# Swine manure on the control of damping-off in beetroot seedlings

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## ABSTRACT

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The aim of this study was to evaluate the effect of swine manure (SM) doses applied to soils showing pH of 4.8 and 7.2 on the control of damping-off in beetroot seedlings caused by *Rhizoctonia solani*. To set the trial, plastic bags were filled with 4 kg soil (pH levels of 4.8 and 7.2) and 15 g *R. solani* inoculum kg soil$^{-1}$. This mixture was moistened, homogenized and kept in a greenhouse for seven days. Following this period, SM doses (0, 5, 10, 15 and 20%) were incorporated into the bags, which were again stored in a greenhouse. After seven days, part of the soil from each plastic bag was separately placed into 16 cells of a 128-cell polystyrene tray, and two beetroot seeds were sown per cell. Seedling emergence and damping-off were evaluated for 21 consecutive days. The other part of the soil was used for microbial activity quantification based on the $CO_2$ release method at 7, 14 and 21 days. The trial was conducted in a completely randomized design, with four replicates per treatment, and repeated twice. All tested SM doses reduced the number of damped-off beetroot seedlings in both trials, and the greatest disease control was provided by treatments that had SM doses of 15% and 20% applied to soil showing pH level of 7.2. In addition, regardless of the pH level, all tested SM doses increased soil microbial activity.

**Keywords**: soil borne pathogen, organic matter, *Beta vulgaris*.

## RESUMO

Butrinowski, I.T.; Dallemole-Giaretta, R.; Santos, I.; De Bortoli, B.B.; Steilmann, P.; Baldin, R.. Chorume de suíno no controle de tombamento de plântulas em beterraba. ***Summa Phytopathologica,*** v.45, n.4, p.393-398, 2019.

O objetivo deste estudo foi avaliar o efeito de doses de chorume de suíno (CS), aplicadas em solos com pH 4,8 e 7,2, no controle do tombamento de plântulas de beterraba causado por *Rhizoctonia solani*. Para montagem do ensaio foram acondicionados em sacos plásticos, 4 g de solo (pH 4,8 e 7,2) e 15 g de inóculo de *R. solani* kg de solo$^{-1}$. Em seguida, essa mistura foi umedecida, homogeneizada e mantida em casa de vegetação por sete dias. Após esse período, em cada saco foram incorporadas, as doses do CS (0, 5, 10, 15 e 20%), sendo os sacos novamente armazenados em casa de vegetação. Após sete dias, parte do solo de cada saco plástico foi acondicionada separadamente em 16 células de bandeja de isopor de 128 células, e semeadas duas sementes de beterraba por célula. A emergência e o tombamento das plântulas foram avaliados por 21 dias consecutivos. A outra parte do solo foi utilizada para quantificação da atividade microbiana, por meio do método de desprendimento de $CO_2$, aos 7, 14 e 21 dias. O ensaio foi conduzido em delineamento inteiramente casualizado, com quatro repetições por tratamento, e repetido duas vezes. Todas as concentrações de CS testadas reduziram o número de plântulas de beterraba tombadas, em ambos os ensaios, tendo o maior controle da doença sido obtido nos tratamentos com as doses 15 e 20% de CS, em solo com pH 7,2. Além disso, independentemente do pH, todas as doses de CS testadas aumentaram a atividade microbiana do solo.

**Palavras-chave**: Patógeno habitante de solo, matéria orgânica, *Beta vulgaris*.

*Rhizoctonia solani* Kunh is a soil borne necrotrophic pathogen that causes damping-off and root and collar rot in adult plants of numerous crops of economic importance (1). In addition, diseases caused by this fungus are difficult to control due to its high saprophytic ability and sclerotium production in the soil (1).

Incorporation of organic compounds into the soil is an alternative method for the management of soilborne pathogens (3). Potential organic compounds include products derived from composted plants (6), composted or non-composted forest substrates (7) and swine manure (5, 13).

