Efficacy of Some Agriculture Wastes in Controlling Root Rot of *Glycine max* L. Induced by *Rhizoctonia solani*

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ABSTRACT

This study was conducted to examine the antifungal activity of some agriculture wastes (rice straw, maize and cotton wastes) against *Rhizoctonia solani* which is the causal agent of root rot of soybeans. For this target, it is logical start first to test the effects of different agriculture wastes extracts on the growth of *R. solani* under laboratory conditions, while most of the tested agriculture wastes extracts were exhibited antifungal activities against *R. solani* on Potato Dextrose Agar medium, so we applied them under greenhouse conditions. Furthermore, we determined the relation between the phenols, polysaccharides and protein content of the tested agriculture wastes and their antifungal activities. The results showed that maize wastes were the most active of all the tested wastes. It could be concluded from the obtained data the use of the tested biotic factors can be fruitful for controlling soybean root rot induced by *R. solani*.

Key words: Agriculture wastes, antifungal activity, biocontrol, *Rhizoctonia solani*, soybeans growth

INTRODUCTION

Biological control agents for plant diseases are currently being examined as alternatives to synthetic pesticides due to their perceived increased level of safety and minimal environmental impacts (Brimmer and Boland, 2003) reduce the disease and are perceived as less harmful than conventional fungicides (Washington *et al.*, 1999). It has long been recognized that the biological control became recently an effective strategy for fighting plant pathogens and biocontrol agents are safe and environmental friendly alternatives than pesticides in agriculture application (Bai *et al.*, 2008). Rhizoctonia root rot and hypocotyl rot caused by *R. solani*, is a common disease of soybean (Bradley *et al.*, 2002). Seed rot and damping-off caused by *R. solani* is the most important disease of bean (Bohlooli *et al.*, 2005).

Most soil-borne pathogens are difficult to control by conventional control measures such as the use of resistant cultivars and synthetic fungicides (Weller *et al.*, 2002) although the use of fungicides, besides being expensive and involving risks to the environment associated with the application of chemicals, is not totally affective and may lead to the appearance of new, resistant strains of pathogens (Soylu *et al.*, 2005). Unexpected results derived from applications of chemicals to soils led to investigations of the soil biology, discovery of the rhizosphere, developing disease suppressive soils by introducing organic amendments and crop residue management takes time but
the benefits accumulate across successive years improving soil health and structure (Bailey and Lazarovits, 2005). In this context, Moyer and Huang (1997) stated that residues of canola, lentil, oat and barley have potential for reducing herbicide use in winter wheat production. Lopez-Llorca et al. (1999) showed that plant waste has a potential compounds with antifungal activity. In recent Aba AlKhall (2005) confirmed that plant extracts can be used as natural fungicides to control pathogen fungi to reduce the dependence on the synthetic fungicides. Pattaranawadee (2007) developed a new products such as medicines, food additives, feed additives for animal farms and crop protectants from raw or waste materials from the agriculture/horticulture. Velickovic et al. (2008) stated that plant material and solid waste residue may contain biological compounds which could be exploited as secondary raw material for obtaining different bioactivities. The objective of study was the investigation of antifungal activity induced by some agriculture wastes against the pathogenic fungus Rhizoctonia solani in vitro and under greenhouses conditions.

MATERIALS AND METHODS

Biological agents

- **Agriculture wastes:** Rice straw, maize and cotton wastes were collected from fields in 2009 in Mahelet Roh at Tanta city in El Gharbia Governorate, Egypt.
- **Fungi:** The pathogenic fungus (Rhizoctonia solani Kühn) was obtained from Plant Pathology Department, El-Gemmeizea Agricultural Research Station, (Agricultural Research Center, Egypt) and incubated on Potato Dextrose Agar (PDA) (Jong and Edwards, 1991) slants and plates at 28±1°C to establish growth then stored at 5°C in refrigerator.
- **Host plant:** Soybean (Glycine max L. Merrill) seeds, cultivar (Giza 111) were kindly supplied by the Legumes Department, El-Gemmeizea Agricultural Research Station and Agricultural Research Center, Egypt. All experiments were carried out in Mycology laboratory and greenhouses of Botany Department, Faculty of Science, Tanta University, Tanta, Egypt.

