integrity Research Journals

Journal of Bioscience and Biotechnology Discovery
Volume 7(3), pages 42-46, October 2022
Article Number: 78A5D68D3
ISSN: 2536-7064
https://doi.org/10.31248/JBBD2022.168
https://integrityresjournals.org/journal/JBBD

Full Length Research

Biocontrol potential of *Gliocladium virens* against damping off inducing pathogens in *Amaranthus hybridus*

Habiba Maikudi Muhammed*, Aliyu Danjuma Aliyu, Deborah Yageh Stephen

Department of Biological Sciences, Ibrahim Badamasi Babangida University, Lapai Niger State, Nigeria.

*Corresponding author. Email: mmhabiba@ibbu.ed.ng, habibamaliyu@gmail.com; Tel: +2348065647965.

Received 9th September 2022; Accepted 29th September 2022

ABSTRACT: Damping-off disease is caused by several soil-borne fungi such as *Pythium ultimum* and *Rhizoctonia solani,* which infect seedlings and cause them to 'damp off' or collapse and decay. The aim of this study was to evaluate the biocontrol potential of *Gliocladium virens* against damping off inducing pathogens in *Amaranthus hybridus*. Infected leaves of *Amaranthus hybridus* were collected from some selected farmlands in Lapai Local Government Area of Niger State, Nigeria. The fungal species were isolated from the plants with damping-off symptoms using the agar pour plate method. The isolation of fungal species from goat dung and chicken droppings was conducted using the serial dilution agar plate method. Isolates were identified using morphological characteristics features from the mycological atlas. *Pythium ultimum* and *Rhizoctonia solani* were associated with the plants and upon testing the pathogenicity of these isolated fungi on the plants, the severity of the disease was 85 to 100%. The fungus *Gliocladium virens* was identified from the two composted manures. *Amaranthus hybridus* seeds treated with *Gliocladium virens* produced plants that have significantly increased plant height (45.67cm) than untreated seeds (27.10cm). Higher stem girth was observed when the seeds were treated with *Gliocladium virens* (8.21cm). This was significantly different from the stem girth in plants from untreated seeds (4.12cm). Number of leaves produced per plant was significantly increased in plants from treated seeds (23.54) and decrease in plants from untreated seeds (13.17). From the result of this study, it could be concluded that, *Gliocladium virens* when seed coated, is a potential biological agent in controlling damping-off diseases and this will help the general public towards eradicating vegetable diseases.

Keywords: Antagonists, biocontrol, chicken droppings, goat dung, pathogens.

INTRODUCTION

Damping-off is a disease of seedlings caused by several different fungi (Vanti *et al*., 2020). This disease causes emerging seedlings to collapse, often submerged in a mass of white fungal growth, (Al-Hussini *et al*., 2019). Damping-off diseases in bedding plant production are commonly encountered in the greenhouse and are primarily caused by the ubiquitous pathogen mainly *Pythium ultimum* and *Rhizoctonia solani* (Nofal *et al*., 2021; Chandrashekara *et al*., 2012). Among several *Pythium* species that cause damping-off disease, *P. ultimum* is the most consistently virulent and the most frequently isolated (Lamichhane *et al*., 2017). Additionally, *R. solani* was commonly isolated from bedding plants in midwestern greenhouses (Sureshkumar and Kavitha, 2019; Lumsden and Locke, 1989). Control of damping-off traditionally has emphasized proper sanitation and manipulation of the environment (Lamichhane *et al*., 2017). Disease control has been improved by the recent introduction of soilless potting is significant, and reliance on chemical fungicides was an accepted means of disease control for several important reasons. Fungicides are heavily regulated and vary from country to country in their usage and registration. Additionally, they can cause environmental pollution and may induce pathogen resistance in young seedlings (Kazan and Gardiner, 2018). The effectiveness of chemical fungicides may vary if they interact chemically with the planting material. The genus *Gliocladium* consists of several species of fungi that

are antagonistic to plant pathogens. *G. virens* has been shown to control soil-borne plant disorders, such as cotton seedling disease caused by *P. ultimum* (Mukherjee *et al*., 2014; Gupta *et al*., 2021) through the production of several antibiotics (Walter and Lumsden, 2019). *G. virens* also produces an endochitinase that inhibited spore germination and damaged the cell walls of exposed *Botrytis cinerea* Pers (Prajapati *et al*., 2020).

