Effect of *Lasiodiplodia pseudotheobromae* Isolates, a Potential Bioherbicide for *Amaranthus hybridus* L. in Maize Culture

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Abstract

The use of phytopathogenic fungi in biological control of weeds may represent a promising alternative to the use of chemicals and may lead to the sustainability of agro-ecosystems. In the current study, strain C1136 was developed into various formulations and their activities were compared with a chemical herbicides using glyphosate in a screen house, applying standard procedure. All the bioherbicide treatments gave significantly higher yield component on the following parameters: tasselling, number of cobs, day of silking, number of ears, number of grain/cob, weight of 100 grains, number of cob, weight of cob, and length of cob from maize when compared to the chemical-treated herbicides and weedy cheek. Moreover, the bioherbicidal formulation coded BH4 (32 g of semolina + 6 g kaolin + 20 mL of glycerol + mutant strain of *Lasiodiplodia pseudotheobromae* + glucose+ sucrose + fructose + dextrose + lactose sugar + peptone) showed the highest activities when compared to other formulations. Conclusively, this study revealed that *Lasiodiplodia pseudotheobromae* C1136 strain has bioherbicidal activity and could therefore be exploited for large scale production of bioherbicides for weed control in conventional farming, to improve yield and enhance food security.

Keywords: bioherbicides, *Lasiodiplodia pseudotheobromae*, yield components, weeds, pasta granules

Introduction

The synthetic pesticides used to control pests may find their way into food chain through residues in grains consumed by man and animals (Maton *et al.*, 2016; Marvel and Sarah, 2016). Hence, there is the need for safe, eco-friendly and effective bio-pesticides. Maize is commonly used as food for man and for feeding livestock, constituting about 50% of monagastric animal feed. Pests, such as weevils, rodents and weeds are important factors militating against maize cultivation, thereby intensifying the keen competition between man and animals for cereals (Bankole and Mabekoje, 2004; Ofor *et al.*, 2009). Nigerian agriculture is dependent on the use of synthetic pesticides which has resulted in reduced body weight gain and liver dysfunction in animals (Adejumo *et al.*, 2014; Adejumo *et al.*, 2015). There is an obvious need to source for cheap and safe pesticides through the use of agro-industrial wastes.

The "bioherbicide strategy" is a microbial approach used to control weeds in agronomic crops (Charudattan, 1991). This strategy involves treating weed-infested crops with an inundative application of microbial propagules of highly aggressive, host-specific pathogens of the target weed (Jackson and Schisler, 1995; Jackson *et al.*, 1996). Constraints to the commercial development of these agents include the lack of low-cost production methods, stable microbial formulations with extended shelf life, and consistent weed control under field situations whenever it is applied. The development of low-cost methods for mass producing stable bioherbicidal propagules is a critical step in the commercialization of these products (Bowers, 1982).

Weed control strategies using microbial agents have received considerable attention in recent years due to the mounting expense for registration of chemical herbicides, ban on the use of chemical herbicides and public demands for reduced chemical uses (Auld, 1991; Charudattan, 1991). There is a worldwide resurgence of interest in the use of indigenous ecofriendly and host specific fungal pathogens, as

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herbicides (myco-herbicides) and a significant advance in mass production and fermentation of some of them, have been observed (Eilenberg *et al.*, 2001; Adetunji and Oloke, 2013).

Lasiodiplodia pseudotheobromae emerged from a recent separation of cryptic species originally identified as *L. theobromae* (Alves *et al.*, 2008). The species is known from Africa, Europe and Latin America, where it has been described from forest and fruit trees. Growing evidence suggests that *L. pseudotheobromae*, like *L. theobromae,* has a worldwide distribution and a wide host range (Begoude *et al.*, 2010; Mehl *et al.*, 2011).

Amaranthus species are listed among the most herbicide resistance-prone annual broadleaf weeds because of their high genetic variability, high production of rapidly germinating seeds, and efficient pollen and seed distribution (Lovell *et al.*, 1996). *Amaranthus* species including smooth pigweed (*Amaranthus hybridus* L.) have been reported to be resistant to ALS-inhibiting herbicides (Heap, 2015). *A. hybridus* and redroot pigweed (*A. retroflexus* L.) interference have been studied in a variety of field crops as major weeds that affect the productivity of beans (*Phaseolus* spp.) (Aguyoh and Masiunas, 2003; Amini *et al.*, 2014; Mirshekari *et al.,* 2014), soybean (*Glycine max*) (Bensch *et al.*, 2003), corn (*Zea mays*) (Knezevic *et al.*, 1994; Sheibany *et al.*, 2009; Ghanizadeh *et al.*, 2014) cotton (*Gossypium hirsutum*) (Buchanan and Burns, 1971; Buchanan *et al.*, 1980) and yield of corn (Knezevic *et al.*, 1995).

