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EFFECTS OF *Trichoderma asperellum* [T1] ON *Botrytis cinerea* [PERS.: FR.], GROWTH AND YIELD OF ORGANIC STRAWBERRY

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Abstract. Fungi *Trichoderma* spp. are present in nearly all soils and other habitats. Most of *Trichoderma* strains are efficient for control of some pathogens. Some strains induce plant native defence mechanisms. *Botrytis cinerea* [Pers.: Fr.] causes economic losses on a wide range of cultivated plants and stored fruits. In presented work *Trichoderma asperellum* [strain T1] isolated from commercial product was selected to apply in the field experiments. The trade bioproduct containing 5×10^8 of *Trichoderma* conidium per one gram was used in organic field strawberry. Treatments with microorganism-antagonist at concentration $10 \text{ g} \cdot \text{l}^{-1}$ were performed as a foliar spraying, three times during the growing season. Applied *Trichoderma* caused bigger and more vigorous growth. The insignificant presence of *B. cinerea* on harvested and stored fruits was found. The possibility of use of *T. asperellum* as biological control agent for post-harvest diseases and for prolongation of time storage was observed until 7 days after harvest. In the second season of research, statistical significantly higher yield of the treated plants (30.2%) was obtained compare to the untreated plants. Also the weight of aboveground part of plants (24.0%) and number of runners were increased.

Key words: Trifender WP, cold keeping of strawberries, foliar treatment, growth and development of plant

INTRODUCTION

Strawberries are growing in many countries and the area of their cultivation is spread quickly. In organic production protection and fertilization treatments are very important. Inorganic fertilizers are not allowed to use. Biofertlizers are an alternative to mineral for increasing soil productivity and plant growth. Plant growth promoters are used for better plant growth and better management of nutrients. These promoters play

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a major role in seed germination, fruit ripening, enhance uptake of nutrients and help to overwhelming stress, reduce flower and fruit drop.

Grey mold *Botrytis cinerea* [Pers.: Fr.] is one of the most important pathogen in strawberry production causing losses on plants and fruits. This infection causes decay of mature fruit during storage. Key strategies for managing of fruit rot caused by *B. cinerea* include suppression of conidial production by the pathogen in inoculum sources and protection of flowers against infection by conidia of the fungus. Control of grey mold is usually carried out by the application of fungicides. In organic fruit production only a few plant protection agents are permitted to use. These products are less efficient than chemical. Additionally chemical treatments have produced increasing numbers of fungicide-tolerant grey mold strains. This problem supports the need for alternative methods. Therefore, the search for biological control agents has been investigated and some microorganisms with high activity have been identified [Jacometti et al. 2010].

The sources of grey mold are mycelium in dead strawberry leaves, mummified strawberry fruits, straw mulch and weed [Sutton 1995]. The pathogen can survive and sporulate in dead strawberry leaves for several months on the field [Braun and Sutton 1988]. Conidia can disperse from inoculum source and infect flowers, than the pathogen invades fruits [Bristow et al. 1986]. Biological methods can be developed as an alternative to chemical fungicides for fruit rot control [Peng and Sutton 1991, Jacometti et al. 2010]. Leaves and flowers at the bud stage are highly susceptible to infection by grey mold, therefore the beneficial microorganism should be used in early time of growing season.

For many years *Trichoderma* isolates have been used in different fields of production and protection in agriculture [Nelson and Powelson 1988, Wisniewski et al. 1991, Edwards and Seddon 1992, Blakeman 1993, Elad 1995, Elad 1996, Howell 2003, Korolev et al. 2008]. According to Report of SANCO from 14 May 2008 *Trichoderma viride* strain ICC080 (currently identified as *Trichoderma gamsii* ICC080), *Trichoderma viride* strain T-25 (currently identified as *Trichoderma asperellum* T11) and *Trichoderma viride* strain TV1 (currently identified as *Trichoderma asperellum* TV1) do not have any harmful effects on human or animal health or on groundwater or any unacceptable influence on the environment. Many companies used these strains to produce of biological plant protection products. Others processors produce plant growth promoters based generally on *Trichoderma* spp.