Preparation of antifungal extracts of agriculture wastes: Agriculture wastes were thoroughly washed with water then surface sterilized with 10% sodium hypochlorite solution, rinsed with sterile distilled water and air dried at room temperature. The samples were ground into a fine powder. Ten grams of air dried powder of agriculture wastes were taken in 100 mL of petroleum ether in conical flask (plugged with cotton wool), then kept on rotary shaker at 190-220 g for 24 h. The supernatant was discarded and evaporated. The dry powder was then taken in 100 mL of 70% solvent (acetone, chloroform, methanol or sterilized water) in a conical flask plugged with cotton wool and kept on rotary shaker at 190-220 g for 24 h. The extracts were centrifuged at 4000×g for 10 min. The supernatant was collected and then the solvents were evaporated. The dry extract was stored at 4°C in air tight bottles. The extraction was carried out at least five times for each agriculture wastes (Vaghiasiya and Chanda, 2007).

Antifungal assay by the agar disc method: Petri dishes (9 cm in diameter) contains 15 mL of PDA medium were divided into two equal halves, the first half was inoculated with a disk (0.5 cm in diameter) of R. solani and the second half was inoculated with a disk (0.5 cm in diameter) of agriculture wastes extracts (Bauer et al., 1996; Nair et al., 2005). The percentage of inhibition (I%) was calculated after 4 days of incubation at 28±1°C according to Topps and Wain (1957) as follows:
\[ I\% = \frac{A-B}{A} \times 100 \]

Where:
\( I\% \) = Percent of inhibition
\( A \) = Mean diameter growth in the control
\( B \) = Mean diameter growth in a given treatment

**Determination of the total phenolic contents of agriculture wastes:** The total phenolic contents of the tested agriculture wastes were determined as described by Jindal and Singh (1975).

**Determination of the polysaccharides contents of agriculture wastes:** Polysaccharides of the tested agriculture wastes were extracted and determined as the method described by Shi et al. (2007).

**Determination of the protein content of agriculture wastes:** The total protein contents of the agriculture wastes were determined as described by Bradford (1976) methods after pretreatment by Wood and Goodenough (1977) method.

**Pathogen inoculation:** Pathogenicity test is primary test for determination of the suitable concentration of \( R. \ solani \) under greenhouse conditions in early May 2009. The inoculum was prepared by dispensing 100 g of mixture wheat bran and sand (2:1) in bottles then moistened with water. Contents of bottles were autoclaved for 20 min at 1.5 atm then inoculated with \( R. \ solani \) which had been grown on PDA for one week and incubated at 28±1°C for 14 days. Autoclaved soil was placed in greenhouse and infested with inocula of \( R. \ solani \) one week before sowing at the rates of 10, 30, 50, 70 and 90 g kg\(^{-1}\) soil. Pre emergence damping-off was recorded using the following equation after 15 days of sowing as percentage of infected plants:

\[ \text{Damping-off} \% = \frac{\text{Number of infected plant}}{\text{Total plant numbers}} \times 100 \]

During the season (late May to June 2009), sterilized soil was placed into 25 cm diameter plastic pots, each pot contained 3 kg soil. Soil infestation was carried out one week before sowing at the rate of 30 \( R. \ solani \) inoculum and 20 g kg\(^{-1}\) sterilized agriculture wastes (size 0.0-0.2 cm) and kept moist. Twenty sterilized soybean seeds were sown in each plastic pot and replicated five times for each particular treatment. Post-emergence damping off was recorded after 45 days of sowing for each treatment as mention above.

**Plant analysis:** Measurement of soybeans growth included post-emergence damping-off, surviving seedlings, plant height, fresh and dry weights of shoot and roots, carbohydrate content (Nelson, 1944; Naguib, 1964), protein (Bradford, 1976), nitrogen (Naguib, 1969) and phosphorous content (Allen et al., 1974) after 45 days of sowing.

