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Use of Waste Vegetable Biomasses Treated by Steam Explosion for the Horticultural Crop Protection

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Abstract: The purpose of this study was to assess the suppressive effect of Waste Vegetable Biomasses (WVBs) treated by the Steam Explosion technique in a continuous plant, against soil-borne plant pathogens. In order to assess their disease suppression, five WVBs (*Miscanthus* biomass, durum wheat straw, rice straw, corn stalk and wood shaving) and commercial compost were tested *in vivo* at three different doses (10, 20 and 30% of potting mix) on seven horticultural pathosystems plant/fungus: tomato/*Phytophthora nicotianae*, cucumber/*Pythium ultimum*, lettuce/*Fusarium oxysporum* f. sp. *lactucae*, melon/*Fusarium oxysporum* f. sp. *melonis*, bean/*Rhizoctonia solani*, eggplant/*Verticillium dahlie* and fennel/*Sclerotinia sclerotiorum*. The results showed that the corn stalk was more efficient respect to *Miscanthus*, compost, wheat straw, rice straw and wood shaving in all the pathosystems and at all the doses tested. The corn stalk suppression ranged from 97% in eggplant/*V. dahliae* to 35% in lettuce/*F. oxysporum* f. sp. *lactucae*, and it was significantly higher with respect to the other substrates. In general, the wheat straw, rice straw and wood shaving were statistically found less efficient as suppressive substrate with respect to corn stalk, *Miscanthus* and compost at the 30% dose in four pathosystems. In particular, the wood shaving suppressiveness ranged from 48% in eggplant/*V. dahliae* to 12% in lettuce/*F. oxysporum* f. sp. *lactucae*. The different suppressiveness observed could be attributed to different concentration of the microbial inhibitory substances (furfurals, organic acids and lignosulfonates) produced during the processing of fresh biomass.

Key words: Compost, disease suppression, soil-borne plant pathogen, steam explosion, waste vegetable biomass.

1. Introduction

The wide spread diseases of horticultural crops are caused by several soil-borne plant pathogens, which are responsible for symptoms on roots, steams and vascular tissues in presence of susceptible hosts and under favourable environmental conditions [1]. Synthetic fungicides and chemical fumigants are usually applied in horticulture to limit these drawbacks, although their indiscriminate use may induce resistance in the plants and accumulation of toxic residues in food, water, air and soil. Therefore, more emphasis should be given to valid alternative methods to help make the environment less disease favourable and host plant more resistant or less susceptible to the diseases caused by soil-borne pathogens [2, 3].

The studies carried out on the suppressive substrates used in horticulture against soil-borne pathogens, have been the object of great interest since the 1980s [4]. The suppression of soil-borne fungal diseases with organic amendments (i.e. compost and other bio-fertilizers) has been widely studied in Italy [5], and their suppressive effect attributed to the microbial activity related to decomposition of soil organic matter [6].

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Recent studies have demonstrated that the Steam-Exploded Biomass of *Miscanthus sinensis* L. var. *giganteus* (SEB), a renewable energy source considered also for the ethanol industrial production by technological process of Steam Explosion (SE), could be used in horticulture as valid alternative to use of compost in greenhouse [7]. In fact, the SEB has been already efficiently used on three plant-fungus pathosystems very important for the Italian horticultural market, because it possesses suppressiveness properties against some soil-borne plant pathogens [8, 9].

Among the different processes of biomass energy conversion, the SE has been the object of great interest since the 1990s for the ethanol industrial production from vegetable wastes [10]. The production of 2nd generation biofuels obtained from energy crops (belonging to annual or perennial and herbaceous or wooding species) and microalgae, is considered very interesting in Italy. The SE is a thermo-mechano-chemical processing performed in different steps (Fig. 1). The saturated steam penetrates the lignocellulosic structures by diffusion in the reactor, it partially or completely condenses at the contact with cold biomass, thereby wetting the material, thus hydrolysing the acetyl groups of hemicellulose fraction, forming furfurals and organic acids; such as acetic, formic and uronic acid. The treatment ends with a sudden decompression to atmospheric condition which mechanically defiberized the biomass, because of the instantaneous vaporization of the liquid water. The process parameters, temperature and time, typically range between 180-220 °C and 2-6 minutes respectively.

