Evaluation of four plants compost teas against fungi responsible for Corn damping-off in Côte d’Ivoire

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Abstract—Corn (Zea mays L.) is one of the most important cereals in Côte d’Ivoire. However, Corn seeds and seedlings are susceptible to infection by a number of soilborne fungi which caused seeds decay before or after germination. The objective of this study is to evaluate the suppressive effect of Four plants compost teas (Chromolaena odorata, Ricinus communis, Nicotiana tabacum, Azadiracta indica) on Corn damping off. In vitro assays showed a most suppressive effect of C. odorata and R. communis compost teas on mycelial growth of pathogenic fungi. Results of in vivo trials showed significant reduction of Corn seedlings diseases incidence and high seed germination percent after treatment with C. odorata, R. communis and A. indica compost teas. No efficiency effect was noted with N. tabacum compost tea. This study demonstrated the usefulness of compost tea as an efficient biological tool for the control of fungi responsible of Corn damping-off.

Keywords—Corn, damping-off, fungi, compost tea.

1. INTRODUCTION

Corn (Zea mays L.) is an annual tropical herbaceous plant of the family Poaceae and it is one of the most important cereal grains grown worldwide. Corn is the world’s top most cereal crop in terms of total production and productivity after wheat and rice (FAO, 2006). Corn is the second most important food crop in Côte d'Ivoire after rice with an annual production of about 600,000 tones (FAO, 2014). This commodity is widely grown for its starch-rich grains. It is also used as a forage plant in some localities. Corn is also the principal staple cereal diet of most of the Ivorian people who mainly lives in the North. In the last twenty years, Corn cultivation has experienced a considerable development of its market with urbanization and especially the establishment of food industries (Boone et al., 2008).

However, several diseases are observed on Corn and cause significant yield reduction (Sétamou et al., 1998). This decrease in yield is linked to the action of certain fungi that can have adverse effects on the aerial organs (ears, leaves and stems) or on the root system thus reducing the productivity of the plant. According to Harvey et al. (2006), fungi such as Aspergillus sp., Pythium sp., Fusarium sp. and Rhizoctonia sp. would be responsible for root rot and seeding of corn.

Synthetic fungicides remain the most widely used control measure against fungal plant diseases. Although relatively effective, synthetic fungicides have two major drawbacks: their generally widespread lack of long-term efficacy caused by the development of resistance in plant pathogens (Avis, 2007). The fungicides, however, can have a negative effect on human health and the environment (Perez-Garcia et al., 2011).

A possible alternative to synthetic chemical fungicides is to exploit the antimicrobial activities of compost teas. The potential of compost tea in the suppression of plant pathogens has been demonstrated (Litterick et al., 2004). Recently, compost teas from sheep manure compost showed antimicrobial activities against phyllosphere (Koné et al., 2010) and rhizosphere (Dionne et al., 2012) pathogens of tomato (Solanum lycopersicum L.) plants. In addition, Compost teas was considered safer for health and the environment (Siddiqui et al., 2009).

The objective of this study is to identify the fungi responsible of seed-borne fungi in Corn seeds collected from different areas and evaluate compost tea of four plants on some fungal pathogens.

1. MATERIALS AND METHODS

1.1. SAMPLING AND ISOLATION OF FUNGI

Samples of ungerminated corn kernel showing rot symptom were removed 15 days after planting from Corn fields in three cultivation areas of Northern Côte d’Ivoire (Katiola, Korhogo, Didenne). Samples were transported to the laboratory for fungi isolation. To obtain fungal population from rotten Corn kernel, samples were disinfested in 2% sodium hypochlorite for 3 min, rinsed twice in distilled water for 5 min and air-dried at 26°C for 1 to 2 h in a sterile Lamina flow hood. Sterilized kernels were placed into Petri dishes contained PDA medium, each
medium containing 3 kernels was incubated at 28±2°C. Mycelial growths from the corn kernel were transferred (picked with a flame needle from the periphery of growth) to fresh PDA plates. Sub-culturing was carried out to obtain pure isolates which were maintained on PDA. To confirm the identification of isolated fungi, microscopic observation of the morphological characteristics of the isolates, grown for two weeks in Petri dishes containing Potato Dextrose Agar (PDA), was performed using identification keys (Botton et al. 1990, Barry and Barnett, 1972). The frequency of each isolated fungi was calculated by using the following formula (1)

\[ F(\%) = \frac{Nc}{Nt} \times 100 \]  