**Statistical analysis:** The presented results are the Means±SD (standard deviation) of at least five readings. One way analysis of variance (ANOVA) was applied using the SAS (1996) program version 6.12.

Table 1: Antifungal activity of some agriculture wastes against Rhizoctonia solani on PDA medium

<table>
<thead>
<tr>
<th>Biological agents</th>
<th>Acetone</th>
<th>Chloroform</th>
<th>Methanol</th>
<th>Water</th>
<th>LSD at 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice straw</td>
<td>31.1±2.2</td>
<td>37.8±1.1</td>
<td>43.3±0.6</td>
<td>0.0</td>
<td>11.25**</td>
</tr>
<tr>
<td>Maize wastes</td>
<td>0.0</td>
<td>46.3±0.6</td>
<td>14.4±0.5</td>
<td>0.0</td>
<td>5.78**</td>
</tr>
<tr>
<td>Cotton wastes</td>
<td>0.0</td>
<td>36.7±0.3</td>
<td>37.0±0.6</td>
<td>0.0</td>
<td>9.8**</td>
</tr>
<tr>
<td>LSD at 5%</td>
<td>6.16**</td>
<td>3.92**</td>
<td>2.75**</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

Values represent Means ± Standard deviation (n = 5). ** = p<0.05

Table 2: Total phenol contents, polysaccharides and protein content of tested agriculture wastes (mg g⁻¹ dry weight)

<table>
<thead>
<tr>
<th>Agriculture waste</th>
<th>Total phenol contents (mg g⁻¹ DW)</th>
<th>Polysaccharides content (mg g⁻¹ DW)</th>
<th>Protein content (mg g⁻¹ DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice straw</td>
<td>1.29±0.01</td>
<td>68±0.06</td>
<td>9.2±0.1</td>
</tr>
<tr>
<td>Maize wastes</td>
<td>1.25±0.04</td>
<td>71±1</td>
<td>9.6±0.1</td>
</tr>
<tr>
<td>Cotton wastes</td>
<td>1.03±0.02</td>
<td>63.7±1.6</td>
<td>4.7±0.3</td>
</tr>
<tr>
<td>LSD at 5%</td>
<td>0.53**</td>
<td>1.55**</td>
<td>0.38**</td>
</tr>
</tbody>
</table>

Values represent Means ± Standard deviation (n = 5). ** = p<0.05

RESULTS

Antifungal activity of the tested extracts of some agriculture wastes: The results in Table 1 show that chloroform and methanol extracts of all tested agriculture wastes exhibited antifungal activity against R. solani. The obtained results show that the acetone extract of rice straw showed antifungal activity, whereas, the acetone extract of other tested wastes had no antifungal activity against R. solani. On the other hand, water extracts of the tested agriculture wastes and the pure solvents showed no antifungal activity against R. solani. The results show that the tested agriculture wastes showed significant differences at p = 5% in their activity against R. solani. The highest antifungal activity was exhibited by chloroform extract of maize wastes.

The antifungal activity of the tested agriculture wastes was studied in relation to their phenol, polysaccharides and protein contents. The results indicate that all tested agriculture wastes contain variable amounts of total phenol contents. The highest values of phenol content were detected in maize wastes (1.25 mg g⁻¹ DW). Phenol content of rice straw came in second after phenol content of maize while cotton wastes showed the lowest value of total phenol as compared to other tested wastes. The obtained results show that the phenol contents of tested agriculture wastes increased, the antifungal activity increased (Table 2).

Data recorded in Table 2, revealed that the polysaccharides content of the tested agriculture wastes showed different values according to the agriculture waste type. The amount of polysaccharides of maize wastes had more or less similar value as rice straw content, whereas the polysaccharides content of cotton wastes were lower than the other tested agriculture wastes. Also, the protein content of agriculture wastes is different according to wastes type, thus maize wastes have higher amount of protein than other tested wastes. It could be observed that the antifungal activity of the tested agriculture wastes was increased as the level of their phenol, polysaccharides and protein increased.