Swine manure (SM) is used in agriculture as a source of nutrients and organic matter. Its application for plant disease control is rarely studied; however, SM is known to have potential action in the management of soil borne pathogens due to the release of volatile compounds such as fatty acids, nitrous acid and ammonia (5, 20).

Considering the socioeconomic and environmental importance of the final destination of SM, its contribution to the control of soil borne pathogens and the few studies on its application to soils of different pH levels to control damping-off caused by *R. solani*, this study evaluated the effect of different SM doses applied to soils showing pH levels of 4.8 and 7.2 on the control of rhizoctoniosis in beetroots under greenhouse conditions.

## MATERIAL AND METHODS

### Inoculation of the fungus

To obtain *R. solani* inoculum, ten disks of seven-day-old culture of the pathogen (5 mm diameter), previously grown in potato-sucrose-agar medium (PSA) at ± 24 °C, were placed in a glass Erlenmeyer flask containing 150 g rice and 200 ml distilled water, which were previously autoclaved for 20 minutes at 120 °C. The flasks were kept in a growth chamber at ±24 °C for 15 days. Then, the substrate was removed from the Erlenmeyer flasks and allowed to dry on plastic trays (35 x 20 cm), at room temperature (~ 24 °C), for eight days. After drying, the rice substrate containing the fungus was ground in a blender (Britania®, slow speed) for 2 minutes and stored in flasks kept in the dark, at 10 °C, until use.

### Obtaining the soil

The soil used in the experiments was collected from an area under no-tillage, at a depth of 0 to 20 cm, and classified as "Latossolo Vermelho Distroférrico", according to Embrapa classification (9). Chemical analysis of the soil showed the following values: pH in water of 4.8, organic matter of 50.93 $g.dm^{-3}$, nutrients: Mg (2.0 $cmol_c^{(+)}$) $dm^{-3}$, K (0.35 $cmol_c^{(+)}$) $dm^{-3}$, P (3.28 mg).$dm^{-3}$, Cu (3.15 mg).$dm^{-3}$ and Zn (4.46 mg).$dm^{-3}$, and soil base saturation of 59%, according to the methodology described by Pavan et al. (17).

### Swine manure

Swine manure (SM) was obtained from "Universidade Tecnológica Federal do Paraná", located in Dois Vizinhos, Paraná State, Brazil. The chemical components of SM were analysed according to the method described by Embrapa (8). The following nutrient values were found in the sample: nitrogen (N) 5.30%, phosphorus (P) 0.75%, potassium (K) 0.51%, calcium (Ca) 1.72%, magnesium 0.46% and dry matter (Ms) 7%.

### In vivo test

The assay was conducted in a greenhouse, in a completely randomised 2 x 5 bi-factorial design, using two soil pH levels (4.8 and 7.2) x five SM doses (0, 5, 10, 15 and 20%, calculated as a function of total soil weight per treatment), and four replicates per treatment.

To adjust the soil pH to 7.2, for each treatment, 5 kg sieved soil and 50 g dolomitic limestone $kg^{-1}$ soil were added to a 50-L plastic bag. Before the assay was set up, the pH of the soil was tested again to confirm that it had the desired pH (4.8 and 7.2).

To set the trial, 4 kg dry unsterilized soil (pH 4.8 and 7.2), 15 g *R. solani* inoculum $kg^{-1}$ soil and 200 mL water were placed, separately, in 50-L black plastic bags. Then, they were manually homogenized and the mixture was stored in a greenhouse at ± 25 °C.

After seven days, the respective SM doses were added to separate plastic bags, manually homogenized and stored again in a greenhouse for seven days. Then, half of the soil of the respective treatments was placed in polystyrene trays containing 128 cells, of which 16 cells represented one experimental unit. In each cell, two seeds (monogerm) of 'Katrina' beetroot were sown and irrigated when needed.

Emergence and damping-off were daily evaluated for 21 days, and their percentages were calculated based on the number of seeds that were sown and the number of seedlings exhibiting damping-off.

