MÍRIAN RABELO DE FARIA

CONTRIBUIÇÃO DE RESÍDUOS ORGÂNICOS NAS PROPRIEDADES DO SOLO E SOBREVIVÊNCIA DE *Stenocarpella* EM COLMOS DE MILHO

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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia/Fitopatologia, área de concentração em Fitopatologia, para a obtenção do título de Mestre

Orientador

Dr. Wagner Bettiol

LAVRAS, MG

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MÍRIAN RABELO DE FARIA

CONTRIBUTION OF ORGANIC AMENDMENTS TO SOIL PROPERTIES AND SURVIVAL OF *Stenocarpella* ON CORN STALK

(CONTRIBUIÇÃO DE RESÍDUOS ORGÂNICOS NAS PROPRIEDADES DO SOLO E SOBREVIVÊNCIA DE *Stenocarpella* EM COLMOS DE MILHO)

Thesis presented at the Federal University of Lavras to obtain the degree of Master in Agronomy. Area: Plant Pathology.

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DEDICATION

To God for giving me faith and strength, and for all the wonderful that gave me.

To my parents for teaching, trust and love.

To my friends for the encouragement and companionship.

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RESUMO

Com a implementação do sistema de plantio direto, principalmente, em monocultivo de milho, houve aumento da incidência de doenças causadas por patógenos necrotróficos como *Stenocarpella* spp. Uma das estratégias para reduzir o tempo de sobrevivência destes patógenos é estimular a degradação dos restos de cultura. O presente estudo avaliou o efeito da aplicação de resíduos orgânicos e nutrientes no solo na sobrevivência de *Stenocarpella* spp. em colmos de milho. Nos ensaios foram conduzidos nas fazendas experimentais da UFLA/Lavras e da Embrapa Milho e Sorgo/Sete Lagoas, MG num delineamento de blocos casualizados. Os colmos de milho, previamente inoculados com o patógeno, foram mantidos no campo por um período de três meses após a aplicação dos resíduos orgânicos (cama de frango, esterco suíno, hidrolisado de peixe e lodo de esgoto) e ureia. Como controles, foram mantidos os colmos infestados com o patógeno, porém não tratados na superfície ou incorporados ao solo. As avaliações da atividade microbiana indireta (β-glicosidase, hidrólise de diacetato de fluoresceína (FDA) e urease), análise química do solo e a sobrevivência do patógeno, por meio de qRT-*PCR,* foram realizadas no final do período. Em Lavras e Sete Lagoas verificou-se aumento no teor de cálcio com a aplicação de cama de frango. A aplicação de cama de frango e lodo de esgoto aumentou a atividade de β–glicosidase e a hidrólise de FDA nas duas localidades A dinâmica da população do patógeno por qPCR, quando comparado com a porcentagem de Ct do tratamento com colmo na superfície, nos dois locais, os colmos enterrados apresentaram a maior redução do patógeno, seguido por lodo de esgoto em Lavras e, hidrolisado de peixe e lodo de esgoto em Sete Lagoas. Hidrolisado de peixe e lodo de esgoto são promissores na indução de supressividade, pois elevaram a atividade enzimática do solo e reduziram a quantidade de patógeno.

Palavras chave: *Zea mays*, resteva, manejo alternativo de doenças.

ABSTRACT

No-tillage systalks, especially in corn monoculture, contribute to the incidence of diseases caused by necrotrophic pathogens such as *Stenocarpella* spp. One strategy to reduce the survival of such a pathogen is through the application of organic matter and nutrients in the soil. The aim of this study was to evaluate the effect of the application of organic wastes and urea on the survival of *Stenocarpella* spp. in corn stalks. The assays were conducted in experimental area of Lavras and Corn Sete Lagoas. Corn stalks, previously infested with the pathogen, were laid on the floor in the field for a period of three months after application of the organic wastes (Poultry litter, swine manure, fish hydrolyzed, and sewage sludge), urea a negative control with untreated stalks and a positive one with buried stalks. The indirect microbial activity (β-glucosidase and hydrolysis of fluorescein diacetate (FDA) and urease), chemical soil analysis and dynamics of pathogen population, through qPCR, were evaluated at the end of the period. The calcium content increased with application of Poultry litter in both areas. Poultry litter and sewage sludge increased β-glucosidase activity and hydrolysis of FDA at both locations. The pathogen population dynamics, the buried stalkstalks reduced the pathogen at both locations, the second highest reduction was obtained by sewage sludge in Lavras and fish hydrolyzed and sewage sludge in Sete Lagoas. Therefore Fish hydrolyzed and sewage sludge were promising to induce suppressiveness, since increased the suppressivity-realted soil enzymatic activity and reduced the pathogen population.

Key words: *Zea mays*, stubble, alternative management

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SUMMARY

1 INTRODUCTION

Corn is one of the most important crops in Brazil and several factors cause economic losses. With the widespread adoption of the no-tillage systalk, the saprophytic fungal community builds up, some of those fungi are corn pathogens, such as *Stenocarpella* spp. (CASA; REIS; ZAMBOLIM, 2004).

In cornfields, the fungi *Stenocarpella macrospora* and *Stenocarpella maydis* are found causing stalk rot, white ear rot and leaf spots (CASA; REIS; ZAMBOLIM, 2006). These pathogens survive in crop residues and therefore, the no-tillage system, especially associated with corn monoculture, increases its inoculums, leading to ear and/or stalk rot epidemics (CASELA; ZAMBOLIM; PINTO, 2006).

In order to evaluate the survival of *Stenocarpella* spp., microscopical visualization and quantification of pycnidia and conidia are performed on corn Stalk. However, the presence of the pathogen mycelium or early picnicium formation are not taken into account (SARTORI, 2003). Thus, molecular primers can be developed to evaluate the survival of necrotrophic pathogens in plant debris over time (KÖHL et al., 2015) and has already been used to detect *Stenocarpella* spp in corn Stalk (XIA; ACHAR, 2001).

When the inoculum *Stenocarpella* spp. is detected in the area, it is necessary to prevent its establishment on crop Stalk (ROMERO; WISE, 2015). Crop rotation is suggested for the control these diseases, because there are no resistant hybrids for *Stenocarpella* spp. in Brazil (CASELA; ZAMBOLIM; PINTO, 2006). Fungicides have been used as seed treatments (CARVALHO et al., 2004), as well as in the field. However, the best disease management strategy combines multiple disease control tools and none has yet been proposed to reduce the pathogen (BETTIOL et al., 2002). The amendment of stalks to stimulate the decomposition of plant residues and/or increase the buildup of

antagonist microorganisms can contribute to the reduction in the pathogen survival during overwintering in Stalk (CASA; REIS; ZAMBOLIM 2003).

Studies with amendment of organic matter into soil have been carried out in order to evaluate its effect in the control of plant diseases. This technique has shown significant results against various pathogens and can become a viable alternative to control *Stenocarpella*. Bettiol et al. (2009) observed that the amendment of sewage sludge, chicken manure, or swine manure to commercial pine bark-based commercial potting mix, reduced Fusarium wilt in chrysanthemum. The organic residues amended to the soil are sources of carbon, macro-and micronutrients for plants while also contribute to change in pH, electric conductivity and stimulate macro-and microflora of the soil (BETTIOL et al., 2009; GOMES et al., 2005; PIRES et al., 2008). Incorporation of organic matter contributes to the increase in the activities supressivity-related enzymes, which play an important role in the decomposition of crop residues and also cell wall of many plant pathogens (EIVAZI; TABATABAI, 1990; ZANG, 2015).

There is presently no report of contribution of soil amendment to the chemical properties of corn Stalk residues and its link to the survival of *Stenocarpella* sp. Thus, the aim of this study was to evaluate the effect of the application of organic wastes and urea on the survival of *Stenocarpella* spp. in corn stalks.

2 REFERENCIAL TEORICO

2.1 Perspectiva atual do milho

O milho é uma das *commodities* agrícolas mais importantes e apresenta variações de preço devido ao comportamento cíclico ou sazonal. Essas alternâncias ocorrem devido a diversos fatores que podem influenciar os preços, como o clima, as previsões de colheitas, os estoques do grão e até mesmo especulação nas Bolsas Futura de Mercadorias (INSTITUTO MATO-GROSSENSE DE ECONOMIA AGROPECUÁRIA - IMEA, 2015).

