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INFLUENCE OF TWO *PASTEURIA PENETRANS* ISOLATES AT DIFFERENT APPLICATION TYPE ON ROOT-KNOT NEMATODES *(Meloidogyne* spp.)

Mahdy, M. E.[1]; Mousa, E. M.[1] and Heba Y. Al-Sisi[2]

[1]Dept. of Agric. Botany ,Fac. Of Agric., Minufiya Univ., , Shebin El-Kom, Egypt

[2]Dept. Of Nematode Res., Inst. Of Plant Pathol., Agric. Res. Centre, Giza, Egypt

ABSTRACT

Two *P. penetrans* isolates namely: Pp Japanese and Egyptian were used at different three types: pp infected root powder, spore suspension and attached larvae as a biocontrol agent against root-knot nematodes, Meloidogyne spp. on tomato plants under greenhouse conditions. Results revealed that the effective treatment obtained with Pp Japanese isolate as a pp root infected powder, where all nematode parameters significantly reduced compared to plants treated with nematode alone. Egyptian isolate appeared promise result when applied also as a pp root infected powder and as a spore suspension. The lowest effect was noticed with Pp Japanese isolate when applied as attached larvae. Results indicate that the highest Pp infected females recorded with Pp Japanese isolate as a root powder, followed by Pp Egyptian isolate as a spore suspension, whereas the lowest Pp infected females recorded with Pp Japanese isolate as a attached larvae.

Key words: Root-knot nematodes *Meloidogyne* spp.; *Pasteuria penetrans*; Tomato (*Lycopersicon esculentum* Mill).

INTRODUCTION

The root-knot nematodes (*Meloidogyne* spp.) are sedentary endoparasites and are among the most damaging agricultural pests, attacking a wide range of crops (Sikora and Grico, 1993). The infection starts with root penetration of second stage juveniles hatched in soil from eggs encapsulated in egg masses laid by the females on the infected roots (Barker *et al*., 1985).

Chemical control is becoming more and more expensive because of increased costs in the synthesis of new compound and their use is increasingly undesirable because of environmental hazards associated with their application. Now some attention has been given to biological control of plant-parasitic nematodes with the use of natural enemies as a safe and cheap alternative method to chemical control (Gowen and Ahmed, 1990).

Encouraging results were obtained with the use of *Pasteuria penetrans* as a biological control agent of nematodes on different crops (Zaki and Maqbool, 1991 a). *P. penetrans* is an endospore-forming, gram-positive bacterium that is considered an important parasite of several of the agriculturally important *Meloidogyne* spp. Within this bacterial species exist strains that are specific to species within the genus *Meloidogyne* even down to the host race level of the nematode (Chen and Dickson, 1998). The bacterium is an obligate parasite of root-knot nematode, and so it has not been successfully cultivated on artificial media although numerous researchers have made such attempts (Chen and Dickson, 1998). The Pp infected females was distinguished by their opaque dull creamy white to amber color compared to white healthy females of *Meloidogyne* spp. as described by Mankau and Imbriani (1975); Mankau and Prasad (1977). Infected females of *Meloidogyne* spp. can contain up to 2.5 million non-motile endospores (Hewlett and Dickson, 1993) that are released into the soil

*corresponding author e-mail:Mahdymagdy@yahoo.com

environment upon degradation of the nematode carcass.

The objective of this work was to evaluate both *P. penetrans* isolates at three different types application against root-knot nematodes, *Meloidogyne* spp. on tomato plants under greenhouse conditions.

MATERIALS AND METHODS

In our research two *Pasteuria penetrans* isolates i. e Pp Japanese and Egyptian were evaluated against root-knot nematodes on tomato plants under greenhouse conditions. The bacteria isolates were applied at three types as follow: pp infected root powder, spore suspension as well as attached larvae. This experiment was carried out at the Experimental farm of Faculty of Agriculture, Minufiya Univ, Shebin El-Kom.

Experiment was carried out in plastic pots (15 cm in diam.) filled with sandy-clay mixture soil (2:1, v/v). Three weeks old tomato seedlings (*Lycopersicon esculentum* Mill cv. GS) were transplanted into plastic pots (one plant/ pot) and all the treatments were applied simultaneously at transplanting time. One thousand nematode larvae either attached or healthy were inoculated/plant by pipetting into 3 holes made around the plant roots. Each treatment was replicated three times. Plants were daily watered and fertilized weekly with 5 ml of 2g/L of N:P:K (20:20:20), International Egypt Company for Agricultural and Industrial Developing.

