Environmental fate and effects of granular pesta formulation from strains of *Pseudomonas aeruginosa* C1501 and *Lasiodiplodia pseudotheobromae* C1136 on soil activity and weeds

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HIGHLIGHTS

- This work has discovered a novel alternative for chemical herbicides.
- Formulation BH4 showed the best evolution of CO2 and organic carbon content.
- Increased in microbial composition from bioherbicidal treated soil when compared to chemical herbicides.
- Absence of antagonism on Solanum lycopersicum seeds and seedlings when compared to the observed effect on tested weeds.
- All the formulations developed and tested during the study improve and sustain enzymatic activity.

ARTICLE INFO

ABSTRACT

This work investigated the effect of variably formulated pesta granules containing wild and UV mutated *Pseudomonas aeruginosa* and *Lasiodiplodia pseudotheobromae* on the rate of CO_2 evolution, organic carbon content, enzymatic activity (acidic and alkaline phosphatase, dehydrogenases, urease and protease) and representative soil microorganisms in the soils using different assay techniques. After the 35th day period of experiment, the pesta granule formulation BH4 showed the best evolution of CO_2 (824 ± 6.2 mg CO_2 kg^{-1} soil hr^{-1}) as against control treatment (689 ± 3.7 mg CO_2 kg^{-1} soil hr^{-1}). Enzymes activities, organic carbon content of 3.8% on the 15th day of study and stable representation of microorganisms that include actinomycetes, fungi, heterogenous as well as soil nitrogen-mediatory bacteria were equally at their maximum level BH4 treatments. The phytotoxic assay showed no inhibitory effect on *Solanum lycopersicum* seeds and seedlings compared to the observed growth inhibition on the tested weeds (*Amaranthus hybridus* and *Echincholoa crus-galli*) which corresponds with positive control glyphosate treatment. The glyphosate treated soil had the least critical results on parameters investigated during the study. The order of bioherbicidal activity is BH4>BH2>BH6>BH3>BH1>BH5>positive control. Results from this study confirmed the target efficacy of variably formulated pesta granules which is sustainable, cheap, ecologically suitable and recent. This is in addition to recognizing the microbial-derived formulations as characteristically potent alternative to chemical herbicides utility in agrosystems practice. Further study of the underlining factor responsible for the bioherbicidal performances of the variably formulated pesta granules and field trials are critical for their future commercialization.

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1. Introduction

Significant efforts have gone into the development and commercialization of microbial bioherbicides (bacteria, fungi, and virus) over the past two decades (Bailey, 2010; Bailey and Falk, 2011; Bailey et al., 2010). This is due to the rising environmental nuisance and agro-menace caused by unwanted grass and broadleaf weeds in many global arable communities (Ash, 2010; Charudattan and Dinoor, 2000; Hynes and Boyetchko, 2006; Glare et al., 2012; Adetunji and Oloke, 2013; Adetunji, 2015; Adetunji et al., 2017a). The growing concern on the negative

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impacts from the continuous use of chemical or synthetic herbicides on the environment, human health, food safety and ecosystem functionality cannot be downplayed albeit shifting attention to bioherbicides (Glare et al., 2012). Furthermore, rising research efforts into bioherbicides formulation and application was premised on the emergence of acquired resistance response of weeds to prolong synthetic herbicide treatments further encouraging them as viable, cost effective alternatives to controlling pestilent weeds (Beckie et al., 1999; Heap, 2015).

Mycoherbicides which are derived from phytotoxic fungi e.g. Collego (*Collectotricum gloeosphoroides*), ABG 5003 (*Cercospora rodmanii*), CASET* (*Alternaria cassia*) and DeVine (*Phytophthora palmivora*) are logically more popular than bacterioherbicides in the classical as well as augmentative biological control of weeds, particularly in agriculture (van Lenteren, 2012). In contrast, living bacteria are less successfully applied in the fields as bioherbicides because their sensitivity to changing ambient environmental conditions. In addition, their inability to produce abundant exospsores and maintain relatively stable genetic integrity in storage also affected their popularity in bioherbicide formulations (Mejri et al. 2013). Ash (2010) defined formulated bioherbicides as a mixture of the active ingredients, a carrier or solvent that delivers an active ingredient to the target weeds. Boyette et al. (1991) as well as Hynes and Boyetchko (2006) observed that such formulation must be synergetic with a spectrum of chemical adjuvant that improves their survival and potentiate their infectivity even in adverse environmental conditions.

Mycoherbicides (*Fusarium oxysporum*, *Lasiodiplodia pseudotheobromae*) and bacterial herbicides (*Pseudomonas fluorescens*, *Pseudomonas aeruginosa*) have been formulated into pesta in previous studies (Daigle et al., 2002; Shabana et al., 2003; Elzein et al., 2008; Kohlschmid et al., 2009; Adetunji and Oloke, 2013; Yang et al., 2014; Adetunji et al., 2017a,b,c).

Chemical Herbicides are used widely in the management of weeds. Glyphosate is one of them and is the most widely utilized chemical herbicide in the management of weeds. The worldwide global market for glyophosate has been projected to 1.35 million metric tons (Global Industry Analysts, 2011). The adverse effects of glyphosate on the environment has been documented and they are found to be the major agent for altering and reducing the biodiversity of various microbial communities both in aquatic an terrestrial environment. (Thammavongs et al., 2008; Relyea, 2005). Thus, there is a need to critically examine the efficacy of glyphosate and the newly formulated bioherbicides on some key soil microorganisms involved in edaphic properties as actinomycetes, fungi, and soil nitrogen-mediatory bacteria (Prashar et al., 2014). These microorganisms play an important role in the enhancement of biogeochemical processes, durable soil sustainability, and eventually support for ecosystems for sustainable agriculture (Newman et al., 2016)

This study therefore evaluated the non-target effect of bioherbicidal formulations on soil microbiota measuring critical parameters like enzymatic activity, carbon dioxide emission and organic carbon contents comparison to a commercial chemical herbicide (glyphosate).

2. Material and methods

2.1. Source of microorganism

The wild fungus (*Lasiodiplodia pseudotheobromae* A.J.L. Phillips, A. Alves & Crous) used in this study was isolated from *Tridax procumbens* leaves. The inter transcribe spacer D1/D2 region characterization of the bioherbicidal strain as *Lasiodiplodia pseudotheobromae* and coded C1136 with accession number KY432690

while *Pseudomonas aeruginosa* was isolated from the rhizosphere of wheat plants in a previous study by Adetunji and Oloke (2013). The isolated bacterium was identified as *Pseudomonas aeruginosa* and coded C1501with accession number KF976394.

