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Differential Toxicity Effects of Herbicides on the Growth of Soil Bacteria and Fungi

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Abstract: ***The aim of the present study is to determine the in-vitro responses of selected bacteria and fungi to different concentrations of commonly used herbicides (paraforce and glyphosate). The inhibitory effects of five different concentrations (1.0, 0.5, 0.25, 0.125, and 0.062 standard field dose (sfd)) of herbicides (paraforce and glyphosate) on the growth of two bacteria (Staphylococcus aureus and Pseudomonas cepacia) and two fungi (Aspergillus niger and Penicillum citreonigrum) were assessed using spectrophotometer at 620 nm and diameter of growth of fungi were measured on potato dextrose agar after incubation, respectively. The glyphosate inhibited the growth of both S. aureus and P. cepacia after eight hour irrespective of the concentrations used while the growth of the two bacteria are unaffected by paraforce irrespective of the concentrations. For the A.niger and P. citreonigrum, the diameter of growth increased with time, irrespective of the concentrations of glyphosate while the diameter of growth of fungi decreased with increased concentration of the paraforce compared to glyphosate and controls.The effects of herbicides on soil bacterial and fungal growth depend on the chemicals composition, concentrations and microbial species.Herbicides has differential effects on soil bacteria and fungi.***

Keywords: glyphosate, paraforce, bacteria, fungi, growth, soil

1. Introduction

The rising use of herbicides in farming has not only raised concerns about their negative effects on soil, human health, and agricultural sustainability. There is widespread worry about herbicide contamination, which can result in soil and water pollution (Juhler*et al.*, 2001), reduced biodiversity, and loss of soil heterotrophic bacteria (including denitrifying bacteria) and fungi (Song *et al*., 2013; Bello, 2021). Understanding the effect of herbicides in soil, on the other hand, is required for an accurate assessment of their activity and potential environmental harm (Gianelli*et al*., 2014). According to previous research, the majority of herbicides penetrate the cell walls of soil-dwelling bacteria that are not their targets (non-target organisms), interfering with their metabolism and eventually causing cell death (Sattler *et al*., 2006).

Herbicides are thus seen as a significant danger to soil microbiota and soil health, affecting natural habitats in the soil (Sattler *et al*., 2006; Bello, 2021; 2022). Herbicide treatment alters the soil microbial community in both quantitative and qualitative ways (Raj and Syriac, 2017; Bello, 2022).In today's environment, the microbial population in soil serves as an indicator of agricultural performance. Soil microorganisms are a crucial link between the soil, plant, herbicide, fauna, and man relationships because they play a critical role in herbicide breakdown (Raj and Syriac, 2017). Herbicide application causes quantitative and qualitative changes in soil microbial growth (either stimulating or depressive) and enzymatic activities, depending on the herbicide's phytotoxic nature (type and concentration), microbial species, and environmental conditions (Maheswari and Ramesh, 2019; Bello, 2022).

Furthermore, these non-target effects on soil microbes may impair the performance of essential soil activities such as organic matters (OM) decomposition, the nitrogen cycle, and methane oxidation (Sebiomo*et al.*, 2011). It is critical to note that the use of herbicides reduces the microbial population, which includes bacteria, fungi, actinomycetes, and protozoa, disrupting the soil ecological balance between plant pathogenic and beneficial organisms, allowing disease-causing microorganisms to proliferate (Kalia and Gupta, 2004).

However, repeated applications of 2, 4-D, trifularin, paraforce, and glyphosate reduced the bacterial population in the soil significantly (Breazeale and Camper, 1970; Bello, 2021; 2022). Previous research found that herbicides have less of an effect on soil bacteria when sprayed in prescribed amounts (standard field dose) (Imfield and Vuilleumei, 2012). However, there are limited data to back up this assertion because microorganisms may react differently to different herbicides. The purpose of this research is to determine the inhibitory effect of common herbicides (paraforce and glyphosate) on the growth of various bacterial and fungal species.