Eight weeks after nematode inoculation, nematode parameters i.e. total number of galls/root system, number of egg masses/root system, number of eggs/egg masses, number of juveniles/250 g soil, number of infected and non infected females/root system, number of attached and nonattached larvae/root system, final nematode population (P_f) and reproduction factor (R_f) (nematode build-up). Vegetative growth parameters i.e. shoot and root length, fresh shoot and root weights, shoot dry weight as well as root volume were also determined.

Egg masses were assessed by dipping the root system into Phloxin-B staining solution (0.015%) for 20 minutes according to Daykin and Hussey (1985). Females of *Meloidogyne* spp. were collected by cutting the root system of each plant into 2 cm pieces and soaking in glass beaker full of tap water for 4 days under room temperature until they became soft. The roots were then washed through 500 and 250 µm sieves to separate the females from the root debris (Mahdy, 2002).

Final nematode population (PF) was assessed according to the equation:

PF = (No. of egg-masses × No. of eggs/ egg-mass) + No. of females + No. of developmental stages + No. of juveniles in soil. The reproduction factor of root-knot nematode (RF) was also recorded according to Norton (1978).

Soil nematode population was enumerated by extraction root-knot nematode juveniles (J_2S) by using the tray modification of Baerman funnel as described by Barker (1985).

The chemical constituents i.e. membrane permeability in roots calculated according to Leopold *et al*., (1981), carbohydrate and total phenols were determined in dry leaves according to Dubois *et al*., (1956) and Snell and Snell (1953) respectively. Total amino acid in dry leaves calculated according to Rosen (1957), peroxidase, phenoloxidase and catalase enzymes activity in fresh leaves were also determined. Catalase enzyme activity was measured according to the method described by Bach and Oparin (1968). Phenoloxidase was measured according to the method described by Broesh (1954). Peroxidase activity was measured according to the method described by Fehrman and Dimond (1967) .

Statistical Analysis:

Data were statistical analyzed according to standard analysis of variance by a one way ANOVA with the software statgraphics (Statistical Graphics, Crop, Rockville, MD).

Source of *P. penetrans* Isolates:

The Japanese isolate of *P. penetrans* was obtained from Dept. of Nematology, Fac. Of Agric., Reading Univ., England.

The Egyptian isolate was detected on J_2S and mature females of root-knot nematodes in soil and root samples, respectively only in Kom Hamada region, El-Behira governorate **(Bakr *et al*., 2011)**.

P. penetrans application type:

Both *P. penetrans* isolates were applied to soil pots at three different type as follow:

I- Attached larvae

Larvae were extracted from infected roots according to Oosenbrink (1960). Larvae were exposed to spore suspension in Petri dishes (3cm in diam.) each contain 1ml of spore suspension ($1x10^6$ spore/ml) plus 1ml distilled water contain 500 freshly hatched larvae. Petri dishes were incubated for 12 hours under laboratory conditions. Number of attached larvae were counted under stereomicroscope at magnification 400x and adjusted to the optimum density of spores/larvae (10-15 spore/larvae after 12 hrs.) as shown in Figure (1).

