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Influence of Herbicides on Symbiotic Bacteria Survival, Growth and Nodulation of Legume Niébé (*Phaseolus vulgaris*) in Center-West of Côte d'Ivoire

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Abstract **– We studied the influence of six selected Herbicides on the survival of rhizobia *in vitro* and on the growth and nodulation of legume Niébé (*Phaseolus vulgaris*) under field conditions in Daloa (Center-West of Côte d'Ivoire). 14 strains isolated from nodules of common bean differed significantly (P <0.01) in their tolerance towards the rates of the six herbicides added in YEM medium. Roundup, Bibana and Rapid max had adverse effect on rhizobia survival. Whereas, Killer, Herbo Select and Detru Herb at 50 % and recommended rates were moderately toxic to rhizobia and above 40 % of strains grew well at the label concentrations. The average number and mass of nodules formed varied significantly (P < 0.01). At 60 DAS, the higher values of nodule from control plots were observed with the means of 23 nodules.plant^{-1}, 393 and 40 mg.plant^{-1} respectively for weight of fresh and dry nodules, but the herbicides treatment decreased dramatically those parameters (8 to 17 nodules.plant^{-1}, 8.4 to 40 and 1.63 to 11.6 mg.plant^{-1} for weight of fresh dry nodules). At normal dose of herbicides, the plant growth analyzed through the height, stem circumference, folio length, leaf surface and biomass of plant was significantly and adversely affected (P < 0.01). However, Killer, Detru Herb, Herbo Selec and Rapid Max at 50 % of label rate had safe toxicity to the both symbiosis partners Rhizobia - *Phaseolus vulgaris* and considered relatively suitable for treatment of agricultural fields.**

Keywords **– Herbicides, Rhizobia, Niébé (*Phaseolus Vulgaris*), Tolerance, Survival, Growth.**

I. INTRODUCTION

Face to high growth of the world populations and continual demand of food supply, several countries oriented their agricultural policy to legume products such as soybean, greengram, pea, chickpea, groundnuts, common beans, [6, 10 and 12]. Thus, one of the most practiced strategies to increase the agricultural productivity and to maintain the soil fertility is the cultivation of annuals legumes either alone or in a rotation cropping [2, 14 and 15].

Legume common bean or called Niébé (*P. vulgaris*) is considered to be an important component of vegetation for environmental, economic and social reasons. In Côte d'Ivoire, it is produced in all areas notably for household need and considered as food or the 'meat of the poor', due to its low cost relative to animal products. Additionally, Niébé constitute one of the main source of plant protein and provides a rich combination of carbohydrates (60-65 %), proteins (21-25 %), fats (less than 2 %), vitamins and minerals [11] and used as an important plant medicine for treatment of many diseases such as diabetes [7, 22 and 25].

Legumes are the most important group of plants playing a key role in the sustaining and increasing N pool of soil by forming symbiosis nodules with rhizobia which fix and transform atmospheric N_2 into other compounds utilizable by host plants [17 and 29]. Rhizobia-legume symbiosis is the primary source of fixed nitrogen in land based systems and can provide well over half of the biological source of fixed nitrogen [32, 35 and 38]. However, the plant protection measures with herbicides pose a potential menace to the survival of various benefic rhizobial strains, to the nodulation, to the symbiotic efficiency and to the soil fertility [5, 14, 21 and 27].

The extensive use of herbicides under field conditions to weeds control may be detrimental to both legumes and its symbiotic partner [18 and 38]. Several studies showed the toxic effects of various herbicides on legume and revealed that their toxic effects depends primarily on the type and dose of compound, duration of exposure, species and age

of plants and other environmental factors [38]. In fact, the soils applied of pesticides had been identified as national problem requiring research into the effect of herbicides on various biological systems in the field [19].

There is limited information in the literature on herbicides effects on symbiosis establishment and growth of common bean (*P.vulgaris*) in West Africa and particularly in Côte d'Ivoire. Thus, in this study we aim to report the influence of some herbicides commonly used in Côte d'Ivoire agricultural systems with legumes (i) on the survival of native rhizobia *in vitro* and (ii) on the nodulation and growth of Niébé (*P.vulgaris*) in field conditions.

II. Materials and Methods

The research was conducted in the experimental fields of the Jean Lorougnon Guede University, Daloa (Center-West of Côte d'Ivoire).