Regarding to chemical composition of exploded vegetable biomass, it varies widely depending on the raw materials and treatment severity. The following substances have been usually detected in the exploded vegetable biomass: carbohydrate [glucose (30-40%), xylose (12-28%), arabinose (3-8%) and galactose (2-5%)]; lignin (15-20%); proteins (1-4%); ash (8-10%); organic acids (3%); furfurals (1-3%) and light solvents (about 0.6%).

The present research work has been aimed for to investigate the possibility to reduce the amount of synthetic fungicides and chemical fumigants widely used in horticulture, since they are pollutant for the environment and harmful for the consumers health. Therefore, the purpose of this study was to assess the disease suppressiveness of four different types of Waste Vegetable Biomasses (WVBs), usually used for the ethanol industrial production by SE treatment in a continuous plants, with respect to commercial compost and SEB.

2. Materials and Methods

2.1 Waste Vegetable Biomasses

Five different types of renewable energy sources (*Miscanthus* SEB, durum wheat straw, rice straw, corn stalk and wood shaving) and the commercial compost usually used in greenhouse for the cultivation of horticultural crops, were compared in order to their disease suppressive effect against soil-borne fungal diseases.

The biomasses were collected from different Regions located at Southern Italy (Apulia, Calabria and Basilicata) during the common agricultural practises in field and in greenhouse.

The commercial compost, usually used in horticulture for controlling some soil-borne plant pathogens in greenhouse [11, 12], was obtained from the biodegradable fraction of biomass, such as “Municipal Solid Wastes”, green wastes and urban sludge. Thus, it was produced by an accelerated composting process lasting 3 months, and then used for the trials within 3 months after the end of the composting process.

2.2 Steam Explosion Treatment

The SE treatments of fresh biomass of *Miscanthus*, wheat straw, rice straw, corn stalk and wood shaving, were carried out in a continuous pilot plant (Mod.