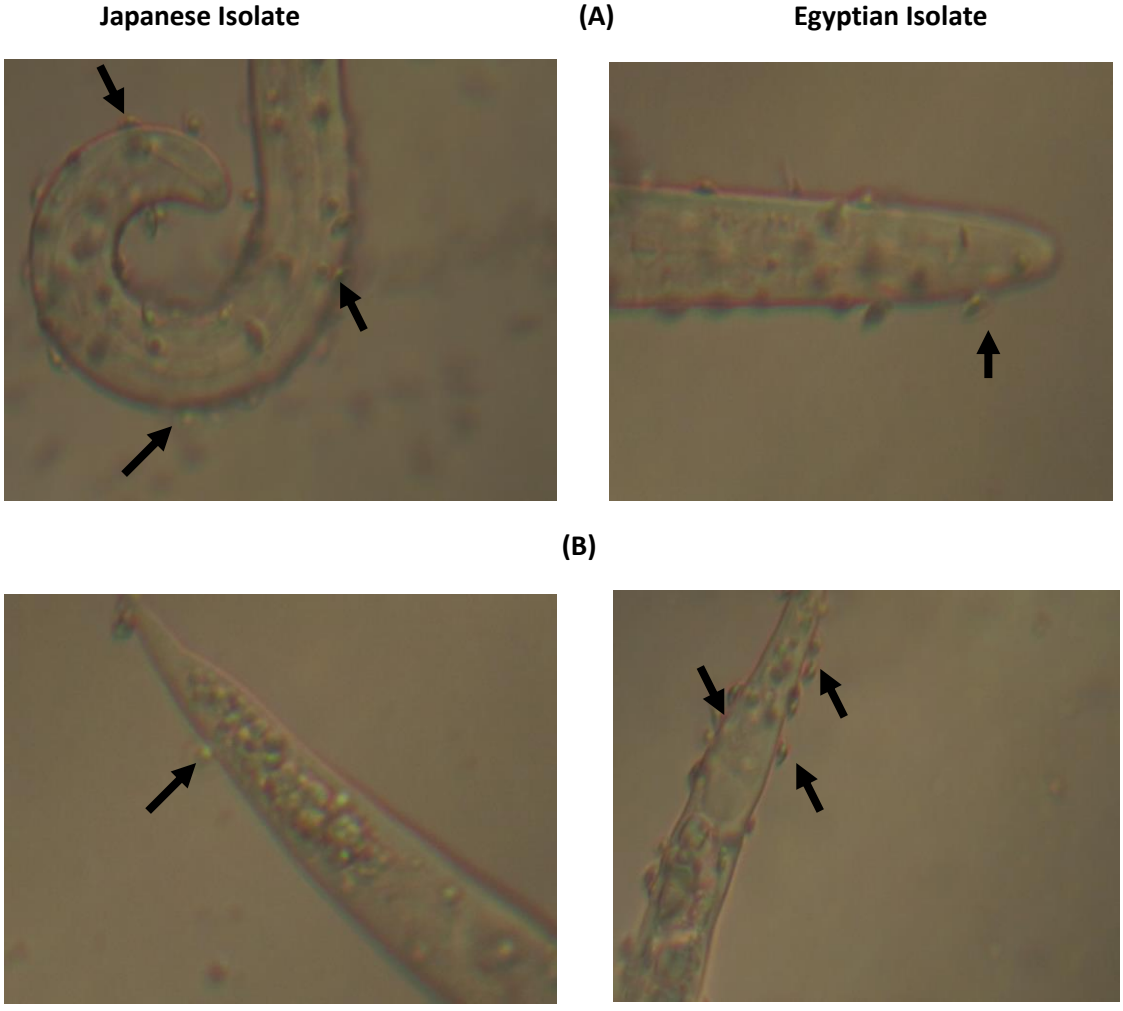

Figure (1): Spores of *P.penetrans* Japanese isolate attached to *Meloidogyne* spp. larvae (A) Head region (B) Tail region.

II- Spore suspension

Spore suspension (Figure 2) of both Pp isolates was prepared by adding 0.1 gm of Pp infected root powder of each isolate to a small amount of tap water in pestle and mortar as described by Stirling and Wachtel (1980). Number of spores/ml was counted on a haemicytometer slide and five ml of spore suspension ($1x10^6$ spore/ml) of each isolate was inoculated per plant in the root zone.

