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Scientific Article

Alternative management strategies reduced the incidence and severity of root rot of melon

Estratégias de manejo alternativo reduz a incidência e severidade da podridão da raiz do melão

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ABSTRACT - Melon (*Cucumis melo* L.) is one of the most economically important fruit crops in the Northeastern region of Brazil, nearly all production is exported to European countries. Because of the indiscriminate use of monoculture, the incidence of soilborne pathogens in melon fields is on the rise, resulting in increasing losses in fruit production. The objective of this study was to investigate if the incorporation of different vegetable materials (*Crotalaria juncea* L. and *Pennisetum glaucum* L.) in the soil, combined with polyethylene mulch, and the application of commercial products (Compost-Aid® + Soil-Set®) can efficiently control soilborne pathogens in melon fields. Two greenhouse experiments were identically set up using soil naturally infested with various phytopathogenic fungi, including *Fusarium* spp. and *Macrophomina* spp. The experimental design was completely randomized, with seven treatments and seven replications. The pathogens' occurrence, disease incidence, and severity were evaluated, as well as fruit quality indicators (weight, firmness, and Brix Degree). Two treatments showed great potential for decreasing disease incidence, severity, and the occurrence of pathogens. One of the treatments had pearl millet (*Pennisetum glaucum*) incorporated into the soil that was covered with polyethylene mulch. The other treatment was when crotalaria was incorporated into the soil and covered with polyethylene mulch. Commercial products (Compost-Aid® and Soil-Set®) were applied in high temperatures and lower humidity, in both treatments. Plants submitted to these treatments also yielded fruits with higher weight and Brix Degree than the control treatment.

Keywords: *Fusarium*. *Macrophomina*. *Cucumis melo* L. Polyethylene mulch. Mulching.

RESUMO - Melão (*Cucumis melo* L.) é uma das frutas mais importantes da região Nordeste do Brasil e quase toda a produção é exportada para países europeus. Com o uso indiscriminado da monocultura, aumenta a incidência de patógenos de solo nas lavouras de melão, resultando em perdas crescentes na produção de frutas. O objetivo deste estudo foi investigar se a incorporação de diferentes materiais vegetais (*Crotalaria juncea* L. e *Pennisetum glaucum* L.) no solo, combinado com mulch de polietileno, e a aplicação de produtos comerciais (Compost-Aid® + Soil-Set®) pode controlar eficientemente patógenos de solo na produção de melão. Dois experimentos em casa de vegetação foram montados de forma idêntica usando solo naturalmente infestado com *Fusarium* spp. e *Macrophomina* spp. O delineamento experimental foi inteiramente casualizado, com sete tratamentos e sete repetições. Foram avaliadas a ocorrência dos patógenos, a incidência de doenças e a severidade, bem como as características de qualidade dos frutos (peso, firmeza e brix). Dois tratamentos mostraram grande potencial para diminuir a incidência e a severidade da doença, bem como a ocorrência dos patógenos. Um tratamento foi o milheto (*Pennisetum glaucum*) incorporado ao solo e coberto com mulch de polietileno. O outro tratamento foi quando a crotalária foi incorporada ao solo coberto com mulch de polietileno. Produtos comerciais (Compost-Aid® e Soil-Set®) foram aplicados em alta temperatura e baixa umidade, em ambos tratamentos. Esses tratamentos também produziram frutos com maior peso e brix do que o tratamento controle.

Palavras-chave: *Fusarium*. *Macrophomina*. *Cucumis melo* L. Filme de polietileno. Mulching.

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INTRODUCTION

The cultivation of melon (*Cucumis melo* L.) is extremely important for the Brazilian economy, as it is the country's third most exported fresh fruit (FAO, 2022). Brazil is the fifth largest producer of melon in the world (FAO, 2022). However, the continuous practice of monoculture by many Brazilian farmers has directly influenced the surge of soilborne pathogen populations in melon fields and consequently increased the reduction in fruit quality, yield, and fruit production - jeopardizing the ability of growers to meet export contracts (HUANG et al., 2013).

Fusarium solani (Mart.) and *Macrophomina phaseolina* Tassi (Goid.) are among the major pathogens that cause diseases in melon plant root systems (PORTO et al., 2016). They produce resistance structures, chlamydospores (*F. solani*) and sclerotia (*M. phaseolina*), which can help the pathogens to survive in the soil for long periods of time, threatening crop production (PORTO et al., 2016). Therefore, it is necessary to adopt and combine different integrated disease management strategies to help control those persistent soilborne pathogens.

Several control techniques are widely used to manage soilborne pathogens in crop fields (e.g. chemical, biological control, and the use of natural products). However, for melon crop, there is no chemical registered in the Brazilian Department of Agriculture (Ministério da Agricultura, Pecuária e Abastecimento) to control *F. solani* and *M. phaseolina* in the field (AGROFIT, 2023). The only recommendations are to use resistant varieties and cultural practices to manage these pathogens in the field (AGROFIT, 2023). However, as Sales Júnior et al. (2017) stated, all these measures are superficial and the development of new techniques to complement these management strategies is extremely important, even if all of them need to be used concomitantly. Additionally, melon-importing countries, mostly European, have restricted regulations in place that limit the use of chemicals, which adds another layer of complexity to soilborne pathogen control in melon fields. Therefore, there is an eminent need to develop soilborne pathogen management strategies that use fewer chemicals, are sustainable, and are suitable to the Northeastern region of Brazil that melon growers can readily adopt.

One of the complementary techniques used is the application of green manure, which can be deposited over the ground or incorporated into the soil, to efficiently manage phytopathogens (BARRADAS, 2010). This technique has been successfully tested to reduce the inoculum of soilborne pathogens in melon crop (DANTAS et al., 2013; PORTO et al., 2016). However, optimal results are achieved when green manure is incorporated into the soil with cover crops, such as crotalaria (*Crotalaria juncea*) (FONSÊCA NETO et al., 2016) and pearl millet (*Pennisetum glaucum*) (ASMUS; INOMOTO; BORGES, 2016). When applied alone, green manure application yields slow results over time, and thus it is not attractive to growers to adopt.

