

AGRO-MORPHOLOGICAL RESPONSE OF CORN (*Zea mays* L.) TO A BIOSTIMULANT FROM THE SOUTHEASTERN ALGERIA

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Abstract: This study highlights the effect of a biostimulant based on *Trichoderma*, indigenous antagonistic fungus from the region of Touggourt, on the development of a local variety of corn. This study was conducted at the experimental station of National Institute of Agronomic Research (Sidi Mehdi, Touggourt) as a complete randomize block design. The examined agro-morphological parameters showed that the application of this bioproduct stimulates vegetative growth of the plant. Besides, better germination and earliness of seeds. The results showed more interesting values were obtained in the treated plots (height = 135.9 cm, diameter = 28.9 mm, surface of leaves = 318 cm², Pan = 10.2 panicles/plant) compared to those of the controls (height = 98.6 cm, diameter = 23.7 mm, surface of leaves = 260.4 cm², Pan = 7.4 panicles/plant). Statistical analyzes confirmed the existence of a very highly significant difference between treated and control subjects.

Keyword: Biostimulant, *Trichoderma*, corn, vegetative growth, Touggourt, Algeria.

I. INTRODUCTION

In Algeria, corn production has dropped considerably with values of 3687 and 2634 tons recorded respectively in 2016 and 2017 [1]. This decrease is due to the reduction of devoted areas of crop which are about 817 ha and 658 ha in the same period [1]. The improvement of food security, which implies, among other things, the increase in corn production, comes up against production constraints such as falling in soil fertility, lower adaptability of genotypes to climates, irregularity of rains, diseases, soil acidity and particularly aluminum toxicity [2]. Organic farming is a mode of production limited by a regulation that prohibits the use of synthetic chemical products (fertilizers, pesticides, etc...) and encourages the use of physical and biological means [3,4].

In addition, intensive use of chemicals in agriculture is leading to biological soil depletion, groundwater pollution and development of resistance of plant pathogens and pests. To move towards a more sustainable mode of production, two categories of micro-organisms are of interest, present in the soil and can be applied at the level of the root system to contribute in growth of surrounding plants. These are mainly bacteria and antagonistic fungi [5]. Since a long time, *Trichoderma harzianum* species have been known as biological control agents that antagonize phytopathogenic agents as well as their biofertilizing ability to stimulate shoot and root growth, to produce antibiotics and promote plant defense mechanisms [6-8]. These stimulation products are sometimes called alternative insofar as they are differentiated by their indirect action through the plant or the soil [9]. In general, biostimulants are known by synthesis of important metabolites that approve natural processes of increasing nutrient efficiency uptake, tolerance to abiotic stresses, and crop quality when applied on plants or rhizosphere. This study comes to evaluate the potential of a biostimulant made by some strains of indigenous fungus, from the southeastern Algeria, of *Trichoderma* genus on the performance of a local variety of corn.

II. MATERIAL AND METHODS

A. Study area

This study was carried out at the experimental station of Sidi Mehdi (Touggourt), National Institute of Agronomic Research of Algeria (INRAA). This is located at a longitude of 06 ° 05 '49 "East, a latitude of 33 ° 04' 13" North and an altitude of 85m.

B. Plant material

The bio stimulant used in this study is innovated and formulated by our laboratory team of bio-pesticides production of INRAA Touggourt, as part of collaborating mixed project between this institute, General Direction of Scientific Research and Development of Technology (DGRSDT) and socio-economic partner SINAL of Oran. This product is a wettable powder based on several indigenous strains of *Trichoderma*. It is used throughout the vegetative cycle of the plant at a rate (2kg/ Ha) of one treatment every 15 days.

C. Experimental device

Six elementary plots, 4.8 m² for each, were created according to a complete random block model with 3 plots/treatment (control and treated by the bio-stimulant). The biological and physical properties of the experimental site are presented in the table 1.

