BIOLOGICAL CONTROL OF BOTRYTIS GRAY MOULD ON TOMATO CULTIVATED IN GREENHOUSE

F. FIUME & G. FIUME
Sezione di Biologia, Fisiologia e Difesa - Istituto Sperimentale per l'Orticoltura (C.R.A.)
Via Cavalleggeri 25, I-84098 Pontecagnano (Salerno), Italy
E-mail: orticoltura.fiume@tiscali.it

SUMMARY

Research was carried out to evaluate the effectiveness of the biological control of the Botrytis gray mould, caused by *Botrytis cinerea* Pers., one of the most important fungal diseases of the tomato (*Lycopersicon esculentum* Mill.). Biological control was performed by using *Trichoderma harzianum* Rifai, an antagonist that is a naturally occurring fungus found on some plants and in the soil worldwide. *Trichoderma spp.* are fungi diffused in nearly all agricultural soils and in other environments such as decaying wood. The object of this research is to find control strategies to reduce chemical treatments that cause damage to the environment and increase the pathogen resistance, applying the biological control by using *T. harzianum* against *B. cinerea.* A commercial product containing a natural isolate of *T. harzianum* is trichodex (Makhteshim Chemical Works, LTD).
The research was performed in laboratory and in greenhouse. In laboratory, radial growth reduction of *B. cinerea*, in presence of *T. harzianum*, was calculated in relation to the growth of the pathogen control, by using a specific formula that measures the percentage of the inhibition of the radial mycelial growth. In greenhouse, starting from the tomato fruit setting, the research was carried out comparing, by a randomized complete block experiment design, replicated four times, the following treatments:1) untreated control; 2) pyrimethanil (400 g/L of a.i.), at 200 cc/hL of c.i. (pyrimidine fungicides); 3) trichodex at 100g/hL (1 kg/ha); 4) trichodex at 200 g/hL (2 kg/ha); 5) trichodex at 400 g/hL (4 kg/ha). Before fruit setting, the plots were all treated against Botrytis gray mould with iprodione 50% (100 g/hL), procymidone 50% (100 g/hL) and switch (Novartis plant protection) at 80 g/hL.
In dual culture, the inhibition of *B. cinerea* radial mycelial growth was 76%. No inhibition halo was observed between *B. cinerea* and *T. harzianum* colonies but, after 3 days, the pathogen colony radius resulted no more than 1.8 cm (towards *T. harzianum*). *T. harzianum* grew quickly and after 5 days the medium surface was completely colonized covering the *B. cinerea* colony. The laboratory tests were confirmed in greenhouse trials. Trichodex at 400g/hL gave the best results, decreasing the disease over 50% compared to untreated control and over 70% compared to chemical control. After fruit setting, *T. harzianum* was the best in the control of Botrytis gray mould, allowing to avoid the use of the chemical fungicides that can be applied only up to the fruit setting, obtaining high quality tomato fruits.

INTRODUCTION

The tomato cultivation is diffused in the entire world, occupying an esteemed surface of about 8 millions of hectares and with a production of 217 millions of tons.
Tomato is the second most consumed vegetable in the world behind only the potato. It is eaten fresh or processed and can be stewed, fried, baked, or used as juice. In addition to this versatility, tomatoes are also nutritional. They are low in calories (20 calories per average size fruit) and they are an excellent source of iron and vitamins A and C. They also contain carote-

noids, such as lycopene with antioxidant properties, and small amounts of the B complex vitamins thiamin, niacin, and riboflavin (Fanasca *et al.*, 2006).

The Italy is placed in the first places in the worldwide rating and it is one of the most important countries of the European Community producing tomatoes. In Italy, in 2002, the surface of the Table tomato is about 31.000 hectares of which about 7.600 in protected cultivation, while the fruit production is about 550.000 tons, with about 71 tons per hectare of unitary production.