Where, F: Frequency (%), Nc: Number of genus or species isolated, Nt: total number of isolates

### 1.2. PATHOGENICITY TEST

#### 1.2.1. INOCULATION OF SEEDS

Isolated fungi were analysed for their capacity to inhibit seed germination and the development of roots. Briefly, kernels corn was surface-disinfected for 3 min in 2% sodium hypochlorite, rinsed twice with distilled water. Corn kernels were immersed for 1 minute in a 10^6 mL^-1 micro conidial suspension and placed into Petri dishes on sterile water saturated filter paper. three dishes containing sterile water were used to determine if they were significantly different at 0.05.

#### 1.2.2. EFFECT OF COMPOSTS TEAS ON THE MYCELIAL GROWTH OF CORN DAMPING-OFF FUNGI

Anti-fungal activity of compost tea was evaluated using three fungi cultured on agar plates at concentration 60 g L^-1 and unamended media were used as controls. The prepared composts were added to conical flasks containing previously sterilized and cooled agar medium. After thorough mixing, 15 mL of media were poured into sterilized Petri dishes 9 cm in diameter. Fungal plugs (0.5 mm in diameter) were removed with a cork borer from the growing margin of each fungus colony and placed at the center of the test plate. Five replications were made for each treatment and the cultures were incubated at room temperature. Colony diameter was measured in two directions daily until the fungus covered the whole of the agar in the control plate. Data were expressed as growth rate (mm/day) relative to control. Percentage inhibition (mycelial growth) was determined.

### 1.3. EVALUATION OF ANTIFUNGAL ACTIVITY OF COMPOST TEA

#### 1.3.1. PREPARATION OF COMPOST TEA

Four extracts prepared from different compost (C1, C2, C3, and C4) were used (Table1). Original composts were produced according to an aerobic process (Znaidi, 2002). Extract production consists on suspending composts in tap water (1:5, v/v) in 20 litre plastic container and stirring the mixture daily for about 10 min during an extraction period of 5 days (Weltzein, 1992). After the incubation period, the mixture was filtered through cheesecloth (250 µm) and the obtained extract were stored at 4°C

| Table1: compost ingredients |
|-----------------------------|
| Compost | Compositions |
| C1 | 20% Cm + 80% Chromolaena odorata leaves |
| C2 | 20% Cm + 80% Ricinus communis leaves |
| C3 | 20% Cm + 80% Azadirachta indica leaves |
| C4 | 20% Cm + 80% Nicotiana tabacum leaves |

Cm : Cattle manure

#### 1.3.2. EFFECT OF COMPOSTS TEA ON THE MYCELIAL GROWTH OF CORN DAMPING-OFF FUNGI

Composting was carried out in a growth chamber at 27°C with a 12-hour photoperiod. For each treatment 15 pots were used and 3 seeds were planted per pot. Seedlings were watered daily. As a measure of disease severity, seedling stands were counted 25 days after inoculation. Low percent seedling stands indicated high disease severity, whereas high percent-seedling stands indicated high disease suppressiveness. Percent-seedling stands were calculated and the mean value of five replications was considered.

### 1.4. STATISTICAL ANALYSIS

Means and standard error of the mean were calculated for the mycelial growth inhibition and germinated seeds after composts teas treatment measured for the three sets of experiments in each case. These means were statistically compared using the LSD Fischer test was used to determine if they were significantly different at P < 0.05.
II. RESULTS

2.1. Identification and frequency of isolated fungi

In total, seven fungal genera associated with corn kernel rot was isolated on PDA medium and were identified based on the morphologic and cultural characteristics as Aspergillus sp, Colletotrichum sp, Fusarium sp, Pythium sp, Rhizoctonia sp, Rhizomucor sp, Trichoderma sp. Trichoderma sp was the most commonly isolated fungal species among all of the isolates obtained (48.06%) followed by Fusarium sp (34.73%) and Rhizoctonia sp (13.53%). Aspergillus sp (3.42%) and Colletotrichum (6.23%) had the lowest isolation rates (Figure 1).