TABLE (1) : BIOLOGICAL AND PHYSICAL PROPERTIES OF THE EXPERIMENTAL SITE

Analyses	Profile 1	Profile 2	Profile 3	Profile 4
PH	7.67	7.70	7.77	7.62
EC mmhos/cm	4.43	2.76	3.14	2.68
Chloride meq/l	17.5	6.5	7.5	5.5
Sulfate meq /l	32.53	36.38	34.67	34.24
Carbonate meq/l	1.00	1.00	0.5	1.00
Bicarbonate meq /l	00	00	00	00
Nitrogen %	0.093	0.093	0.093	0.00
Gypsum %	15.20	16.53	6.78	2.81
Organic material %	0.10	0.07	0.07	0.00
Organic carbon %	0.04	0.03	0.03	0.00
Total limestone %	6.70	6.70	5.76	2.04
Actif limestone %	4.50	4.37	//	//
Phosphore P2O5 g/kg	0.04	0.02	0.08	0.08
Total fungi CFU/g	4 x 10 ⁴	0.2 x 10 ²	00	00

D. Morphological parameters

Control and treated plant samples were randomly taken in order to study the stimulatory response of corn to the tested bio-stimulant. According to BBCH scale of notation (2001), quantitative measurements (table 2) were taken from the 1st principal stage to the end of the 6th stage (10 to 69).

TABLE (2): QUANTITATIVE MEASUREMENTS TOOK FROM THE MAIZE PLANTS

Stage of maize	Measurements
1 st principal stage - 6 th stage	Length
	Diameter
	Surface of leaves
	Internodes space
6 th stage	Panicles
	Ears
	Tillers

E. Estimation of leaves area

Area (S) of leaves was calculated according to the classical formula adopted by [10]:
 $S = \text{Length} \times \text{Diameter} \times 0.75$

F. Application modalities of the biostimulant

In order to have a good efficiency, the bio-stimulant was applied by irrigation or spraying (2 to 4 bars) under controlled conditions where temperatures are more or less high. Four treatments were carried out during the different stages of development of the plant (stage standards established by BBCH) with the recommended dose (02 kg/ha). Procedures to apply these treatments were shown in table 3.

TABLE (3): APPLICATION MODALITIES OF BIOSTIMULANT TREATMENTS ON THE CORN PLANTS

Application dates	Development stages of corn (BBCH)	Weather situation	Application methods
15 days before seeding 30/ 08/2018	Before transplantation	30.4° C 43%	Powder spreading (2kg/ha)
Principal stage 1: Leaves development			Incorporation in irrigation water (2kg/ha)
27/09/2018	Stage 15 5 spread leaves	22.4° C 56%	
Principal stage 5: Appearance of the inflorescence			
18/10/2018	Stage 51 The terminal inflorescence (panicle M) starts to come out, it is discernible at the end of the main stem	21.2° C 54%	
06/11/2018	Stage 59 The terminal panicle is completely out and its branches are spread out	17.7° C 52%	

G. Statistical study

Two groups (control and treated) are shown in the present study which allow us to compare means of 60 plants in every treatment by MANOVA analysis of variance ($P \leq 0.05$) using the SPSS statistical program (version 20.0.). For this, two hypotheses have been developed:

- Null hypothesis (H0): there is no difference between studied treatments;
- Alternative hypothesis (H1): the applied biostimulant treatment actually changes significantly the plant behavior.

III. RESULTS AND DISCUSSION

After one week of seeding, treated seedlings registered an emergence rate of the order of 90.5% (95/105 seeds) while that of the control plants represented only 28.6 % (30/105 seeds), this reached 89.5 % in the second week. Likewise, it shows that the length of treated plants is 135.9 cm while that of the controls is only 98.6 cm. Also, tested biostimulant appears to have a significant effect on diameter of treated plants that is of 28.9 mm and only 23.7 mm for the one of control. In addition, surface of leaves in treated plants is very important because it is about 318 cm² at

the time when the control one is only about 260 cm². Although the internodes space in treated plants is very important with 9.1 cm and it didn't even achieve only 7 cm in control.

