EFFECTS OF COMMON HERBICIDES ON SOIL FUNGI IN A MAIZE (*Zea mays*) FARM

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ABSTRACT
The increasing use of pesticides in Agriculture have raised concerns over the unintended consequences, especially multiple applications of various forms of pesticides on the same farm land during a particular production period. This field trial was conducted to evaluate the effect of common farmland application of herbicides i.e. atrazine, xtravest , 2,4-D, on the species and population of soil fungi. Soil samples were collected thrice at a three month interval (July, September and December 2010) from a herbicide trial site located in Iddo town, Iddo Local Government area of Oyo State, Nigeria. The herbicides were applied at recommended rates either singly or in combination over the 2009 / 2010 growing seasons on a maize field. Their effects were tested on the fungal species present and their abundance using the soil dilution method. Data collected were subjected to descriptive and inferential statistics. The results showed the presence of different fungal genera on all the treated plots including the control. The fungi populations observed include *Aspergillus niger, Lasidiodiplodia theobromae, Nattrassia mangiferae, Trichoderma spp, Fusarium verticillum, Fusarium oxysporum, Rhizopus stolonifer, Macrophomina phaseolina and Colletotrichum gleosporioides. Lasidiodiplodia theobromae* occurred the most (15/16) times among the treatments, across the sampling periods while *Rhizopus stolonifer* occurred the least (1/16) number of times among the treatments through the sampling periods. Although, there were reductions in the number of isolated fungi across all treatment periods, however, the reductions were not consistent. In conclusion, the application of herbicide(s) may not exclude the use of other forms of pesticides in the management of soil microorganisms on the farm.

Keywords: Herbicide, Soil fungi, Treatment, Maize

INTRODUCTION
Crop production is constrained by a number of factors which include pests, diseases and weeds. These factors can act either singly or in combination to limit productivity (FiBL, 2011). Herbicides are chemicals used to kill or inhibit weed growth and they may be specie-specific or non-specific. Herbicides can be classified into a number of ways. These include mode of action, method of application, differential
response among plants, pathway of herbicide movement in plant and others (DiTomaso, 2002; Varshney and Sondhia, 2010; Pennsylvania State University Extension, 2015). Herbicides commonly used on maize farms include 2, 4-D, atrazine, glyphosate, paraquat, xtravest, and a host of others.

Herbicides Researchers and Scientists have raised concerns over the unintended consequences of herbicides on non-target organisms in the soil including soil microorganisms (Karthikeyan et al., 2003; Prado et al., 2009). Most soil organisms proliferate rapidly in the soil and their diversity is largely due to the limitless variety of food and wide range of habitat found in the soil environment (FAO, 2002). Soil microorganisms can either be of beneficial or harmful effects to plants (Domergues et al., 1980). Specialized fungi can be pathogenic on the plant tissues while others form mutually beneficial relationships (mycorrhizae) with plants and assist in direct nutrient supply to the plants (Domergues et al., 1980).

Fungal hyphae, push their way between soil particles, roots, and rocks and are usually only several thousandths of an inch (a few micrometers) in diameter. A single hypha can span in length from a few cells to many yards when a few fungi, such as yeast, are single celled. Their number in soil varies from a few thousand to a few million per gram (Hoorman, 2011). They cannot synthesize their own food and they are dependent on complex organic substances (Hoorman, 2011). Fungi are dominant in acid soils because acidic environments are not conducive and or suitable for the existence of bacteria and actinomycetes (Bhoopander et al., 2005). Fungi require an optimum pH range of 4.5 – 6.5. They are also present in neutral and alkaline soils and some can even tolerate a pH of up to 8.0 (Wood, 1989; Bhoopander et al., 2005). Soil fungi are part of the extremely diverse group of microorganisms. Their biodiversity has been confirmed in soils (Hoorman, 2011). Fungi are vital in the degradation and decomposition of cellulose, starch, pectin, lignin and hemi-cellulose in the organic matter added to the soil. They also serve as food for bacteria. They play an important role in soil aggregation and in the formation of humus. Some fungi form a mycorrhizal association with the roots of higher plants (symbiotic) and help in the mobilization of phosphorus and nitrogen, while some are parasitic in nature and cause a number of plant diseases as wilts, rots, seedling blights, and so on and so forth. (NSW Department of Primary Industries, 2005). Fungi can be classified into three general functional groups based on how they get their energy. They can either be decomposers, mutualists or pathogens / parasites. Pathogens cause reduced production or death when they colonize the roots and other organisms. Root-pathogenic fungi as *Rhizoctonia* cause major economic losses in agriculture each year. Fungal diseases often reduce crop yield and lower crop quality by producing toxins which are harmful to human health (Placinta et al., 1999 and NSWDPI, 2005). Some of the most commonly observed fungi in the soil are *Alternaria, Aspergillus, Cephalosporium,*
Mucor, Gliocladium, Penicillium, Trichoderma, Cladosporium, Botrytis, Monilia, Chaetomium, Pythium, Cladosporium, and so on and so forth (Brady et al. 1999).

