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Artigo Científico

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Incorporation of plant materials in the control of root pathogens in muskmelon

Incorporação de materiais vegetais no controle de patógenos radiculares em meloeiro

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Abstract - The effect of plant materials [Sunn Hemp (*Crotalaria juncea),* Castor Bean (*Ricinus communis* L.), Cassava (*Manihot esculenta* Crantz) and Neem (*Azadirachta indica*)] and the times of incorporation of these materials in regards to the incidence of root rot in melon was evaluated in Ceará state, Brazil. The experiment was conducted in a commercial area with a history of root pathogens in cucurbitaceae. The randomized block design was used, in a 5 x 3 factorial arrangement with four repetitions. The treatments consisted of a combination of four plant materials (sunn hemp, castor beans, cassava and neem) and a control with no soil incorporation of plant material and three times of incorporation (28, 21, and 14 days before the transplanting of the seedlings). Lower incidence of root rot was observed in practically all of the treatments where materials were incorporated at different times, with variation between the materials, corresponding with the time of incorporation, in relation to the soil without plant material. The pathogens isolated from the symptomatic muskmelon plants were *Fusarium solani, Macrophomina phaseolina, Monosporascus cannonballus* and *Rhizoctonia solani, F. solani* being encountered most frequently.

Key words - *Fusarium solani. Macrophomina phaseolina. Monosporascus cannonballus.* Organic material. *Rhizoctonia solani*.

Resumo - Avaliou-se o efeito de materiais vegetais [Crotalária (*Crotalaria juncea),* Mamona (*Ricinus communis* L.), Cassava (*Manihot esculenta* Crantz) e Nim (*Azadirachta indica*)] e do tempo de incorporação destes sobre a incidência de podridões radiculares no meloeiro, no estado do Ceará, Brasil. O experimento foi conduzido em área comercial com histórico de patógenos radiculares em cucurbitáceas. O delineamento utilizado foi de blocos casualizados, em esquema fatorial 5 x 3, com quatro repetições. Os tratamentos consistiram da combinação de quatro materiais vegetais (crotalária, mamona, mandioca brava e nim) mais uma testemunha com solo sem incorporação de material vegetal e três tempos de incorporação (28, 21, e 14 dias antes do transplantio das mudas). Menor incidência de plantas com podridão radicular foi observada em praticamente todos os tratamentos onde se incorporou materiais nos diferentes tempos, com variação entre os materiais, conforme o tempo de incorporação, em relação ao solo sem material vegetal. A crotalária proporcionou menor incidência quando a incorporação foi de 14 dias e à medida que aumentou o tempo, houve acréscimo na porcentagem de plantas com podridão. Quando se utilizou a mandioca, houve redução da podridão, proporcionalmente ao tempo de incorporação. Os patógenos isolados das plantas de melão com sintomas foram *Fusarium solani*, *Macrophomina phaseolina, Monosporascus cannonballus* e *Rhizoctonia solani,* sendo o *F. solani* o de maior frequência.

Palavras-chave - *Fusarium solani. Macrophomina phaseolina. Monosporascus cannonballus.* Material orgânico. *Rhizoctonia solani*.

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Introduction

Rio Grande do Norte stands out on the national scene as one of the main muskmelon producing states (*Cucumis melo* L.). Additionally, the municipality of Mossoró possesses a cultivated area of approximately 6,200 hectares, and attained an output of 198,400 tons in the year 2011 (IBGE, 2012).

However, the growth of the cultivated area associated with intensive cultivation has contributed to the increase in the incidence of disease (SANTOS *et al*., 2000). Among these, root rot caused by the soil-inhabitant pathogens *Fusarium solani* f.sp. *cucurbitae* Snyder and Hansen, *Lasiodiplodia theobromae* (Pat.) Grif. And Maubl., *Macrophomina phaseolina* (Tassi) Goid., *Monosporascus cannonballus* Pollack and Uecker, *Myrothecium roridum* Tode and *Rhizoctonia solani* Kühn (SANTOS *et al.*, 2000; SALES JR. *et al*., 2003) were highlighted.