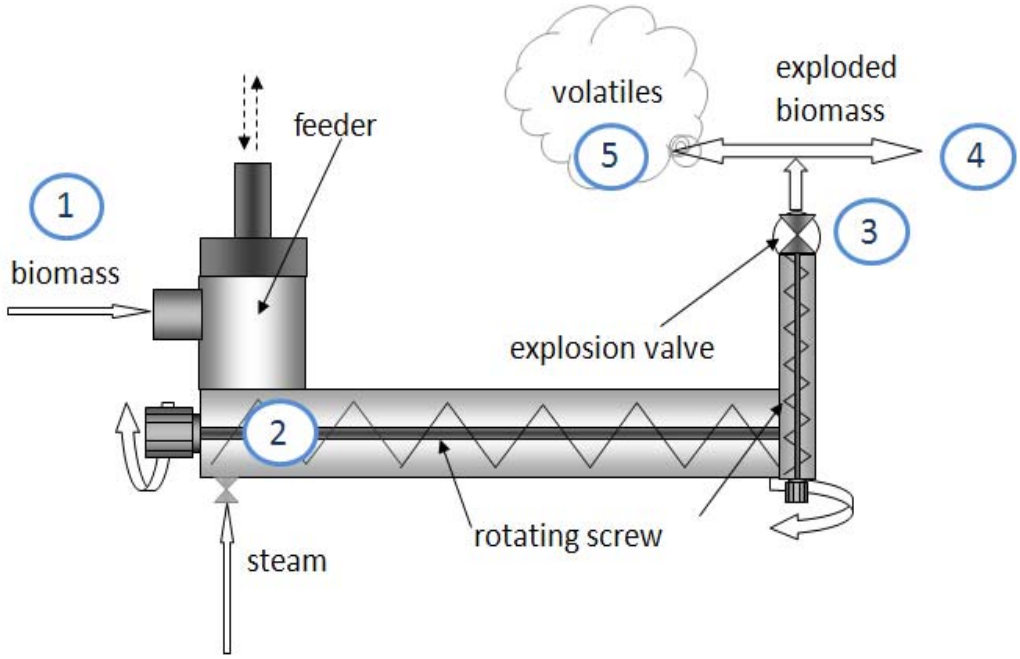

Fig. 1 Treatment of fresh biomass by Steam Explosion in a continuous pilot plant: the biomass, eventually humidified up to 50 wt% (1), is continuously fed into the pressurized reactor where water steam is introduced at the saturated state (2); the residence time is tuned by the rotation rate of the internal screw, typically 2-6 minutes, temperature and pressure is set at the external boiler conditions (180-220 °C); a fast opening and closure of the explosion valve involves the instantaneous decompression (3); the exploded biomass is recovered as wet solid (4) in which are entrapped some degradation compounds while part of volatiles (5) are conveyed to the abatement systems.

StakeTech System Digester) located at ENEA–Trisaia Research Centre (Rotondella, Matera, Italy) (Figs. 2a, 2b).

It was done by processing of 150-200 kg/h of dry biomass, to which water was added to raise the intrinsic humidity up to 50%. A mild severity parameter was chosen to reduce the hemicelluloses degradation, ensuring an adequate cellulose fiberization [13]. The processing times depending on the different WVBs considered: the single treatment was carried out at 215 °C for 3 minutes, while the whole process was terminated after 5-7 hours.

2.3 Assessment of Disease Suppression

2.3.1 Experimental Trials

The experimental trials were carried out *in vivo* in greenhouse with seven plant/fungus pathosystems very important in many Italian horticultural cropping systems [tomato/*Phytophthora nicotianae* Breda de Haan, cucumber/*Pythium ultimum* Trow, lettuce/*Fusarium oxysporum* Schl. f. sp. *lactucae* (Matuo & Motohashi), melon/*Fusarium oxysporum* Schl. f. sp. *melonis* (Leach & Currence) Snyd. & Hans., bean/*Rhizoctonia solani* Kühn, eggplant/*Verticillium dahlie* Kleb. and fennel/*Sclerotinia sclerotiorum* (Lib.) de Bary].

Six plots of potting mixes containing separately compost, *Miscanthus* SEB (Figs. 3a, 3b), wheat straw, rice straw, corn stalk and wood shaving, were statistically compared for their disease suppressiveness in relation to each pathosystem. A single suppressive substrate was manually mixed to peat substrate at three different doses [10, 20 and 30% (w/w)] of potting soil. The peat, commonly used in horticulture, was disinfested at 121 °C for 30 minutes and used in the trials for comparative testing.

2.3.2 Artificial Infestation of the Potting Mixes

The potting mixes and peat substrate (control) were artificially infested seven days before the transplanting of the susceptible hosts with seven soil-borne pathogen

Fig. 2 External (a) and internal (b) view of the continuous Steam Explosion pilot plant located at ENEA – Trisaia Research Centre.

strains (*P. ultimum*, *P. nicotianae*, *F. oxysporum* f. sp. *lactucae*, *F. oxysporum* f. sp. *melonis*, *R. solani*, *V. dahliae* and *S. sclerotiorum*), provided by the German Laboratory (Collection of Microorganisms and Cell Cultures, DSMZ, Braunschweig, Germany).

Inoculum of each fungal strain was manually mixed into substrates and maintained for 7 days under plastic bag at 22 ± 4 °C. The *P. ultimum* and *P. nicotianae* fungi were propagated in flasks on wheat plus hemp (200 g of wheat kernels and 100 g of hemp kernels in 320 mL of distillate water and autoclaved at 120 °C for 30 min) and incubated for 10-12 days at 20 °C. Inoculation dosage was 2 g/g of potting mix or peat control. The *F. oxysporum* f. sp. *lactucae* and *F. oxysporum* f. sp. *melonis* fungi were incubated in flasks on casein liquid medium for 10-15 days on rotary shaker, then centrifuged and mixed with talc for to produce chlamydospores [14]. Inoculation dosage