2.2. PATHOGENICITY TESTS

The pathogenicity test with fungi associated with corn kernel rot was carried out. Results based on the in vitro seeds inoculation test showed significant pathogenic effects of Pythium, Fusarium and Rhizoctonia on seeds germination and seedlings health due to high infection. Pythium sp and Rhizoctonia reduced significantly seeds germination with percentage germination of 5 and 2% respectively. Seeds infected by Fusarium sp showed percentage germination of 55%. The seed samples inoculated with Aspergillus sp, Colletotrichum sp, Rhizomucor sp and Trichoderma sp showed similar percentage germination with the control seeds (Figure 2).

2.3. SUPPRESSION OF MYCELIAL GROWTH BY PLANTS COMPOST TEA

Results showed that the tested compost teas induced a significant interaction with the tested phytopathogenic fungi. The radial growth of Pythium, Rhizoctonia and Fusarium noted after 7 days of incubation, was significantly (p < 0.05) reduced in comparison to the controls. In fact, Chromolaena odorata (C1) and Ricinus communis (C2) compost tea reduced significantly mycelial growth of all tested fungi with inhibition rates ranged from 85 to 100%. The compost tea C3 was also effective against Rhizoctonia sp and Fusarium sp with inhibition ratios of 94.09 and 95.78 % respectively. Compost tea C4 showed the less inhibitory effect reducing mycelial growth of Pythium and Fusarium by approximately 45 and 50% respectively (Table 2).

Table 2: In vitro effect of compost teas on mycelial growth of Pythium, Rhizoctonia, Fusarium

| Compost tea | Mycelial inhibition rate (%) |
|-------------|------------------------------|
|             | Pythium | Rhizoctonia | Fusarium |
| C0          | 34.59±5.30<sup>a</sup> | 22.36±2.63<sup>ab</sup> | 43.33±2.88<sup>b</sup> |
| C1          | 92.82±1.93<sup>a</sup> | 94.09±1.93<sup>b</sup> | 97.46±0.00<sup>b</sup> |
| C2          | 85.23±1.93<sup>b</sup> | 96.62±1.46<sup>b</sup> | 100.0±0.00<sup>b</sup> |
| C3          | 65.40±7.30<sup>c</sup> | 94.09±1.46<sup>b</sup> | 95.78±1.46<sup>b</sup> |
| C4          | 45.56±3.34<sup>d</sup> | 100.0±0.00<sup>b</sup> | 50.21±6.97<sup>b</sup> |
| F           | 10.42   | 8.40       | 10.35     |
| P           | 0.015   | 0.038      | 0.015     |

Within a column, means with the same letter are not significantly different according to Fisher’s LSD test (P = 0.05).

2.4. IN VIVO EVALUATION OF THE EFFECT OF COMPOSTS ON CORN KERNELS GERMINATION

The percentage germination of corn kernels varied significantly according to compost tea treatments. In fact, the lowest percentage germination ranged from 16.29 to 53% was observed on the inoculated soil without compost tea treatment (T0) and soil treated with nicotiana tabacum compost (T4) (Table 3). In these subtract seedling showed disease symptoms include rotted seed that is soft and brown, rotted roots with a wet and slimy appearance. Above ground symptoms include damping-off after emergence and seedlings that turn yellow, wilt and die. Results recorded on inoculated soil treated with C. odorata and R. communis compost showed highest percentage germination (100%). C. odorata and R. communis exhibited remarkable potency in suppressing seeds rot and seedling blight (Figure 3).
Table 3: In vivo effect of compost teas on corn kernels germination.

| Compost teas treatment | Germination rate (%) |
|------------------------|----------------------|
|                        | Pythium | Rhyzoctonia | Fusarium |
| T₀                     | 44.44±8.11a         | 16.29±3.39a | 43.40±10.02 |
| T₁                     | 100a            | 100a         | 100a       |
| T₂                     | 100a            | 100a         | 100a       |
| T₃                     | 91.11±8.01b         | 94.07±5.59a | 95.55±5.87a |
| T₄                     | 53.12±5.87          | 43.70±5.59  | 47.44±6.78  |

Fig. 3: Corn seedlings diseases incidence after treatment with compost teas

T₀: Inoculated soil without compost tea treatment; T₁: C. odorata compost tea treatment; T₂: R. communis compost tea treatment; T₃: A. indica compost tea treatment; T₄: N. tabacum compost tea treatment

Within a column, means with the same letter are not significantly different according to Fisher's LSD test (P = 0.05).