Trichoderma is able not only to produce toxic compounds with a direct antimicrobial activity against pathogens, but also generates fungal substances which are able to stimulate plant to produce its own defense metabolites. *Trichoderma* isolates are known for their ability to control plant pathogens [Elad and Freeman 2002]. Isolate T-39 of *T. harzianum* (Trichodex®) was the first biocontrol agent commercialized. *T. harzianum* isolates were also reported to control strawberry grey mold [Tronsmo and Dennis 1977]. *T. asperellum* as a biocontrol agent was successfully developed by many companies. Some strains induce plants to change their native defence mechanisms. *Trichoderma* spp. possesses innate resistance to most agricultural chemicals, including fungicides, although individual strains are differ in their resistance. The ability of these fungi to increase the rate of plant growth and development has been known for many years. These beneficial microorganisms compete with plant pathogens. It is a nature-friendly, ecological approach to overcome the problems caused by standard chemical methods of plant protection and it can be introduced in organic system of food production [Harman et al. 2004, Kowalska 2010]. Mostly, microbial plant growth promoters are applied by mixing with soil before planting, but also can be applied around the roots of plants, or are recommended to use by dipping the roots.

The main objectives of paper were to evaluate *T. asperellum* as strawberry growth promoter (i) applied in foliar spraying during the growing season, (ii) the control effects of *T. asperellum* on *B. cinerea,* (iii) the influence of this microorganism on strawberry yield and (iiii) the influence of *Trichoderma* treatments on storage of strawberries fruits.

MATERIALS AND METHODS

Field trials. The experiment was located in northern of Wielkopolska region, Poland. In 2009–2010, field trials were carried out, in the first and the second year of strawberry cultivation (cv. 'Honeoye'). Type soil was noted as sandy loam. The experimental design was completely randomized with 4 repetitions. Each plot contained 30 strawberry plants with 5 rows with 6 plants in each, spaced 60 × 35 cm. Foliar applications were done three times during the season. In the experiment the suspension of Trifender WP® (product of Biovéd Co., Hungary) was used as microbial plant growth promoter. One gram of the product contained 5×10^8 of conidium isolate T1 (NCAIM 68/2006). Product was applied in water solution at a concentration of 10 $g \cdot l^{-1}$ by shoulder-carried sprayer "Kwazar" (vol. 10 L), on sunny days in the evenings. Treatments were done as followed: (1) at the beginning of the growing season (April), (2) during plant flowering time, (3) one week before harvest. Time of treatments was chosen according to strawberry commercial cultivation time in Poland. Plants were not irrigated, hand weeding was made. Strawberry plants treated by Trifender WP were assessed towards effect on development and condition of plants. The total fruit yield harvested from 10 selected plants from each plot was evaluated. One month after harvest the aboveground parts of 10 plants from each plot were cut and weighed. Their numbers of runners were counted, too. Control plots were treated only with water, no chemical fungicides were used because of lack of permitted fungicides for organic system.

Weather conditions (2009–2010). Data on weather conditions are originated from meteorological observation station around the experimental fields (tab. 1). In 2009, monthly temperature ranged from 12 to 15^{o}C (April – June). In growing season in region precipitation was much diversified, ranged from 2 to 123 mm and relative humidity (RH) was from 65 to 85%. In 2010, monthly temperatures were noted in range 8–17°C (April – June). Precipitation in two months was relatively steady (33 and 42 mm, respectively in April and June), RH not exceeded 83%. The weather conditions, especially temperature, were conductive to set applied antagonist and for development of pathogen.

