# A SUSTAINABLE MANAGEMENT OF CORKY ROOT AND ROOT-KNOT NEMATODES BY THE BIOCONTROL AGENT *APHANOCLADIUM ALBUM* ISOLATE MX-95

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**Abstract**: *A trial was carried out in a plastic house on tomato to assess the efficacy of the chitinolytic fungus Aphanocladium album isolate MX-95 (AA MX-95) against the soil borne pathogen Pyrenochaeta lycopersici and the root-knot nematode Meloidogyne incognita. Treatments were: a) AA MX-95 applied in sub irrigation at 2.5 L/plot (1.2 x $10^7$ CFU/mL, conidial suspension) in pre transplant (20 days), transplant and in post transplant (2 times every 20 days) + root dipping (5 min – conc. 1.2 x $10^7$ CFU/mL) at transplant); b) as before indicated without treatment in pre transplant; c) root dipping and d) dazomet (chemical control) applied 30 days before transplant at 600 Kg/ha. Untreated plots served as control. A significant yield increase and a reduction of severity of corky root and nematode attacks were observed in AA MX-95 and dazomet treated plots in comparison to control. High positive correlations were found between the symptoms caused on tomato roots by M. incognita and P. lycopersici.*

**Key words**: *Pyrenochaeta lycopersici, Meloidogyne incognita, chitinolytic fungus, bio-nematicide, plant protection.*

## INTRODUCTION

The recent European Legislation on the use of pesticides on agricultural crops has strongly reduced synthetic formulates available for pest control and plant protection and has imposed the search of new alternative control strategies joining a satisfactory biocidal performance to environmental and economical sustainability. The sustainable management of soil borne plant pathogens and parasites is a key factor for a quanti-qualitative improvement of crop yield both in open field and plastic house conditions, for environmental safety.

During the last decade, research on low environmental impact alternatives to pesticide has considered a wide range of options including agronomic strategies (amendments, biofumigations, crop rotations, grafting, green manure, mycorrhization, resistant or tolerant cultivars) [1; 11; 15; 16; 18; 22], physical methods (soil solarization, steam sterilization and ozone treatments as gas or ozonated water) [12; 17; 20; 25], use of biocidal plants [14] and biological control agents (BCAs) [19; 23; 24].

Biological control of plant diseases by antagonistic microorganisms is one of the most important alternative methods to the use of pesticide in agriculture.

Since 1972, it was observed by Biali *et al*. (1972) [3] that various isolates of the hyphomycetous fungus *Aphanocladium album* (Preuss) W. Gams grown on uredia of a number of rust species (*Puccinia coronata*, *P. hordei*, *P. graminis* f.sp. *avenae* and *P. recondita* f.sp. *triticina*) when host plants were kept under high humidity and they didn't grown on unrusted plants. Uredia were adversely affected and apparently normal teliospores developed earlier on detached or undetached rusted leaves of young oat plants inoculated with *A. album* than on non-inoculated rusted leaves. *A. album* induced telial formation in some isolates of rust species that had rarely or never produced telia. So it was demonstrated the importance of *A. album* as a working tool in rust research and as a possible means for biological control of rust. Four years later Forrer (1977) [7] hypothesized that the induction of teliospore was probably due to metabolic products of *A. album* and used the fungus as biological control method of rusts.

Some years ago one isolate, signed as Mx-95, of the fungus *A. album* (patent N° MI2006A 000503 21 march 2006, inventor Prof. Ciccarese *et al*.) has shown an appreciable effects in the biological control of powdery mildew on tomato, squash and cucumber caused by *Oidium lycopersici* (= Oidium lycopersicum Cooke and Mass.) and *Sphaerotheca fusca* Blumer, respectively [5; 10].

*A. album* is characterized for its capacity to survive for a long time and to sporulate rapidly also on poor substrates. It is a necrotrophic mycoparasite able to produce hydrolytic enzymes as protease, -gluconase and chitinase [9]. These enzymes are responsible of total or partial degradation or demolition of cell walls of numerous phytoparasitic fungi or other biotic elements.