Amaranthus hybridus (Amaranth) is a nutritional vegetable largely grown and consumed in Niger State, and Nigeria as a whole. The viscosity of these leaves tends to lose its value due to pathogenic infection. *Pythium ultimum* and *Rhizoctonia solani* are among the pathogens that caused damping-off diseases in *Amaranthus hybridus*. The control of the disease is presently achieved using chemicals, the residuals of which are dangerous to human health and beneficial to microorganisms. Therefore, there is a need to come up with standard methods of assessing healthy seeds as well as treating infested seeds before sowing. This treatment of infested seeds is what this study has set out to achieve using pathogens present in the composts. The objective of this study is to identify potential antagonists from composted poultry droppings and goat dung that will effectively control *Pythium ultimum* and *Rhizoctonia solani* causing damping-off diseases under greenhouse conditions in Lapai Metropolis, Nigeria.

MATERIALS AND METHODS

Isolation of pathogenic fungi

The infected seedlings *Amaranthus hybridus* with symptoms of the damping-off disease were obtained from selected farmlands in Lapai, Niger State, in North Central Nigeria and brought to the Department of Biological Sciences Laboratory, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria for isolation and identification of the samples.

The infected samples of *Amaranthus hybridus* were rinsed in tap water and the necrotic portions were excised and cut into 1 mm pieces and surface sterilized with 10% sodium hypochlorite for 30 seconds and rinsed in four successive changes of sterile distilled water and the sections were then blotted dry on clean, sterile paper towels. They were then plated on Potato Dextrose Agar media (obtained from Titan Biotech Limited, India) in triplicates and incubated for 36 hours at 32±2°C under 12 hours photoperiod (Muhammad and Amusa, 2003; Muhammed and Muhammad, 2013).

Purification of isolates

The fungal colonies were recognized in the mixed-culture and were sub-cultured aseptically by transferring them onto fresh culture plates of Sabroud Dextrose Agar media (obtained from Titan Biotech Limited, India) three times before a single pathogen was obtained in a plate. The sub-culturing was done by using a sterilized loop and needle to obtain pieces of growing mycelium of fungi from parts and edges of the mixed cultures (Muhammed and Muhammad, 2013).

Pathogenicity of the isolates

The mycelia suspension of the isolates was produced in V8 broth medium in 250 mL conical flasks for 6 days. The mycelium of each isolate was filtered through the cheesecloth, gently pressed to remove excess liquid and blended for 3 seconds in a warring blender at the rate of 5 g of mycelium per millilitre of sterile deionized water. The resulting suspensions were used as inoculum. The inoculum was freshly prepared before the applications. Three weeks old vegetable seedlings growing in oven-sterilized topsoil (0.5 cm) contained in 15 cm diameter pots were inoculated with the mycelia suspension of the fungal isolates. The plants were then placed on benches in the greenhouse and observed for symptoms of the disease. A numerical scale of 0 to 5 was used to determined the disease severity (%) as follows; 0% = No infection; 1-20% = Mild infection; 21-40% = Moderate infection; 41-60% = High infection; 60% & above = Severe infection as described by Bem *et al.* (2010). The pathogens were later re-isolated from the inoculated plants and compared with the initial isolates (Muhammed and Muhammad, 2013).

Preparation of composts

The composts were prepared using goat dung and chicken droppings, as described by Abo and Badr (2001). The separate polythene bags were weighed with 30 kg of goat dung, and chicken droppings each. The polythene bags were sealed and a small hole was made to allow decomposition and microbial activity to take place. Each of the composts was watered daily and allowed to decompose for 21 and 24 days respectively (Muhammed and Muhammad, 2013).