Another promising technique for the control of pathogens that affect plant root systems is soil solarization, which has great efficiency when associated with the incorporation of vegetal materials, mainly because it helps raise the soil temperature (ROCHA; CARNEIRO, 2016), in addition to releasing volatile and non-volatile compounds, which can be toxic to soilborne pathogens. Polyethylene mulch, unlike other types of plastics used in solarization, can be of various colors (black-white, black-silver, and black-black), the choice of color is determined by the variation of the planting season and the place of cultivation (LAMBERT et al., 2017). This technique is already being used to reduce weeds, increase irrigation efficiency, decrease nutrient losses through leaching caused by rain, accelerate plant development, improve fruit hygiene and quality, and also to increase productivity (LAMBERT et al., 2017). In the Northeast region of Brazil, white-black polyethylene mulch with holes for transplanting seedlings has been widely used and it is recommended for melon cultivation.

Soilborne pathogens are very common in melon production fields in the Northeast region of Brazil. It is possible that the incorporation of plant materials into the soil associated with polyethylene mulch may reduce the inoculum potential of these pathogens. This approach can accelerate the decomposition of organic matter, raise the soil temperature, and release compounds from plant materials that are toxic to pathogens. However, during the treatment, the polyethylene mulch should not contain holes in order to stimulate and harness the benefits of controlling soilborne pathogens. Therefore, in addition to the incorporation of plant material and treatment time, the used polyethylene mulch with predrilled holes, which is widely used by melon growers to facilitate the transplantation of seedlings, will have to be replaced with one without holes.

The overall goal of this study was to develop new alternatives to manage soilborne pathogens in melon fields. We hypothesized that the incorporation of vegetal materials in the soil combined with polyethylene mulch and with the application of commercial products would reduce soilborne pathogens's initial inoculum in the soil. We tested this hypothesis in soil naturally infested with soilborne pathogens, and added inoculum of *F. falciforme* and *M. phaseolina*, in order to reduce the damage caused by these fungi throughout the melon cycle, from planting to harvest.

MATERIAL AND METHODS

Experiment setup

In order to achieve preliminary results for field application and mainly to ensure high infestation and viability of the studied pathogens, the experiments were performed in a controlled environment (greenhouse). Two experiments were conducted concomitantly, the first one was implanted at the end of March, and the second experiment was implanted 30 days later, using soil that has long been cultivated with muskmelon, with up to three crop cycles per year in the same field, and also with a long history of natural infestation of soilborne pathogens. The soil had the following chemical characteristics: pH(H_2O)=6.10, P(mg dm^{-3})=101.00, sum of bases (SB) (cmolc dm^{-3})=2.99, K^+(mg dm^{-3})=85.10, Mg^{+2} (cmolc dm^{-3})=0.50, Al^{+3}(cmolc dm^{-3})=0.00, cation exchange capacity (CEC) (cmolc dm^{-3})=3.65, O.M=3.56 (g Kg^{-1}), and base saturation (V%)=82.00. The experiments were conducted in 14L pots, with a diameter of 0.28m, in a greenhouse located in the city of Mossoró in the state of Rio Grande do Norte, Brazil (5° 11' 17" South, 37° 20' 39" West). Both trials had the same treatments.

Experimental design

A completely randomized design with seven treatments and seven replications was used. The treatments were: (C) - Control (pots were not covered with polyethylene mulch and neither with vegetal material), (M) - polyethylene mulch (pots were covered with polyethylene mulch but not covered with vegetal material), (C+M) - incorporation of *Crotalaria juncea* L. + polyethylene mulch, (P+M) - incorporation of *Pennisetum glaucum* L. + polyethylene mulch, (M+CS) - polyethylene mulch + (Compost-Aid® + Soil-Set®), (C+M+CS) - incorporation of *Crotalaria juncea* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®), and (P+M+CS) - incorporation of *Pennisetum glaucum* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®).

Inoculum

Inoculum was prepared by cultivating the fungi in flasks containing sand organic substrate (LEFÈVRE; SOUZA, 1993). The substrate was composed of three parts of cow manure, one part of washed sand, and 2% of oats (v/w); 20mL of distilled water was added to each 100mL of substrate. Then, the substrate was autoclaved twice, at 24 hours intervals, for one hour each at 1.27 Kg/cm^2 (18 psi) and 121° C. Subsequently, five 5mm diameter discs were transferred in a laminar flow cabinet from the colonies growing in Petri dishes to the flasks containing the sand organic substrate. The fungi used were, *M. phaseolina* (CMM-1531, deposited in the Coleção de Culturas de Fungos Fitopatogênicos "Prof^a. Maria Menezes", at the Universidade Federal Rural de Pernambuco, Brazil, GenBank code MN136199) and *F. falciforme* (CML 3946, deposited at the *Coleção* micológica de Lavras, at the Universidade Federal de Lavras, Brazil, GenBank code MH709261). The soil, naturally infested with soil pathogens, was also artificially infested on the same day that the pots were filled and after 20 days of growth in the sand organic substrate in the laboratory, 54g of substrate from each fungus per pot, totalizing 108g of *M. phaseolina* and *F. falciforme* inoculated per pot.

The incorporation of the vegetal materials (leaves and branches) and the covering of the pots with the polyethylene mulch were done 17 days before transplanting. The plant materials, crotalaria (*Crotalaria juncea* L.) and pearl millet (*Pennisetum glaucum* L.), were incorporated in the first 10cm of the soil at the amount of 4kg/m² of plant material per pot, the pots were kept for 15 days in the greenhouse (AMBRÓSIO, 2003). After the treatment period (15 days), holes were drilled in the polyethylene mulch to remove toxic gases and lower the soil temperature. The melon seedlings were transplanted two days later. Seedlings were cultivated in trays using topsoil mix and hybrid yellow melon Goldex Topseed seeds, the seedlings were transplanted 12 days after sowing.