2.2. Mutagenesis

Using the protocol elucidated by Adetunji and Oloke (2013), sterile plates each containing mycelia plugs of *Lasiodiplodia pseudotheobromae* were exposed for 30, 60 and 90 min respectively to UV light at 300 nm wavelength and 30 cm to the plates. Thereafter, 5 mycelia plugs were extracted and used as inoculants of potatoes dextrose broth. The wild strain for the purpose of the study was coded WLp (control) while the mutant strains were variably coded as Lp 30, Lp 60 and Lp 90 respectively.

Similarly, a wild strain of *Pseudomonas aeruginosa* was cultured by the modified method of Evans et al. (2004). Bacterial cultures were streaked on the entire surface of the Kings agar plates in triplicates and later exposed to UV light of 254 nm at a distance of 30 cm for 30, 60, and 90 min respectively. After the exposure, the plates were incubated at 37 °C for 48 h and the most distinct colonies observed were selected for further investigation. Irradiated suspensions were later plated within 1 h of their exposure. Further, their viability was determined by a formula (CFU remaining/by initial CFU x 100). Control cultures unexposed to UV radiation were also subjected to similar viability test. The wild strain was coded WPa (control) while the mutants were variably coded as Pa30, Pa60 and Pa90 respectively.

Mutagenesis was carried out in order to determine whether there will be an improvement in the activities of phytotoxic metabolites from strains used in this study.

2.3. Preparation of pesta granule formulations

The procedure for the formulation of granular pesta was as developed by Connick et al. (1991) and modified by Adetunji and Oloke (2013) while the optimization level was done according to Elzein et al. (2004). All the reagents were of analytical grade and high purity. They were purchased from Lab trade Nigeria Ltd, Ilorin, Kwara State, Nigeria. The semolina used in this study was obtained from a supermarket and its a product of Dangote Flour Mills Plc, Nigeria. The formulated granular pesta consists of the bioherbicidal strains in addition to semolina (wheat flour, vitamins, and minerals), kaolin ($H_2Al_2Si_2O_8$–H_2O.) and the adjuvants [glucose ($C_6H_{12}O_6$); glycerol ($C_3H_8O_3$); sucrose ($C_{12}H_{22}O_{11}$); sucrose ($C_6H_{12}O_6$); dextrose ($C_6H_{12}O_6$) and lactose ($C_{12}H_{22}O_{11}$). The formulations were also coded BH1, BH2, BH3, BH4, BH5, and BH6 respectively.
Their individual composition was as follows:

BH1 = semolina (32 g) + kaolin (6 g) + glycerol (20 ml) + WLp + Pa30 + glucose + sucrose + fructose + dextrose + lactose sugar + peptone.
BH2 = semolina (32 g) + kaolin (6 g) + glycerol (20 ml) + Lp 90 + Pa 90.
BH3 = semolina (32 g) + kaolin (6 g) + glycerol (20 ml) + Lp 30 + glucose + sucrose + fructose + dextrose + lactose sugar + peptone + Pa 30.
BH4 = semolina (32 g) +kaolin (6 g) + glycerol (20 ml) + Lp90 + glucose + sucrose + fructose + dextrose + lactose sugar + peptone + Pa 90
BH5 = semolina (32 g) + kaolin (6 g) +glycerol (20 ml) + WLp + WPa.
BH6 = semolina (32 g) + kaolin (6 g) +glycerol (20 ml) + Lp60 + Pa60 + glucose + sucrose + fructose + dextrose + lactose sugar + peptone.
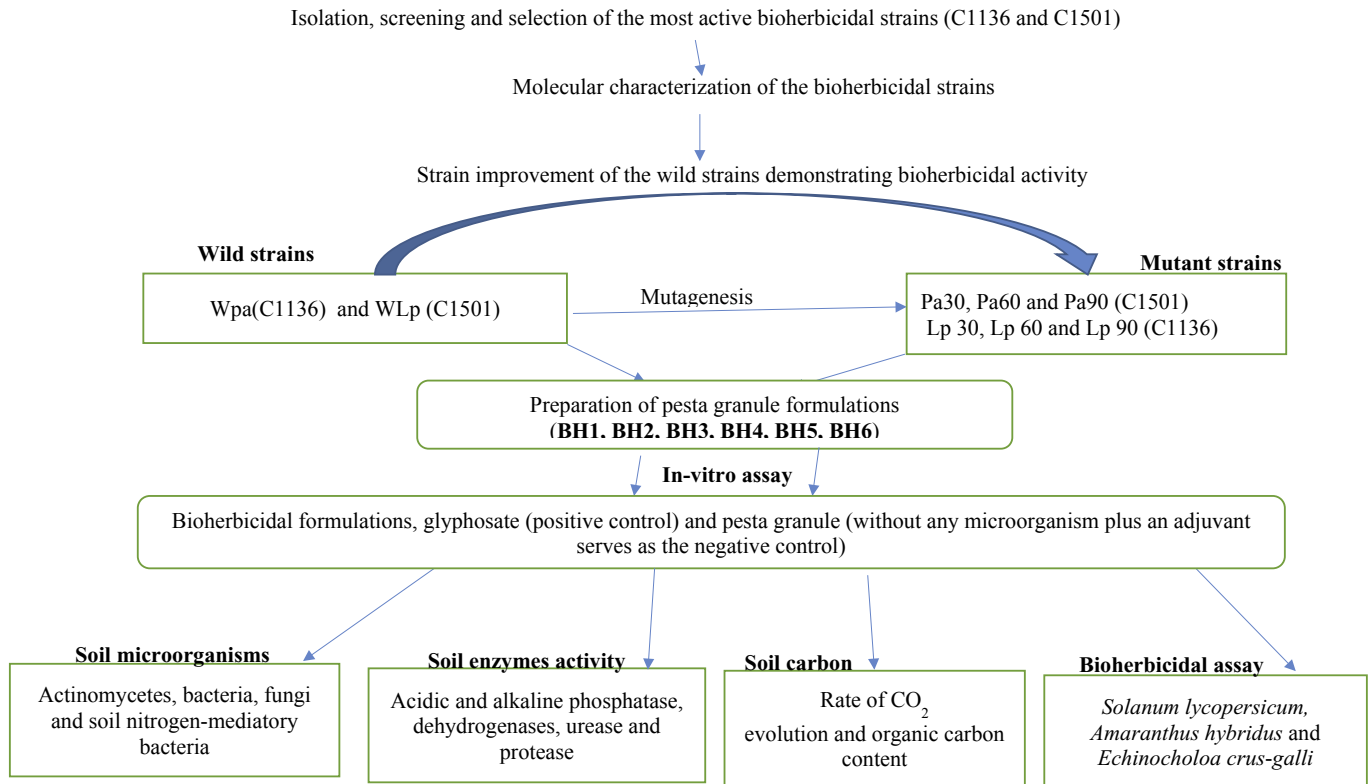
Isolation, screening and selection of the most active bioherbicidal strains (C1136 and C1501)