2. 1. Treatment with Herbicides

Herbicides tested were Glyphosate [Killer 480SL, Detru-herb 360SL, Rapid max 750WG, Roundup 360SL, Bibana 680SG] and Haloxyfop-R-methyl [Herbo select 108EC]. Plots were arranged in randomized block system with two replications. The selected herbicides were applied at recommended and half (50 %) of rate doses to soil surface using a backpack sprayer delivering a volume of approximately 300 L/ha. All herbicides contained Ghyphosate were applied to soil surface at post emergence 7 days before common bean sowing and Haloxyfop-R-methyl (Herbo select 108EC) was applied at pre-emergence 14 days after sowing (DAS).

2. 2. Tolerance of common bean symbiotic bacteria to herbicides

A total of 14 native rhizobial strains were isolated from nodules of Niébé (*P. vulgaris*) plant cultivated in the untreated control plots. The isolation was realized on YEM agar medium [36]. The symbiotic bacteria were tested further for their tolerance/sensitivity to six herbicides according to protocol described by Khan *et al.* [18] with a slight modifications [21]. The freshly sterilized solid complete mediums were separately amended with various herbicides at following concentrations: Killer, 10 and 20 µl/ml; Detru-herb, 5 and 10 µl/ml; Rapid max, 13,5 and 27 µl/ml; Roundup, 13,5 and 27 µl/ml; Bibana, 27 and 54 µl/ml and Herbo select, 1,7 and 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.

2. 3. Effect of selected herbicides on plant growth and nodulation

To evaluate the plants growth parameters (height, stem circumference, folio length and leafy surface), Ten plants were randomly sampled from each plot at 60 days after seeding (DAS).

At 30, 45 and 60 DAS, the plants were randomly harvested from each plot to determine the toxic effect of herbicides on the nodulation of Niébé. The number and fresh weights of nodules were recorded and the dry weight was taken after incubation at 70 °C for 48 h. The weight of both shoots and roots dried were also registered.

2. 4. Statistical analysis

The data of measured parameters recorded were pooled together and subjected to statistical analysis using the STATISTICA program (7.1). The strain tolerance was subjected of Chi^2 of Pearson and plant growth parameters were subjected by analysis of variance method. The difference between the herbicides treatments means were evaluated at the 5 % level of significance using Fisher's LSD test.

III. Results

3. 1. Tolerance of native symbiotic strains

Six selected herbicides tested *in vitro* affected considerably the survival of the 14 *Rhizobium spp* strains (coded RHC) isolated from nodules of common bean (*Phaseolus vulgaris*) cultivated under field conditions at Jean Lorougnon Guede University in Daloa (Center-West of Côte d'Ivoire). The strains differed significantly ($P < 0.01$) in their tolerance towards the rate of herbicides added in solid complete medium (Table 1). The herbicides Roundup, Bibana and Rapid max had adverse toxic effect on the survival of rhizobia strains and none of them survived in the presence of lower concentrations. Whereas, Killer, Herbo Select and Detru Herb at the both rate (half and recommended) were moderately toxic to rhizobia and above 40 % of strains grew well at the recommended concentrations (Fig. 1).

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Table 1: Tolerance of common bean symbiotic bacteria to six herbicides tested in culture medium: 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**
RHC 1	+	+	+	+	-	-	-	-	-	-	+	+
RHC 2	+	+	+	+	-	-	-	-	-	-	-	-
RHC 3	-	-	-	-	-	-	-	-	-	-	-	-
RHC 4	+	-	+	+	-	-	-	-	-	-	-	-
RHC 5	-	-	-	-	-	-	-	-	-	-	-	-
RHC 6	-	-	-	-	-	-	-	-	-	-	-	-
RHC 7	+	-	+	+	-	-	-	-	-	-	+	+
RHC 8	-	-	-	-	-	-	-	-	-	-	+	+
RHC 9	-	-	+	+	-	-	-	-	-	-	+	+
RHC 10	-	-	-	+	-	-	-	-	-	-	+	+
RHC 11	+	-	+	-	-	-	-	-	-	-	-	-
RHC 12	+	-	+	-	-	-	-	-	-	-	-	-
RHC 13	+	-	+	+	-	-	-	-	-	-	+	+
RHC 14	+	-	+	-	-	-	-	-	-	-	-	-