Estima-se que a produção global de milho para a safra 2015/2016 seja de 989,3 milhões de toneladas, com destaque para EUA, China e Brasil, países que detém 65,62% da produção mundial (IMEA, 2015).

A safra 2015/2016, no Brasil, se destaca pela previsão de diminuição na área de plantio, ou seja, uma redução na produção final. Apesar da redução, a Companhia Nacional de Abastecimento (Conab) estima que a área a ser plantada deva ficar em torno de 5,728 milhões de hectares. Desta forma, o Brasil continua como o terceiro maior produtor mundial do grão, com a previsão de 75 milhões de toneladas para safra 2015/16 (COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB, 2015).

2.2 Gênero *Stenocarpella*

Os fungos *Stenocarpella maydis* (Berkeley) Sutton [Sin. *Diplodia maydis* (Berkeley) Saccardo; *Diplodia zeae* (Schweinitz) Leveille] e *Stenocarpella macrospora* (Earle) Sutton [Sin. *Diplodia macrospora* Earle] pertencem a Ascomycetes/Dothideales. As duas espécies apresentam no seu

ciclo de vida somente a fase imperfeita e podem provocar podridões da base do colmo ou podridão da espiga (CASA; REIS; ZAMBOLIM, 2006).

S. maydis foi relatada pela primeira vez em 1884 (SACCARDO, 1944) e *S. macrospora* foi primeiramente relatada, sobre colmos de milho, em 1896 nos Estados Unidos (EARLE, 1897). A primeira ocorrência de *S. macrospora* no Brasil foi em São Paulo relatada por Johann (1935) causando podridão em sementes.

Tanto *S. maydis*, como *S. macrospora* apresentam picnídios subepidérmicos, globosos ou alongados, com coloração marrom-escura a preta, paredes grossas e um ostíolo protuberante papilado (SUTTON; WATERSTON, 1966a; SUTTON; WATERSTON, 1966b; SUTTON, 1980). A distinção entre as duas espécies pode ser feita pelo tamanho dos conídios que em *S. macrospora* mede 44-82 x 7,5-11,5 μm e pode apresentar 1-2 septos, sendo 2 -3 vezes maiores que o os esporos da *S. maydis* (SUTTON, 1980).

Stenocarpella macrospora e S. maydis são no Brasil causador em milho da doença anteriormente, chamada Diplodia, também conhecida por promover a podridão da espiga. Atualmente, estes fungos podem ser identificados nas regiões onde se produz milho levando ao surgimento de sintomas de podridão de diplodia do colmo, podridão branca da espiga e mancha de macrospora nas folhas (CASA; REIS; ZAMBOLIM, 2006). Além disso, esses fungos produzem micotoxinas e relatos atribuem a toxina diplodiol a *S. macrospora* (CUTLER et al., 1980).

Stenocarpella macrospora parece estar restrita a regiões tropicais, localizada em áreas quentes e úmidas, ao passo que *S. maydis* é largamente distribuída onde se cultiva o milho (SUTTON; WATERSTON, 1966). Segundo Costa, Cota e Silva (2013) após a polinização a ocorrência de temperaturas altas

entre 28 e 30ºC e alta umidade favorecem estes patógenos. Ao passo que as podridões de colmo têm maior ocorrência na safra de verão do que na safrinha.

Em relação à disseminação das duas espécies, Carvalho et al. (2004) citam que a semente é de grande importância na transmissão e disseminação dessas espécies. Além de ser a responsável pela introdução do fungo em novas áreas. Os restos culturais deixados sobre o solo após a colheita do milho são fontes importantes de inoculo, pois *S. macrospora* e *S. maydis* são necrotróficos, ou seja, sobrevivem como parasitas na planta viva e saprófitas nos restos culturais (CASA; REIS; ZAMBOLIM, 2006). Nas condições de clima quente e úmido os conídios são liberados dos picnídios que se encontram sobre os restos culturais sendo formados os cirros que serão disseminados pelos respingos de água da chuva e pelo vento (SARTORI, 2003).

Os sintomas podem ser observados no colmo, na folha e na espiga. No colmo são observadas lesões, sendo possível ver pontos (picnídios) pequenos. À medula do colmo fica com coloração marrom, podendo se desintegrar, os vasos lenhosos continuam inteiros, mas é possível observar a presença de picnídios (CASA; REIS; ZAMBOLIM, 2006). O desenvolvimento da planta é comprometido, levando à quebra do colmo, acamamento e morte prematura da planta (REIS; CASA, 1996; SHURTLEFF, 1992).

Quando os sintomas ocorrem na espiga, é possível observar a presença de um crescimento micelial denso e compacto entre os grãos na cor branca que se inicia na base das espigas (CASA; REIS; ZAMBOLIM, 2006). As espigas acometidas são mais leves e podem ser totalmente apodrecidas. Podem ser observados picnídios nos grãos e nas raques das espigas, estes picnídios servem como fonte de inoculo para os próximos plantios (COSTA; CASELA; COTA, 2009). As folhas normalmente são atacadas por *S. macrospora.* As primeiras lesões apresentam-se na forma de tecido clorótico seguido de necrose em alguns

híbridos. Nas áreas necrosadas é possível observar picnídios subepidérmicos, isolados ou agrupados (CASA; REIS; ZAMBOLIM, 2006).

Dentre as medidas de manejo, o genético seria a melhor opção. Entretanto, na literatura não existe resultados claros a respeito do comportamento dos híbridos quanto à resistência a esses patógenos (CASA; REIS; ZAMBOLIM, 2006). Em relação ao controle químico existe pouca informação sobre a eficiência dos fungicidas sobre *Stenocarpella* spp. (APROSOJA, 2015). Esses fungos sobrevivem e se multiplicam na palhada em decomposição e o milho é o seu único hospedeiro (SHURTLEFF, 1992; REIS; CASA, 1996). Dessa forma, a rotação de culturas é extremamente importante para redução do nível do inoculo primário do fungo na área de cultivo (CASA; REIS; ZAMBOLIM, 2006). O tratamento de sementes com fungicidas é outra medida de grande importância no combate a essa doença, por causa da capacidade dos fungos de infectarem e serem transmitidos via sementes (COSTA; COTA; SILVA, 2013).

2.3 Supressividade do solo

Alguns solos apresentam a característica de controlar, de forma natural, alguns fitopatógenos que o habitam. Esse fenômeno é chamado de supressividade e o solo que apresenta este atributo é denominado de solo supressivo. Desta forma, os solos que não são supressivos são conhecidos como solos conducentes (BETTIOL et al., 2009).

Existem solos que suprimem os patógenos por meio da diminuição da densidade de inoculo e suas atividades saprofíticas, ao passo que existem solos que suprimem a doença, promovendo a redução da severidade, mesmo em alta

densidade de inoculo e capacidade de sobrevivência do patógeno (COOK; BAKER, 1983).

A supressividade do solo pode estar relacionada à atividade microbiana, mas também com as propriedades físicas e químicas do solo, como substâncias indutoras de resistência, o tipo de argila, a estrutura e textura do solo, disponibilidade de nutrientes, pH, a condutividade elétrica e a umidade atuando sobre os patógenos e plantas (HORNBY, 1983).

A supressividade do solo pode ser determinada de várias formas. Rodrigues et al. (1998) avaliou a indução de supressividade em diferentes classes de solo de forma indireta, por meio do desenvolvimento da doença utilizando uma escala de notas e o índice de doença. Outra forma de avaliar a supressividade de um solo é através da transferência de porções de solo como feito no trabalho de Eloy et al. (2004) onde se avaliou a severidade da murcha de fusário em Caupi cultivado em uma mistura de duas porções de solo previamente esterilizado e infestado com patógeno. A taxa de extinção relativa da população também pode ser utilizado para caracterização da supressividade dos solos (ALVARADO et al., 2007).

A determinação da atividade enzimática no solo pode ser um indicativo da natureza da supressividade de um solo já que é uma forma de medir a atividade da microbiota deste ambiente. A atividade enzimática do solo também está relacionada com a matéria orgânica, propriedades físicas e com a atividade e biomassa microbiana, podendo ser utilizada como um indicador biológico da qualidade do solo (DICK, 1997).