Research has been carried out on the effect of herbicides on soil microbiota and some researchers (Milicic, 1987; Schuster and Schroder, 1990; Radosevich et al., 1995; Milosevic et al., 2002) suggested that the rate of herbicide decomposition in the soil is influenced by several factors like the properties of the herbicide applied, its dose as well as by the physical and chemical soil properties, soil moisture and temperature, plant cover, soil cultivation method and the types of microorganisms present. Some other researchers also pointed out that the toxicity level of an organic pollutant will depend on the soil characteristics such as the organic matter and clay content of the soil (Bossio et al., 1998 and Labud et al., 2007). This means that soil microorganisms in different environments will react differently to different herbicide applications. The intensive use of these herbicides has caused environmental concern, especially unforeseen consequences on soil microbes (Karthikeyan et al., 2003). Since herbicides can potentially be toxic to organisms other than their intended target. Determining their impact on non-target organisms (such as microbes) in the soil has been of considerable interest (Bending et al., 2007 and Nur Masirah et al., 2013). Also, knowledge on the effects of on non-target organisms may cause a reduced application of other pesticides.

This study was therefore set up to assess the effect of common herbicide treatments on soil fungi population dynamics and diversity.

MATERIALS AND METHODS
The experiment was conducted on a maize farm at Iddo, Iddo Local Government Area of Oyo State. The farm used for the experiment lies on latitude 7.3964° North and longitude 3.9167° East. Soil samples collected from the herbicide trial site were first analyzed for their routine physical and chemical properties as presented in Table 1. Selection of the herbicides used were based on the extent of their use in Agriculture (Table 2). Soil samples were collected every three months (July, September and December 2010). The experimental design was a randomized complete block design (RCBD) with six treatments. Samples were collected randomly from three spots within each plot using a 10 cm soil auger. The samples collected from each plot were mixed thoroughly for uniformity, labelled and kept in individual bags for further analysis.

Sterilization of materials
All glassware (Petri dishes, beakers, pipette, conical flasks, test tubes) used for the experiments were washed using detergent, rinsed and allowed to dry. The glassware were afterwards sterilized in a hot air oven (Gallenkamp Hotbox Oven, Gallenkamp, UK) at 200°C for one hour. Conical flasks were filled with water and plugged with cotton
wool at the top to avoid contamination. They were then sterilized in the oven (Gallenkamp Hotbox Oven, Gallenkamp, UK) at 200°C for one hour. Inoculating needles, spatula, cock borers were sterilized by dipping them in 70% ethanol and flaming them until red hot. The work benches were sterilized by swabbing with cotton wool soaked in 70% ethanol.

**Preparation of growth media**

Potato dextrose agar (PDA) was used as a medium for the growth and maintenance of the fungal isolates all through the experiment. The preparation of the PDA was done according to the manufacturer’s recommendation. Exactly 39g of dehydrated PDA (Difco Laboratories, Michigan, USA) was dissolved in 1 litre of distilled water in a 1000 ml conical flask. 15ml of bacterial agar was also added for quick solidification of the PDA. The solution was autoclaved at 121°C (1.05kg/cm²) for 15 minutes and allowed to cool to 45-50°C. The molten PDA was poured into sterile Petri dishes, 9 cm in diameter in such a way that the PDA filled the whole bottom of the Petri dishes. The mouth of the conical flask was flamed every time before and after pouring the PDA into the Petri dishes. The PDA was slowed to solidify and preserved in the refrigerator.