The weight of the Italian production of Table tomato becomes difficult to estimate in consideration of the possibility of the dual use of the tomato (for processing and fresh market) and for the frequent destination of the open field production (usually used for processing) to the vegetable market for cookery use, at familiar level. Referring to tomato yield in protected cultivation, in Italy, the tomato fruits sent to fresh market each year are about 2.5 millions of tons.

United States have developed a fundamental role in the Table tomato production in greenhouse. Tomatoes are the leading greenhouse vegetable crop in the United States and Canada. In the U.S., the total acreage in greenhouse tomato production increased by 40% between 1996 and 1999. Statistics for 1999 show that the U.S. had about 800 acres (324 hectares) in greenhouse vegetable production, with tomatoes accounting for 750 of those acres. The leading States in greenhouse vegetable production are California, Florida, Colorado, Arizona, Ohio, Texas, and Pennsylvania, each with more than one million square feet (9.3 hectares) in production (Snyder, 1993; Cook and Calvin, 2005). The vast majority of greenhouse tomatoes are produced in greenhouses using conventional production systems.

Referring processing tomato, comparing the Italian regional productive areas, the Apulia produces 42.5%, the Emilia and Romagna 28.7%, follows the Campania with 6.7%, the Calabria, Lazio, Lombardy, Basilicata pool with 14.5%, Sicily and Tuscan together with 4.0%, Venetia with 1.2% and the remaining Regions with 2.4% of the total (about 6.219.532 tons). Comparing the tomato worldwide productive areas, the United States are at the first place with 10.3 millions of tons, follows the China with 8.9 millions of tons, the Turkey with 6.3, the ex-URSS with 5.6, Egypt with 4.6, Spain with 3.1, Brazil with 2.5, Greece with 1.8 and Mexico with 1.5. However, beyond the production, Italy is the second tomato processing country of the world, after United States, with 9.7 million of tons.

When completely ripe, the tomato fruits are fiery red colour, with an edible part of over 96%. The remaining part (4%) is composed of peel, seeds and part fibrous-wooden not digestible. On average 100 g of fresh tomato they are constituted from 93% of water, 2,9% of carbohydrates, 0,2% of fat, 1% of proteins and 1,8% of fibres with an energy value of about 100 KJ (20 Kcal).

Botrytis cinerea Pers. is a ubiquitous pathogen which causes severe losses in many fruit, vegetable and ornamental crops and which can be especially dangerous in greenhouse production.

Botrytis cinerea can cause a characteristic symptomatology named Botrytis gray mould that is a common and often serious fungal disease which regards also the tomato plants (*Lycopersicon esculentum* Mill.) cultivated in open field and in greenhouses. Once established it is difficult to control and it may be

present in greenhouse crops for all the year, causing serious reduction in yield. Severe infection of stems can often kill the plants.

Botrytis gray mould can occur on all above-ground parts of the tomato plant and it often starts at a point of damage or on any decaying tissue. Fallen flower petals resting on leaves, and pruning wounds on stems are examples of infection points. The most characteristic symptom is a grey-brown furry mould, which are masses of spores of the gray mould fungus, covering the infected area. When shaken, clouds of spores are released from these infected areas. The infected areas can expand rapidly covering whole stems, leaves or petals. Stem infections can girdle the whole stem and cause wilting of the plants above the infected area. Flower petals are particularly susceptible. The fungus may grow from the infected petals into the fruit. Another symptom of gray mould is the production of halo or ghost rings on the fruit. These are caused by partial infections which stop developing before the fruit is rotted.

The gray mould fungus survives on plant debris and in the soil. The source of contamination to a new crop is from spores carried by wind coming from host plants outside the greenhouse or spores spread on air currents in the greenhouse. Botrytis gray mould development is favoured by cool and humid conditions. These conditions also stress the plants, making them susceptible to disease. Spores are developed only under very humid conditions. Four to six h of free water on the plant surface is required for spores to germinate and infect the plant. Water condensation on the plant surface is the most common source of free water in a greenhouse. Condensation occurs when the plant surfaces are colder than the surrounding air. The chance of condensation increases with high humidity of the air and when the air temperature decreases quickly. Condensation may also form on the interior surfaces of the greenhouse and to drip on the plants.

