MYCOHERBICIDES FROM CONSORTIUM CULTURE OF RHIZOSPHERE FUNGAL ISOLATES: EFFECTS ON SOIL CHEMICAL AND BIOLOGICAL PROPERTIES

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ABSTRACT

The quest for organic replacement for chemical herbicides has attracted attention from researchers over the past two decades owing to the deleterious effect of chemical herbicides on soil properties. This study investigated how mycoherbicides affected soil chemical and biological properties. Some metabolites were produced from consortium culture of fungal isolates obtained from rhizospheres of Panicum maximum, Ipomoea involucrata and Amaranthus viridis. The isolates were co-cultured using Czapek broth, and incubated for 28 days. The consortium combinations were: 1 - Aspergillus welwitschiae and Trichoderma hamatum, 2 - Aspergillus welwitschiae and Aspergillus aculeatus, and 3 - Aspergillus aculeatus and Trichoderma hamatum, with glyphosate as the control. Five millilitres (5 mL) of the crude extract containing 200, 400, 800 and 1600 mgL^{-1}, respectively, was added to experimental pots containing weeds of interest (Panicum maximum or Ipomoea involucrata) for 6 weeks in the screenhouse. The soils were evaluated for pH, soil organic carbon (SOC), total nitrogen (TN), available phosphorus and soil biological indicators like microbial respiration, soil microbial biomass carbon (SMBC), total heterotrophic bacteria and fungi (THF and THB). Results showed that combination 2 increased SMBC by 290% while combination 3 increased SOC from 1.87% to 3.04%. Available phosphorus uptake in combination 3 was 15.61%. There was a reduction in TN from 1.42 gkg^{-1} to 0.91 and 0.94 gkg^{-1} for consortia 1 and 2, respectively. The mycoherbicides stimulated the growth of THF at a rate of 0.96 fungi/week ($p \leq 0.05$). It was concluded that mycoherbicides hold beneficial effects on soil biological and chemical properties.

Keywords: ***Soil microbial biomass carbon, soil organic matter, total heterotrophic fungi, consortium culture, mycoherbicides.***

INTRODUCTION

Biopesticides are living organisms or products of living organisms (like phytochemicals, and microbial products) or byproducts (semichemicals) that can be used to manage pests that cause damage to plants (Ortiz and Sansinenea, 2023). Biopesticides could be bacterial, fungal, viral, or protozoan origin, respectively and can control different kinds of pests with the most negligible negative impact on nontarget pests and are environmentally friendly. Biopesticides have an important role in crop protection, however, they are mostly combined with other tools, including chemical pesticides, as part of bio-intensive integrated pest management (Sarwar, 2015). They are known to have no hazardous residual effects on the treated commodities and are less harmful to the environment and end consumers. When developed at a broader scale, these may be found cheaper, extra efficient, longer, persistent, and recyclable than chemicals. Classical examples of microbial insecticides (type of biopesticide) that are in use include *Trichoderma* species,

viruses (Baculovirus), and entomopathogenic bacteria (*Bacillus thuringiensis*) with their metabolites usually, entomopathogenic nematodes and protozoans (Lacey *et al*., 2015; Sarwar, 2015).

Biopesticides of fungal origin are classed based on the spectrum of action. Some are insecticidal (mycoinsecticide, e.g., *Isaria fumosorosea*), herbicidal (mycoherbicide, e.g., *Fusarium oxysporum*), nematicidal (myconematicide, e.g., *Fusarium oxysporum*) etc. (Singh *et al*., 2019).

Humans at the beginning were accustomed to eradicating weeds by hand. The transition of weed control has witnessed a massive impact on the efficiencies and productivity of crops from about 6000 B.C. till date (Timmons, 2005). This progress could be tracked from the era of manual weed control through the era of the use of chemical substances to the current era of biological products (Vats, 2015). The momentum gained at bio-pesticides, semiochemical interaction signals, and similar substitute controls were first witnessed in the post-1960s era and have been consequently propagated (Auger-Rozenberg and Roques, 2008). For biological weed control agents to compete with chemical ones in the market, they must be reasonably priced, effective and reliable or have significant eco-toxicological advantages (Heiny and Templeton, 2018). In complement to the comparative advantages, their application to soils should positively impact soil properties. Among these soil properties is the soil organic matter which is an index of soil fertility. Microbial biomass carbon derived from bioherbicide-based microorganisms could be easily mineralized to SOM carbon than plant residues and hence, the carbon derived from microbes contributes quantitatively more to SOM formation (Simpson *et al*., 2007; Schmidt *et al*., 2011). Soil microbial biomass carbon (SMBC) being a fraction of soil organic matter is made up of carbon reserve from consortia of bacteria, fungi, actinomycetes, protozoa, algae and yeast. Evaluation of the SMBC could provide a means of inspecting responses from microorganisms resulting from changes in soil management practices (Chaudhari *et al*., 2020). These microbial forms of life are noted to be involved in the regulation of elemental-carbon (C) transfer from land-dwelling ecosystems to the mesosphere, stratosphere and troposphere via the mineralization of mineral-bound elements in once living or living components in soil (Dooley and Treseder, 2012). Several other biological and chemical properties of soil as affected by chemical herbicides are recessively investigated. This study therefore investigated the effect of mycoherbicides and glyphosate on some biochemical properties of soil.

