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Host status and disease threshold of *Rhizoctonia solani* and *Fusarium solani* on *Salvia hispanica* L. and its suitability as a rotational crop to *Nicotiana tabacum* L.

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ABSTRACT

The study was done following the continued outbreaks of problematic diseases in the production of tobacco (*Nicotiana tabacum* L.) in Zimbabwe which amongst the problematic ones are *Rhizoctonia solani* and *Fusarium solani*. The experiment was conducted at Marondera University of Agriculture Science and Technology in Zimbabwe, aiming to evaluate the suitability of Chia as a rotational crop to tobacco. Chia is a seed oil producing legume crop which is regarded the most suitable in rotation with tobacco at the moment because of its monetary benefit to the farmer hence will encourage crop rotation. An experiment was set up with two trials in three growing seasons arranged in a complete block design. *Fusarium solani* five treatments were used one control and four other different rates of inoculum 2.5g, 3.5g, 5.5g and 7.5g/10ml of Distilled water and *Rhizoctonia solani* five treatments were used with a control and four other different inoculations rates inoculated at 0.1g, 0.2g, 0.4g and 0.6g/10ml Distilled water. Data collection was based on the disease severity scale and index of Chia, and also histochemical parameters which were hydrogen peroxide content and malonaldehyde content. There was significant ($p < 0.05$) differences between the different inoculation rates of all the measured parameters under the distinct diseases. Hence Chia cannot be rotated with tobacco even under low levels of disease threshold within the soil, unless proper and intensive management practices are put in place.

KEYWORDS: Tobacco, chia, rhizoctonia solani, fusarium solani, hydrogen peroxide, disease severity

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INTRODUCTION

In Africa Zimbabwe is the number one producer of tobacco and the world's fourth-largest producer of flue-cured tobacco, after China, Brazil and the United States of America [1]. The country does not have a large tobacco manufacturing industry and produces only enough cigarettes to supply domestic demand and provide a relatively small volume for export. Tobacco production makes an important contribution to GDP, export revenue, and plays a major role in the national economy [2]. The major challenge of concern in tobacco production, has been the outbreaks of diseases across the major growing regions in Zimbabwe. Regardless of the synthetic chemicals which have been approved for disease management, the world environmentalist has advocated for green practices that are eco-friendly and for that reason, crop rotation was seen to be the

most ideal practice, hence the need to evaluate on the possible rotational crops which will alternate with tobacco.

Crop rotation is one of the most effective and inexpensive methods known to increase the efficiency of flue-cured tobacco production [3]. Crop rotation has long been an important practice for maintaining and improving soil fertility and condition, minimizing erosion and slowing down the build-up and spread of pests, diseases, and weeds [4]. It is therefore a corner-stone of integrated pest management (IPM) programs and for achieving sustainable crop production [5]. Continuous tobacco culture, even in the best of fields, promotes erosion of the soils and compromises the soil structure, which will eventually reduce the capacity of plants in such fields to obtain enough plant food and water for maximum production [6]. In addition, crop rotation has proved to be the most appropriate

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and beneficial practice for control of tobacco diseases, insects, and weeds [7,8,9]. Not only does crop rotation reduce losses in yield and quality to these pests, but it also reduces the need for expensive pesticides, thus reducing production costs [10,11]. Crop rotation can, therefore be advantageous by increasing net economic returns to producers, increasing the yield and quality from each field and lastly reducing the costs of producing flue-cured tobacco.

In tobacco production, crop rotation is practiced mainly to assist in the control of the common most prevalent disease causing micro-organisms such as soil borne fungi and nematodes, to a lesser extent, to assist in control of insect pests, broom rape and weeds [12]. In Zimbabwe the tobacco sector is encouraging farmers to diversify into other high value export crops such as chia, an oil seed, to boost their income [13]. “Chia is a non-traditional crop, it does and good yields are obtainable. Chia is also resistant to diseases and pests that affect many other crops hence there is no need for chemical use. Also Chia is an ideal crop for diversification since the optimum time for growing it is between January-April which normally is during the tobacco marketing season.

