Environment, Development and Sustainability
https://doi.org/10.1007/s10668-018-0273-1

Effect of carbon-to-nitrogen ratio on eco-friendly mycoherbicide activity from *Lasiodiplodia pseudotheobromae* C1136 for sustainable weeds management in organic agriculture

Charles Oluwaseun Adetunji[1] · **Julius Kola Oloke**[2] · **Gandham Prasad**[3]

Received: 16 March 2018 / Accepted: 10 October 2018

Abstract
The utilization of bioherbicides in place of chemical herbicides has been described as one of the sustainable eco-friendly technologies that can improve agricultural outputs. This work investigated the impact of different carbon and nitrogen sources on the bioherbicidal efficiency of *Lasiodiplodia pseudotheobromae* (C1136). The preliminary screening of various carbon and nitrogen sources was carried out, out of which sucrose and peptone were selected for further C:N ratio investigations using strain C1136. The fungus produces a different quantity of mycelium biomass and spores in varied ratios of C:N when compared to the basal medium. Also, the effect of C:N ratio was tested by evaluating for leaf necrosis assay, mortality rate and the dry-matter biomass from the aerial parts of *Chromolaena odoratum and Echinochloa crus-galli* under guided laboratory and screen house conditions. Both the 7.5:1 and 10:1 ratios produced optimal spores count of 6.1×10^7 and 5.9×10^7 CFU/mL, respectively ($p = 0.05$) when compared to the control containing potatoes dextrose broth without any carbon or nitrogen amendment produced a spores count of 1.1×10^7 CFU/mL. Also, the phytotoxic metabolite amended with C:N of 10:1 showed a strong herbicidal, high mortality and a significant decrease in dry weight on the seedling on the tested weeds. This demonstrated hypothetically the optimization influence of carbon (sucrose)–nitrogen (peptone) manipulations either in variable combination or as single nutrient based on the cultural development of strain C1136. Spores, mycelium biomass and biocontrol efficacy of the fungus all experienced accretion in sucrose and peptone nutrient environment and likewise support their use as an attractive alternative in the optimization of mycoherbicidal assays.

Keywords Mycelium biomass · Nitrogen · Carbon · Sporulation · C:N ratio · Mycoherbicide

✉ Charles Oluwaseun Adetunji
charliguitar@yahoo.com; adetunjicharles@gmail.com

Extended author information available on the last page of the article

Published online: 16 October 2018

1 Introduction

One of the greatest challenges encountered in sustainable Agriculture is weed management because they affect the ecosystem and consequently lead to reduction in profitable food production (Barberi 2002; DeDecker et al. 2014; Adetunji et al. 2017a, b, c). Constant usage of chemical pesticides has led not only to weed resistance, but also, bioaccumulation and biomagnification of the chemicals with subsequent health hazard and environmental pollution thereby disrupting the normal green house (Adetunji et al. 2017b). In view of the aforementioned, there is a need to embrace organic Agriculture system which supports the ecological and biological facets of farming system that could ensure the successful delivery of management goals required for sustainable organic agriculture (FAO 1999; Usama and Siddiqui 2016; Meng et al. 2017).

Carbon and nitrogen are critical to a healthy terrestrial ecosystem and plant cellular functioning. This is in addition to their popular use as a basic recipe for the optimization of media to permit mass production of spores or vegetative propagules (Gao et al. 2007). The coordinated manipulation to sustain optimal plant development and growth, and soil microbial metabolisms is what constitute carbon/nitrogen ratio or balance (Yang et al. 2011). Similarly, their dynamics have implication for numerous naturally arising biogeochemical cycles and by extension plant resilience, soil fertility via residue decomposition, weeds accretion as well as phytopathogenicity (Zheng et al. 2009). Moreover, it is hypothetical that increase in the utilization of carbon from decomposing organic matters of different origin result in the corresponding elevation of soil nitrogen (Mojdeh et al. 2013). In recent decades, there have been more emerging reports of experiments that investigate the influence of C:N ratio on the development, sporulation and mycelia biomass of fungi with biopesticidal potential (Tapia et al. 2012; Azim et al. 2014; Wang et al. 2014; Bushra and Narzish 2016). Conversely, empirical data obtained from the impact of carbon and nitrogen balance on the intrinsic effectiveness of biological prevention of weeds in agroecological systems remain inadequate (Alexandra and José 2005).

In arable environments, weeds compete vigorously with economic crops for space, nutrients and solar energy thereby reducing yield and interfere with harvesting operations in forests, farm fields, and plantations. Additionally, weeds act as alternate or collateral hosts to potentially harmful insect pests and pathogens also undermine the quality of harvested products (Barreto 2009). Crop losses due to weeds invasion amount to 30–40% of tropical agricultural production (Lorenzi 2009; Soltani et al. 2014, 2016). Uncontrolled weeds interference equally has consequences for business returns on crop production and remained a major constraint to food production in many regions of the world (Adetunji 2015).

Bioherbicides are the specialized type of microbial-based pesticide used in the prevention and management of undesirable plants or weeds (Hoagland 1990). In many cases, bioherbicides are formulated from mainly potent fungal organisms compared to bacteria (Weissmann et al. 2003) and viruses (Ferrell et al. 2008). Moreover, these microbes are normally manipulated or engineered and expressed with several techniques to potentiate their bioherbicidal property and expand their target range.

Culturing conditions such as culture medium constituents, level of aeration and lightening regimes are critical factors influencing the production of phytotoxins from pathogenic or rhizospheric microbes (Stierle et al. 1992; Adetunji et al. 2017a, b). Phytopathogenic fungi produce low molecular weight phytotoxins that are destructive to

host plants in low concentrations and can induce necrosis symptoms on their affected host plants (Wheeler 1981).

The production methods for bioherbicidal which include metabolic profiling of the agents must be low-cost and high yielding of viable inocula. The nutritional composition of the medium can have a dramatic effect on propagule attributes such as phytotoxin expression, the potency of phytotoxicity and viability during storage (Adetunji and Oloke 2013a, b).

Mostly, the culture media for fungi growth encompass carbon (C) and nitrogen (N) sources, and most fungi need some precise elements for development and multiplication (Walker and White 2005; Gao et al. 2007). Furthermore, numerous forms of C and N sources could be utilized by fungi due to their ability to produce extracellular enzymes that could help them in degrading a larger substrate into smaller and simple molecules (Lee et al. 2007). The nature and ratio, sources, C and N ratio and micronutrients are the vital factors in medium composition and optimization for the enrichment of fungal growth, sporulation, and metabolome expression as well as the enhancement of bioactivity (Zahra et al. 2011). Therefore, the present study was undertaken to screen various C and N sources and their combinations on the mycelial development and sporulation of strain C1136. Additionally, the fungal strain was assayed for phytotoxic metabolite and its bioherbicidal property on *Chromolaena odorata* (L.) R.M.King & H.Robinson (Siam weed) and *Echinocholoa crus-galli* (L.) P. Beauv. (barnyard grass) was also carried out.