These pathogens are considered to be difficult to control due to the specialized resistance structures, which guarantee survival under adverse conditions and a wide range of hosts, and also because of the complexity of the environment in which they are found (BEDENDO, 2011). Chemical control is not economically viable and presents a series of restrictions from an environmental standpoint (LIMA *et al*., 2005).

Research involving the addition of organic material to the soil has been developed as an alternative for the management of these pathogens (BLOK *et al.*, 2000; BUENO *et al*., 2004; GHINI *et al*., 2006; MORALES *et al*., 2007; PINTO *et al*., 2010; KLEIN *et al.*, 2011; TOMAZELI *et al.*, 2011; WONG *et al*., 2011).

The incorporation of this kind of material in the soil contributes to the increase in the activity of the microbial community, stimulating the development of antagonists that will limit the action of the phytopathogens. Aside from this, such compounds also provide improvement in the chemical characteristics of the soil (GHINI *et al*., 2006). During the process of decomposition, the organic residues may yet allow the release of volatile and nonvolatile compounds that are toxic to the pathogens (AMBRÓSIO *et al*., 2008).

The objective of the present work was to evaluate the effect of different plant materials, and the time of incorporations of these materials, on the incidence of root rot in muskmelon.

Materials and methods

General considerations

The experiment was conducted in the period from November 2011 to February 2012, at Fazenda Agrícola Famosa, in Ceará state, Brazil, located in a commercial area with a proven history of phytosanitary problems arising from root pathogens in cucurbitaceas. The analyses were conducted in the Universidade Federal Rural do Semi-árido, in the Laboratory of Microbiology and Phytopathology, in the Plant Health sector of the Department of Plant Sciences.

Experimental design

The design used was one of randomized blocks, with the treatments fixed in a 5 x 3 factorial arrangement, with four repetitions. Four plant materials were used as well as one control with no soil incorporation of plant material and three times of incorporations.

Plant materials

Leaves and branches of Sunn Hemp (*Crotalaria juncea),* Castor Bean (*Ricinus communis* L.), Cassava (*Manihot esculenta* Crantz), and Neem (*Azadirachta indica*) were used. The materials were collected and crushed in the same farm where the work was conducted and placed in the planting furrow, in the proportion of 4kg of fresh mass per linear meter (AMBRÓSIO *et al*., 2004). Subsequently, they were incorporated into the soil at 10 cm depth, with the employment of a rotary tiller. The plant materials were incorporated in the following times: 28, 21, and 14 days before the transplanting of the muskmelon, the greatest time of incorporation (28 days) being the first to be conducted, followed by the other times (21 and 14 days respectively) so the transplanting was conducted in the same day for all of the treatments.

The experimental plot consisted of three lines of 18.0 m of length. The spacing used was of 2.0 m between lines and 0.3 m between plants in the lines. The internal area of the experimental unit was made up of plants in the central line, disregarding both plants from either end. In this study, the melon hybrid cultivar Platinum Life of the group Cantaloupensis was used. The seeding was conducted in expanded polystyrene trays with 200 cells containing the commercial substrate Plantmax®, and the seedlings were transplanted nine days after the seeding. In the preparation of the area, a disking and plowing was carried out following the incorporation of the plant materials in the furrow and covering of the same.

The conduction of the culture was carried out in accordance with the management adopted in the region, in which a drip irrigation system, a white plastic covering (mulching), a thermal blanket and manual weedings were used. Following the collection, 62 days after transplanting, the plants from the internal area were collected and evaluated for incidence of root pathogens.

Evaluation of the incidence of phytopathogens

The percentage of plants with visible symptoms of the disease in relation to the total stand was considered for the evaluation of the incidence. All plants of the internal area were collected, taken to the laboratory of Microbiology and Phytopathology of DCV/UFERSA and, subsequently washed and evaluated for presence of symptoms of rot in the roots, stem and vascular systems. Isolations of the pathogens in all of the symptomatic plants were conducted through the removal of fragments from the adjacent area, which were subjected to surface disinfection (alcohol 70%, sodium hypochlorite 2% and sterilized distilled water). Next, they were plated on PDA (Potato-dextrose-agar) culture medium, plus tetracycline (0.05 g L^{-1}) for removing bacteria. The plates were kept in a B.O.D. incubator at 28 ± 2 °C for seven days, with a photoperiod of 12 hours. Following this period, the pathogens that occurred in the symptomatic plants were transferred to PDA culture medium to obtain pure cultures. The identification of the fungi was performed based on the vegetative and reproductive structures observed in the optical microscope and compared with the identification key of Barnett and Hunter (1998). The percentage of the incidence of each pathogen in relation to the total number of plants was identified.

