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EVALUATION OF EFFICACY OF *PAECILOMYCES LILACINUS* AS BIOLOGICAL CONTROL AGENT OF *MELOIDOGYNE INCOGNITA* ATTACKING TOMATO

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Abstract: The efficacy of *Paecilomyces lilacinus* strain UP1 as biological control agent of *Meloidogyne* incognita attacking tomato was evaluated under screenhouse condition pot experiments. *P. lilacinus* was formulated on rice substrate in powder form. Root weight, gall index rating, number of galls, egg masses and nematodes per one gram root sample were determined and per cent reduction in gall number was computed. Root weight and gall index ratings were significantly higher in untreated plants than those with *P. lilacinus* and with the commercial fungicide Nemacur. Number of galls, nematodes and egg masses per one gram root sample were significantly reduced by the application of *P. lilacinus* at all levels and this was comparable with Nemacur. However, egg mass count in plants treated with the lowest concentration of the biocontrol agent was not significantly different from the uninoculated control. Per cent reduction in gall number was the highest at treatment with 7.92 x 10^6 spores per ml of *P. lilacinus*.

Key words: biocontrol, *Paecilomyces lilacinus*, *Meloidogyne incognita*, root knot disease, tomato

INTRODUCTION

In the Philippines, tomato is the second most important vegetable crop in terms of area and volume of production. However, tomato production is beset by many production constraints, one of which is the root knot disease caused by *Meloidogyne incognita* (Kofoid and White) Chitwood. It was reported to cause 85% yield loss in the coastal plains and 20–30% in the highland (Valdez 1979). Infection is characterized by root swellings often called knots and galls that contain the enlarged saccate female nematode. Galls affect the conductivity of roots, so that in later stages, roots become deformed and very much reduced (Taylor and Sasser 1978). As a consequence of galling and reduced root system, the efficiency of the roots decreases and ultimately causes stunting of the plant.

Government restrictions on the use of chemicals by small farmers and the increasing cost and hazards of the application have stimulated research towards the development of a cheaper and effective control measures that could be recommended to all crop growers in the country. The use of biological control agents to manage plant parasitic nematodes may provide the best alternative to pesticides.

The discovery of nematophagous fungus *Paecilomyces lilacinus* in 1979 by Jatala *et al.* at the International Potato Center in Lima, Peru greatly stimulated 16 years study on biological control of plant parasitic nematodes in the Philippines. Two genera of nematode-trapping fungi, *Arthrobotrys* sp. and *Dactyllela* were also tried against plant parasitic nematodes, however, results were not generally convincing until the use of *P. lilacinus* which was quite successful (Cortado and Davide 1968; Reyes and Davide 1975). Davide and Zorilla (1983) revealed that *P. lilacinus* isolates from Peru and the Philippines were comparatively effective in controlling the cyst nematode and their use resulted in a significant increase in potato yield.

The objectives of the study were: 1) to evaluate the efficacy of formulated *P. lilacinus* against *M. incognita*; 2) to determine the level of formulated *P. lilacinus* which is effective against *M. incognita* and 3) to compare its effectiveness with that of Nemacur, a commercial nematicide.

MATERIALS AND METHODS

Mass production of *P. lilacinus*

P. lilacinus strain UP1 grown in sterile rice powder was used. *P. lilacinus* UP1 is a strain from the original *P. lilacinus* culture used by Villanueva and Davide (1984), Generalao and Davide (1986), Orolfo and Davide (1986).

Inoculation with *M. incognita*

Galled roots were collected from the culture of plants and washed gently with water to remove soil particles. The roots were cut into 1–2 cm segments and placed in a jar containing 200 ml of 0.5% NaOCl solution. The jar was shaken vigorously for 2–4 minutes. The suspension

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was passed through 60 and 325 mesh sieves nested over the 500 mm mesh sieve which collected the eggs. The eggs in the sieve were placed quickly under stream of cold water to remove the residual NaOCl. The eggs were then transferred into a beaker with the aid of a wash bottle. The number of eggs per unit volume was standardized and the desired number of eggs (approximately 500 eggs/pot) were placed in separate vials. The egg suspension was introduced by pouring into the sterilized potted soil.

Application of treatments

Pots filled with sterilized soil were randomly arranged in the screenhouse. The desired concentrations of *P. lilacinus* were prepared and placed in separate vials. The treatments were as follows: T0-uninoculated; T1 – *M. incognita* alone; T2 – *M. incognita*+*P. lilacinus* ($1.584x10^5$ spores/ml; T3 – *M. incognita*+*P. lilacinus* ($7.92x10^6$ spores/ml); T4 – *M. incognita*+($3.96x10^8$ spores/ml); T5 – *M. incognita*+Nemacur. Each treatment was replicated four times. The application was done through soil drenching one day after inoculation with the nematode. Tomato seedlings were then transplanted and allowed to grow for 37 days.