Chia (*Salvia hispanica* L) is an oleaginous, annual and summery plant, which adapts well to the Zimbabwean geography climate. It belongs to the *Lamiaceae* family, native to southern Mexico and northern Guatemala [14,15]. It is a staple food of the Central America civilizations in pre-Columbian period, along with corn, beans and amaranth [16].

Chia seeds are rich in oil, mucilage, dietary fibers and protein. Its oil accounts for 33% of its seed weight, with 68% being α -linolenic acid the highest percentage recorded in oilseeds [17,18]. Seed consumption has not shown any of the problems associated with other sources of omega-3 fatty acids, such as fishy taste, weight loss in animals or digestive problems [19]. Chia contains 25% dietary fibre (10% soluble fibre of very high molecular weight) and 20% content of gluten-free proteins, which makes it suitable for people suffering from celiac disease [20].

However, looking at all those aforementioned facts and information about Chia, there is a need to evaluate on the potential capability of Chia to precede tobacco in every marketing season, hence the needs to carry out disease pressure studies against the following objectives.

Main Objective

- To investigate the host status of Chia to two important tobacco soil borne diseases.

Specific Objectives

- Determination of the host status of Chia to *Rhizoctonia solani* and *Fusarium solani*
- To determine the disease index of Chia as exposed to different disease pressure of the respective pathogens
- To determine the Malonaldehyde and hydrogen peroxide concentration of Chia after exposure to the fungal pathogens under study

Hypothesis

- There is no significant difference in the host status of Chia to *Rhizoctonia solani* and *Fusarium solani*.
- There is no significant difference on the disease index of Chia as exposed to different disease pressure of the respective pathogens
- There is no significant difference on the Malonaldehyde and hydrogen peroxide concentration of Chia after exposure to the fungal pathogens under study.

MATERIALS AND METHODS

Description of Site

The study was carried out at Marondera University of Agriculture Science and Technology located 72km East of Harare at latitude 18° 19' S and longitude 31° 55' E with an altitude of 1479 m above sea level.

Rhizoctonia Solani Isolation

Rhizoctonia solani isolate, *R. solani* AG 4, was obtained from the Kutsaga Research Station Plant Pathology Division culture collections. The isolate was originally isolated from a tobacco seedling stem base affected by damping-off using Potato-Dextrose Agar. The isolate was stored under oil and maintained at room temperature before being revived and checked for viability by growing on PDA and pathogenicity by inoculating tomato seedlings.

Fusarium solani Production and Isolation

Fusarium solani isolate was obtained from the Kutsaga Research Station Plant Pathology Division culture collections. The isolate was originally isolated from a tobacco stem base affected by Fusarium wilt using Potato-Dextrose Agar. Barley seed was used to prepare pathogen inoculum for infestation of growing substrate. Seeds (3 kg) were washed and soaked overnight and autoclaved at 121°C for 15 min [21]. Barley seeds were inoculated with agar plugs from a 3 day old PDA culture and placed into an incubator at 28°C for 5 days. Inoculated seeds were dried under laminar flow for 3 days before being ground using a hammer mill and stored in paper bags at room temperature. The isolate was stored under oil and maintained at room temperature before being revived and checked for viability by growing on PDA and pathogenicity by inoculating tomato seedlings.

Treatments

The treatments for this experiment were laid according to the inoculum densities, for both *R. solani* and *F. solani*. The untreated control had 10ml of distilled water applied to it, followed by 0.1g, 0.2g, 0.4g, 0.6g of *R. solani*/10ml of distilled water applied as a drench, and 2.5g, 3.5g, 5.5g, 7.5g of *F. solani*/10ml of distilled water applied as a drench.