In treatments (M+CS), (C+M+CS) and (P+M+CS), Compost-Aid® and Soil-Set® (Table 1) were applied once on day one after transplanting at the dosage of 3 kg ha^{-1} and 2 L ha^{-1}, respectively. Those two products were applied twice again, at 7 and 14 days after transplanting, at the concentrations of 2 kg ha^{-1} (Compost-Aid®) and 1.5 L ha^{-1} (Soil-Set®) - considering a population of 12,500 plants ha^{-1} and one plant per pot for each experiment.

Table 1. Composition of biofertilizers used in the study to evaluate the effect on *Fusarium* spp. and *Macrophomina* spp. in melon.

	Compost-Aid®	
Bacteria	UFC g^{-1}	Enzymes
Lactobacillus plantarum	1.25 x 10^8	Protease
Bacillus subtilis	1.25 x 10^8	Cellulase
Enterococcus faecium	1.25 x 10^8	Xylanase
	Soil-Set®	
Minerals	%	g L^{-1}
Sulfur	3.70	45.51
Zinc	3.2	39.36
Copper	2.00	24.60
Iron	1.60	19.68
Manganese	0.8	9.84

*Compost-Aid® and Soil-Set® are trade names of compounds produced by Alltech Crop Science.

Plants were watered by drip irrigation and fertirrigation was conducted according to soil analysis to meet the crop needs. The maximum temperature of the soil in each pot was measured by a mercury thermometer and the maximum temperature and humidity of the air was measure by digital hygro-thermometer, every day at 1.00 p.m.

Disease evaluation

At the end of the cycle of the melon, all plants were collected to evaluate the occurrence of root rot. Fragments from the border of the disease lesions were removed from all plants that presented symptoms. Five fragments from each plant were surface disinfested. The five fragments were placed in a Petri dish containing potato dextrose agar (PDA) + antibiotic (tetracycline 0.05 g L^{-1}) and incubated for seven days, in a BOD at 28 ± 2 °C. Then, the plates were evaluated for the presence of the pathogens, if the pathogen was present in the five fragments, it was evaluated as 100% occurrence; if it was present in just one fragment, it was evaluated as 20% occurrence. For instance, it is possible to have on the same plate an 80% occurrence of *Fusarium* sp., 40% occurrence of *Macrophomina* sp., and 40% occurrence of *Rhizoctonia* sp. Pathogens were identified using standard identification keys, according to their morphological characteristics (BARNETT; HUNTER, 1998; LESLIE; SUMMERELL, 2008).

The incidence of the disease was assessed by the percentage of plants showing symptoms. In our case, we evaluated seven plants, if the seven showed symptoms, it meant 100% disease. If there were four plants with symptoms, it totaled 57% of disease in this treatment. Disease severity was determined by using a disease note scale (AMBRÓSIO et al., 2015), following the classifications: (0) - asymptomatic, (1) - less than 3% of infected tissues, (2) - 3-10% of infected tissues, (3) - 11-25% of infected tissues, (4) - 26-50% of infected tissues, and (5) - more than 50% of infected tissues. All steps involved in the experiments are depicted in Figure 1.

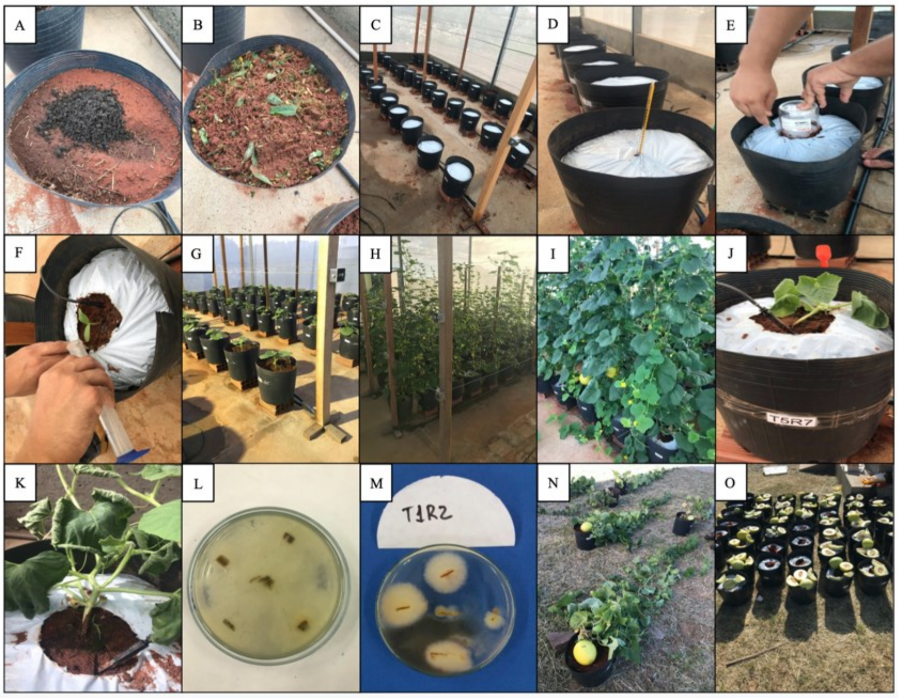

Figure 1. Photographs showing the steps depicting the implementation, conduction, and evaluation of the experiments to evaluate the effect on *Fusarium* spp. and *Macrophomina* spp. in melon. A – Fungal inocula being added to the soil. B – Incorporation of plant material. C - Pots covered with polyethylene mulch and going through the 15-days soil treatment period. D – Recording the soil temperature. E – Drilling holes on the polyethylene mulch. F – Seedlings planting and application of Compost-Aid® + Soil-Set®. G, H, and I – Different growth stages of melon plants during the 60 days of the experiment duration. J and K – Plants that did not survive the high disease pressure used in the experiments. L and M – Petri dishes illustrating the fungal isolation assay from infected plant tissue for the evaluation of incidence and occurrence of soilborne pathogens. N and O - End of the experiments, last evaluations, and harvesting.

Fruits evaluation

Fruit weight, firmness, and Brix Degree content were measured respectively, using a digital hook scale, an analogical penetrometer PTR 100, and a portable refractometer RT-30 ATC.