The current study aimed to study the screen house evaluation of myco-herbicides from *Lasiodiplodia pseudotheobromae* against *Amaranthus hybridus.*

Materials and Methods

Source and maintenance of fungal isolate

C1136 strain was isolated from a small chlorotic and necrotic lesions on leaves of *Tridax procumbens* from Ogbomoso and Ilorin environments. The most active strain showing herbicidal properties from the authors' previous work was coded as "C1136" (Adetunji and Oloke *et al.*, 2013). The inter transcribe spacer D1/D2 region characterization of the bioherbicidal strain identified it as *Lasiodiplodia pseudotheobromae* with an accession number KY432690. The fungal isolates were incubated on potatoes dextrose agar (PDA) for 7 days at 25 ± 1 °C in BOD incubator. At the end of each incubation period, the colonies were sub-cultured onto fresh media maintained on slants of PDA and stored at 4 °C in the refrigerator.

Exposure of Lasiodiplodia pseudotheobromae to UV light to induce random mutation

This experiment was carried out in order to observe whether by mutation it can improve the amount of phytotoxic metabolites in the medium. This was carried out by preparation of fresh PDA plate, to grow the organisms. After the growth of the organisms, cork borer was used to obtain several mycelia plugs from the culture into a sterile PDA plate. The sterile plate containing several mycelia plugs were placed under UV lamp at 300 nm wavelength at a distance of 30 cm to the plates. At different time interval (30, 60 and 90 minutes), 5 mycelia plugs were withdrawn and used as

inoculants. The mycelia plugs from the wild type culture served as the control. The wild strain was coded WLp, while the mutants were coded Lp 30, Lp 60 and Lp 90. The mycelia plugs from the domesticated type culture serve as the control (Adetunji and Oloke, 2013).

Preparation of pasta granules

The procedures developed by Connick *et al.* (1991), Adetunji and Oloke (2013) and optimized by Elzen *et al.* (2004) were used. Thirty-two grams (g) of semolina, a coarse durum wheat flour, 6 g kaolin, 2 g sucrose, 20 mL of fungal inoculum serving as active ingredient and 3 mL of deionized water were thoroughly mixed in a dish. The dough was then rolled through a small, hand-operated pasta machine (Marcato Model Ampia 150, Padova, Italy) into sheets, which were folded and extruded 10-15 times at different roller gap settings until it became homogeneous. The dough sheets were then extruded, without refolding, at a narrow setting to yield a 1-mm thick sheet. The sheets were then placed on aluminum foil and air-dried at ambient laboratory conditions [28 ± 2 °C, 33 ± 2% relative humidity (RH)]. The dried sheets were grounded in a grinder into granules and sieved to specific sizes (501- 2,000 μm). Their initial viability was determined by serial dilution method, by plating the different dilution on nutrient agar and potatoes dextrose agar.

The various pasta granules formulated into various bioherbicides were designated as followed:
- (a) BH1 = 32 g of semolina + 6 g kaolin + 20 mL of glycerol + wild strain of *Lasiodiplodia pseudotheobromae* (WLp) + glucose + sucrose + fructose + dextrose + lactose sugar + peptone
- (b) BH2 = 32 g of semolina + 6 g kaolin + 20 mL of glycerol + mutant strain of *Lasiodiplodia pseudotheobromae* (Lp 60)
- (c) BH3 = 32 g of semolina + 6 g kaolin + *Lasiodiplodia pseudotheobromae* (Lp 30) + glucose + sucrose + fructose + dextrose + lactose sugar + peptone
- (d) BH4 = 32 g of semolina + 6 g kaolin + 20 mL of glycerol + mutant strain of *Lasiodiplodia pseudotheobromae* (Lp 90) + glucose+ sucrose + fructose + dextrose + lactose sugar + peptone
- (e) BH5 = 32 g of semolina + 6 g kaolin + 20 mL of glycerol + wild strain of *Lasiodiplodia pseudotheobromae* (WLp)
- (f) BH6 = 32 g of semolina + 6 g kaolin + 20 mL of glycerol + mutant of *Lasiodiplodia pseudotheobromae* (Lp30)