Data Collection

Disease Severity (DS)

After eight weeks following pathogen inoculation, the chia plants were uprooted and washed thoroughly before *R. solani* root and stem lesion assessments were done. All plants were assessed for the disease. Assessments were done by visually observing the roots and stems of the plants and assigning disease scores with score 0 = no damage, 1 = 25% root and stem discoloration, 2 = <25% of root system rotted away, slight wilting of the plant, 3 = 50 – 75% of roots discoloured, 25 – 50% of root system rotted away, moderate wilting of the plant, 4 = > 75% of roots discoloured, >50% of root system rotted away, severe wilting of the plant and 5 = Plant dead

Disease Index (DI)

Disease index (DI) and relative resistance index (RRI) were calculated by the following formulas.

$$DI = \frac{(n_0 \times 0) + (n_1 \times 1) + (n_2 \times 2) + (n_3 \times 3) + (n_4 \times 4) + (n_5 \times 5)}{(Y \times 5)} \times 100$$

where n_x = number of seedlings in severity class x and Y = total number of tubers:

where, n_0 = number of seedlings in 0 rating; n_1 = number of seedlings in 1 rating; n_2 = number of seedlings in 2 rating; n_3 = number of seedlings in 3 rating; n_4 = number of seedlings in 4 rating; n_5 = number of seedlings in 5 rating.

Determination of H_2O_2 content

Hydrogen peroxide (H_2O_2) content was measured according to [22]. In summary, about 0.5 g of Chia tissue was ground in 1 ml of 0.1% (w/v) triochloactive acid (TCA) on ice and centrifuged at $13,000 \times 20$ min. 1 cm^3 of potassium phosphate buffer and 1 cm^3 of potassium iodide (KI) was added to 0.5 cm^3 of the supernatant. The absorbance of the supernatant was measured at 390 nm and H_2O_2 content was calculated using a standard curve.

Determination of Malondialdehyde (MDA)

Malondialdehyde (MDA) content was measured and determined by the method described in [23]. Chia tissue (0.5 g) was ground in 5 cm^3 of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at $10,000 \times g^{-1}$ for 20 min. To the 1 cm^3 of the supernatant, 4 cm^3 of 0.5% thiobarbituric acid (TBA) in 20% TCA was added. The mixture was heated at 95°C for 30 min and cooled in an ice bath. The absorbance was calculated at 532 nm and 600 nm, immediately after centrifugation at $12,000 \times g^{-1}$ for 20 min. The value for non-specific absorption at 600 nm was then subtracted from that of 532 nm. MDA concentration was calculated using an absorption coefficient of $155 \text{ Nm}^{-1} \text{ cm}^{-1}$

Data analysis

Data was analyzed using Genstat 18th edition. Separation of means was done using Fisher's unprotected least significant difference test.

Trial Design, Treatments and Maintenance

A complete randomized design of with five treatments was used. Each treatment was replicated five times and each plot consisted of three plants.

RESULTS

Pathogenicity of *Rhizoctonia Solani* (Kühn (Teleomorph: *Thanatephorus Cucumeris* (Frank) Donk), to Chia (*Salvia hispanica. L*) at Different Rates of Inoculation

Chia was exposed to *R. solani* AG 4 disease pressure and the results gathered indicated that Chia is prone to fungal infection and pressure. There was significant ($p < 0.05$) differences between the different rates of the inoculated *R. solani* with *R. solani* (0.6g/10ml of distilled water) having the highest severity on assessments (Figure 1). The untreated control had the lowest severity in terms of disease infestation, but there was some infection, most probably due to cross contamination amongst the pots. There were also some comparable similarities which were quantified, with 0.2g/10ml treatment giving statistically some similarities in terms of disease severity to Chia. Rates of inoculum indicated that in as small a quantity as 0.2g/10ml of *R. solani* inoculation, Chia succumbed to disease pressure.