The possibilities of control of the disease are numerous. Good ventilation conditions, both day and night, to keep plants dry and air humidity low are critical in preventing and controlling the gray mould. Chemicals should not be relied on for control because without proper environmental controls the gray mould will continue to develop and spread. The vents are fundamental in greenhouse when temperatures rise to replace warm moist and warm air with cool air. Greenhouse should be kept hot at night to reduce humidity. In fact when heated, air absorbs moisture. Plant transpiration (releasing water vapour) at night causes high humidity levels in greenhouses. To maintain low humidity a cycle of heating and venting must be practised during the night. Control of the ventilation is best achieved by the installation of a humidity sensor to allow automatic opening and closing of the vents.

The maintenance of the good air circulation in the greenhouse to improve ventilation and reduce the chance of water condensation forming, moving air through the crop helps keep the plant and air temperatures the same. For this purpose, fans can be used to move air within the greenhouse. Besides, it is necessary to choose a plant density that will allow good air movement between the plants, to prune regularly to remove laterals and old and dense leaves, to assist air movement through the plants, to avoid plant wetness by never spraying crops in the afternoon or at any time.

Optimum hygienic conditions are very important to gray mould control. This is obtained by cutting out stem infections and removing dead plant parts

before pruning operation beginning. To prevent the build up of spores and the spread of infection, it is useful cutting out stem infections before the whole stem is damaged, saving the plant from dying by removing all plant debris from the previous crop because this material carries gray mould spores that can infect the next crop, disposing plant residues away from the greenhouse, covering the floor of the greenhouse for avoiding to have bare soil because the gray mould fungus survives in the soil and become a source of infection for the next crop and last but not least, washing down walls and floors after each crop.

Chemical control of Botrytis gray mould is an other strategy against the phytopathogenic fungus. Therefore, chemicals alone cannot be relied on to give control of gray mould. It is difficult to spray all surfaces of the plant where infection may occur. The fungicides currently registered are protective types and do not have a systemic action of control. Fungicides only provide a protective barrier to the outside part of the plant that discourages fungal spore development. They do not cure the disease once it has developed. Chemicals help reduce the risk of infection along with good ventilation and hygiene.

Chemicals used on tomato plants in greenhouse are iprodione, procimidone, pyrimentanil, ciprodinil + fludioxonil. It would not be necessary to exceed 1 or 2 treatments per year, trying to change the products and intervening in presence of initial hotbeds.

Because *B. cinerea* has developed resistance to several fungicides (Moorman and Lease, 1992), there is concern that selection for additional fungicide resistance in the pathogen will occur. Some products will not be registered for greenhouse use by the manufacturer because the development of pest resistance in a confined space is much more likely to occur, and it is to the manufacturer's benefit to preserve the effectiveness of the product as long as possible. In addition, many fungicides labelled for gray mould on greenhouse tomato are under scrutiny as potential carcinogens. Future availability of fungicides for greenhouse use on vegetables may be limited because of concerns for the environment and human safety. The emergence of fungicide resistance and increasing consumer demands for reduced residues on fruit emphasises the need for alternative disease control strategies.

Another possibility of control of Botrytis gray mould in greenhouse is represented by the use of biological means. Biological control, as an alternative strategy, has the advantage of greater public acceptance and reduced environmental contamination.

There has been a strong research and development thoughts in the area of the biological control of plant pathogens (Elad and Evensen, 1995; O'Neill *et al.*, 1996; Gielen *et al.*, 2004). Many products and microrganisms have been discovered that discourage the growth and survival of plant pathogens, and these antagonists are now arriving in the marketplace. The organisms tested for effectiveness, are now mass-produced and are processed to ensure shelf life.

