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Sorghum sudanense AS COVER CROP AND BIOLOGICAL PRODUCTS ON INCIDENCE OF *Sclerotinia sclerotiorum* AND YIELD OF TOBACCO

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ABSTRACT

Brazil is the world's second largest producer of tobacco (*Nicotiana tabacum*) with part of its production concentrated in Santa Catarina. The incidence of *Sclerotinia sclerotiorum* in tobacco areas has promoted a decrease in productivity, and control alternatives are needed due to the limited use of agrochemicals for the crop. With the hypothesis that the sudan grass (*Sorghum sudanense*) and *Trichoderma harzianum* reduce the attack of *S. sclerotiorum* in tobacco crops on the coast of Santa Catarina; the objective here was to test the incidence of *S. sclerotiorum* and the use of sudan grass associated with biological products on tobacco yield. A study with application of straw from sudan grass (SG); SG + *Trichoderma harzianum* (TH); SG + TH + *Melaleuca alternifolia* essential oil; SG + TH + *Bacillus subtilis* & *Bacillus licheniforimis*; and SG + TH + *Bacillus subtilis* in a conventional system of tobacco were carried out in São João do Sul and Santa Rosa do Sul, Santa Catarina. Results showed that in São João do Sul, there was no difference between the treatments in yield and incidence of *S. sclerotiorum*. However, in Santa Rosa do Sul, the association of SG + TH, SG + TH + *B. subtilis* & *B. licheniforimis*, and SG + TH + *B. subtilis* presented greater yields than others. In both sites, sudan grass and associations promoted a higher financial return compared to control. More studies are requested to elucidate the physiological effect of biological products and sudan grass as a cover crop on tobacco.

Keywords: Sudan grass, *Bacillus subtilis, Trichoderma harzianum, Nicotiana tabacum*

Sorghum sudanense COMO PLANTA DE COBERTURA E PRODUTOS BIOLÓGICOS NA INCIDÊNCIA DE *Sclerotinia sclerotiorum* E PRODUÇÃO DE TABACO

RESUMO

O Brasil é o segundo maior produtor mundial de tabaco (*Nicotiana tabacum*) com parte da produção concentrada em Santa Catarina. A incidência da *Sclerotinia sclerotiorum* em áreas de tabaco tem promovido à diminuição de produtividade e alternativas para o controle são necessárias, uma vez que o uso de agroquímicos é limitado para a cultura. Esse estudo foi desenvolvido com a hipótese que a introdução de boas práticas agrícolas como a semeadura do capim sudão (*Sorghum sudanense*) e a aplicação de *Trichoderma harzianum* auxiliam na redução do ataque da *S. sclerotiorum* nas lavouras de tabaco do litoral catarinense. Dois estudos com aplicação de palhada de capim sudão (CS); CS + aplicação da *Trichoderma harzianum* (TH); CS + TH + óleo essencial de *Melaleuca alternifolia*; CS + TH + *Bacillus subtilis* & *Bacillus licheniforimis*; e CS + TH + *Bacillus subtilis* foram desenvolvidos em um sistema convencional de produção de tabaco em São João do Sul and Santa Rosa do Sul, Santa Catarina. Resultados mostraram que em São João do Sul, não houve diferença entre os tratamentos na produção e incidência de *S. sclerotiorum*. Porém, em Santa Rosa do Sul, a associação de SG + TH, SG + TH + *B. subtilis* & *B. licheniforimis* e SG + TH + *B. subtilis* apresentou rendimentos maiores que os demais tratamentos. Em ambos os locais, o capim sudão e as associações promoveram um maior retorno financeiro em comparação com o controle. Mais estudos são necessários para elucidar o efeito fisiológico de produtos biológicos e do capim sudão como cultura de cobertura do tabaco.

Palavras-chave: Capim sudão, *Bacillus subtilis, Trichoderma harzianum, Nicotiana tabacum*

INTRODUCTION

Brazil is the world's second largest producer of tobacco (*Nicotiana tabacum*) with a production of 685 thousand tons in a produced area of 297,000 hectares in the 2017/2018 harvest. China ranks the first position in the world, with a production of around 2,160 million tons (AFUBRA, 2018). Brazil is the world largest exporter with the production destined for five continents, reaching 457 thousand tons exported (SINDITABACO, 2019).

The Brazilian tobacco production is concentrated in the south region of Brazil with 95% of the planted area, followed by the northeast region which concentrates 4% of production. In the south region of Brazil, 50% of Brazilian tobacco is produced in Rio Grande do Sul, 28% in Santa Catarina, and 22% in Paraná. Tobacco production is present in 556 cities with 150 thousand producers in the south of Brazil (AFUBRA, 2018).