MATERIALS AND METHODS

Production and Extraction of Mycoherbicides

These samples were collected from Teaching and Research Farm, Faculty of Agriculture, Obafemi Awolowo University (latitude 7° 30′–7° 35′ N and longitude 4° 30′–4° 35′ E), Ile-Ife, Nigeria. The location has an altitude of about 200 meters above the mean sea level, and a bimodal annual rainfall pattern, spanning approximately eight months (March–October) with peaks in June and September. The four-month duration, usually, November–February (subject to some irregular rainfall distribution pattern over the years, constitutes the dry season (Ojetade et al. 2016). The mean annual precipitation is about 1400 mm. Soil samples from the Iwo soil series (Ultisol) were collected from a secondary forest that had not received fertiliser or herbicide treatment in recent times. The soil samples were air-dried and sieved through a 5 mm sieve. Five hundred grams (500g) were transferred into a micropot. Rhizosphere soils of infected plants: green amaranth, weeds (guinea grass

and morning glory) and cacao pod samples were the sources of the test fungi. These isolates were characterized by molecular techniques (Alwakeel, 2017). Isolates of *Aspergillus* spp. and *Trichoderma* spp. were combined thus: consortium 1 (*Aspergillus welwitschiae* and *Trichoderma hamatum*), consortium 2 (*Aspergillus welwitschiae* and *Aspergillus aculeatus*), and consortium 3 (*Aspergillus aculeatus* and *Trichoderma hamatum*). Five-day-old fungal cultures of the isolates were inoculated into the production medium. The medium for the production of the metabolites from different fungal isolates was Czapek Dox broth supplemented with malt extract (El-Banna *et al.,* 1987). The composition of Czapek broth included sucrose, 20.0 gL^{-1}; $NaNO_3$, 2.0 gL^{-1}; $MgSO_4 \cdot 7H_2O$, 0.5 gL^{-1}; KCl, 0.5 gL^{-1}; $FeSO_4$, 0.01 gL^{-1}. One thousand millilitres (1000 mL) of the broth medium was prepared and distributed in sterilised 250 mL Erlenmeyer flasks. The broth media were inoculated with 20 mm of the fungal cultures in triplicates and incubated in a rotary incubator at 30 rpm for 28 days at 28^oC. Treatments were collected and centrifuged at 2500 rpm for 10 minutes to separate the mycelia (Abdul-Manan and Webb, 2018) from the culture broth. The aliquot from the preliminary screening of fungal isolates was transferred into a sterile flask and stored in the refrigerator at -4^oC for further analyses. The mycoherbicides were applied at the rates of 200, 400, 800 and 1,600 mg L^{-1}, respectively to the weeds as earlier described by Osunde *et al*. (in press). The experimental design was a completely randomised design.

Determination of Soil Physical and Biological Properties

Soil pH was determined using the method of Peech *et al*. (1953) in all treatments including glyphosate and the control. Available P in the Control and mycoherbicide treatments was determined according to Bray and Kurtz (1945). Soil organic carbon (SOC) and Total N (TN) contents of the control and herbicide treatments were determined by adopting the chromic acid digestion method of Walkley-Black (1934) and using the Kjeldahl method (Bremner, 1996), respectively. Particle size distribution of the soil was determined using the modified Bouyoucos hydrometer method (Bouyoucos, 1965) as reported by Gee and Or (2002), and exchangeable cations were determined using the 1 N ammonium acetate solution (pH 7) (Soil Survey Staff, 2010). Soil microbial biomass carbon was determined according to Brookes and Joergensen (2005) and Kumar *et al*. (2020). The evolved carbon dioxide (CO_2) was determined using Stotzky *et al*. (1958) double acid titration method. The total heterotrophic bacterial and fungal counts were done by serial dilution of the soil samples from which significant dilutions were selected.