To control; T₁: C. odorata compost tea treatment; T₂: R. communis compost tea treatment; T₃: A. indica compost tea treatment; T₄: N. tabacum compost tea treatment

III. DISCUSSION

Based on morphological characteristics, seven fungal genera were identified in total after isolation from corn kernels samples showing rot symptoms. These genera include Aspergillus sp, Colletotrichum sp, Fusarium sp, Pythium sp, Rhyzoctonia sp, Rhizomucor sp, Trichoderma sp. In this present study most of the obtained fungus genera (Colletotrichum sp, Rhizomucor sp, Trichoderma sp, Aspergillus sp) were saprophytic. Survey conducted by Niaz et al. (2009) showed that these fungi were frequently found in corn kernel. The abundant growth of saprophytic fungi on Corn seeds implies that storage problems should also be studied. It is now imperative that efforts should be made to continuously evaluate the seed health of Corn seeds produced in Côte d’ivoire. This current survey showed that Fusarium sp, Pythium sp and Rhyzoctonia sp. induced corn kernel rot and seedling blight. Recent study conducted by Tesfaye and Dawit (1998) in Ethiopia showed that several phytopathogenic species included Fusarium spp were found to be associated with damaged corn kernel. (Girma, 2009) also identified Fusarium species associated with Corn grain in Ethiopia. Kommedahl (1981) observations revealed that symptoms caused by these pathogens are: failure to emerge, wilting, chlorosis or yellowing, root rot and poor rot development. Concerning Pythium sp, Zhang et al. (2000) confirms that a variety of Pythium spp. have the capacity to reduce germination and cause lesions on roots of corn and soybean seed and seedlings. Investigations conducted by Dorrance et al. (2004) showed that Pythium spp. frequently are associated with seed and seedling diseases, and commonly have been isolated from corn.

The aqueous extracts of composts leaf of Chromolaena odorata, Azadiracta indica and of Ricinus communis showed a more significant antifungal activity against Pythium sp., Rhyzoctonia sp. and Fusarium sp. Compost treatments significantly reduced the incidence of the disease on seed germination. No disease was observed in soils treated with Chromolaena odorata and Ricinus communis composts. Our results are in agreement with those obtained by Scheuerell and Mahaffee in 2004. These authors have shown that the irrigation by compost extract of a culture substrate inoculated with Pythium ultimum at reduces the effect of cucumber blight caused by this pathogen. The study conducted by El-Masry et al. (2002) showed that the compost extract can control several pathogenic fungi like Pythium debaryanum, Sclerotium bataticola and Fusarium oxysporum f. sp. lycopersici. In addition, Khaled et al. (2005) showed that the treatment of the growing medium, used for growing tomato plants, by the various compost extracts interfered with F. oxysporum infection. F. sp. radicis-lycopersici and its expression, which greatly reduced the incidence of the disease. Compost tea effectiveness could be explained by antifungal substances contained in leaves of each plant. Monisha et al. (2013) and Koumaglo et al. (2009) suggested that the aqueous extract of Chromolaena odorata and Ricinus communis leaves had antifungal properties due to the presence of compounds such as phenols, tannins and flavonoids. Le Page and Bousquet (2007) showed that several organic chemicals present in compost or released by compost inhabiting microorganisms provided disease suppressive effects, including phenolic compounds, volatile fatty acids and salicylic acid.

To summarize the present study showed that compost tea prepared from various plants leaves had negative effect on corn kernel pathogenic fungi (Fusarium sp, Pythium sp, Rhyzoctonia sp). They could constitute a promising alternative for a biological control of seed and seedling diseases and reduces the abusive use of synthetic fungicides. However, a more field comprehensive survey is needed to confirm our findings in the field under natural conditions.

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