The other half of the soil was placed in separate 1-kg pots and sown with the beetroot seeds to quantify microbial activity based on $CO_2$ release, according to the methodology described by Grisi (11). Thus, 100 g soil was collected from each pot at 7, 14 and 21 days after the incorporation of SM. Each soil sample was sieved and placed in separate 2-L plastic pots. A Petri dish containing 10 mL potassium hydroxide (KOH), at 0.5 normal (N), was deposited on the soil of each pot. The pots were hermetically sealed to prevent both air ingress and egress and stored in the dark, for 15 days, at 24 ºC. Two pots containing only Petri dishes with KOH left under the same conditions were used as control. The trial was conducted in a completely randomised design, using four replicates per treatment.

Subsequently, the KOH in the dishes was titrated with 0.1 Mol hydrochloric acid (HCl), using phenolphthalein and methyl orange as indicators, two drops/sample, respectively. The obtained results were employed in the following formula: (Treatment - Control) * Molecular weight $CO_2$ * mol HCL, yielding the quantity of mg $CO_2$/100g soil. Both experiments were repeated twice.

### Statistical analysis

The obtained data were verified according to analysis of variance and transformed if necessary. Analysis of variance was performed at 5% probability of error. If an interaction between factors was significant, regression analysis of SM doses was carried out for each pH level (4.8 and 7.2); in the absence of interaction, F test was used to verify the difference between the means of levels, while polynomial regression analysis was adopted for SM doses.

## RESULTS

There was a significant interaction between pH levels x SM doses for beetroot seedling emergence in both trials (Tables 1 and 2). In the first trial, emergence percentage was highest for SM doses of 6.16% and 5.65% in soil showing pH of 4.8 and 7.2, respectively: 97.41% and 96.85% beetroot seedling emergence. On the other hand, the highest SM dose reduced beetroot seedling emergence by more than 45% at both pH levels (Figure 1A). In the second trial, SM doses of 10.18% and 6.49% in soil showing pH of 4.8 and 7.2, respectively, resulted in maximum beetroot seedling emergence: higher than 99.40% (Figure 2A).

For the variable damping-off, there was no significant interaction between pH levels x SM doses. However, a difference between crops was observed for the isolated factors (Tables 1 and 2). In trial I, the lowest percentage of beetroot seedling damping-off (17.37%) was obtained for the SM dose of 13.56% (Figure 1B). In trial II, the fit of the equation was linear, and beetroot seedling damping-off decreased from SM dose of 10% (Figure 2B).

Considering the different pH levels, soil showing pH of 7.2 had 21.84% and 16.56% less beetroot seedling damping-off in trials I and II, respectively, compared to soil showing pH of 4.8 (Table 3).

For microbial activity, the factor SM dose was only significant at 7 and 14 days (trials I and II) and at 21 days (trial II) (Tables 1 and 2). In trial I, microbial activity (8.42 mg $CO_2$/100 g soil) was maximal for SM dose of 13.64% at 7 days and (8.85 mg $CO_2$/100 g soil) for SM dose of 12.24% at 14 days (Figure 1C and 1D).

However, in the second trial, there was a significant interaction between factors (pH x SM dose) at 7 days, when all tested SM doses, regardless of the soil pH, increased microbial activity. SM doses of 17.14% and 13.20% led to $CO_2$ releases of 8.43 and 8.52 mg $CO_2$/100 g soil at pH levels of 4.8 and 7.2, respectively (Figure 2C). At 14 days, the highest microbial activity (8.89 mg $CO_2$/100 g soil) was obtained for SM dose of 13.1% applied to the soil (Figure 2D). At 21 days, SM dose of 12.50% had the best result for microbial activity (8.73 mg $CO_2$/100 g soil) (Figure 2E).