Isolation and identification of microorganisms developed from the compost

Exactly ten grams (10 g) of soil compost samples were taken from the greenhouse. The samples were serially diluted to 10^{-10} to isolate the secondary microorganisms. One ml of the dilution at 10^{-2} was plated on PDA in triplicates, using the pour plate method. After 48 hours of incubation at 32°C, the Petri dishes were examined for the pattern of growth of microorganisms. Colonies with clear zones of inhibition of the growth of other microorganisms

around them were then subcultured on PDA until a pure culture was established. The secondary microorganisms isolated were identified as described by Barnett and Hunter (1996).

Preparation of fungal inoculum and seed coating

The isolate of *Gliocladium virens* that was identified from the composts was multiplied with the host plant of *Amaranthus hybridus* in a multi-spore pot culture containing a mixture of zeolite and expanded clay (1:1; v:v) for six months. The inoculum was sieved through a 250 µm mesh and mixed with the coating material silicon dioxide and starch (1:1:1 w/w). The most probable number (MPN) (Porter, 1979) value of the inoculum was ca. 400,000 infective propagules (IPs) per kg. *Amaranthus hybridus* seeds were surface-sterilized with 0.5% (v/v) sodium hypochlorite for 10 minutes. After spraying with sterile distilled water, seeds were coated by gradually adding inoculum-coating material mixture according to the pan coating method (Oliveira *et al*., 2016) and then air dried at 25°C for 24 hours. This resulted in ca. 9 IP per plant and a buildup of 50% of seed weight as described by Ma *et al*. (2019).

Soil treatment with the antagonistic fungus

Seeds of *Amaranthus hybridus* were planted in unsterile soil infested with *Pythium ultimum* and *Rhizoctonia solani*. *Gliocladium virens* inoculum was added as an in-furrow treatment at the rate of 6 g/m per pot. Control plantings were treated with ground peat moss. The experiment was done in a screen house at 14 hours photoperiod and temperatures of 22°C for *Rhizoctonia solani* and 20°C for *Pythium ultimum*. After fourteen (14) days of incubation, counting was conducted for seedling emergence and death. All treatments were replicated in triplicates (Howell, 1991).

Statistical analysis

Analysis of variance (ANOVA) was performed following a completely randomised design to test the significant effects and means compared using the LSD test (p≤0.05) as outlined by Duncan(1955).

RESULTS

Isolation of the fungi

Isolation from the infected seedlings revealed that *Pythium ultimum* and *Rhizoctonia solani* are associated with the plants and upon testing the pathogenicity of these isolated fungi, the severity of the disease was 85 to 100% from day 6 to 11 days, as presented in Table 1. The fungus *Gliocladium virens* was the most dominant identified from the two composted manures as presented in Table 2.

Table 1. Number of Days and the disease severity (%) of infected *Amaranthus hybridus* seedlings.

Days	Disease severity (%)
6	85
7	85
8	87
9	90
10	100
11	100

Table 2. Fungi species isolated from the composted wastes.

Composts	Fungi species isolated
Goat dung	*Gliocladium virens*
Chicken droppings	*Gliocladium virens*

Effect of *Gliocladium virens* as bio-control agent against damping-off disease on the performance of *A. hybridus* seedlings

Mean squares for the effect of the bio-control agent of damping-off on the performance of *Amaranthus hybridus* was presented in Table 3. The table showed that treatment had a significant effect (p≤0.05) on plant height, stem girth and number of leaves per plant. On the other hand, treatment was highly significant for (p≤0.01) the number of branches per plant and stem girth.

The mean effects of the bio-control agent of damping-off on the performance of *A. hybridus* was shown in Table 4. The table indicated that *Amaranthus hybridus* treated seeds with *Gliocladium virens* produced plants with an increase in plant height of 45.67 cm, which was significantly different from untreated seeds that produced a plant height of 27.10 cm. A higher stem girth of 8.21 cm was observed with treated seeds, this was significantly different from the stem girth of untreated seeds (4.12cm). The mean value of the number of leaves produced per plant in treated seeds (23.54) was significantly different from untreated seeds (13.17). There was an increased number of branches produced per plant in treated seeds (7.65). These were significantly different from untreated seeds (2.65) which produced a decreased number of branches per plant. Similarly, the leaf area in treated seeds produced (85.67cm^2) these value was significantly different from untreated seeds which produced a smaller leaf area of 48.01cm^2.