Statistical analysis

The values for weight, firmness, and Brix Degree of the fruits were analyzed with the generalized linear mode using the glm function (R CORE TEAM, 2019). Because incidence and severity were not normally distributed, a nonparametric Kruskal-Wallis test was used to analyze those variables (R CORE TEAM, 2019). Pairwise correlation analyses were performed on the dataset with the nonparametric Kendall's τ rank correlation coefficient to measure the strength of the relationship between each type of symptom using the packages Hmisc and corrplot (R CORE TEAM, 2019). All statistical analyses and plotting for data visualization were performed in R program version 3.1.1 (R CORE TEAM, 2019).

RESULTS AND DISCUSSION

The maximum soil temperature in all treatments was higher than the greenhouse air temperature in both experiments (Figure 2). However, at the end of experiment 2 – starting at 41 days after the holes were punctured on the polyethylene mulch - both temperatures (air and maximum soil temperature) had similar measurements (Figure 2D). In both experiments, the maximum soil temperature did not exceed 41 °C and it didn't go below 32 °C, except in (C+M), (P+M), (C+M+CS) and (P+M+CS) at 7 days of treatment which reached 42 °C (Figure 2A). Overall, all treatments had similar maximum soil temperature throughout the experiments (Figure 2), with the exception of the control (C) treatment in the first 15 days of the experiment 1, it had lower maximum soil temperature in comparison to the other treatments, with average of 36.63 °C and all other treatments between 37.11 and 37.47 °C. The relative air humidity trend was similar until day 41 in both experiments, then it became higher in experiment 1 in comparison to experiment 2 (Figure 3).

The maximum soil temperature achieved in all treatments throughout phase one, before drilling a hole on the polyethylene mulch, in both experiments (Figure 2), was above the optimal temperature for the development of *Macrophomina* spp. (30-35 °C) (GHOSH et al., 2018) and *Fusarium* spp. (28-30 °C) (PAPIZADEH et al., 2018). However, the treatment period of only 15 days may have caused just a fungistatic effect (fungal growth inhibition) on the root rot pathogens. Experiments conducted at temperature ranges close to what we achieved in this study concluded that the fungicide effect in *Fusarium* spp. is achieved only after 21 days of treatment and the same temperature range had little to no effect on *Macrophomina* spp. Development (BASSETO et al., 2011).

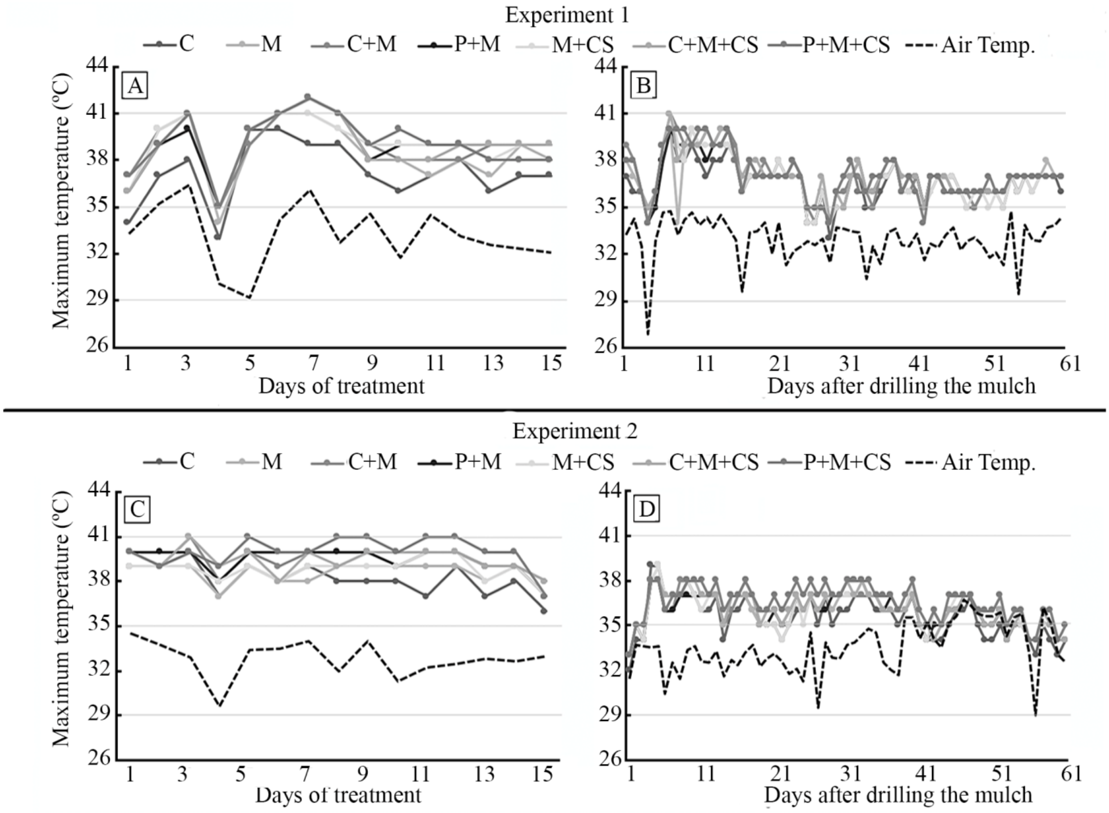

Figure 2. Maximum soil temperature measured in experiment 1 (A and B) and in experiment 2 (C and D). Graphs A and C display soil temperatures measured during the curing treatment (15 days before holes were drilled on polyethylene mulch). Graphs B and D represent temperature measured after the curing treatment. Treatments: (C) – Control, (M) - polyethylene mulch, (C+M) - incorporation of *Crotalaria juncea* L. + polyethylene mulch, (P+M) - incorporation of *Pennisetum glaucum* L. + polyethylene mulch, (M+CS) - polyethylene mulch + (Compost-Aid® + Soil-Set®), (C+M+CS) - incorporation of *Crotalaria juncea* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®), and (P+M+CS) - incorporation of *Pennisetum glaucum* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®).