(+) = Tolerant, (-) = Sensible

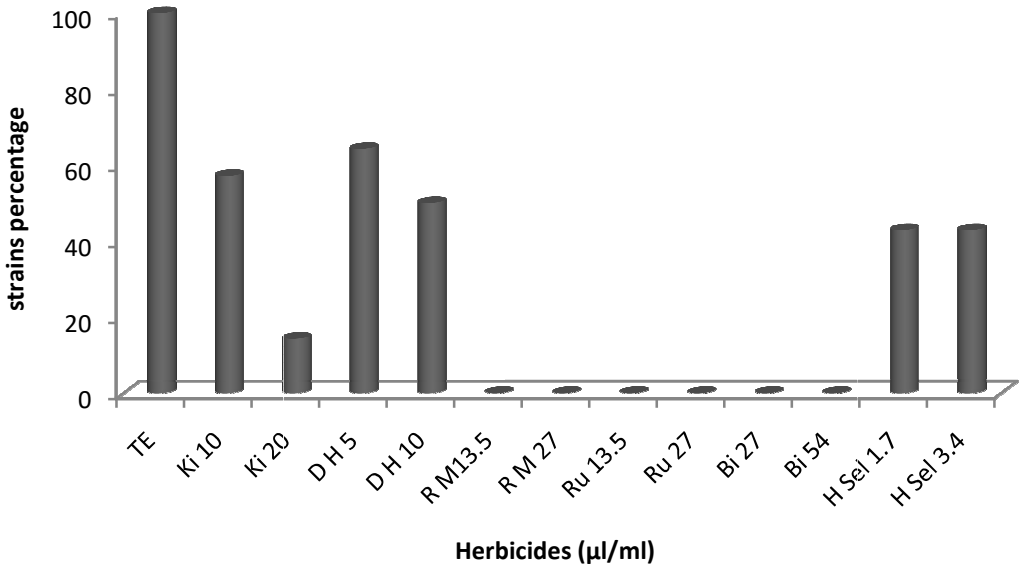

Fig.1. Tolerance of symbiotic bacteria strains to different doses of herbicides

3. 2. Nodulation of Niébé

The data showed that average number and mass of nodules formed in a subsequent experiment by Niébé plant varied significantly ($P < 0.01$) throughout different herbicides treatments. In the untreated control plots, the number, fresh and dry weight of nodules increased with Niébé plant age up to the beginning of flowering stage (Table 2). The higher values of nodule from control plots were observed at 60 DAS with the means of 23 nodules.plant^{-1}, 393 and 40 mg.plant^{-1} respectively for weight of fresh dry nodules.

However, all Herbicides treatments decreased significantly the number, fresh and dry weight of nodules comparing to control samples at all stage of common bean growth. The application of recommended and half (50 %) of herbicides rates to soil surface reduced the number of nodules on common bean. At 60 DAS and comparatively to control plots, the treatment with Killer (5 µl.ml^{-1}), Detru Herb (10 µl.ml^{-1}) and Rapid max (13.5 µl.ml^{-1}) revealed a moderate decrease of nodule but significant ($P < 0.01$). In contrast, Rapid max (27 µl.ml^{-1}) and all doses of Roundup (13.5 and 27 µl.ml^{-1}), Bibana (27 and 54 µl.ml^{-1}) and Herbo Select (1.7 and 3.4 µl.ml^{-1}) dramatically decreased the number of nodules per plant at 30, 45 and 60 DAS (Table 2).

Table 2: Effect of recommended and half of dose of killer, Detru Herb, Rapid Max, Roundup, Bibana and Herbo Select on Common bean nodulation. Data registered at 30, 45 and 60 days after sowing (DAS).

Herbicides	Dose (µl.ml^{-1})	Nodules number.Plant^{-1}			fresh weight (mg.plant^{-1})			Dry weight (mg.plant^{-1})		
		30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
Killer	10	11b	14b	17b	20	40	45bc	4.9b	8.8b	10.4cd
	20	10	12c	14c	16	20c	40c	3	5	8.4e
Detru Herb	5	11b	14b	17b	30	32	49.6b	5b	10.4	11bc
	10	8c	10de	15c	6.1bc	20c	42c	1c	3.3c	9.4de
Rapid Max	13.5	11b	13bc	15c	7b	30b	60.4	1c	6.4	11.6b
	27	9	10de	10ef	2f	30b	33	0.2f	3c	8.3e
Roundup	13.5	8c	11d	12d	10	18.7c	43.7bc	0.94cd	8.3b	10cd
	27	4	9e	11de	4de	8d	18.2d	0.3f	1d	9.6d
Bibana	27	8c	9e	10ef	4.7cd	10.3	16.1de	0.9cde	8.5b	9.7d
	54	6	7f	9g	4.2de	7de	8.4e	0.5cdef	0.95d	5.1f
Herbo Select	1.7	5d	7f	12d	3.82de	6.7de	10de	0.41def	1d	5.3f
	3.4	5d	6f	8g	2.89ef	5.6e	8.82e	0.36ef	0.93d	1.63
Control		19	20	23	110	130	393	19	30	40
LSD (0.05)		**0.7**	**1.51**	**1**	**1.4**	**1.4**	**7.7**	**0.53**	**0.5**	**1**