2 Materials and methods

2.1 Source of microorganism

The wild fungus used in this study was isolated from *Tridax procumbens* leaves. The molecular characterization was done by the amplification of 18srRNA gene using universal primers. The bioherbicidal strain was identified as *Lasiodiplodia pseudotheobromae* and coded as strain C1136 with an accession number KY432690 (Adetunji 2015).

2.2 Inoculum preparation

The basal medium used for the inoculum growth was prepared with glucose 3.0%, malt extract 3.0% and peptone 0.2% (w/v), and it was later sterilized by autoclaving. The bioherbicidal strain C1136 was inoculated to the basal medium by removing out 0.9-mm-diameter agar disks from a 7-day-old culture of strain C1136 grown on potatoes dextrose agar. Three of these mycelia agar disks were used to inoculate 90 mL of the basal liquid medium in a 250-mL flat-bottom flask at 25 °C and the culture was kept for shaking inside an incubator at 180 rpm for 72 h.

2.3 Preliminary screening of carbon and nitrogen sources

The basal medium for the study contained 5.0 g of KH_2PO_4, 0.5 g of $MgSO_4{\cdot}7H_2O$, 0.5 g of $CaCl_2$, 0.05 g of $FeCl_3.6H_2O$ and 0.05 g of $CuSO_4$ in 1 L of sterilized water. Eight C sources that included maltose, glucose, sorbitol, glycerol, mannose, dulcitol, rhamnose, sucrose were used for the study. Ten grams (10 g) from the sugars were investigated for

their influence to enhance the production of the fungus mycelium. The mixtures were autoclaved at 121 °C for 15 min using 250-mL flat-bottom flask containing 100 mL basal medium. The flask was then inoculated with 10% (v/v) of the inoculum from strain C1136 after which the flask was shaken for 1 week at 25 °C on a rotary shaker at 180 rpm. At the end of the submerge fermentation, the fresh mycelium was harvested and their weight was taken on a digital weighing balance, and the estimation of the dry biomass was also determined after drying the wet mycelia at 60 °C. The C source that enhanced and gave the maximum mycelium yield was selected for C:N ratio test. The same procedure used for investigating the significance of various C-sources on the development of the experimental fungus was repeated for the nine nitrogen (N) sources (ammonium sulfate, ammonium nitrate, ammonium chloride, trypsin from bovine, arginine, sodium nitrate, tryptone, peptone, and asparagine) used for the study. The N-source that formed the highest mycelium yield was also selected for C:N test. Having identified sucrose and peptone as the mutually exclusive optimal carbon and nitrogen nutrients bases that potentiate mycelium yield, and they were primed at different concentrations (0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4.0 g/L), were used independently as nutrient base for the development of strain C1136 and bioassayed for phytotoxicity on *Chromolaena odorata* and *Echinocholoa crus-galli* leaves.

2.4 Effect of carbon/nitrogen ratio on mycelium biomass and spores production

The best media containing sucrose (C) and peptone (N) that showed the highest activities form the two previous experiments were selected for C:N ratio studies. Sucrose and peptone were then differentially mixed to form 5:1, 7.5:1, 10:1, 15:1, 20:1, 40:1 and 1:45 ratios and added to 90 mL basal medium in 250-mL flat-bottom flasks in triplicate for each ratio. This is prior to the inoculation of each flask with 10% (v/v) of the inoculum development and incubation at 25 ± 2 °C for 5 days. These treatments were carried out under alternate fluorescent light and dark regime (12 h light: 12 h dark) in the first 2 days. The number of colony forming units from each ratio was assessed by a hemocytometer, while the biomass was determined as described above.

2.5 Bioherbicidal assay

The leaves of *Chromolaena odorata and Echinocholoa crus-galli* were surface disinfected with 70% ethanol and later rinsed with sterile distilled water for three times. The leaf-bioherbicidal bioassay was performed to determine the influence of different C and N sources and their ratios on the bioherbicidal potency of the experimental fungus on two target weeds using the 2 µl of active metabolite derived from the bioherbicidal strain C1136. The tested weeds were wounded on the surface by the injection of the phytotoxic metabolites using a sterile needle and transferred into Petri plates containing sterilized filter paper. The development of necrotic lesions on the tested weeds was observed after 3 days of inoculation (Adetunji et al. 2017b).

2.6 Effect of different C and N ratios on phytotoxin from strain C1136 on target weed in the screen house

Sucrose and peptone in various ratios (5:1, 7.5:1, 10:1, 15:1, 20:1, 40:1 and 1:45) were added to 90 mL basal medium in 250-mL flat-bottom flasks in quadruplicate for each ratio.

Thereafter, eighteen germinable seeds of *Chromolaena odorata* and *Echinochloa crus-galli* were planted per potted soil. These were then thinned to 13 seedlings per pot prior to inoculating with 2 µl suspension of strain C1136. The phytotoxic metabolites obtained from the different sucrose and peptone ratio treatments were sprayed at the first leaf stage of *Chromolaena odorata* and *Echinochloa crus-galli*, respectively. The experiment was performed in a screen house and later incubated in at 25 °C for a 12 h photoperiod. The control was, however, sprayed with basal medium unamended with C:N ratio. The treatments were assessed daily for pathological signs by totaling the sum of dead leaves. The dry biomass in the two tested weeds was determined after 7 days. Symptomatic severity was evaluated using the rating scale of 0–5 scale as described by Smith (2003). This scale is as follows: 0 = absence visible symptoms, 1 = Presence of small necrotic flecks on the leaves, absence of lesions on the stem (0–5%), 2 = distinct lesions and wilting on leaves and/or stem (6–25%); 3 = more prominent and distinct lesions and wilting on leaves and/or stem (27–75%); 4 = complete leaf necrosis and serious stem wilting (76–99%), and 5 = total plant damage.

2.7 Effect of different C and N ratios on phytotoxin from strain C1136 on biomass of the tested weeds

The phytotoxin obtained from strain C1136 when C and N ratios were varied were determined on wet and dry weights of *Chromolaena odorata* and *Echinochloa crus-galli. This* evaluated at the end of the experiment. The stems of *Chromolaena odorata* and *Echinochloa crus-galli* were excised at the soil line and oven dried for 48 h at 60 °C in the laboratory to constant weight.