**Table 1.** Degrees of freedom (DF) and mean squares of analysis of variance for the variables: emergence (%) and damping-off (%) of beetroot seedlings, and microbial respiration at 7, 14 and 21 days after the incorporation - DAI (mg $CO_2$/100 g of soil), in a bi-factorial trial, using two soil pH levels (4.8 and 7.2) and five swine manure (SM) doses (0, 5, 10, 15 and 20%) for the first trial conducted in completely randomised design.

| Causes of variation | DF | Mean squares | | GL | Mean squares | | |
|---|---|---|---|---|---|---|---|
| | | | | | Microbial respiration (mg $CO_2$100g $^{-1}$soil$^{-2}$) | | |
| | | Emergence[1] | Damping-off | | 7 DAI | 14 DAI | 21 DAI |
| pH | 1 | 0.0006$^{ns}$ | 466.8989$^{*}$ | 1 | 0.9118$^{ns}$ | 0.8875$^{ns}$ | 0.0975$^{ns}$ |
| Swine manure dose | 4 | 0.5060$^{*}$ | 1266.3326$^{*}$ | 4 | 7.1475$^{*}$ | 2.4151* | 0.5883$^{ns}$ |
| pH x SM dose | 4 | 0.0479$^{*}$ | 33.3858$^{ns}$ | 4 | 0.7316$^{ns}$ | 0.1124$^{ns}$ | 0.0990$^{ns}$ |
| Residual | 30 | 0.0169 | 28.3752 | 20 | 0.3324 | 0.3739 | 0.2082 |
| Overall mean | | 81.25 | 27.85 | | 7.63 | 8.28 | 8.36 |
| C.V. (%) | | 11.00 | 19.12 | | 7.56 | 7.38 | 5.45 |

[1] Arcosene transformation. * Significant at 5% probability of error. $^{ns}$ Not significant at 5% probability of error.

**Table 2.** Degrees of freedom (DF) and mean squares of analysis of variance for the variables: emergence (%), damping-off (%) of beetroot seedlings and microbial respiration at 7, 14 and 21 days after the incorporation - DAI (mg $CO_2$/100 g of soil), in a bi-factorial trial, using two soil pH levels (4.8 and 7.2) and five swine manure (SM) doses (0, 5, 10, 15 and 20%) for the second trial conducted in completely randomised design.

| Causes of variation | DF | Mean squares | | DF | Mean squares | | |
|---|---|---|---|---|---|---|---|
| | | | | | Microbial respiration (mg $CO_2$100g $^{-1}$soil$^{-2}$) | | |
| | | Emergence[1] | Damping-off | | 7 DAI | 14 DAI | 21 DAI |
| pH | 1 | 0.00000$^{ns}$ | 161.8855$^{*}$ | 1 | 0.4060$^{ns}$ | 1.3062$^{ns}$ | 0.0740 $^{ns}$ |
| Swine manure dose | 4 | 0.00017$^{*}$ | 501.9845$^{*}$ | 4 | 6.7578$^{*}$ | 4.3314$^{*}$ | 30.524$^{*}$ |
| pH x SM dose | 4 | 0.00005$^{*}$ | 58.1467$^{ns}$ | 4 | 1.2317$^{*}$ | 0.3674$^{ns}$ | 0.3529$^{ns}$ |
| Residual | 30 | 0.00001 | 31.5516 | 20 | 0.3133 | 0.4040 | 0.2272 |
| Overall mean | | 93.59 | 22.32 | | 7.56 | 8.12 | 8.14 |
| C.V. (%) | | 3.12 | 25.17 | | 7.40 | 7.83 | 5.86 |

[1] Arcosene transformation. * Significant at 5% probability of error. $^{ns}$ Not significant at 5% probability of error.

**Table 3.** Comparison of means of the soil pH levels for the variable (%) damping-off in beetroot seedlings (Trial I and II), in a bi-factorial experiment, using two soil pH levels (4.8 and 7.2) and five swine manure (SM) doses (0, 5, 10, 15 and 20%), conducted in a completely randomised design.

| Soil pH | % Damping-off | |
|---|---|---|
| | Trial I | Trial II |
| pH 4.8 | 31.27 a$^{*}$ | 24.33 a |
| pH 7.2 | 24.44 b | 20.30 b |

* Means not followed by the same letter, in the column, differ from each other at 5% probability of error, according to F test.

