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**"Research Note"**

# The Effects of *Paecilomyces lilacinus* on the Pathogenesis of *Meloidogyne Javanica* and Tomato Plant Growth Parameters

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**ABSTRACT-** The present study is based on the investigation of a soil hyphomyces, *Paecilomyces lilacinus*, an opportunistic bio-control agent, in controlling root-knot nematode *Meloidogyne javanica* on tomato in greenhouse conditions. *P. lilacinus*, effectively promoted the growth of plants inoculated with *M. javanica* by suppressing its pathogenesis as root galling by the nematode and egg mass production was greatly reduced. The fungus was most effective when the fungus and the nematode were inoculated simultaneously or the fungus preceded the nematode in sequential inoculation. Conversely, when the nematode preceded the fungus, the improvement in plant growth and reduction in root galling and egg mass production were not substantial. A great number of nematode eggs were infected by *P. lilacinus*, inhibiting juvenile development. The interior of eggs, were devoid of juveniles and filled by the fungus mycelium. Developed juveniles were found attacked and/or killed and showed mycelial growth over their bodies. Simultaneous inoculation or sequential inoculation in which the fungus was added prior to the nematode was more effective in controlling the nematode than when nematodes preceded the fungus. *P. lilacinus* was, therefore, effective in controlling the root knot nematode on tomato and suppressing its population growth.

**Keywords: Egg mass, Eggs parasiting fungi, Larvae parasiting fungi, *Lycopersicon esculentum*, Root galling**

## INTRODUCTION

Tomato (*Lycopersicon esculentum*) is regarded as a most favorable host for root-knot nematodes. All the 4 major species of *Meloidogyn*e and their known races readily attack tomato crops in indoor and outdoor cultivations. Root-knot nematodes cause as high as 85% suppression in the yield of tomatoes [14].

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Application of chemicals and the use of host resistance have been two major strategies for management of root-knot nematodes. Some tomato cultivars show resistance, but the resistance is mainly vertical [3]. The existence of races with varying pathogencity and race-specific resistance have complicated the efforts to breed cultivars resistance to major species/races of root-knot nematodes. Chemical control methods have been successful, but have initiatd problems related to the pollution hazards involved in their manufacturing and residues left in the consumable parts of the treated plants.

At present, bio-control seems to be the most relevant and practically demanding approach for the control of root nematodes. Some of the opportunistic bio-control agents like soil hyphomycetes have shown great promise [2, 6, 7, 8, 10,]. *Paecilomyces lilacinus* is one such fungus, which was sent to several nematologists of 60 countries through the International *Meloidogyne* Project for trial. *Paecilomyces* species are listed by the Hawaii State Quarantine Branch as non-restricted microorganisms [17]. Data obtained from several countries indicated that this fungus adapts well in varied climatic conditions and is effective in controlling root-knot nematodes [5, 9].

The trial of this fungus is in progress at various parts of the world [1, 4, 12, 15, 16, 17]. Effective bio-control can only be achieved with a full understanding of the microbial pest control agent.

In the present study, we attempted to investigate the efficacy of *P. lilacinus* in various times of application in controlling root-knot nematode, *Meloidogyne javanica* on tomato in greenhouse conditions.

## MATERIALS AND METHODS

**Nematode culture:** After identification based on perineal pattern characters and North Carolina host differential test, field population of *M. javanica* (Treub) Chitwood, was purified by single Egg mass inoculation of young tomato seedlings. Sub-culturing was done subsequently by inoculating new tomato seedlings with at least 15 Egg masses obtained from pure culture in order to maintain sufficient inoculum for the experiment [18].

**Fungal culture:** The activated culture of *Paecilomyces lilacinus* on *M. javanica* in sterilized pots on tomato roots was recultured under a set of defined conditions to produce aerial spores on agar plates. It was then grown in a liquid medium. Czapek's liquid medium was used for culturing in order to obtain mycelium for blending to make mycelial suspension for inoculations.

**Plant culture:** Seedlings of tomato *Lycopersicon esculentum* cv. Pusa Ruby were grown in sterilized soil after surface sterilization of the seeds. Three-week old seedlings were transplanted (one/pot) in 30 cm clay pots filled with autoclaved field soil.

