highest resistance towards attacks from nematodes. In all three plots, the seedlings were able to gain some form of ground establishment.

Seenivasan *et al*. (2012) [36] reports that *Pseudomonas fluorescens* strains PF1, TDK1 and PY15 were tested individually as well as in combinations with *Meloidogyne graminicola* in rice under in *vitro*, in a glasshouse and in field experiments. Individual or in combination, the filtrates of the above strains suppressed egg hatching and resulted in the mortality of the juvenile of *M. graminicola* under in *vitro* conditions. This efficiency was even more pronounced when filtrates of the strain applied were in forms of mixtures rather than single strains. Drenching of seed with bacterial suspensions of single or combined cultures of *P. fluorescens* was successful for reducing *M. graminicola* infection on wheat plants. The enzyme peroxidase activity and chitinase activity were enhanced by *P. fluorescens* mixture than plants treated strains, two strain mixture and control plants. When applied on rice, the tale formulations of the *P. fluorescens* strains were investigated individually and in a mixture with each other as well as in combination with the chemical separately for the seed treatment and/or the soil treatment and when both seed and soil were treated. Both the seed + soil and seed treatment of the three strains together resulted to the highest percentage germination as identified in the experimentation. Thus, the introduced strains of *P. fluorescens* were found capable of becoming endophytes on roots of rice plants. The highest grain yield of rice was obtained where *P. fluorescens* mixture, PF1 + TDK1 + PY15 was applied through seed + soil at 27.3% higher than the control and seed treatment with of *P. fluorescens* mixture, PF1 + TDK1 + PY15 was at 24.7% higher than the control.

Khatamidoost *et al*. (2015) [20] identified the ability of some Iranian strains of *Pseudomonas sp* to control *Meloidogyne incognita* and their capability to biocontrol pistachio root tissues. According to in *vitro* experiments, all the tested bacterial species exert significant pathogenicity against *M. incognita* and all the strains presented the ability to kill *M. incognita* juveniles: in detail, the mortality reached the 99% at the end of the 72h incubation for the strain VUPf428. In this experiment, *in vivo* treatments revealed that the overall favorite strains that could establish high population densities in the termite soil foci that were infested with nematodes are VUPfS, VUPf52 and VUPf205. They also displayed the highest reduction in galling and nematode multiplication in a greenhouse test, but all the Iranian native strains were also able to colonise pistachio roots in pots. Selected strains may release secondary metabolites like siderophores, proteases and volatile metabolites and high population levels.

Role of PGPRs in plant disease management

Siddiqui *et al*., (2003) [37] declared that the antimicrobial metabolites were 2,4-diacetylphloroglucinol (2,4-DAPG) and pyoluteorin that increase the biocontrol capability of Pseudomonas fluorescens strain CHAO for diseases caused by the soil pathogenic fungi. Out of the three *Pseudomonas fluorescens* strains; the CHAO, the genuine CHA89, which lacked antibiotic production, and the transgenic strain CHA0/pME3424 which overproduced antibiotics, screening for root-knot nematode *Meloidogyne javanica* biocontrol was carried out. Exposure of root-knot nematode under in *vitro* condition to *P. fluorescens* reduced the egg hatch in culture filtrates to the maximum level and about 50% mortality of *M. javanica* juveniles was achieved. The effects of the utilization of NBY with 2% glucose or 1 mM EDTA on the hatch inhibition activities of the strain CHAO and the modified strains, CHA0/pME3424 and CHA89, were observed in this experiment. However, the germination of NBY agar medium supplemented with glucose enhanced the nematicidal effect of strain CHA0/PME3424 significantly. This experiment showed that neither glucose nor EDTA altered the nematicidal properties of either the strains CHAO or CHA89. To analyze the mode of action of the nematode suppression the split root bioassay was conducted during the current investigation. A split-root system provided the necessary physical separation between CHAO or CHA0/pME3424 inducing agent and the recalcitrant *M. javanica* pathogen; in results, tomato roots treated with the cell suspension of CHAO or CHA0/pME3424 imparted significant SAR to *M. javanica* in tomato, in the non-inoculated nematode half. The findings discussed here give ample evidence in support of the view that, 2,4-DAPG antibiotic from *P. fluorescens* CHAO are the systemic resistance inducer in tomato root tissues. Cultures at CHAO and its derivatives reduced by 10 fold at first and fourth stopover (0-21 days after inoculation). However, bacterial populations increased at final harvest (28 days after application) showing a contrary result to the observed trend of maize plants.