Molecular characterization of the bioherbicidal strains

Strain improvement of the wild strains demonstrating bioherbicidal activity

Wild strains

Wpa(C1136) and WLp (C1501)

Mutagenesis

Mutant strains

Pa30, Pa60 and Pa90 (C1501)
Lp 30, Lp 60 and Lp 90 (C1136)

Preparation of pesta granule formulations
(**BH1. BH2. BH3. BH4. BH5. BH6**)

In-vitro assay

Bioherbicidal formulations, glyphosate (positive control) and pesta granule (without any microorganism plus an adjuvant serves as the negative control)

Soil microorganisms

Actinomycetes, bacteria, fungi and soil nitrogen-mediatory bacteria

Soil enzymes activity

Acidic and alkaline phosphatase, dehydrogenases, urease and protease

Soil carbon

Rate of CO_2 evolution and organic carbon content

Bioherbicidal assay

Solanum lycopersicum, Amaranthus hybridus and *Echinochloa crus-galli*

Fig. 1. Schematic model showing the experimental design performed for pesta granules application in soil.

The pesta granules were each later applied at the rate of 1.0 g granules/pot. Glyphosate (N-phoshonomethyl-glycine) was used as a positive control after diluting the stock concentration to a final glyphosate concentration of 1 g while a pesta granule without any microorganism plus an adjuvant serves as the negative control. Pots of 20 cm height x 10 cm width dimension were filled up to 2/3 of its volume with previously prepared soil for the study.

The experimental design performed for pesta granules application on soil activity and weeds is shown in Fig. 1.

2.4. Non-target effect of biological herbicide formulations on critical soil microbial parameters

Soil samples which are loamy-sandy in nature were randomly collected from Ladoke Akintola University Farm site at a depth of 20 cm with a hand trowel. The soil samples in triplicate were analyzed for pH (9.5), organic matter (0.83%) and organic carbon (0.48%) immediately after collection. Thereafter, the soil samples were sieved (2 mm sieve) and moistened prior to carrying out the laboratory incubation experiments to evaluate their microbial respiration rate, soil organic carbon and qualitative soil microbial representation of each bioherbicide formulation (Anderson et al., 1993).

2.4.1. 2.4.1microbial respiration rate (CO_2)

Microbial respiration was measured according to Anderson et al. (1993) by the rate of CO_2 production during incubation. Fifty grams (50 g) each of the sieved soil samples was collected in 500 ml conical flask, amended with the pesta formulation and thoroughly mixed using a stirrer. Chemical herbicides of Glyphosate and pesta granules without the experimental soil microorganisms were used as positive and negative control respectively. A neutral control composed by soil without any input (neither glyphosate nor pesta

granules or adjuvant). Each treatment was arranged in triplicate, thus a total of 24 flasks were involved in the experimentation. A 10 ml of 0.3 M NaOH solution in a glass vial was carefully suspended in each experimentation flask with a string and sealed with a rubber bung. A blank was also run to evaluate the quantity of CO_2 in the flask. The flasks were incubated at 35 °C, taken out at 1, 5, 10, 15, 20, 25 and 35 days of incubation respectively with the vial content carefully exchanged for new one each time while its collected content is analysed. Added to the collected NaOH was 10 ml of 1 M $BaCl_2$ solution and a few drops of phenolphathalein. This was titrated against 0.1 M HCl solutions until the pink colour disappeared. During the reaction 1 mol of CO_2 neutralizes 2 mol of NaOH. The amount of CO_2 produced was calibrated as g/g of moist soil/h (Anderson et al. 1993).

2.4.2. Soil organic carbon content

Soil organic carbon (OC) in the different bioherbicides treated and control soil samples was determined by the partial oxidation method of Walkley-Black procedure on 7th, 15th, 25th and 35th day respectively. Using the same 50 g of soil and formulated bioherbicides mixture (60-mesh) in an Erlenmeyer flask, 10 ml of 1 N $K_2Cr_2O_7$ and 10 ml of concentrated H_2SO_4 were added. After 30 min, 50 ml of deionized water, 3 ml of concentrated H_3PO_4 and 0.5 ml of 1% diphenylamine indicator were also added. The filtrate was later titrated slowly with 1 N $FeSO_4$ solution until a green colour end point was observed (Loeppert and Suarez, 1996).

2.4.3. Soil microbial representation

After day 1, 7, 15 and 35 respectively, bioherbicides treated soil and chemical glyphosate control soils were evaluated for total number of heterotrophic bacteria, fungi and N-bacteria using selective media. This was carried out by dissolving 1 g of each soil samples in 10 mL of distilled water and was serially diluted to

10^{-7}.0.1 mL of the dilution was plated on nutrient agar plates using a sterilized L shaped glass spreader and incubated at 37 °C for 48 h for bacterial growth. Similarly, nutrient casein agar plates incubated at 30 °C for 72 h were used for promoting the growth of Actinomycetes. 0.1 mL soil dilution on potato dextrose agar with 100 μg/g streptomycin and incubation at 28 °C for 5 days was used for fungal growth (Jahnel et al., 1999; Monkiedje and Spiteller, 2002; Araujo et al., 2003; Geetha and Jyothi, 2017).

The nitrifying and denitrifying bacteria medium were prepared using a protocol developed by Wang et al. (2007). 1 L of medium containing the following; 1 g of $NaNO_2$, 1 g of Na_2CO_3, 1 g of NaH_2PO_4, 0.25 g of $MnSO_4 \cdot 4H_2O$, 0.01 g of; $MgSO_4 \cdot 7H_2O$, 0.03 g of; K_2HPO_4 and amended with 0.75 g of 2% agar with pH of 7 was used for isolating nitrifying bacteria. Similarly, 1 L of medium containing 0.5 g of potassium sodium citrate, 2.0 g of KNO_3, 0.5 g of KH_2PO_4, 0.2 g of $MgSO_4$ with pH 7.0 was used for isolation of denitrifying bacteria. Incubation was carried out at 30 °C. The different media used for the isolation of different microorganisms in this study was pasteurized at 121 °C for 30 min.