On the other hand, the effect of this bio-stimulant on the number of leaves allowed recording a mean of 10.1 leaves/ plant while that of control is only 8.9 leaves/ plant. Concerning the number of ears of corn, it shows an important rate of 2.40 ears/ plant for the treated plants while the control gives 2.07 ears/ plant. Same case is noted with number of panicles where treated plants present 10.2 panicles/ plant unlike that of control (7.4 panicles/ plant). In addition, results reveal a positive effect on the number of tillers of treated plants with 0.9 tillers/ plant against 0.4 tillers/plant of control. Statistical analysis allowed as confirming that the bio stimulant tested has a positive significant effect on the growth of corn (Table 4).

TABLE(4): STATISTICAL ANALYSES CONFIRMING THE SIGNIFICANCE EFFECT OF THE BIO-STIMULANT ON THE GROWTH OF MAIZE

Measurements	Treated plants	Control	Sum of squares Type III	DL	Square means	D	Sig.	Eta of squared partial
Length	135.9 cm	98.6 cm	12539,941	1	12539,941	47,140	,000	,285
Diameter	28.9 mm	23.7 mm	653,333	1	653,333	42,537	,000	,265
Surface of leaves	318 cm ²	260.4 cm ²	99413,028	1	99413,028	27,632	,000	,190
Panicles	10.2 pan/plant	7.4 pan/plant	229,633	1	229,633	14,116	,000	,107
Ears	2.40 ear/plant	2.07 ear/plant	3,333	1	3,333	8,912	,003	,070
Tillers	0.9 tillers/plant	0.4 tillers/plant	9,633	1	9,633	15,431	,000	,116

In recent years, agricultural practices were emphasized the sustainability of various components of environment by limiting the use of chemical fertilizers and pesticides. Soil organic amendments offer promising alternatives for minimizing the deleterious effects of chemical fertilizers [11]. The ability of microorganisms to produce and release various metabolites that influence plant growth and health is considered to be one of the most important factors in soil fertility [12]. Several studies were carried out to evaluate the effect of antagonistic strains (as biostimulant) on many cultures in the world. Indeed, several works demonstrated the growth stimulating effect after using species *T. harzianum*.

The biostimulant (based on several species of *Trichoderma*) that is tested in this study showed a very strong positive effect on all the tested parameters of corn *i.e.* the length, diameter, leaf surface, internodes space, number of leaves, of panicles, of ears and tillers of plants, although, treated subjects recorded a significant effect on these parameters unlike the control. Our results are confirmed by those advanced by [13] who note that all strains of *T. harzianum* induce a better response of stimulation of tomato plant growth parameters. So did [14], in studying the biostimulant effect of *T. harzianum* on corn, found that height of plant, length of roots, number of leaves/ plant, area of leaves, fresh and dry weight of shoots and roots were greater in treated plants compared to control ones. Also, [15], who selected two strains of *T. harzianum*, among 100, that exerted a stimulating effect on germination rate, dry matter and number of flowers of tomato as of cucumber plants. Another study, conducted by [16], found that *T. harzianum* has a positive effect on the stimulation of tomato biomass in the presence of *Fusarium* wilt, where this growth was observed in aerial and underground parts. This phenomenon is attributed by [17] who revealed the inhibition of minor pathogens, thus inducing high growth and nutrient supply. Although the mechanisms for stimulating plant growth by *Trichoderma* are not yet well understood, they are often attributed to their ability to combat plant diseases. In this sense, [18] explained the stimulation of melon development following the application of *T. harzianum* due to activation of plant's defense system and increase in chitinase and peroxidase activity as in enzymatic activity in leaves which induce systemic resistance in these plants. Treatment of corn with *Trichoderma harzianum* resulted a 63% of increase in growth compared to the control, so the nitrogen content of the aboveground cowpea biomass was highest with this treatment [19]. So mentioned [20], weight and root length of corn treated with *T. harzianum* T22 were almost twice of that of controls.