In particular the endochitinase, produced in large amount by *A. album* [2], is responsible of the hydrolysis of the chitin, a long chain polymer of N-acetyl-D-glucosamine (GlcNAc), one of the most common polysaccharides in nature, produced by many members of both the plant and animal kingdom, including fungi, algae and protozoans. Chitin is also an important constituent of the exoskeleton and egg shells of nematodes [4].

The degradation of the chitin, a nitrogenous material, by chitinase gave a series of monomer and dimer useful to *A. album* as nutritive substrate [8; 9; 21].

Therefore, considering that severe corky root symptoms caused by *Pyrenochaeta lycopersici* Schneider *et* Gerlach have frequently been found in association with *Meloidogyne* attacks [13] a trial was carried out on tomato in plastic house to investigate a) the possibility of the use of the chitinolytic activity of *A. album* isolate MX-95 (AA MX-95) also in nematode biological control as bio-nematicide at lower environmental impact which could be alternative to pesticide, and b) the interaction between the soilborne pathogen and the root-knot nematodes.

## MATERIALS AND METHODS

A plastic house of 280 $m^2$ at Valenzano (province of Bari, Apulia region, Southern Italy) (41° 03' 06'' N, 16° 90' 02'' E), with sandy soil naturally infested by *Meloidogyne incognita* (Kofoid *et* White) Chitw. and *P. lycopersici*, of which severe symptoms were

evident in the previous tomato crop cycles, was selected. The soil was deeply ploughed, rotavated and subdivided in 6 m x 2 m plots and distributed in a randomized block design with four replications for each treatment. A sub-irrigation system (depth 0.2 m) was performed in each plot by PVC drip lines ( 1.6 cm) equipped with water emitters (flow rate 4 l/h) every 30 cm to allow different treatments (Fig. 1). Mycelium of AA MX-95 was dissolved in sterile water, sown in PDA in plastic Petri dishes, incubated at 24°C for 7 days and then homogenized in sterile water with a tensioactive to disperse the strongly hygroscopic conidia. Concentration of fungal inoculum was determined and diluted to obtain a 1.2 x $10^7$ CFU/mL standard conidial suspension. Suspension was then applied by sub-irrigation.

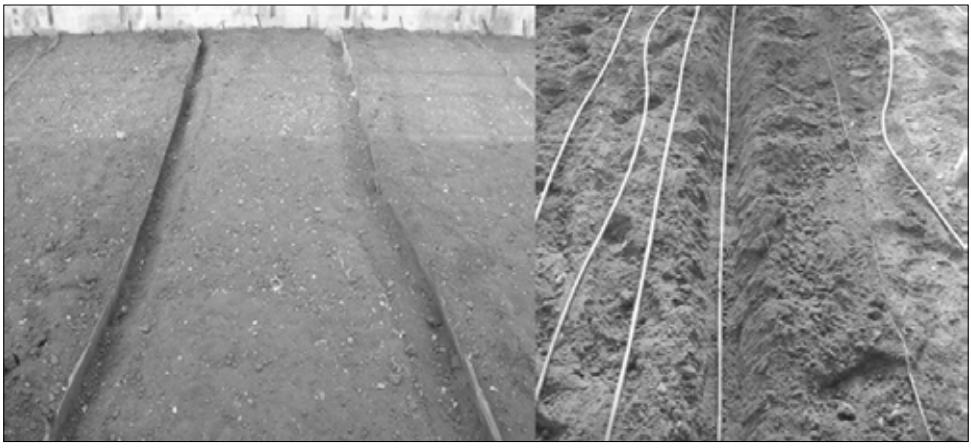

Fig. 1. Plots with the sub-irrigation system

Treatments were: a) AA MX-95 applied at 2.5 L/plot (1.2 x $10^7$ CFU/mL, conidial suspension) in pre transplant (20 days before transplant), transplant and in post transplant (2 times every 20 days) + root dipping (5 min. – conc. 1.2 x $10^7$ CFU/mL) at transplant (Fig. 2); b) as before indicated without the AA MX-95 treatment in pre transplant; c) AA Mx-95 root dipping as above reported; d) dazomet (nematicide, chemical control) at 600 Kg/ha applied 30 days before transplant and covering the plots with a plastic film (Fig. 3). Untreated plots were used as controls. In each plot, tomato seedlings (cv. Super Marmande) were transplanted in 3 rows with 7 plants for each row.

