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ORIGINAL ARTICLE

Maize Kernal Rot Management: Evaluating the Antifungal Strategies Against *Fusarium verticillioides*

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ABSTRACT

Background: Maize is one of the major staple and export crops in several countries. However, many pests and diseases severely reduce its yield. *Fusarium verticillioides* is a major cause of maize kernel yield loss globally.

Objectives: This research provides practical solutions to *Fusarium* kernel rot infections via the application of biological, chemical, and botanical agents.

Methods: Three experiments/methods (i.e. biocontrol, chemical, and botanical) were employed to control the kernel rot pathogen *in vitro*. Each experiment was laid out using a completely randomized design with each treatment being replicated thrice. The data collected included radial growth of the pathogen.

Results: The three experiments yielded the following outcomes. Firstly, the results of the biocontrol experiment revealed that the most effective isolates were *Trichoderma harzianum* isolate AIPT26, *T. virens* isolate BGMZ2 followed by *T. hamatum* isolate ZXPB. Their effects were at par. Secondly, chemical experiment showed that the highest control was from Ridomil (at 50% and 100%), Mancozeb (at 50% and 100%), and Itraconazole (at 50% and 100%). Thirdly, the plant extract experiment revealed that the highest pathogen control was obtained using *Ricinus communis* (i.e. Castor soap) (at 50% and 100%). The *Aframomum melegueta* (i.e. Alligator pepper) (at 100%), then *Panax ginseng* (i.e. Ginseng) (at 100%), *A. melegueta* (at 50%), and lastly *P. ginseng* (at 50%) was effective.

Conclusion: All the agents (i.e. biocontrol, chemical, and botanical) were highly successful in inhibiting *Fusarium verticillioides.* It is recommended that research should continue on integration of these three control measures so as to reduce chemical pesticides usage.

Keywords	*Address of Correspondence	Article info.
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INTRODUCTION

Maize (*Zea maize* L.*) is the* third most important cereal crop after wheat and rice [1,2]. It serves as a staple and export commodity [3,4] which accounts for approximately 75% of the global food supply. Unfortunately, maize is often infected by many disease-causing agents among which are major fungal diseases that cause pervasive ear and stalk rots (resulting in circa 11% maize grain yield loss globally) [1, 2, 5, 6].

The ear rots result in contamination of the grains with mycotoxins like fumonisins and deoxynivalenol. The major producers of these mycotoxins are *Stenocarpella maydis, Fusarium verticillioides,* and *F. graminearum*. Some *Aspergillus* species can produce aflatoxin [7-12] on maize grains. *Fusarium* species invade more than half of the maize cobs in the field which induce mycotoxicity [13,14].

The primary ear rots include namely *Diplodia* kernel rot (by *Stenocarpella maydis,* and *Stenocarpella macrospora),* and pink kernel rot (by *Fusarium verticillioides*). Another kernel rot is the Red kernel rot, as well as

Aspergillus kernel rot [5, 15, 16]. Fumonisins are harmful to warm-blooded animals [17]. *Gibberella* kernel rot induces direct yield loss as well as the production of the deoxynivalenol and zearalenone [18]. These ear rots also affect seed germination and seedling health [19].

The control of pests and diseases has become more challenging nowadays due to the reduced efficacy of available synthetic pesticides as pests and pathogens develop resistance. Moreover, some pesticides have been banned due to the negative effects they have on the environment, human and animal health. The efficacy of these pesticides varies with the environment and pest/pathogen progeny. This calls for more research to assess the efficacy of available pesticides and the development of environmentally friendly alternatives.

Some of the alternative pesticides have proven that they can match the performance of the synthetic chemicals. For instance, the extract of mancozeb, eucalyptus, neem and mahogany inhibited the growth of the *Phytophthora colocasiae*. Moreover, Eucalyptus-100% (induced 94.4−100.0% inhibition) which was comparable to Mancozeb (100% inhibition) of the *P. colocasiae* [20]. However, breakthroughs on alternative pesticides are long overdue.

Also, the extracts of Eucalyptus (50 and 100%) and Mancozeb (50 and 100%) completely inhibited the radial growth of the *A. flavus.* The Eucalyptus aqueous extract was more effective than neem extract and synthetic Copper-I-oxide+Metalaxyl [21]. Regrettably, insects followed by fungi are the main cause of the corn yield loss globally [1, 13].

The kernel rots are not new to corn producers, yet very little is known about their management. With this insufficiency of know-how on corn ear rot management, this research was conceived to tender practical solutions (employing biological, chemical, and botanical agents) to *Fusarium* kernel rot management.