2. Materials and Methods

Top soil sample (about 0-4cm in depth) in Adekunle Ajasin University Akungba Akoko (AAUA) was collected aseptically into a sterile ziplock polyethylene bag using a sterile spatula. The collected soil samplehad known history of herbicide application (paraforce and glyphosate). The sample was taken to Microbiology laboratory for microbial examination and subsequent analysis. Bacteria (*Staphylococcus aureus* and *Pseudomonas cepacia*) and fungi (*Aspergillus niger* and *Penicillum citreonigrum*) isolated using culture media (nutrient agar and potato dextrose agar (Oxoid, Uk), respectively) and identified according to Bello (2021; 2022).The herbicides (Glyphosate and Paraforce) used in present study were obtained from local agrochemical dealer in Ikare AkokoOndo State Nigeria,based on their frequent usage by farmers in the study area. Approximately 4.9ml each of glyphosate or Paraforcesolution was added to 1000ml each of prepared

nutrient broth that have been cooled to 47^{o}C to achieve a standard field dose (sfd) of 78.5 ml of herbicide per 16 liters of water according to Armstrong and Lancaster (2017). This resulted in 1.0sfd concentration, which was diluted down to create five different concentrations (1.0, 0.5, 0.25, 0.125, and 0.062 sfd) with the addition of sterilized nutritional broth in sterile McCartney bottle. Each McCartney bottle contained approximately 25 ml solution of herbicides and brothwere inoculated with 1 ml of standardized (0.5 McFarland standards) pure bacterial culture according to Oyeleke*et al.* (2008), with the control (culture media with herbicide only).For bacteria, 3 ml of the pure culture sample of bacteria were taken aseptically at each time and measured at 0h, 8h, 16, 24 and 36hours (h) of incubation using spectrophotometer (X RITE, England) at 620 nm wavelength to determine the bacteria cell concentration using Beer-Lambert's equation (A=cεb);where A is the absorbance, c is the molar concentration of the herbicide, ε is the molar absorptivity coefficient that gives light absorbed by 1 mole of a molecule and b is the length that light travels in the solution (1.0 cm).

However, for fungimolten Potato dextrose agar (PDA) was used for the dilution of the herbicides to achieve five different concentrations (1.0, 0.5, 0.25, 0.125, and 0.062 sfd) instead of nutrient broth. About 20 ml of PDA and herbicides solutions at different concentrations were poured into sterile Petri-dish and allowed to set. The set plates were inoculatedat center of the plate with fungi and incubated at room temperature (28^{o}C) for 196 hours. The diameter of growth in millimeters (mm) was measured with ruler and recorded accordingly.Data obtained were plotted against the time (in hours).

3. Results

The toxicity effect of the used herbicides (para-force) on the growth of both *S. aureus*and *P. cepacia*showed thatat 8 h of sampling, the concentration of *S. aureus*and *P. cepacia*cell decreased from about 2.5 x10^{7}at time 0 h to 1.7 x10^{7} or less across all the five concentrations (1.0, 0.5, 0.25, 0.125, and 0.062 sfd) compared to the control, which showed no decrease in cell concentration at 8 h of sampling (figure 1). Moreover, there was increase in the cell concentrations of both *S. aureus*and*P. cepacia*after 8 h of sampling, irrespective of the herbicide concentrations.But the concentrations of *S. aureus*and *P. cepacia*cell decreased with an increase in concentration of the herbicide (paraforce). However, the concentration ofboth *S. aureus*and *P. cepacia*decreased to 0 after 8 h of sampling irrespective of the concentration of herbicide (glyphosate) compared to the control sample (figure 1).