Table 3. Mean squares for effect of bio-control agent of damping off on *Amaranthus hybridus.*

Sample	PLT HT (cm)	STM girth (cm^2)	NOL/PLT	NOB/PLT	Leaf area (cm^2)
Treatment	28.96*	31.56*	37.40*	67.02**	32.52**
Error	31.11	23.12	11.31	23.89	12.10

Key: PLT HT = Plant height; **NOL/PLT=** Number of leaves per plant; **NOB/PLT =** Number of branches per plant; **STM GIRTH=** Stem girth; ** = Highly significant at (p≤0.01); * = Significant at (p≤0.05).

Table 4. Mean Effect of bio-control agent of damping off on *Amaranthus hybridus.*

Treatment	PLT HT (cm)	STM girth (cm)	NOL/PLT	NOB/PLT	Leaf area (cm^2)
Seeds+ *Gliocladium spp*	45.67a	8.21a	23.54a	7.65a	85.67a
Seeds without *Gliocladium spp*	27.10b	4.12b	13.17b	2.65b	48.01b

*Means with different letter in a column are significantly different from each other. **Key: PLT HT =** Plant height; **NOL/PLT =** Number of leaves per plant**; NOB/PLT =** Number of branches per plant**; STM GIRTH =** Stem girth.

DISCUSSION

The study revealed the microorganisms associated with damping-off disease in *Amaranthus hybridus*. The presence and isolation of these microorganisms revealed that they are the causative agents responsible for the death of such an economical and nutritional plant. In the present study, *Pythium ultimum* and *Rhizoctonia solani* are the biotic agents associated with damping-off disease of *Amaranthus hybridus* in the field, which are similar to those isolated from the inoculated seedlings suggesting that they were probably seed-borne pathogens. Similar fungal species have been isolated from damping-off disease in *Amaranthus hybridus* plants. Lamichhane *et al*. (2017) reported that damping-off diseases can be caused by a number of biotic factors which prevent seeds to germinate or seedlings to emerge notably those living in soil which are *Rhizoctonia solani* and *Pythium ultimum* destroying seeds and seedlings of almost all species of fruit, vegetable, ornamental, and forestry crop. Also, Gleń-Karolczyk *et al*. (2021) in their study had a similar report supporting the association of these fungi with the damping-off disease.

The fungus *Gliocladium virens* isolated from the manure in this study have earlier been reported to be soil-borne and they are saprophytic organisms causing the decay of organic matter. Their isolation from organic compost was in agreement with the reports of Walter and Lumsden (2019), who isolated *G. virens* from some organic material.

The pathogenicity test of the fungus isolated from the infected plants confirmed that they are the causative organisms of damping off disease of *Amaranthus hybridus* plants. This was earlier reported by Al-Hussini *et al*. (2019) and Dube *et al*. (2018) that pathogens become active when seeds are sown, which may result in seed decay or damping-off disease of vegetables.

In this study, the treatments had some significant effects as bio-control agent of damping-off disease on the performance of *A. hybridus* under a greenhouse environmental condition. This might be on the relatively controlled and are reasonably uniform, the ecological interaction between the pathogen- antagonist and host. This relationship was earlier reported by Lumsden and Locke (1989). The control of several vegetable seedling diseases caused by soil borne pathogens was reported by Papavizas (1985) and Lamichhane *et al*. (2017). The use of antagonists such as *Gliocladium virens* to control vegetable seedling diseases caused by soilborne pathogens was also reported by Maheshwary *et al*. (2020), this may augment the current chemical methods of control.