RESULTS AND DISCUSSION

Antecedent properties of the soil used for the study

The antecedent properties of the soil (Table 1) indicated a textural class of sandy loam, a total nitrogen content of 1.42 gkg^{-1} which is within the medium fertility range (Adepetu *et al.,* 2014), high available phosphorus (29.67 $mgkg^{-1}$) according to Adepetu *et al.* (2014), and moderately acidic pH value (5.8) in $CaCl_2$ solution. The organic carbon was medium (3.07 gkg^{-1}) according to Adepetu *et al.* (2014). Exchangeable potassium and sodium were low with values of 0.02 and 0.01 $cmolkg^{-1}$, respectively, while exchangeable calcium (8.51 $cmolkg^{-1}$) and magnesium (1.99 $cmolkg^{-1}$) levels were high (Adepetu *et al.* 2014).

Table 1: Antecedent Properties of the Soil Used for the Study

PROPERTIES	Values
Particle size distribution (gkg^{-1})	
Sand	710
Silt	140
Clay	150
Texture	Sandy loam
pH in 0.01 M $CaCl_2$ solution	5.8
Organic Carbon (gkg^{-1})	3.07
Available P ($mgkg^{-1}$)	29.67
Total Nitrogen (gkg^{-1})	1.42
Exchangeable Cations ($cmolkg^{-1}$)	1.42
Ca	8.51
Mg	1.99
K	0.02
Na	0.01

Effects of the Herbicides on Soil Chemical and Biological Properties

Data on the effects of mycoherbicides and glyphosate on some of the soil parameters are presented in Table 2. The pH of the soil increased by about 5% when glyphosate was applied compared with the control ($p \leq 0.05$). The percentage decreases in pH of soils that received extracts from the culture of *Aspergillus aculeatus* and *Trichoderma hamatum* (5.8), *Aspergillus welwitschiae* and *Trichoderma hamatum* (5.8) and *Aspergillus welwitschiae* and *Aspergillus aculeatus* (5.7) were generally lower than when glyphosate (5.9) was applied ($p \leq 0.05$). The microbial biomass carbon of soil that was treated with extract from the culture of *Aspergillus welwitschiae* and *Aspergillus aculeatus* was observed to increase by 289.2% when compared with the control (soil without any form of herbicide applied). However, extracts from the culture of *Aspergillus welwitschiae* and *Trichoderma hamatum*, and *Aspergillus aculeatus* and *Trichoderma hamatum* significantly increased the soil microbial biomass carbon by 173.3% and 147.1%, respectively, when compared with the control ($p \leq 0.05$). The organic carbon (gkg^{-1}) of the control soil was observed to be lower (1.87 gkg^{-1}) when compared with other treatments. The available phosphorus in the soil was observed to have decreased in all treatments when compared with the control. This might be due to high phosphorus fixing by aluminium, and other interactive interferences by the components of the different classes of herbicides applied. The control sample had its available phosphorus

to be 13.00 $mgkg^{-1}$ while herbicide extracts of *Aspergillus welwitschiae* and *Trichoderma hamatum* had 10.97 $mgkg^{-1}$. Glyphosate application led to the highest reduction in the available phosphorus of the soil from 13.00 $mgkg^{-1}$ to 7.28 $mgkg^{-1}$. The microbial respiration (4.81 $mgkg^{-1}$) of the soil was higher when no application was made. The total nitrogen in soils was not significantly different among the control and glyphosate-treated variant and consortium 3 but significantly higher than consortiums 1 and 2.

Table 2: Effects of Herbicides on Soil Chemical and Biological Properties

Sample	pH	SMBC, (μgg^{-1})	Org. C., (gkg^{-1})	Avail. P., ($mgkg^{-1}$)	M.R., ($mgkg^{-1}$)	T.N., (gkg^{-1})
Consortium 1	5.8ab	277.5ab	2.36c	10.97b	3.91ab	0.91b
Consortium 2	5.7b	395.28a	2.65bc	9.44c	3.31bc	0.94b
Consortium 3	5.8ab	250.91ab	3.04a	8.48d	2.68bc	0.97ab
Control	5.6b	101.55b	1.87d	13.00a	4.81a	1.42ab
Glyphosate	5.9a	157.04b	2.69b	7.28e	2.34c	1.98a

Means followed by different letters in a column are significantly different ($p< 0.05$) according to Fisher's LSD.
SMBC = Soil microbial biomass carbon; Org. C. = Organic carbon; Avail. P. = available phosphorus; M.R. = Microbial respiration; T.N. = Total nitrogen
Consortium 1: Extract from the culture of *Aspergillus welwitschiae* and *Trichoderma hamatum*
Consortium 2: Extract from the culture of *Aspergillus welwitschiae* and *Aspergillus aculeatus*
Consortium 3: Extract from the culture of *Aspergillus aculeatus* and *Trichoderma hamatum*

The lower pH value of the soil that received the application of mycoherbicides when compared with that of glyphosate might be due to the presence of acid-forming radicals whose utilisation by fungal species resulted in the production of the acidic medium that stimulated the growth of fungi (Yadav *et al*., 2018).