Each value represents the mean of two replicates. Mean values within each column followed by the same letter are not significantly different from each other at 5 % level of probability according to LSD test.

Table 3: Effect of different doses of killer, Detru Herb, Rapid Max, Roundup, Bibana and Herbo Select on Common bean growth at flowering (60 DAS)

Plant Parameters	Control	Killer		Detru Herb		Rapid Max		Roundup		Bibana		Herbo Select		Test	
		10	20	5	10	13.5	27	14	27	27	54	1.7	3.4	**P**	**LSD**
plant height (cm)	51	43.5 bc	35.5 fg	42.5 cd	35 g	42 cd	37 f	40 e	32h	44.5b	35.4g	41.5 de	32h	**<0.01**	**1.5**
Stem circumference (cm^2)	3.71	3.1 b	2.7 ef	3.05 bc	2.65 ef	2.9 cd	2.8 de	2.62 f	2.3	2.91cd	2.6 f	2.91 cd	2.6f	**<0.01**	**0.15**
Leaves number/plant	53	40b	26d	38c	27.2 d	35	26 d	38c	26d	46	41b	32	30	**<0.01**	**1.2**
foliar surface (cm^2)	59.87	48.7	44.7	57.3	53.4	37.5	30.1	40.1b	37	41.8	40	40.7	33	**<0.01**	**0.1**
petiole length	13.8	10b	8.2cd	12	10.1 b	8.02 de	7.79d	8de	6.4	8.1 de	8.1 de	8.5c	8.2cd	**<0.01**	**0.3**

Each value represents the mean of two replicates. Mean values within each column followed by the same letter are not significantly different from each other at 5% level of probability according to LSD test

3. 3. Plant growth and biomass

All herbicides tested in this study affected and reduced significantly ($P < 0.01$) the height, the stem circumference, the folio length and the leaf surface of Niébé cultivated in field conditions. However, the toxic effects of those pesticides at 50 % of the recommended rate on the plant height were moderate except Roundup. At flowering, the stem circumference, foliar surface and petiole length were rather affected by both Killer and Detru Herb at half of rate but the recommended dose of herbicides had adverse inhibition on foliar surface and the petiole length. All rates of herbicides decreased considerably the leaves number except Rapid Max at 50 % of recommended rate (Table 3). The biomass production of common bean plant (*Phaseolus vulgaris*) decreased significantly ($P < 0.01$) after 30 and 45 DAS of the treatment with six selected herbicides. Especially at 60 DAS, the biomass of the two organs, roots and shots, were adversely inhibited by the different doses of the herbicides used except Killer, Detru Herb and Rapid Max that had safe toxicity at the half of recommended rate (Table 4).

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Table 4: Effect of different rates of killer, Detru Herb, Rapid Max, Roundup, Bibana and Herbo Select on Common bean biomass product 30, 45 and 60 days after sowing.