Disease rating and analysis of data

Galling index was determined using the following rating scale: 1 – no galling; 2 – trace (1–25% galling); 3 – slight (26–50% galling); 4 – moderate (51–75% galling) and 5 – severe (76–100% galling). Population density of nematodes in roots, number of galls and egg masses were determined in one gram root sample stained with acid fuchsin lactophenol. Counting was done with the aid of a dissecting microscope and a hand tally counter. Percentage reduction in gall number was computed using the formula:

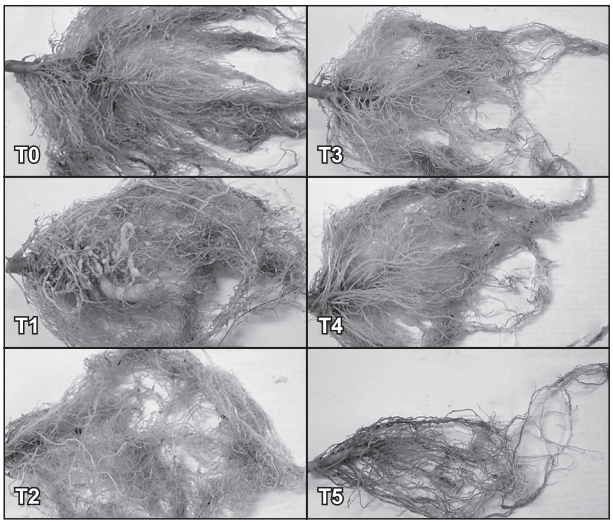
$$\text{Percentage reduction} = \frac{\text{no. of galls (untreated)} - \text{no. of galls (treated)}}{\text{no. of galls (untreated)}} \times 100$$

Completely randomized design was used in this experiment. Data were subjected to analysis of variance (ANOVA) using the MSStat Sofware.

RESULTS AND DISCUSSION

Root weight

The weight of roots was significantly higher in plants treated with *M. incognita* alone (Table 1) which was attributed to the presence of larger galls in the roots (Fig. 1.). On the other hand, plants treated with *P. lilacinus* showed reduced root weight and this was comparable with the uninoculated control. Lowest root weight was observed in plants treated with Nemacur which was probably due to phytotoxic effect of the chemical owing to small pots used in the experiment.



T0 – uninoculated control; T1 – *M. incognita* alone; T2 – *M. incognita*+*P. lilacinus* ($1.584x10^5$ spores/ml); T3 – *M. incognita*+*P. lilacinus*($7.92x10^6$ spores/ml); T4 – *M. incognita*+*P. lilacinus* ($3.96x10^8$ spores/ml.) T5 – *M. incognita*+Nemacur

Fig. 1. Roots of tomato inoculated with *M. incognita* and *P. lilacinus*

Table 1. Effect of *P. lilacinus* on *M. incognita* attacking tomato 37 days after inoculation

Treatments[1]	Root weight	Gall index rating[2]	No. of galls[3]	No. of nematodes[3]	No. of egg masses[3]	Per cent reduction
Uninoculated control	6.025 b	1.0 c	0	0	0	–
M. incognita alone	9.225 a	5.0 a	32.5 a	245.0 a	31.3 a	–
M. incognita+*P. lilacinus* ($1.584 x 10^5$ spores/ml)	3.175 bc	2.3 b	23.5 b	23.5 b	19.0 ab	89.89
M. incognita+*P. lilacinus* ($7.92 x10^6$ spores/ml)	5.725 b	2.0 b	6.3 b	6.3 b	4.5 bc	97.31
M. incognita+*P. lilacinus* ($3.96x10^8$ spores/ml)	4.725 bc	2.0 b	10.8 b	10.8 b	9.0 bc	95.38
M. incognita+Nemacur	1.575 c	1.0 c	0	0	0	100.0

[1] average of four replicates. Means followed by the same letter are not significantly different
[2] per root system
[3] per one gram root sample

Gall index

Gall index rating of the whole root system showed trace galling when treated with *P. lilacinus* but it was not comparable with the effect of Nemacur. However, it was significantly lower as compared to the inoculated check. This suggests that the biocontrol agent was able to suppress gall formation in tomato roots. Davide and Zorilla (unpublished) revealed that the greenhouse test in tomato using the drenching method of application showed that the fungus grown in different substrates had considerably controlled the root knot nematodes showing trace to slight galling index rating as compared to severe gall index in the check.

Number of galls and nematodes

Gall and nematode counts per one gram root sample showed the same trend. These were significantly reduced in plants treated with *P. lilacinus* when compared with those treated with *M. incognita* alone. The values were comparable with those treated with Nemacur and uninoculated control plants. This implies that *P. lilacinus* is as effective as a nematicide in reducing nematode population.