A variance analysis was performed to evaluate the characteristics using the SISVAR software (FERREIRA, 2000). The data for *Macrophomia phaseolina* was transformed for incidence +10. Tukey's test was used to compare between the averages of the plant materials studied. The response curve adjustment procedure for the factor time was conducted using the software 'Table Curve Package' (JANDEL SCIENTIFIC, 1991).

Results and discussion

Lower incidence of melon plants with root rot symptoms were observed in practically all of the treatments where plant materials were incorporated at different times when compared to soil without the incorporation of plant material, with the exception of the sunn hemp at 28 days of incorporation. It was found that there was variation between the plant materials used, in accordance with the time of incorporation, with respect to efficiency in reducing root rot (Table 1).

Sunn hemp yielded smaller incidence of disease when the time of incorporation was 14 days, however, corresponding to the increase of this time, there was an increase in the percentage of plants with root rot, especially in the time of 28 days (Figure 1A).

The contrary effect was observed when using cassava, as it reduced root rot as it increased the time of incorporation (Figure 1B). This can be explained by the Carbon/Nitrogen (C/N) relationship of these materials, as the sunn hemp features a smaller C/N (26/1) than the cassava (40/1) (KIEHL, 1985) which provides for a more accelerated decomposition, releasing nutrients and chemical compounds more rapidly, which promotes a faster reduction of disease, while cassava, having a higher C/N, decomposes more slowly and consequently, the control of disease is more delayed.

Botelho *et al.* (2001), also obtained positive results when the effect of the incorporation of different plant materials was studied, among them sunn hemp (*Crotalaria juncea*) in inducing suppressiviness to *R. solani* in bean plants and found that, at 60 days of incorporation there is a reduction in root rot, independent of the plant material used. This can be justified by the presence of the compounds released during decomposition, which promote the increase of the natural microbial activity, limiting the damage caused by phytopathogens, and encourages the action of antagonistic microorganisms (ROBBS, 1991). These results also corroborate those found by Garrido *et al.* (2008), when assessing the effect of the incorporation of sunn hemp and pigeon pea into the soil, both separately and together, in tomato and yam crops, to inhibit nematodes.

The other materials (castor beans and neem) (Figure 1C e 1D) and the soil without the addition of plant materials (Figure 1E) did not present the same trend in the increase or decrease of disease throughout the time of incorporation. However, these materials also provide smaller incidence of disease in relation to the control, a fact also noted by Silva and Pereira (2008) when they studied the effect of the incorporation of fresh neem leaves into the soil to control *Fusarium oxysporum* f. sp. *vasinfectum,* and *Meloidogyne incognita* (Kofoid and White) Chitwood breed 1, in seedlings of okra cv. Santa Cruz where they obtained a reduction in the incidence of disease in all of the treatments that featured the addition of plant material.

The pathogens isolated from the muskmelon plants that presented symptoms of root rot were *Fusarium solani*, *Macrophomina phaseolina, Monosporascus cannonballus,* and *Rhizoctonia solani* with *F. solani* being the most frequently encountered (Table 2).

These pathogens were also identified by Andrade *et al.* (2005) when they conducted a survey of the fungal species associated with the roots of muskmelon plants, with symptoms of collapse, cultivated in the states of Rio Grande do Norte and Ceará, noting the elevated prevalence of *F. solani* and *M. phaseolina,* the latter being noted to have been encountered the least frequently in this work (Table 2). There was no interaction between the plant materials tested and the times of incorporation when one evaluated the occurrence of the pathogens, however it was

Table 1 – Percentage incidence of root rot in muskmelon plants at the different times of incorporation of plant materials.