**Isolation and identification of fungi from soil samples**

The soil dilution method also known as the serial dilution method was used for the isolation of the different fungal species in the different soil treatments. One gram of each soil sample was weighed into sterile test tubes and 9 ml of sterile distilled water was added to each of the test tubes. This tube was thoroughly mixed to give a stock suspension. Fungal cultures were plated into freshly prepared PDA using 1:10⁴, 1:10⁵, 1:10⁶, 1:10⁷ with the aid of individual sterile pipettes. A sterile glass rod was used to gently spread the suspension across the face of the solidified PDA. Inoculated Petri dishes were covered and sealed with paper tape. The dishes were turned upside down to avoid accumulation of condensation. They were incubated at 27°C. After 72 hours, plates were examined daily for the development of fungal mycelium. Fungi growing on PDA were counted and each fungal specie found on each plate were isolated and sub-cultured. Flame sterilized needles were used to transfer the fungi isolates into a new sterile plate containing fresh PDA so as to obtain a pure culture for each of the fungal species. The Petri dishes were also sealed with paper tape to prevent any contamination and were incubated at 27°C for 72 hours to further examine the fungal growth on the PDA. The various fungal isolates from each sample were sub-cultured again onto a fresh PDA to obtain a pure culture for identification. The identification was done in the laboratory of the Department of Crop Production, Soil and Environmental Management of Bowen University, Iwo and confirmation through assistance of Advanced Phytopathology Laboratory of International Institute of Tropical Agriculture (IITA) Ibadan Nigeria.
Effects of common herbicides on soil fungi in a maize farm

compound microscope was used in the identification and all guides laid down by the International mycological Institute were adhered to.

Statistical analysis
Data collected were subjected to both descriptive and inferential statistical analysis using SAS (2001). The descriptive statistics include frequency count and tables. Frequency of occurrence of each fungal isolate were recorded and expressed as a percentage of the total isolation made from each plot.

RESULTS AND DISCUSSION
Isolation and identification of fungi from soil samples.

The results showed that varying genera and population of fungi were observed in the plots treated with different herbicides as well as the untreated plot (control) throughout the sample period. *Lasidiodiplodia theobromae* had the highest frequency of occurrence (%) through all the treatments.

Treatment with atrazine and 2, 4-D mix (Treatment E) had the most restraining effect on the fungal community as no single genera of fungi was isolated at the end of the third sampling period. A similar experiment was conducted by Namita and Deep (2008) and it showed that 2,4-D had the most toxic effect on soil mycoflora. Plots treated with xtravest (Treatment D) also had just one genera of fungi (*L. theobromae*) isolated at the end of the third sampling period (Table 3c). Despite atrazine’s (Treatment B) persistence in the soil for long periods as reported by several scientists, plots treated with atrazine for four planting seasons through 2009 and 2010 had the largest number of fungi genera at the end of the third sampling period. This finding agrees with the report of Rogers et al. (1994) that pesticides applied at recommended levels and intervals seldomly have marked effects on soil microorganisms. Radosevic et al. (1995) reported that microbes may use atrazine as a source of carbon. Cook and Hutter (1981) also pointed out that herbicides may be a source of nutrition for microbes in which case they significantly affect microbial growth and multiplication as in when they are used as a source of nitrogen. Smith et al. (1976) reported similar findings. *Aspergillus* spp and *Peniculium* spp. were dominant in soil treated with paraquat. Ceballos et al. (2006) reported that (4-Chloro-2-methylphenoxy) acetic acid increased the severity of *Fusarium* root rot in red clover seedlings. Ali (1990) had shown that the fate of pesticides in soils is greatly affected by the presence of organic matter in the soil by aiding their disappearance. Ceballos et al.
Table 1. Soil physical and chemical properties of the experimental site.

<table>
<thead>
<tr>
<th>Soil property</th>
<th>Value obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (H$_2$O)</td>
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</tr>
<tr>
<td>Organic carbon (g/kg)</td>
<td>2.18</td>
</tr>
<tr>
<td>Total N (g/kg)</td>
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</tr>
<tr>
<td>Available P (mg/kg)</td>
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</tr>
<tr>
<td>Ex. Bases (c/mol/kg)</td>
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</tr>
<tr>
<td>Ca</td>
<td>1.37</td>
</tr>
<tr>
<td>Mg</td>
<td>2.42</td>
</tr>
<tr>
<td>Na</td>
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<tr>
<td>K</td>
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<td>Ex. Acidity</td>
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</tr>
<tr>
<td>CEC (mol/kg)</td>
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<tr>
<td>Base sat. (c/mol/kg)</td>
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</tr>
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<td>Sand (g/kg)</td>
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</tr>
<tr>
<td>Silt (g/kg)</td>
<td>174</td>
</tr>
<tr>
<td>Clay (g/kg)</td>
<td>148</td>
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</tbody>
</table>