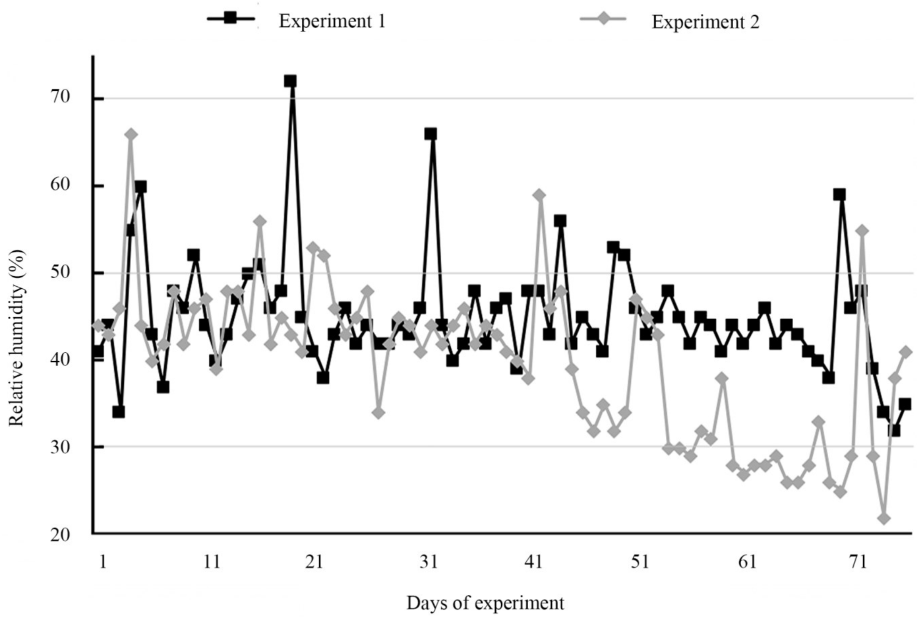

Figure 3. Relative humidity measured inside the greenhouse during experiments 1 and 2 to evaluate the effect on *Fusarium* spp. and *Macrophomina* spp. in melon.

Studies have shown that polyethylene mulch can be used in several crops for leaf disease suppression and the choice of its color is according to the planting season and the place of cultivation (LAMBERT et al., 2017). In the Northeast region of Brazil, white-black polyethylene plastic has been widely used and it is recommended for melon cultivation. There is evidence that polyethylene mulch can also be used for weed control and soil moisture retention (SILVA; FELIPE, 2014). Soil solarization, preferably transparent plastic, is mainly used to control pathogens and weeds, but this technique is not used in melon crops in Brazil, due to the large areas of production that make the use of such technology difficult to be adopted by melon growers. However, the use of polyethylene mulch, which is already used in Brazil for melon production, can be adapted to reduce soil inoculum potential, which has been increasing year after year due to the adoption of monoculture. Furthermore, because this technique is similar to solarization and widely used in the control of soilborne pathogens, it may be beneficial to microorganisms living in the soil, especially those that thrive in high temperatures (ROCHA; CARNEIRO, 2016).

Soilborne pathogen control efficacy achieved by using polyethylene mulch can be boosted by concomitantly incorporating organic material. Organic material has some natural antipathogen compounds that are released into the soil and may help to suppress pathogen growth (DANTAS et al., 2013). Besides, the incorporation of organic material helps to elevate the soil temperature higher than when polyethylene mulch is used alone (WONG; AMBRÓSIO; SOUZA, 2011). However, we did not observe a significant increase in the maximum soil temperature using polyethylene mulch with or without the incorporation of organic materials. The maximum soil temperature was similar among all treatments throughout the experiments, implanted and evaluated from March, even in the control (C) treatment where the soil was not covered with polyethylene mulch or vegetal material (Figure 2). Comparing several studies, Pramanik et al. (2015) demonstrate that the coloration of plastics is fundamental for soil temperature increase, especially transparent and black plastics. Furthermore, Ibarra-Jiménez et al. (2011) concluded that white plastic, used in our experiments, helps to increase productivity, but causes minimal increase in soil temperature, which corroborates with our soil temperature results.

In this study, we infested the soil with the same amount of inoculum of *M. phaseolina* (GenBank MN136199) and *F. falciforme* (GenBank MH709261) and intentionally used soil from a field with previous history of melon root rot, so that other pathogenic fungi could occur during the assessments. *Fusarium* occurred more frequently in both experiments in comparison to other fungi isolated, with less occurrence in experiment 2 than in experiment 1. Overall, *Macrophomina* occurred with less frequency in experiment 1 than in experiment 2, but it did occur in low incidence in experiment 2 in most treatments. Furthermore, we isolated *Rhizoctonia* spp. in (P+M) and (C+M+CS) treatments in experiment 1, but we did not detect this fungus in any of the treatments in experiment 2 (Figure 4).

