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RESEARCH ARTICLE

# EFFECT OF HERBICIDES ON RHIZOBACTERIA DENSITY IN EXPERIMENTAL FIELDS AND ON BACTERIAL STRAINS SURVIVAL *IN-VITRO*

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ABSTRACT

We evaluated six selected commercial herbicides commonly used for the control of several weeds in Daloa (Côte d'Ivoire), for their toxicity against density of rhizobacteria in experimental field conditions, and against survival of symbiotic and associative bacteria *in vitro*. Herbicide applied under dose recommended and reduced to 50 %, decreased the soil bacteria density from $2,83.10^{10}$ to $3,7.10^{6}$ $CFUg^{-1}$soil, respectively for control soil and spots treated. The loss of rhizobacterial population was estimated to be more than 36 % ($5,75.10^{3}$ $CFUg^{-1}$soil) after a week and a month of herbicides application. All six herbicides have shown differences concentrations in their toxicities on bacterial strains survival in culture medium. The inhibitory effects with more than 80 % were observed at concentrations within the range of at 13,5-27 µl/ml, 13,5-27 µl/ml, 27-54 µl/ml, 1,7-3,4 µl/ml respectively for Rapid max, Roundup, Bibana and Herbo select. But, Killer and Detru-herb were less toxic (40 %) even at label concentration and were considered suitable for field application.



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## INTRODUCTION

Soil is an important resource not only for agricultural production and food security but also towards maintenance of most life processes. It is considered as storehouse of microbial activity (Tilak *et al.,* 2005). Soil microorganisms like rhizobacteria, fungi, algae, protozoa, actinomycetes and some nematodes have a vital role in maintaining the soil health and productivity (Ali *et al*., 2014). Naturally, the diversity and community structure of microorganisms such us rhizobacteria in the rhizosphere are influenced by both plant and soil type (Latour *et al.,* 1996; Tilak *et al.,* 2005; Konate, 2007; Manimekalai *et al.,* 2015). However, current agricultural practices using mineral fertilizers (Umesh *et al.,* 2015) or chemical control like herbicides can considerably affect soils health and consequently the human, animal and plant health.

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Herbicides are commonly used in modern agriculture for the control of weeds in crops to increase yield and quality. Due to extensive and injudicious application, most of the unused fractions of herbicides act as pollutant can effect soil, groundwater and environment (Ahemad and Saghir, 2011; Ali *et al*., 2014). The plant protection measures with herbicides pose a potential threat to the survival and metabolic activities of rhizobacteria and consequently, could indirectly affect soil fertility (Ahemad *et al*., 2009; Ahemad and Saghir, 2011). Several research have shown some herbicides are toxic to soil microorganisms and may directly affect rhizobiums growth, survival, recognition of the host plant and nodule formation (Michiel *et al*., 1994; Singh and Wright, 1999; Anderson *et al*., 2004; Fox *et al*., 2004; Ali *et al*., 2014) but results are restricted to Mediterranean and South African conditions. No wide range study about the effects of herbicides on the survival and growth of rhizobacterial community in Côte d'Ivoire has been reported. The objective of this study was to evaluate the direct effect of six selected commercial herbicides commonly used in Daloa (Côte d'Ivoire) on (i) the rhizobacteria density in treated soil of experimental field, and (ii) the survival and growth of rhizobacteria, symbiotic and associative bacteria in culture medium *in vitro*.

## MATERIALS AND METHODS

### Bio-toxic effect of herbicides on rhizobacterial density in soils

The study was conducted in the experimental fields of the University Jean Lorougnon Guédé, Daloa, Côte d'Ivoire (6,91° N, -6,44° W). Herbicides tested were Killer 480SL (Glyphosate), Detru-herb 360SL (Glyphosate), Rapid max 750WG (Glyphosate), Roundup 360SL (Glyphosate), Bibana 680SG (Glyphosate) and Herbo select 108EC (Hexalofop-R-methyl). Plots were arranged in simple block system with 12 treated and 2 untreated control plots. The selected herbicides were applied at recommended dose and half (50 %) of the recommended rates to soil surface. Soil sample was collected 7 and 30 days after treatment from each plot to determine rhizobacterial density. Bacterial enumeration was carried out using the indirect method of Colony Forming Unit (CFU). 10 g of soil sample were dissolved in 100 ml of sterile water and then diluted several times. A volume of 10 µl (Vs) of each dilution was spotted on solid complete medium and incubated at 30 °C. Two repetitions (n=2) were performed for each dilution. The dilutions (D) generating a colony number (CN) between 30 and 300 cells were selected for bacterial enumeration according to the following formula:

$$CFU.ml^{-1} = \frac{\Sigma\, CN}{n \times Vs} D$$

The bacterial strains were tested further for their sensitivity to six herbicide according to protocol described by Khan et *al*. (2006) with a slight modifications using solid complete medium amended with these herbicides separately in order to sterile agar at following concentrations: Killer, 10-20 µl/ml; Detru-herb, 5-10 µl/ml; Rapid max, 13,5-27 µl/ml; Roundup, 13,5-27 µl/ml; Bibana, 27-54 µl/ml and Herbo select, 1,7-3,4 µl/ml. After that, plates were spot inoculated with 10 µl of heavy concentration of each strain used. Each experiment was replicated two times and incubated at 30 °C for 72 hours.

## RESULTS

### Bio-toxic effect of herbicides on rhizobacterial density in soils

The half and normal recommended dose of six herbicides tested showed a significant decrease on bacterial density in the treated plots. 7 days after herbicides applications, the bacterial enumeration performed on the solid complete medium in presence of 50 % of recommended dose of each herbicide, registered a density of $2,83.10^{10}$ $CFUg^{-1}$soil for control soil and much lower densities 3,7 to $5,6.10^{6}$ $UFCg^{-1}$soil, respectively for treated soil with Killer and Herbo select. Hence, important loss of bacterial population ranging from 35, 41 to 37, 13 % was observed (Table 1, A1). Compared with control spot, this loss is slightly increased when the recommended dose is used for each herbicide with a gap of 35, 98 to 37, 51 % of population number (Table 1, B1). Furthermore, the analysis performed after a month of herbicides applications, showed persistence of toxic effects of the tested herbicides in soils reducing soil rhizobacteria density. Indeed, the latter results were very similar to the previous with slight positive changes in bacterial population in treated plots with Killer (Table 1. A2, B2).

**Table 1. Variation of the soil bacteria number (BN) and the loss of bacteria population in control soil (CS) and spots treated with herbicides: Killer 480SL (Ki), Detru-herb 360SL (D.H), Rapid max 750WG (R.M), Roundup 360SL (Ro), Bibana 680SG (Bi) and Herbo select 108EC (H.S). The results registered after a week and a month of application with half (A1, 2) and recommended dose (B1, 2)**