Table 2. Herbicide treatments

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Treatment description</th>
<th>First season 2009</th>
<th>Second season 2009</th>
<th>First season 2010</th>
<th>Second season 2010</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>Atrazine</td>
<td>Atrazine</td>
<td>2,4-D</td>
<td>2,4-D</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Atrazine</td>
<td>Atrazine</td>
<td>Atrazine</td>
<td>Atrazine</td>
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</tr>
<tr>
<td>C</td>
<td>Xtravest</td>
<td>Xtravest</td>
<td>Atrazine + 2,4-D</td>
<td>Atrazine + 2,4-D</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Xtravest</td>
<td>Xtravest</td>
<td>Xtravest</td>
<td>Xtravest</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Atrazine + 2,4-D (Mix)</td>
<td>Atrazine + 2,4-D</td>
<td>Atrazine + 2,4-D</td>
<td>Atrazine + 2,4-D</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>No herbicide</td>
<td>No herbicide</td>
<td>No herbicide</td>
<td>No herbicide</td>
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</tr>
</tbody>
</table>
Table 3a. Frequency of isolation (%) of fungal pathogen from herbicide treated soil and untreated at three seasons

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fungus</th>
<th>Frequency of isolation</th>
<th>Frequency of occurrence</th>
<th>Mean ± Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Aspergillus niger</td>
<td>4</td>
<td>4</td>
<td>20.000 ± 12.94</td>
</tr>
<tr>
<td></td>
<td>Lasidiodiplodia theobromae</td>
<td>31</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Natrassia mangiferae</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rhizopus stolonifer</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Fusarium oxysporum</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Macrophomina phaseolina</td>
<td>13</td>
<td>13</td>
<td>14.429 ± 6.68</td>
</tr>
<tr>
<td></td>
<td>Natrassia mangiferae</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trichoderma spp</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fusarium oxysporum</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aspergillus niger</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lasidiodiplodia theobromae</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fusarium verticillum</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Natrassia mangiferae</td>
<td>20</td>
<td>20</td>
<td>25.000 ± 14.47</td>
</tr>
<tr>
<td></td>
<td>Aspergillus niger</td>
<td>30</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Fusarium oxysporum</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trichoderma spp</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Lasidiodiplodia theobromae</td>
<td>50</td>
<td>50</td>
<td>33.333 ± 14.43</td>
</tr>
<tr>
<td></td>
<td>Trichoderma spp</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Collectotrichum gleosporioides</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Fusarium oxysporum</td>
<td>25</td>
<td>25</td>
<td>33.333 ± 14.43</td>
</tr>
<tr>
<td></td>
<td>Lasidiodiplodia theobromae</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Trichoderma spp</td>
<td>25</td>
<td>25</td>
<td>33.333 ± 14.43</td>
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<tr>
<td></td>
<td>Fusarium verticillum</td>
<td>17</td>
<td>17</td>
<td>16.667 ± 11.67</td>
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<tr>
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<td>Natrassia mangiferae</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lasidiodiplodia theobromae</td>
<td>39</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trichoderma spp</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Macrophomina phaseolina</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fusarium oxysporum</td>
<td>8</td>
<td></td>
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</tr>
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</table>
Table 3b. Frequency of isolation (%) of fungal pathogen from herbicide treated soil and untreated at three seasons