Entre as enzimas encontradas no solo, as hidrolases tais como β – glicosidase, urease e a fosfatase são as mais estudadas, devido ao fato de que catalisam a quebra de substratos em compostos orgânicos de menor massa molar, facilitando sua mineralização (BURNS, 1983). Acosta-Martıinez e

Tabatabai (2000) relataram a sensibilidade da enzima β-glicosidase a alterações de pH. Esta característica pode ser um bom indicador bioquímico para medir modificações ecológicas resultantes da acidificação do solo em um contexto que envolve a atividades desta enzima. A urease apresenta uma atividade maior com o aumento da temperatura, isto sugere que as temperaturas mais elevadas aumentam a atividade desta enzima (SHUKLA; VARMA, 2011).

A hidrólise do diacetato de fluoresceína é outra medição indireta da atividade microbiana do solo Isso porque este substrato pode ser hidrolisado por diferentes enzimas, por exemplo, as proteases, as lípases e as esterases (SCHNÜRER; ROSWALL, 1982). Estas enzimas são liberadas em quantidades significativas por decompositores primários como bactérias e fungos (LUNDGREN, 1981; SODERSTROM, 1977).

Diversos trabalhos mostraram como a atividade enzimática pode ser afetada, pelo sistema de manejo do solo ou aplicação de resíduos. Matsuoka (2006) concluiu em seu trabalho que as enzimas β-glicosidase, fosfatase ácida, urease e amidase são sensíveis à modificação do sistema de manejo da cultura, de tal forma que indicam alterações no solo. Ji et al. (2014) observaram que o cultivo profundo e o retorno da palha aumentou a microbiota do solo e a maioria das atividades enzimáticas.

2.3.1 Indução de supressividade

A indução de supressividade pode ser conseguida por meio de práticas como rotação de culturas, adição de matéria orgânica que estimulem os antagonistas, modificação do pH para nível de favorecimento dos antagonistas e desfavorecimento dos patógenos, utilização de sistema de cultivo que beneficiem a estruturação do solo e favoreçam antagonistas e hospedeiro, incorporação de matéria orgânica, introdução massal de antagonistas e manejo

correto de irrigação também são maneiras de se induzir supressividade (BAKER; COOK, 1974).

Em se tratando de incorporação de matéria orgânica os resíduos orgânicos são usados para melhorar a qualidade do solo, podendo colaborar para a indução da supressividade por meio da adição da biomassa e da atividade microbiana do solo (BAKER; COOK, 1974). Esses resíduos apresentam em sua composição frações ricas em carbono lábil, que serve como fonte de energia para os microrganismos, além de conter antagonistas. No entanto, com relação à supressão de doenças tem apresentado diferentes resultados (STEINBERG et al., 2007; TERMORSHUIZEN et al., 2006).

Vários resíduos orgânicos têm sido trabalhados em pesquisas, buscando-se avaliar seu efeito nas propriedades do solo e na indução de supressividade a fitopatógenos. Dentre estes o lodo de esgoto, o hidrolisado de peixe, a cama aviaria, esterco suíno. Pinto, Bettiol e Morandi (2010) avaliaram o efeito de casca de camarão, hidrolisado de peixe e quitosana no controle da murcha de *Fusarium oxysporum* f. sp. *chrysanthemi* em crisântemo. A casca de camarão e o hidrolisado de peixe foram incorporados a um substrato à base de casca de Pinus em concentrações diferentes e 50% do volume de água necessário para atingir a capacidade de campo do substrato. Os autores verificaram que a casca de camarão suprimiu a doença na concentração de 4% e, além disso, promoveu o crescimento da planta. No entanto, na concentração de 5% foi fitotóxica. O hidrolisado de peixe não induziu a supressividade à murcha de Fusarium, de forma que a severidade se mostrou maior com aumento da sua concentração no substrato. A quitosana não apresentou uma resposta padrão com resultados variáveis.

Outro resíduo de destaque é o lodo de esgoto derivado do tratamento de águas residuais com potencial para uso na agricultura. O lodo de esgoto

apresenta alta concentração de matéria orgânica e elementos essenciais para as plantas (NASCIMENTO et al., 2004). Guedes et al. (2006) destacaram que o uso deste resíduo como fertilizante e condicionador de solos favorecendo o crescimento de plantas, aumentando a produtividade das culturas, diminuindo o uso de fertilizantes minerais. Oliveira et al. (2002) relataram os efeitos de doses crescentes de lodo de esgoto sobre propriedades de um latossolo amarelo distrófico cultivado com cana-de-açúcar e o aumento nos teores de C-orgânico, condutividade elétrica e pH do solo em dois anos agrícolas.

Com relação à cama aviária e esterco suíno, Santos, Tomazeli e Morales (2009) observaram que a associação desses resíduos orgânicos com solarização no controle de *Sclerotium rolfsii* no feijoeiro é uma alternativa viável. A cama aviária obteve resultados satisfatórios na redução da incidência, severidade e tombamento de plantas causado pelo patógeno.

Podemos observar nos trabalhos relacionados à indução de supressividade de solo resultados variáveis. Desta forma, novos estudos são necessários para mostrar com clareza os benefícios do uso de estratégias que favoreçam tal fenômeno.

3 MATERIAL AND METHODS

The experiments were conducted in a randomized complete block design with four replications in two experimental areas: Federal University of Lavras, in Lavras, and Embrapa Corn and Sorghum, in Sete Lagoas, Minas Gerais state. The concentration, application methods and attributes of each organic residue and urea are shown in chart 1 and table 2. The hybrid DKB 390 VT PRO 2 (DEKALB) was used to obtain infected stalk with *Stenocarpella* spp. This hybrid is susceptible to the disease caused for *Stenocarpella* spp., and is recommended for cultivation in Minas Gerais.

Corn seeds were sowed in areas under 5 years no-tillage system, fertilization was in accordance with recommendation for corn (ALVES et al., 1999) and weed control using glyphosate (before sowing) and atrazina + soberan at the stage V6. Plants were not sprayed with fungicide. For inoculation of stalks, the isolate CML 698 (mycological collection of plant pathology department of UFLA) was replicated in oatmeal agar (VTEC). The plates were incubated at 25°C, when the pathogen abundantly sporulated, plates were flooded with distilled water and the fungal matt was scraped to release the conidia. One ml of suspension of *S. maydis* CML 698 with 10^6 conidia/mL was inoculated with a needle in the second internode of corn 70 days after sowing.

Thirty days after inoculation, the stalks were harvested, transported to the laboratory; and the severity of corn stalk rot was evaluated using the scele proposed by Costa et al. (2014). Half of those stalks were grouped according to the severity of the disease and maintained under refrigeration (-18 °C) until use. The other half was used to determine the initial inoculum.

In the experimental fields, part of the stalks was laid on ground, grouped in blocks according to the disease severity level. The organic materials and urea were immediately applied over the stalks. The evaluation of pathogen population dynamics was carried out after 3 months (KOHL et al., 2015).

Chart 1 Treatments performed in studies in Federal University of Lavras and Embrapa Mayze and Sorghum to evaluate the effects of organic matter, in the inoculum of *Stenocarpella* spp.

Treatment Organic waste / Biocontrol agent	**Concentration and method to apply**	**References**
Poultry litter	960 g (in total area)	Santos; Tomazeli; Morales (2009)
Swine manure	544 g (in total area)	Lazarovits et al. (2009)
Fish hydrolyzed (FishFértil)	0.32 mL (sprayed on the soil surface)	Manufacturer's Recommendation
Sewage sludge	21.3 g + K2O (in total area)	Araújo and Bettiol (2009)
Urea	0.32 g/25,5 mL (sprayed on the soil surface)	Bellotte et al. (2009)
Buried stalk (positive Control)	Buried stalk at 10 cm depth	Casa; Reis e Zambolim (2003)
Stalk in soil surface (negative Control)	Stalks laying on the ground without any treatment	_

Table 2 Composition of Poultry litter, swine manure, sewage sludge , fish hydrolyzed and urea.