Pathogenicity of *Fusarium solani* (Mart.) Sacc. (teleomorph = *Nectria haematococca* (Berk. & Br.) to Chia (*Salvia hispanica. L*) at different rates of inoculation

There was significant ($p < 0.05$) differences between the different inoculation rates of *Fusarium solani* on Chia. The untreated control had the least disease severity as

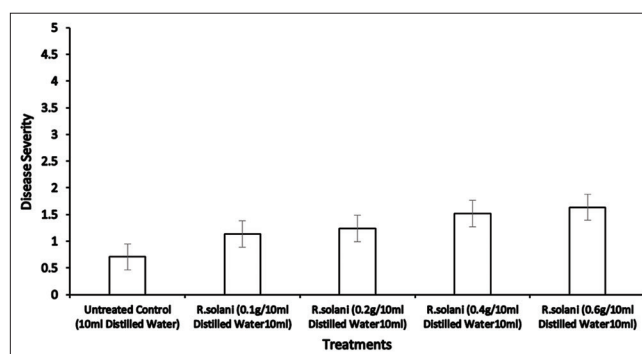


Figure 1: Pathogenicity of *Rhizoctonia solani* (Kühn (teleomorph: *Thanatephorus cucumeris* (Frank) Donk), to Chia (*Salvia hispanica. L*) at different rates of inoculation Bars are LSDs ($P=0.05$) for comparisons between "non-control" points

compared to the 7.5g/10ml of distilled water inoculated treatments. There was a significant increase of 44% disease infestation and severity after inoculating with the highest rate (7.5g/10ml). The rest of the other treatments (2.5g up to 5.5g/10ml) were comparable, with no significant statistical differences (Figure 2).

Effect of *Fusarium solani* (Mart.) and *Rhizoctonia solani* (Kühn) on the histochemical content of Malonaldehyde (MDA) in the tissues of Chia (*Salvia hispanica. L*)

The MDA content of Chia was measured to determine the level of lipid peroxidation upon infection by the two pathogens. Results obtained showed a significant ($p < 0.05$) increase in MAD following the increased rates of disease pressure on Chia (Figure 4.3). MDA is considered a general indicator of lipid peroxidation [24]. The MDA produced during lipid peroxidation is an indicator of cellular damage at the cell membrane by pathogenic infection [25]. The MDA content was statistically similar in the control plants for both

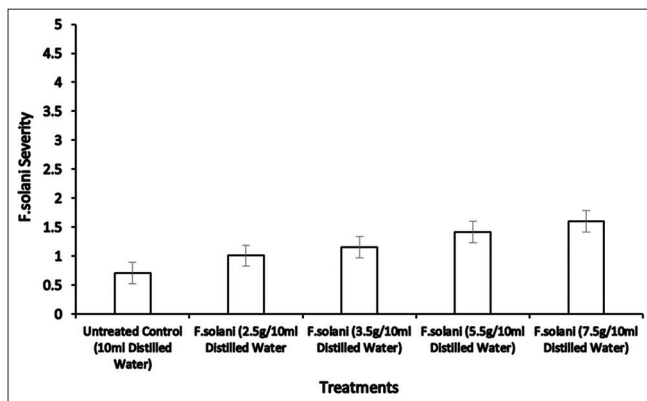


Figure 2: Pathogenicity of *Fusarium solani* (Mart.) Sacc. (teleomorph = *Nectria haematococca* (Berk. & Br.) to Chia (*Salvia hispanica. L*) at different rates of inoculation. Bars are LSDs ($P=0.05$) for comparisons between “non-control” points

Fusarium solani and *Rhizoctonia solani* experimental plots. The extent of the cellular damage caused by the oxidative stress related to the plant response against the pathogen infection can be estimated by the products of the peroxidation of membrane lipids [25]. In the present study, MDA levels were found to be significantly increased in inoculated leaves of Chia (Figure 3).

Effect of *Fusarium solani* (Mart.) and *Rhizoctonia solani* (Kühn) on the histochemical content of Hydrogen Peroxide (H_2O_2) in the tissues of Chia (*Salvia hispanica. L*)

There were significant differences ($p < 0.05$) between the different administered treatments on the hydrogen peroxide content of Chia as shown in Figure 4. The administered different rates of inoculum showed that Chia’s response to pathogenicity results in constitutive synthesis of hydrogen peroxide as a signalling compound and also as a systemic defence mechanism. The highest inoculum concentrations (0.6g per plant for *R. solani* and 7.5g per plant for *F.solani*) had significantly high concentrations of Hydrogen peroxide in their tissues. The untreated control had the lowest concentration of hydrogen peroxide and the rest of the other treatments were comparable for both pathogens under this study.