## DISCUSSION

Addition of SM to the soil controlled damping-off caused by *R. solani* in beetroots and increased microbial activity in the soil; besides, SM doses up to 15% did not affect beetroot seedling emergence.

Swine manure (SM) is an organic compound rich in nitrogen, which leads to release of ammonia when added to soil showing alkaline pH (5, 13). On the other hand, in soils showing acidic pH, it leads to the release of volatile fatty acids, especially acetic acid, propionic acid and isobutyric acid (19). These compounds were responsible for reducing the damping-off caused by *R. solani* in the beetroots of the present study when the highest SM doses were added to the soil, particularly soil showing pH of 7 (Figures 1 and 2; Table 3). Antifungal activity of SM was also reported against the pathogens *Verticillium dahlia* Kleb (4, 5), *Sclerotium rolfsii* Sacc (16) and *Pythium aphanidermatum* (Edson) Fitzp (15).

Swine manure (SM) also stimulated microbial activity in the soil, especially when applied at higher doses (Figure 1C, 1D and Figure 2C, 2D and 2E). The increased microbial activity promoted antagonism among the microorganisms present in the soil, which is another factor responsible for reducing the damping-off caused by *R. solani* in the beetroot seedlings of the present study (Figures 1B and 2B). Antagonistic effect on this pathogen is reported for numerous microorganisms such as actinomycetes (3), *Pseudomonas flurences* (21), *Bacillus subtilis* (12) and *Trichoderma* sp. (14).

Therefore, based on these results, SM can be employed by farmers in the management of areas infested with this pathogen. In small farms, in areas cultivated with vegetables, the farmer can apply SM with watering cans or hoses coupled to reservoirs. However, in larger areas, pig SM can be applied to the soil with a liquid manure distributor coupled to a tractor or tanker truck. Thus, repeated SM application to the soil enhances the control of soil borne pathogens by multiple