Santa Catarina is the second most important state in tobacco production in Brazil. In the 2017/2018 harvest, tobacco production generated income for 41,818 families, who produced the equivalent of 192 thousand tons on 83,289 hectares. The tobacco production in the state of Santa Catarina is stratified into four production regions: Coast, Alto Vale, North Plateau, and Coast and West of Santa Catarina (SINDITABACO, 2019).

Indirect contamination with pesticides is currently a challenge in tobacco production: one example is the contamination with 2.4 D herbicide and Carbendazim fungicide derived from other commercial crops. Both products are not registered and recommended for tobacco (MAPA, 2007), but lots contaminated with 2.4 D have been detected and isolated from commercial crops, and producers have been instructed to install green curtains to protect their crops from future contamination (CORESTA, 2018).

The incidence of *Sclerotinia. Sclerotiorum* has been a problem in tobacco production. *S. sclerotiorum* survives as black, hard, and oblong sclerotia in plant tissue or the soil, considered a fungus widely distributed in the world and affecting several plant species (SILVA et al. 2015). Alternatives related to the use of sudan grass (*Sorghum sudanense*) and the application of *Trichoderma harzianum* have been proposed to reduce the incidence of *S. sclerotiorum* in tobacco areas, and studies are requested to understand the efficiency of these uses.

With the hypothesis that the sudan grass and the application of *Trichoderma harzianum* reduce the attack of *S. sclerotiorum* in tobacco crops in Santa Catarina; the objective here was to test the incidence of *S. sclerotiorum* and the use of sudan grass associated with biological products on tobacco yield.

MATERIAL AND METHODS

Area characterization and experimental design

The study was carried out in a tobacco farmer, in São João do Sul (29° 10' 59.55" S and Long. 49° 50' 20.94" W) and Santa Rosa do Sul (29° 8' 49.63" S and 49° 45' 46.11" W), located in the coastal region of Santa Catarina, Brazil.

The coastal region of Santa Catarina has a humid subtropical climate according to the Köppen classification, with well-distributed rainfall, hot summers, and an average temperature of 21° C. The development and harvest of tobacco occur between July and December, characterized by high temperatures and air humidity, factors that favor the incidence and proliferation of *S. sclerotiorum.*

The study was developed using an experimental design in randomized blocks with three replications. The treatments were straw from sudan grass (SG); SG + *Trichoderma harzianum* (TH); SG + TH + *Melaleuca alternifolia* essential oil (MA-oil); SG + TH + *Bacillus subtilis* & *Bacillus licheniforimis* (BB); and SG + TH + *Bacillus subtilis* in a conventional system of tobacco. A control treatment was installed without sudan grass and biological products.

Experimental management

The experimental units were sowed with sudan grass, 150 days before tobacco planting, using a density of 100 kg ha^{-1}. After 120 days of sudan grass planting, the plants were desiccated using 3.5 L ha^{-1} of glyphosate. The soil for tobacco planting was managed according to the agronomic recommendation. Fertilizer applications were carried out using doses of 750 kg ha^{-1} of the formulated 10-10-10 at planting, and 500 kg ha^{-1} of the formulated 15-03-15 in coverage (applied twice, in doses of 250 kg ha^{-1} at 15 days and 30 days after transplantation), using ammonium nitrate, triple superphosphate, and potassium chloride, respectively.

Tobacco seedlings (AOV 911 cultivar) were planted in a float system, a system in which the trays are floating under a layer of water. The seedlings were pruned three times before transplanting, to reduce the leaf area and increase the stem diameter. Two days before transplantation, the seedlings received an application of Imidacloprid + Beta Cyfluthrin, following the agronomic recommendations. The seedlings were transplanted 70 days after sowing, using a spacing of 1.20 meters between rows and 0.45 meters between plants.

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Thrichoderma harzianum (Koppert®) was applied at the time of transplantation using a dose of 1 L ha^{-1} sprayed onto the ridge. The *Melaleuca alternifolia* (Timorex Gold®) was applied 30 days after transplanting using a dose of 2 L ha^{-1}, sprayed on the leaf area of the plants. *Bacillus subtilis* & *Bacillus licheniforimis* (Quartzo®) and *Bacillus subtilis* (Serenade®) were applied weekly until transplanting, receiving the last application in the field at the time of transplanting. *B.subtilis* & *B. licheniforimis* was applied using a dose of 0.3 g per tray weekly and 200 g ha^{-1} in the transplant.