2.8 Data analysis

The obtained data were expressed as mean ± standard error mean and were analyzed using SPSS software 21. The null hypothesis of equality of mean effect was tested using the two-way ANOVA table at $p = 0.05$, and means of significant treatments were separated using Duncan's multiple range tests.

3 Results

3.1 Effect of carbon and nitrogen sources on biomass production from strain C1136

Mycelium biomass production of strain C1136 was boosted in basal medium amended by any one of sucrose, glucose, glycerol, mannose, maltose, dulcitol, rhamnose and sorbitol. Sucrose amended basal medium exhibited the highest wet (14.6 g) and dry biomass (4.4 g), while sorbitol formed the lowest wet of 1.2 g and dry weight of 0.1 g, respectively ($p = 0.05$) (Fig. 1A). Wild strain C1136 showed different levels of increase in mycelium biomass production in basal medium amended with various N sources. Peptone, however, formed the maximum mycelium biomass compared to the other nitrogen sources used. Peptone produced the highest wet (16.8 g) and dry biomass (6.0 g), while arginine was observed with the lowest wet (2.5 g) and dry biomass (0.8 g), respectively, after 7 days of inoculation ($p = 0.05$) (Fig. 1B).

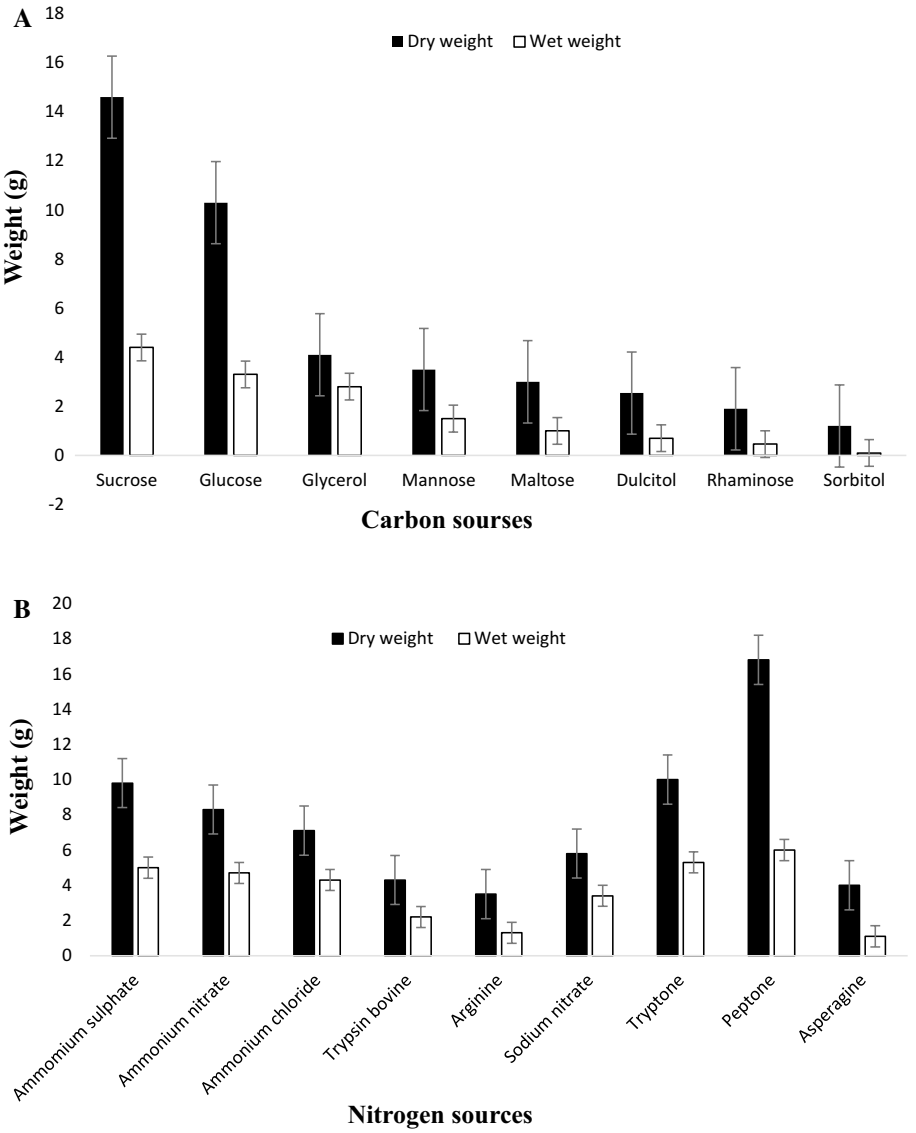

Fig. 1 Effect of different carbon sources (**A**) and (**B**) nitrogen sources on the wet and dry mycelia biomass of the strain C1136. Each value is the mean for three replicates, and vertical bars indicate the standard errors

3.2 Effect of different concentrations of peptone and sucrose on phytotoxic metabolite production from strain C1136

The concentration 2.0 g/L peptone was found to produce maximum growth and phytotoxic metabolite by the test fungus. At that concentration (2.0 g/L), it produced a necrotic area of 4.8 mm^2 on *Chromolaena odoratum and* 3.0 mm^2 on *Echinochloa crus galli* leaves,