These considerations have induced us to carry out researches with the purpose to identify some alternative strategies against tomato gray mould in greenhouse such as the biological control. Objective of this research is to test in commercial greenhouses the biological control compared with chemical treatments. This research based on biological control application needs to provide answers to questions frequently asked by horticulturalists and to

supply the data necessary to increase producer confidence in fungicide alternatives.
The current paper reports the results of the laboratory and greenhouse trials to the purpose to observe the behaviour of *Trichoderma harzianum* Rifai against a local isolate from tomato plants of *B. cinerea* in dual culture and to evaluate the effectiveness of tricodex, a biological fungicide, compared with some chemicals.
T. harzianum is an efficient biocontrol agent that is commercially produced to prevent development of several soil pathogenic fungi. Different mechanisms have been suggested as being responsible for their biocontrol activity, which include competition for space and nutrients, secretion of chitinolytic enzymes, mycoparasitism, production of inhibitory compounds, light spectral quality (Haram *et al.* 1996; Zimand *et al.* 1996; Paul *et al.* 2005).

MATERIALS AND METHODS

The research was performed in laboratory and in greenhouse from January to June 1999.
The ability and effectiveness of *T. harzianum* to control specific isolate of *B. cinerea* were studied in the laboratory of the Research Institute for Vegetable Crops of Pontecagnano (Salerno).
B. cinerea was isolated from gray mould grown on stems of diseased tomato plants, "Nikita" cultivar, cultivated under greenhouse.
T. harzianum was isolated from powder of trichodex that is a natural biological fungicide containing a natural isolate of mycelium and conidia of the fungus.
After purification by using the technique of the monoconidic culture, *B. cinerea* and *T. harzianum* were both grown on potato dextrose agar (PDA, Difco) plates (diameter 9.2 cm) to produce the necessary inoculum.
All incubations were performed at 20°C under constant fluorescent light. Ultrapure H_2O (quality >18MW) was used in all experiments.
A mycelial disc (0.9 cm diameter), obtained from the peripheral region of 4 day old cultures of *B. cinerea* on PDA, was placed on a fresh PDA plate (3 cm from the centre). Then, a 0.9 cm diameter mycelial disc, obtained from the periphery of a 3 day old culture of *T. harzianum*, was placed 3 cm from the plate centre, opposite the inoculum of the pathogen. The two fungi (pathogen and antagonist) were inoculated at a distance of 6 cm to each other and at 1.6 cm from the border of the plate. Each fungus could occupy the dish for an available maximum radius of 7.6 cm, from the inoculum point up to the opposite border. The plates were incubated at 28°C, and daily measurements of the radial growth were taken.
In the control experiment, a sterile agar disc (0.9 cm diameter) was placed in the plate instead of *T. harzianum*. The sterile agar disc and the disc of *B. cinerea* were both placed at a distance of 6 cm each from the other and at a distance of 1.6 cm from the border of the plate.
In the dual culture (*Botrytis* and *Thichoderma*), for each colony, the radial growth was measured on the segment which connected the origin points (inoculum points) of the two opposite colonies.
The radial growth was measured each day, until the dishes were completely colonized by the fastest mycelium. Radial growth reduction of *B. cinerea*, in

presence of *T. harzianum*, was calculated in relation to the growth of the pathogen, by using the following formula that measures the percentage of the inhibition of the radial mycelial growth:

$$\text{Inhibition of the mycelial radial growth (\%)} = \frac{C - T}{C} \times 100$$

where:
C is the radial growth measurement of the pathogen in control and
T is the radial growth of the pathogen in the presence of *T. harzianum*.