MATERIALS AND METHODS

Isolation and Identification of the Two Fungi Utilized

The *F. verticillioides* and *Trichoderma* spp. were cultured on Acetate Differential Agar® enriched with dextrose and identified using standard methods [22-25].

Preparation of the Plant Extracts

The plant extracts (alligator pepper (sweet type) - *Aframomum melegueta*, castor bean soap (white powder type) - *Ricinus communis, and* African ginseng (root powder) - *Panax ginseng* used for bio-assay were selected based on their indigenous spice and medicinal uses. The plant tissues were utilized at the rate of 111.1 g L^{-1} H_2O to give the full-strength level. The plant extracts were utilized at the dose rates of 0.0, 50, and 100%.

Biocontrol of *F. verticillioides* Using Various Trichoderma Species

The completely randomized design (CRD) experiment (replicated three times) had five treatments which included four *Trichoderma* isolates and a control. The agar medium was inoculated with discs of the fungal agents.

Effects of Man-Made Pesticides on *F. verticillioides*

The completely randomized design (CRD) experiment (replicated three times) had the following treatments: a negative control, Mancozeb® (0, 50, and 100%), Ridomil Gold Plus 66 WP® (0, 50, and 100%), Itraconazole (0, 50, and 100%), and Ciprofloxacin (0, 50, and 100%).

Effects of Botanical Extracts on *F. verticillioides*

The *in vitro* CRD trial was carried out using seven treatments, which included a control, alligator pepper (50% and 100%), castor soap (50% and 100%)*, and* ginseng (50% and 100%).

Data Collection and Analysis

The radial growths (cm) were measured, the data was subjected to the analysis, and the means were separated using (DMRT) Duncan's new Multiple Range Test ($P≤0.05$) in IBM SPSS version 23.

RESULTS

Results of the Biocontrol Experiment

The means of the treatments with all the *Trichoderma* isolates were lower compared to the control at 24, 48, and 72 hours after inoculation (HAI), (Figure **1**). Among the isolates, *T. harzianum* AIPT26 consistently had lower mean pathogen radial growth, followed by *T. hamatum* ZXPB, then *T. virens* BGMZ2, and finally *T. harzianum* AIPT26. Thus, these isolates were different compared to the control and amongst themselves. All the biocontrol agents were able to successfully control the pathogen.

In order to determine the scientific significance of these findings, the results were subjected to ANOVA and DMRT procedures. The letters placed above each bar indicate the significant difference ($p \leq 0.05$) between corresponding mean and others in that series. The results of the separation of the means showed significant difference between all the *Trichoderma* species and the control, (Figure **1**). The topmost efficient treatment was *T. harzianum* (AIPT26) followed by *T. virens* isolate (BGMZ2) and *T. hamatum* (ZXPB). The *T. harzianum* (AIPT25) was the least effective biocontrol agent compared to the other isolates. This pattern was observed (at 24h, 48h, and 72 HAI) to be the same with minor changes.

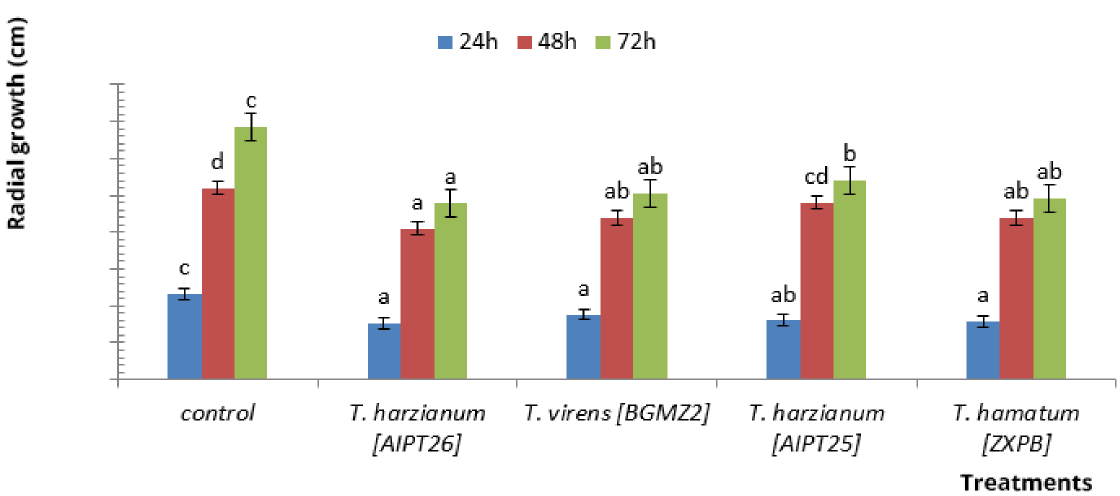
Results of The Chemical Fungicides Experiment