Further, the dissociative nature of glyphosate to form carboxylate, phosphonic acid and amine with pKa of 2.3, 6 and 11, respectively (EPA, 1993) could be the reason for increased soil pH associated with glyphosate.

The increase in microbial biomass carbon by the mycoherbicide extracts when compared with the control could be attributed to the high amount of polyphenol in the form of organic carbon as well as fungal cellular carbon present in the extract of *Aspergillus aculeatus* and *Trichoderma hamatum* (Song *et al*., 2019). This increase in the organic carbon content of the soil due to applications of herbicide extracts of *Aspergillus aculeatus* and *Trichoderma hamatum* and glyphosate could be attributed to the addition of organic matter from the dead organic materials. This is supported by the findings of Olayinka (1996) and Atere *et al*. (2020) who reported that the addition of organic amendment to the soil tends to increase the organic carbon content of such soil. This reduction in the available phosphorus of soils treated with the herbicides when compared with the control might be due to the effect of phosphorus adsorption by organic substances introduced into the soil by the herbicides. This result correlates with the finding of Yang and Yang (2019) who reported that the adsorption capacity of SOM has a great impact on the soil available phosphorus because soil phosphorus is easily adsorbed in combination with SOM.

The lack of significant difference in the total nitrogen contents of the treatments and the control could arise because organic

herbicides, favouring larger microbial communities could have nitrogen volatilized and temporarily immobilised, resulting in a decrease in the soil nitrogen content. This outcome is in line with the findings of Li *et al*. (2020) which reported that both bacteria and fungi can immobilise soil inorganic nitrogen from the immediate surroundings.

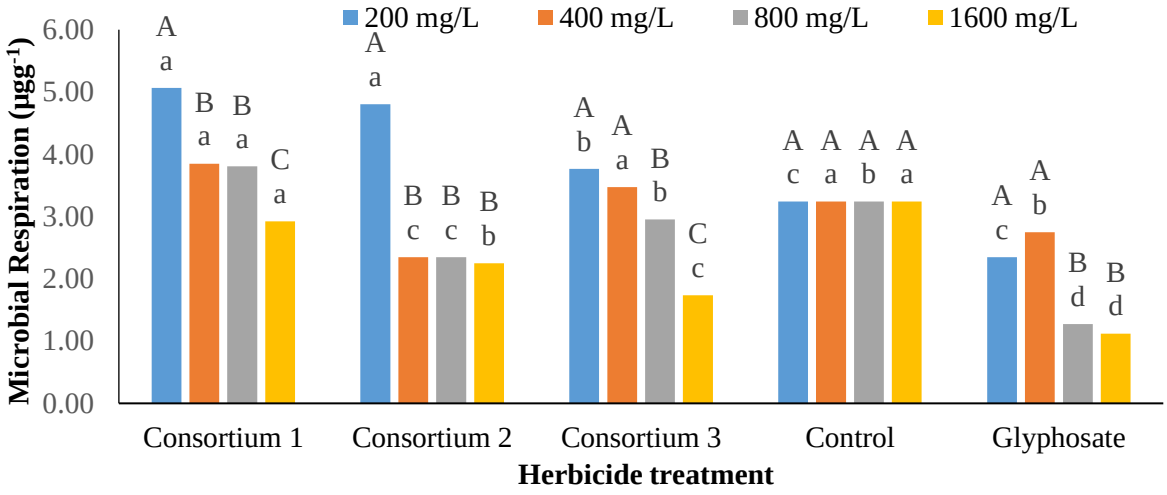

Figure 1: Effects of Herbicides' Application on Soil Microbial Respiration

Means followed by different letters in a column are significantly different (p< 0.05) according to Fisher's LSD.
Letters with uppercase show contrast within herbicides, while letters with lowercase show contrast between doses among herbicides
Consortium 1: Extract from the culture of Aspergillus welwitschiae and Trichoderma hamatum
Consortium 2: Extract from the culture of Aspergillus welwitschiae and Aspergillus aculeatus
Consortium 3: Extract from the culture of Aspergillus aculeatus and Trichoderma hamatum

Effects of herbicides' application on total heterotrophic fungi (THF)

To ascertain the effect of the application of the different herbicides used, the coefficient of determination which is the sole measure of the effect caused by the application of the herbicides was employed. By this model, the effect caused by the application of these herbicides was determined.