All the experiments in laboratory were replicated four times.
In greenhouse, the research was carried out at the Valcalcer P. horticultural farm, in St. Lucia locality, Battipaglia (Salerno). The following treatments were compared by a randomized complete block experiment design, replicated four times, starting from tomato fruit setting: 1) untreated control; 2) pyrimethanil (400g/L of a.i.), 200 cc/hL of c.i. (pyrimidine fungicides); 3) trichodex, 100 g/hL (1 kg/ha); 4) trichodex, 200g/hL (2 kg/ha); 5) trichodex, 400g/hL (4 kg/ha). Each plot was treated two times, on May 15th and May 28th, 1999.
Trichodex (Makhteshim Chemical Works, LTD) is a commercial product containing a natural isolate of *T. harzianum* with fungal mycelium and conidia, minimum $1x10^9$ per gram CFU (Colony Forming Units) of T-39 isolate.
The Nikita F_1 cultivar, of the S & G Sandoz Seed, was transplanted on January 30th under greenhouse large 5.0 m, high 3.8 m (maximum) and 1.6 m (minimum, on the sides). The trial was performed on a surface of 500 m^2, where 20 plots were randomized. Each plot was 25.0 m^2, 10.0×2.5 m^2, with 60 plants for everyone and density of 2.4 plants/m^2. Irrigation and fertigation systems were performed with automatic mechanisms.
Before fruit setting, the plots were all treated against Botrytis gray mould with iprodione 50% (100g/hL), procymidone 50% (100g/hL) and switch (Novartis Plant Protection) at 80 g/hL. The treatments were applied on March 18th (iprodione), April 12th (procymidone) and April 28th, 1999 (switch). Therefore, the control strategy of *B. cinerea* was carried out according to the integrated control technology: the chemical treatments were executed for all the plots in the same way, from the transplant up to the fruit setting. Then, from the setting to the beginning of the fruit harvest, the untreated, the chemical treatment by pyrimenthanil and the biological control by trichodex (with three dosages) were compared.
The phytopathological observations started on May 15th continued with weekly cadence. A this date almost all the plants were healthy, the chemical treatments pre-setting were stopped from 17 days, the treatments post-fruit setting began because the environmental conditions became favourable to *B. cinerea* development, with the reappearance of the early symptoms of gray mould on pruning wound. The gray mould, developed from the necrotic lesion on the tomato stem, showed erect and cylindrical conidiophores. The microscopic exam of the conidiophores showed that they were 0.5-1.5 mm tall, ending with 2-3 very short branches (each furnished of sterigmas) to which ovoid and hyaline, 8-15 × 6-10 µm, conidia (3-5 per branch, forming a compact glomerulus) were attached.

For each plot, the number of diseased plants was registered. The symptom severity was estimated for each plant and all the plants were grouped into infection classes, calculating the frequencies. The tomato gray mould severity was rated according the following evaluation scale: 0 = no symptoms, healthy plants; 1 = less 10% of infected stems and leaves with lesions for no more 10% of shoot length; 2 = less 20% of infected stems and leaves with lesions for no more 20% of shoot length; 3 = less 40% of infected stems and leaves with lesions for no more 50% of shoot length; 4 = less 80% of infected stems and leaves with lesions for no more 80% of shoot length; 5 = infected areas covering whole the stems and leaves causing wilting and death of plants.
Severity and diffusion of infection were obtained by resorting to the McKinney index (McKinney, 1923). The McKinney index (I) was obtained by using the following formula:

$$I = \frac{\Sigma (f \times v)}{N \times X} \times 100$$

where:
f = infection class frequencies
v = number of plants of each class
N = total of observed plants
X = highest value of the evaluation scale.

Phytopathological data were transformed to angular values, before analysis of variance (ANOVA), and compared using Duncan's test for multiple comparison among treatments with Mstat statistical analysis program (Mstat, 1987).

RESULTS

The single culture of *T. harzianum* and of *B. cinerea* on PDA in Petri dishes showed that the growth of the antagonist fungus was faster than the growth of the pathogen. The radius of the *B. cinerea* colony ranged from 0.21 cm to 4.58 cm, with daily increases of the mycelial radius of 0.58, 1.06, 1.21 and 1.52 cm (Figure 1).