The means of the treatments using the synthetic fungicides were lower compared to the control at 24, 72, and 120 HAI, (Figure **2**). The fungicides consistently had lower mean pathogen radial growth compared to the control with time. Ridomil (50% and 100%) and Mancozeb (50% and 100%) completely inhibited the *F. verticillioides* up to 120 HAI. This perfect control was followed by Itraconazole 100% with barely more than 1.5 cm colony growth at 120 HAI. Itraconazole 50% controlled Fusarium species to a lesser extent compared to Itraconazole 100%, but it was better than Ciprox 100%. The least effective fungicide rate was Ciprox 50%. Nevertheless, all these fungicides induced lower radial growth rate of the pathogen with time compared to the control.

In order to determine the objective significance of these findings, the results were subjected to ANOVA and DMRT procedures. The letters placed above each bar indicate the significant difference between that mean and others in that series. The separation of the means showed significant difference ($p \leq 0.05$) between all the chemical fungicides and the control (Figure **2**). The most effective control agent was Ridomil 100%, Ridomil 50%, Mancozeb 100%, Mancozeb 50%, Itraconazole 100%, and Itraconazole 50% (N.B: the effects of these agents were significantly at par statistically). The least effective agent was Ciprofloxacin 100% followed by Ciprofloxacin 50%. However, Ciprofloxacin was significantly different when compared to the control throughout.

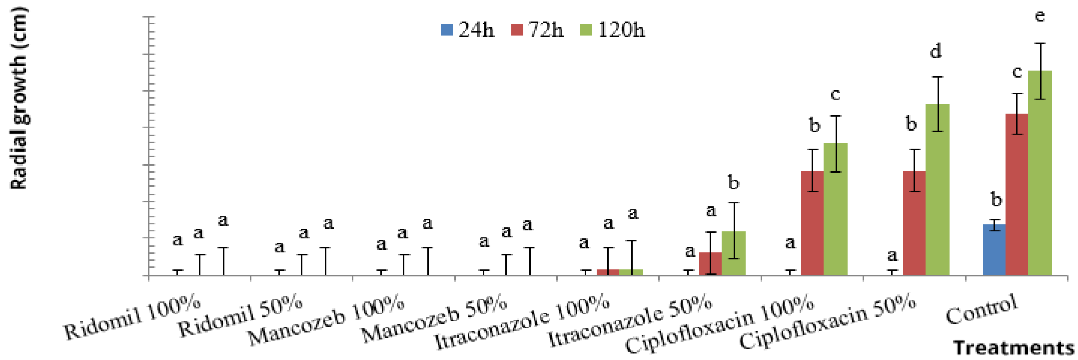
Results of the Plant Extracts Experiment

The means of the treatments with the botanical pesticides were lower compared to the control at 48, 96, and 120 HAI, (Figure **3**). The botanicals consistently had lower mean pathogen radial growth compared to the control with time. Castor soap (50% and 100%) completely inhibited the *F. verticillioides* up to 120 HAI throughout. This perfect control was followed by Alligator pepper 100% and Ginseng 100% which controlled the pathogen significantly. Alligator pepper 50% and Ginseng 50% controlled Fusarium species less compared to the other rates of the botanical agents. Nevertheless, all these fungicides permitted lower radial growth rates of the pathogen with time compared to the control.



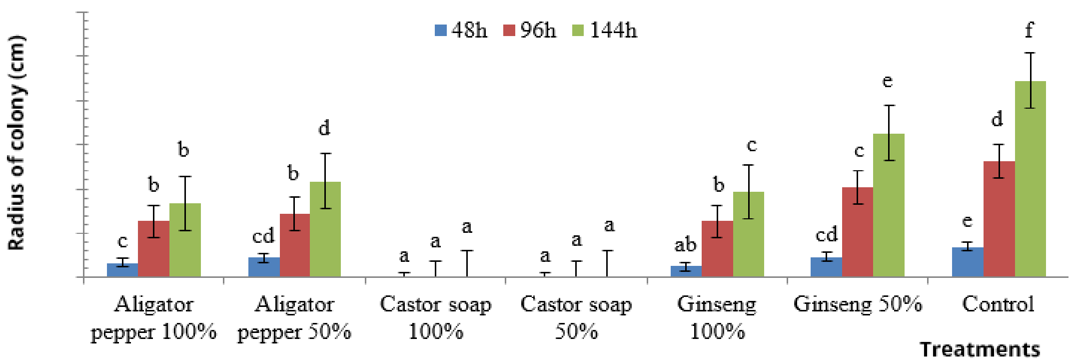
Means over which the same letter(s) are shown are statistically similar based on means separation using DMRT ($p \leq 0.05$).