Composition	Poultry litter	Swine manure	Sewage sludge	Fish hydrolyzed	Urea
N(g/Kg)	41.30	40.50	34.8	-	-
P (g/Kg)	26.01	64.48	26.73	-	-
K (g/Kg)	36.71	18.21	3.64	-	-
Ca (g/Kg)	49.4	41.11	20.02	-	-
Mg (g/Kg)	11.56	28.82	3.44	-	-
S (g/L ou g/Kg)	30.97	29.85	15.51	-	-
Cu (g/L ou l/Kg)	366.48	200.69	1018.88	-	-
Fe (g/L ou l/Kg)	4341.48	1915.10	48647.17	-	-
Mn (g/L ou l/Kg)	743.43	1039.48	873.43	-	-
Zn (g/L ou l/Kg)	588.21	1956.87	2574.55	-	-
Total Organic Carbon (g/L)	-	-	-	92	-
Calcium Soluble in water (g/L)	-	-	-	11.5	-
Fish and cane molasses	-	-	-	*	-
Nitrogen Total (%)	-	-	-	-	46

Analyses made in the Plant Nutrition Laboratory - Department of Soil Science (UFLA)

The plots consisted of plastic rings (64 cm-diameter containing 8 stalks cut longitudinally each) (Figure 1) arranged in the distance of 2 m between blocks and 1m between treatments.

Figure 1. Plot on the field where the *Stenocarpella* sp. survival was evaluated in the Federal University of Lavras. Stalk before treatments.

After the distribution of the stalk in the plots and application of organinc amendments and again three months later, the soil surface was cleared off but scrapping the soil surface and two soil samples (250 g / sample) were collected per plot and analyzed a the Soil Fertility Laboratory at UFLA following standard procedures.

3.1 Disease assessments

The inoculated stalks were maintained in the field for 3 months following the methodology described by Köhl et al. (2015). After that period, the stalks were collected for the pathogen DNA quantification by qPCR. The samples were kept under refrigeration at -80°C until DNA extraction.

In the plots with Poultry litter, swine manure, fish hydrolyzed, sewage sludge, urea, and control, samples (100 g) were collected for enzymatic analysis (hydrolyze of FDA, Beta glucosidase and Urease). These samples were sieved (2 mm mesh) and stored in refrigerator for evaluation.

3.1.1 Hydrolysis of fluorescein diacetate (FDA)

For FDA analysis the samples were weighed in a glass container and 20 mL of phosphate buffer and 0.2 mL FDA stock solution (2 mg.mL-1) were added to start the reaction and right after, the samples were placed in a shaker (160 rpm) at 25°C. After 20 minutes, 20 ml of acetone were added to each container in order to stop the reaction, and then each sample was filtered in test tubes through Whatman filter paper, number 1. The absorbance of the filtrate was determined in a spectrophotometer at 490 nm. The absorbance values were compared to a standard curve that was made in duplicates for each treatment. Different FDA concentrations of 0, 100, 200, 300 and 400ug and 5 ml of phosphate buffer solution were added to threaded tubes, which were closed and incubated in a water bath for 60 minutes. At the end of FDA hydrolysis, 5 g of soil were placed into an Erlenmeyer flask for each of the five concentrations and 10 ml of phosphate buffer and 5 ml of the hydrolyzate was added to it. The flasks were shaken (160 rpm) and incubated at 25 ° C for 20 minutes. At the end of the incubation period, acetone was added and the solution was filtered through Whatman filter paper number 1 and absorbance read at 490nm. The FDA activity was estimated according to the obtained standard curve (BOEHM; HOITINK, 1992; GHINI; MENDES; BETTIOL, 1998).

3.1.2 Determination of activity β –glycosidase

For the B-glucosidase analysis, 4.0 mL of MUB buffer, pH 6.0 was mixed in a 10mL screw cap test tube to 1g of each soil sample after drying out. Then, the suspension was added to 1 mL of PNG (4-Nitrophenyl β-D-glucopyranoside) Sigma (N7006) 25 mMand incubated (37°C) for 60 minutes, when it was amended with $CaCl_2$ 0.5 M (1mL) and Tris 0.1 M pH = 12 (4.0 mL). Vials were shaken and the suspension was filtered through Whatman paper number 2. Then the absorbance of the filtrate of each sample was read 400 nm using the blank of each treatment to set the equipment. The amount of p-nitrophenol released per gram of dry soil was calculated based on a standard curve. The standard curve was made from a stock solution [100 mL of water + 1 mL of p-nitrophenol solution ($1g.L^{-1}$)]. From this solution, dilutions were made to obtain the concentrations of 0.001 to 0.005 mg mL^{-1} (EIVAZI; TABATABAI, 1988; TABATABAI, 1982).

3.1.3 Urease Activity in soil

For the determination of urease activity in the soil it was used Tabatabai and Bremner (1972) adapted methodology. In 5 g of the soil was added 9 mL of Tris buffer 50 mM. The contents were mixed and afterwards 1 mL of urea solution 200 mM was added. Then it was stirred and incubated in a water bath (37°C) for 2 hours. After the incubation period, 40mL of KCl-Ag_2SO_4 solution was added and the samples were placed to decant for about 30 minutes. For the control, first 40 mL of KCl-Ag_2SO_4 solution was added, and then the urea solution. After this process, the distillation of 20 mL of the obtained extract was made, the distilled solution was subjected to titration with 0.005 M H_2SO_4 and titration volumes for the samples and controls were obtained. To estimate the release of μg NH_4-N/g of dry soil/hour, the following formula was used:

$$\text{Urease} = ((V_t - V_b) \,.\, 50 \,.\, 70 \,.\, F_c) \,.\, (m_{dry} \,.\, 20)^{-1}$$

Where, Vt and Vb mean the volumes of distillation samples and control respectively; m_{seca}=5.Ps/Pu; Fc = correction factor obtained in the titration of 0,005 $_M$ H_2SO_4 solution.

3.1.4 Organic carbon in soil

For soil organic carbon (OC), 1g of the dry soil samples was added to 15mL screw-cap glass digestion tubes mixed to 4 mL of dichromate and 6 mL of sulfuric acid were added. The tubes were incubated at the digester block at 150°C for 40 minutes. In each analysis three heated and three non-heated controls were used. Then the tubes were let cool down on the lab bench at room temperature, the solution was transferred to glass jars in which eight drops of indicator (ferroin) were added and then it was made the titration of the samples using ferrous ammonium sulfate solution [$Fe(NH_4)_2(SO_4)_2$.6H2O] 0.2 mol L^{-1}. Titration of unheated control samples was used to estimate the ferrous ammonium sulfate concentration (NELSON; SOMMERS, 1996; RHEINHEIMER et al., 2008).

3.1.5 Nitrogen and Carbon in soil Total

The analyzes of total carbon and nitrogen were made by the Dumas combustion method, which is the total sample oxidation in the presence of oxygen at elevated temperatures, followed by reduction of nitrogen oxides produced and detection of molecular nitrogen (RIBEIRO, 2010).

3.1.6 DNA extraction and real-time PCR for the presence of *Stenocarpella* sp.

The stalks used for real-time PCR analysis were cut into ca. 2 mm-long segments and milled to obtain ca. 1mm diameter particles and 40 mg was stored at – 18C ° until DNA extraction. DNA extraction was performed using the genomic DNA Purification Wizard® kit (PROMEGA, MADISON, WI) following the manufacturer's recommended protocol.

The test SYBR Green PCR were performed in Rotor Gene 6500 (Corbett Research, Montlake, Australia). For each reaction, a sample was mixed to 2.0µL reaction mixture containing 23µL 12.5µL SYBR Green PCR Kit (Qiagen), 0.75µM of each forward and reverse primer. P1/P2 primers described by Xia and Achar (2001), specific for the genus *Stenocarpella following the protocol* previsouly described for in situ detection. The cycle consisted of an initial 95°C for 3 min, denaturation at 94°C for 30 seconds, annealing at 60°C for 1 minute and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes for a total of 40 cycles. A dilution series of 5 times ranging from 20 ng to 2 pg of DNA isolated from *S. maydis* CML 698 was included in each assay for reference. The analyzes were calculated from the values derived from the threshold cycle (Ct values) for the series of PCR DNA dilutions and the DNA extracts of plant samples (SIQUEIRA et al., 2014).

3.1.7 Climate and geographical data

Lavras located at latitude 21 ° 14 '43 south and longitude 44 ° 59 '59 west, with an altitude of 919 m and Embrapa Corn and Sorghum (Sete Lagoas) with latitude 19º28'S, 44º15'W longitude and altitude of 732 m. The climate information was shown in Table 3.

Table 3 Average temperature, rainfall and humidity data recorded for Lavras and Sete Lagoas during the experiment. (June to Aug., 2015).