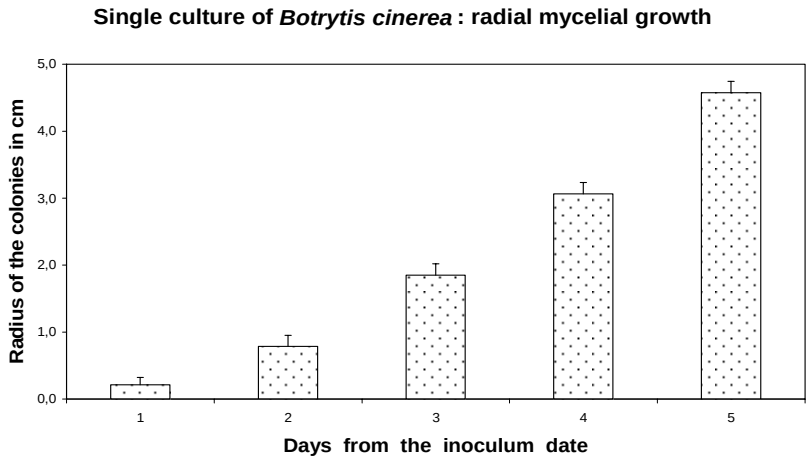

Figure 1. Radial mycelial growth of *B. cinerea* on PDA Petri dishes

The radius of the *T. harzianum* colony ranged from 0.93 cm to 4.43 cm, with daily increases of the mycelial radius of 1.57 and 1.93 cm. (Figure 2). The *T. harzianum* growth was much faster than *B. cinerea* growth. In three days, the surface of the plate medium was totally colonized by the antagonist fungus.

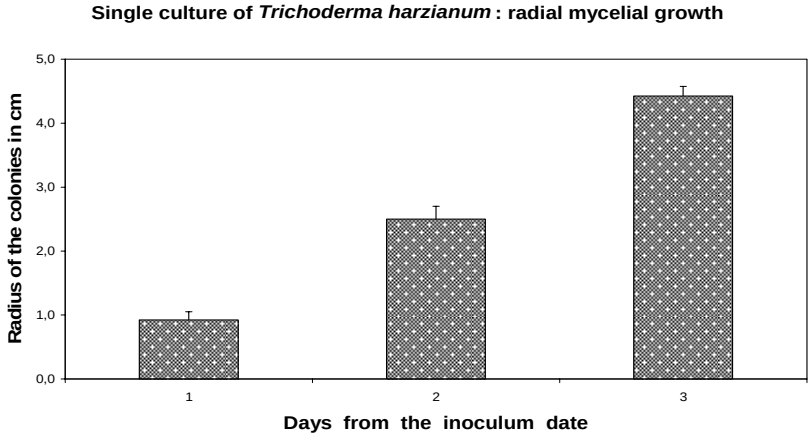

Figure 2. Radial mycelial growth of *T. harzianum* on PDA Petri dishes

In dual culture, *T. harzianum* showed inhibitory effects on the mycelial growth of *B. cinerea* (Figure 3). *B. cinerea* radial growth did not overgrow 3 cm because the development was blocked by the antagonist fungus. In fact, growing quickly, *T. harzianum* reached the periphery of the *B. cinerea* colony, preventing the development of the phytopathogen fungus not over 3 cm. The development of *T. harzianum* was so bursting out, massive and quick that it

grew above *B. cinerea* colonizing in 5 days all the surface of the plate. The radius of *T. harzianum* toward the opposite colony was over 7 cm after 5 days (Table 1).

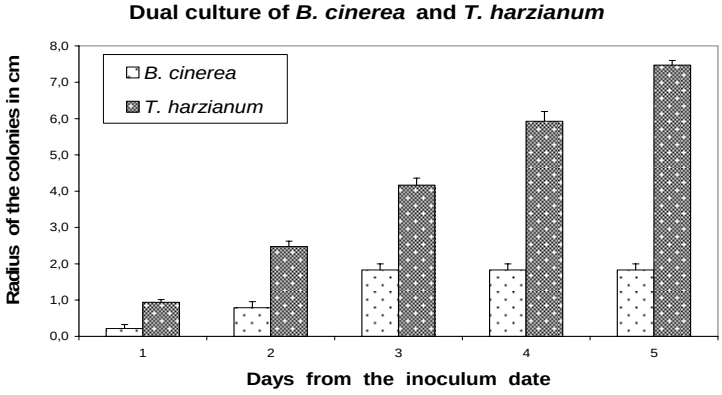

Figure 3. Mycelial radial growth in dual culture of *B. cinerea* and *T. harzianum* on PDA Petri dishes