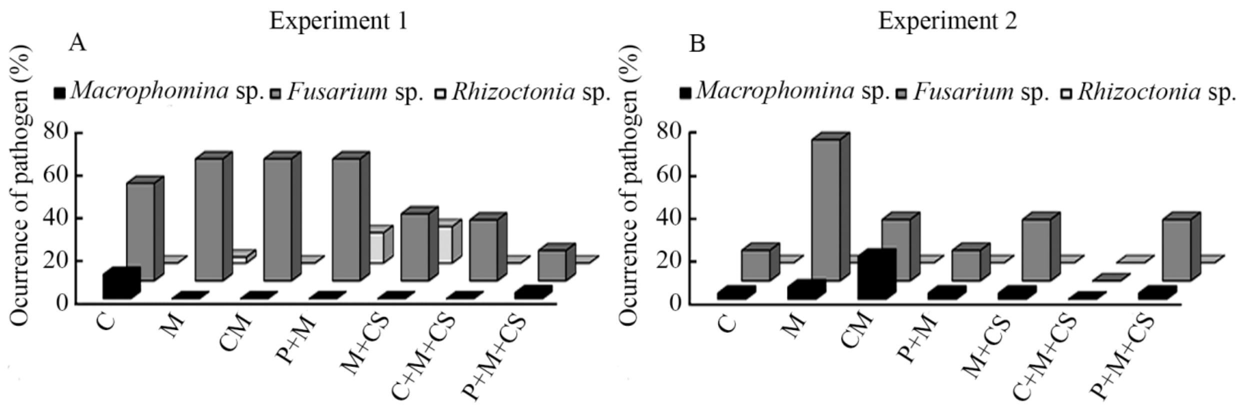

Figure 4. Occurrence of pathogens isolated from muskmelon plants in experiment 1 (A) and experiment 2 (B). Treatments: (C) – Control, (M) - polyethylene mulch, (C+M) - incorporation of *Crotalaria juncea* L. + polyethylene mulch, (P+M) - incorporation of *Pennisetum glaucum* L. + polyethylene mulch, (M+CS) - polyethylene mulch + (Compost-Aid® + Soil-Set®), (C+M+CS) - incorporation of *Crotalaria juncea* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®), and (P+M+CS) - incorporation of *Pennisetum glaucum* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®).

The low occurrence of *M. phaseolina* found in our experiments can be explained by the environmental conditions (Figure 4). In both experiments, the soil temperature and air humidity were high (Figure 3). The soil moisture was also high because of the drip irrigation system that was activated hourly. It is possible that this microclimate was unfavorable for the development of the pathogen. Although *M. phaseolina* thrives in high temperature (30–35 °C) (GHOSH et al., 2018), it develops better in low humidity (LINHARES et al., 2016). On the other hand, *Fusarium* spp. develops better in high humidity (PANWAR et al., 2016), which is exactly what we observed in our first experiments – high humidity and high levels of occurrence of this pathogen. Moreover, in the second experiment, the air relative humidity was lower than registered in experiment 1 towards half of the duration of the experiment (Figure 3). This helps to explain why we observed a lower occurrence of *Fusarium* spp. in experiment 2 than registered in the first experiment (Figure 4).

Overall, the disease incidence was higher in experiment 1 than in experiment 2, except for the treatments (M) and (P+M+CS), which had higher disease incidence in experiment 1 than in experiment 2 (Figure 5). The disease incidence in (M) treatment was consistently high in both experiments. In experiment 1, the treatments (C+M+CS) and (P+M+CS) had the lowest disease incidence but only (P+M+CS) was statistically different from the other treatments (p<0.05). No disease was observed in (C+M+CS) treatment in experiment 2 (Figure 5).

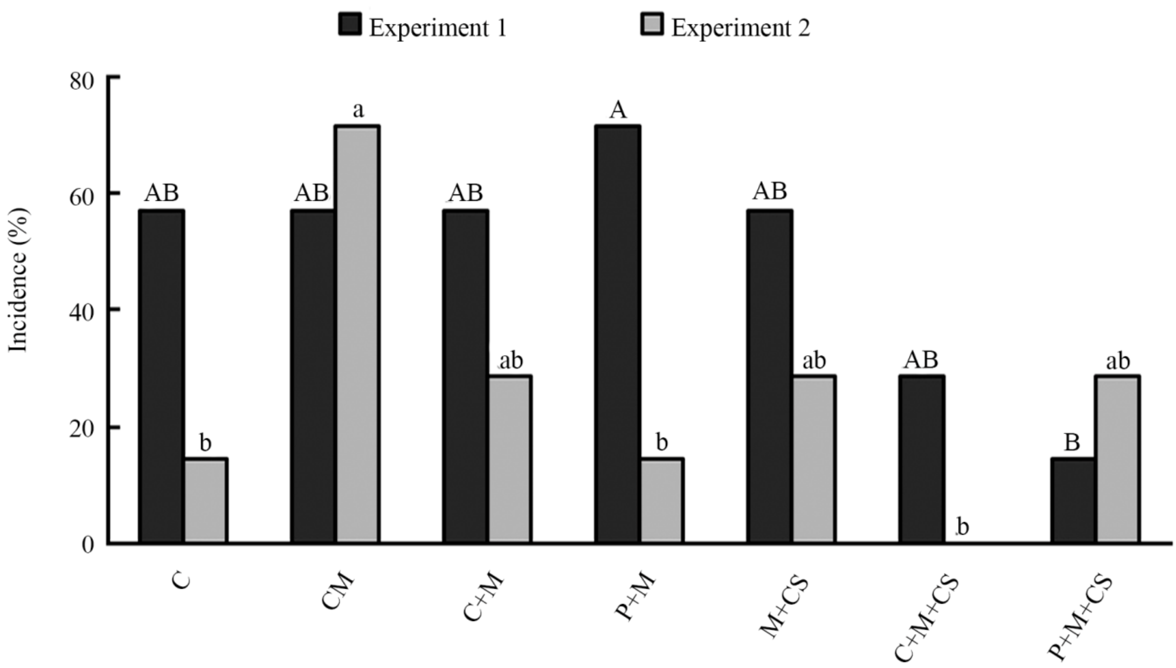

Figure 5. Root rot disease incidence on melon plants in different treatments. Treatments: (C) – Control, (M) - polyethylene mulch, (C+M) - incorporation of *Crotalaria juncea* L. + polyethylene mulch, (P+M) - incorporation of *Pennisetum glaucum* L. + polyethylene mulch, (M+CS) - polyethylene mulch + (Compost-Aid® + Soil-Set®), (C+M+CS) - incorporation of *Crotalaria juncea* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®), and (P+M+CS) - incorporation of *Pennisetum glaucum* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®). Bars with the same letter in the same experiment do not differ statistically by Kruskal-Wallis test (p<0.05), the upper case in experiment 1 and the lower case in experiment 2.

The efficacy of the incorporation of vegetal material in controlling melon root rot was boosted by the addition of Compost-Aid® and Soil-Set®. It is possible that the bacteria that compose the product (Table 1) had a direct effect on inhibiting the growth of the root rot pathogens in our experiments. Studies have demonstrated that the application of Compost-Aid® on the soil can successfully control *Meloidogyne javanica* (MIAMOTO et al., 2017) and when it is applied together with Soil-Set® decreases the germination rate of *Cercospora coffeicola* more than 20% (LABORDE, 2014). According to our results, the best use of the formulations (Compost-Aid® and Soil-Set®) is to apply them simultaneously with the incorporation of vegetal material and polyethylene mulch. The results achieved by this combination emphasize the current managing plant pathogens recommendations, that is, the efficiency in controlling plant pathogens is maximized when several techniques are combined (KIMATI, 1995).