Growth responses of infected soybeans: The infection of soybean with inoculum of R. solani (3 g kg⁻¹) was found to cause a significant reduction in the measured soybean growth parameter (survival, root depth, shoot length, fresh and dry weights) amounted by 77.22, 45.8, 31, 48.3 and 51.1%, respectively below the healthy control at p<0.001 after 45 days of sowing under greenhouse conditions (Fig. 1A-D).
Fig. 1: Effect of the tested agriculture wastes on, (A) post damping off and survival percentage (I%), (B) length; (C) fresh; (D) dry weight; (E, F) carbohydrate; (G) protein and (H) nitrogen contents of infected Glycine max L. with Rhizoctonia solani after 45 days of sowing under greenhouse conditions.
The infection of soybean with inoculum of *R. solani* (3 g kg⁻¹) was found to cause a significant reduction in the soybean survival rate by 77.22 and caused a highly significant increase in the post-emergence damping off by 83.3% after 45 days of sowing at p<0.001 (Fig. 1A).

*R. solani* caused a highly significant decrease soybean root depth and shoot length amounted by 31 and 48.3%, respectively below the healthy control at p<0.001 (Fig. 1B).

The fresh and dry weights of infected soybeans with *R. solani* exhibited progressive decreases throughout the cultivation period up to 45 days by about 48.3 and 51%, respectively at p<0.001 (Fig. 1C and D).

It is inferred from Fig. 1E and F that 3% *R. solani* treatment caused highly significant reduction in carbohydrate contents (DRV, TRV and sucrose) of soybean shoot system amounted by 24.8, 45.8 and 66.1%, respectively. The same treatments caused also decrease in root system DRV, TRV and sucrose by 57.8, 26.8 and 14.6% below the control value under greenhouse conditions at p<0.001. An improvement in growth parameter (severity, length, weights and carbohydrate contents) with respect to the infected control was observed in the presence of 2% (w/v) of each tested agriculture wastes with different percentage according to types of tested wastes. The most detectable increases in shoot length and root depth amounted by 37.3, 109%, fresh and dry weights by 55.1, 90.5% and DRV, TRV and sucrose contents of shoot system by 32.2, 48.9, 55.2% and root system by 66.8, 60.5, 43.4%, respectively of infected soybeans was recorded by maize wastes treatment as compared with infected control.

Compared to healthy plants, *R. solani* infected soybean induced significant increase in the shoot and root soybeans protein by 57.2, 6.9% and nitrogen contents by 58, 42.6%, respectively after 45 days of sowing under greenhouse conditions (Fig. 1G).

The addition of 2 g kg⁻¹ sterilized agriculture wastes (rice straw, maize wastes and cotton wastes) caused significant decreases in protein by 42.7, 38.5 and 29.2%, respectively (F = 26.14, p<0.001) and nitrogen contents by 12.3, 7.8 and 12.3% (F = 26.87, p<0.001) of infected shoot system as compared with infected plant but higher than uninfected control (Fig. 1H). On the other hand, the rice straw, maize wastes and cotton wastes caused non significant decreases in the protein contents of soybean root system by 7.5, 14.7 and 16.2% (F = 20.15, p<0.001) and nitrogen contents by 4.9, 8.2, 8.2% (F = 20.38, p<0.001), respectively below the infected soybeans under greenhouses conditions.