**Inoculations:** Inoculations of the seedlings in pots with the nematode and the fungus were done simultaneously as well as sequentially, according to the following treatment scheme:

T1 = Control (without any inoculation)
T2 = Inoculated with *M. javanica* alone
T3 = Inoculated with *P. lilacinus* alone
T4 = Inoculated with *M. javanica* and *P. lilacinus* simultaneously
T5 = Inoculated with *M. javanica* followed by *P. lilacinus*, after one week
T6 = Inoculated with *P. lilacinus* followed by *M. javanica*, after one week

The level of inoculum of *M. javanica* was 2000 (freshly hatched J2/pot) and of *P. lilacinus* 1g mycelium/pot (in the form of suspension). In control pots, no organism was added. All treatments were replicated five times. After inoculations, pots were kept in a greenhouse (25-27°C) in a randomized block design. Plants were allowed to grow for 2 months.

**Parameters:** Sixty days after inoculations, plants from each treatment were uprooted and the roots were washed. The following plant parameters and nematode indexes were considered.
1. Plant length (root + shoot); 2. Plant fresh weight (root + shoot); 3. Plant dry weight (root + shoot), 4. Gall index; 5. Egg mass index and 6. Percentage of eggs infected (IE) with *P. lilacinus*.

Plant length and fresh and dry weights (IGR) were determined by standard methods, and mean values were then calculated. Gall index (GI) and Egg mass index (EMI) were rated on a scale of 0-5 [18].

To determine the percentage of eggs infected with *P. lilacinus*, randomly selected Egg masses from the roots of plants in T4, T5 and T6 were stained with cotton-blue in lacto phenol. Egg mass was transferred gently onto a clean glass slide and a drop of sodium hypochlorite was added. The Egg mass was pressed under a cover slip, so that the eggs were separated and spread. The number of eggs infected with *P. lilacinus* was counted under a light compound microscope and the percentage of infected eggs was calculated. In addition, deformity and abnormal development of juveniles and their egg infection were also observed and recorded.

The data were subjected to analysis of variance, and mean comparison was conducted using the LSD test. Pearson‘s correlation coefficients were calculated on the plant parameters (IGR) and nematode indexes. The data were also subjected to cluster analysis according to Ward’s minimum variance method, using the cluster procedure of SAS computer software [19].

## RESULTS

**Plant growth:** Tomato plants inoculated with *M. javanica* showed significant reduction in their growth ( Table 1). Plant length and fresh and dry weights were significantly poor (P= 0.05) in comparison to un-inoculated control. When tomato plants were inoculated with *P. lilacinus*, there was no significant difference in fresh and dry weights of the plants in comparison to un-inoculated control. In simultaneous inoculation of *M. javanica*, and *P. lilacinus* plant length significantly differed (P= 0.05) from the control. When compared to plants inoculated with *M. javanica* alone, plant length was significantly greater. When *P. lilacinus* preceded *M. javanica* by one week, plant lengths differed significantly from the control. However, when *M. javanica* preceded *P. lilacinus*, significant reduction was observed as compared to

control and plant length differed from plants inoculated with *M. javanica* alone (Table 1). There was a high correlation (***p***=0.01) between plant length (pl) and two of nematode indexes (Table 2).

**Table 1. Effects of *Paecilomyces lilacinus* on the growth parameters of tomato and nematode indexes (*Meloidogyne javanica )***

| Treatments | Plant parameters | | | Nematode indexes† | | |
|---|---|---|---|---|---|---|
| | Length (cm) | fresh weight (g) | dry weight (g) | GI | EMI | Infected eggs (%) |
| **Control** | **69.0 a** | **41.8 a** | **6.5 a** | **0** | **0** | **0** |
| ***M. javanica*** | **46.0 e** | **25.0 c** | **2.5 c** | **5** | **5** | **0** |
| ***P. lilacinus*** | **63.8 b** | **38.9 ab** | **6.0 a** | **0** | **0** | **0** |
| **Simultaneous inoculation** | | | | | | |
| ***M. javanica* + *P. lilacinus*** | **59.3 c** | **35.7 b** | **4.8 b** | **2** | **1** | **68** |
| **Sequential inoculation** | | | | | | |
| ***M. javanica* + *P. lilacinus*‡** | **48.6 d** | **28.3 c** | **2.8 c** | **4** | **4** | **55** |
| ***P. lilacinus* + *M. javanica*§** | **59.3 c** | **36.8 b** | **5.1 b** | **2** | **1** | **72** |
| **L.S.D. (0.05)** | **1.1** | **4.3** | **1.02** | | | |