2.5. Enzymatic activity

Soil samples from each experimental pot were instantly assayed for enzymatic activities. This was carried out by mixing 5 g of soil samples that has been exposed for 15days to various bioherbicidal formulations (BH1, BH2, BH3, BH4, BH5, and BH6), positive control and negative control. One gram of the soil sample from these treatments were then mixed with 25 ml of MilliQ water and incubated for 1.5 h at 30 °C under continuous stirring (250 rpm). An aliquot from the resulting solution was then utilised for the various soil enzymatic quantification. Urease activity was performed by ammonium release assessment (Nannipieri et al., 1979) while the Soil phosphatase activity was determined using modified universal buffer solution, and separated into acid phosphatase (pH 6.5) and alkaline phosphatase (pH 11) on p-nitrophenylphosphate substrate. After 1 h at 37 °C, the development of p-nitrophenyl was determined by spectrophotometer at 410 nm (Tabatabai, 1982). Soil dehydrogenase activity was evaluated according to Garcia et al. (1993) while the protease activity of each soil sample was by the protocol of Ladd and Butler (1972).

2.6. Phytotoxicity assessment of bioherbicidal formulations

Fifty mL of sterile water was added to the different samples from each potted experimental soil and mixed thoroughly. Extractions were performed at 50 °C in an orbital shaker at the speed of 130 g for 24 h by centrifugation, treated with 50 mL ethyl acetate and centrifuged again at 6000 g for 10 min. The ethyl acetate was later removed from the supernatant using a separating funnel and dried over sodium sulphate prior to vaporizing at 40 °C in rotary evaporator. The crude extracts were then prepared into 1, 1.5 and 2 g L^{-1} concentrations respectively and tested for pre-emergence bioherbicidal activity on Solanum lycopersicum L (tomato), Amaranthus hybridus L (pigweed) and Echinocholoa crus-galli (L.) P. Beauv. (barnyard grass).

Fifteen seeds each of Solanum lycopersicum, Amaranthus hybridus and Echinocholoa crus-galli were immersed in 100 mL 5% sodium hydrochlorite solution for 30 min, rinsed repeated (3×) with sterilized water and placed separately on top of sterile Whatman No. I filter paper (7 cm diameter) in triplicate set inside Petri dishes. The measurement of germination rate was calculated using the formula: Germination rate (%) = (Number of germinated seeds in treatment/Number of germinated seeds in control) × 100. The radicle elongation was determined using a calibrated meter rule.

2.7. Statistical analysis

The mean values were subjected to an analysis of variance using SPSS (Version 21). Significant means were analyzed using Duncan's multiple range tests at α = 0.05.

3. Result

3.1. Effect of bioherbicidal formulations on soil microbial respiration (CO_2 release)

There was no significant difference (p = 0.05) in soil CO_2 emission rate (965 mg CO_2 kg^{-1} soil hr^{-1}) from the 1st to 5th day. After the 35th day, BH4 treated soils produced the highest quantity of CO_2 per kilogram soil per time (824 mg CO_2 kg^{-1} soil hr^{-1}) compared to the negative control soil treatment that had 689 mg CO_2 kg^{-1} soil hr^{-1}. There was a gradual reduction in CO_2 release from the formulation treated soil with increasing treatment days. The glyphosate treated soil (positive control) had the least CO_2 emission of 133 mg CO_2 kg^{-1} soil hr^{-1} and generated significantly different (p = 0.05) values compared to the treated soils (Table 1).

Table 1
Rate of CO2 emission (mg CO2 kg-1 soil hr-1) in bioherbicides soil treatment at different incubation periods (days).

|	Period (Days)						
	1	5	10	15	20	25	35
	965 ± 6.8^a	965 ± 4.2^a	965 ± 7.1^a	717 ± 5.3^e	738 ± 5.1^e	613 ± 3.2^e	468 ± 4.1^f
BH2	965 ± 3.5^a	965 ± 3.3^a	965 ± 7.2^a	953 ± 4.1^b	961 ± 4.12^b	824 ± 3.5^a	795 ± 8.1^b
BH3	965 ± 6.2^a	960 ± 3.6^a	867 ± 4.5^b	738 ± 5.9^d	767 ± 3.7^d	538 ± 4.3^f	497 ± 5.3^e
BH4	965 ± 3.7^a	965 ± 4.1^a	965 ± 7.2^a	968 ± 3.4^a	997 ± 3.9^a	833 ± 5.8^a	824 ± 6.2^a
BH5	965 ± 6.5^a	965 ± 5.4^a	761 ± 2.8^c	703 ± 3.5^e	712 ± 5.6^f	694 ± 8.1^d	406 ± 4.7^g
BH6	965 ± 6.2^a	965 ± 3.7^a	965 ± 4.8^a	923 ± 3.2^c	945 ± 6.8^c	812 ± 6.3^b	763 ± 5.1^c
PC	965 ± 3.8^a	860 ± 6.1^b	650 ± 3.6^d	586 ± 5.1^f	592 ± 4.3^g	338 ± 4.6^g	133 ± 2.8^h
NC	965 ± 6.2^a	965 ± 4.3^a	965 ± 3.7^a	715 ± 4.9^e	775 ± 4.3^d	769 ± 5.8^c	689 ± 3.7^d

Positive control = Negative control treatment with chemical glyphosate, Negative Control = Positive control without treatment. Effect of various formulated bioherbicides on soil organic carbon (%) over different incubation periods. Values are means ± standard error in triplicates (n = 3). Means with different superscripts within the same column are significantly different (P=0.05). BH1 = semolina (32 g) + kaolin (6 g) + glycerol (20 ml) + WLp + Pa30 + glucose + sucrose + fructose + dextrose + lactose sugar + peptone; BH2 = semolina (32 g) + kaolin (6 g) + glycerol (20 ml) + Lp 90 + Pa 90; BH3 = semolina (32 g) + kaolin (6 g) + glycerol (20 ml) + Lp 30 + glucose + sucrose + fructose + dextrose + lactose sugar + peptone + Pa 30; BH4 = semolina (32 g) +kaolin (6 g) + glycerol (20 ml) + Lp90 + glucose + sucrose + fructose + dextrose + lactose sugar + peptone + Pa 90; BH5 = semolina (32 g) + kaolin (6 g) +glycerol (20 ml) + WLp + WPa; BH6 = semolina (32 g) + kaolin (6 g) +glycerol (20 ml) + Lp60 + Pa60 + glucose + sucrose + fructose + dextrose + lactose sugar + peptone.