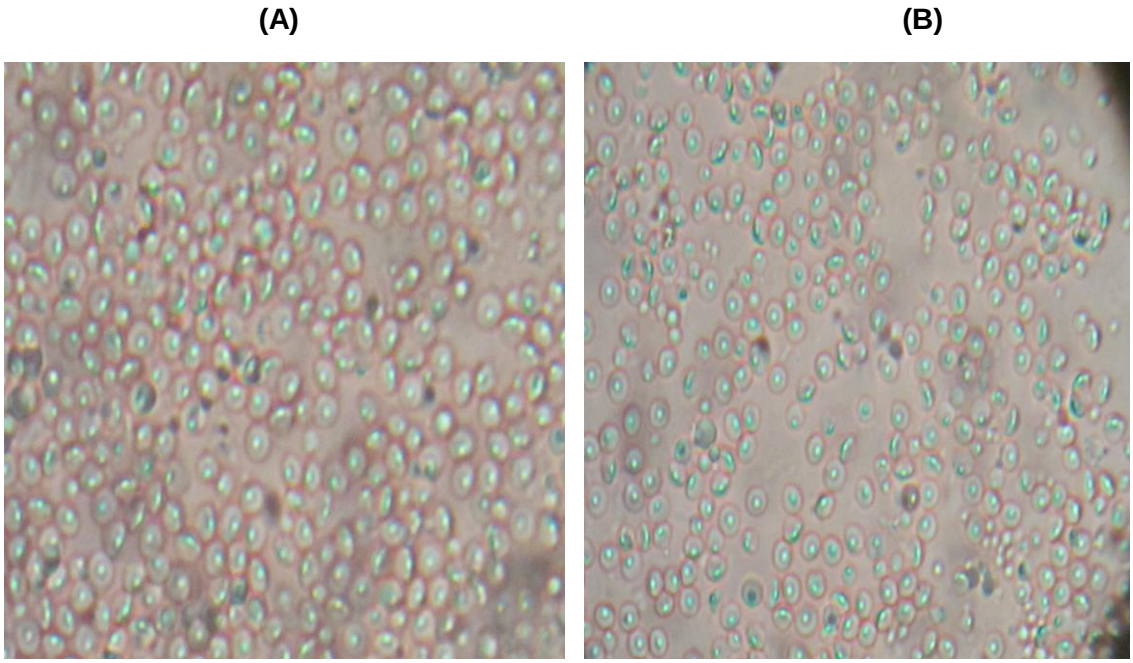

Figure (2): Spore suspension of *P. penetrans* isolates (A) Japanese isolate (B) Egyptian isolate.

Plant powder was obtained by drying Pp infected root aerobically and milled well in a pestle and mortar, Half gram of each Pp powder were added per plant at the same time of tomato seedlings transplanting and healthy nematode larvae inoculation.

RESULTS

Both *Pasteuria penetrans* isolates either the Japanese or the Egyptian one was significantly reduced the nematode related parameters compared to the treated plants with nematode alone. against root-knot nematode *Meloidogyne* spp. Results illustrated in figure (3) showed that the percentage of galls reduction failed between 41 and 93%. The highest reduction recorded with Pp Japanese powder by 93, followed by 88% for the Pp Egyptian powder, whereas the lowest one recorded with Pp Japanese attached larvae by 41%.

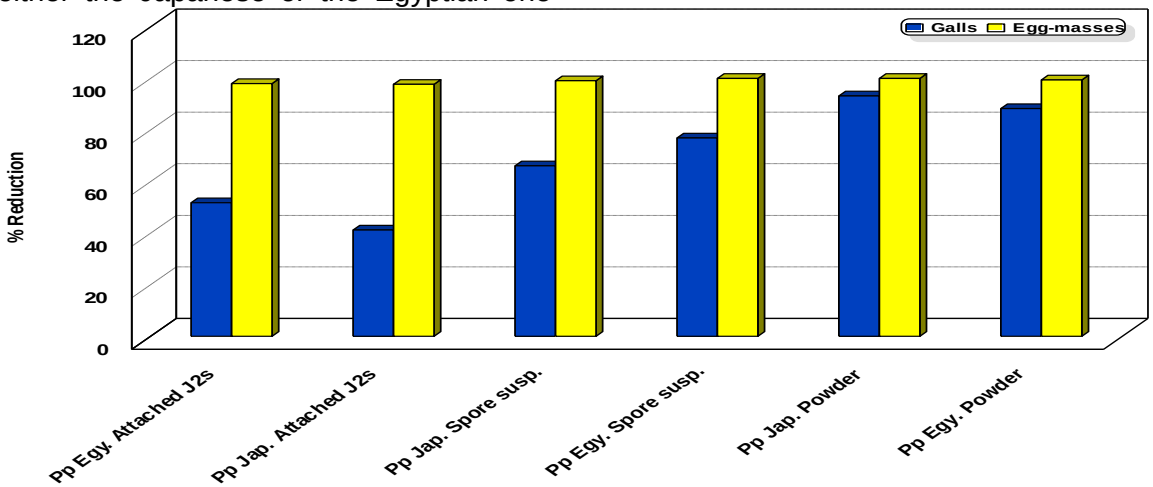

Figure (3): Percentage of galls and egg masses reductions as affected by application of both *P. penetrans* isolates on tomato.