Plant materials	Days after incorporation		
	14	21	28
	Incidence of root rot (%)		
Sunn hemp	39.0 b	60.0 ab	70.0 a
Castor beans	31.2 b	79.0 a	57.0 ab
Cassava	51.2 ab	42.0 b	37.5 b
Neem	48.0 ab	39.7 b	60.2 ab
Control	77.2 a	86.2 a	67.5 ab
CV (%)	26.7	26.7	26.7

Averages followed by the same lowercase letter in the column did not differ between themselves by Tukey test at level of 5% of probability

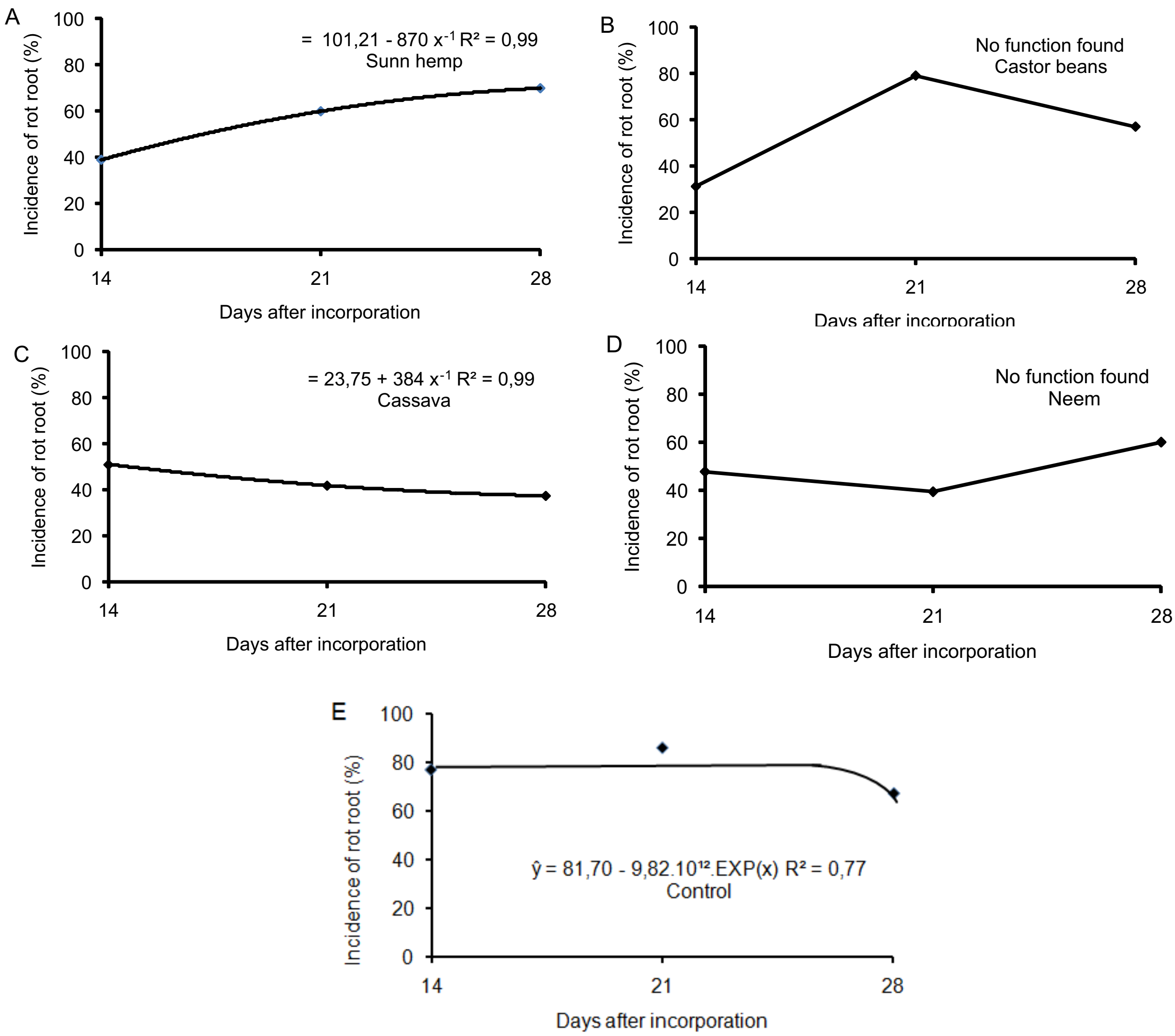

Figure 1 – Incidence of root rot (%) in muskmelon plants at the different times of incorporation of plant materials: Sunn hemp (A), Castor beans (B), Cassava (C), Neem (D) and Control (E).