	Lavras		
	Temperature	**Rain fall**	**Relative humidity**
1º June	18.1	0.0	76.5
2º July	17.4	0.0	77.0
3º Aug	18.6	0.0	63.3
	Sete Lagoas		
	Temperature	**Rainfall**	**Relative humidity**
1º June	19.2	0.0	66.6
2º July	19.3	0.0	58.3
3º Aug	22.0	0.0	54.1

4 RESULTS

From the soil analysis a significant effect was obtained for the organic amendedments and is presented in details at APPENDIX A (Tables 7-10). The details on the effect of each organic amendment on each nutrient level is presented below.

4.1 Effect on soil property

The application of organic waste resulted in Calcium content increase in Lavras (p = 0.0019908) and Sete Lagoas (p = 0.00066) (Figures 2 and 3).

The organic amendments sewage sludge (44% increase), poultry litter (38%) and swine manure (36%) in Lavras and poultry litter (25%) in Sete Lagoas were the treatments that contributed to the significant increases in Ca content in the soil.

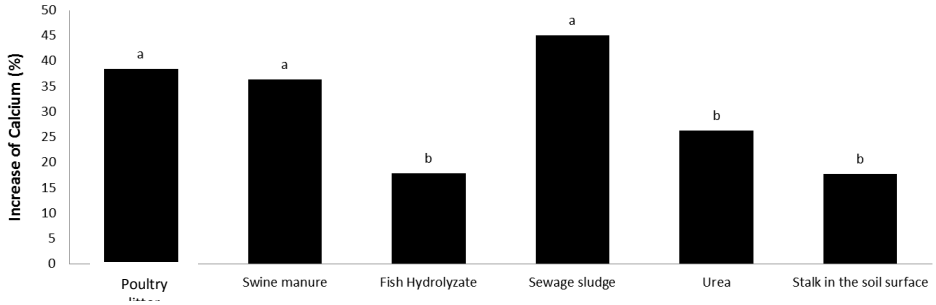

Figure 2 Effect of poultry litter, sewage sludge, swine manure, fish hydrolyzed and urea on the calcium content in soil after three months of application in Lavras. Means followed by the same letter does not differ in level of 5% probability by Scott-Knott (n = 4).

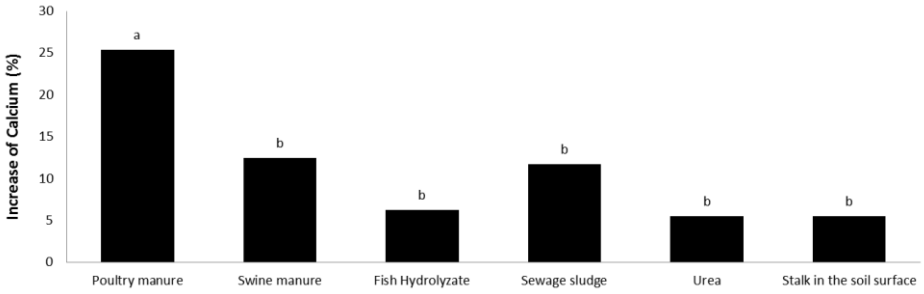

Figure 3 Effect of poultry litter, sewage sludge, swine manure, fish hydrolyzed and urea on the calcium content in soil after three months of application in Sete Lagoas. Means followed by the same letter does not differ in level of 5% probability by Scott-Knott (n = 4).

Table 4 Soil properties after three months to incorporated organic wastes.

Soil Attributes Analyzed		
Soil Properties	**Lavras- MG**	**Sete Lagoas-MG**
	p – value	**p – value**
pH	0.32717	0.64408
K	0.41050	0,53970
Available P	0.42992	0.93970
Mg	0.27563	0.09822
H+Al	0.59703	0.55247
OM	0.54210	0.51448
Remaining P	0.54178	0.98648
Sum of Bases	0.36884	0.88417
CEC at pH 7.0 (T)	0.67713	0.98719
Base Saturation (V)	0.53911	0.31601
Effective CEC (t)	0.34291	0.89784

4.2 Soil organic carbon, total nitrogen and carbon Total

There was no effect on the organic carbon content at either location (data not presented). The soil organic carbon is only expected to increase after a longer period incubation time according to Turco, Kennedy and Jawson (1994), which can take over two years to occur. Thus, the test period may not have been enough for the occurrence of noticeable changes in organic carbon content of the soil.

For the total nitrogen content there was a significant effect at both locations. In Lavras (p= 0.00122) and Sete Lagoas (p= 0.00006). The significant nitrogen content increases were obtained for poultry litter amendment (21%) in Lavras and poultry litter (23%) and fish hydrolysate (21%) for Sete Lagoas (Figure 4 and 5).

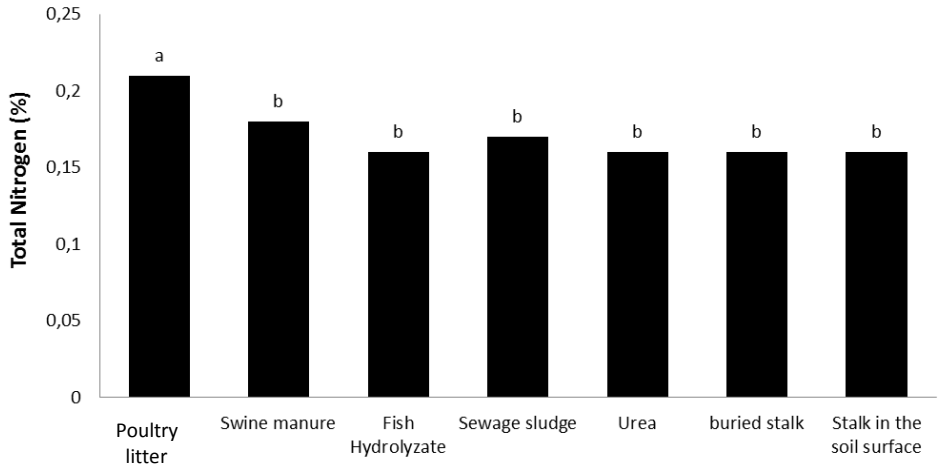

Figure 2 Total Nitrogen in the soil (0-10 cm depth) three months after application swine manure, poultry litter, sewage sludge, fish hydrolyzed, urea in Lavras. Means followed by the same letter does not differ in level of 5% probability by Scott-Knott (n = 4).

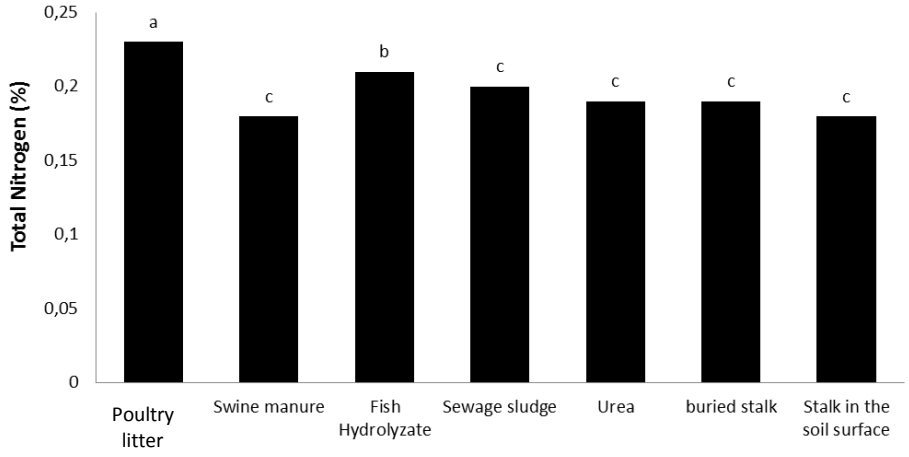

Figure 5 Total Nitrogen in the soil (0-10 cm depth) three months after application swine manure, poultry litter, sewage sludge, fish hydrolyzed, urea in Sete Lagoas. Means followed by the same letter does not differ in level of 5% probability by Scott-Knott (n = 4).

For carbon content, only in Sete Lagoas it was possible to observe significant increases (p= 0.00034) for poultry litter (3.20 %) (Figure 6).