3.2. Effect of various formulated bioherbicides on organic carbon contents of soil

Organic carbon content was observed to vary with different bioherbicide soil treatment (Fig. 2). The result showed increase in the amount of organic carbon content in bioherbicide soil treatments compared to the chemical treated soil (positive control) on the 7th (2.0%), 15th (2.1%), 25th (1.9%) and 35th (1.5%) day respectively. BH4 however had the highest organic carbon value of 3.8% on day 15 of incubation compared to the 2.4% determined for the untreated soil (negative control).

3.3. Non target effect of various formulated bioherbicides on soil microorganisms

The plate-count results showed high representations of heterotrophic bacteria, actinomycetes, fungi and non-heterotrophic bacteria categories in the bioherbicidal formulation treated soils compared with the controls (Fig. 3A–E). BH4 formulation was however observed to have different soil microbial presence composed of heterotrophic bacteria, N-fixing bacteria and actinomycetes. Fungi were the least represented with a colony formed unit (CFU) of $13 \times 10^6\,g^{-1}$ in all treated soils while heterotrophic bacteria and actinomycetes were more dominant with CFU of $100 \times 10^6\,g^{-1}$ soil and $48 \times 10^6\,g^{-1}$ respectively. The negative control soil treatments showed insignificant level of microbial representations over the course of study.
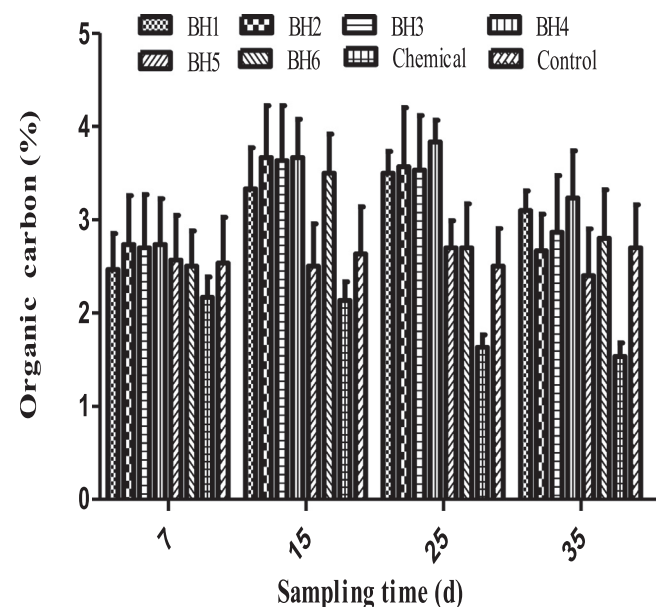
Fig. 2. Effect of various formulated bioherbicides on soil organic carbon (%) over different incubation periods. Error bars represent the standard error of the mean (n = 3). Effect of various formulated bioherbicides on soil organic carbon (%) over different incubation periods. Error bars represent the standard error of the mean (n = 3). BH1 = semolina (32 g) + kaolin (6 g) + glycerol (20 ml) + WLp + Pa30 + glucose + sucrose + fructose + dextrose + lactose sugar + peptone; BH2 = semolina (32 g) + kaolin (6 g) + glycerol (20 ml) + Lp 90 + Pa 90; BH3 = semolina (32 g) + kaolin (6 g) + glycerol (20 ml) + Lp 30 + glucose + sucrose + fructose + dextrose + lactose sugar + peptone + Pa 30; BH4 = semolina (32 g) +kaolin (6 g) + glycerol (20 ml) + Lp90 + glucose + sucrose + fructose + dextrose + lactose sugar + peptone + Pa 90; BH5 = semolina (32 g) + kaolin (6 g) +glycerol (20 ml) + WLp + WPa; BH6 = semolina (32 g) + kaolin (6 g) +glycerol (20 ml) + Lp60 + Pa60 + glucose + sucrose + fructose + dextrose + lactose sugar + peptone.

3.4. enzymatic assay of bioherbicidal formulations

Biological Herbicide formulation 4 (BH4) exhibited more dehydrogenase, urease, phosphatases (alkaline and acidic) and protease activities compared to both the positive and negative control samples among all the mutant strains investigated. However the negative control involving soil treated with chemical glyphosate was observed with the least hydrogenase, urease and protease activities. BH4, BH2, BH6 showed decreasing order of enzyme activities which is above those noted for the negative control (Fig. 4A–E).

3.5. effect of bioherbicidal formulations on non-target and target plants

BH4 compared to other formulations and relative to the negative control treatments showed no recognizable inhibitory effect on the seed germination rate of *Solanum lycopersicum* at different concentrations as against the positive control with complete pre-emergence inhibition. The highest radicle length at 1 (3.5 cm), 1.5 (2.3 cm) and 2.0 mg/L (1.8 cm) concentrations respectively were observed for tomato seedlings treated with BH4 bioherbicidal formulation while complete inhibition of radical elongation was evident for seeds of pigweeds and barnyard grass. Furthermore, the effect of BH4 on tomato was comparable to the result obtained for the negative control (mixture of soil and pesta granules only). (Fig. 5A). Similarly, the seeds of the target weeds used in this study (*Amaranthus hybridus* and *Echinocholoa crus-galli*) showed repressed germination activity at the various concentrations of BH4 treatment. The germination values for *Amaranthus hybridus* are 1 mg/L (18.4%), 1.5 (12.6), and 2.0 (9.3%) while 1 mg/L (16.2%), 1.5 mg/L (10.1%) and 2.0 mg/L (8.2%) were observed for *Echinocholoa crus-galli* (Fig. 5B).

4. Discussion

Bioherbicides are majorly phytopathogenic microorganisms or microbial phytotoxins useful for the biological control of weed. It is therefore logical to expect that certain level of allelopathic phenomena would underscore their biochemical relationship with economically beneficial and non-beneficial ambient organisms (Hoagland et al., 2007). It was on this premise that scientific focus shifted to investigating the non-target and target interactions of phytopathogens, phytotoxins and allelochemicals of microorganisms on weeds (Charudattan, 2001, 2005).