Figures 2 (i-v) show that there was a strong positive relationship between the different herbicides applied and the time it takes to stimulate the growth of total heterotrophic fungi ($p \leq 0.05$). There was a significant effect when extract from the culture of *Aspergillus welwitschiae* and *Aspergillus aculeatus* was applied as presented in Figure 2 [ii] with an R^2 value of 0.9578. This was closely followed by soil to which no herbicide was applied which accounted for 94.94% (R^2 = 0.9494) stimulation in the growth of THF when compared with other external factors as shown in Fig. 2 [iv]. Other effects as influenced by the application of extract from the culture of *Aspergillus aculeatus* and *Trichoderma hamatum,* accounted for 91.24% (R^2 = 0.9124) stimulation in the growth of THF while application of the extract from the culture of *Aspergillus welwitschiae* and *Trichoderma hamatum* accounted for 76.35% (R^2 = 0.7635) stimulation in the growth of THF. Upon the application of the reference herbicide, glyphosate, it accounted for 84.09% stimulation in the growth of THF. The increase observed in the THF was due to

the capacity of fungal cells to assimilate most of the components from the extracts as biomass for cellular carbon formation. This is likened to their ability to detoxify contaminants through mechanisms like bio-sorption, bio-absorption, biotransformation, and degradation (Magnoli *et al*., 2023).

Effects of herbicide application on total heterotrophic bacteria (THB)

Contrary to Figure 2 (i-v), Figure 3 (i-v) showed that there was a strong negative correlation between total heterotrophic bacteria and the different classes of herbicides applied ($p \leq 0.05$). The strongest negative relationship was observed with the extract from the culture of *Aspergillus welwitschiae* and *Trichoderma hamatum* with a bacterial growth inhibition rate of 0.5338 bacteria/week. Closely followed by the application of extract from the culture of *Aspergillus welwitschiae* and *Aspergillus aculeatus* with an antibacterial potential of 0.6792/week. Application of glyphosate and extract from the culture of *Aspergillus aculeatus* and *Trichoderma hamatum* was noted to have inhibited bacterial growth by 0.0618 bacteria/week and 0.1391 bacteria/week, respectively. This slight reduction in the THB population observed over the period under investigation might be due to the bactericidal or bacteriostatic nature of the extracts applied which tend to inhibit their growth or destroy them (Desbois and Smith, 2010).

Effects of application of herbicides' doses on total heterotrophic fungi (THF)

The effects of the application of doses of herbicides on the growth of total heterotrophic fungi are shown in Figures 4 (i-iv). The figure shows that as the herbicide's dose applied increases, the fungi-stimulating effect of the mycoherbicides increases with weeks after application. For instance, upon the application of 800 mgL^{-1}, an increase in the fungal population by 0.3288 fungi/week was observed to which the herbicide applied was accountable for 77% ($R^2 = 0.77$) of the growth stimulation. The study also showed that upon the application of 1600 mgL^{-1}, the rate of fungal growth was stimulated by 0.27 fungi/week to which this dose accounted for 98% ($R^2 = 0.98$) of such stimulation in fungal growth. On application of 400 mgL^{-1} and 200 mgL^{-1} of herbicides, it was noted that the doses applied caused a stimulation in fungal growth by 0.113 fungi/week and 0.2138 fungi/week, respectively, with each doses accounting for 97% ($R^2 = 0.97$) and 85% ($R^2 = 0.85$) of the stimulation in fungal growth. For the control, the fungal population was stimulated by 0.17 fungi/week with R^2 of 0.95. The varying trends observed for effect of dose on THF in this study might be due to the ability to reversely regulate the effects of mycoherbicides on the soil environment by degrading the harmful active substances of herbicides (Chen *et al*., 2021).