Variables analyzed

In both areas, yield, quality index, financial return, and alkaloid contents were monitored following morphological development. Five harvests were carried out at 70, 93, 111, 124, and 133 days after the transplant, and average yield was presented here.

After the last harvest, leaf samples were collected and sent to Alliance One's Research, Development & Training Center, for the classification of the leaves following the Normative Instruction 10/2007 of the Ministry of Agriculture, Livestock and Supply (MAPA, 2007). The number of tobacco plants attacked by the fungus *S. sclerotiorum* was monitored every two weeks with visual assessments from 45 to 111 days after transplantation.

Data were compiled and correlation analysis (Sigmaplot®, Pearson correlation; $P<0.05$) was performed. The data set was tested according to the analysis of variance (ANOVA), F test, when significant the averages were compared according to the Duncan test ($P<0.05$).

RESULTS AND DISCUSSION

Incidence of *S. sclerotiorum*

In Santa Rosa do Sul and São João do Sul, there was no significant difference of plants attacked by *S. sclerotiorum* using sudan grass associated with *T.harzianum*, oil-MA, *B. subtilis & B. licheniforimis*, and *B. subtilis* (Table 1). Possibly, there was no significant effect due to monitoring at the end of the cycle.

Table 1. Tobacco plants attacked (un) by *S. sclerotiorum* in planting with sudan grass (SG, cover planting) and applications of *Trichoderma harzianum* (TH), *Melaleuca alternifolia* essential oil (MA-oil), *Bacillus subtilis* & *Bacillus licheniforimis* (BB), and *Bacillus subtilis* (BS) in São João do Sul and Santa Rosa do Sul, Santa Catarina State, Brazil.

Cover crop	São João do Sul [Ns]	Santa Rosa do Sul [Ns]
Control	2,67	2,00
SG	2,00	1,67
SG+TH	1,67	1,67
SG+TH+oil-MA	2,67	2,00
SG+TH+BB	0,67	0,33
SG+TH+BS	0,67	0,33

Control: conventional treatment. In the column, the averages were compared with the Duncan test (P<0.05). Ns: no significant effect.

There was a negative correlation between the incidence of *S. sclerotiorum* and yield (r: -0.55; p<0.06), indicating that the increase of *S. sclerotiorum* reduced the yield (Figure 1). This result was expected because the *S. sclerotiorum* attacks on the stem, close to the ground, causing a dark brown lesion and leaving the leaves yellow, and consequently reducing the yield. However, the incidence of *S. sclerotiorum* did not present a significant correlation with the tobacco quality (Figure 1).

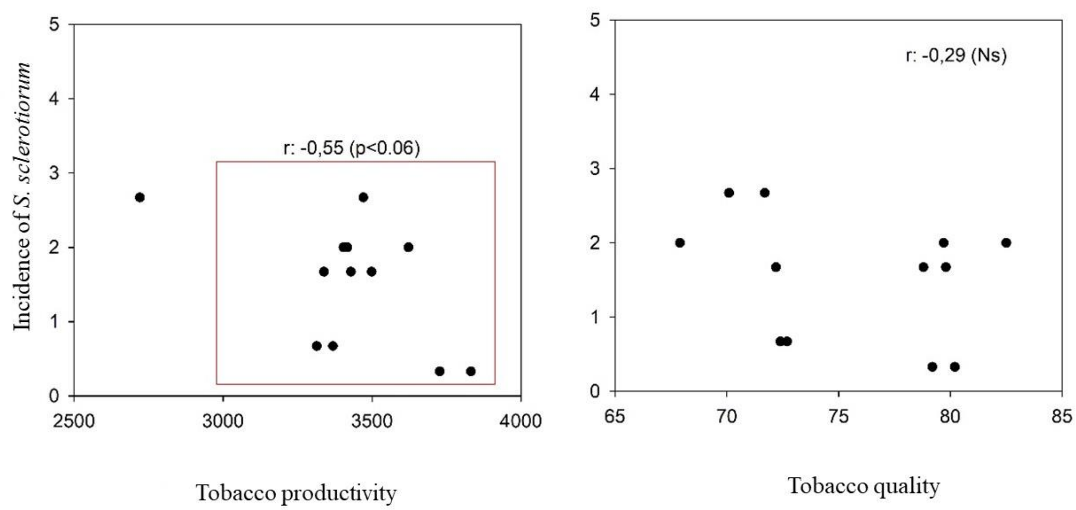

Figure 2. Correlation between the incidence of *S sclerotiorum* and tobacco yield and quality. Correlation values were significant with a P < 0.05; Ns: no significant correlation.