Effect of *Fusarium solani* (Mart.) and *Rhizoctonia solani* (Kühn) on the Disease Index (DI) of Chia (*Salvia hispanica. L*)

The infection of *R.solani* and *F. solani* had a significant impact on Chia as shown by the disease index in Figure 5. The trend was literally the same following some of the measured parameters where the highest rates of inoculum for the two diseases showed highest disease index percentages. Although there was some statistically similar infection percentages on *F.solani*, it can be concluded that Chia succumbs very much to these fungal pathogens even at inoculum rates as low as 2.5g per plant for *R.solani* and 0.2g per plant for *F. solani*.

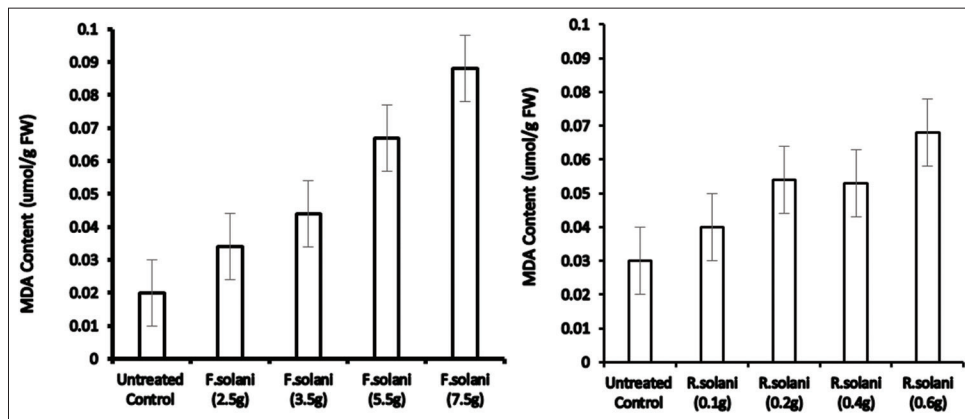


Figure 3: Effect of *Fusarium solani* (Mart.) and *Rhizoctonia solani* (Kühn) on the histochemical content of Malonaldehyde (MDA) in the tissues of Chia (*Salvia hispanica. L*)

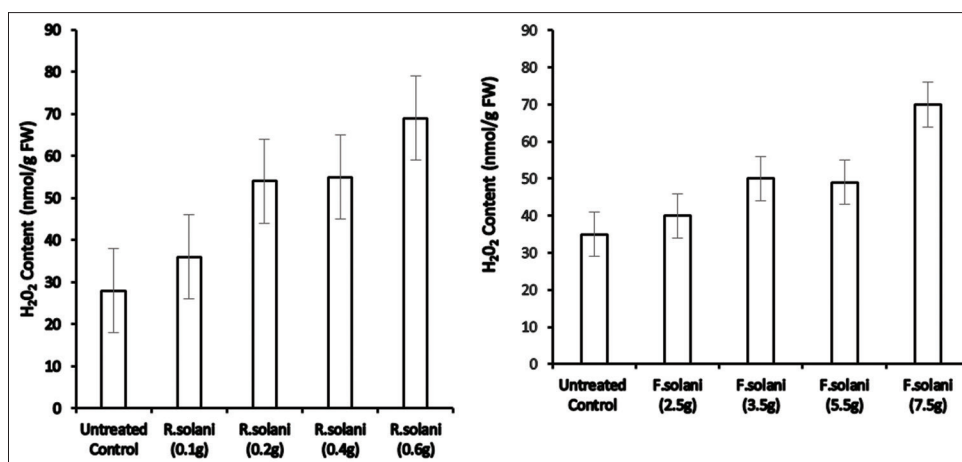


Figure 4: Effect of *Fusarium solani* (Mart.) and *Rhizoctonia solani* (Kühn) on the histochemical content of Hydrogen Peroxide (H₂O₂) in the tissues of Chia (*Salvia hispanica*. L)