| | Treatment | CS | Ki | DH | R.M | Ro | Bi | H.S |
|---|---|---|---|---|---|---|---|---|
| | 50 % of dose | - | 3 L/Ha | 1.5 L/Ha | 1 kg/Ha | 4 L/Ha | 2 kg/Ha | 0.5 L/Ha |
| | BN ($UFC/g^{-1}$soil) | $2,83.10^{10}$ | $3,7.10^{6}$ | $5,2.10^{6}$ | $4,55.10^{6}$ | $4,88.10^{6}$ | $3,87.10^{6}$ | $5,6.10^{6}$ |
| A1 | Log (BN) | 10,45 | 6,57 | 6,71 | 6,66 | 6,69 | 6,59 | 6,75 |
| Week | Loss (%) | - | 37,13 | 35,79 | 36,27 | 35,98 | 36,94 | 35,41 |
| A2 | BN ($UFC/g^{-1}$soil | $2,64.10^{10}$ | $9,4.10^{6}$ | $4,32.10^{6}$ | $3,82.10^{6}$ | $3,63.10^{6}$ | $2,54.10^{6}$ | 3.106 |
| Month | Log (BN) | 10,42 | 6,97 | 6,64 | 6,58 | 6,56 | 6,4 | 6,48 |
| | Loss (%) | - | 33,11 | 36,17 | 36,85 | 37,04 | 38,58 | 37,81 |
| | Normal dose | - | 6 L/Ha | 3 L/Ha | 2 kg/Ha | 8 L/Ha | 4 kg/Ha | 1 L/Ha |
| | BN ($UFC/g^{-1}$soil) | $2,83.10^{10}$ | $3,95.10^{6}$ | $4,45.10^{6}$ | $3,75.10^{6}$ | $3,38.10^{6}$ | $4,13.10^{6}$ | $4,9.10^{6}$ |
| B1 | Log (BN) | 10,45 | 6,6 | 6,65 | 6,57 | 6,53 | 6,62 | 6,69 |
| Week | Loss (%) | - | 36,84 | 36,36 | 37,13 | 37,51 | 36,65 | 35,98 |
| B2 | BN ($UFC/g^{-1}$soil | $2,64.10^{10}$ | $8.10^{6}$ | $4,03.10^{6}$ | $3,1.10^{6}$ | $2,7.10^{6}$ | $3,8.10^{6}$ | $3,49.10^{6}$ |
| Month | Log (BN) | 10,42 | 6,9 | 6,6 | 6,49 | 6,43 | 6,58 | 6,54 |
| | Loss (%) | - | 33,78 | 36,95 | 37,72 | 38,29 | 36,85 | 37,24 |

### Bacterial strains and herbicide sensitivity/tolerance

We used in this test 15 rhizobacteria (RB) isolated from untreated control plots, 10 associative bacteria (BIRC) of cacao and 4 symbiotic bacteria (RVC) of *Vigna subterranea* provided by Laboratory of Host-Microorganism-Environment Interactions and Evolution (LIHME) at Jean Lorougnon Guédé University and 6 reference strains belong to *Rhizobium*, *Sinorhizobium* and *Mesorhizobium* provided by Laboratory of Microbiology and Molecular Biology at Faculty of Sciences, University Mohammed V-Agdal, Rabat, Morocco.

### Tolerance/sensitivity of bacteria strains to herbicides

Herbicides tested may manifest their bio-toxic effect trough loss of survival or growth of bacteria strains. Herbicide sensibility/tolerance of the strains varies depending on the dose, herbicide type and niche of the strains (Table 2). Thus, herbicides Rapid max, Roundup, Bibana and Herbo select were very bio-toxic affecting the strains survival of more than 80 %. But, Killer and Detru-herb were less toxic on the strains survival even at a higher concentration of 10-20 µl/ml with over 40 % of tolerance (Fig 1).

**Table 2. Tolerance/sensitivity of rhizobacteria (RB), cacao associative (BIRC) and legume symbiotic bacteria (RVC and reference Rhizobia) to herbicides present in culture medium. Herbicides used were Killer 480SL, Detru-herb 360SL, Rapid max 750WG, Roundup 360SL, Bibana 680SG and Herbo select 108EC**