Screen house parameters

The screen house parameters were as follows: 29 °C day temp, 25 °C night temp, 16-h day length, with 60 to 90% RH Supplemental lighting, computer controlled. Soil mix consisted of sterilized top soil. The soil was supplemented with commercial fertilizer (14:14:14, N:P:K). Experimental pots of 20 cm height were watered daily until germination. After germination, caution was taken not to get water in the whirl of the seedling. *Amaranthus hybridus* seeds were collected at Nigerian Stored product research institute farm and stored at 5 °C. Immediately after maize planting and at the cotyledon growth stage of maize, pigweed was planted in the same pot with the maize. Dates of weed sowing were selected so that

emergence times coincided with specific maize growth stages within the time frame of the critical weed-free period (Van Acker *et al.*, 1993). The following parameters were then taken into consideration: weed control per pot, weed dry weight and weed control efficiency. In each container fertilizer was applied at 1.5 g fertilizer per pot, while the pasta granules were applied at the rate of 1.0 g granules/pot. The pasta granules were applied when the seedlings were in the cotyledon to first leaf stage. Control pot was treated with pasta granules without fungus.

Procedures for data collection in the screen house

Data regarding days to emergence was recorded from the date of sowing till when 80% of the seedlings emerged in each pot. The data on emergence m^{-2} was recorded from each treated pot accordingly. Silking date was recorded when the silk became visible on the topmost ear of at least 50% of the plants in pots. The number of days from planting to silking was then expressed as days to silking. Days to tasseling were observed by counting the number of days from sowing till when 80% of the plants produced tassels and silks in each pot. Plant height was recorded at the time of physiological maturity from bottom to top excluding tassel. Number of ears plant^{-1} was noted by counting the number of ears in three central rows and were then averaged. The ears harvested from randomly selected plants in each pot were dried and shelled. Number of grains ear^{-1} was counted in selected the thousand grain weights were taken on randomly selected shelled ears of

each pot and then their average weight was recorded. Data regarding fresh and dry weight of weed biomass was recorded 26 d after sowing. After recording the fresh weight, weed samples were oven dried at 80 °C for 24 h and re-weighed for dry weight. The leaf area index (LAI) is the ratio of leaf area per plant to the area occupied by the plant. The assay was carried out in triplicates.

Results

Effect of the different formulated bioherbicides on the growth component of maize at screen house

All the bioherbicide treatments determined a significantly higher growth rate on the following parameters: plant height (cm), leaf area index, stem diameter (cm), emergence of maize when compared to the chemical treated herbicides and weedy cheek (Tables 1 and 2). Moreover, BH4 provided the highest growth rate on the above tested parameters on maize compared to other treatments throughout the sampling period, whereas the least growth parameters of maize tested was recorded in the weedy check.

At 30 DAT and harvest, the fresh weight of shoot obtained from maize treated with formulation from BH4 were 54.34 ± 3.14 g and 172.73 ± 5.01 g respectively, compared to weed check that had 25.39 ± 2.11 g and 96.21 ± 3.31 g at 30 DAT and harvest respectively; the dry weight of shoot treated with the same formulation had values of

Table 1. The growth parameters of maize after application of various treatments

Treatments	Fresh weight of shoot (g)		Fresh weight of root (g)		Dry weight of shoot (g)		Dry weight of root (g)	
	30 DAT	At harvest	30DAT	At harvest	30 DAT	At harvest	30 DAT	At harvest
BH1	35.60±1.88^c	126.73±5.61^c	16.93±2.03^c	68.34±2.15^c	17.67±2.01^c	42.38±3.18^c	6.68±0.62^c	16.21±1.09^c
BH2	46.79±2.13^b	158.39±4.04^b	20.76±2.11^b	34.01±2.10^b	19.78±1.37^b	52.11±2.03^b	8.69±0.91^b	20.67±2.16^b
BH3	37.23±2.67^c	136.32±4.93^c	17.11±1.23^c	70.27±4.04^c	18.17±1.13^c	46.38±1.63^c	8.01±0.58^c	18.67±1.27^c
BH4	54.34±3.14^a	172.73±5.01^a	25.23±3.76^a	89.63±4.23^a	20.67±1.12^a	60.78±3.93^a	9.32±1.07^a	22.69±1.91^a
BH5	33.63±2.26^c	111.29±3.45^c	16.01±1.87^c	65.52±0.94^c	16.34±2.30^c	39.78±2.54^c	6.12±1.02^c	15.98±1.23^c
BH6	42.87±2.93^b	143.28±3.13^b	17.36±1.95^b	76.13±4.31^b	19.23±2.05^b	48.21±2.79^b	8.13±0.99^b	19.66±1.65^b
CC	30.67±2.23^d	101.93±4.39^d	15.98±1.45^e	60.32±3.88^e	13.13±1.70^e	35.96±2.13^e	6.01±0.56^e	15.06±1.48^e
Weedy check	25.39±2.11^e	96.21±3.31^e	13.93±2.67^e	43.27±3.46^e	10.96±1.92^e	30.78±2.15^e	4.29±0.53^e	13.73±2.08^e