Experiment in cold-room conditions. From each plot 1 kg of fruits were collected. Fruits were harvested carefully and put in plastic containers, then were directly transported to the laboratory and were immediately cooled in temperature 4°C in the storage room. During the storage period (7 days, at 4°C and 98% RH) strawberry fruits were

Table 1. Weather conditions in Wielkopolska region in 2009–2010
Tabela 1. Warunki meteorologiczne w rejonie Wielkopolski w latach 2009–2010

Date Data	Month mean temperature Średnia miesięczna temperatura °C	Month sum of precipitation Miesięczna suma opadów mm	Month max. RH Maksymalna miesięczna wilgotność %
2009-04	11.6	1.7	65
2009-05	13.1	69.2	74
2009-06	15.3	123.3	85
2010-04	8.5	32.8	71.5
2010-05	11.7	103.6	82.8
2010-06	17.4	42.8	70.2

Table 2. Symptoms of grey mold on the ripped strawberry fruits
Tabela 2. Objawy szarej pleśni na dojrzałych owocach truskawki

T. asperellum	1 day after harvest Dzień po zbiorze %		7 days of storage Po 7 dniach przechowywania %	
	2009	2010	2009	2010
Treated Traktowane	1	0	1.4*	1.8*
Untreated Nietraktowane	8	4	33	23

* statistically different comparing to untreated – statystyczne róznice w porównaniu z nietraktowanymi

evaluated on first and seventh day of storage. Every appearance of disease and decay symptoms was assessed. Fruits with symptoms of disease were disqualified and removed from the box.

Data analyses. Observations of disease symptoms on harvested and stored fruits are shown in table 2 as mean percentage per plot. Date expressed in percentages were arc sin transformed. Differences in observation between treated and untreated plants were determined using Tukey's multiple range test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Field trials. *Trichoderma* applied three times during season stimulated the growth of strawberry plants. The increase of weight of aboveground of parts of plants and number of runners were observed in the treated plots. Strawberry plants were without symptoms of disease. Optimal conditions of weather in 2009 and 2010 were favored for germination of *Trichoderma* conidia. In experiment conducted by Martinez et al. [2009] *T. asperellum* inoculum was used as substrate to protect against *Phytophtora cactorum* and *Verticilium dahliae* in strawberry. Their results showed that this antagonist can successfully protect strawberry roots. The various isolates of *Trichoderma* were effective in controlling grey mold in strawberry under greenhouses controlled conditions [Freeman et al. 2004]. Microorganism could be applied to soil and in foliar. Obtained results of this work indicate that foliar treatment can stimulate the growth of plants. Time of treatments is very important factor of efficacy; the first application should be done at the beginning of season.

Table 3. Plant growth and fruits yield of the treated and the untreated combinations
Tabela 3. Wzrost roślin i plon owoców w zależności od kombinacji doświadczenia

T. asperellum	Number of runners (n = 10 plants·plot^{-1}) Liczba rozłogów (n = 10 roślin·poletko^{-1})		Plant weight of aboveground part of plants (n = 10 plants·plot^{-1}) Masa nadziemnych części roślin (n = 10 roślin·poletko^{-1}) g		Fruits yield (n = 10 plants·plot^{-1}) Plon owoców (n = 10 roślin·poletko^{-1}) g	
	2009	2010	2009	2010	2009	2010
Treated Traktowane	5	8*	423,1	544,2*	15.010	21. 990*
Untreated Nietraktowane	3	4	367,1	438,7	14.290	16.890

* as in Tab. 3 – tak, jak w tabeli 3

The obtained results showed potential of *T. asperellum* for protection of strawberry plants used in field conditions, not only in greenhouse conditions. In the first year increase of weight of parts of plants, number of runners and weight of fruits yield were observed, but not significant differences were found. The significant differences were confirmed only in the second year of experiment. Application of *T. asperellum* gave higher mean weight of plants parts' comparing to untreated plants (544.2 g and 438.7 g, respectively). It is possibility that treated plants can produce the natural substances which induce defence reaction against pathogens infection and help to take more nutrients from the soil by plants. Production of natural substances which are inducing plant defence was described for *Pythium oligandrum* and its oligandrin by Mohamed et al. [2007]. *P. oligandrum* induces defence reaction in plant by phytohormones stimulation, which are involved in the resistance mechanisms against diseases. *P. oligandrum* has also significant growth stimulation effects which results in the increased yield. This same effect was found in the presented work. In the second year significant higher fruits yield from the treated plots was obtained (21.9 g) compare with the untreated one (16.9 g). Effect of *Trichoderma* on fruits yield was also confirmed by other authors. Significant positive correlation was observed by Porras et al. [2009] between application of *Trichoderma* and strawberry yield. *Trichoderma* applications increased root weight and strawberry yield in 84.9% in second and 17.6% in third year of experiment and the effect on yield was observed since the second year of experiment, similarly to the presented results.