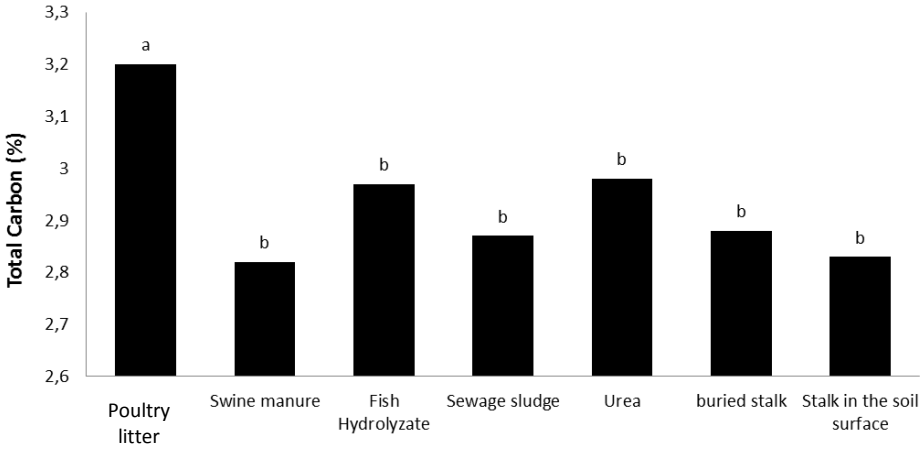

Figure 6 Total Carbon in the soil (0-10 cm depth) three months after application swine manure, poultry litter, sewage sludge, fish hydrolyzed , urea in Sete Lagoas. Means followed by the same letter does not differ in level of 5% probability by Scott-Knott (n = 4).

4.3 Enzymatic activity in the soil

In regard to β-glucosidase activity (p = 0.00001 Lavras and p= 0.00803 Sete Lagoas) and hydrolysis of FDA (p = 0.01781 Lavras and p= 0.03560) a significant effect was obtained and the contribution of each treatment to the obtained result is detailed below (Table 5).

When compared, the treatments for each site, in Lavras the highest β-glycosidase activity was obtained for fish hydrolyzate amendment (16.16 p-nitrophenol µg/g dry soil/h) followed by sewage sludge (15.79 p –nitrophenol µg/g dry soil/h), Poultry litter (p-nitrophenol 14.98 µg/g dry soil/h) and urea (14.02 µg p-nitrophenol/g dry soil/h). The other treatments were similar to the controls. In Sete Lagoas, the its highest activity was induced by poultry litter (14.87 p-nitrophenol µg/g dry soil/h) and urea amendments (p-nitrophenol 12.76 µg/g dry soil/h).

In Lavras, the organic residue poultry litter provided the highest enzyme activity to the soil (1.05 µg FDA hydrolyzed / g dry soil / min), but the control treatment achieved similar results at best average (0.95 µg FDA hydrolysate / g dry soil / min), followed by buried stalk (0. µg FDA hydrolysate / g dry soil / min) and urea (0.86 ug / g of dry soil / min) (Table 9). In Sete Lagoas, treatments that allowed greater FDA activity were poultry, swine manure, urea and fish hydrolyzed with 0.72, 0.61, 0.53, and 0.52 µg FDA hydrolyzed / g dry soil / min, respectively.

Unlike the results found for the other two enzymes, there was no significant effect of treatment for the enzyme urease activity at either location, i.e. Lavras (p-value 0.086866) and Sete Lagoas (p- value 0.91706) (Table 5).

Table 5 Effect of organic matter amendment and urea in the β-glycosidase, urease, and hydrolyze of FDA in soil (deepth 0-10 cm) after three months that were applied.

Treatment	ACTIVITY β-glycosidase p-nitrophenol μg/gdry soil /h		μg of FDA hydrolyzate/g dry soil/ /minute		ACTIVITY UREASE ug of N-NH4/ g dry soil / h	
	Lavras	Embrapa	Lavras	Embrapa	Lavras	Embrapa
Poultry litter	14.98 a	14.87 a	1.05 a	0.72 a	159.00 a	162.71 a
Swine Manure	12.67 b	10.14 b	0.73 b	0.61 a	55.68 a	144.53 a
Fish Hydrolyzed	16.26 a	11.32 b	0.69 b	0.52 a	95.42 a	122.63 a
Sewage Sludge	15.79 a	7.64 b	0.74 b	0.39 b	83.76 a	124.93 a
Urea	14.02 a	12.76 a	0.86 a	0.53 a	90.21 a	130.56 a
Buried Stalk	9.49 c	9.67 b	0.89 a	0.43 b	162.31 a	126.05 a
Stalk in the soil surface	10.77 c	9.34 b	0.95 a	0.33 b	111.92 a	163.70 a
	CV (%):10.7	**CV (%):17.2**	**CV (%):**16.5	**CV (%):**23.1	**CV (%):**45.1	**CV (%):**48.8

Means followed by the same letter in the column do not differ significantly from each other by the Scott-Knott test test the level of 5% probability ($p < 0.05$).

4.4 Real-time PCR

To evaluate the efficacy of qPCR reaction serial dilutions were made with predetermined concentrations of target DNA to establish a standard curve. The Standard curve derived from dilutions of 20 ng to 2 µg of *S. maydis* DNA presented an initial efficiency of 127% for Lavras, slope of -2.809 and R^2 0.98 (Figure 7). Thus, the final reaction observed a known concentration of the log plot of the dilution series against the Ct (threshold cycle) value (Figure 7).

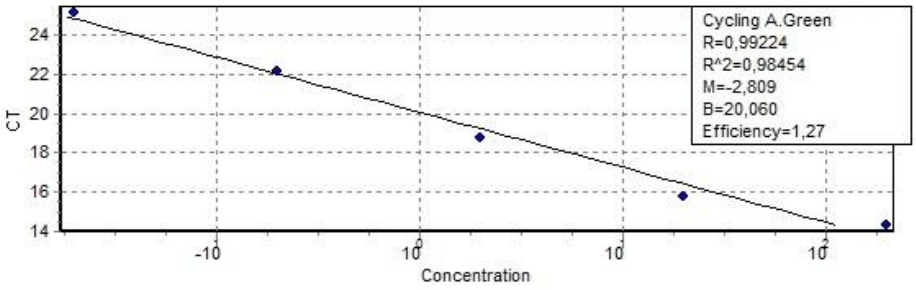

Figure 3 Standard curve DNA for sampling of Lavras and Sete Lagoas. Dilutions of 20 ng to 2 µg of DNA

4.4.1 Detection of *Stenocarpella* spp in corn stalk

The real-time PCR detects the cycle in which the reaction reaches the limit of the exponential phase, this is called the cycle threshold (Ct) (NOVAIS; ALVES, 2004). Thus, lower Ct values indicate a higher concentration of DNA in the sample. The reaction tends to reach the threshold faster than the samples with a smaller amount of DNA. By using the threshold cycle (Ct) obtained by qPCR, it was possible to increase the percentage of the initial collection cycle

for the final collection and if there was a reduction or increase in the DNA of the pathogen.

The increase in the percentage of cycles suggested for the reduction of DNA. Thus, analysis of variance of this variable was significant for both Lavras ($p = 0.04023$) and Sete Lagoas ($p= 0.0100$).

In Lavras buried stalk, stalk in the soil surface and sewage sludge had the largest increase percentage of Threshold Cycle, while in Sete Lagoas buried stalk, fish hydrolyzed, sewage sludge and stalk on the soil surface had the highest Ct treatments (Figures 6 and 7).

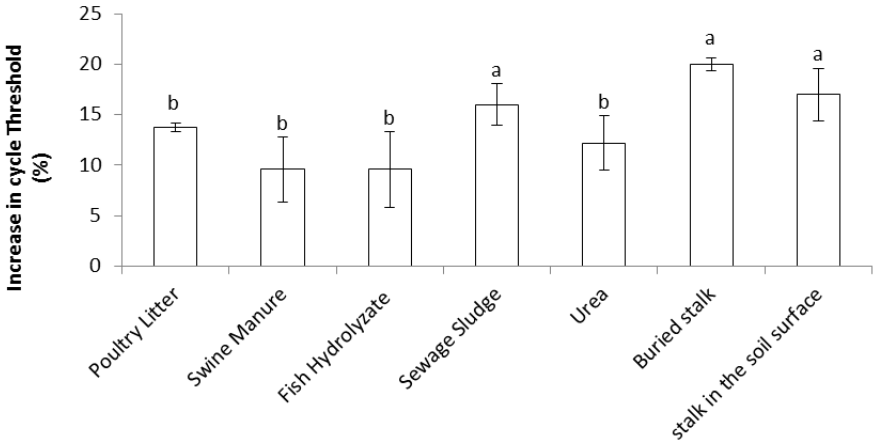

Figure 4 Increase in cycle threshold (%) in number in Lavras comparing samples before each treatment and three months of stalk treatment with organic wastes (sewage sludge, poultry litter, swine manure, fish hydrolyzed) and urea. Means followed by the same letter does not differ in level of 5% probability by Scott-Knott ($n = 4$).