Results in Fig. (3) showed also the same trend as the highest percentage of egg masses reduction obtained with Pp Japanese powder and Pp Egyptian spore suspension by 100%, followed by Pp Egyptian powder by 72.5%, whereas the

lowest one recorded with Pp Japanese attached larvae by 48% compared to the plants treated with nematode alone.

Number of Pp infected females was also significantly affected by both source as they originated and the application type (Fig. 4).

The infection of *Meloidogyne* spp. females with *P. penetrans* spores (Fig.5) was increased when nematodes exposed to Pp Japanese powder followed by Pp Egyptian powder. The least Pp infected females recorded with attached larvae of Pp Japanese as illustrated in figure (4).

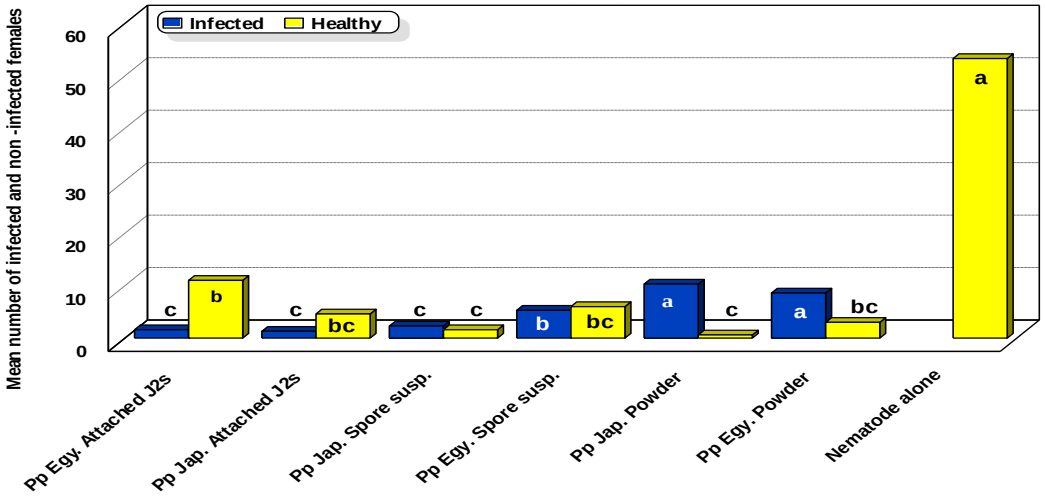

Figure (4): Mean number of infected and non-infected *Meloidogyne* spp. females as affected by application of both *P. penetrans* isolates on tomato.

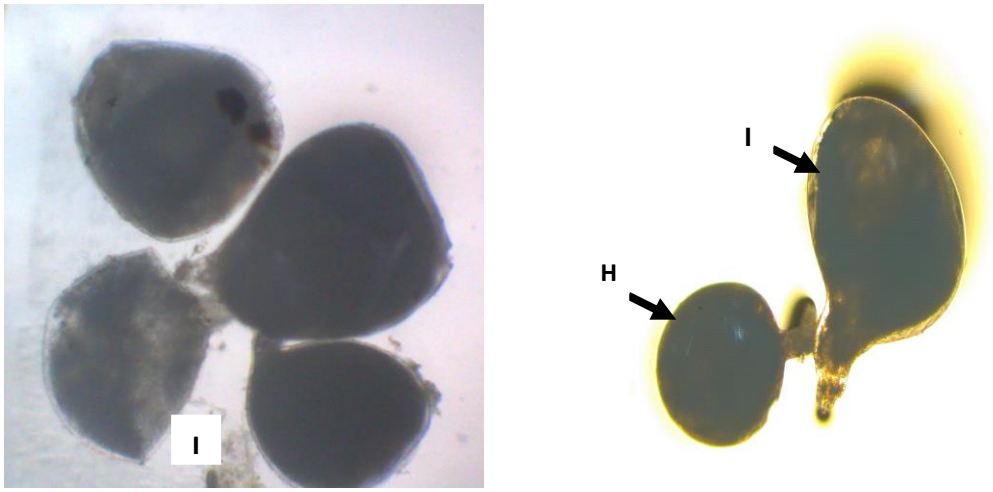

Figure (5): Infected (I) and healthy (H) *Meloidogyne* spp. females as affected by application of both *P. penetrans* isolates on tomato

Results illustrated in figure (6) showed that the percentage of membrane permeability was significantly influenced with all applied bacterial treatments at all three types compared to the treated plants with nematode alone. The lowest damage in cell membrane recorded with Pp Japanese isolate as a powder, followed by Pp Egyptian powder and spore suspension, respectively. The least effect on membrane permeability obtained with attached larvae of Pp Japanese isolate (Fig. 6) compared to nematode only treated plants.