Table 2 – Percentage of occurrence of soilborne pathogens in muskmelon plants at the different plant materials incorporated.

Plant materials	Pathogens identified			
	Fusarium solani	*Macrophomina phaseolina*	*Monosporascus cannonballus*	*Rhizoctonia solani*
Sunn hemp	40.7 ab*	16.4 a	27.3 ab	22.2 ab
Castor beans	46.7 ab	18.2 a	16.0 bc	13.7 b
Cassava	30.5 b	13.2 a	4.1 c	17.1 ab
Neem	38.7 b	12.7 a	19.7 abc	18.4 ab
Control	58.2 a	17.6 a	35.3 a	32.2 a
CV (%)	37.2	51.42	71.8	67.7

*Averages followed by the same lowercase letter in the column did not differ between themselves by the Tukey test at a level of 5% of probability. NS= not significant differences.

found that 14 days after incorporation, lower occurrences of *F. solani*, *M. cannonballus* and *R. solani* were recorded. On the other hand, *M. phaseolina* had a lower occurrence at 28 days after incorporation (Figure 2).

Statistical difference was verified between the plant materials tested so that each material behaved differently in the occurrence of each fungus, with cassava and neem being statistically equal and providing for less of occurrence of *F. solani*, while for *M. cannonballus* and *R. solani* the lowest occurrences were noted with the materials cassava and castor bean respectively. The plant materials and the control did not differ between one another in the occurrence of *M. phaseolina*, while neem and cassava demonstrated the highest tendency to reduce the occurrence of this fungus (Table 2).

Cassava stood out among the materials as when it didn't provide for the lowest occurrences of pathogens it was one of the two materials that provided for lowest incidences of said microorganisms (Table 2). This material has already shown promising evidence for the control of root pathogens (AMBRÓSIO *et al*., 2008; WONG *et al*., 2011; BASSETO *et al*., 2012).

This fact is probably due to certain toxic compounds present in cassava that provide a negative effect in the development of field disease. According to Carvalho and