During the growing season tomato crop received the cultural practices that are common for the area like weed, insect, pathogen control and fertilizer application,. Fruits were harvested (five times) during crop cycle and yield recorded. At the end of the experimental trial, plants were uprooted to estimate root gall index caused by the nematode attack according to a 0-5 scale (0 = health root system and 5 = root system completely deformed by numerous large galls). Severity of corky root symptoms on main and secondary roots was estimated also according to a 0-5 scale (0 = root healthy; 1 = 1-10% affected root surface (a.r.s.); 2 = 11-25% a.r.s.; 3 = 26-50% a.r.s.; 4 = 51-75% a.r.s. and 5 = > 76% a.r.s.). Nematodes were extracted from soil samples of each plot processing 500 mL soil sub-sample with the Coolen's method [6].

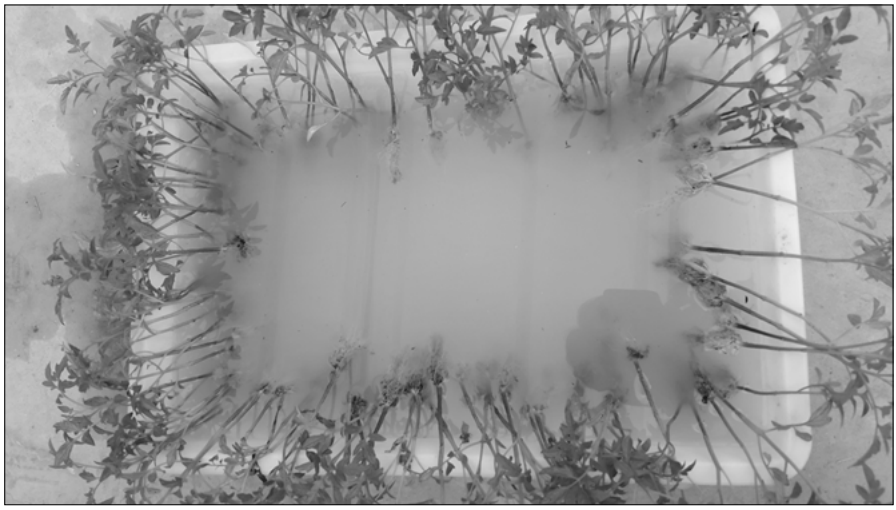

Fig. 2. Tomato root dipping

Fig. 3. Dazomet treatment with plot covered by a VIF plastic film.

Data were subjected to analysis of variance (ANOVA) and means compared by Duncan's Multiple Range Test (P=0.05) by Plot IT software (Ver. 3.2).

## RESULTS AND DISCUSSION

Treatments with AA MX-95 applied by sub-irrigation at transplant and in pre and post transplant in addition to root dipping resulted in a marketable yield significantly higher than that recorded in the untreated control (Table 1) and it was no significantly different from dazomet treatment. AA MX-95 root dipping alone was not significantly different from the untreated control (Table 1).