For the fungi (*Aspergillus niger* and *Penicillum citreonigrum*), the diameter of growth increased with time, irrespective of the concentrations in glyphosate used. However, the diameter of growth of both *A. niger* and *P. citreonigrum*decreased with increased concentration of the paraforce compared to glyphosate and controls (figure 2).*A. niger* and *P. citreonigrum* were inhibited by paraforce at concentrations of 0.5 and 1.0 sfd but growth still occurred at these concentrations (0.5 and 1.0 sfd) with time.

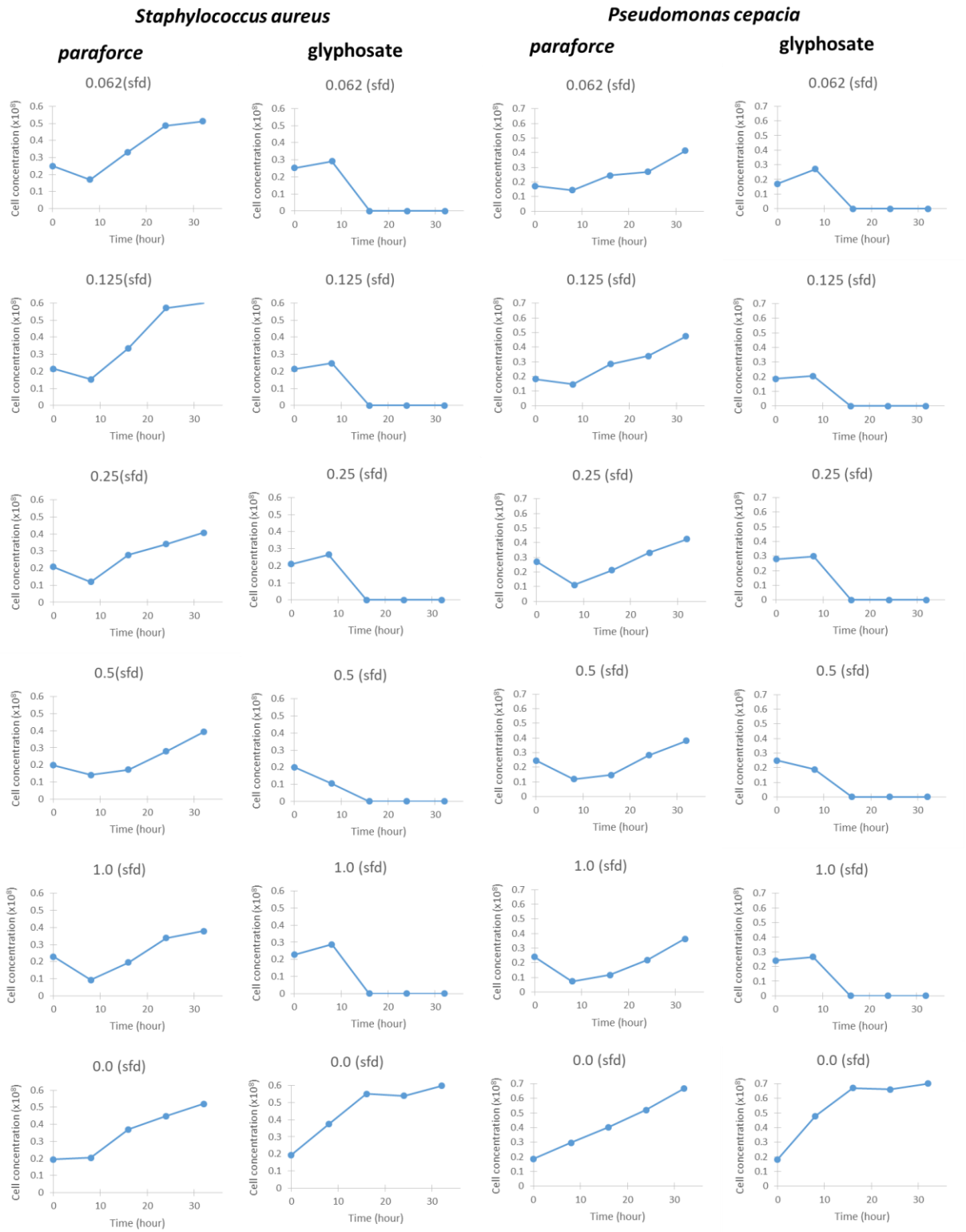

Figure 1: The bacteria (*Staphylococcus aureus* and *Pseudomonas cepacia*) growth curve under five different concentrations (1.0, 0.5, 0.25, 0.125 and 0.062 sfd) of herbicides (paraforce and glyphosate) and control (0.0 sfd). Data represents the mean of triplicate samples.