**DISCUSSION**

The results show that the tested wastes (rice straw, maize and cotton wastes) extracts exhibited different antifungal activity against *Rhizoctonia solani*. The difference in the activities of wastes extracts may be attributed to the presence of different compounds having different polarities. These results are consistent with the observations reported by Lopez-Llorca et al. (1999), who showed that plant waste has a potential compounds having antifungal activity. The antifungal activity of agriculture wastes may be related to their phenol, polysaccharides and/or polysaccharides content (Table 2). This interpretation based on the results concerning the content of these substances in the tested agriculture wastes where their antifungal effect were increased as their polysaccharides and/or phenol content increased. In agreement with our explanation, there are a number of reports on the antifungal activity of phenolic substance e.g., Moure et al. (2001) extracted polyphenols from agricultural and industrial wastes with antimicrobial and antioxidant properties. In the same time Shaukat et al. (2001) demonstrated that caffeic acid at 5 μg g⁻¹ soil caused greater suppression of
_F. solani_ whereas p-hydroxybenzoic acid at 10 µg g⁻¹ resulted in the maximum inhibition of _R. solani_. Shaukat and Siddiqui (2002) stated that phenolic compounds or some other chemicals exuded from the roots of _Lantana camara_ were probably responsible for the suppression of the root infecting fungi ( _F. solani_ and _R. solani_). Sekine _et al._ (2009) detected that phenolic hydroxyl compounds have antifungal activity against white and brown-rot fungi.

With respect to polysaccharides, they are one of the main compounds of plant cell wall which have antifungal activity; the antifungal potency of these compounds was associated with their aglycone moieties and the number and structure of monosaccharide units in their sugar chains (Yang _et al._, 2006). Polysaccharides especially β-d-glucan derivate, glycopeptide/protein complexes have more important role in immunomodulating, antimicrobial and antitumor activities (Moradali _et al._, 2007). Protein play important role as defense mechanism for plant wastes which reflect the antifungal activity of tested agriculture wastes, this observation has been emphasized by Ludwig and Boller (1990) who stated that crude protein extracts from plant tissues have high activities of chitinase and β-1, 3-glucanase which reduced the microbial growth. Plant proteins are glycosidases, thionins, permatins and ribosome-inactivating proteins, all of which exert antifungal activity _in vitro_ (Bowles, 1990).

The results show that the severity of soybean seed sowing in soil infected with _R. solani_ decreased by 77.3% below the control after 45 days of sowing (Fig. 1). Present results support the results obtained by Allen (2000) who mentioned that Rhizoctonia root and stem rot caused by the fungus _R. solani_, is a common early season disease of soybeans and cause extensive damage by 50% losses and causing loss of seedlings (damping-off) in small patches or within rows. Heydari _et al._ (2007) observed that _R. solani_ induced damping off symptoms on all emerged and non emerged cotton seedling. Fayzalla _et al._ (2009) stated that _F. oxysporum, R. solani, Macrophaminaphaseolina and Sclerotium rolfsii_ are common fungal pathogens to soybean causing damping off, root rot and wilt diseases resulting in serious economic losses. As regards to the effect of agriculture wastes on infected soybean seeds which reflected the antifungal activity against _R. solani_ under greenhouse conditions and confirm the previous tests of antifungal activity of agriculture wastes _in vitro_. Very little data have been published on the effect of agriculture wastes on controlling _R. solani_ under greenhouse conditions. One of the earliest reports is that of Mehrotra and Tiwari (1976) who recorded that soil amended with corn straw gave best control to fungal disease. Furthermore, Abawi and Pastor-Corrales (1990) stated that the incorporation of residue, deeply and early enough to promote complete decomposition is effective against _R. solani_ because it reduces inoculum density near the soil surface, where the pathogen is most active. The increase of soybean severity may be related to fertilizer effect of agriculture wastes which stimulated the defense mechanism of seeds. In this connection, Zadrazil and Grabbe (1983) recommended that the agricultural residues if not burned, should be fragmented and added to soil as organic fertilizer. Yuan _et al._ (2004) found that rice straw, pig faeces and wood chip could alleviate Fusarium wilt and Rhizoctonia wilt of cucumber and the effect of rice straw was most significant, followed by pig faeces and wood chip. Liu _et al._ (2008) showed the positive effect of cotton stalk by the returning them to the soil, hence the activities of urease, catalase and invertase increased.