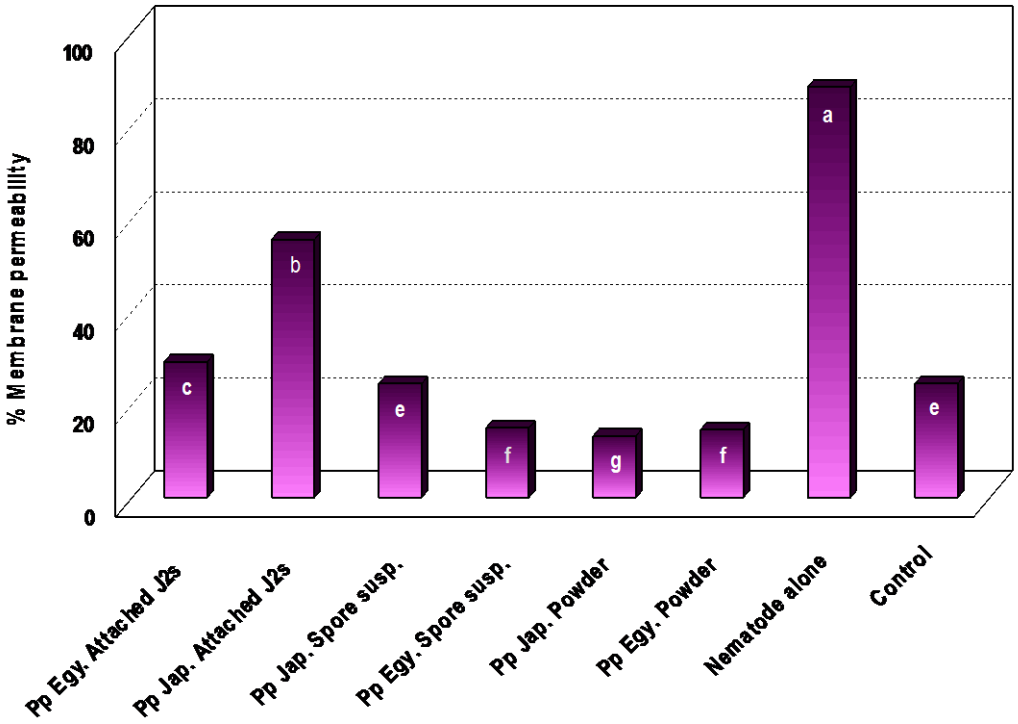

Figure (6): Effect of *P. penetrans* isolates on the percentage of membrane permeability of tomato roots infected with *Meloidogyne* spp.

The chemical components i.e. total carbohydrates, total phenols and total amino acids in tomato dry leaves were also improved with Pp Japanese powder, followed by Pp Egyptian powder as presented in figure (7). The lowest value of total carbohydrate and phenols recorded with Pp Japanese attached larvae, whereas the lowest value of total amino acid recorded with Pp Egyptian attached larvae .