The biocontrol of damping-off diseases of *Amaranthus hybridus* treated seeds in this study have established that *Gliocladium virens* was capable of disease suppression, with increased in some growth parameters and reduced seedling death. Similar reports has been reported by Usman *et al*. (2013) and Sharma and Gothalwal (2017), that seeds treated with *Gliocladium virens* suppressed disease by increasing the growth parameters and reduced seedling death induced by the pathogens for several weeks under the greenhouse.

Conclusion

The fungal species *Pythium ultimum* and *Rhizoctonia solani* were found associated with damping-off diseases of *Amaranthus hybridus* plants. The pathogenicity test revealed they are causative microorganisms with 100% disease severity on the plants. *Gliocladium virens* isolated from the two composts was capable of suppressing the disease in treated seeds of *Amaranthus hybridus* . Its application may assist in the establishment of healthy and better *Amaranthus* seedlings both in field and the greenhouse.

CONFLICT OF INTEREST

The authors declare that they have not conflict of interest.

ACKNOWLEDGMENT

We are grateful to the entire staff of the Laboratory of Biological Sciences Department of Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria for providing technical assistance during this research. We also acknowledge the financial assistance receive from the management of Ibrahim Badamasi Babangida University through Tertiary Education Trust Fund for sponsoring this research (TETFUND).

REFERENCES

Abo, S., & Badr, E. (2001). Antagonistic effects of some compost *Phytopathology,* 68, 183-210.

Al-Hussini, H. S., Al-Rawahi, A. Y., Al-Marhoon, A. A., Al-Abri, S. A., Al-Mahmooli, I. H., Al-Sadi, A. M., & Velazhahan, R. (2019). Biological control of damping-off of tomato caused by Pythium aphanidermatum by using native antagonistic rhizobacteria isolated from Omani soil. *Journal of Plant Pathology*, *101*(2), 315-322.

Barnett, H. L., & Hunter, B. B (1996). *Ilustrated genera of imperfect fungi*. Burgess Publishing Company Minnesota. Pp. 10-100.

Bem, A. A., Oluma, H. O. A., Nwantiki, A. O., & Agede, A. Y. (2010). Some Fungi diseases Associated with Tomato (*Lycopersion esculentum L*.) in Benue State Nigeria. *Biotropic Research International Journal*, 2(1), 51-58.

Chandrashekara, C., Bhatt, J. C., Kumar, R., & Chandrashekara, K. N. (2012). Suppressive soils in plant disease management. In: Singh, A. (ed.). *Eco-friendly innovative approaches in plant disease management.* New Delhi: International Book Distributors. Pp. 241-256.

Dube, P., Paul, C. S., & Ngadze, E. (2018). Seed health tests of traditional leafy vegetables and pathogenicity in plants. *African Journal of Agricultural Research*, 13(15), 753-770.

Duncan, D. B. (1955). Multiple range and multiple F-tests. *Biometrics*, 11:1.

Gleń-Karolczyk, K., Boligłowa, E., Gospodarek, J., Antonkiewicz, J., & Luty, L. (2021). Effect of seed dressing and soil chemical properties on communities of microorganisms associated with pre-emergence damping-off of broad bean seedlings. *Agronomy*, *11*(9), Article number 1889.

Gupta, J., Singh, U. N., Akram, M., & Mishra, R. K. (2021). Potential of biological control agents for the management of soil-borne pathogens in pulse crops. *Journal of Food Legumes*, *34*(3), 149-165.

Howell, C. R. (1991). Biological control of *Pythium* damping-off of cotton with seed-coating preparations of *Gliocladium virens*. *Phytopathology,* 81, 738-741.

Kazan, K., & Gardiner, D. M. (2018). Transcriptomics of cereal–*Fusarium graminearum* interactions: what we have learned so far. *Molecular Plant Pathology*, *19*(3), 764-778.

Lamichhane, J. R., Dürr, C., Schwanck, A. A., Robin, M. H., Sarthou, J. P., Cellier, V., Messéan, A., & Aubertot, J. N. (2017). Integrated management of damping-off diseases. A review. *Agronomy for Sustainable Development*, *37,* Article number 10.