†**GI= Gall index; EMI= Egg mass index**
‡**= *M. javanica* preceded *P. lilacinus* by one week**
§**= *P. lilacinus* preceded *M. javanica* by one week**

The fresh and dry weights of plants in various treatments showed a similar trend as length. Significant reduction occurred in fresh and dry weights due to the infection of *M. javanica*. Inoculation of *P. lilacinus* did not cause an adverse effect on fresh and dry weights of plants. When *M. javanica* and *P. lilacinus* were inoculated simultaneously, fresh and dry weights of plants were significantly greater than plants inoculated with *M. javanica* alone and the weights differed significantly from the control. In sequential inoculations, when *M. javanica* was inoculated one week prior to *P. lilacinus*, fresh and dry weights of plants did not differ from plants inoculated with *M. javanica* alone. On the other hand, when *P. lilacinus* preceded *M. javanica*, fresh and dry weights were significantly greater than plants inoculated with *M. javanica* alone and *M. javanica* followed by *P. lilacinus* (Table 1). There was also a high correlation between fresh and dry weights and two of nematode indexes (***p=0.01***) (Table 2).

**Root-galling and Egg mass production:** The inoculation of *P. lilacinus* reduced root-galling and Egg mass production of the nematode as indicated by GI and EMI as compared to plants inoculated with *M. javanica* alone. In simultaneous inoculation of *M. javanica* and *P. lilacinus* GI / EMI were 2/1 in comparison to 5/5 in *M. javanica* inoculated plants. Similar reduction in GI and EMI values were observed, when *P. lilacinus* preceded *M. javanica* in sequential inoculation. In other sequential inoculations, when *P. lilacinus* followed *M. javanica* GI / EMI were slightly reduced (4/4) (Table 1).

**Infection of eggs:** In all the treatments, where *M. javanica* and *P. lilacinus* were added simultaneously or sequentially, a large number of eggs were infected with *P. lilacinus*. The highest percentage of infected eggs (72%) was found when *P. lilacinus*

was added prior to *M. javanica*. This was followed by the treatment, where both were added simultaneously (68%). The lowest percentage of infected eggs (55%) was noticed when *M. javanica* was followed by *P. lilacinus* in sequential inoculation.

Infected eggs contained mycelium inside as well as over their surface. Most of the infected eggs were devoid of juveniles. In some eggs, juveniles were present, but showed various degrees of deformity and abnormal development. A number of juveniles that emerged from the eggs were infected and showed mycelia growth over their body.

The roots of tomato plants were also tested in the treatments, in which the fungus *P. lilacinus* was inoculated, and fungal hyphae were never detected within the roots, though occasionally they arose from the root surface as a protector.

Pearson's correlation coefficient of plant parameters and nematode indexes (Table 2) showed very high significant correlations (P=0.01). Cluster analysis classified the treatments according to plant parameters and nematode indexes into two distinct groups, in which simultaneous and sequential inoculations were placed in one group and the rest in another (Fig. 1)