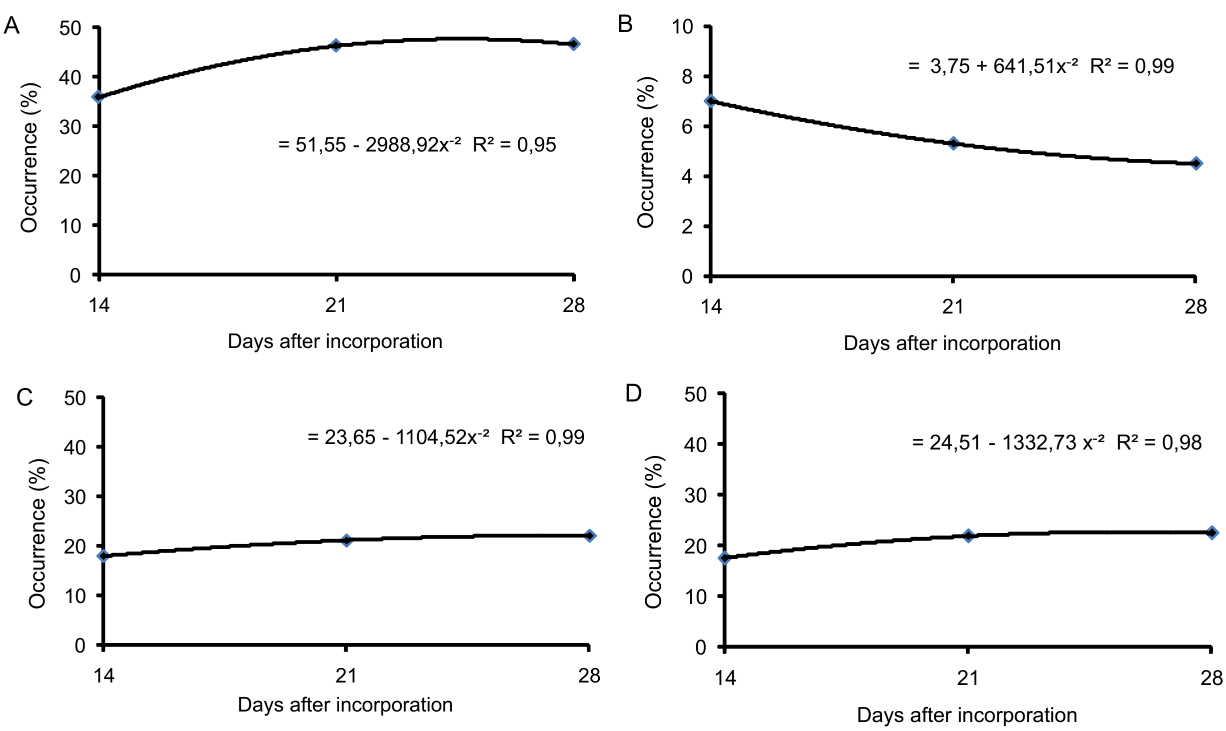

Figure 2 – Occurrence of soilborne pathogens (%) in muskmelon plants at the different times of incorporation (A) *Fusarium solani*; (B) *Macrophomina phaseolina*; (C) *Monosporascus cannonballus* e (D) *Rhizoctonia solani*.

Carvalho (1979) two cyanogenic glycosides (linamarin and methyl lotaustralina) may be responsible for this toxicity. Ponte (2001) reports that the sulfur present in this material, together with ketones, aldehydes, cianalaninas, lectins, and other toxic proteins, all inhibitors of amylases and proteinases, present great efficiency as fungicides. Basseto *et al.* (2012) when they evaluated the effect of the plant materials associated with solarization in the survival of phytopathogens and in the production of volatiles identified in cassava 29 compounds, separated in five groups (alcohols, ketones, acids, esters, and others), all of which can be highlighted as being promising in the control of pathogenic microorganisms.

Neem and castor bean are also materials highlighted in the current work as being effective in the reduction of root rot, possibly due to the fungitoxic substances present and the increase of the antagonists stimulated by the nutrients contained within these materials. The difference observed between the plant materials in the occurrence of each pathogen, is justified by Reis *et al.* (2005) when they emphasized that the quality of the organic material added to soil will determine the increased density of one or several species of microorganisms selected by this substrate and, if a beneficial species is antagonistic to a target phytopathogen control, the damage caused by the same host can be minimized. In Bedendo (2011) although the pathogen can also use the organic material as a substrate, it becomes subject to competition with other microorganisms, resulting in the reduction of pathogenic population. Likewise, the incorporation of organic material can also lead to an increase in the severity of the disease, serving as a food source for the pathogens (BETTIOL; GHINI, 2005). This aspect was observed by Njoroge *et al.* (2008) when they studied the effect of the incorporation of residues of *Brassica* spp. (canola, mustard) in the density of soil pathogens and in the wilting of Fusarium in watermelon (*Citrullus lanatus),* where they noted an increase in the density of *Pythium* e *Fusarium oxysporum* after incorporation.

Therefore, although the use of organic material in agriculture has been linked to longevity of the crops due to the improvement of the physical, chemical and biological conditions, it is of extreme importance to understand the organic material being added to the soil as well as the pathogen that is to be controlled.

Conclusions

Lower incidence of root rot was observed in practically all of the treatments where materials were incorporated at different times, with variation between the materials, corresponding with the time of incorporation.

The use of sunn hemp or castor beans at 14 days of soil incorporation resulted in a lower incidence of root rot. With the use of cassava the lower incidence occurs at 28 days of soil incorporation.

Cassava stood out among the materials as when it didn't provide for the lowest occurrences of pathogens it was one of the two materials that provided for lowest incidences of said microorganisms.

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