Experiment in cold-room conditions. The possible use of *T. asperellum* as biological control agent for post-harvest diseases is also discussed. The treatment during the growing season significantly reduced the incidence of strawberry fruit rots during storage. After 7 days of storage, only 1.4 and 1,8% of the treated fruits was infected in 2009 and 2010, respectively, compared to the untreated fruits, which were infected by mold in 33 and 23% in 2009 and 2010, respectively. Some isolates of *Trichoderma* could be adapted to cold conditions and could be used to reduce diseases during cold storage [Sønsteby 2002]. Wide spectrum of *Trichoderma* spp. activity could be useful for organic producers and processors, who can not use synthetic products.

CONCLUSIONS

T. asperellum applied in foliar was capable to stimulate of the plant growth, our earlier knowledge on this microorganism was concentrated on application to the soil. Increase of yield since the second year of strawberry cultivation after *Trichoderma* treatments was performed. Fungus applied in the field at the beginning of growth season was highly effective against *B. cinerea* on flowering plants, harvested and stored fruits. *T. asperellum* reduced symptoms of *B. cinerea* on the ripped strawberry fruits after harvest and during 7-days-storage by increasing of ability of fresh fruits to storage. It is differ from literature showing the effect of *Trichoderma* in growth, generally. Experiments in cold room indicated usefully *T. asperellum* as preharvest treatments. It is novelty. Consequently, I conclude that this microorganism can be used by organic growers and processors, especially.

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EFEKTYWNOŚĆ *Trichoderma asperellum* [T1] W STOSUNKU DO *Botrytis cinerea* [Pers.] ORAZ WPŁYW ZABIEGU NA WZROST I PLON EKOLOGICZNEJ TRUSKAWKI

Streszczenie. Grzyby *Trichoderma* spp. znajdują się w prawie wszystkich glebach i środowiskach. Wiele szczepów *Trichoderma* zdolnych jest efektywnie ograniczać patogeny. Część z nich wzbudza naturalne procesy obronne w roślinach. *Botrytis cinerea* [Pers.: Fr.] jest sprawcą ekonomicznych strat w wielu uprawach oraz w trakcie przechowywania plonów. W niniejszych badaniach wykorzystano szczep *Trichoderma asperellum* [T1] znajdujący się w produkcie handlowym, który stosowano w truskawkach uprawianych w systemie ekologicznym. Jeden gram biopreparatu zawiera 5×10^8 zarodników grzyba. Zabiegi opryskiwania nalistnego wykonano w dawce środka $10 \text{ g} \cdot \text{l}^{-1}$, trzy razy w trakcie sezonu. Po zastosowaniu produktu obserwowano zwiększony przyrost masy zielonej roślin. Objawy szarej pleśni na roślinach oraz na zebranych i przechowywanych owocach obserwowano incydentalnie. Stwierdzono możliwość wykorzystania *T. asperellum* jako biologicznego czynnika ograniczania zgnilizn pozbiorczych owoców, stwierdzono również jego przydatność do zwiększania zdolności owoców do przechowywania do siódmego dnia po zbiorach. W drugim roku badań stwierdzono statystycznie istotny wzrost plonowania roślin traktowanych (30,2%) w porównaniu do roślin kontrolnych. Ponadto potwierdzono statystyczny wzrost wagi części nadziemnych roślin traktowanych (24,0%) oraz większą liczbę produkowanych przez rośliny rozłogów.

Słowa kluczowe: Trifendr WP, przechowywanie owoców truskawki w chłodni, zabieg nalistny, wzrost i rozwój roślin

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