^{a}Average of the 3 replications. Mean separated by DMRT and column represented with the same letter are not significantly different at 5% level of significant. DAT = Days after planting; CC = chemical control, Control = Pestal granules without any fungus and adjuvant, BH1= 32 g of semolina + 6 g kaolin + 20 mL of glycerol + wild strain of *Lasiodiplodia pseudotheobromae* (WLp) + glucose + sucrose + fructose + dextrose + lactose sugar + peptone, BH2 = 32 g of semolina + 6 g kaolin + 20 mL of glycerol + mutant strain of *Lasiodiplodia pseudotheobromae* (Lp 60), BH3 = 32 g of semolina + 6 g kaolin + *Lasiodiplodia pseudotheobromae* (Lp 30) + glucose + sucrose + fructose + dextrose + lactose sugar + peptone, BH4 = 32 g of semolina + 6 g kaolin + 20 mL of glycerol + mutant strain of *Lasiodiplodia pseudotheobromae* (Lp 90) + glucose+ sucrose + fructose + dextrose + lactose sugar + peptone, BH5= 32 g of semolina + 6 g kaolin + 20 mL of glycerol + wild strain of *Lasiodiplodia pseudotheobromae* (WLp), BH6= 32 g of semolina + 6 g kaolin + 20 mL of glycerol + mutant of *Lasiodiplodia pseudotheobromae* (Lp30).

Table 2. The growth components of maize plant after application of various treatments

Treatment	Plant height (cm)		Leaf area index		Stem diameter (cm)		Emergence of maize
	30 DAT	At harvest	30 DAT	At harvest	30 DAT	At harvest	Days
BH1	36.38±2.09^c	232.11±6.15^c	3.35±0.30^c	3.65±1.01^c	1.32±0.11^b	1.65±0.21^b	8.00±0.32^b
BH2	46.32±2.13^b	286.74±4.38^b	3.37±0.80^b	3.72±0.06^b	1.35±0.23^a	2.00±0.11^a	7.00±0.43^a
BH3	39.75±1.91^c	253.83±4.22^c	3.90±0.31^c	4.00±0.67^c	1.21±0.12^c	1.87±0.60^c	8.00±0.17^b
BH4	49.67±1.30^a	299.35±5.61^a	4.01±0.26^a	4.36±1.50^a	1.38±0.51^a	2.15±0.80^a	7.00±1.02^a
BH5	43.82±1.52^c	219.36±3.29^c	3.23±0.56^c	3.43±0.61^c	1.20±0.90^c	1.48±0.36^c	8.00±0.33^b
BH6	40.21±1.33^b	260.86±4.34^b	3.43±0.94^b	3.86±0.17^b	1.34±0.22^a	1.93±0.14^a	7.00±0.88^a
CC	33.84±2.13^d	210.34±3.41^d	3.12±0.61^d	3.21±0.30^d	1.13±0.15^d	1.35±0.32^d	9.00±1.00^c
Weedy check	30.37±1.56^e	198.86±3.40^e	3.01±0.85^e	3.09±0.12^e	1.07±0.05^e	1.26±0.05^e	10.00±0.18^d

aAverage of the 3 replications. Mean separated by DMRT and column represented with the same letter are not significantly different at 5% level of significant. DAT = Days after planting; CC = chemical control,Control = Pestal granules without any fungus and adjuvant, BH1= 32 g of semolina + 6 g kaolin + 20 mL of glycerol + wild strain of *Lasiodiplodia pseudotheobromae* (WLp) + glucose + sucrose + fructose + dextrose + lactose sugar + peptone, BH2 = 32 g of semolina + 6 g kaolin + 20 mL of glycerol + mutant strain of *Lasiodiplodia pseudotheobromae* (Lp 60), BH3 = 32 g of semolina + 6 g kaolin + *Lasiodiplodia pseudotheobromae* (Lp 30) + glucose + sucrose + fructose + dextrose + lactose sugar + peptone, BH4 = 32 g of semolina + 6 g kaolin + 20 mL of glycerol + mutant strain of *Lasiodiplodia pseudotheobromae* (Lp 90) + glucose+ sucrose + fructose + dextrose + lactose sugar + peptone, BH5= 32 g of semolina + 6 g kaolin + 20 mL of glycerol + wild strain of *Lasiodiplodia pseudotheobromae* (WLp), BH6= 32 g of semolina + 6 g kaolin + 20 mL of glycerol + mutant of *Lasiodiplodia pseudotheobromae* (Lp30).