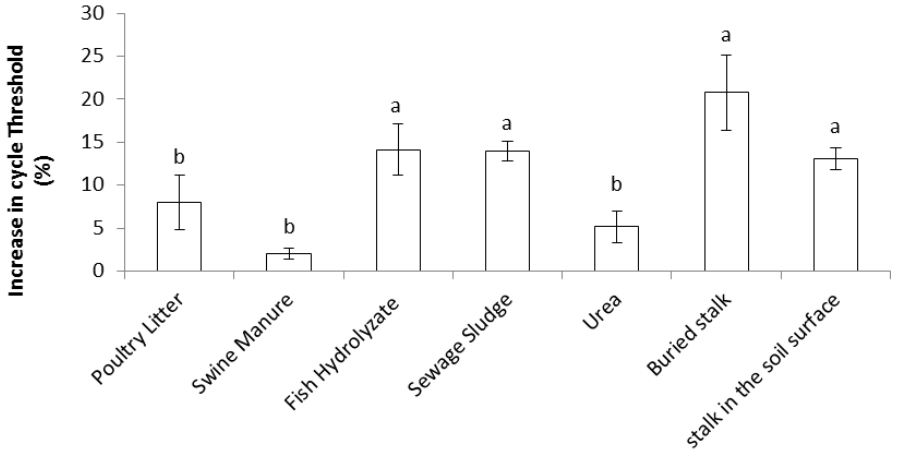

Figure 5 Increase in cycle threshold (%) in number in Sete Lagoas comparing samples before each treatment and three months of stalk treatment with organic wastes (sewage sludge, poultry litter, swine manure, fish hydrolyzed) and urea. Means followed by the same letter does not differ in level of 5% probability by Scott-Knott (n = 4).

4.5 Correlation between the soil properties, enzymatic analysis and percentage of increase in the value of Ct

FDA hydrolysis activity was positively correlated (Table 6) with the Ct increase in Lavras, but in Sete Lagoas there was no significant correlation between these variables (Table 6). The correlation between the increase in the percentage of Ct and Ca were negative for the Sete Lagoas experiment. Correlation between temperature and Ct in Lavras – MG was also negative (Table 6).

For β-glucosidase, carbon, nitrogen and total no significant correlation with the increase in the Ct for any percentage found in site (Table 6).

Table 6 Pearson correlation coefficients between enzymatic activity (β- glyc. and FDA) total carbon, total nitrogen, calcium and sum of bases.

	Lavras		Sete Lagoas	
	Correlation (r)	**p – Value**	**Correlation (r)**	**p – Value**
β –glycosidase	-0.152157	0.3757 ns	-0.096304	0.5763 ns
FDA	0.947566	0.0524 *	0.917433	0.0826 ns
Total C	-0.265125	0.1181 ns	-0.068296	0.6923 ns
Total N	-0.112112	0.5151 ns	0.125465	0.4659 ns
Calcium	-0.013773	0.5210 ns	-0.458386	0.0243 *
Temperature	-0.317785	0.0589 *	-0.084350	0.6247 ns
Humidity	0.201647	0.2383 ns	-0.203670	0.2335 ns

* Significant correlation; ns= non- correlation

5 DISCUSSION

Regarding the influence of the amendments of organic matter on the soil properties, a significant increase in calcium content of soil was observed (Figures 2 and 3). These organic amendments, provided approximately 49 g/kg, 20 g/kg and 71 g/kg for poultry litter, swine manure and sewage sludge, respectively. Santos, Tomazeli and Morales (2009) observed an increase in the Ca, pH, sum of bases, cation exchange capacity and base saturation, a reduction of potential acidity and elevation of P, K, Mg, Cu and Zn. Nascimento et al. (2004) observed an effect of sewage sludge in Ca content. These authors observed also an increase of organic matter, N, P, K, Mg and Na. The effect of swine manure in the soil has been extensively studied. Ceretta et al. (2003) reports a considerable addition of Ca. Gomes et al. (2005) showed an increase of organic carbon, Ca, Mg, K and P in soil amendment of swine manure. However, Queiroz et al. (2004) did not observe changes in Ca contents.

The incorporation of Poultry litter showed an increase in total N and C (Figures 4 and 5). The best poultry litter amendment results for those variables is expected since this organic waste is considered a good source of nutrients, in particular N. While, when used properly can replace, partially or completely, the chemical fertilizer (WATTS; SMITH; TORBERT, 2012). Scherer (1995) and Zarate, Vieira and Cabeças Júnior (1997) reported that poultry litter is rich in nitrogen and its amendment to the soil increased crop yield. Blum, Kothe and Simmler (1999) reported the effect of poultry litter amendment in reducing the survival *Phytophthora capsiciin* the soil. The effect of Poultry litter in total N and C on soil was also observed by Adeli et al. (2007).

The increase in nutrients provided by the application of organic waste, in addition to improving soil quality, which consequently promotes plant development, promotes a reduction of pathogens and promotes microbial biomass (ABBASI; CONN; LAZAROVITS, 2004). Increased activity of β-

glicosidase and FDA was observed upon organic waste amendments tothe soil (Table 5).

Activity of β-glucosidase and FDA at both locations increased the soil amendment with Poultry litter and urea (Table 5). In the experiment of Lavras, an increase in its activity for fish hydrolyzed and sewage sludge. In Sete Lagoas the soil amendment with Poultry litter followed by urea had the highest contribution to the enzyme activity (Table 5). The increase in the enzyme activity after poultry litter amendment to the soil may have an effect on the soil microbial community which in turn would contribute to both the enzyme activity and to the suppressivity to the pathogen (PASSOS et al., 2008).

Fish hydrolyzed increased the β-glucosidase in Lavras and hydrolysis of FDA in Sete Lagoas assays. This product is rich composition which stimulates the activity of soil microbiota and consequently the enzymatic activity (INSTITUTO DE AGRICULTURA BIOLOGICA, 2015).

Similarly to previous report (SOUZA et al., 2009), we observed an increase in β-glucosidase activity and the sewage sludge can also stimulate the microbial activity (FERNANDES; BETTIOL; CERRI, 2005).

Effect on the FDA hydrolysis observed in stalk in the soil surface and buried stalk may be related to the fact that the experiment was done in no-tillage area. In this system the organic material contributed to the deposit to carbon and nutrients to the soil which in turn foster the development of microorganisms and the least fluctuation in temperature and moisture, which is another factor that favors microbial activity (SAFFIGNA et al., 1989). For Poultry litter, a FDA hydrolysis activity indicated the suppressiviness activity to broad range of pathogens.

The positive correlation between FDA activity and increased Ct number indicates that increased hydrolysis of fluorescein occurred reducing the amount of DNA of the pathogen detected. The elevated FDA activity is one of the

indications of microbial activity in the soil (SCHNÜRER; ROSWALL, 1982), which may be related to the reduction in the percentage represented by the pathogen Ct.

Increase in the decomposition of crop residues also was observed by Assis et al. (2003). Then, the plant residues become a source of energy and nutrients to the soil microorganism community, favoring biological activity and improving ecological relationships (POWLSON; BROOKES; CHRISTENSEN, 1987). This may reflect on the rates of enzymatic soil activity.

Several studies highlight the effect of organic wastes in the activity of urease (TRANNIN; SIQUEIRA, MOREIRA, 2007). However, in this study there was no effect of organic residue on the urease activity. The activity values observed are shown variable with values of 55 µg of NH4-N/g dry soil/h to swine manure and 160 µg of NH4-N/g dry soil/h buried stalk in Lavras and 122 µg of NH4-N/g dry soil/h for Fish Hydrolyzed and 163 for stalk in the soil surface in Sete Lagoas. Klose and Tabatabai (1999) also observed this variation in urease activity in the microbial biomass (23 and 146 µg of NH4-N/g^{-1} soil/2 h).