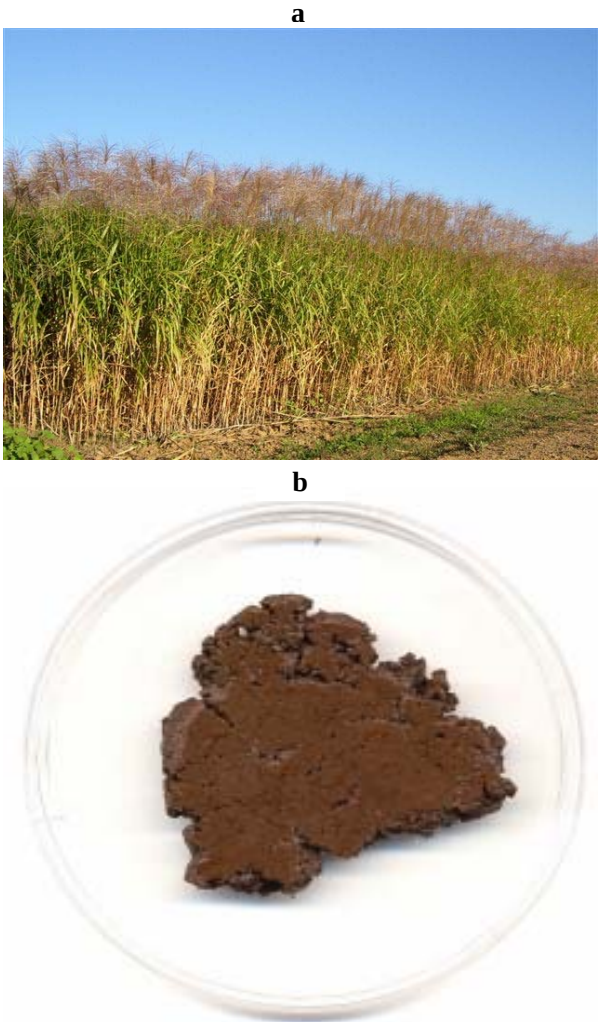

Fig. 3 Crops of *Miscanthus sinensis* var. *giganteus* (a) and Exploded Biomass (b) obtained by Steam Explosion.

was 10^4 colony forming unit/g of potting soil or peat control. Inoculum of *R. solani* and *V. dahliae* were obtained from fungal cultures grown on Potato-Dextrose-Agar (PDA) in 10 cm Petri dishes for 7-8 days in the dark, homogenised in sterile water, propagated in flasks on wheat (300 g of wheat kernels in 400 mL of distillate water and autoclaved at 120 °C for 30 minutes) and incubated for 8-15 days at 25 °C. Inoculation dosage was 3 g/g of potting mix or peat control. Inoculum of *S. sclerotiorum* was obtained from colonies grown on PDA in 10 cm Petri dishes for 4-6 days in the dark. The sclerotia were collected from plates and stored into a climatic room at 22 ± 2 °C and 45 ± 3% R.U. for one week. Inoculation dosage was 20-25 sclerotia/kg of potting soil or peat control.

2.3.3 Experimental Design

Healthy plants of *Lycopersicon esculentum* Miller (cv. Rutgers), *Cucumis sativus* L. (cv. Market), *Lactuca sativa* L. (cv. Iceberg), *Cucumis melo* L. (cv. Rugoso di Cosenza), *Phaseolus vulgaris* L. (cv. Sarconi), *Solanum melongena* L. (cv. Black beauty) and *Foeniculum vulgare* Miller (cv. Provenza) were used as susceptible hosts in the bioassays. They were sowed in the paper pots (96 holes) and transplanted at the 5-8 days after the start germination in the plastic pots (60 × 130 cm) containing the potting mixes infested by pathogens.

All the experimental plots were maintained in greenhouse at 24 ± 3 °C and 85 ± 3% R.U. during the bioassays, together with other plots containing peat without pathogens. Each trial was repeated four times with three replications of 10 plants each. A randomized complete block design was used and the data regarding the number of diseased plants were recorded at 15–20 days after the transplanting.

For each pathosystem, the different disease levels were studied in relation to those of peat control in order to compare the suppression level among the trials. The "Percentage Disease Suppression" index (DS) was calculated as follows: $DS = [(NP - NM) / NP] \times 100$ (NM = average of number of diseased plants in the plots containing the substrates at the three doses tested and in the plots containing peat without pathogens; NP = in the plots containing peat control).