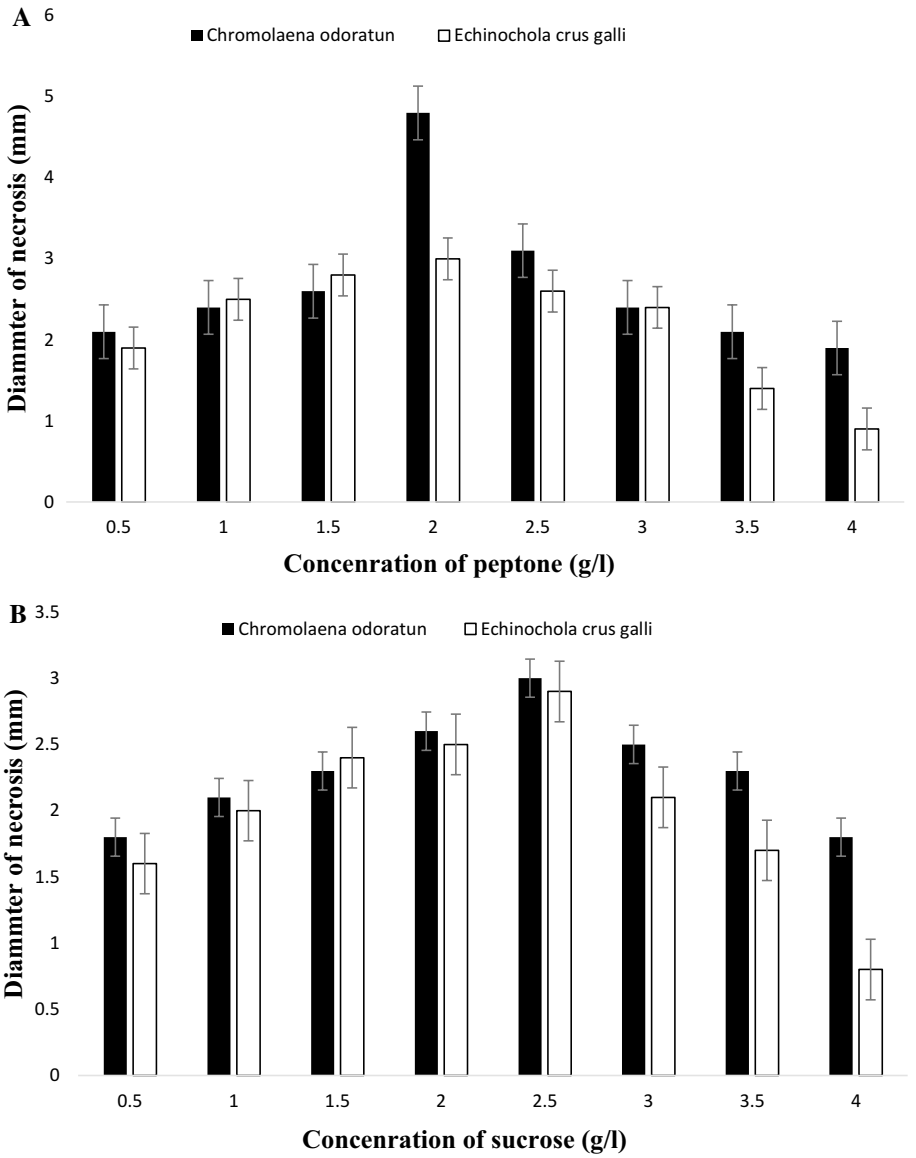

Fig. 2 Effect of different concentrations of (**A**) peptone and (**B**) sucrose on production of phytotoxic metabolite from strain of C1136 on *Chromolaena odorata* and *Echinochloa crus-galli*. Each value is the mean for three replicates, and vertical bars indicate the standard errors

respectively ($p=0.05$) (Fig. 2A).The concentration of 2.5g/L sucrose yielded the maximum growth and phytotoxic metabolite yield by strain C1136. Similarly, necrotic area of 3.0 mm^2 on *Chromolaena odoratum* and 2.9 mm^2 on *Echinochloa crus-galli* leaves, respectively, were observed at the concentration of 2.5g/L ($p=0.05$) (Fig. 2B).

Table 1 Effect of different C:N ratios on mycelium biomass of strain C1136 in fixed carbon and nitrogen basal salt media, respectively

C:N ratio	Mycelial biomass (g/100 mL)	
	Fixed carbon	Fixed nitrogen
5:1	6.505 ± 0.488^{e}	8.439 ± 0.451^{d}
7.5:1	8.285 ± 0.648^{f}	8.782 ± 0.479^{d}
10:1	8.068 ± 0.768^{f}	6.356 ± 0.342^{c}
15:1	2.761 ± 1.078^{d}	4.101 ± 0.296^{b}
20:1	2.351 ± 0.188^{cd}	6.283 ± 0.737^{c}
40:1	6.283 ± 0.737^{c}	6.244 ± 0.479^{c}
45:1	1.57 ± 0.0459^{b}	6.433 ± 0.398^{c}
Control	0.444 ± 0.169^{a}	3.031 ± 1.217^{a}

Values are mean ± standard error. Means with different superscripts within the same column are significantly different ($p = 0.05$)

3.3 Effect of different carbon and nitrogen sources on phytotoxic metabolite, biomass and spore production for the assessments of the severity of disease from strain C1136

Ratio 5:1 produced the highest mycelium biomass with 8.439 ± 0.451 g compared to the control that showed 0.444 ± 0.169 g ($p = 0.05$) (Table 1). Sporulation of strain C1136 was significantly enhanced by the C:N ratios (Table 2). Both the 7.5:1 and 10:1 ratios produced optimal spores count of 6.1×10^7 CFU/mL and 5.9×10^7 CFU/mL, respectively ($p = 0.05$) when compared to the control containing potatoes dextrose broth without any carbon or nitrogen amendment produced a spores count of 1.1×10^7 CFU/mL. The result obtained from the disease severity showed that the phytotoxic metabolite amended with C:N of 10:1 showed the highest disease severity of 85.2 and 76.4% on *Chromolaena odorata* and *Echinochloa crus-galli*, respectively when compared to the control that demonstrated no effect of disease severity ($p = 0.05$) (Fig. 4). It was observed that the phytotoxic metabolite amended with C:N of 10:1 gave the highest dry biomass of 0.9 and 0.5 g, respectively, on *Chromolaena odorata* and *Echinochloa crus-galli*, respectively, when compared to the control that 3.4 g and 2.9 g, respectively, on *Chromolaena odorata* and *Echinochloa crus-galli* ($p = 0.05$) (Fig. 3A and B).

4 Discussion

Optimization techniques are often critical processes employed in bioproducts and biopharmaceutical research (Bushra and Narzish 2016). It involves an ordered manipulation of a basal medium or fermentation systems to generate direct or indirect interactions among introduced (supplemented) and extant components that sustainably maximize the yield of an assay. Suffice to say that optimization techniques generally potentiate selected nutrient factors to permit organismal growth or bioproduct yields in fermentation and biopesticidal studies (Gresham and Inamine 1986; Pathak and Martirosyan 2012). Human preference for increasing food production attracts a corresponding research need for alternative bioresources to combat food loss to phytopathogenic microorganisms, weeds as well as pests. Optimization technologies have equally had positive implications for health, ecological and environmental risk mitigating solutions in agriculture as well as horticulture. While weeds

Table 2 Effect of C:N ratios on spores production of Strain C1136 in fixed carbon and nitrogen basal salt media, respectively