Lumsden, R. D., & Locke, J. C. (1989). Biological control of damping-off caused by *Pythium ultimum* and Rhizoctonia solani with Gliocladium virens in soilless mix. *Phytopathology*, *79*(3), 361-366.

Ma, Y., Látr, A., Rocha, I., Freitas, H., Vosátka, M., & Oliveira, R. S. (2019). Delivery of inoculum of Rhizophagus irregularis via seed coating in combination with Pseudomonas libanensis for cowpea production. *Agronomy*, *9*(1), Article number 33.

Maheshwary, N., Gangadhara Naik, B., Amoghavarsha Chittaragi, M., Naik, S. K., & Nandish, M. (2020). Compatibility of *Trichoderma asperellum* with fungicides. *The Pharma Innovation Journal, 9*(8), 136-140.

Muhammad, S., & Amusa, N. A. (2003). *In vitro* inhibition of growth of some seedling blight inducing pathogens by compost-inhabiting microbes. *African Journal of Biotechnology*, *2*(6), 161-164.

Muhammed, H. M., & Muhammad, S. (2013). *In vitro* inhibition of growth of *Aspergilus niger*, *Fusarium oxysporum* and *Rhizopus nigricans* by antagonistic microorganisms isolated from composted wastes. *Journal of Phytopathology and Plant Health*, *2*, 83-90.

Mukherjee, M., Mukhopadhyay, A. N., & Mukherjee, P. K. (2014). *Trichoderma virens*- An old warhorse in the battle against plant pathogens. *Annual Review of Plant Pathology (Vol. 6)*, 367-386.

Nofal, A. M., Abdelghany, T. M., & Abd-EL-Hamed, W. F. (2021). Significance of local Trichoderma isolates in controlling *Pythium ultimum* and *Rhizoctonia solani* on Bean in Egypt. *Egyptian Journal of Phytopathology*, *49*(2), 131-140.

Oliveira, R. S., Rocha, I., Ma, Y., Vosátka, M., & Freitas, H. (2016). Seed coating with arbuscular mycorrhizal fungi as an ecotechnologicalapproach for sustainable agricultural production of common wheat (Triticum aestivum L.). *Journal of Toxicology and Environmental Health, Part A*, *79*(7), 329-337.

Papavizas, G. C. (1985). *Trichoderma* and *Gliocladium*: Biology, ecology, and potential for biocontrol. *Annual Review of Phytopathology*, *23*(1), 23-54.

Porter, W. M. (1979). The' Most Probable Number' method for enumerating infective propagules of vesicular arbuscular mycorrhizal fungi in soil. *Australian Journal of Soil Research 17*(3), 515-519.

Prajapati, S., Kumar, N., Kumar, S., & Maurya, S. (2020). Biological control a sustainable approach for plant diseases management: A review. *Journal of Pharmacognosy and Phytochemistry*, *9*(2), 1514-1523.

Sharma, P. K., & Gothalwal, R. (2017). *Trichoderma*: a potent fungus as biological control agent. In: *Agro-environmental sustainability* (pp. 113-125). Springer, Cham.

Sureshkumar, P., & Kavitha, S. (2019). Bioprospecting potential of mangrove fungus from vellar estuary, southeast coast of india for biocontrol of damping off on mustard. *Research Journal of Biotechnology*, *14*, 72-78.

Usman, F., Shaukat, S. S., Abid, M., & Hussain, F. (2013). Rhizosphere fungi of different vegetables and their antagonistic activity against pathogenic fungi of brinjal and spinach. *International. Journal. Biological. Biotechnology*, 10, 255-259.

Vanti, G. L., Masaphy, S., Kurjogi, M., Chakrasali, S., & Nargund, V. B. (2020). Synthesis and application of chitosan-copper nanoparticles on damping off causing plant pathogenic fungi. *International Journal of Biological Macromolecules*, *156*, 1387-1395.

Walter, J. F., & Lumsden, R. D. (2019). *Gliocladium* and biological control of damping-off complex. In: *Ecological Interactions and Biological Control* (pp. 254-267). CRC Press.