Many empirically tested bioherbicides posed no risk to the environment, humans, and their immediate ecosystem hence they have successfully complemented integrated weed management systems (Hoagland et al., 2007; Adetunji et al., 2017b). Contrary to popular report, Vey et al. (2001) noted that the phytochemicals of some microorganisms used as bioherbicides may have a toxicity level that could be harmful to humans. Microorganisms are reported as major soil decomposers and contributor to ecosystem service delivery by their direct participation in the breaking down of organic matter, nutrient cycling and improvement of plant survival through asymptomatic symbiosis (Devare et al., 2007; Khan et al., 2007). Growing concerns on the ecological implications of chemical herbicide applications that may include soil pollution from petrochemical wastes and residues on soil ecosystem functions as well as diversity underscore the non-target concept of bioherbicides. The understanding of the non-target effect of such chemical and biological herbicides on soil microbial activities as well as other economic crops is a necessary biological indicator that guaranties the acceptability of herbicidals (Muñoz-Leoz et al., 2013; Zhao et al., 2013). Therefore, agricultural practises including
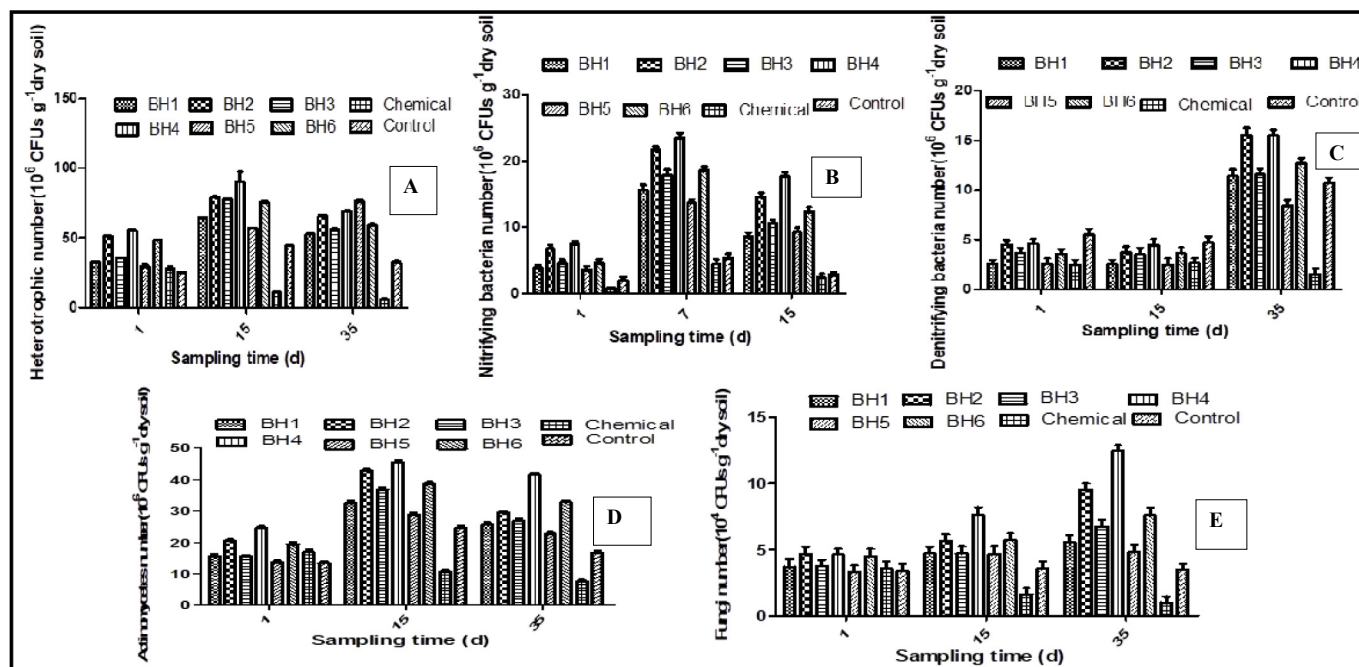
Fig. 3. A—E: Effect of different bioherbicidal formulations on non-target beneficial soil microorganisms: (A) heterotrophic bacteria (B) = nitrifying bacteria (C) = denitrifying bacteria (D) actinomycetes (E) soil fungi. The mean values and standard error of three replicates are presented.

diseases, pests and weeds control must aimed at preserving significantly the activities of soil microorganisms' biological and ecological services capable of increasing economic crops resilience. Suffice to say that selected agrosystem should biologically mitigate the expediency of impact, production and application of bio-herbicides on agroecosystems (Beulke and Malkomes, 2001; Cycon et al., 2006; Yao et al., 2006).

During this study, we have utilized culture enrichment methods in evaluating the effect of formulated pesta granules on available soil microorganisms. We followed the culture enrichment methods as previously carried out by Jahnel et al., 1999; Monkiedje and Spiteller, 2002; Araujo et al., 2003; Geetha and Jyothi, 2017. We couldn't employ the sequencing approaches for understanding the soil microbial biota after application bio herbicides as previously carried out by Liu et al., 2007; Li et al., 2014; Merlin et al., 2015; Romdhane et al., 2016. However, Romdhane et al. (2016), suggested the utilization of traditional enrichment culture method together with sequencing approach to give a better knowledge of soil composition and microbial diversity available in the environment where pesticides have been applied.

Also, the formulated bioherbicides involving the tested wild and mutant microbes produced no non-target effects on the diversity of microorganisms responsible for soil nitrogen economy (Cycoń and Piotrowska-Seget, 2007). Consequently, this caused increase in the production of CO_2 compared to the chemically treated (glyphosate) soils at the end of the experiment. The elevated rate of soil microbial metabolic activities and microbial respiration as confirmed by the communities of representative actinomycetes, Fungi, heterotrophic bacteria, nitrifying and denitrfying bacteria may have accounted for this. Similar results were observed in separate works by Dhillion et al. (1996) and Kandeler et al. (1998) who reported increase in soil microbial respiration from the application of formulated bioherbicides. The non-target effects of many chemical herbicides on critical soil geochemical and microbial functions have equally been reported in literature (Kalia and Gupta, 2004; Sebiomo et al., 2011; Zain et al., 2013). This

corresponds with observations from this study with glyphosate. Additionally, glyphosate and 2,4—Dichlorophenoxyacetic acid (2,4-D) herbicidal applications in agronomic doses, were reported to be potent enough to deplete soil microbial respiration and causes unstable microbial population (Wardle and Parkinson, 1990; Busse et al., 2001; Zabaloy et al., 2008).