**Table 1. Effect of dazomet and different *Aphanocladium album* treatments on marketable yield of tomato (cv. Super Marmande) in a plastic-house infested with *Pyrenochaeta lycopersici* and *Meloidogyne incognita***

| Treatment | Dose | Mode and Application time | | | Marketable yield (q/ha) | | |
|---|---|---|---|---|---|---|---|
| | | Pre transplant (20 days) | Transplant | Post transplant | | | |
| 1. AA MX-95 | Conidial suspension 1.2 x $10^7$ CFU/mL | 2.5 L/plot | 2.5 L/plot + Root dipping (5 min.) | 2.5 L/plot (2 times – 20 days) | 212* | b** | B |
| 2. AA MX-95 | Conidial suspension 1.2 x $10^7$ CFU/mL | | 2.5 L/plot + Root dipping (5 min.) | 2.5 L/plot (2 times – 20 days) | 232 | b | B |
| 3.AA MX-95 | | | Root dipping (5 min) | | 139 | a | A |
| Dazomet | 600 Kg/ha | | | | 237 | b | B |
| Untreated Control | --- | | | | 107 | a | A |

* Each value is an average of four plot replications (21 plants/replication);
** Data flanked in each column by the same letters are not statistically different according to Duncan's Multiple Range Test (small letters for P=0.05; capital letters for P=0.01).

**Table 2. Effect of dazomet and different *Aphanocladium album* treatments against *Pyrenochaeta lycopersici* and *Meloidogyne incognita* on tomato (cv. Super Marmande) in a protected crop**

| Treatment | *Pyrenochaeta lycopersici* | | | | | | *Meloidogyne incognita* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Infestation index (0 – 5) | | | | | | Root gall index (0 – 5) | | | Final population/ mL soil | | |
| | Main root | | | Secondary root | | | | | | | | |
| 1.AAMX95 | 2.2* | b** | B | 2.0 | b | B | 3.3 | b | B | 12 | b | B |
| 2.AAMX95 | 2.5 | b | B | 2.5 | b | B | 3.7 | b | B | 10 | b | B |
| 3.AAMX95 | 3.1 | b | B | 3.2 | b | B | 4.3 | b | B | 10 | b | B |
| Dazomet | 1.9 | c | C | 1.4 | c | C | 3.5 | b | B | 5 | b | B |
| Untreated control | 4.5 | a | A | 4.0 | a | A | 4.9 | a | A | 23 | a | A |

* *Each value is an average of four plot replications (21 plants/replication);*
** *Data flanked in each column by the same letters are not statistically different according to Duncan's Multiple Range Test (small letters for P=0.05; capital letters for P=0.01).*

Compared to untreated control, treatments with AA MX-95 significantly reduced corky root symptoms both on main and secondary roots, without any significant differences among them (Table 2). However, the lowest corky root infestation was observed on main and secondary roots treated with dazomet (Table2). Significantly higher root gall index and final nematode population density were observed in the untreated control compared to all other treatments (Table 2). The relationship between root gall index and severity of corky root symptoms showed positive correlations between the

two parameters both on main ($y = 1.431+0.0623^x$, $r^2=0.97$, P=0.01) and secondary roots ($y = -0.175+0.175x^2$, $r^2=0.881$, P=0.01) as indicating that severity of *P. lycopersici* attack increases by the increase of nematode attack (Fig.4).

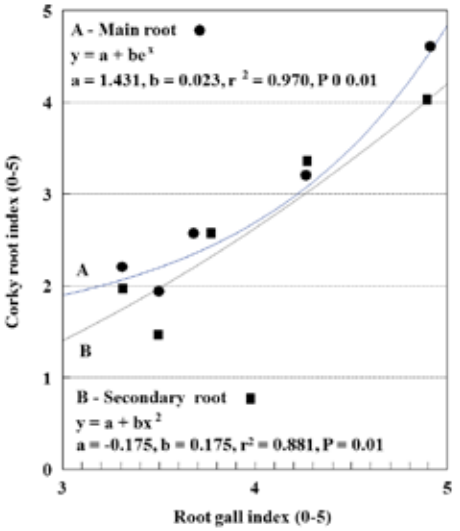


Fig. 4. Relationship between root gall index and severity of corky root

## CONCLUSIONS

On the base of results of this experiment, AA MX-95 treatments, applied by a sub-irrigation system, seem to be an effective alternative control method to chemical against simultaneous *P. lycopersici* and root-knot nematodes attacks and its use could be considered favorable to prevent soil pollution and to protect the environment health from a massive use of fungicides and nematicides.

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