It was also observed in *P. lilacinus* treated plants that only one female (mostly adult) nematode occupies one gall owing to low population, thus they were able to establish on separate infection sites. On the other hand, more than one nematode can be found in one gall in the inoculated check. Juvenile stages of *M. incognita* were also observed. Presence of the juveniles indicates that nematodes which were inoculated have already laid eggs which have hatched and penetrated the roots. The total life cycle of *M. incognita* is only 25 days so at harvest (37 days), there were juveniles already. Due to high level of nematodes, it was possible that the nematodes were able to penetrate closer sites causing the formation of larger galls and this attributes the presence of the same nematode stages in one gall. The absence of juveniles and the presence of adult stages only in *P. lilacinus* treated plants suggested that most probably there was a delay in the life cycle or development of the nematodes due to the presence of the parasite.

Egg Mass Count

Egg mass count per one gram root sample was reduced by *P. lilacinus*, however, no significant differences were observed between plants with the lowest concentration of the biocontrol agent and those with *M. incognita* alone. Low egg mass count with nematode alone reveals that some of the eggs have already hatched in the presence of the juveniles. In case of treated plants, low count could be due to the effect of the parasite. Means among plants treated with *P. lilacinus* showed insignificant differences.

Gall reduction

The numbers of galls was reduced by 89.89%, 97.31% and 95.38% by the application of 1 594^5x10^5 spores per ml, 7.92x10^6 spores per ml and 3.96x10^8 spores per ml, respectively. This concurs with the study conducted by Davide and Zorilla (1986) in which root galling in okra treated with *P. lilacinus* remained at trace and slight levels. Moreover, *P. lilacinus* applied as seed treatment on undelinted and delinted cotton seeds significantly reduced root galling (Davide and Batino 1985). Reduction in the number of galls, nematode and egg masses in plants introduced with *P. lilacinus* is attributed to the antagonistic effect of the parasite on nematode. The fungus might have killed or inactivated the newly hatched larvae due to a toxic material it possibly produced. According to Morgan-Jones *et al.* (1984), the main types of destructive activity of *P. lilacinus* are thought to be enzymatic disruption of nematode structural elements such as egg shells, larval cuticles and physiological disturbances brought about by biosynthesis of diffusible toxic metabolites. The overall effect seems to involve disruption of embryonic development and death of larvae resulting in the reduction of nematode population (Morgan-Jones and Rodriguez-Kabana 1985). Jatala *et al.* (1985) also mentioned that *P. lilacinus* (in addition to penetrating and killing the eggs and occasionally killing juveniles and females of *M. incognita* and *Globodera pallida*) caused substantial egg deformation in *M. incognita.* These deformed eggs never matured or hatched. Once a hyphal network is in the close proximity of nematode eggs, its diffusible toxic metabolites will either cause some alterations in the egg shell makeup or, by their inward seepage in the egg, and physiological disorders leading to aborted embryonic development.

The ability of *P. lilacinus* to effectively kill or inactivate nematode larvae and reduce nematode infectivity and fecundity implies that the fungus is a potential biological control agent.

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POLISH SUMMARY

OCENA SKUTECZNOŚCI *PAECILOMYCES LILACINUS* JAKO CZYNNIKA BIOLOGICZNEGO ZWALCZANIA *MELOIDOGYNE INCOGNITA* ATAKUJĄCEGO POMIDORY

W doświadczeniu wazonowym prowadzonym w warunkach szklarniowych, oceniano skuteczność *Paecilomyces lilacinus* jako czynnika biologicznego zwalczania *Meloidogyne incognito,* atakującego pomidory. Przygotowano formulację *P. lilacinus* na podłożu z ryżu, w sproszkowanej formie. Określano wagę korzeni, indeks występowania narośli, liczbę narośli, masę jaj i nicieni, w jednym gramie próby korzeni oraz obliczano procent redukcji liczby narośli na korzeniach roślin traktowanych grzybem *P. lilacinus.* Waga korzeni i indeks występowania narośli były istotnie wyższe w przypadku roślin nietraktowanych *P. lilacinus*, w porównaniu do roślin traktowanych tym grzybem, lub fungicydem Nemacur. Liczba narośli, nicieni i masa jaj, w jednym gramie próby, były istotnie zredukowane w wyniku potraktowania ich różnymi dawkami *P. lilacinus*, co było porównywalne z zastosowaniem preparatu Nemacur. Jednak masa jaj po zastosowaniu najniższej dawki czynnika biologicznego zwalczania nie różniła się istotnie od nietraktowanej nim kontroli. Procent redukcji liczby narośli był najwyższy w kombinacji – 7,92 x 10^6 zarodników *P. lilacinus* w 1 mililitrze.