20.67 ± 1.2 g and 60.78 ± 3.93 g respectively, compared to the weed check that had 10.96 ± 1.92 g and 30.78 ± 2.15 g at 30 DAT and harvest (Table 1).

Similarly, the dry weight of root obtained from maize treated with formulation from BH4 were 30 DAT (25.23 ± 3.7 g) and harvest (89.63 ± 4.23 g) respectively compared to weed check that had values of 13.93 ± 2.67 g and 43.27 ± 3.46 g at 30 DAT and harvest respectively, while the dry weight of root treated with the same formulation had values of 9.32 ± 1.07 g and 22.69 ± 1.91 g respectively, compared to the weed check that had 4.29 ± 0.53 g and 13.73 ± 2.08 g respectively at 30 DAT and harvest (Table 1).

Moreover, the following data were obtained from the growth parameter of maize treated with formulation from BH4 and weed check. At 30 DAT and harvest time the plant heights obtained were 49.67 ± 1.30 cm and 299.35 ± 5.61 cm respectively, while the following values were obtained for weed check: 30.37 ± 1.56 cm and 198.86 ± 3.40 cm respectively at 30 DAT and harvest (Table 2). Also, at 30 DAT and harvest the leaf area index obtained were 4.01 ± 0.26 and 4.36 ± 1.50 respectively for BH4 treated maize, while the following values were obtained for the weed check with 3.01 ± 0.85 and 3.09 ± 0.12 respectively at 30 DAT and harvest. The values obtained for stem diameter of BH4 treated maize at 30 DAT and harvest were 1.38 ± 0.51 cm and 2.15 ± 0.80 cm, while the following values were obtained for the weed check with 1.07 ± 0.05 cm and 1.26 ± 0.05 cm respectively at 30 DAT and harvest. Moreover, BH4 treated maize emerged on the 7th day after planting, while the weed check emerged on the 10th day (Table 2).

In addition, BH4, out of all the formulated bioherbicides, determined significant effects on all the weed parameters of maize plant. However, the same formulation had a significant effect on the growth components of maize compared to the chemical treated herbicides and weedy cheek (Table 3). At 30 DAT and harvest, the weed control per treated pot from formulation BH4 were 3.01 ± 0.12 and 4.15 ± 0.04 respectively, compared to weed check that had 12.35 ± 1.01 and 19.21 ± 1.67 at 30 DAT and harvest respectively, while the dry weight of weeds treated with BH4 had values of 0.02 ± 0.01 g and 0.05 ± 0.01 g respectively compared to the weed check that had 1.89 ± 0.90 g and 2.3 ± 0.16 g respectively at 30 DAT and harvest (Table 3). Also, the weed control efficiency obtained from maize treated with formulation BH4 at 30 DAT and harvest were 0.73 ± 3.10% and 72.45 ± 3.70% respectively compared to weed check that had 0% weed control efficiency at 30 DAT and harvest respectively (Table 3).

The cost of production of the various formulated bioherbicides in comparison with herbicides used in the screen house

It was observed that the cost of production of the various bioherbicides formulation was far cheaper when compared to the chemical herbicide used, BH4 being the cheapest, followed by BH2, BH6, BH3, BH5, BH1, glyphosate and weedy check. The control treatment contained only the carrier, which is containing semolina only. BH4 was found to be the best formulation out of all the bioherbicides and its cost of production was $ 0.33, while the cost of production of a bottle of glyphosate was $ 3.17. Moreover, the cost of production of the control treatment which contained only the carrier made of semolina only was found to be $ 0.33 (Table 4).

Effect of the different formulated bioherbicides on the yield component of maize

All the bioherbicide treatments gave significantly higher yield component on the following parameters: tasselling, number of cobs, day of silking, number of ears, number of grain/cob, weight of 100 grains, number of cob, weight of cob, and length of cob from maize when compared to the chemical treated herbicides and weedy cheek (Table 5). Moreover, BH4 gave the highest growth rate on the above tested parameters on maize compared to other treatments throughout the sampling period, whereas the significantly least yield components of maize tested was recorded in the weedy check. The following parameters were obtained from the yield of maize when formulation BH4 was applied. It took 50 days for plants to tassel when BH4 was applied, while it took 60 days for the weed check. Similarly, silking took 55 days when BH4 was applied, while it took 68 days in weed check. The number of cobs/plant for BH4 was 1.89 ± 0.25 compared to weed check that had 1.21 ± 0.08. Also, the number of ears/plant for BH4 was 1.33 ± 0.02 compared to weed check that had 1.10 ± 0.01. Moreover, the values obtained for 100 g weight of cobs, number of grain/cob and cob length (cm) were 23.67 ± 4.23, 1.89 ± 0.06 g and 17.59 ± 2.01 cm, compared to weed check that had the following values respectively 6.68 ± 2.12, 1.21 ± 0.67 g and 10.23 ± 2.43 cm (Table 5).