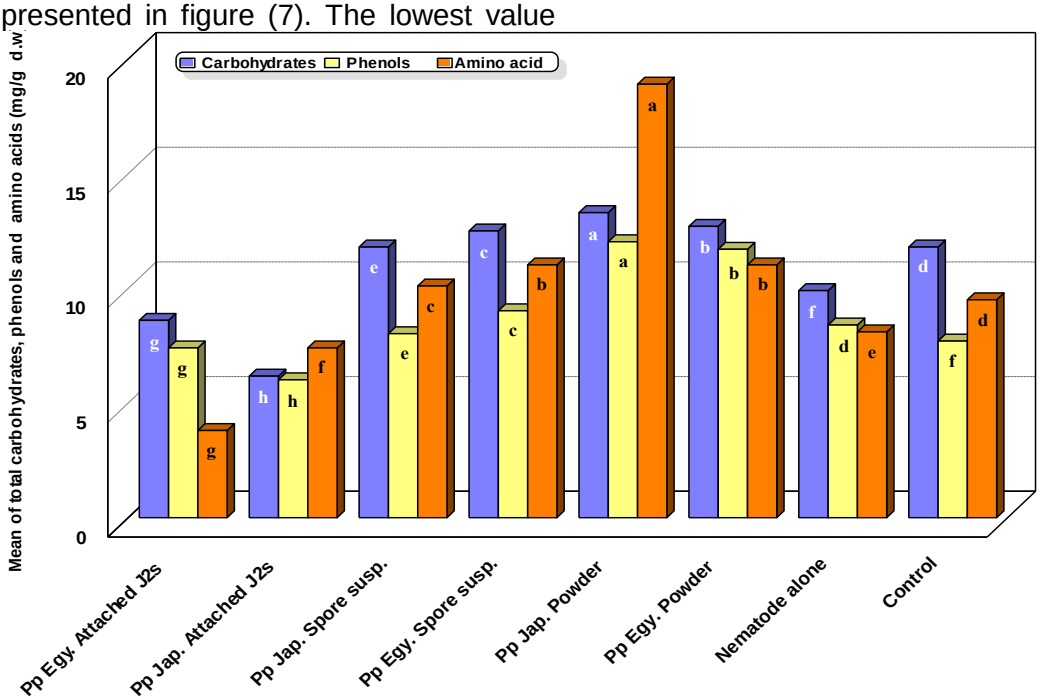

Figure (7): Effect of *P. penetrans* isolates on the total carboydrates, phenols and amino acids of tomato dry leaves.

Data illustrated in figure (8) proved that the activity of antioxidant enzymes i.e. phenoloxidase, peroxidase and catalase recorded with both Pp isolates at all three applied types compared to the plants treated with nematode alone. Japanese Pp powder proved to be the effective one as it significantly enhanced the activity of phenoloxidase enzyme, followed by Pp Egyptian powder (Fig. 8 A).