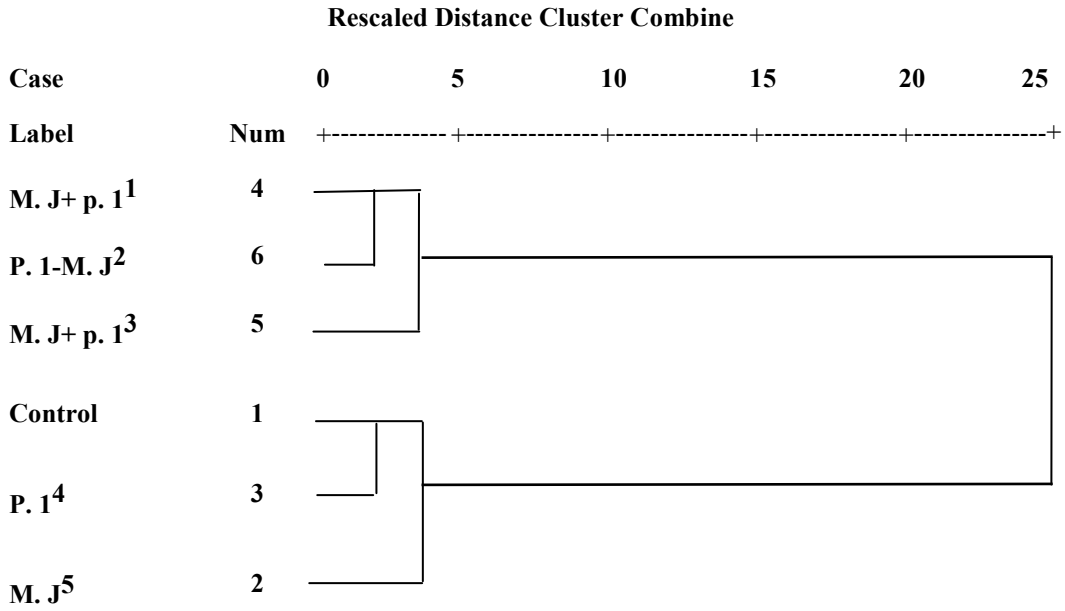
**Table 2. Pearson-s correlation coefficient of plant parameters and nematode indexes**

| Parameters | Plant length | Plant fresh weight | Plant dry weight | GI | EMI | Infected eggs |
|---|---|---|---|---|---|---|
| **Plant length** | **1.000** | **0.992**** | **0.993**** | **-0.977**** | **-0.968**** | **-0.170** |
| **Plant fresh weight** | | **1.000** | **0.991**** | **-0.975**** | **-0.985**** | **-0.067** |
| **Plant dry weight** | | | **1.000** | **-0.985**** | **-0.978**** | **-0.178** |
| **Gall index** | | | | **1.000** | **0.970**** | **0.211** |
| **Egg mass index** | | | | | **1.000** | **0.006** |
| **Infected eggs** | | | | | | **1.000** |

****- Correlation coefficient is significant at the 0.01 level**

## DISCUSSION

*M. javanica* readily infected tomato cv. Pusa Ruby, retarded its growth, and reduced the fresh and dry weight of the plants. In simultaneous inoculations, adverse effects of *M. javanica* were greatly reduced and plant growth was as good as un-inoculated plants. Apparently, *P. lilacinus* was effective in suppressing *M. javanica,* but time of application was important.




1- **Simultaneous inoculation (*M. javanica* + *P. lilacinus*)**
2- ***M. javanica* preceded *P. lilacinus* by one week**
3- ***P. lilacinus* preceded *M. javanica* by one week**
4- ***P. lilacinus***
5- ***M. javanica***

**Fig 1. Dendrogram of the similarities among the treatments, using Word's minimum variance method of cluster analysis**

The fungus was effective in reducing the resulting population of *M. javanica*. This proved the abilty of *P. lilacinum* as a bio-control agent of *M. javanica*. The performance of plants in relation to root-galling and Egg mass production was significantly better in simultaneous inoculation or in inoculation, where *P. lilacinus* preceded *M. javanica*. However, prior inoculation of *M. javanica* followed by *P. lilacinus* was not that effective. This variation was in favor of the application of timing of *P. lilacinus* which is an egg parasite [9]. Its presence in the rhizosphere of roots at the time of penetration may reduce the number of juveniles that could ingress the roots. This finding is in agreement with Holland et al, who stated that *P. lilacinus* colonized the root and protects its surface from root knot nematode attacks [5]. It also reduced the number of viable eggs and juveniles of the second generation during the experimental period. There is also a very high significant (p=0.01) correlation between the plant parameters and two of nematode indexes (Table 2).