| Herbicides | Killer (µl/ml) | | Detru Herb (µl/ml) | | Rapid Max (µl/ml) | | Roundup (µl/ml) | | Bibana (µl/ml) | | Herbo Select (µl/ml) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strains | 10 | 20 | 5 | 10 | 13.5 | 27 | 13.5 | 27 | 27 | 54 | 1.7 | 3.4 |
| RB1 | + | - | + | + | - | - | - | - | - | - | - | - |
| RB2 | + | - | + | - | - | - | + | + | + | - | - | - |
| RB3 | - | - | + | - | - | - | - | - | - | - | - | - |
| RB 4 | + | + | + | + | + | + | - | - | + | + | + | + |
| RB 5 | + | - | + | - | - | - | - | - | - | - | - | - |
| RB 6 | + | - | + | + | - | - | - | - | - | - | + | + |
| RB 7 | + | - | + | - | - | - | - | - | - | - | - | - |
| RB 8 | + | - | + | + | - | - | - | - | - | - | + | - |
| RB 9 | + | - | + | - | - | - | - | - | - | - | - | - |
| RB 10 | - | - | + | - | - | - | - | - | - | - | - | - |
| RB 11 | + | - | + | + | - | - | - | - | - | - | - | - |
| RB 12 | - | - | - | - | - | - | - | - | - | - | - | - |
| RB 13 | - | - | - | - | + | - | - | - | - | - | - | - |
| RB 14 | + | - | + | - | - | - | - | - | - | - | - | - |
| RB 15 | + | - | + | + | + | - | - | - | - | - | + | + |
| RVC1 | + | + | + | + | + | + | - | - | + | + | + | + |
| RVC2 | + | + | + | + | + | - | - | - | + | - | + | + |
| RVC 3 | + | - | + | + | + | - | - | - | - | - | + | + |
| RVC 4 | + | + | + | + | + | + | + | - | + | + | + | + |
| L01 | + | + | + | + | + | + | - | - | + | + | + | + |
| H 152 | + | - | + | + | - | - | - | - | - | - | + | + |
| LO21 | + | + | + | + | - | - | - | - | + | + | + | + |
| CFN | - | - | - | - | - | - | - | - | - | - | - | - |
| H 132 | + | + | + | + | - | - | - | - | - | - | + | + |
| STM | + | + | + | + | + | + | - | - | + | + | + | + |
| BIRC 1 | + | - | + | + | - | - | - | - | - | - | + | - |
| BIRC 2 | + | - | + | + | - | - | - | - | - | - | - | - |
| BIRC 3 | + | + | + | + | + | - | - | - | - | - | + | + |
| BIRC 4 | + | - | + | + | + | - | - | - | - | - | + | + |
| BIRC 5 | + | - | + | + | - | - | - | - | - | - | - | - |
| BIRC 7 | + | - | + | + | + | + | - | - | - | - | - | - |
| BIRC 9 | + | + | + | + | + | - | - | - | - | - | - | - |
| BIRC 13 | + | + | + | + | + | + | - | - | - | - | - | - |
| BIRC 17 | + | - | + | - | - | - | - | - | - | - | - | - |
| BIRC 22 | + | - | + | + | + | + | + | + | - | - | + | + |

(+) = Resistance (-) = Sensible

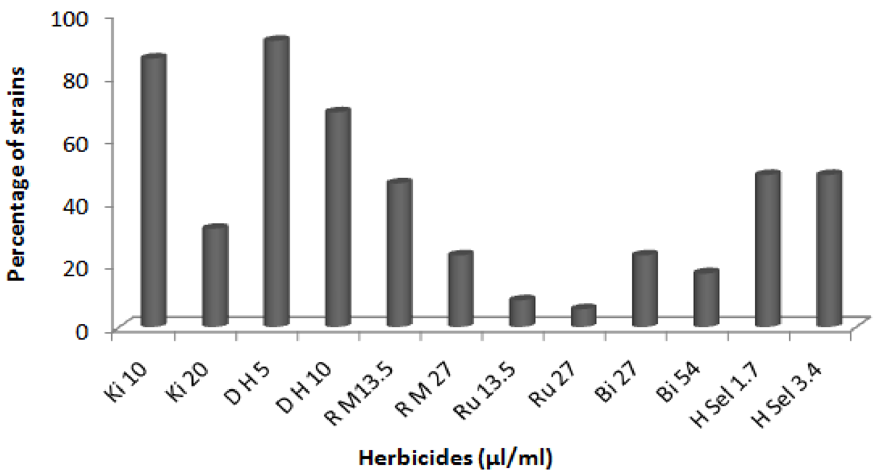


**Figure 1. Tolerance of bacteria strains to different doses of herbicides**

Symbiotic bacteria (RVC and reference strains of Rhizobia) have exhibited a wide tolerance to the herbicides tested except Roundup that impeded the survival of all strains at a concentration of 27 µl / ml (Fig. 2). Associative bacteria (BIRC) isolated from *Theobroma cacao* tree were sensitive to the presence of the selected herbicides (over 70 % of strains), except Killer and Detru-herb including at 50 % of label dose with 100 % of tolerance (Fig. 3).