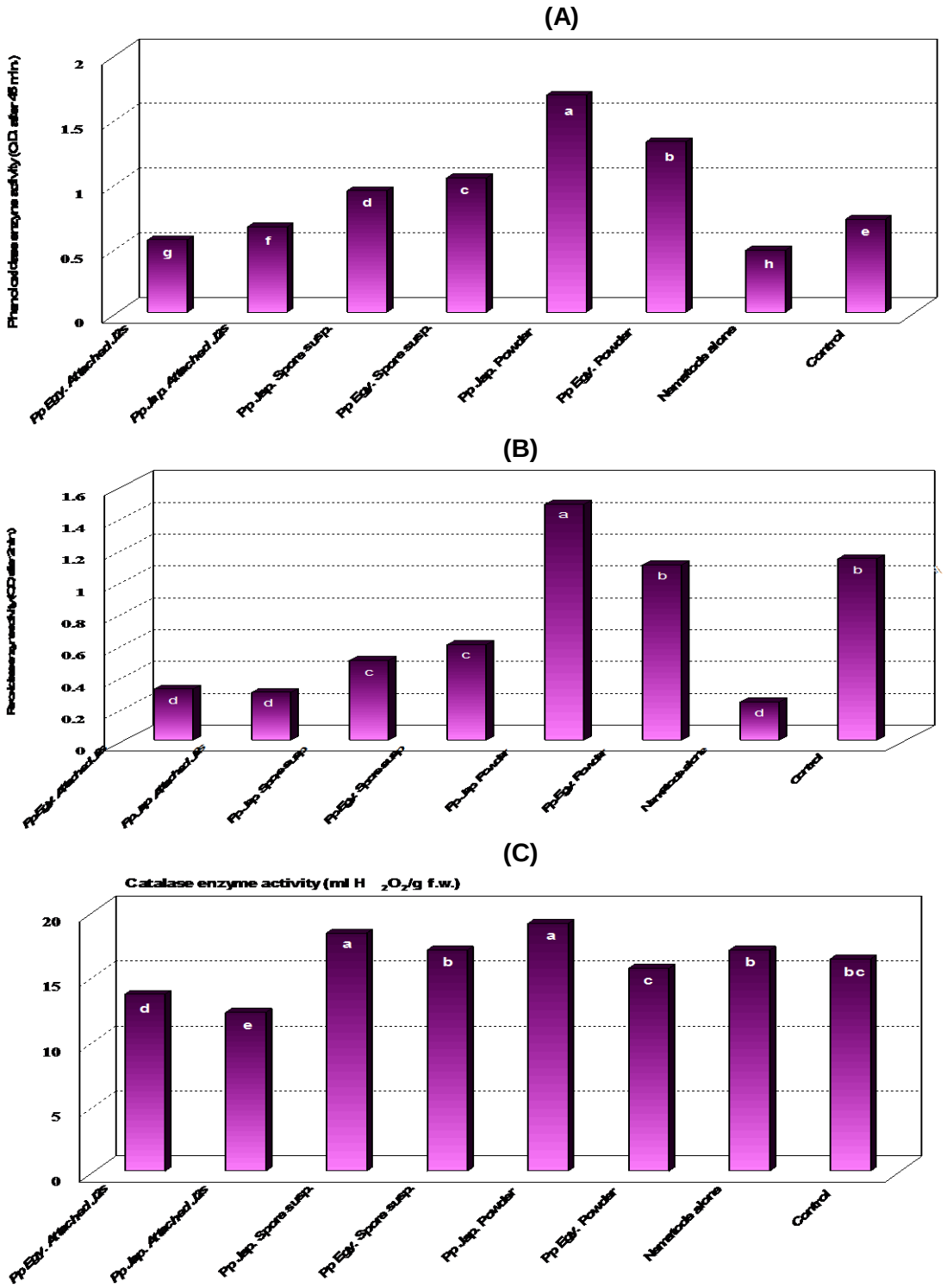

Figure (8): Effect of *P. penetrans* isolates on the antioxidant enzymes activity i.e. phenoloxidase (A), peroxidase (B) and catalase (C) in fresh tomato leaves.

DISCUSSION

The infection of *Meloidogyne* spp. with the endoparasitic bacterium *Pasteuria penetrans* were varied depending on Pp isolates (original source), bacterial densities and application type as well. whereas the Japanese isolate caused high significant reduction in number of galls and egg masses /root system compared to plants treated with nematode alone and also it caused increase in number of Pp infected females. Japanese isolate also increased number of root knot nematodes attached juveniles extracted from infected roots when it applied as a powder.

Whereas the Egyptian isolate applied as a powder caused high reduction in number of galls and egg masses /root system, but lower than the obtained with Japanese

one. These results means that the isolates of *Pasteuria penetrans* are highly specific and the greatest attachment of spores occurred when they were exposed to the species of *Meloidogyne* from which they were originally isolated as described by Davies *et al*., (1988); Espanol *et al*., (1997) and Regina *et al*., (1999).

Our results were consistent with those obtained by Mahdy (2002) since he used a seven different isolates from *P. penetrans* and he found that three of the seven isolates attained a 50% higher different attachment rate to *Meloidogyne* juveniles and the other four isolates had lower attachment rate.

Two isolates of *P*. *penetrans* namely: El-Behera (PpB) and Kafer El- Sheikh governorate (PpG) were evaluated for their potential against *M. incognita* infected tomato plants. Results found that treatment with spore suspension of PpB from infested soil caused significant reduction in number of nematode root galls and egg masses, more than that of isolate PpG. Treatments with spore suspension of PpB from powdered roots caused considerable reduction, followed by the treatment with PpG as confirmed by El-Saedy and Asmaa Mokbel (2007).

Zareen *et al*., (2002) evaluated different *P. penetrans* isolates i. e UK1, PK1 and PK2 at three different concentrations as a spore suspension against root-knot nematodes *Meloidogyne* spp. juveniles. Results revealed that there was significant differences in root invasion by nematodes exposed to the different isolates and concentrations. Variable spore infection of root knot nematode was observed with each test bacterial isolate (UK 1, PK 1 and PK 2). They found that also reduced number of bacterial spores to single nematodes by all three bacterial isolate may attributed to their compatibility with nematode host.

The variation in our results due to the different Pp isolates and application type. So, Zareen *et al*., (2002) reported that the variation in spore attachment of the different bacterial isolates may be attributed to difference in the surface composition of nematode species, race, population and heterogeneity of the endospore surface.

Our results revealed that inoculation the Pp attached larvae observed a low effect on the nematode related parameters and low numbers of Pp infected females. These results may be due to that the juveniles carrying larger number of bacterial endospores, so become less mobile in the rhizosphere and consequently can not invade the plant root tissues (Stirling and White, 1982). Zareen *et al*., (2002) found that nematode penetration was suppressed with such treatment, which were receiving nematodes with heavy spore load.

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