C:N ratio	Spore yield × (10^8)	
	Fixed carbon	Fixed nitrogen
5:1	60.716 ± 0.543^{f}	30.440 ± 0.69^{b}
7.5:1	55.533 ± 0.629^{d}	60.114 ± 0.818^{f}
10:1	50.602 ± 0.654^{b}	59.054 ± 0.139^{e}
15:1	57.089 ± 0.873^{e}	35.868 ± 0.57^{c}
20:1	53.817 ± 0.292^{c}	37.920 ± 0.648^{d}
40:1	56.307 ± 0.913^{de}	37.833 ± 0.617^{d}
45:1	55.562 ± 0.511^{d}	37.805 ± 0.497^{d}
Control	20.41 ± 0.553^{a}	11.31 ± 0.397^{a}

Values are mean ± standard error. Means with different superscripts within the same column are significantly different ($p = 0.05$)

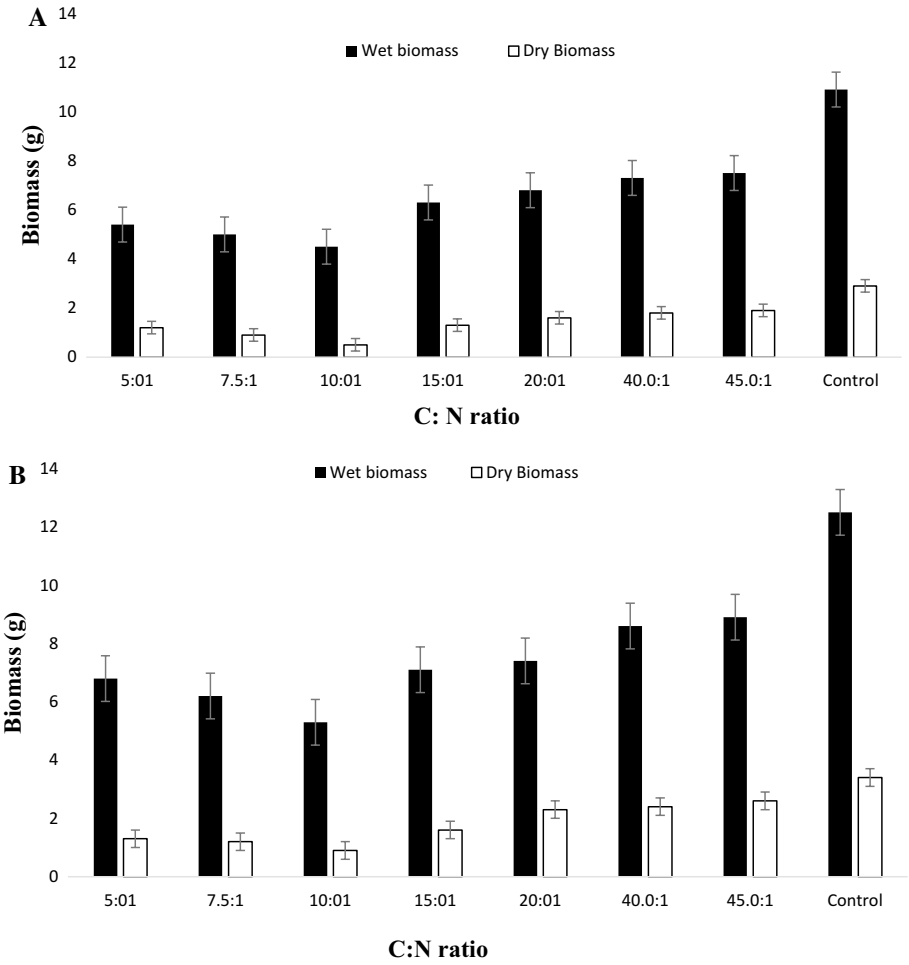

Fig. 3 Effect of C:N amendment on the phytotoxic metabolites produced from strain C1136 on biomass (g) of *Chromolaena odorata* (**A**) and *Echinochloa crus-galli* (**B**)

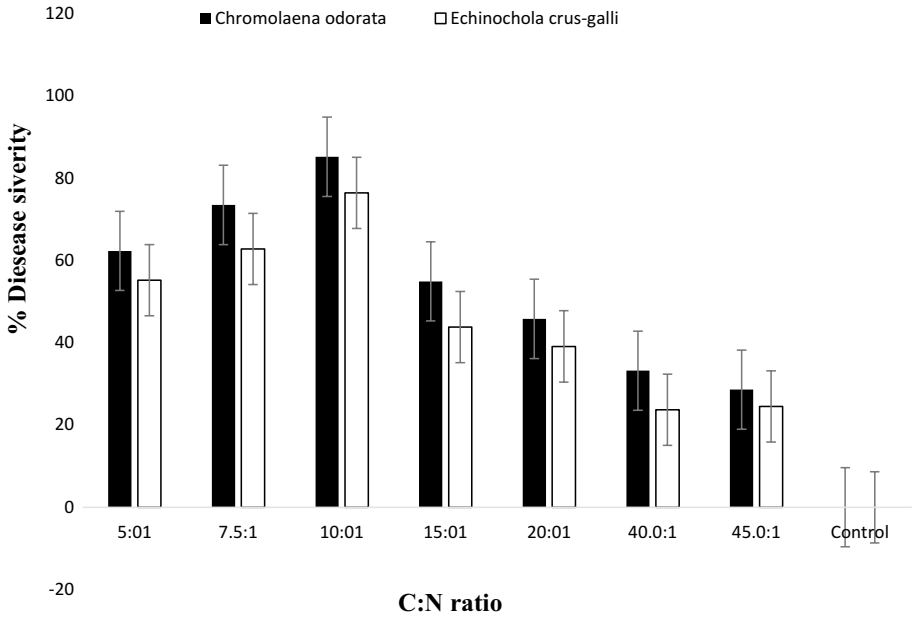

Fig. 4 Effect of C:N amendment on the phytotoxic metabolites produced from strain C1136 on disease severity of *Chromolaena odorata* and *Echinochloa crus-galli*

ranked behind arthropods in devastating crop production, the inexpensive development and commercialization of bioherbicides rely on a suitable scientific method that optimizes viable, virulent inoculum production and sufficient biomass which can store within dry formulations (Jonsbu et al. 2002). In this process, the selection of a suitable basal medium (defined and undefined) which optimizes growth is the first step in bioherbicidal inoculum production.