<table>
<thead>
<tr>
<th>Second sampling</th>
<th>Treatments</th>
<th>Fungus</th>
<th>Frequency of occurrence</th>
<th>Mean ± Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Lasidiodiplodia theobromae</td>
<td>61</td>
<td>33.333 ± 25.42</td>
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</tr>
<tr>
<td></td>
<td>Natrassia mangiferae</td>
<td>28</td>
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<tr>
<td></td>
<td>Aspergillus niger</td>
<td>11</td>
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</tr>
<tr>
<td></td>
<td>Lasidiodiplodia theobromae</td>
<td>35</td>
<td>20.000 ± 10.61</td>
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</tr>
<tr>
<td></td>
<td>Macrophomina phaseolina</td>
<td>25</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Fusarium moniliforme</td>
<td>10</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Trichoderma spp</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fusarium oxysporum</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Lasidiodiplodia theobromae</td>
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<td>20.000 ± 12.79</td>
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</tr>
<tr>
<td></td>
<td>Fusarium verticillum</td>
<td>81</td>
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<td>Collectotrichum gleosporioides</td>
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<td>71</td>
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<td>Trichoderma spp</td>
<td>6</td>
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</tr>
<tr>
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<td>Aspergillus niger</td>
<td>33</td>
<td>33.333 ± 0.58</td>
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</tr>
<tr>
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<td></td>
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<tr>
<td></td>
<td>Lasidiodiplodia theobromae</td>
<td>34</td>
<td></td>
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<td>E</td>
<td>Fusarium oxysporum</td>
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<td>33.333 ± 16.50</td>
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<td></td>
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</tr>
<tr>
<td>F</td>
<td>Trichoderma spp</td>
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<td>Trichoderma spp</td>
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<tr>
<td></td>
<td>Lasidiodiplodia theobromae</td>
<td>33</td>
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</tr>
</tbody>
</table>

(2011) again reported stimulating effects on fungus growth of haloxyfop-methyl. In another experiment, 4-(4-Chloro-2-methylphenoxy) butanoic acid, Bentazon, 4-(4-Chloro-2-methylphenoxy) butanoic acid + Fluozifop-p-butyl, Bentazon+Flu-ozifop-p-butyl, Metribuzin, Flouzifop-pbutyl+Metribuzin, Cycloxydin, and Sethoxydin were shown to increase the population of soil fungi, 4 to 10
Table 3c. Frequency of isolation (%) of fungal pathogen from herbicide treated soil and untreated at three seasons

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fungus</th>
<th>Frequency of isolation</th>
<th>Mean ± Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Aspergillus niger</td>
<td>33</td>
<td>33.333 ± 0.58</td>
</tr>
<tr>
<td></td>
<td>Lasidiodiplodia theobromae</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Fusarium verticillum</td>
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</tr>
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<td>Aspergillus niger</td>
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<td>Fusarium oxysporum</td>
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<td></td>
<td>Natrassia mangiferae</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Aspergillus flavus</td>
<td>29</td>
<td>20.000 ± 8.22</td>
</tr>
<tr>
<td></td>
<td>Trichoderma spp</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lasidiodiplodia theobromae</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fusarium verticillum</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aspergillus niger</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Lasidiodiplodia theobromae</td>
<td>100</td>
<td>100.020 ± 0.01</td>
</tr>
<tr>
<td>E</td>
<td>Nil *</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Aspergillus niger</td>
<td>17</td>
<td>50.000 ± 46.67</td>
</tr>
<tr>
<td></td>
<td>Lasidiodiplodia theobromae</td>
<td>83</td>
<td></td>
</tr>
</tbody>
</table>

*Fungi not isolated*

times compared with the control (Ali et al., 2014). Although Nur Masirah et al. (2013) reported growth inhibitions of soil microorganisms treated with common herbicides in oil palm plantations in Malaysia. Herbicides have been found to affect microbes physiologically by changing their biosynthetic mechanism (a change in the level of protein biosynthesis is reflected on the ratio of extracellular and intracellular enzymes), by affecting protein biosynthesis (induction or repression of synthesis of certain enzymes), by affecting the cellular membranes (changes in transport and excretion processes), by affecting plant growth regulators (transport of indolacetic acid, gibberellin...
synthesis and ethylene level) or application in high doses which may kill the organisms (Milosevic et al., 2002). Even though the xtravest treatment (Treatment D) gave the second least number of genera of fungi at the end of the third quarter, it had the highest mean frequency of occurrence across all the treatments. It is pertinent to note that the control plot (Treatment F) had the second least number of fungi genera isolated at the end of the third sampling period.

CONCLUSION
In conclusion, the application of tested herbicides may not exclude the use of other forms of pesticides in the management of soil microorganisms on the farm. However, indiscriminate use of chemical pesticides should be avoided because of the long term effects on the environment. Soil microorganisms should be effectively managed using an integrated pest management approach to reduce their adverse effects on the different stages of crop production.

REFERENCES


Milicic, B. (1987): Transformacija S-triazinskih herbicida i njihov uticaj na mikrofloru u zemljištima. Doktorska disertacija, Poljoprivredni fakultet, Sarajevo. pg 78-84