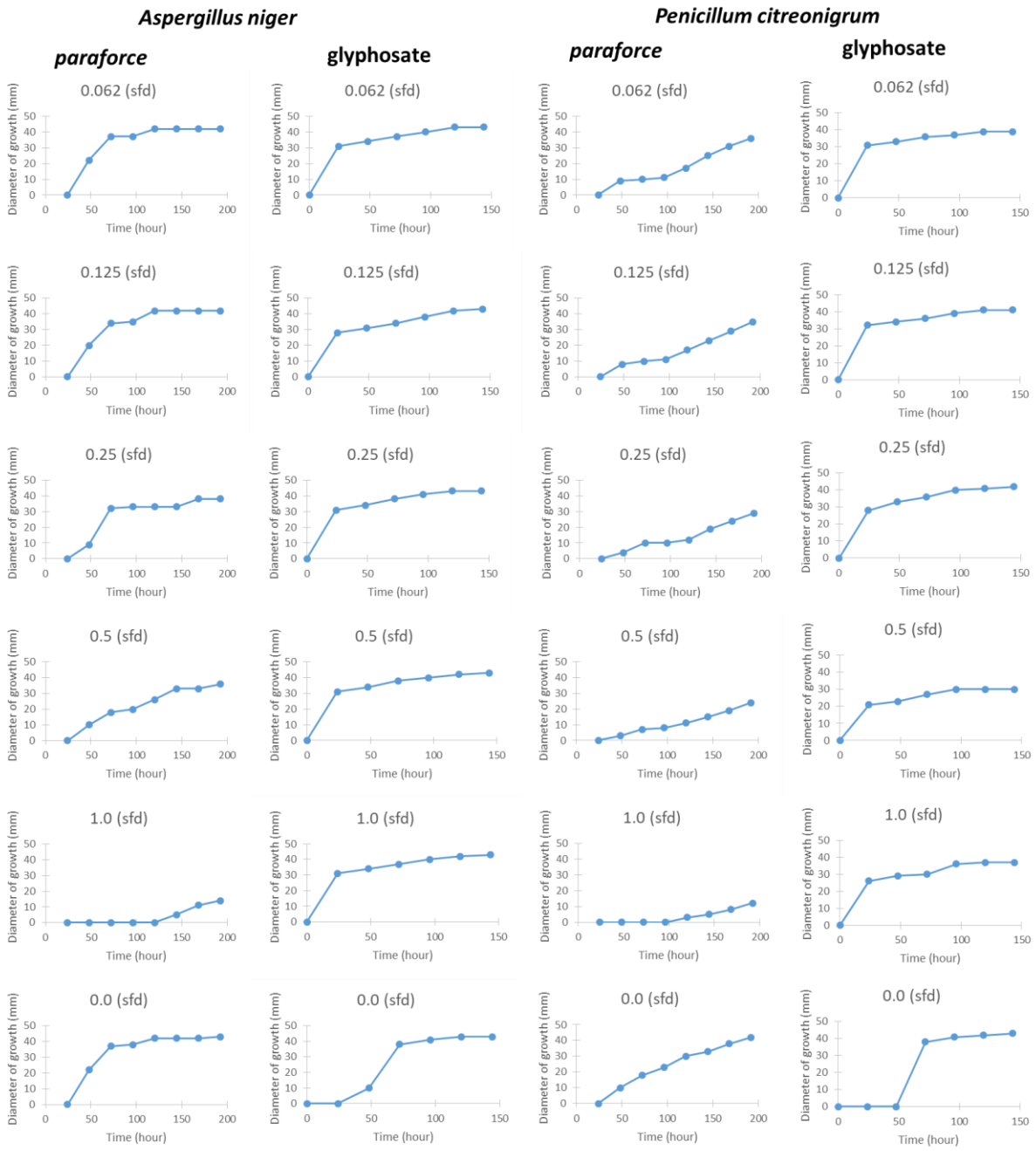

Figure 2: The fingi (*Aspergillus niger* and *Penicillum citreonigrum*) growth curve under five different concentrations (1.0, 0.5, 0.25, 0.125 and 0.062 sfd) of herbicides (paraforce and glyphosate) and control (0.0 sfd). Data represents the mean of triplicate samples.

4. Discussion

The cytotoxicity (toxicity) effect of herbicide on the growth of isolated bacterial and fungi wereevaluated in the present study. The initial decreased in the number of bacteria cell after 8 hours of sampling which increased afterward across all concentration of paraforce used irrespective of the bacteria (*Staphylococcus aureus* and *Pseudomonas cepacia*) tested indicates that the paraforce showed mild toxicity and the bacteria cell adjusted to the mild toxicity of paraforce and multiply afterward. The results obtained from the present study agrees with previous study by Adomakoand Akyeampong (2016) and Tyagi *et al.* (2018) who observed thereduction in the bacterial population in soil treated with paraforceafter ten days which later increased after three monthsin their independent studies.

Additionally, the decreased in the cells of *Staphylococcus aureus* and *Pseudomonas cepacia* to zero (0) after 8 hours irrespective of the concentration of the glyphosate used indicate that glyphosate inhibits the growth of bacterial. The results also shows the high cytotoxicityof glyphosate to the bacterial cell.The results obtained in the present study corroborates previous studies by Zain *et al.* (2013), Gianelli, *et al*. (2014) and Balasubramanian, (2017) who in their independent studies demonstrated that bacteria species were highly sensitive to glyphosate when they observed a decreased in bacteria population after the application of glyphosate into soil.