In order to effectively simulate the nutrient conditions in the natural habitat of potential bioherbicidal microorganisms, the different identified growth factors would have to be variable manipulated in one-factor-at-a-time in a basal medium for optimal assay of propagule yield, fitness and stability potential in bioherbicidal formulations. Many microorganisms naturally utilize nutritional factors such as organic and inorganic matters, trace metals and vitamins, and respond to their spontaneous proportionality that reflects a dynamic equilibrium. This underscores the concept underlying optimization techniques (Jonsbu et al. 2002; Alexandra and José 2005). Fungi had the innate versatility advantage over other microorganisms to grow on various organic and inorganic carbon sources as substrates, and utilize them for energy generation (Moore-Landecker 1996). While this may improve understanding on their saprophytic competitiveness expressed in fast mycelia growth, spores production and extensive enzyme systems, it may also be the reason for the observed growth and performance of strain C1136 on the various carbon sources used independently in compounding the culture medium (Silva et al. 2005; Khalid et al. 2006).

Screening all the carbon sources in this experiment, sucrose and glucose were observed to produce superior levels of mycelial biomass, respectively. Similarly, the peptone and tryptone yielded the highest mycelia biomass. While the underlying physiological processes responsible for the rapid assimilation of sucrose, glucose, peptone and tryptone, respectively, into body mass and energy is unclear, it may be philosophically connected with

the simplicity of their chemical nature or the innate potency of fungal expressed enzymes *ceteris paribus* (Yan et al. 2012; Nurliyana et al. 2017). It may be logically assumed that based on chemical complexity, the microbial assimilation of sucrose for biomass formation would be slower than that of glucose metabolism in contradiction to the observation made from this study. This demonstrates that the strain C1136 could possibly have a higher innate nutritional preference for sucrose compared to glucose. Suffice to say that the nutrient recipe for cell growth is species dependent (Gao et al. 2007). Sucrose-, glucose-, peptone-, and casein-derived tryptone equally optimizes phytotoxic metabolite activity of strain C1136 on the targeted weeds (*Chromolaena odorata* and *Echinochloa crus-galli*) at concentrations between 2.0 to 2.5g/L. This observation concurred with Vahidi et al. (2004) and Arotupin (2007) in a related study. The reason for the high phytopathology susceptibility of the weeds to the fungus phytotoxic metabolite exudations from sucrose- and glucose-amended media is unclear and requires further investigation. Vahidi et al. (2004) and Gao et al. (2007) reported good growth and biological control efficacy with the use of complex organic nitrogen sources such as yeast extract and peptone) compared to inorganic nitrogen sources (NH_4Cl and $NaNO_3$) in inoculum development. This suggests that the sugar and nitrogen bases used in this study may have potentially invigorated the fungus herbicidal potential by hydrolytically acquiring mineral components, growth, and energy boosters that impart phytotoxic strength (Mansour et al. 1996).

Carbon concentration and C:N ratios were observed from this study to impact spore yield and pathogenicity. There are significant increase in the level of conidiation from strain C1136 which might be linked to the different nutritional environments investigated (Jackson and Bothast 1990). It, therefore, implies that experimental attempts to optimize spore production of potential biocontrol agents of weeds must take into consideration both the quantity and efficacy of the spores targeted for mass production. C:N ratio 5:1 produced the highest amount of mycelium biomass and spores. In other words, the biomass production reduced when the supply of nitrogen decreases and complete lack of nitrogen might have stopped the growth of the bioherbicidal agent. Therefore, if additional carbon were to be added in a high C:N ratio, nitrogen may compete with temperature in limiting fungal growth (Wang et al. 2014).

In a nitrogen-scarce situation, the wild strain C1136 changes from vegetative growth to sporulation (Yu et al. 1998), suggesting the fundamentality of a balanced proportionality of carbon–nitrogen in the optimization of biological assays. Jackson and Schisler (1992) and Krasniewski et al. (2006) made similar observation supporting that nitrogen limitation and calcium supply prompt sporulation at a higher rate when compared to carbon starvation. The ratio of C:N engaged in this study to improve the sporulation of strain C1136 contained great quantity sucrose and small amount of peptone. The capability of fungi to sporulate was linked to a refined interactive organization of temporal and spatial regulatory gene expressions, cell specialization, and intercellular communication (Adams et al. 1998; Yan et al. 2012).

Our result shows that the medium composition containing peptone and sucrose maximized the amount of mycelium biomass produced, phytotoxic metabolite expressed via fermentation, and bioherbicidal efficacy as indicated by the extent of phytopathological damage to target weeds (Stierle et al. 1992). A complex physical tripartite (fungus, nutritional environment, and target weeds) interaction and chemical communications or signaling are fundamental to fully understand the observation made from this study (Vera-Estrella et al. 1994; Zheng et al. 2009). How this interactive complexity has invigorated the virulence of strain C1136 is giving to logical assumptions that linked to enhanced phytotoxicity strength or adaptive modification of inocula for improved pathogenicity and inoculums threshold

potential (Baker et al. 1997). Fungi and other microorganisms that produce such phytotoxic biochemicals are attractive for potential biocontrol agents study. Literature abounds on the diverse strength of fungi as bioherbicidal of various weeds through the same principles used in this present study (Charudattan and Rao 1981; Zhang and Watson 1999; Yoshida et al. 1999).

5 Conclusion

In this research work, the effect of C:N ratio greatly influences the rate of pathogenicity, induction of disease symptoms of the phytotoxic metabolites of strain C1136 when performed under liquid fermentation. Moreover, it was observed that sucrose (C) and peptone (N) exhibited the maximum activity (mycelium biomass, spores production, necrosis activity) among other carbon and nitrogen sources. The phytotoxic metabolite amended with C:N of 10:1 exhibited the maximum disease severity on *Chromolaena odorata* and *Echinochloa crus-galli* weeds.

Acknowledgements The authors are grateful to Council of Scientific and Industrial Research, New Delhi, India, and The World Academy of Science, Italy, for providing the fund with necessary facilities to carry out this work. FR number: 3240267282. (2013).

References

Adams, T. H., Wieser, J. K., & Yu, J. H. (1998). Asexual sporulation in Aspergillus nidulans. *Microbiology and Molecular Biology Reviews, 62*, 35–54.

Adetunji, C. O. (2015) Mycoherbicidal potentials of *Lasiodiplodia pseudotheobromae* and *Pseudomonas aeruginosa* formulation in the control of weeds in Cowpea-Maize field. PhD Thesis, pp. 1–389.

Adetunji, C. O., & Oloke, J. K. (2013a). Effect of temperature and storage time on shelf life of bioherbicidal formulations from the multi-combination of wild and mutant strain of *Lasiodiplodia pseudotheobromae and Pseudomonas aeruginosa*. *International Journal of Agricultural Science and Research, 3*(1), 147–160.