2.3.4 Statistical Analysis

Analyses of variance (ANOVA) of DS means were carried out with the statistical programme SPSS version 12.0 (Statistics Base™, Chicago, IL, USA).

Tukey's HSD test was applied when ANOVA revealed significant differences ($P < 0.05$) among the means.

3. Results and Discussion

The disease suppressiveness of the WVBs tested has been statistically analyzed and compared to those observed with the compost at the same doses. The results obtained are reported in Table 1.

Table 1 Statistical analyses of the disease suppressiveness (% Ds) of six substrates treated by Steam Explosion, added to peat at three different doses, on seven horticultural plant-fungus pathosystems.

Suppressive substrate (SS)	Peat + pathogen	Peat – pathogen	Peat +10% SS	Peat + 20% SS	Peat + 30% SS
Cucumber/*Pythium ultimum*					
Commercial compost	0^{a}	100^{a}	45^{bc}	61^{c}	66^{c}
Miscanthus SEB	0^{a}	100^{a}	65^{ab}	72^{b}	78^{b}
Durum wheat straw	0^{a}	100^{a}	34^{cd}	41^{d}	42^{d}
Rice straw	0^{a}	100^{a}	29^{d}	38^{d}	45^{d}
Corn stalk	0^{a}	100^{a}	73^{a}	88^{a}	95^{a}
Wood shaving	0^{a}	100^{a}	32^{cd}	37^{d}	39^{d}
Bean/*Rhizoctonia solani*					
Commercial compost	0^{a}	100^{a}	19^{b}	24^{c}	27^{c}
Miscanthus SEB	0^{a}	100^{a}	49^{ab}	51^{b}	69^{b}
Durum wheat straw	0^{a}	100^{a}	12^{b}	13^{d}	17^{c}
Rice straw	0^{a}	100^{a}	18^{b}	14^{d}	18^{c}
Corn stalk	0^{a}	100^{a}	55^{a}	77^{a}	88^{a}
Wood shaving	0^{a}	100^{a}	11^{b}	14^{d}	19^{c}
Tomato/*Phytophthora nicotianae*					
Commercial compost	0^{a}	100^{a}	57^{b}	59^{c}	62^{c}
Miscanthus SEB	0^{a}	100^{a}	50^{b}	69^{b}	77^{b}
Durum wheat straw	0^{a}	100^{a}	21^{c}	28^{d}	26^{d}
Rice straw	0^{a}	100^{a}	22^{c}	25^{d}	29^{d}
Corn stalk	0^{a}	100^{a}	64^{a}	78^{a}	91^{a}
Wood shaving	0^{a}	100^{a}	17^{c}	21^{d}	25^{d}
Lettuce/*Fusarium oxysporum* f. sp. *lactucae*					
Commercial compost	0^{a}	100^{a}	12^{b}	14^{b}	16^{b}
Miscanthus SEB	0^{a}	100^{a}	10^{b}	13^{b}	18^{b}
Durum wheat straw	0^{a}	100^{a}	8^{b}	12^{b}	15^{b}
Rice straw	0^{a}	100^{a}	6^{b}	11^{b}	13^{b}
Corn stalk	0^{a}	100^{a}	32^{a}	33^{a}	35^{a}
Wood shaving	0^{a}	100^{a}	5^{b}	8^{b}	12^{b}
Melon/*Fusarium oxysporum* f. sp. *melonis*					
Commercial compost	0^{a}	100^{a}	19^{ab}	25^{b}	28^{b}
Miscanthus SEB	0^{a}	100^{a}	8^{b}	18^{bc}	22^{b}
Durum wheat straw	0^{a}	100^{a}	5^{b}	7^{c}	13^{c}
Rice straw	0^{a}	100^{a}	6^{b}	9^{c}	16^{c}
Corn stalk	0^{a}	100^{a}	25^{a}	31^{a}	42^{a}
Wood shaving	0^{a}	100^{a}	7^{b}	11^{c}	15^{c}
Eggplant/*Verticillium dahliae*					
Commercial compost	0^{a}	100^{a}	48^{c}	59^{c}	64^{c}
Miscanthus SEB	0^{a}	100^{a}	67^{b}	71^{b}	80^{b}
Durum wheat straw	0^{a}	100^{a}	24^{d}	37^{d}	45^{d}
Rice straw	0^{a}	100^{a}	22^{d}	31^{d}	47^{d}
Corn stalk	0^{a}	100^{a}	73^{a}	89^{a}	97^{a}
Wood shaving	0^{a}	100^{a}	28^{d}	32^{d}	48^{d}
Fennel/*Sclerotinia sclerotiorum*					
Commercial compost	0^{a}	100^{a}	8^{c}	11^{cd}	28^{c}
Miscanthus SEB	0^{a}	100^{a}	47^{b}	56^{b}	66^{b}
Durum wheat straw	0^{a}	100^{a}	18^{c}	21^{c}	27^{c}
Rice straw	0^{a}	100^{a}	15^{c}	24^{c}	28^{c}
Corn stalk	0^{a}	100^{a}	65^{a}	77^{a}	85^{a}
Wood shaving	0^{a}	100^{a}	10^{c}	13^{cd}	23^{c}