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Tobacco yield

In Santa Rosa do Sul, the use of sudan grass + T. *harzianum* + B. *subtilis & B. licheniforimis* promoted the highest yield (3,832 kg ha^{-1}), with an increase of 16% in yield compared to the control (Table 2).

The addition of sudan grass straw + *T. harzianum* associated with oil-MA, B. subtilis, or *B. subtilis & B. licheniforimis* showed no differences with average yields of 3,622; 3,727; and 3,832 kg ha^{-1}, respectively (Table 2). The best performance of the intercropping of sudan grass + T. *harzianum*. + *B. subtilis* & *B. licheniforimis* in Santa Rosa do Sul, probably occurred because the study was installed in an area with the occurrence of *S. sclerotiorum* and nematodes (*Meloidogyne*). Lima (2017), demonstrated that the application of *B. subtilis & B. licheniforimis* provided a reduction of *Meloidogyne paranaensis* and a better reconstitution of the root system of the coffee plants. *Trichoderma* has been considered effective in controlling phytopathogens, especially those with resistance structures considered difficult to be attacked by microorganisms, such as spores, sclerotia, chlamydospores, and microsclerotia (MELO, 1996).

Table 2. Tobacco yied (kg ha^{-1}) using sudan grass (SG), *Trichoderma harzianum* (TH), *Melaleuca alternifolia* essential oil (MA-oil), *Bacillus subtilis* & *Bacillus licheniforimis* (BB), and *Bacillus subtilis* (BS) in São João do Sul and Santa Rosa do Sul, Santa Catarina State, Brazil.

Cover crop	**Tobacco yield, kg ha^{-1}**	
	São João do Sul	Santa Rosa do Sul
Control	2,721 b	3,404 c
SG	3,417 a	3,499 b
SG+TH	3,339 a	3,429 bc
SG+TH+oil-MA	3,471 a	3,622abc
SG+TH+BB	3,314 a	3,832 a
SG+TH+BS	3,369 a	3,727 ab

Control: conventional treatment. In the column, means were compared with the Duncan test (P<0.05).

In São João do Sul, the use of sudan grass isolated or associated with *T. harzianum*, oil-MA, *Bacillus subtilis* & *Bacillus licheniforimis*, and *Bacillus subtilis*, showed similar averages ranging from 3,314 to 3471 kg ha^{-1}. With only the sudan grass straw intervention, it resulted in an increase of 20% in yield compared to control (Table 2). This result is due to the greater physical protection of the soil with the addition of sudan grass straw that controls the incidence of *S. sclerotiorum*, acting as a physical barrier and hindering the germination of apothecia. Pacheco et al. (2014) comparing sudan grass and millet showed that sudan has a leaf and stalk blade 3.9 and 1.0% larger than millet. According to Cavalli (2016), sudan grass has been standing out as an alternative to reduce water deficiency during the dry season with a high rustic and drought tolerance.

Another variable to be analyzed refers to rainfall between the transplant and the end of harvest. According to Doorembos and Kassam (1994), tobacco requests between 400 and 600 mm to plant development. In Santa Rosa do Sul, 887 millimeters of rain in 45 days (19.7 mm day^{-1}) were recorded between the period of transplanting and the end of harvest, which was 133 days. While in São João do Sul, 661 millimeters of rain in 31 days (21.3 mm day^{-1}) were recorded during 133 days of the crop cycle, with 1/3 of the total volume accumulated in September 2018. The high rainfall in a short period may be associated with lower yields obtained in São João do Sul, when compared to yields obtained in the Santa Rosa do Sul.

Quality index and alkaloids

The quality index ranged from 67.9 to 82.5, without differences between the treatments (Table 3). Visually, in the field, all plants presented an adequate leaf coloration. Generally, when tobacco is harvested before the ideal maturity point, there are decreases in the levels of starch present in the leaves, resulting in lower percentages of sugar in leaves and lower quality in the post-curing/drying period of the tobacco.

All treatments had alkaloid indices below the control treatment, which presented an average of 3.0 and 3.8% in São João do Sul and Santa Rosa do Sul, respectively (Table 4). For Virginia-type tobacco, the alkaloid levels ranged between 1.5% and 3.5% which varies according to climate, cultivar, and leaf position on the plant. The alkaloid indices are directly linked to the level of stress, which the higher levels of stress increase the alkaloid contents in plant (COLLINS & HAWKS, 1993).