However, rhizobacteria (RB) isolated from the experimental field have exhibited slight tolerance to different doses of six herbicides used with 60 to 95 % of sensitivity (Fig. 4). Moreover, strains such as $RB_4$, $RCV_1$, $RVC_2$, $RVC_4$, *Rhizobium leguminisarum* ($L_{01}$), *Mesorhizobium* sp ($STM_{183}$) and $BIRC_{22}$ were characterized showing a character of multiple herbicide resistance.

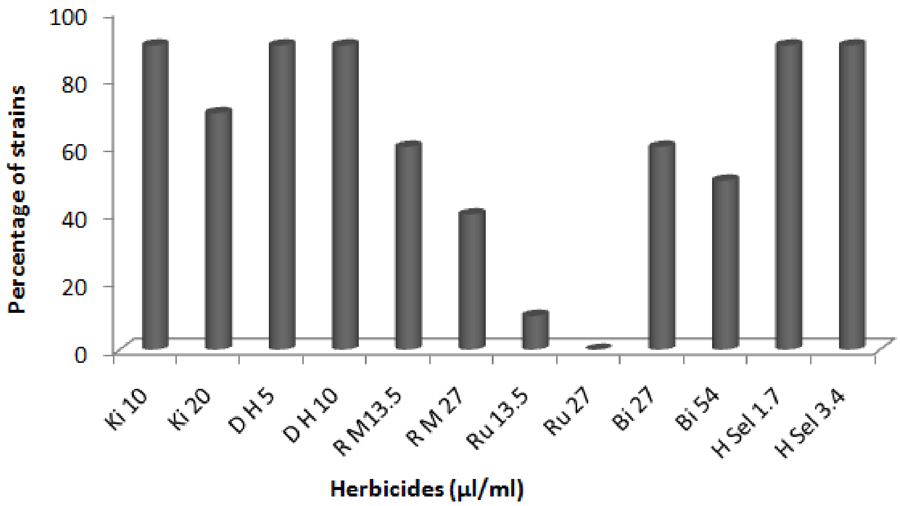


**Figure 2. Tolerance of symbiotic bacteria of *Vigna s*. (RVC) and reference strains of Rhizobia to herbicides**

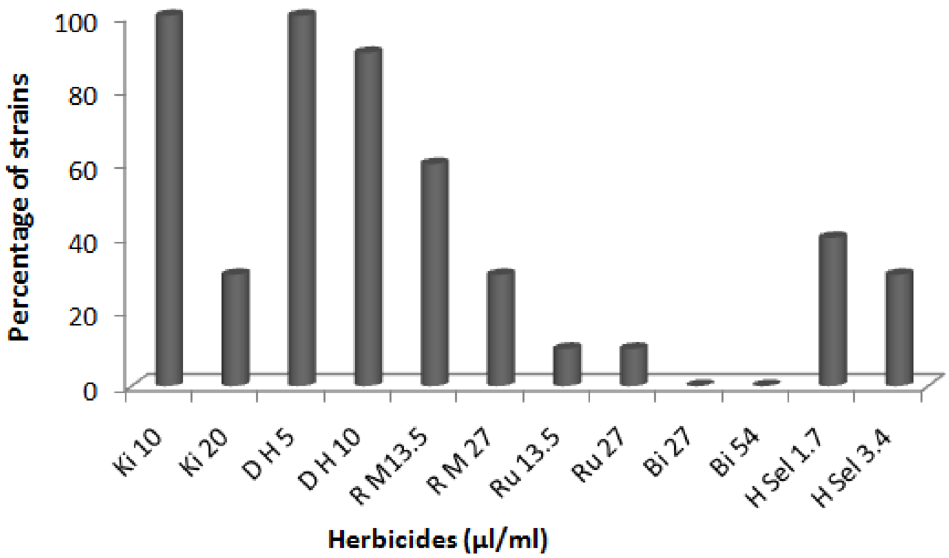


**Figure 3. Tolerance of associative bacteria (BIRC) isolated from cacao tree to herbicides**

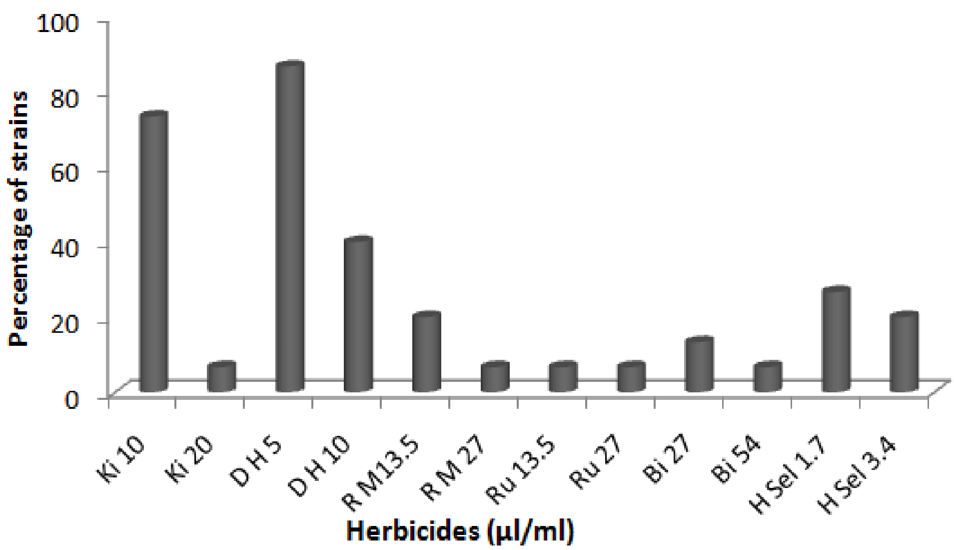


**Figure 4. Tolerance of rhizobacteria (RB) isolated from experimental field to herbicides**

## DISCUSSION

The herbicides are used for weeds control in order to increase agricultural production and quality. However, its toxic action is not limited only to target plants but also affects the survival and diversity of soil microbial populations composing mostly by bacteria. Our results clearly showed the eco-toxicological effects of the six selected herbicides commonly used in Daloa (Côte d'Ivoire) on soil fertility by persistent and progressive decrease in rhizobacteria density. Soil application with Rapid max, Roundup, Bibana, Herbo select, Killer and Detru-herb caused the loss of about 36 % of bacterial populations as observed in this study. These results showed similar finding with research reported by others authors (Bopaiah and Rai, 1979; Quinn *et al.,* 1988). Herbicides are toxic and could pose a potential damage to the survival and metabolic activities on soil microflora such us benefic rhizobacteria and consequently affect soil fertility and induce the spread in plant or animal pathogens (Anderson *et al.,* 2004; Durgo *et al.* 2008; Ahemad *et al.,* 2009; Ali *et al.,* 2014).

The addition of these herbicides on the culture medium revealed a variable toxicity on the survival of strains used. Symbiotic and associative bacteria exhibited respectively high and moderate tolerance (50 to 90 %) to different herbicides. However, rhizobacteria showed slight tolerance to all herbicides. These results are consistent with the reports of some researchers, who demonstrated the tolerance of Rhizobia to a wide range of herbicides (Jansen van Rensburg and Strijdom, 1984; Singh and Wright 2002; Patil *et al.,* 2012). This work allowed to classify herbicides tested in three distinguished categories. The 1st category of high biological and ecological toxicity is composed of Bibana and Roundup. The 2sd category of moderate eco-toxicity is composed of Rapid max and Herbo select. The 3rd group of very low eco-toxicity consists of Killer and Detru-herb, and is considered relatively suitable for treatment of agricultural fields.

### Conclusion

Herbicides toxicity to soil microorganisms can alter their community structure and function. Thus, herbicides have previously to be selected taking into count their effect on soil micoflora, soil health, soil fertility, plant growth, symbiotic efficiency and nitrogen activity according to the agro-forestry ecosystem conditions of each region.

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