However, the fungi tested against herbicides in the present study exhibited different feature from the bacteria as thegrowth of fungal species (*A. niger* and *P. citreonigrum*) werenot inhibited by the glyphosate. Rather, their growth were stimulated by the different concentrations of glyphosate used.On contrary, the growth of *A. niger* and *P. citreonigrum*were inhibited by paraforce (at 0.5 and 1.0 sfd)compared to the control even though growth occurred at these concentrations (0.5 and 1.0 sfd) with time. Additionally, the growth of the two fungi (*A. niger* and *P. citreonigrum*) were stimulated by the herbicides irrespectiveat lower concentrations (0.062 to 0.25 sfd). This results suggest that herbicides can elicit different reactions by different fungi species i.e. certain fungal species are benefitted by herbicide addition in soil, while others are inhibited according to Bollen(1961).The results obtained in the present studies also indicates that paraforce has mild cytotoxicity on fungi while glyphosate stimulates the growth of fungi. This observation from the present study corroborates the previous studies by Sebiomo*et al.* (2011) who reported that some soil microorganisms can degrade the herbicide while some others were adversely affected. The differential effect of herbicides on soil bacteria and fungi could depend on the herbicides application rates, mode of actions and the chemical composition of herbicide used. Therefore, the results obtained from the present study further confirm that the effects of herbicides on soil bacterial and fungal growth depend on the chemicals composition, chemical concentrations and microbial species according to Zain *et al*. (2013). Finally, herbicides has differential effectson soil bacteria and fungi, manufacturer should be encouraged to produce herbicides with lesser toxicity on non-target soil bacteria and fungi.