The highest disease severity was observed in the treatment (P+M) followed by (M+CS) and the lowest in (P+M+CS) treatment, in experiment 1 (Figure 6). However, in experiment 2, the highest disease severity was found in (M) treatment and the lowest in treatment (C+M+CS) followed by (P+M) (Figure 6).

Fruit weight and Brix Degree were lower in control treatment (C) in both experiments in comparison to the other treatments, but Brix Degree was statistically significant just in experiment 2 (p <0.05). On the other hand, fruit firmness was higher in the control treatment (C) in experiment 1 in comparison to the other treatments (p<0.05) (Table 2).

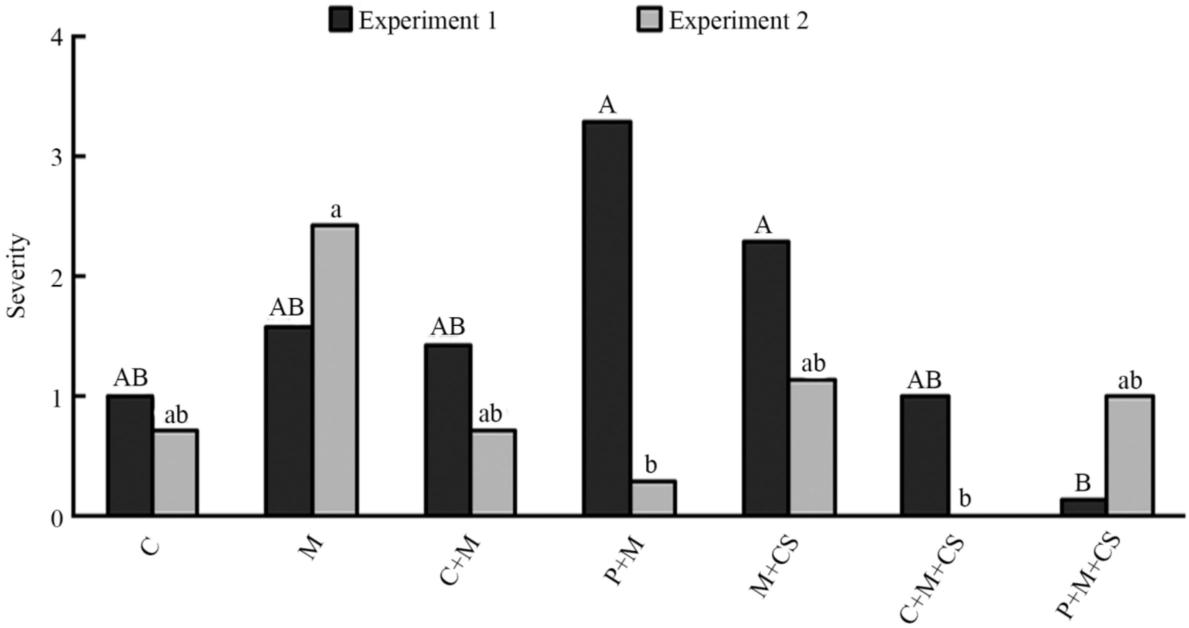

Figure 6. Severity of melon root rot disease in different treatments in melon plants evaluated by a disease rating scale: (0) - asymptomatic, (1) - less than 3% of infected tissue, (2 – 3) - 10% of infected tissue, (3 – 11) - 25% of infected tissue, (4 – 26) - 50% of infected tissue, and (5) - more than 50% of infected tissue. Treatments: (C) – Control, (M) - polyethylene mulch, (C+M) - incorporation of *Crotalaria juncea* L. + polyethylene mulch, (P+M) - incorporation of *Pennisetum glaucum* L. + polyethylene mulch, (M+CS) - polyethylene mulch + (Compost-Aid® + Soil-Set®), (C+M+CS) - incorporation of *Crotalaria juncea* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®), and (P+M+CS) - incorporation of *Pennisetum glaucum* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®). Bars with the same letter in the same experiment do not differ statistically by Kruskal-Wallis test ($p<0.05$), the upper case in experiment 1 and the lower case in experiment 2.

Table 2. Effect of the treatments on the characteristics of the melon fruits at harvest.

Treatments	Experiment 1			Experiment 2		
	Weight (kg)	Firmness (Kgf)	Brix (°Bx)	Weight (Kg)	Firmness (Kgf)	Brix (°Bx)
C	0.88 a[x]	4.54 a	9.57 a	0.50 a	6.60 b	7.14 a
M	1.10 ab	4.11 a	10.71 ab	0.87 b	5.55 a	11.50 d
C+M	1.42 b	4.17 a	11.93 b	0.85 b	5.34 a	10.77 cd
P+M	1.37 b	4.28 a	12.00 b	0.79 b	5.80 ab	9.03 b
M+CS	1.16 ab	3.82 a	10.56 ab	0.77 b	4.92 a	9.31 bc
C+M+CS	1.20 ab	3.83 a	10.69 ab	0.78 b	5.74 a	10.29 bcd
P+M+CS	0.89 ab	3.93 a	10.86 ab	0.70 b	5.15 a	9.83 bcd

Treatments: (C) – Control, (M) - polyethylene mulch, (C+M) - incorporation of *Crotalaria juncea* L. + polyethylene mulch, (P+M) - incorporation of *Pennisetum glaucum* L. + polyethylene mulch, (M+CS) - polyethylene mulch + (Compost-Aid® + Soil-Set®), (C+M+CS) - incorporation of *Crotalaria juncea* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®), and (P+M+CS) - incorporation of *Pennisetum glaucum* L. + polyethylene mulch + (Compost-Aid® + Soil-Set®). [x]Tukey's Honest Significant Difference (Alpha = 0.05). Averages with the same letter within the same column are not significantly different.