According to Habazar *et al* (2021) [17], out of all the different nematodes described, *Meloidogyne sp*., are considered economically most hazardous worldwide. Since the nematodes infect a wide host range, they can only be managed with a lots of challenges. Chemical nematicides are very lethal, thus the need to establish control tactics. As shown in our previous work highlighted some of the Bacillus sp. into biocontrol agents against plant pests and diseases. Many of the products have more than one use, for instance plant growth promoter, phosphate dissolving bacteria among others. The focus of this study was to manage *Meloidogyne sp*. with the selected *Bacillus sp*. to enhance growth and yield of tomato. Treatments were eight strains of *Bacillus sp*., Carbofuran, *Meloidogyne sp.* tomato seedlings and a control. The *Bacillus sp*. have been introduced as seed treatment and seedling treatment prior to planting the crop. The *Meloidogyne sp*. has been presented to 4weeks tomato plants. These were disease development, reproduction of *Meloidogyne sp*., vegetative growth and productivity of tomato plants. This study revealed that *Bacillus sp*. submission reduced galls, egg mass, eggs per egg mass and nematode per 300 g soil compared to the inoculated control but the suppressiveness was less than treatment with nematicide on *Meloidogyne sp*. On the other hand, it was shown that three *Bacillus sp*. enhanced the growth and yield of the tomato plant higher than the Carbofuran. Kumar *et al*., (2015) [24], quantified that in the use of microbial inoculants in agro as well as allied cultivation, PGPR has the ability to enhance plant growth through the means of acquisition of the inorganic resources or through direct alteration of the levels of plant hormones or through regulation of the bacteria in the locale. For intensive agriculture the important intermediate chemicals are the agrochemicals such as the pesticides, insecticides and herbicides has been extensively linked to adverse health effects and environmental degradation. PGPR are legal, biodegradable and non-hazardous natural occurring microbes belonging to plant growth promoting rhizobacteria. This is by production of antibiotics, toxins, bio-surfactants, cell wall degrading enzymes, chitinases, glucanase and siderophores. The *Bacillus sp.* in particular have been shown to suppress soil borne diseases of wheat, rice, maize, chick pea and barley effectively using Antibiosis as a mode of action. Among the bacterial species, *Pseudomonas*, *Bacillus*, *Acetobacter*, *Paenibacillus*, *Streptomyces* biocontrol activity some of the bacterial species have been reported. Workers have recorded successful use of PGPR for the management of soil borne diseases and many bacterial strains could be potential bio-control agent. According to the present article, various types of potential PGPR strains have been identified for antibiotics, antimicrobial agents, toxins, surfactants, enzyme minerals competition, antagonism, and the formation of an efficient systemic resistance.

Sahu *et al*., (2018) [34] noted that PGPR is a diverse group of microorganisms which become more and more valuable for their function in increasing the primary production by promoting plant growth and activating ISR. Since the application of PGPR leads to plant defence, it can play a major role in biocontrol of pests and pathogens that attack plants. Some of the fluorescent *Pseudomonas* are one of the plant growth promoting rhizobacteria the most important major group of bacteria that have enormous impact contribution to the plant growth promotion, induced systemic resistance, and the biological control agents of pathogens. It could therefore be the bacterial siderophores and antibiotics suppression of phytopathogens that gives the material most of its agronomic value. C adapted has important roles in microbial antagonism although both mechanisms generate the elicited resistance. May be the resistance-inducing and antagonistic rhizobacteria could be useful and effective in generating new inoculants, serve as an attractive different approach of ecologically safe bio-control of plant diseases, and where it will yield the most benefit for yield enhancement in integrated cropping systems.

Conclusion

In conclusion, biological control of root-knot nematodes and other plant pathogens which are proposed in this paper are safer and effective solutions to the chemical pesticides. *Pseudomonas fluorescens*, *Bacillus* and *Trichoderma* species of PGPR have been reported to stimulate plant growth, initiate systemic resistance, and reduce nematode pests. As well as more disease free crops that are healthier there are also benefits to the microbe population in the soil and the overall biology of the soil environment. The ability of PGPRs to minimize the application of deleterious chemical applications makes their utilization in sustainable agriculture warrant since food demands ascend and environmental issues arise. The knowledge generated should be extrapolated in future studies to fine tune the application of PGPR in various cropping systems, the development of microbial cocktails and their sustained impact on plant productivity and soil fertility. Through combining bio-control methods such as PGPR, farmers are also able to gain optimum pest control and yield to help in formulating effective green agriculture.

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