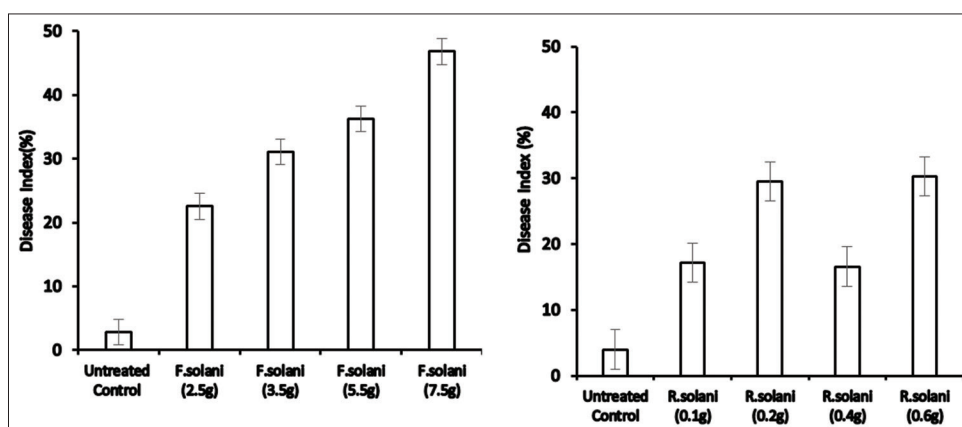


Figure 5. Effect of *Fusarium solani* (Mart.) and *Rhizoctonia solani* (Kühn) on the Disease Index (DI) of Chia (*Salvia hispanica*. L)

DISCUSSION

The effects of the increasing rates of *R. solani*, *F. solani* inoculum on Chia were reasonably consistent across all of the different treatments. The results indicates that Chia when its grown in an open field will definitely succumb to fungal pathogen attack. Differences in overall disease incidence between the different inoculum rates of rhizoctonia and fusarium were precisely due to differences in disease pressure, with the highest rates of the treatments having relatively more infectious spores as compared to the other lower treatments. Although all the treatments caused disease, the untreated control had the least infection. However, an inoculum rate giving intermediate disease incidence, was also observed on the middle treatments. The most probable explanation from the experimental results is that, Chia is solely susceptible to bio trophic fungi.

Biotrophic fungi and oomycetes constitute some of the most destructive pathogens of agriculturally important plants, with a particular mention in this case being tobacco. In susceptible hosts, these pathogens infect and establish a dynamic

relationship with living plant cells through which they redirect plant resources to support pathogen growth and reproduction hence causing a decrease in the production potential of the crop.

Interaction between Chia and *R. solani* and *F. solani* has been clearly explained by the theory called gene-for-gene (GFG) hypothesis [26,27]. This GFG concept was defined as the reaction of the plant's R gene against the pathogen's cognate avr gene [28]. Plants defend fast either to hinder pathogen effects completely or to reduce that, have been called resistant or tolerant plants, respectively. The basis of susceptibility and resistance in plant– pathogen systems depends on the pathogen and the host characters, which agrees with the characteristics of Chia which allows easy invasion by pathogens. [29]. The relative metabolic compounds which were measured showed that, systemically these compounds (MAD and H₂O₂) plays a role in defence mechanisms hence the highest concentrations where there was more disease pressure. The results of this study might help to explain a long-known phenomenon characteristic of the first phase of disease caused by *R. solani* on potato. [30-33].

CONCLUSIONS AND RECOMMENDATIONS

The results of the study showed that Chia is susceptible to fungal pathogens *R. solani* and *F solani* especially at the highest inoculum rates. This was shown by highest disease incidences at the highest inoculum rates and also the incremental production of signalling compounds and reactive oxygen species in the tissues of Chia upon infection, hence an intercrop between Chia and Tobacco will not be productive. For a rotational crop management which will involve Chia, will also require proper disease management as well.

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