Means followed by different letters within each column were significantly different according to Tukey's HSD test ($P < 0.05$).

The plants grown in the pots containing peat without pathogens remained healthy and without any disease symptom within the end of bioassays in all the experimental trials.

The disease incidence of the pathosystems cucumber/*P. ultimum*, tomato/*P. nicotianae* and eggplant/*V. dahliae* was significantly reduced by adding of corn stalk, *Miscanthus* SEB and compost at the 30% dose. When the corn stalk was mixed to potting soil, the percentage of disease suppression was 95, 91 and 97% for cucumber/*P. ultimum* (Fig. 4a), tomato/*P. nicotianae* and eggplant/*V. dahliae*, respectively. The adding of *Miscanthus* SEB reduced the disease incidence of cucumber/*P. ultimum*, tomato/*P. nicotianae* and eggplant/*V. dahliae* of 78, 77 and 80%, respectively. Finally, the suppressiveness of cucumber/*P. ultimum* (Fig. 4b), tomato/*P. nicotianae* and eggplant/*V. dahliae* was 66, 62 and 64% by adding of compost, respectively. The other substrates (wheat straw, rice straw and wood shaving) showed in these three pathosystems a less suppressive activity with respect to corn stalk, *Miscanthus* SEB and compost at the highest dose tested.

The disease incidence of the bean/*R. solani* and fennel/*S. sclerotiorum* was significantly reduced by adding of corn stalk and *Miscanthus* SEB at the highest dose tested. When the corn stalk was added to potting soil, the percentage of disease suppression was 88 and 85% for bean/*R. solani* and fennel/*S. sclerotiorum*, respectively. The adding of *Miscanthus* SEB suppressed the disease of bean/*R. solani* and fennel/*S. sclerotiorum* of 69 and 66%, respectively. Finally, the other substrates (compost, wheat straw, rice straw and wood shaving) showed in these two pathosystems a less suppressive activity with respect to corn stalk and *Miscanthus* SEB at the highest dose tested.

The disease incidence of the melon/*F. oxysporum* f. sp. *melonis* and lettuce/*F. oxysporum* f. sp. *lactucae* was significantly reduced by adding of corn stalk at the 30% dose. The percentage of disease suppression was 42 and 35% in melon/*F. oxysporum* f. sp. *melonis* and lettuce/*F. oxysporum* f. sp. *lactucae*, respectively. The other substrates (*Miscanthus* SEB, compost, wheat straw, rice straw and wood shaving) showed in these two pathosystems a less suppressive activity with respect to corn stalk at the same dose.

In consideration of these results, we can maintain that the corn stalk resulted efficient as suppressive substrate more than the *Miscanthus* SEB, compost, wheat straw, rice straw and wood shaving in all the pathosystems at the 30% dose. The percentage of corn stalk disease suppression was often significantly higher with respect to the suppressiveness of the other substrates tested at the 10 and 20% doses.