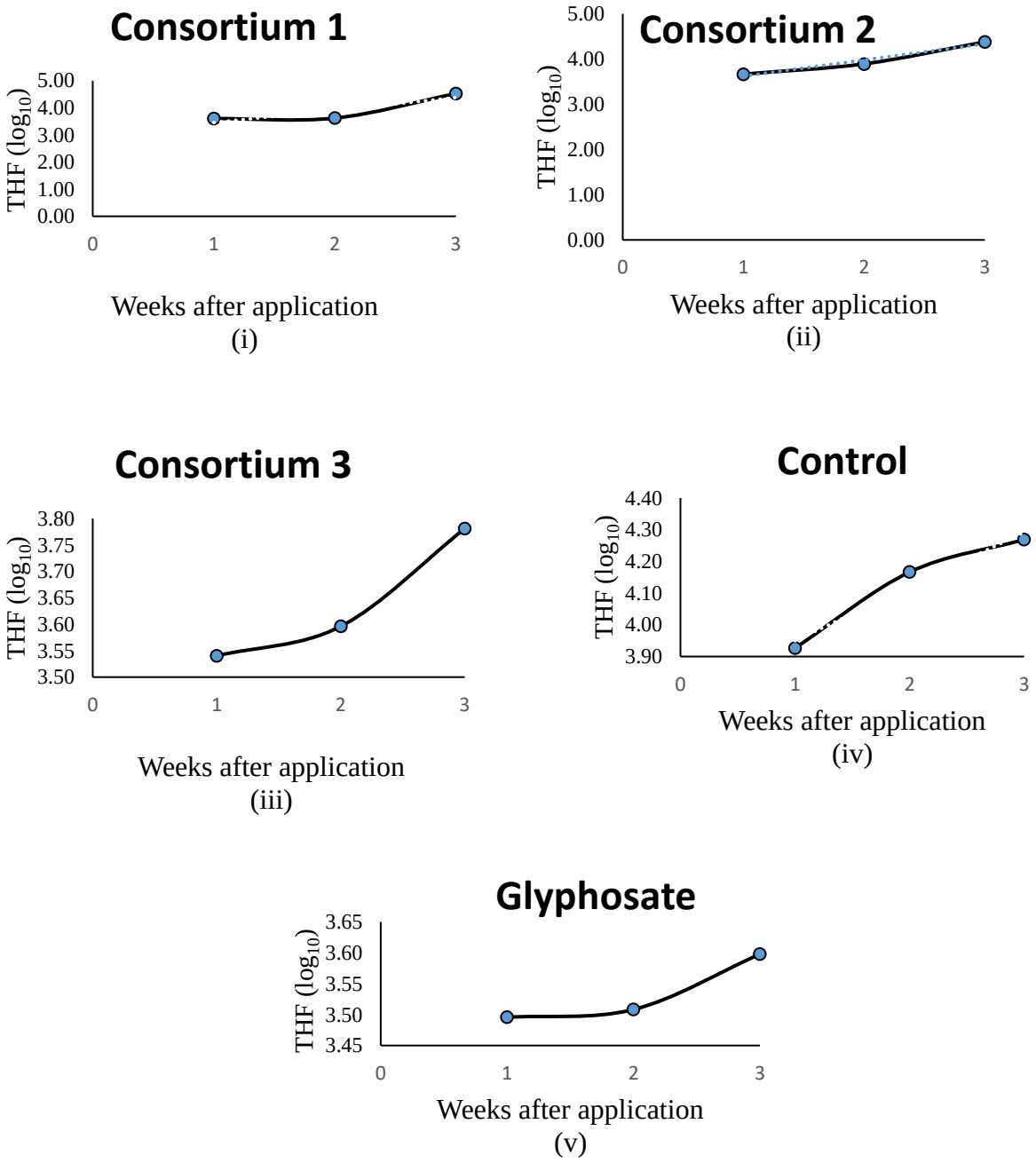

Figure 2: Regression Analysis of the Effect of Herbicides on Total Heterotrophic Fungi with Time after Application

THF = Total heterotrophic fungi; Consortium 1: Extract from the culture of *Aspergillus welwitschiae* and *Trichoderma hamatum*; Consortium 2: Extract from the culture of *Aspergillus welwitschiae* and *Aspergillus aculeatu*; Consortium 3: Extract from the culture of *Aspergillus aculeatus* and *Trichoderma hamatum*; 1 = Week 1; 2 = Week 2; 3 = Week 3; 4 = Week 4

Effects of application of herbicides' dose on total heterotrophic bacteria

Figure 5 (i-iv) shows the relationship between bacterial growth and doses within the length of time under different herbicides' doses ($p \leq 0.05$). It shows that there was a negative relationship between bacterial growth and dose after application throughout the study. The figures show that as the doses applied increased, the rate of bacterial growth decreased. For instance, Figure 5 (iii, iv and v) showed that the application of herbicide doses of 400 mgL^{-1}, 800 mgL^{-1} and 1600 mgL^{-1} caused a reduction in the bacterial population by 0.3786 bacteria/week, 0.5457 bacteria/week and 0.6051/week, respectively, and that these doses applied accounted for 99%, 91% and 87%, respectively, of the bacterial growth inhibition observed. Shortly after application of herbicides 15 days after application significant differences in the population of soil microorganisms (bacteria) were noticed as compared to their population before herbicide application which conformed with the results of Jing *et al*. (2010).

Effects of herbicide and dose application on total heterotrophic fungi (THF) and total heterotrophic bacteria (THB)

The overall effect of herbicide application showed that their application resulted in a reduction in THB but caused an increase in the THF. This trend was observed for the doses used among bacterial and fungal growth. This implies that all of the herbicides applied, both the organic and glyphosate, are bactericidal or bacteriostatic.