Table 3. The weed parameters of maize plant after application of various treatments

Treatments	Weed control per pot		Weed dry weight (g)		Weed control efficiency (%)	
	30 DAT	At harvest	30 DAT	At harvest	30 DAT	At harvest
BH1	5.32±0.23[c]	7.70±1.01[c]	0.05±0.01[c]	0.75±0.26[c]	29.82±2.33[e]	31.67±2.63[f]
BH2	3.06±0.10[b]	6.21±0.09[b]	0.03±0.01[b]	0.06±0.05[b]	42.75±3.17[b]	51.23±2.54[c]
BH3	4.02±0.05[c]	6.78±0.82[c]	0.04±0.02[c]	0.07±0.02[c]	33.29 ±2.72[d]	40.23±3.14[d]
BH4	3.01±0.12[a]	4.15±0.04[a]	0.02±0.01[a]	0.05±0.01[a]	50.73±3.10[a]	72.45±3.70[a]
BH5	6.16±0.25[c]	7.76±1.03[c]	0.06±0.02[c]	0.87±0.31[c]	20.36±2.34[f]	33.13±2.41[e]
BH6	3.06±0.09[b]	6.83±0.06[b]	0.03±0.01[b]	0.06±0.02[b]	40.13±3.45[c]	52.31±3.10[b]
CC	8.37±1.00[d]	12.67±1.20[d]	0.91±0.11[d]	1.07±0.49[d]	7.32±1.04[g]	10.93±1.13[g]
Weedy check	12.35±1.01[e]	19.21±1.67	1.89±0.90[e]	2.3±0.164[e]	0.00±0.00[h]	0.00±0.00[h]

[a]Average of the 3 replications. Mean separated by DMRT and column represented with the same letter are not significantly different at 5% level of significant. DAT = Days after planting; CC = chemical control,Control = Pestal granules without any fungus and adjuvant, BH1= 32 g of semolina + 6 g kaolin + 20 mL of glycerol + wild strain of *Lasiodiplodia pseudotheobromae* (WLp) + glucose + sucrose + fructose + dextrose + lactose sugar + peptone, BH2 = 32 g of semolina + 6 g kaolin + 20 mL of glycerol + mutant strain of *Lasiodiplodia pseudotheobromae* (Lp 60), BH3 = 32 g of semolina + 6 g kaolin + *Lasiodiplodia pseudotheobromae* (Lp 30) + glucose + sucrose + fructose + dextrose + lactose sugar + peptone, BH4 = 32 g of semolina + 6 g kaolin + 20 mL of glycerol + mutant strain of *Lasiodiplodia pseudotheobromae* (Lp 90) + glucose+ sucrose + fructose + dextrose + lactose sugar + peptone, BH5= 32 g of semolina + 6 g kaolin + 20 mL of glycerol + wild strain of *Lasiodiplodia pseudotheobromae* (WLp), BH6= 32 g of semolina + 6 g kaolin + 20 mL of glycerol + mutant of *Lasiodiplodia pseudotheobromae* (Lp30).

Table 5. The effect of various formulations from *Lasiodiplodia psedotheobromae* on yield component of maize