Adetunji, C. O., & Oloke, J. K. (2013b). Effect of wild and mutant strain of *Lasiodiplodia pseudotheobromae* mass produced on rice bran as a potential bioherbicide agents for weeds under solid state fermentation. *Journal of Applied Biology & Biotechnology, 1*(2), 018–023.

Adetunji, C., Oloke, J., Kumar, A., Swaranjit, S., & Akpor, B. (2017a). Synergetic effect of rhamnolipid from *Pseudomonas aeruginosa* C1501 and phytotoxic metabolite from *Lasiodiplodia pseudotheobromae* C1136 on *Amaranthus hybridus* L. and *Echinochloa crus-galli* weeds. *Environmental Science and Pollution Research.*. https://doi.org/10.1007/s11356-017-8983-8.

Adetunji, C. O., Oloke, J. K., Prasad, G. S., & Adejumo, I. O. (2017b). Effect of *Lasiodiplodia pseudotheobromae* Isolates, a potential bioherbicide for *Amaranthus hybridus* L. maize culture. *Notulae Scientia Biologicae, 9*(1), 131–137. https://doi.org/10.15835/nsb9110018.

Adetunji, C. O., Oloke, J. K., Prasad, G., Bello, O. M., Osemwegie, O. O., Mishra, P., et al. (2017c). Isolation, identification, characterization and screening of rhizospheric bacteria for herbicidal activity. *Organic Agriculture*. https://doi.org/10.1007/s13165-017-0184-8.

Alexandra B, José B (2005) *The importance of soil organic matter: Key to drought-resistant soils and sustained food and production*. FAO Soil Bulletin (No. 80). XCVII pages.

Arotupin, D. J. (2007). Microbiology and pectinase activity of fungi associated with soils cultivated with different crops. Ph.D Thesis, F. U. T., Akure, Nigeria.

Azim, K., Ouyahia, K., Amellouk, A., Perissol, C., Thami-Alami, I., & Oudi, B. (2014). Dynamic composting optimization through C/N ratio variation as a start-up parameter. In G. Rahmann, & U. Aksoy (Eds.). *Proceedings of the 14th ISOFAR scientific conference*. Istanbul Turkey.

Baker, B., Zambryski, P., Staskawicz, B., & Dinesh-Kumar, S. (1997). Signaling in plant-microbe interactions. *Science, 276,* 726–733.

Barberi, P. (2002). Weed management in organic agriculture: Are we addressing the right issues? *Weed Research, 47,* 177–193.

Barreto, R. W. (2009). Controle biológico de plantas daninhas com fitopatógenos. In W. Bettiol & M. A. B. Morandi (Eds.), *Biocontrole de doenças de plantas: uso eperspectivas* (pp. 101–128). Jaguariúna: Embrapa Meio Ambiente.

Bushra, K., & Narzish, M. A. (2016). Optimization of fermentation media and growth conditions for microbial xylanase production. *3 Biotech, 6,* 122.

Charudattan, R., & Rao, K. V. (1981). Bostrycin & 4-Deoxy bostrycin: Two non specific phytotoxins produced by *Alternaria eichorniae*. *Applied and Environmental Microbiology, 43,* 846–849.

DeDecker, J. J., Masiunas, J. B., Davis, A. S., & Flint, C. G. (2014). Weed management practice selection among 396 Midwest U.S. organic growers. *Weed Science, 62,* 520–531.

FAO. (1999). *Organic agriculture*. Rome: Food and Agriculture Organization of the United Nations.

Ferrell, J., Charudattan, R., Elliott, M., & Hiebert, E. (2008). Effects of selected herbicides on the efficacy of tobacco mild green mosaic virus to control tropical soda apple (*Solanum warm*). *Weed Science, 56,* 128–132.

Gao, L., Sun, M. H., Liu, X. Z., & Che, Y. S. (2007). Effects of carbon concentration and carbon to nitrogen ratio on the growth and sporulation of several biocontrol fungi. *Mycological Research, 111*(1), 87–92.

Gresham, R., & Inamine, E. (1986). Nutritional improvement of process. In A. L. Demain & N. A. Solomon (Eds.), *Manual of industrial microbiology and biotechnology* (pp. 41–48). Washington: ASM.

Hoagland, R. E. (1990). Microbes and microbial products as herbicides—An overview. *ACS Symposium Series, 439,* 2–52.

Jackson, M. A., & Bothast, R. J. (1990). Carbon concentration and carbon-to-nitrogen ratio influence submerged-culture conidiation by the potential bioherbicide *Colletotrichum truncatum* NRRL 13737. *Applied and Environment Microbiology, 56*(11), 3435–3438.

Jackson, M. A., & Schisler, D. A. (1992). The composition and attributes of Collectotrichum truncatum spores are altered by the nutritional environment. *Applied Environmental Microbiology, 58*, 2260–2265.

Jonsbu, E., McIntyre, M., & Nielsen, J. (2002). The influence of carbon source and morphology on nystatin production by *Streptomyces noursei*. *Journal of Biotechnology, 95,* 133–144.

Khalid, M., Yang, W., Kishwar, N., Rajput, Z. I., & Arijo, A. G. (2006). Study of cellulolytic soil fungi and two nova species and new medium. *Journal of Zhejiang University Science B, 7*(6), 459–466.

Krasniewski, I., Molimard, P., & Feron, G. (2006). Impact of solid medium composition on the conidiation in *Penicillium camemberti*. *Process Biochemistry, 41*(6), 1318–1324.

Lee, C. L., Hung, H. K., Wang, J. J., & Pan, T. M. (2007). Improving the ratio of monacolin K to citrinin production of *Monascus purpureas* NTU 568 under dioscorea medium through the mediation of pH value and ethanol addition. *Journal of Agricultural and Food Chemistry, 55*(16), 6493–6502.

Lorenzi, H. (2009). *Plantas daninhas no Brasil*. Nova Odessa: Plantarum.

Mansour, F. A., El-Shirbiny, S. A., & El-Metwaly, N. A. (1996). Demethyltetracycline biosynthesis by *Streptomyces aureofaciens* sub-species *viridulans* as influenced by medium composition. *Egyptian Journal of Microbiology, 31,* 221–235.

Meng, F., Qiao, Y., Wu, W., Smith, P., & Scott, S. (2017). Environmental impacts and production performances of organic agriculture in China: A monetary evaluation. *Journal of Environmental Management, 188,* 49–57.