While the reason for the high performances of BH4 bioherbicide formulation in this study is not fully understood, it may be hypothesized to be due to the intricacy of biomodification or underlying influence of UV irradiation relative to the exposure time. The use of mutagenic UV lamp in genetically modifying *Pseudomonas aeruginosa* and *Lasiodiplodia pseudotheobromae* to potentiate bioherbcidal activity might be due to their genetically improvement of their cellular components (Valero et al., 2007; Braga et al., 2015). The range of enzyme activities observed during the study is according to Sebiomo et al. (2011) and Hoagland et al. (2007) a good reflection of soil healthiness and nutrient recycling activities. This involves the transformation of organic macromolecules into biolabile and bioaccessible plant beneficial nutrients. The level of hydrogenase, phosphatase and urease in the various experimental soils treated with the bioherbicidal formulations and their observed phytotoxicity assessment results. Poor sensitivity of economically valuable *Solanum lycopersicum* in this study buttresses the efficacy and specificity of these pesta formulations as weeds control agents. This recognized effect may hypothetically translate to all other non-target crops and soil microbiota with ecologically safe result. Furthermore, the enzymes, especially hydrogenase serve as the best biomarkers of soil oxido-reduction activities compared to the result obtained from the positive control experiment.

Among all the formulated bioherbicides, BH4 treated soils showed the highest amount of organic carbon compared with the others. The efficient delivery of soil ecological functions require settled communities of microorganisms, dominant representation of soil beneficial rhizopheric microbes. This population is often hypersensitive and vulnerable to different levels of accumulated
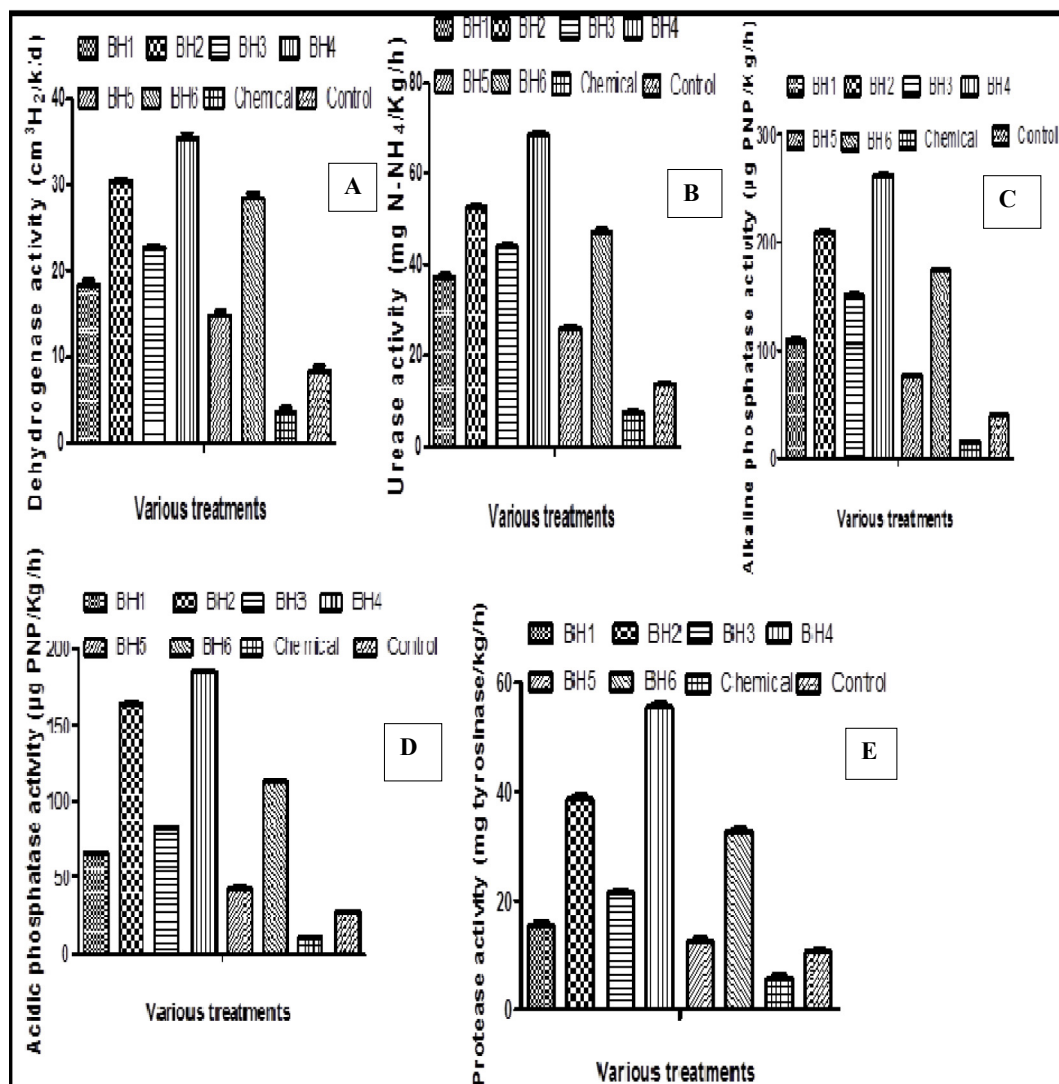
Fig. 4. A–E. Enzyme activities of investigated soils containing various treatment. Error bars represent the standard error of the mean (n = 3): (A) dehydrogenase (B) urease (C) alkaline phosphatase (D) acidic phosphatase (E) protease.

toxic as well as recalcitrant chemical pollutants. The status of soil organic matter which is critical to the thriving of promoting soil microbial richness was reported to deplete with constant herbicide treatments (Wardle and Parkinson, 1991; Savonen, 1997; Rath et al., 1998; Sebiomo et al., 2011). Startton and Stewart (2002) recorded similar non-target effect with glyphosate on soil biomass in Canadian coniferous forests. Furthermore, Radivojević et al., (2008) in another study observed transitory effects of atrazine on soil biomass-C while Fraser et al. (1994) noted 10–26% increase in microbial biomass under organic management treatment i.e. compost, farm yard manure (FYM) of soils (Leita et al., 1999; Cerny et al., 2008). Stimulation of microbial biomass and activities by organic carbon inputs has been well documented in literature (Ali, 1990; Ayansina and Oso, 2006; Tu et al., 2003).