CONCLUSION

Co-culturing of rhizospheric fungi as a viable technology for the production of organic compounds among which are herbicidal in nature. The study showed that mycoherbicides obtained from co-culturing of rhizospheric isolates stimulated soil fungal growth and enhanced general soil microbial (microbial biomass carbon, microbial respiration and soil organic matter). While soil pH remained largely the same among mycoherbicides treatments, glyphosate and the control, the available P reduced in mycoherbicides relative to the control, but increased relative to the glyphosate-treated soils. Mycoherbicides formulation technology, therefore, possesses high potential as an environmentally friendly alternative to chemical herbicides in sustainable agriculture.

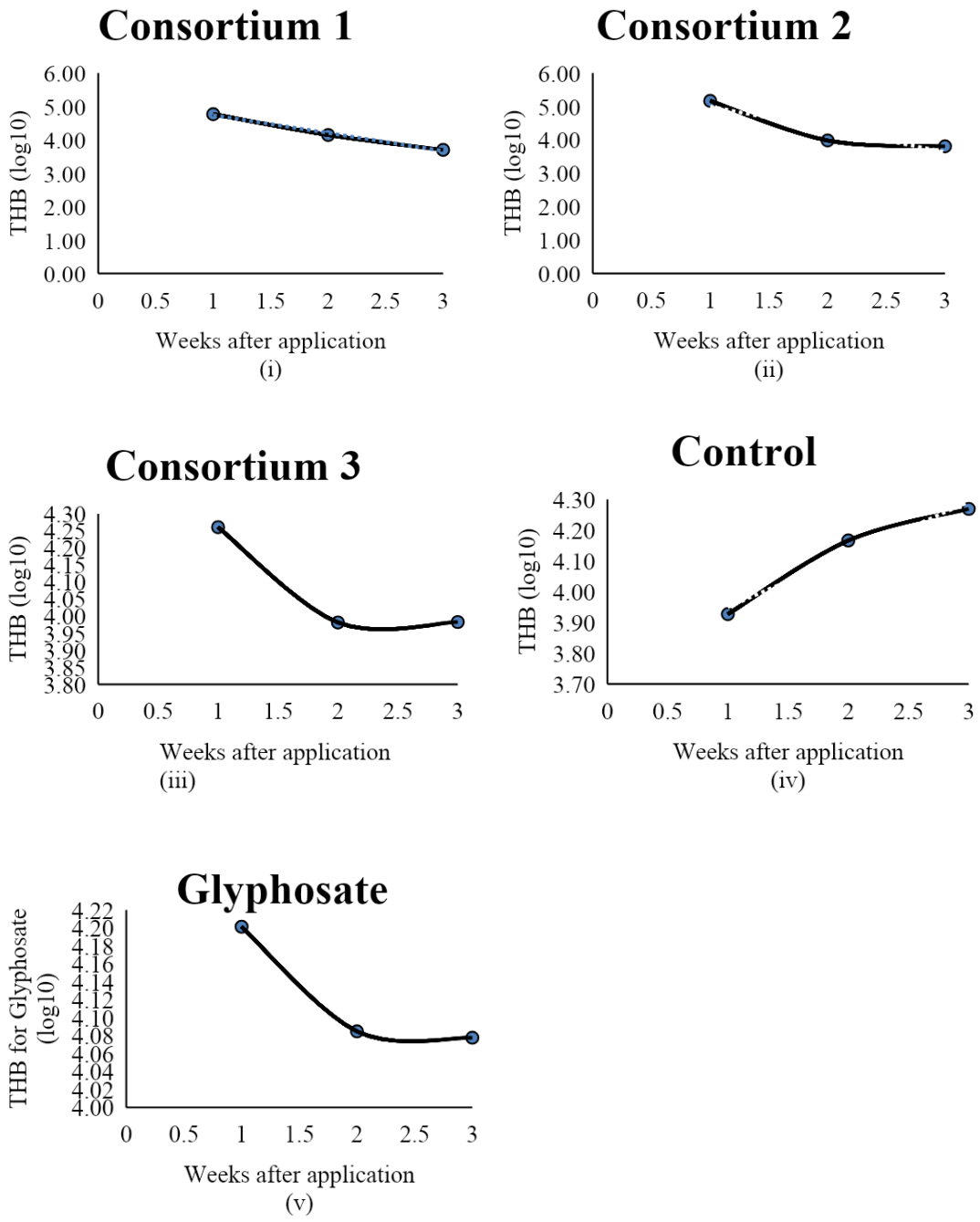

Figure 3: Regression Analysis of the Effect of Herbicides on Total Heterotrophic Bacteria with Time after Application