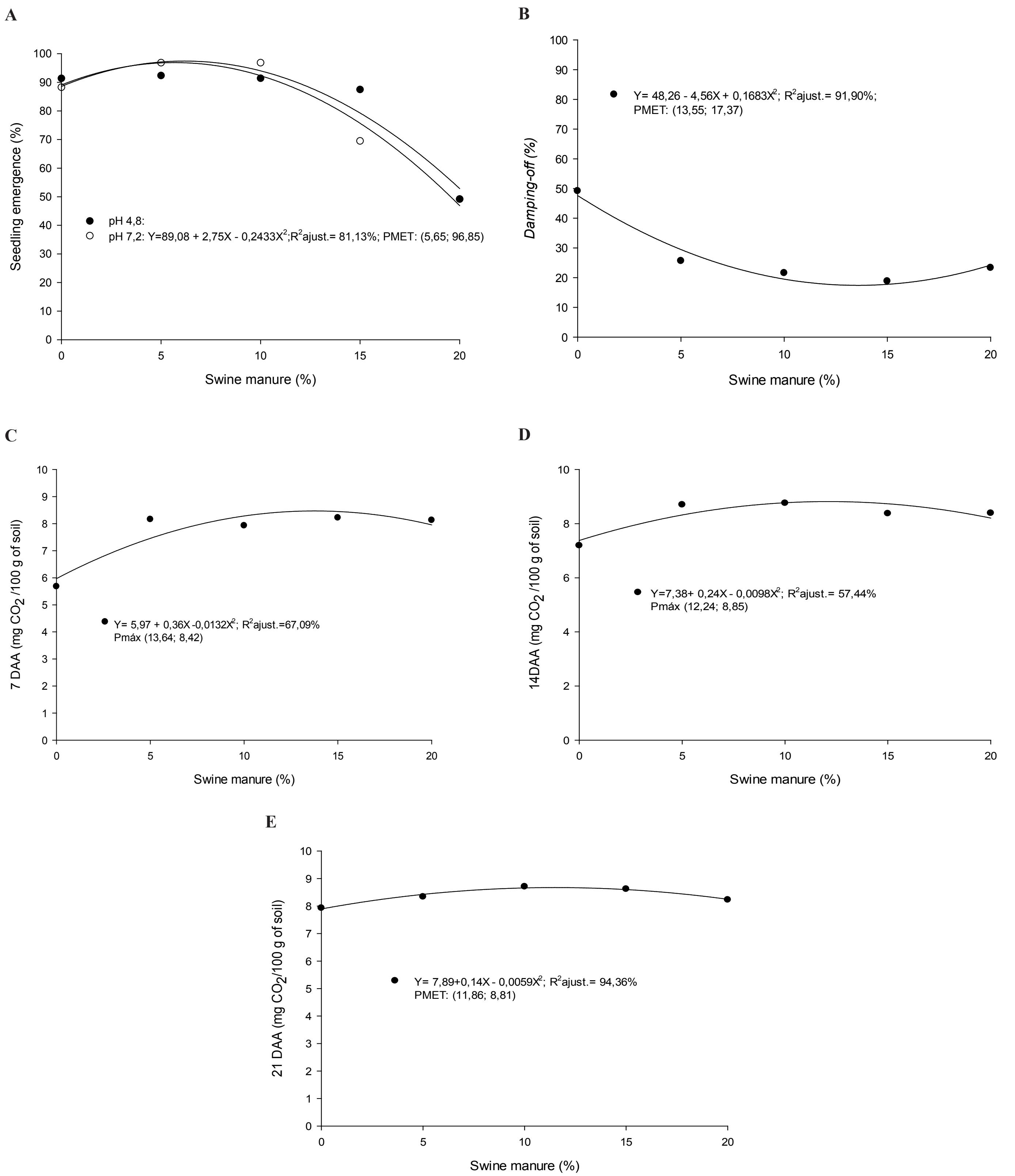


**Figure 1.** Emergence (%) of seedlings (A), damping-off (%) of seedlings (B), microbial respiration (mg $CO_2$/100 g of soil) at 7 (C), 14 ( D) and 21 days after swine manure incorporation - DAI (E), using five swine manure doses (0, 5, 10, 15 and 20%) and two soil pH levels (pH 4.8 and 7.2) for the first trial conducted in completely randomised design.