Figure 1. Impact of the application of *Trichoderma* species on the radial growth of kernel rot agent.



Means over which the same letter(s) are shown are statistically similar based on means separation using DMRT ($p \leq 0.05$).

Figure 2. Influence of agro-chemicals on the radial growth of kernel rot pathogen.



Means over which the same letter(s) are shown are statistically similar based on means separation using DMRT ($p \leq 0.05$).

Figure 3. Consequences of applying botanical extracts on the radius of the colony of the kernel rot pathogen.

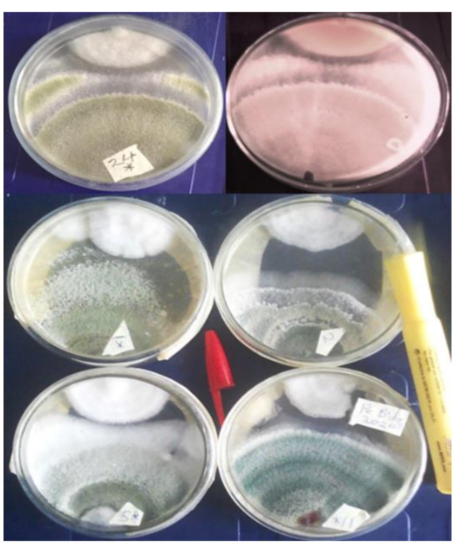


Figure 4. Some illustrations of the bio-control agents right at the point of contact of the colonies when myco-parasitism starts between the microbes* in the plates.

*Colony at the top of each plate shows the pathogen and colony at the bottom of each plate shows the bio-control agent. Top plates: right - view, left back view of the plate. Middle plates: right - *T. virens*, left - *T. hamatum.* Bottom plates: *T. harzianum* isolates. Mycoparasitism could be seen as conidia formation on top of the pathogen colony at later stages of competition but the images were not very good for use. No zone of inhibition was formed at the point of contact.

In order to determine the scientific significance of these outcomes, the results were subjected to ANOVA and DMRT procedures. The letters placed above each bar indicated the significant difference between that mean and others in that series. The results of the separation of the means revealed that the botanical extracts were significantly different ($p \leq 0.05$) compared to the control.

Castor soap was the most effective agent, (Figure **3**). The next top performers were Alligator pepper (at 100% concentration), then Ginseng (at 100% concentration), Alligator pepper (at 50%), and lastly Ginseng (at 50% concentration). However, from 48-144h, the trend shows that castor bean led the pack followed consistently by Ginseng (at 100% concentration) and then Alligator pepper (at 100% concentration). In the figures similar means are over-shadowed by the same letter(s) in each series. Figures **4** and **5** shows the in vitro dual culture of the biocontrol agent and the pathogen. The focus was on the point where the colonies me*et al*though the fungi compete using different mechanisms like mycoparasitism, competition for limiting resources and use of volatile exudates/gases, etc. The mycoparasitism sets in when limited resources are exhausted, thus the biocontrol may first have a concave looking front before the appearance of the conidia later over the pathogen's colony.

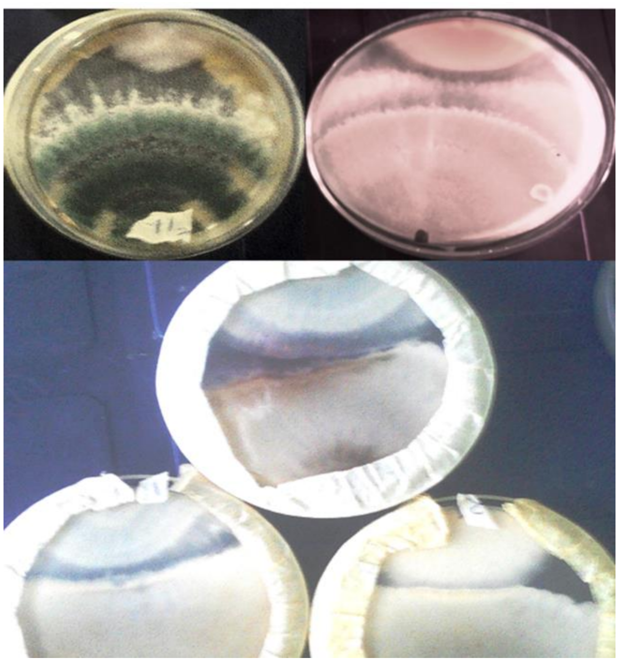


Figure 5. Close-up view of the dual culture set-up immediately at the point of contact of the colonies when myco-parasitism starts between the microbes* in the plates.