Consortium 1: Extract from the culture of *Aspergillus welwitschiae* and *Trichoderma hamatum*
Consortium 2: Extract from the culture of *Aspergillus welwitschiae* and *Aspergillus aculeatus*
Consortium 3: Extract from the culture of *Aspergillus aculeatus* and *Trichoderma hamatum*
1 = Week 1; 2 = Week 2; 3 = Week 3; 4 = Week 4; THB = Total heterotrophic bacteria

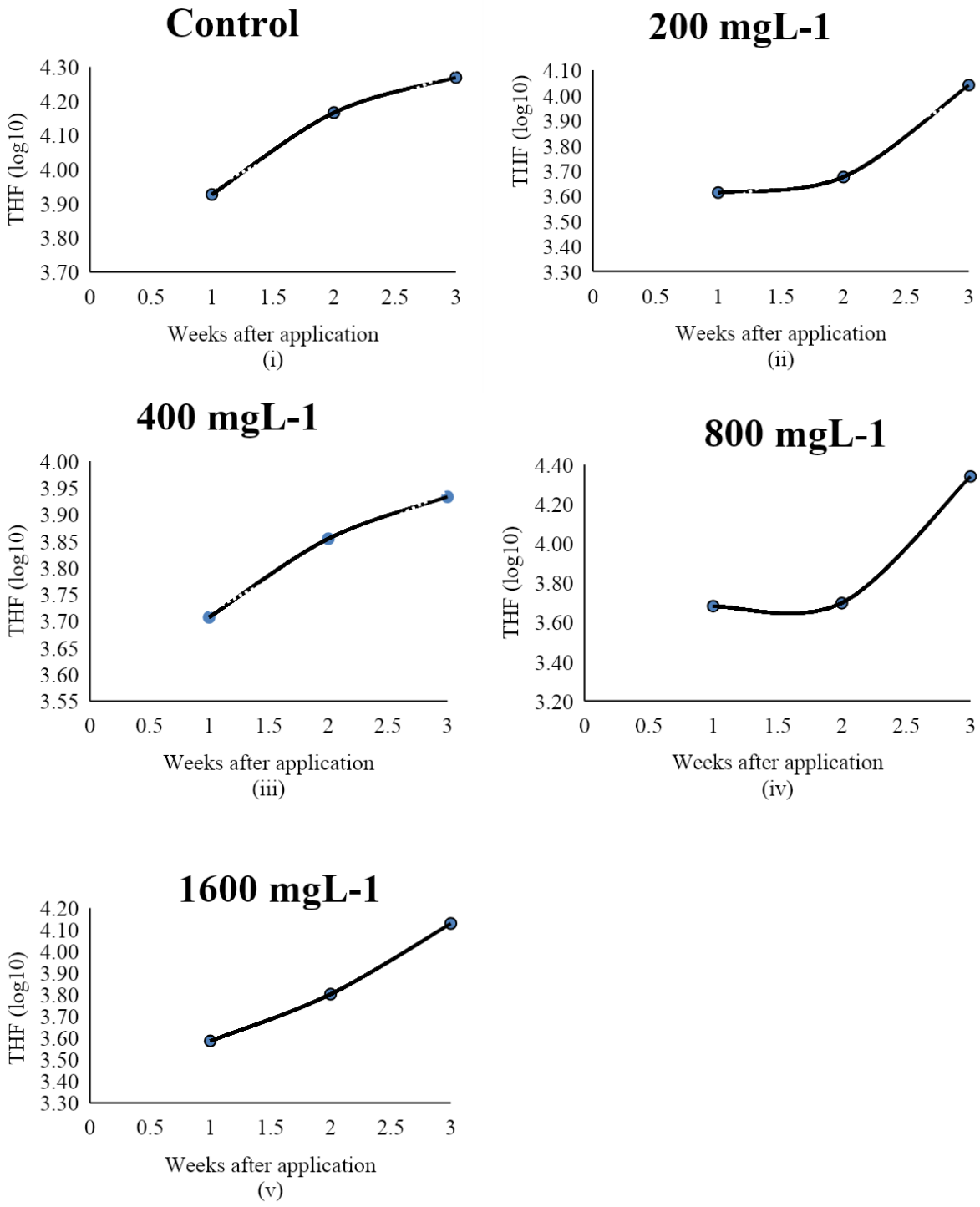

Figure 4: Regression Analysis of the Effect of Herbicides' Dose on Total Heterotrophic Fungi with Time after Application

1 = Week 1; 2 = Week 2; 3 = Week 3; 4 = Week 4

THF = Total heterotrophic fungi

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