Therefore, it is plausible to expect that the presence of *P. lilacinus* before the nematode attack would offer greater protection to plants. *P. lilacinus,* a saprophytic soil-inhabitant is not expected to cause any harm to plant roots in general and is not a plant entophyte, as was true in these trials too. But, when *M. javanica* eggs, Egg masss and juveniles were present, it attacked and destroyed them to a great extent, thereby ameliorating plant growth. It is clear that, fungal hyphae of *P. lilacinus* penetrate eggshells of *M. javanica* with enzymes and pressure following the formation of a simple appressorium. The entire contents of the egg are then used as a food resource by the fungus, completely destroying the embryo/larva in the process. Eggs containing embryos or larvae can then become infected by the fungus [2].

In the present study, it was found that *P. lilacinus* penetrated the eggs and developed profusely inside and over the eggs completely inhibiting juvenile development. Some juveniles were attacked and deformed. This happened also when *M. javanica* preceded *P. lilacinus* in sequential inoculation. A high percentage (55%) of Egg masses was infected, though the root-galling and Egg mass production were not poor (Table 1 and Fig. 1). This was primarily because the fungus was not present in the soil at the time the juvenile penetrated the roots. Consequently, plant growth suffered, but the population growth of the nematode was suppressed (Table 2).

It is also important to know what will happen to a bio-control agent after it has been applied to the soil. The persistence of *P. lilacinus* after application to the soil has been estimated and results indicated that, levels fall after application and after a few months, it is difficult to isolate the fungus from the soil. This suggests that *P. lilacinus* will only cause short-term disturbances to the soil biota and will not have any long-term effect as other bio-control agents do [11, 13].

Therefore, the fungus is a potential bio-control agent attacking the infective units of the root-knot nematodes, checking the initiation of the disease and reducing the inoculum potential for successive crops. This two-pronged effect of the fungus is its most significant attribute.

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**"مقاله کوتاه"**

# بررسی اثر قارچ آنتا گونیست *Paecilomyces Lilacinus* در روند بیماری زایی نماتد *Meloidogyne javanica* و پارامترهای رشد گوجه فرنگی

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**چکیده -** بررسی های انجام شده در این تحقیق روی قارچ انگل اختیاری *Paecilomyces lilacinus* از گروه هیفومیست ها در کنترل نماتدهای ریشه گرهی گیاه گوجه فرنگی در شرایط گلخانه می باشد. این قارچ به طور موثری نماتد ریشه گرهی *Meloidogyne javanica* را کنترل نموده و باعث افزایش رشد گیاه گوجه فرنگی مورد آزمون گردیده و اثر معنی داری را در مقایسه با تیمار شاهد آلوده شده به نماتد ریشه گرهی در برداشت. تعداد گره ریشه و نیز تولید کیسه های تخم به شدت کاهش یافت. قارچ آنتاگونیست مورد آزمون اثر بیشتری را در تیمار مایه زنی همزمان قارچ و نماتد و نیز در تیمار مایه زنی قارچ قبل از نماتد در کنترل نماتد *M. javanica* از خود نشان داد. تخم های تولید آلوده به قارچ فاقد هر گونه نوزاد سن دو بوده و توسط قارچ مربوطه اشغال شده بودند ، برعکس وقتی که نماتد ریشه گرهی قبل از قارچ مایه زنی شده بود رشد گیاه کاهش و تعداد گال و تولید کیسه تخم شدت یافت. تعداد بی شماری از تخم های تولید نماتد توسط قارچ آنتاگونیست *P. lilacinus* آلوده شده و رشد و تولید نوزادهای سن دو را محدود ساخت. تخم های آلوده عاری از هرگونه نوزاد بوده و توسط قارچ مورد آزمون احاطه شده بودند. لاروهای سن تولیدی نیز توسط قارچ مربوطه مورد حمله قرار گرفت و از بین رفته و ریسه های قارچ در طراف آنها قابل مشاهده بود. مایه زنی همزمان قارچ و نماتد و نیز مایه زنی قارچ قبل از نماتد اثر بسیار بالا و معنی داری در کنترل نماتد نسبت به مایه زنی قارچ پس از نماتد داشت. لذا ، قارچ آنتاگونیست *P. lilacinus* در کنترل نماتد ریشه گرهی در گیاه گوجه فرنگی موثر واقع گردیده است.

**واژه های کلیدی: کیسه تخم، قارچ انگل تخم، قارچ انگل نوزاد، *lycopersicom esculentum* گال ریشه**

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