Many economic plants are negatively affected by the fastidious and more competitive use of necessary growth promoting soil factors. They equally depend on different types of soil microorganisms to mediate soil nitrates economy through associations. This suggests an interaction that promotes the coexistence of actinomycetes, nitrogen fixing and denitrifying bacteria in the ecological management of the soil nutrient resources. The susceptibility of microorganisms

that manage the economy of soil nitrates and their eventual assimilatory process under agro-chemical applications distress is well documented (Singh and Singh, 1989; Frankenberger et al., 1991; Arias and Fabra, 1993; Tözüm-Çalgan and Sivaci-Güner, 1993; Martens and Bremner, 1993; Santos and Flores, 1995; Pell et al., 1998; Fabra et al., 1997).

It was further observed that bio-herbicidal formulations from this study exhibited comparatively different degree of target or phytotoxicity effect on the seeds and seedlings of pigweeds and barnyard grasses. The factor or mechanism responsible for this non-target behaviour is currently unclear and may require further studies. While various levels of seed and seedling inhibitory effects of the BH formulations on *Amaranthus hybridus* and *Echnocholoa crus-galli* were apparently comparable to that of chemical glyphosate treatments, *Solanum lycopersicum* observably exhibited very high germination rate and seedling development values. The herbicidal performance of BH4 among all the formulations is interesting and further investigation may be required to fully understand the underlying biological phenomenon. It is philosophical to infer once again that the alternate damage by UV exposure time of 90 min and repair from raw materials provided by
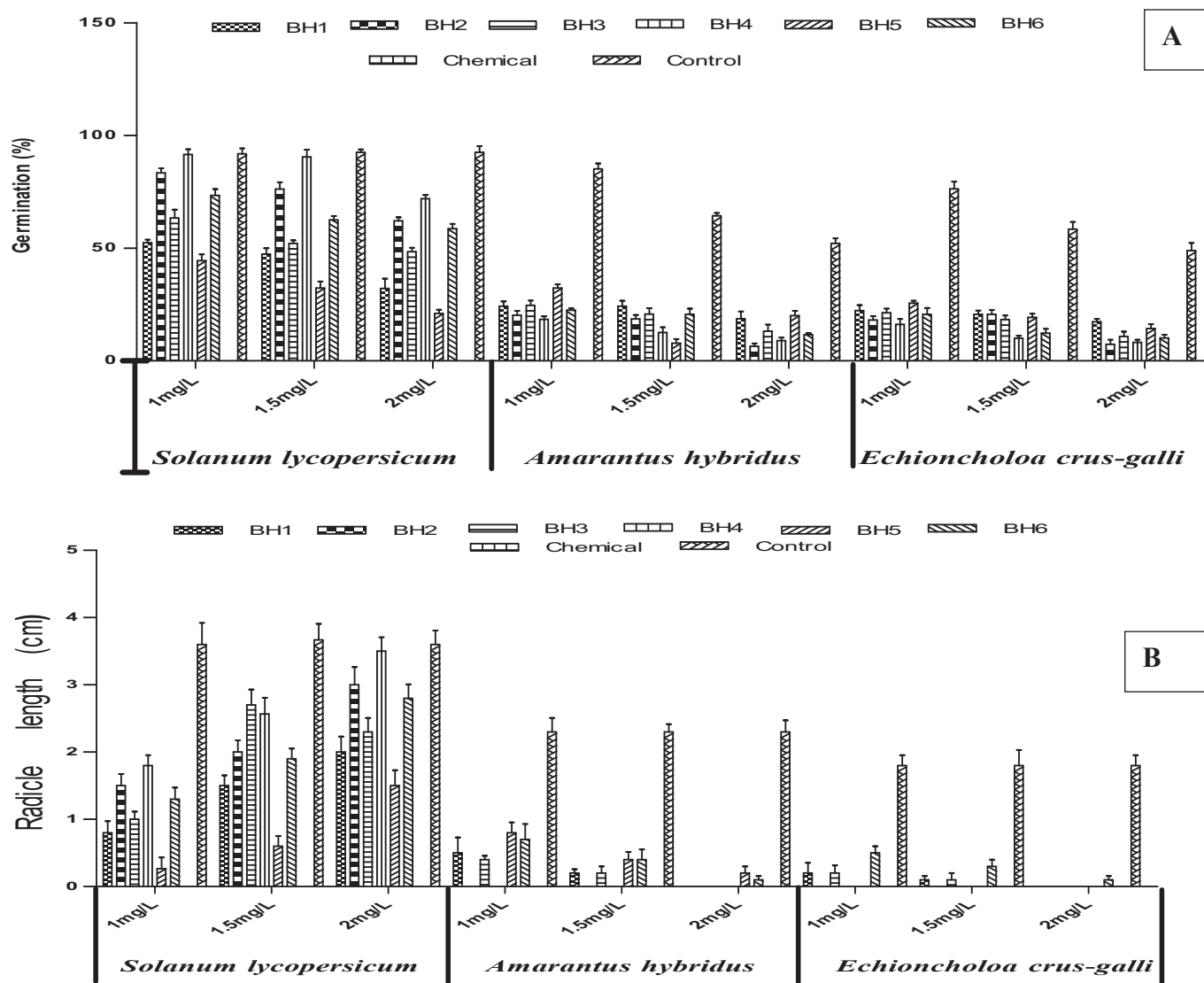
Fig. 5. A—B: Effect of bioherbicidal formulations on (A) Germination (%) and (B) Radicle length (cm) of non-target Solanum lycopersicum and target (Amaranthus hybridus and Echioncholoa crus-galli). Error bars represent the standard error of the mean (n = 3).

the diverse sugars moieties by wild *Lasiodiplodia pseudotheobromae* and the rhizospheric *Pseudomonas aeruginosa* accounted for the effectiveness in the BH4 formulation. This study has therefore identified the bio-herbicidal potency of different combination of phytopathogenic *Lasiodiplodia pseudotheobromae* and rhizospheric *Pseudomonas aeruginosa* as potential target-specific bioherbicide formulation. It also projects the bioherbicidal formulations as cheap, commercially amenable, environmental-friendly, alternative weed control option that could compliment as well as integrate with existing weeds management systems.

Compliance and ethics

Authors declare that no competing interest exists concerning this manuscript.

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