Treatments	Days to tasseling	No of cobs/plant	Days to silking	No of ears/ plant	100 grains weight of cobs	No of grain/ cob	Cob length (cm)
BH1	56.00±2.01[abc]	1.43 ±0.03[ns]	58.00±2.46[b]	1.32 ±0.78 [ns]	16.43±0.03[e]	1.43 ±0.24[ns]	13.32±2.46[e]
BH2	52.00±2.14[cd]	1.76 ±0.22[ns]	55.00±2.18[b]	1.33 ±0.45 [ns]	21.86±3.21[b]	1.76 ±0.38[ns]	15.89±2.35[b]
BH3	55.00±2.10[abcd]	1.53 ±0.24[ns]	57.00±3.11[b]	1.22 ±0.23 [ns]	17.36±3.02[d]	1.53 ±0.21[ns]	14.78±2.21[d]
BH4	50.00±3.02[d]	1.89 ±0.25[ns]	55.00±4.01[b]	1.33 ±0.02 [ns]	23.67±4.23[a]	1.89 ±0.06[ns]	17.59±2.01[a]
BH5	56.00±1.97[abc]	1.38 ±0.2 [ns]	58.00±2.59[b]	1.14 ±0.35 [ns]	15.32±3.17[f]	1.38 ±0.26[ns]	13.1 ±2.53[f]
BH6	53.00±2.06[bcd]	1.63 ±0.03[ns]	56.00±2.62[b]	1.22 ±0.68 [ns]	19.67±3.19[c]	1.63 ±0.32[ns]	14.98±2.21[c]
Chemical control	58.00±4.05[ab]	1.41 ±0.06[ns]	65.00±3.00a	1.11 ±0.76 [ns]	12.39±2.53[g]	1.41 ±0.43[ns]	12.97±2.56[g]
Weedy check	60.00±3.16[a]	1.21 ±0.08[ns]	68.00±2.98[a]	1.10 ±0.01 [ns]	6.68 ±2.12[h]	1.21 ±0.67[ns]	10.23±2.43[h]

[a]Average of the 3 replications. Mean separated by DMRT and column represented with the same letter are not significantly different at 5% level of significant. DAT = Days after planting; ns = non-significant; CC = chemical control,Control = Pestal granules without any fungus and adjuvant, BH1= 32 g of semolina + 6 g kaolin + 20 mL of glycerol + wild strain of *Lasiodiplodia pseudotheobromae* (WLp) + glucose + sucrose + fructose + dextrose + lactose sugar + peptone, BH2 = 32 g of semolina + 6 g kaolin + 20 mL of glycerol + mutant strain of *Lasiodiplodia pseudotheobromae* (Lp 60), BH3 = 32 g of semolina + 6 g kaolin + *Lasiodiplodia pseudotheobromae* (Lp 30) + glucose + sucrose + fructose + dextrose + lactose sugar + peptone, BH4 = 32 g of semolina + 6 g kaolin + 20 mL of glycerol + mutant strain of *Lasiodiplodia pseudotheobromae* (Lp 90) + glucose+ sucrose + fructose + dextrose + lactose sugar + peptone, BH5= 32 g of semolina + 6 g kaolin + 20 mL of glycerol + wild strain of *Lasiodiplodia pseudotheobromae* (WLp), BH6= 32 g of semolina + 6 g kaolin + 20 mL of glycerol + mutant of *Lasiodiplodia pseudotheobromae* (Lp30).

Table 4. Cost of production of each treatment used for the control of weed in maize culture

Treatments	Cost of production ($)
BH1	0.43 ± 0.25[b]
BH2	0.38 ± 0.16[b]
BH3	0.41 ± 0.09[b]
BH4	0.33 ± 0.16[b]
BH5	0.44 ± 0.05[b]
BH6	0.40 ± 0.05[b]
Weedy check	3.17 ± 0.38[a]
CC	0.32 ± 0.12[b]

CC = chemical control; weedy check (control) containing only carrier without any bioactive carrier and adjuvants, BH1= 32 g of semolina + 6 g kaolin + 20 mL of glycerol + wild strain of *Lasiodiplodia pseudotheobromae* (WLp) + glucose + sucrose + fructose + dextrose + lactose sugar + peptone, BH2 = 32 g of semolina + 6 g kaolin + 20 mL of glycerol + mutant strain of *Lasiodiplodia pseudotheobromae* (Lp 60), BH3 = 32 g of semolina + 6 g kaolin + *Lasiodiplodia pseudotheobromae* (Lp 30) + glucose + sucrose + fructose + dextrose + lactose sugar + peptone, BH4 = 32 g of semolina + 6 g kaolin + 20 mL of glycerol + mutant strain of *Lasiodiplodia pseudotheobromae* (Lp 90) + glucose+ sucrose + fructose + dextrose + lactose sugar + peptone, BH5= 32 g of semolina + 6 g kaolin + 20 mL of glycerol + wild strain of *Lasiodiplodia pseudotheobromae* (WLp), BH6= 32 g of semolina + 6 g kaolin + 20 mL of glycerol + mutant of *Lasiodiplodia pseudotheobromae* (Lp30).

Discussion

Due to the development of herbicide resistance and a trend towards a chemically free environment, biological control using plant pathogens (bioherbicides) has been considered (Hoagland, 2007). The phytotoxic metabolite from *Lasiodiplodia pseudotheobromae* used during the current study had no adverse effect on the tested crop, but showed a bioherbicidal effect on all the tested weeds. Some authors have also reported similar results from the use of fungal phytotoxic metabolite (Evidente *et al.*, 2003).