References

[1] Adomako, M.O. and Akyeampong, S. (2016). Effect of Some Commonly Used Herbicides on Soil MicrobialPopulation. *Journal of Environment and Earth Science*, 6(1): 2224-3216.

[2] Balasubramanian, A. (2017). Soil microorganisms. *Univ. Mysore*. Springer, Dordrecht,pp.5-22.

[3] Bello, M.O. (2021). Prolong usage of Herbicides Reduces Heterotrophic Aerobic Bacteria and Fungi Population and Alters soil physiochemical parameters. *Journal of Advances in Microbiology*, 21(4): 63-75; Article no. JAMB.67297 ISSN: 2456-7116.

[4] Bello, M. O**.** (2022). Prolonged Use of Herbicides Increased the Resistance of Soil Aerobic Heterotrophic Bacteria and Fungi to Antibiotics. *International Journal of Current Microbiology and Applied Science*,11(06): 281-291.

[5] Bollen, W.B. (1961). Interactions between pesticides and soil microorganisms. *Annual Review Microbiology*, 15:69-92.

[6] Breazeale, F.W. and Camper, N.D. (1970). Bacterial, fungi and actinomycete populations in soil receiving repeated applications of 2,4-Dichlorophenoxyacetic acid and trifluralin. *Applied Microbiology*,19:379-380**.**

[7] Gianelli, V.R., Bedmar, F. and Costa, J.L. (2014). Persistence and sorption of imazapyr in three Argentinean soils. *Environmental Toxicology and Chemistry*,33: 29-34.

[8] Imfeld, G., and Vuilleumier, S. (2012). Measuring the effects of pesticides on bacterial communities in soil: a critical review. *European Journal of Soil Biology*, 49: 22-30.

[9] Juhler, R. K., Sørensen, S. R. and Larsen, L. (2001). Analysing transformation products of herbicide residues in environmental samples. *Water Reserve*, *35*(6): 1371-1378.

[10] Kalia, A. and Gupta, R.P. (2004). Disruption of food web by pesticides. *Indian Journals of Ecology*, 31:85-92**.**

[11] Maheswari, S.T. and Ramesh, A. (2019). Fate and persistence of herbicide residues in India. In: Sondhia S, Choudhury PP, Sharma AR (eds) Herbicide residue research in India. *Springer, Singapore*, pp 1-28.

[12] Oyeleke, S. B., Dauda, B. E. N. and Boye, O. A. (2008). Antibacterial activity of *Ficuscapensis*: *African Journal of Biotechnology*,7 (10): 1414-1417.

[13] Raj, S.K. and Syriac, E.K. (2017). Herbicidal effects on the bio-indicators of soil health - a review. *Journal of Applied National Science*,9:2438-2448**.**

[14] Sattler, C., Kächele, H., and Verch, G. (2007). Assessing the intensity of pesticide use in agriculture. *Agriculture, ecosystems & environment*, 119(3-4): 299-304.

[15] Sebiomo, A., Ogundero, V. W., Bankole, S. A. (2011). Effects of four herbicides on microbial population, organic matter and dehydrogenase activity. *African Journal of Biotechnology*, 10:770-778.

[16] Song, J., Gu, J., Zhai, Y., Wu, W., Wang, H., Ruan, Z., Shi, Y. and Yan, Y. (2013). Biodegradation of nicosulfuron by a *Talaromycesflavus*LZM1. *Bio resources Technology*,140: 243-248.

[17] Tyagi, S., Mandal, S. K., Kumar, R. and Kumar, S. (2018). Effect of Different Herbicides on Soil Microbial Population Dynamics in Rabi Maize (*Zea mays* L.).*International Journal of Current Microbiology and Applied Sciences*,7:3751-3758.

[18] Zain, N.M.M., Mohamad, R.B., Sijam, K., Morshed, M.M. andAwang, Y. (2013). Effects of selected herbicides on soil microbial populations in oil palm plantation of Malaysia: A microcosm experiment.*African Journal of Microbial Research*, 7:367-376.