The *Miscanthus* SEB was efficient as suppressive substrate more than the compost, wheat straw, rice straw and wood shaving in five pathosystems (cucumber/*P. ultimum*, bean/*R. solani*, tomato/*P. nicotianae*, eggplant/*V. dahliae* and fennel/*S. sclerotiorum*). The percentage of SEB disease suppression was statistically higher with respect to the suppressiveness of the substrates above cited, when they were mixed to a potting soil at the 30% dose.

The compost showed a very good suppressive effect with respect to wheat straw, rice straw and wood shaving in four pathosystems (cucumber/*P. ultimum*, tomato/*P. nicotianae*, melon/*F. oxysporum* f. sp. *melonis* and eggplant/*V. dahliae*). The percentage of compost disease suppression was significantly higher with respect to the suppressiveness of the substrates above mentioned, when they were added to a potting mix at the highest dose tested. Moreover, the compost used in these trials showed more suppressiveness in cucumber/*P. ultimum*, tomato/*P. nicotianae* and eggplant/*V. dahliae*, as compared to bean/*R. solani* and lettuce/*F. oxysporum* f. sp. *lactucae*. Instead, a medium level of inhibitory effect was found in melon/*F. oxysporum* f. sp. *melonis* and fennel/*S. sclerotiorum*. These results have been also partially confirmed by the studies carried out by Pugliese *et al*. (2007, 2008) [11, 12].