Plant height is one of the important growth parameters of any crop, as it determines or modifies the yield contributing characters and finally shapes the grain yield (Reddy and Reddi, 2002). It was observed that the formulated bioherbicides enhanced the plant height of the maize, out of which BH4 had the best height of maize, because it enhanced plant height by a better soil aeration and increased the organic carbon content of the soil compared to others (Vijayakumar *et al.*, 2006). Variation in plant height of maize could be attributed to the various effect of treatment used for controlling weeds. These

results are in line with Akhtar *et al.* (1998) and Hussain *et al.* (1998), who stated that the maximum plant height was in treated pots compared with untreated ones.

The variations in LAI (leaf area index) are an important physiological feature that determines crop yield (Evans and wardlaw, 1976). The LAI is a determinant of dry matter production, and hence increased the total dry matter production results in increased grain yield for a given variety (Yoshida, 1972). There was no significant difference in leaf area per plant among the bioherbicide treatments when compared with the control.

The production of total dry matter per unit area is the prerequisites for higher production. The amount of dry matter production depends on the effectiveness of photosynthesis of the crop, which in turn depends on large and efficient assimilating area for adequate supply of solar radiation and carbon dioxide and favourable environmental condition (Reddy and Reddi, 2002). The total yield of dry matter accumulation is the total amount of dry matter produced, less than photosynthates used for respiration. Finally, the amount of economic yield depends on the manner in which the net dry matter produced is distributed among the different parts of the plants, which will determine the magnitude of economic yield (Aron, 1972).

Fageria *et al.* (1997, 2006) reported a similar reduction in shoot dry weight of upland rice from flowering to physiological maturity, as is was observed in the current study.

The number of grain rows per cob directly affected cob weight and ultimately grain yield of maize. These results are in agreement with the results obtained by Singh and Singh (1989) and Sulewska *et al.* (2006), who reported that weed control practices resulted in increased number of grain rows per cob. The significant variation for 1,000-grain weight in weed control treatments was due to vigorous growth and development of maize plants, which resulted in more photosynthates assimilation in grains.

These results are in line with those of Tanveer *et al.* (1999), Hussain *et al.* (1998) and Baye and Bouchache (2007) who concluded that 1,000-grain weight were greater in various controlled treatments than in weedy check in maize. The longer the cob length, the more would be the number of grains per cob and consequently higher yield in the form of grains. The data regarding to this parameter revealed that all weeds control treatments significantly affected the cob length

compared with weedy check. The significantly minimum cob length was recorded in weedy check plot. The cob length was significantly high in bioherbicides, whereas the longest was from BH4 treatment, which could be attributed to timely and efficiently weed control and thus, less weed competition period within the treatment, which allowed the maize plant to produce more photosynthetic material by using available nutrients. These results are similar to the findings of Singh and Singh (2003) and Stefanovic *et al.* (2004) who observed greater cob length in weed controlled treatments and smallest cob length in weedy check plots.

Grain yield is a function of the cumulative behaviour among various yield, influencing different components namely the number of cobs per plants, cob length, number of grains per cob and 1,000-grain weight, which showed variations by prevailing growing conditions and various crop management practices. This was mainly because of more number of grain rows per cob, number of grains per cob and 1,000-grain weight over weedy check. The lowest grain yield recorded in weedy check could be attributed to the maximum weed density which suppressed the growth and development of maize plants by competing for moisture, light and nutrients. The efficiency of various chemicals and other weed control practices in enhancing grain yield had also been observed by Stefanovic *et al.* (2004). The total number of grains per cob is an important yield component parameter of maize which indicated that all weed control practices significantly affected the total number grains per cob. The highest number of grains per cob in bioherbicides treated maize could be due to less weeds and consequently more photosynthesis available for plant growth and development. These results are confirmatory to Tanveer *et al.* (1999). They concluded that all weeds control treatments significantly increased the number of grain rows and number of grains per cob.

Conclusions

This study has provided information on the effectiveness of the local isolates of *Lasiodiplodia pseudotheobromae* used as bioherbicide. The pasta formulation proved to be an easy and cost-effective delivery system for the tested fungal isolate and has showed a high efficacy in weed control and consequently improved maize performance under screen house conditions. Moreover, more researches on the action way, non-target effect and field trial has to be carried out in different agro-ecological area to establish the mycoherbicidal potential of *L. pseudotheobromae* in different environmental conditions.

Acknowledgements

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