Table 1 reports the radial growth of *B. cinerea* and *T. harzianum* together inoculated at a distance of 6 cm to each other and at 1.6 cm from the border of the plate. Therefore, each colony may be described with two sizes: one towards the nearer border of the plate (maximum 1.6 cm) and the other (that we detected) towards the further, where the opposite fungus was inoculated (maximum 7,6 cm). In the control, *T. harzianum* was not inoculated in the plate and at its place a PDA sterile disc was placed. The radius of *B. cinerea* without *T. harzianum* ranged from 0.21 to 7.53 cm, in 7 days. The radius of *B. cinerea* in presence of *T. harzianum* did not exceed 1.83 cm and stopped after 3 days. After 5 days, all the plate was completely occupied with *T. harzianum* that grew quickly and covered the *B. cinerea* colony.

The data of the radial growth of *B. cinerea* in single and in dual culture with *T. harzianum* allowed to build the graph of the daily per cent inhibition of the development *in vitro* of the tomato pathogen (Figure 4). No inhibition of the *B. cinerea* growth was observed until the third day from the inoculation, when *T. harzianum* reached the periphery of the pathogen fungus colony. The noticed inhibition percentage ranged from 4.7% (third day) up to 75.76% (seventh day). No inhibition halo was observed between *B. cinerea* and *T. harzianum* colonies. That means that the antagonist effects of *T. harzianum* are based on the competition for the vital space and for nutrients and not on a chemical aggressiveness or classic antibiosis.

Table 1. Radial growth of *B. cinerea* and *T. harzianum* in dual culture (1).

Pathogen or antagonist	Days from the date of the inoculum						
	I	II	III	IV	V	VI	VII
B. cinerea: control (2)	0.21 ± 0.1	0.79 ± 0.2	1.85 ± 0.1	3.06 ± 0.1	4.58 ± 0.1	5.93 ± 0.2	7.53 ± 0.1
B. cinerea: dual culture (3)	0.21 ± 0.1	0.79 ± 0.2	1.83 ± 0.2	1.83 ± 0.2	1.83 ± 0.2	1.83 ± 0.2	1.83 ± 0.2
T. harzianum: dual culture (4)	0.94 ± 0.1	2.48 ± 0.1	4.16 ± 0.2	5.93 ± 0.3	7.48 ± 0.2	–	–

1. The radius is measured in cm and it is detected towards the point of inoculum of the opposite colony
2. *B. cinerea* opposite to sterile agar disc
3. *B. cinerea* opposite to *T. harzianum*
4. *T. harzianum* opposite to *B. cinerea.*

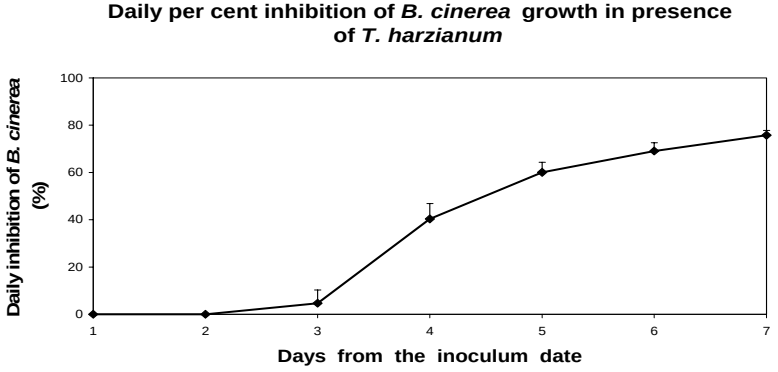

Figure 4. Daily per cent inhibition of the *B. cinerea* development in presence of *T. harzianum* in dual culture on PDA in petri dishes

Table 2. Percentage of tomato plants affected by *B. cinerea* in greenhouse trials