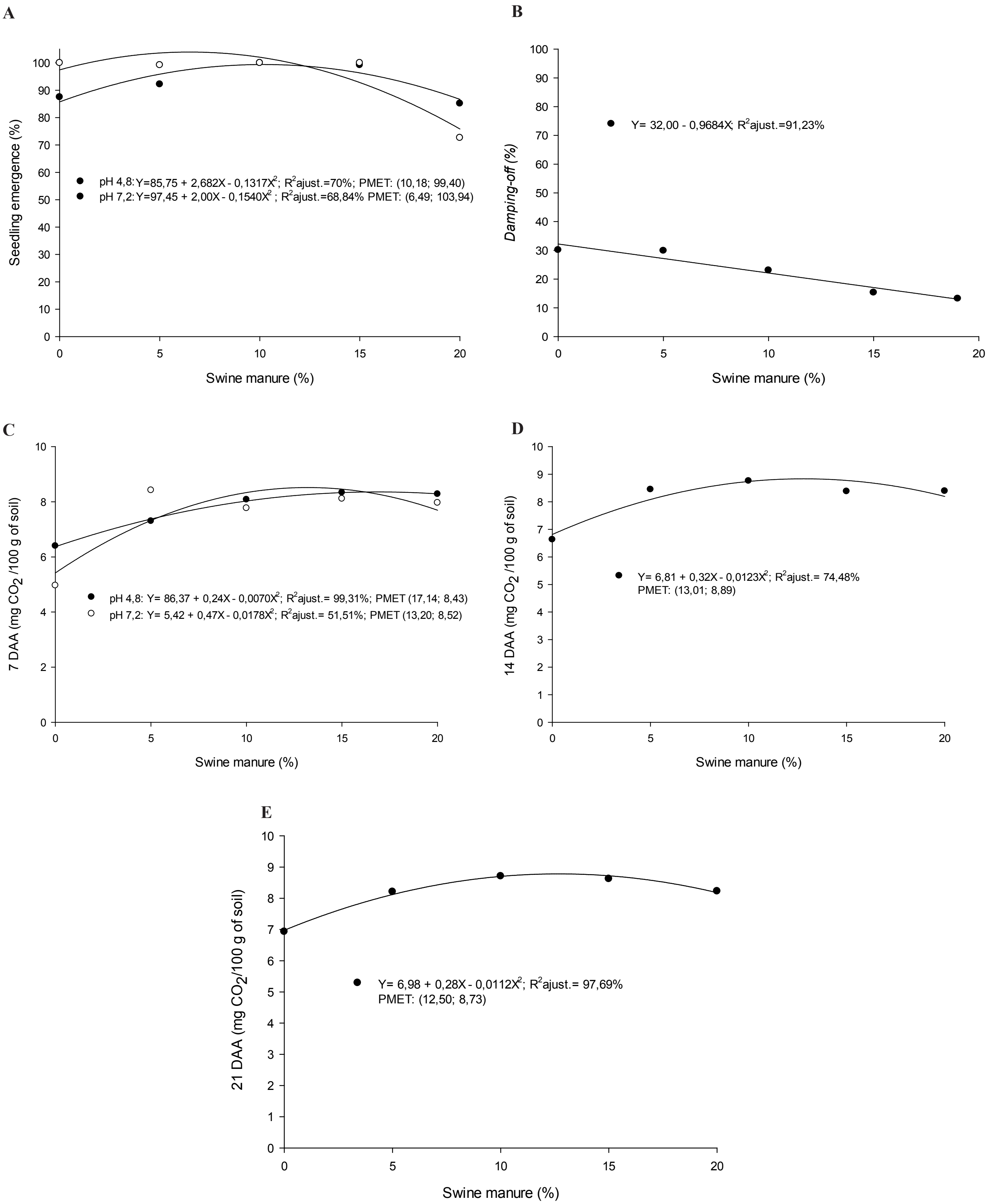


**Figure 2**. Emergence (%) of seedlings (A), damping-off (%) of seedlings (B), microbial respiration (mg $CO_2$/100 g of soil) at 7 (C), 14 (D) and 21 days after swine manure incorporation - DAI (E), using five swine manure doses (0, 5, 10, 15 and 20%) and two soil pH levels (pH 4.8 and 7.2) for the second trial conducted in a completely randomised design.

mechanisms of action.

On the other hand, although the highest SM doses applied to the soil were more efficient in reducing beetroot seedling damping-off, there was a reduction in seedling emergence in both trials (Figure 1A and 2A). Similar results were obtained by Manteli (15) for cucumber when doses higher than 10% were added to soils showing pH levels of 4.8 and 6.3, in the management of *P. aphanidermatum*, under greenhouse conditions. The reduced emergency observed in this study may have been due to the excess K (potassium) and N (nitrogen) present in the used SM (5.30% N and 0.51% K), which consequently decreased water absorption, germination of seeds, and development of seedlings. Salt excess may impair plant growth (2, 10, 18). In addition, the reduction in beetroot seedling emergence was also due to the surface sealing of the soil. In addition, the reduction in beetroot seedling emergence was also due to the surface sealing of the soil.

Based on the obtained results, application of SM at doses up to 10%, regardless of the soil pH, is efficient for rhizoctoniosis management, without affecting beetroot seedling emergence. Swine manure (SM) was not only a viable option for rhizoctoniosis control but also an inexpensive and easily accessible product, especially for regions where pig farming is predominant.

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