*No inhibition zone is left at the end of the growth and the plates look more like a carpet of mycelia with no distinction of zones of fungal growth. This stage still shows different colonies.

DISCUSSION

This is easier to discuss the outcomes of these three experiments since most sources cited combined the agents they used for control of pathogens. Here, we have discussed all the experiments clearly. In the current research, all the three; botanical, chemical, and biological control agents successfully controlled the pathogen in vitro. Firstly, this corroborated the finding that *Bacillus subtilis* and plant extracts were effective against an oomycetes' agent [26]. Application of Eucalyptus, Mahogany, and Cashew resins caused significant percentage inhibition (5–100%) of *F. oxysporum*.

Application of 100% Eucalyptus followed by the Mancozeb (both 50% and 100%), Eucalyptus 50%, and Mahogany 100% controlled *F. oxysporum* significantly more than all other treatments [27]. These findings on use of plant extracts concurred with the findings of this current trial using plant extracts.

Secondly, extracts of Eucalyptus (83.8-89.2%) and Neem (20.3–28.4%) successfully inhibited the growth of *A. flavus in vitro* [21]. This agrees with the findings that *Chromolaena odorata* and *Vernonia amygdalina* completely inhibited the *Botryodiplodia theobromae. Chromolaena odorata* extract produced the highest inhibition on all the fungi tested in this experiment [28].

Moreover, extracts of *M. oleifera* pods inhibited eight bacteria [29]. This is similar to the findings that *Zingiber officinale* (1.28 cm radial growth reduction) then *Allium sativum* (1.62 cm), *Datura stramonium* (2.17 cm), *Eucalyptus globulus* (2.50 cm), and *Azadirachta indica* (2.76 cm radial growth reduction compared to the Control) successfully controlled the tested pathogenic fungi experimented *in vitro* [30].

Besides, eucalyptus gum, plum seeds, bark of African locust bean tree, and orange seed extracts inhibited the growth of the *A. rolfsii* (8-100% inhibition) [31]. It was revealed that some plant extracts significantly (P≤0.05) controlled the test fungus more than the control which affirmed the present findings [32].

Thirdly*,* the *T. harzianum* isolates significantly (*P*≤0.05) inhibited the *Colletotrichum alatae,* thus proving that bio-control of fungal agents is feasible [32]. Percentage inhibition of *F. oxysporium* using *Trichoderma* isolates (16–87%

inhibition) was attained. Less area of the medium was covered by the pathogen due to the presence of the *Trichoderma* species compared to the control [27].

The percentage inhibition (between 28-82%) of *A. rolfsii* by *Trichoderma* and *Cladosporium* spp was achieved [31]. It was clearly shown that the biocontrol agents significantly inhibited the pathogen.

Fourthly, Copper-based fungicides-controlled the fungi more than other treatments [24]. Both 50 and 100% concentrations of mancozeb gave 100% inhibition of the *A. flavus* [21]. While, all the synthetic fungicides (especially mancozeb) induced 10-90% inhibition of *A. flavus* [31]. These findings confirmed the present positive outcomes of the sub-trial on the use of man-made fungicides.

Evidently the percentage inhibition attained from using Mancozeb and Copper (I) oxide + Metalaxyl was between 26–100%) [31]. In the field under *Fusarium oxysporum* f. sp. melongenae infection, the disease incidence reduced to 21.0% for Shincar, 31.9% for Topsin-M, and 35.9% for Flumax [30]. These outcomes clearly exhibited that application of biological, chemical, and botanical agents against fungal pathogens can potentially aid the farmers to achieve less yield loss.

CONCLUSION

Maize (*Zea mays*) is a staple food crop that suffers from numerous diseases induced by fungi, bacteria, nematodes, viruses, abiotic agents, and so forth. The kernels of this crop are severely infected by toxigenic spoilage agents like *F. verticillioides.* The biocontrol (using *Trichoderma virens, T. harzianum*, and *T. hamatum* isolates) effectively subdued *F. verticillioides*. Synthetic fungicides (Ridomil, Mancozeb, Ciprofloxacin, and Itraconazole) effectively controlled *F. verticillioides*. All botanical agents (Castor soap, Ginseng, and Alligator pepper) successfully inhibited the growth of *F. verticillioides.* These three groups of control agents are strongly recommended for the management of *F. verticillioides.* Furthermore, research on integration of these control agents and reduction of the synthetic fungicides is highly recommended.

CONFLICT OF INTEREST

All authors have no potential conflict of interest.

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