Herbicides	Dose (µl/ml)	30 DAS			45 DAS			60 DAS		
		Root (g)	Shoot (g)	Biom T (g)	Root (g)	Shoot (g)	Biom T (g)	Root (g)	Shoot (g)	Biom T (g)
Killer	10	0.29cd	3.14	**3.43**	0.52b	4.82	**5.34**	0.93a	6.79	7.72*a
	20	0.12	2.64b	2.76	0.44e	4.13b	4.59b	0.7	5.5	6.2
Detru Herb	5	0.35b	1.67d	1.82	0.29f	3.48c	3.77c	1.12a	6.55b	7.67*a
	10	0.15f	1.33	1.68c	0.25	2.05	2.3f	1.08a	5.92	7
Rapid Max	13.5	0.27d	1.1e	1.37	0.46de	3.37cd	3.83c	0.83c	6.56b	7.39*a
	27	0.19e	1e	1.19	0.28f	2.14f	2.42f	0.76d	4.3	5.06c
Roundup	13.5	0.2e	2.17c	2.37	0.51bc	3.97b	4.48b	0.77d	4.95c	5.71b
	27	0.15f	1.47	1.62c	0.35	2.51ef	2.86e	0.34	3.27	3.61
Bibana	27	0.27d	2.73b	3b	0.49cd	3.9b	4.36b	0.95a	5.67	6.6
	54	0.18e	2.73b	2.91b	0.45e	2.18f	2.67e	0.58	4.54d	5.12c
Herbo Select	1.7	0.37b	2.16c	2.53	0.64	3.15d	3.61cd	0.92a	4.86c	5.78b
	3.4	0.31c	1.68d	1.99	0.46de	2.69e	3.43d	0.65	4.53d	5.18c
Control		0.6	4.79	5.39	0.74	5.27	5.91	0.86c	6.97	7.83
LSD (0.05)		**0.02**	**0.1**	**0.09**	**0.03**	**0.44**	**0.23**	**0.04**	**0.09**	**0.44**

Each value represents the mean of two replicates. *Indicates not significant difference from control at $P \leq 0.05$. Mean values within each column followed by the same letter are not significantly different from each other at 5 % level of probability according to LSD test

IV. Discussion

4. 1. Tolerance of native symbiotic strains

The present data showed differences in the toxicity of the various herbicides to the 14 symbiotic strains isolated from nodules of common bean (*Phaseolus vulgaris*) and tested *in vitro*. No strain survived in the presence of Roundup, Bibana and Rapid max while Killer at the half recommended rate. Herbo Select and Detru Herb at the both (half and recommended) rates affected moderately the growth of the stains. Several authors demonstrated the various sensibilities of Rhizobacteria such as rhizobia to a wide range of herbicides [16, 21, 28 and 33]. However, others research has shown that the effect of herbicides on the survival and growth of rhizobia can be quite different depending upon the methods used [24 and 18].

4. 2. Plant nodulation, growth and biomass

The results of this study showed clearly that toxicity of the six herbicides tested on plant growth parameters and nodulation varies widely and are the most damaging to both *Rhizobium* and legume. Generally at 50 % of recommended dose, Killer and Detru Herb had little toxic effect on the growth, the biomass and nodulation of the plant. Herbicides therefore affect the viability of rhizobia and thus affect the mechanism involved in *Bradyrhizobium* – greengram symbiosis [38]. Since number of nodules indicates the number of infection sites by rhizobia, it can thus be concluded that availability and infectivity of rhizobia are not adversely affected by lesser concentration of herbicides [19]. Konate *et al.* [21] identified those two herbicides for their lowest eco-toxicity and considered them as relatively suitable for treatment of agricultural fields.

These phenomena, therefore, suggest that some herbicides or the lower rates might have persisted in the soil for few duration and their rapid mineralization, after which the viable cells of rhizobium strains recovered and multiplied rapidly leading thereby to the establishment of an effective symbiosis with the host plant [2, 8, 18, 30 and 34].

The reduction observed in growth and biomass of common bean (*Phaseolus vulgaris*) plant after herbicide applications could be due to the adverse effects of glyphosate and haloxyfop-R-methyl on plant organs, especially the function of nodules, which, consequently, diminishes N_2 fixation [3]. Various studies have shown that herbicides may inhibit the plant nodulation [9, 13, 23 and 37] and affect differently the growth and biomass of legume plants [4, 26, 31 and 38]. Such inhibitory effect following herbicide applications might possibly be due to the inhibition of enzymes involved in growth and metabolisms [37] or due to disruption of signaling between legume (host) plant-derived phytochemicals and *Rhizobium* Nod D receptors that is necessary for initiation of nodulation and N_2 fixation [13]. Most researchers attribute also the inhibitory effect of pesticides on plant growth to the suppression of growth-promoting micro-organisms in the rhizosphere [1 and 20].

V. Conclusion

Herbicides are used in modern agriculture for weeds control and to increase the crops yield and quality.

However, they have previously to be selected taking into account their eco-toxicological effect. This study allow us to select Killer, Detru Herb, Herbo Selec and Rapid Max for their safe effect on the both symbiosis partners Rhizobia - *Phaseolus vulgaris* and may be relatively suitable for treatment of agricultural fields at lower concentrations.

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