The significantly higher fruit firmness and lower Brix Degree obtained in the control treatment (C) in comparison to the other treatments evaluated during the two experiments (Table 2), corroborate other studies designed to evaluate productivity and fruit quality. This may be due to the absence of the polyethylene mulch, since the only treatment that it was not used in was the control treatment (C) (SILVA; FELIPE, 2014; LAMBERT et al., 2017).

Root rot incidence and severity were negatively correlated with fruit weight, dry matter, and fresh matter in both experiments. Fruit firmness was negatively correlated with root rot incidence and severity in experiment 2, but it had a low correlation in experiment 1 with those symptoms. It was noteworthy that the higher the fruit firmness the lower the Brix Degree of the fruit is, and that *Fusarium* sp. was negatively correlated to *Macrophomina* sp. in experiment 1, but the correlation was positive between those fungi in experiment 2 (Figure 7).

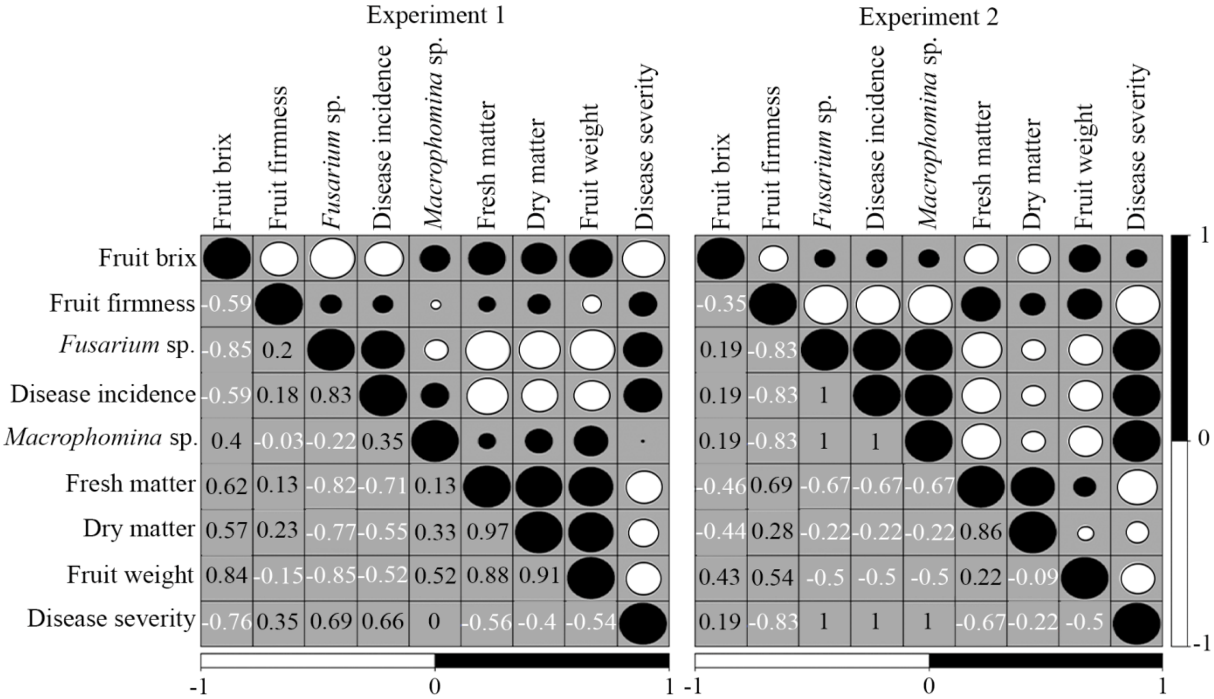

Figure 7. Pairwise correlation analyses using the nonparametric Kendall's t rank correlation coefficient to measure the strength of the relationship between each variable in experiments 1 and 2. Positive correlations are displayed in black and negative correlations in white. Circle size is proportional to the correlation coefficients.

Based on the disease incidence and severity, which were positively correlated (Figure 7), the treatments that achieved the overall best control of melon root rot in both experiments were (C+M+CS) and (P+M+CS). However, the microclimatic conditions in the greenhouse, which were different in the two experiments, seemed to play an important role in the efficiency of each treatment in controlling melon root rot disease. For instance, the lower relative humidity observed throughout experiment 2 in comparison to experiment 1 seems to create the optimum condition for the efficacy of (C+M+CS) as no disease developed in this treatment (Figure 4B).

It is noteworthy that the treatments with vegetal material incorporation achieved the best results overall. This may be due to the release of antifungal compounds from the vegetal material, as found by Linhares et al. (2016), when using pearl millet as coverage there was a low survival of *M. phaseolina*. Furthermore, it has also been found that when pearl millet is used as coverage there is a significant reduction of the pathogen *Sclerotium rolfsii* in bean plants. The reduction is due to the increase in the natural population of *Pseudomonas* (PEREIRA NETO; BLUM, 2010). Therefore, these research results support the low pathogen survival observed in the treatments with *Crotalaria juncea* in our studies.

CONCLUSION

Collectively, our research shows that the incorporation of plant material (crotalaria or pearl millet), associated with the use of polyethylene mulch and commercial products (Compost-Aid® and Soil-Set®), greatly reduces the incidence and severity of melon root rot and the occurrence of its causing pathogens. However, in conditions of high soil temperature and high relative humidity, the combination containing pearl millet yields the best results. On the other hand, when the soil temperature is high and the relative humidity is low, the combination in which crotalaria is incorporated into the soil, yields the lowest disease incidence, severity, and pathogens' occurrence.

Finally, the greenhouse experiments conducted in this study are extremely important, as the results achieved can now be easily transferred and tested in field conditions. Moreover, in this research, we focused on testing technologies that are cost-efficient and readily available to melon growers in the Northeastern region of Brazil to adopt. With these promising results in hand, we will be able to efficiently communicate our research to stakeholders to offer them more alternative strategies to control this devastating disease in melon crops. The obtaining of alternative strategies will support the achievement of our ultimate goal: to shrink the gap between lab research and farm application to strengthen the "lab to farm" concept.

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