The total plant length (root depth and shoot length), weights (fresh and dry weight) and carbohydrate content decreased by infection with _R. solani_ inoculum under greenhouse conditions. This may be due to the effect of some growth inhibitors produced by _R. solani_. These results are in conformity with Haikal (2008) who showed that filtrates of _A. niger, F. culmorum, Penicillium sp._ and _R. solani_ inhibited seed germination and seedling development of soybean due to their toxic
metabolites in the media in which they were grown. These metabolites inhibit and reduce the percentage of seed germination and also retard seedling growth. De Paula Junior (2002) found that *R. solani* consistently reduced bean weight. Hwang *et al.* (2009) recorded that the height, shoot vigour and shoot dry mass of *Rhodiola rosea* were significantly reduced by *R. solani* infection.

Also, *Rhizoctonia solani* may act as inhibitor for carbohydrate biosynthesis in soybeans, due to its inhibitory action on plant metabolism or the fungus may utilize the plant carbohydrate content as carbon sources for growth. These finding are in accordance with Jeun and Hwang (1991) who reported that carbohydrates increase the severity of the infection and may serve as easily metabolized carbon substrates for the pathogen. El-Daly and Haikal (2006) detected that the presence of *R. solani* in soil lowered the lipid, total carbohydrates and protein content of corn flour. Abdullah (2008) stated that *R. solani* decreased total carbohydrate content of wheat and barley.

The obtained results showed that treatment with maize wastes caused the highest increase in soybean length and weight than other tested wastes, which may reflect that the enhancement of soybean growth correlated directly with the degree of antifungal activity of these wastes, while maize wastes caused significant reduction in *R. solani* growth. Our results are in agreement with Jobidon *et al.* (1999b) who stated that the straw treatments enhanced shoot and stem-diameter growth of *Picea mariana* seedlings. This stimulatory effect of agriculture wastes on carbohydrate content of infected soybeans may be attributed to the nature of their chemical compound. Plant residues are suspected to largely govern the fate of their constitutive carbon in soil e.g., soluble sugar and starch (Girardin *et al.*, 2009). Gangwar *et al.* (2006) stated that the incorporation of crop residues increased soil organic carbon.

The protein and nitrogen contents of soybeans increased as affected by *R. solani* infection under greenhouse conditions. Thus, it could be concluded that this increase act as the defense mechanism of plant against pathogen, hence the proteins serve as defense mechanisms of rice when infected with *R. solani*. In this context, Caruso *et al.* (1999) stated that the pathogenesis-related PR2 and PR3 proteins, besides their involvement in plant defense, may play a role also in the normal process of seed germination and have antifungal activity (Coperale *et al.*, 2004; Bortolotti *et al.* (2005) stated that PR proteins were involved in the defense response of maize plants against fungal pathogens.

On the other hand, the protein and nitrogen content of infected soybeans shoot and root systems had different pattern of changes in response to treatment with agriculture wastes. Thus, the shoot systems protein and N contents of treated soybeans decreased as compared with untreated shoot infected control. On the other hand, the protein and N contents of root system showed non significant increase with respect to infected control under greenhouse conditions depending on the wastes types. This different in shoot and root systems protein and N contents may depend on the fungal infection and plant defense mechanism, since the root system is the first line of plant defense mechanism, so plant take and accumulate a large amount of protein in root systems than shoot systems. The plant responses to agriculture wastes were extremely variable in the quantity according to the type of tested agriculture wastes, since legumes, such as alfalfa, peanuts, soybeans and clover, benefit less by manure and sludge additions because they fix their own nitrogen (Awmif, 1996). These results are more or less similar to that reported by Lovett and Jessop (1982) found that application of mature straw resulted in the lowest N accumulation by harvested sugar beet. Jobidon *et al.* (1989a) stated that the nitrogen content of red raspberry (*Rubus idaeus* L.) from the treated plots with straw was significantly lower than in seedlings from the control plots (mean N content 2.24%).

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CONCLUSION
This work is endeavor for utilization of some agriculture wastes as antifungal agent against
*R. solani in vitro* and greenhouses conditions. The antifungal activity induced by such biotic factors
may be related to their phenol, polysaccharides and protein content. Our study suggests that the
antifungal activity of agriculture wastes depended primarily on type, concentration and chemical
structure of each wastes.

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