Treatments	Date of the phytopathological observations (*)									
	May 15th		May 20th		May 28th		June 3rd		June10th	
Untreated control	1.25	Aa	11.67	Aa	20.00	B b	32.08	B b	47.92	B b
Chemical control	0.80	Aa	12.08	Aa	37.92	A a	47.92	A a	80.42	A a
Trichodex 1 kg/ha	1.70	Aa	10.42	Aa	14.17	BC c	29.58	BC b	40.00	B b
Trichodex 2 kg/ha	1.20	Aa	7.92	Aab	9.17	CD d	23.75	C c	39.58	B b
Trichodex 4 kg/ha	0.83	Aa	2.50	A b	4.58	D d	12.92	D d	24.17	C c

(*) Values separation in columns by Duncan's multiple range test (P≤0.01 capital letters, P≤0.05 small letters).

The research led in greenhouse showed a good behaviour of trichodex in the control of tomato gray mould, in particular when it is used at 4 kg/ha (Table 2). With dose of 4 kg/ha, trichodex was always significantly different from the controls and other treatments, showing the lowest values of diseased plants that ranged from 0.83% to 24.17%. Only for the observations in May, the doses of 2 e 4 kg/ha of trichodex were not significantly different.

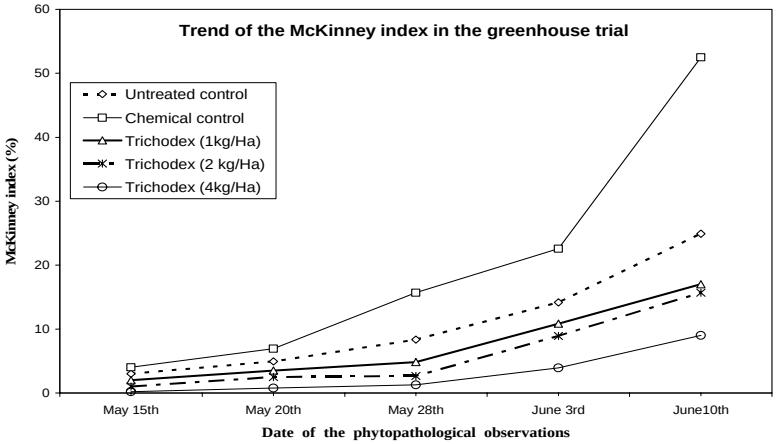

Figure 5. Trend of the McKinney index in the research carried out in greenhouse

Analysing the trend of McKinney index, the treatment with 4 kg/ha of trichodex gave the best results in the control of gray mould. McKinney index ranged from 0.20% to 9% (Figure 5). The index showed higher values for the other trichodex doses but always beneath those exhibited by the untreated and chemical controls. The chemical control gave a very bad result exhibiting a McKinney index up to 52.5%. The disease was supported by the wet caused on the plants by the chemical treatment and by pathogen resistance to active ingredient.

DISCUSSION AND CONCLUSIONS

Laboratory trials showed the antagonist ability of *T. harzianum* against *B. cinerea*. The antagonism is based on quick occupation of the vital space of *T. harzianum*, therefore it is a competition for the vital space and nutritive elements. The non-appearance of the inhibition halo proves the absence of antibiosis based on the synthesis of chemical substances.

The research carried out in greenhouse showed that two treatments with 4 kg/ha of trichodex after the fruit setting controlled with very effectiveness the gray mould. Before fruit setting, if necessary, the tomato plant protection against Botrytis gray mould can be done with iprodione 50% (100g/hL) or procymidone 50% (100g/hL) or switch (Novartis Plant Protection) at 80 g/hL by 1 or 2 treatments.

The resistance of new strains of *B. cinerea* towards an always increasing number of fungicides, the necessity to obtain tomato yields of high quality, the concerns for the environment and the human safety, the possibility to reduce and control the environmental pollution, the request to safeguard the man's health and respect of the regional and national laws that impose reduction of chemical treatments, all that should induce the farmers to choose biological interventions.

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