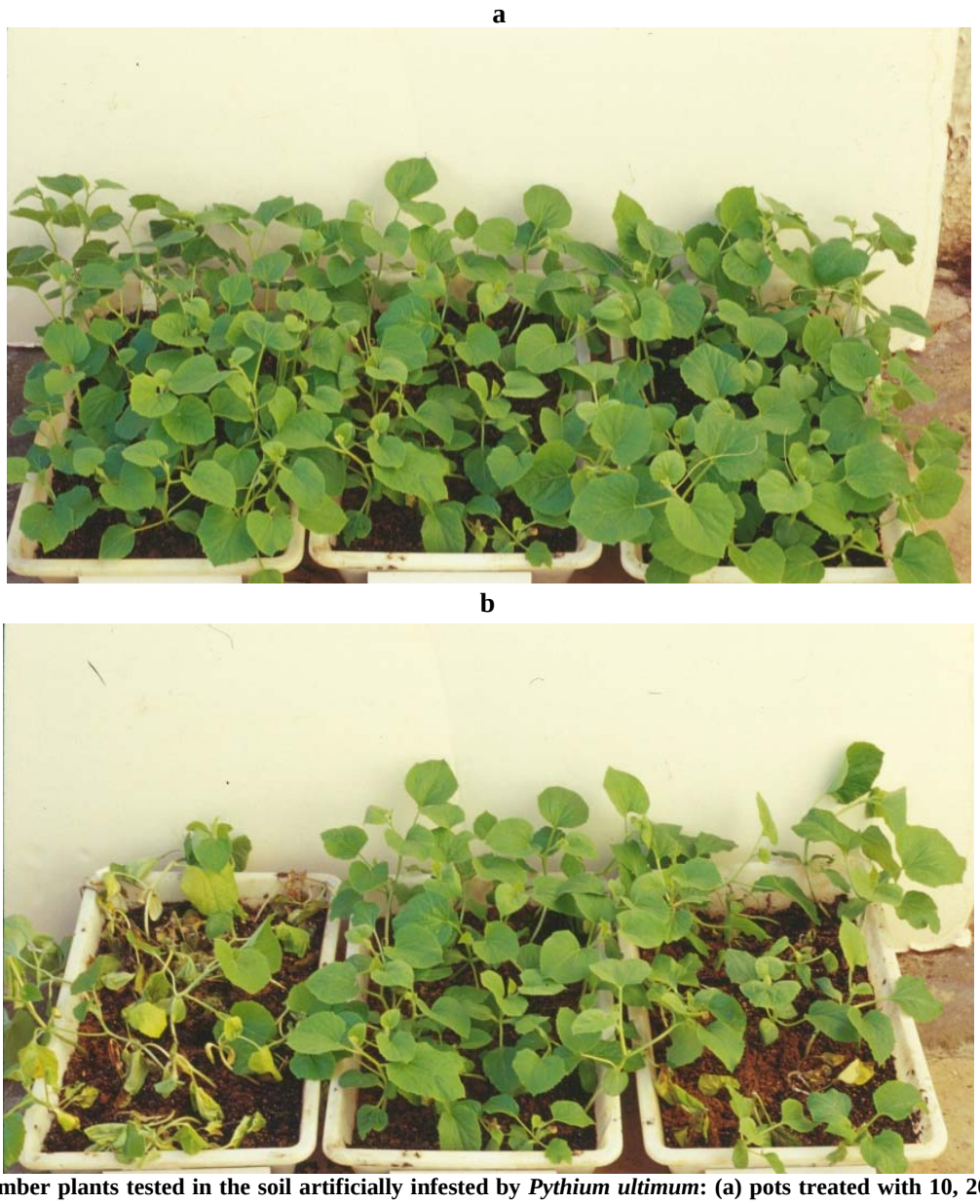

Fig. 4 Cucumber plants tested in the soil artificially infested by *Pythium ultimum*: (a) pots treated with 10, 20 and 30% of exploded biomass of corn stalk (from left to right respectively), (b) pots treated with 10, 20 and 30% of commercial compost (from left to right respectively).

Finally, the remaining substrates tested (wheat straw, rice straw and wood shaving) resulted very similar in relation to their suppressive activity. The minor efficiency of them, with respect to corn stalk, *Miscanthus* SEB and compost, was found in four pathosystems (cucumber/*P. ultimum*, tomato/*P. nicotianae*, melon/*F. oxysporum* f. sp. *melonis* and eggplant/*V. dahliae*). The disease suppression percentages were statistically lower with respect to the suppressiveness of corn stalk, *Miscanthus* SEB and compost on the pathosystems above cited, when they were mixed to a potting soil at the 30% dose.

It is therefore evident that the suppressive effect of the WVBs tested increase with 30% dose in the potting mix. Their suppressiveness might be attributed to inhibitory effect of the antimicrobial substances (i.e. furfurals, organic acids and lignosulfonates) produced during the processing of fresh biomass in a SE

continuous plant [9, 15]. The literature reports also that high treatment severity promotes the chemical degradation of the fermentable sugars (i.e. xylose and glucose) to furan molecules (furfuraldehyde and 5-hydroxymethylfurfural), organic acids (acetic, formic and uronic acid) and lignosulfonates [16]. These substances have also an evident toxic effect on several microorganisms' growth, particularly on the yeasts *Pichia stipitis* and *Saccaromyces cerevisiae*, commercially used for the bioconversion of different types of waste vegetable biomasses to ethanol [17]. Therefore, these microbial inhibitors are particularly undesirable in case of the fermentation systems used for the ethanol industrial production from mixed sugar syrups and corn stalk hydrolysed [17].

The different suppressive effects observed could depend on by different concentrations of fermentable sugars present in the lignocellulosic matters. In fact, the fresh biomass of corn stalk and *Miscanthus* SEB have an higher content of xylose and glucose more than the wheat straw, rice straw and wood shaving [18, 19]. Therefore, their suppressive effect was significantly different and could be related to the different concentration of furan molecules, organic acids and lignosulfonates produced during the SE treatment in a continuous plant, though more detailed studies of this topic are required.

4. Conclusions

The use in horticulture of new fungicides and fumigants at low dosage, integrated to use of compost and WVBs (i.e. corn stalk and *Miscanthus* SEB) treated by SE, is highly recommended to potentially reduce the disease incidence of several soil-borne pathogens (i.e. *P. ultimum*, *R. solani*, *P. nicotianae*, *V. dahliae* and *S. sclerotiorum*) commonly transferred in the crops through the soil.

It is very important, to help the marketing of these news suppressive substrates on commercial scale, to be sure that the natural suppressiveness provided is really able to partially replace the use of traditional fungicides and fumigants.

A main consequence of our research is to help make of research programs and new projects a long term basis for the management of soil-borne fungal diseases very dangerous in the horticulture. These studies are fundamental for their potential applications in the sustainable agriculture.

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