The increase in the number of cycles threshold (Ct) indicated a reduction in the sample DNA concentration. When analyzing the amount of DNA by RT-PCR we found that at both locations the positive control increased the percentage of Ct. The stalks were incorporated into the soil during higher humidity providing greater microbial activity stimulating the decomposition, which can be explained by Summerel and Burgess (1989) suggest that when there is partial incorporation of crop residues as the conventional tillage system, there is greater contact with this soil due to mechanical breakdown of the Stalk favoring the decomposition of this waste. In addition, the increased contact of the Stalk buffers the temperature and humidity fluctuations, favoring microbial activity.

There was a reduction of the pathogen in infected plots with stalk without application of residues. This is frequently found in corn planting areas where no tillage is performed and the Stalk is left on the soil surface until the next planting (SILVA et al., 2009). The reduction in the amount of pathogen DNA in this treatment may be related to several factors such as tillage system that maintains a continuous supply of organic matter to the soil (SILVA et al., 2009) can act in the suppression to the pathogen.

Climatic parameters have also a strong influence on plant pathogens and, in this case, the temperature has a direct link to the DNA concentration detected by qPCR. The temperature in Lavras ranged from 12.9 to 25°C and in Sete Lagoas 12.9 to 26°C during the assays. Low moisture has been observed at both locations. These observed temperatures during the three months timeframe in which the experiment was conducted were not the ones reported as favorable to *Stenocarpella* spp mycelial growth (23 to 28 ° C) and sporulation (26 to 33°C) (CASA, 2000).

Reduction of pathogen detection in soil amendment with sewage sludge and fish hydrolyzed was observed at Sete Lagoas. Sewage sludge present potential to control of soil-borne diseases (LEWIS et al., 1992; LUMSDSEN; MILLNER; LEWIS, 1986, SANTOS; BETTIOL, 2003). This suppression may be related to changes in soil microbial activity (CRAFT; NELSON, 1996; LUMSDSEN; MILLNER; LEWIS, 1986).

Fish hydrolyzed is a byproduct for marine fish base with a rich nutrient composition (Table 2). This product is based on fermented fish waste and has the potential to control plant pathogens directly, in addition to inducing resistance in the host or stimulate natural biological control agents (ABBASI; CONN; LAZAROVITS, 2004). At the studied timeframe (3 months), the fish hydrolizate did not reduced the pathogen survival compared to the controls but reduced it compared to other treatments. We hypothecized that although the fish

hydrolizate has the potential for suppressiveness to pathogens (TENUTA; CONN; LAZAROVITS, 2002) and an expected one to *Stenocarpella* sp, it also supplied the pathogen with essential nutrients that are lacking or limiting in the corn stalk such as calcium and therefore, at short term, the contribution to the pathogen nutrition and multiplication is higher than the eventual suppressiveness which resulted in a result similar to the untreated stalk.

6 CONCLUSION

Poultry litter, swine manure and sewage sludge incorporated into the soil increased calcium and Poultry litter increased nitrogen and carbon contents in the soil.

The activity of β-glucosidase increased when amendment the soil of Poultry litter, fish hydrolyzed, sewage sludge and urea in Lavras. In Sete Lagoas Poultry litter and urea increased this activity. All organic wastes, except for sewage sludge, increased hydrolysis of FDA, as well as treatments with buried stalk and stalk in the soil surface.

Sewage sludge and fish hydrolyzed showed action on reduction of the pathogen population compared to other amendments but not to the untreated controls, while poultry litter, swine manure and urea showed lower reduction of its population.

Fish hydrolyzed and sewage sludge are promising in induction of suppressiveness since they fostered the increase in the soil enzymatic activity and reduced the pathogen population.

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APPENDIX

APPENDIX A - Soil analysis of the initial collection and final collection (Lavras – MG and Sete Lagoas – MG)

Table 7 Soil analysis of the first sample in Lavras - MG

Collect 1						
LAVRAS						
	Poultry Litter	**Swine Manure**	**Fish Hydrolyzate**	**Sewage Sludge**	**Urea**	**Stalk on the surface**
pH	5.55	5.75	5.70	5.55	6.00	5.60
k	119.00	138.00	110.00	147	127.00	126.00
P	20.82	14.02	38.24	20.08	20.14	26.04
Ca	2.40	2.45	2.55	2.2	2.25	2.30
Mg	0.40	0.30	0.40	0.5	0.25	0.30
H+Al	3.83	3.88	3.88	3.24	4.07	4.28
SB	3.00	3.02	2.95	3.56	2.69	2.825
t	3.10	3.17	3.10	3.66	2.84	3.01
T	7.11	6.89	6.39	6.995	6.90	7.365
V	43.96	44.16	42.25	50.91	41.12	40.34
MO	2.42	2.48	2.23	2.36	2.11	2.23
P-rem	24.37	24.38	23.53	27.905	22.61	21.55
S	13.6	12.61	11.68	10.785	22.6	20.73

Table 8 Soil analysis of the first sample in Sete Lagoas - MG

Collect 1						
SETE LAGOAS						
	Poultry Litter	**Swine Manure**	**Fish Hydrolyzate**	**Sewage Sludge**	**Urea**	**Stalk on the surface**
pH	6.25	6.5	6.9	6.3	6.85	6.67
k	155,00	120,00	117,00	130,00	121,00	120.5
P	34.33	27.81	32.72	24.83	30.86	29,33
Ca	8.1	7.65	8.25	8.2	9.1	8.22
Mg	1.1	0.9	1.1	1.1	1.15	1.1
H+Al	3.68	3.97	2.33	3.56	2.45	3.01
SB	9.57	8.83	9.65	9.63	10.61	9.64
t	9.67	8.88	9.75	9.73	10.71	9.74
T	13.25	12.93	12.49	13,64	13.13	13.03
V	72.73	69.65	80.66	74.87	79.52	77.2
MO	2.80	3,00	2.93	2.93	2.87	2.93
P-rem	20.14	19.11	19.68	18.64	19.04	19.08
S	8.85	7.22	6.63	8.68	6.06	6.92

Table 9 The final sample of soil analysis in Lavras - MG

Collect 2						
LAVRAS						
	Poultry Litter	**Swine Manure**	**Fish Hydrolyzate**	**Sewage Sludge**	**Urea**	**Stalk on the surface**
pH	5.4	5.75	5.35	5.45	5.6	5.1
k	210.00	192.00	195.00	179.00	196.00	159.00
P	48.04	25.17	17.84	7.76	22.48	20.14
Ca	3.4	3.55	2.85	3.15	2.75	2.7
Mg	0.7	0.85	0.6	0.65	0.5	0.5
H+Al	4.07	3.6	4.19	4.42	4.12	5.34
SB	4.68	4.96	4,00	4.24	3.74	3.66
t	4.78	5.06	4.1	4.34	3.85	3.76
T	8.69	8.32	8.33	8.4	7.86	8.89
V	54.06	58.77	48.88	48.98	47.62	40.96
MO	2.61	2.87	2.54	2.42	2.54	2.48
P-rem	16.41	16.45	14.17	14.82	14.38	14.82
S	16.65	8.64	11.73	8.02	9.75	13.96

Table 10 The final sample of soil analysis in Sete Lagoas - MG

Collect 2						
SETE LAGOAS						
	Poultry Litter	**Swine Manure**	**Fish Hydrolyzate**	**Sewage Sludge**	**Urea**	**Stalk on the surface**
pH	5.9	6.00	6.15	6.15	6.25	6.15
k	169.00	174.00	147.00	128.00	152.00	140.00
P	40.41	33.35	52.07	30.39	33.23	48.36
Ca	9.83	8.81	9.17	9.41	9.77	9.58
Mg	1.04	1.13	1.08	1.16	1.17	1.11
H+Al	3.11	3.20	3.13	2.78	2.73	3.27
SB	11.45	10.54	10.61	11.01	11.39	11.13
t	11.45	10.54	10.61	11.01	11.39	11.13
T	14.53	14.04	13.75	13.76	14.01	14.26
V	78.58	75.96	77.18	79.82	80.36	77.26
MO	3.34	3.63	3.63	3.41	3.55	3.7
P-rem	12.93	11.75	12.53	11.56	12.24	11.94
S	10.39	9.71	8.23	10.45	7.25	8.43