Mojdeh, D., Malahat, R., Malihe, M., Seyed, D. J., Mohsen, Z., Sahar, A., et al. (2013). Effect of C/N ratio and media optimization through response surface methodology on simultaneous productions of intra and extracellular inulinase and invertase from *Aspergillus niger* ATCC 20611. *Biomed Research International*. https://doi.org/10.1155/2013/508968.

Moore-Landecker, E. (1996). *Fundamentals of Fungi* (4th ed., pp. 279–310). Upper Saddle River, NJ: Prentice Hall Press.

Nurliyana, M., Lai-yee, P., & Suraini, A. (2017). Optimization of metallo-keratnase production by *Pseudomonas* sp. LM19 as potential enzyme for feather waste conversion. *Biocatalysts and Biotransformation, 35*(1), 41–50.

Pathak, M., & Martirosyan, D. (2012). Optimization of an effective medium for culturing probiotic bacteria for applications in stict-vegetarian food products. *Functional Foods in Health and Disease, 2*(10), 369–378.

Silva, R., Lago, E. S., Merheb, C. W., Macchione, M. M., Park, Y. K., & Gomes, E. (2005). Production of xylanase and CMCase on solid substrate fermentation in different residue by *Thermoascus aurantiacus* Miehe. *Brazilian Journal of Microbiology, 36,* 235–241.

Smith, D. A. (2003). Evaluation of *Microsphaeropsis amaranthi* as a bioherbicide for the control of waterhemp (*Amaranthus tuberculatus*). M.S. Thesis, Purdue University, West Lafayette, IN. 47907.USA.

Soltani, N., Brown, L. R., Cowan, T., & Sikkema, P. H. (2014). Weed management in spring seeded barley, oats, and wheat with prosulfuron. *Weed Technology, 30*(4), 979–984.
Soltani, N., Dille, A. J., Burke, I. C., Everman, W. J., Van Gessel, M. J., & Davis, V. M. (2016). Potential corn yield losses due to weeds in North America. *Weed Technology, 30,* 979–984. https://doi.org/10.1614/WT-D-16-00046.1.
Stierle, A., Strobel, G., Stierle, D., & Sugawara, F. (1992). Analytical methods for phytotoxins. In H. F. Linskens & J. F. Jackson (Eds.), *Methods of plant analysis* (pp. 1–32). Berlin: Springer.
Tapia, E. V., Anschu, A., Carodini, A. L. V., Franco, T. T., & Deckmann, A. C. (2012). Optimization of lipid production by the oleaginous yeast *Lipomyces starkeyi* by random mutagenesis coupled to cerulenin screening. *AMB Express, 2,* 64.
Usama, M., & Siddiqui, M. J. A. (2016). Organic farming—An approach to sustainable development. *International Advanced Research Journal in Science, Engineering and Technology, 3,* 119–121.
Vahidi, H., Kobarfard, F., & Namjoyan, F. (2004). Effect of cultivation conditions on growth and antifungal activity of *Mycena leptocephala*. *African Journal of Biotechnology, 3,* 606–609.
Vera-Estrella, R., Barkla, B. J., Higgins, V. J., & Blumwald, E. (1994). Plant defense response to fungal pathogens. *Plant Physiology, 104,* 209–215.
Walker, G. M., & White, N. A. (2005). Introduction to fungal physiology. In K. Kavanagh (Ed.), *Fungi: Biology and application*. New York: Wiley.
Wang, X., Lu, X., Li, F., & Yang, G. (2014). Effect of temperature and carbon-nitrogen (C/N) ration on the performance of anaerobic co-digestion of dairy manure, chicken manure and rice straw: Focusing ammonia inhibition. *PLoS ONE, 9*(5), e97265. https://doi.org/10.1371/journal.pone.0097265.
Weissmann, R., Uggla, C., & Gerhardson, B. (2003). Field performance of a weed suppressing *Serratia plymuthica* strain applied with conventional spraying equipment. *BioControl, 48,* 725–742.
Wheeler, H. (1981). Role in pathogenesis. In R. D. Durbin (Ed.), *Toxins in plant disease* (pp. 477–494). New York: Academic Press.
Yan, S., Liang, Y., Zhang, J., & Liu, C. M. (2012). *Aspergillus flavus* grown in peptone as the carbon source exhibits spore density-and peptone concentration-dependent aflatoxin biosynthesis. *BMC Microbiology, 12,* 106.
Yang, Y., Luo, Y., & Finzi, A. C. (2011). Carbon and nitrogen dynamics during forest stand development: A global synthesis. *New Phytologist, 190*(4), 977–989.
Yoshida, S., Hiradate, S., Fujii, Y., & Shirata, A. (1999). *Colletotrichum dematium* produces phytotoxins in Anthracnose lesions of Mulberry leaves. *The American phytopathological Society, 90,* 285–291.
Yu, X., Hallett, S. G., Sheppard, J., & Watson, A. K. (1998). Effects of carbon concentration and carbon-to-nitrogen ratio on growth, conidiation, spore germination and efficacy of the potential bioherbicide *Colletotrichum coccodes*. *Journal of Industrial Microbiology and Biotechnology, 20,* 333–338.
Zahra, A., Afshin, E., Musaalbakri, A., Muhajir, H., Rosfarizan, M., & Arbakariya, B. (2011). Nutritional requirements for the improvement of growth and sporulation of several strains of *Monascus purpureus* on solid state cultivation. *Journal of Biomedicine and Biotechnology, 2011,* 1–10.
Zhang, W., & Watson, A. K. (1999). Isolation and partial characterization of phytotoxins produced by *Exserohilum monocreas*, a potential bioherbicide for control of *Echinochloa* species. In *Proceedings of the X international symposium on biological control of weeds*, pp. 125–130.
Zheng, L., Lv, R., Hsiang, T., & Huang, J. (2009). Host range and phytotoxicity of *Stemphylium solani*, causing leaf blight of garlic (*Allium sativum*) in China. *European Journal of Plant Pathology, 124,* 21–30. https://doi.org/10.1007/s10658-008-9387-x.

Affiliations

Charles Oluwaseun Adetunji[1] · Julius Kola Oloke[2] · Gandham Prasad[3]

[1] Applied Microbiology, Biotechnology and Nanotechnology Laboratory, Department of Microbiology, Landmark University, Omu-Aran, Nigeria

[2] Department of Pure and Applied Biology, Ladoke Akintola University of Technology, P.M.B 4000, Ogbomoso, Oyo State, Nigeria

[3] Department of Molecular Biology and Microbial Type Culture Collection and Gene Bank(MTCC), Institute of Microbial Technology, Sector 39A, Chandigarh, India