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Table 3. Quality index of tobacco using sudan grass (SG), *Trichoderma harzianum* (TH), *Melaleuca alternifolia* essential oil (MA-oil), *Bacillus subtilis* & *Bacillus licheniforimis* (BB), and *Bacillus subtilis* (BS) in São João do Sul and Santa Rosa do Sul, Santa Catarina State, Brazil.

Cover crop	São João do Sul ^{Ns}	Santa Rosa do Sul ^{Ns}	Média ^{Ns}
Control	71,7	82,5	77,1
SG	67,9	79,8	73,9
SG+TH	72,2	78,8	75,5
SG+TH+oil-MA	70,1	79,7	74,9
SG+TH+BB	72,7	79,2	76,0
SG+TH+BS	72,4	80,2	76,3

Control: conventional treatment. Ns: no significant effect according to the Duncan test (P<0.05).

Table 4. Alkaloid contents of tobacco productivity (kg ha^{-1}) using sudan grass (SG), *Trichoderma harzianum* (TH), *Melaleuca alternifolia* essential oil (MA-oil), *Bacillus subtilis* & *Bacillus licheniforimis* (BB), and *Bacillus subtilis* (BS) in São João do Sul and Santa Rosa do Sul, Santa Catarina State, Brazil.

Cover crop	São João do Sul ^{Ns}	Santa Rosa do Sul	Média ^{Ns}
Control	3.81	3.00 a	3.41
SG	3.54	3.00 a	3.30
SG+TH	2.59	2.59 b	3.06
SG+TH+oil-MA	2.93	2.93 ab	3.38
SG+TH+BB	2.79	2.79 ab	3.24
SG+TH+BS	2.87	2.87 ab	3.17

Control: conventional treatment. Ns: no significant effect according to the Duncan test (P<0.05).

Sudan grass + *T. harzianum* showed the lowest concentration of total alkaloids, considered 14% lower than control in Santa Rosa do Sul (Table 4). This result indicates that the tobacco plant under sudan grass and *T. harzianum* cover conditions present a better balance, providing lower alkaloid indices.

Financial return

In São João do Sul, sudan with *T. harzianum*, oil-MA, *B. subtilis & B. licheniforimis*, and *B. subtilis* presented a superior financial return than the control treatment, varying between US$

7,113 and 7,565 ha^{-1}, and with an average increase of 21%. A similar result was verified with the average of the two areas with an average increase of 12% (Table 5).

Table 5. Financial return of tobacco using sudan grass (SG), *Trichoderma harzianum* (TH), *Melaleuca alternifolia* essential oil (MA-oil), *Bacillus subtilis* & *Bacillus licheniforimis* (BB), and *Bacillus subtilis* (BS) in São João do Sul and Santa Rosa do Sul, Santa Catarina State, Brazil.

Cover crop	**São João do Sul**	**Santa Rosa do Sul**	**Média**
	Financial retorn (US$ ha^{-1})		
Control	5,948 b	8,334 ab	7,141 b
SG	7,113 ab	8,448 ab	7,781ab
SG+TH	7,458 a	8,148 b	7,803 a
SG+TH+ oil-MA	7,399 a	8,878 ab	8,139 a
SG+TH+BB	7,565 a	9,225 a	8,395 a
SG+TH+BS	7,549 a	9,052 ab	8,301 a

Control: conventional treatment. Ns: no significant effect according to the Duncan test (P<0.05).

While, in Santa Rosa do Sul, sudan grass associated with *T. harzianum* and *Bacillus subtilis* & *Bacillus licheniforimis* presented higher financial return (US$ 9,225), considered 12% greater than sudan grass associated with *T. harzianum* (Table 5). The lower financial return of sudan grass + *T. harzianum* is due to the inferior quality index compared with the control treatment, as shown in Tables 3 and 4, resulting in a lower financial return.

CONCLUSIONS

In São João do Sul, there was no clear difference in tobacco yield and incidence of *S. sclerotiorum* using a cover plant with sudan grass and biological products. However, in Santa Rosa do Sul, the association of sudan grass + *T. harzianum* with *B. subtilis* & *B. licheniforimis* or *B. subtilis* or *Melaleuca alternifolia* essential oil presented greater yields with lower alkaloid contents due to lower levels of stress. In both sites, sudan Grass and associations promote a higher financial return compared to control. The additions of the cover crop with sudan grass and biological products increase the tobacco yield and quality. More studies are requested to elucidate the physiological effect of biological products and sudan grass as a cover crop on tobacco.

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