With regard to the development of root system with production of some organic acids in rhizosphere, such as gluconic acid, citric acid and/or fumaric acid, which increase pH of soil, could be due to an increase in solubility of

nutrients by *Trichoderma* species [21]. In another study on the bio stimulant effect of *T. harzianum* on corn cultivation, a significant stimulation is observed on fresh and dry weight of roots and shoots of plants inoculated with *T. harzianum* compared with control plants [22]. [6] showed that the microorganisms used in this study (*T. harzianum*) stimulated seedling growth, with a size gain of 7 to 26% compared to the control at the 5th week. Our results are also similar to those of [23], who confirmed that *T. harzianum* and *T. viride* showed a shoot length, a higher root weight, a higher pod weight per plant and a higher number of nodules per plant than those treated with other species. Results of [24] demonstrated a stimulating effect of *T. harzianum* MAUL-20 on development of tomato and its root system, which results a significant aerial development and improved competitiveness to plant when confronted with different pathogenic agents (*Pythium ultimum*, *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *radicis lycopersici*). Similarly, in a greenhouse experiment, [25] showed that *Trichoderma* isolates show different levels of growth stimulation and increase the emergence of rice seedlings.

III. CONCLUSION

This study was conducted, in the region of Touggourt, to evaluate the effect of a bio stimulant, based on *Trichoderma harzianum*, on the development of corn (*Zea mays* L.). A difference of one week showed that emergence of treated seedlings with this product is greater than that of the controls which implies that the bioproduct has a positive effect on precocity of germination. In the same way, morphological parameters showed a significant improvement of treated subjects than those of control. Besides, better growth, more leaves, ears, panicles and tillers as well as a larger vegetative area are recorded. This work leads to the conclusion that these product is recommended to farmers in order to improve the yield of their crop and reduce using chemical fertilizers, which is very useful for preserving human health and preventing pollution of the environment.

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REFERENCES

- [1] FAO STAT, 2019. <http://www.fao.org/faostat/fr/#data/QC/visualize>. Accessed in 15 Mars 2019; 15:07.
- [2] Mapiemfu-Lamaré, D., T.E. Tsoata, C. Zonkeng and Y.C. Mfopou Mewouo. 2011. «Inhibition de la croissance et du rendement du maïs (*Zea mays* L.) en sols très acides au Cameroun, et identification de critères précoces de tolérance à la toxicité». *Tropicicultura* 29(2), 94-100.
- [3] Sylvander, B., M. François and J.M. Morin. 2005. *Les bases de l'agriculture biologique: définitions, réglementations, histoire et état des lieux*. (IRD éditions).
- [4] Chambre d'Agriculture Rhone-Alpe. 2015. *Convertir son exploitation à l'agriculture biologique : les premières informations pratiques*. (CORABIO).
- [5] Pedrazzi, S., Y. Rechka, P. Pelleteret, R. Chablais, J. Crovadore and F. Lefor. 2016. «Evaluation de biostimulants commerciaux en culture de tomate en sol». *Horticulture* 48 (6), 358-364.
- [6] Harman. G.E., 2000. «Myths and dogmas of biocontrol - Changes in perceptions derived from research on *Trichoderma harzianum* T-22». *Plant Disease* 84, 377-393.
- [7] Bais, H.P., T.L. Weir, L.G. Perry, S. Gilroy and J.M. Vivanco. 2006. «The role of root exudates in rhizosphere interactions with plants and other organisms». *Annu. Rev. Plant Biol.* 57, , 233-266.
- [8] Adams, P., F.A. De-Leij and J.M. Lynch. 2016. «*Trichoderma harzianum* Rifai 1295-22 mediates growth promotion of Crack willow (*Salix fragilis*) saplings in both clean and metal contaminated soil». *Microb Ecol.* 54, 2007, 306-313.
- [9] F. Ludovic, and C. Tostivint. «Les produits de stimulations en agriculture : un état des connaissances». *NESE* 40, 7-39.
- [10] Ruget, F., R. Bonhomme and M. Chartier. 1996. «Estimation simple de la surface foliaire de plantes de maïs en croissance» *Agronomie* 16(9), 553-562.
- [11] Bashan, Y. 1998. «Inoculants of plant growth promoting bacteria for use in agriculture». *Biotechn. Adv.*15, 729-770.
- [12] Ping, L. and W. Boland. 2004. «Signals from the underground: bacterial volatiles promote growth in *Arabidopsis*». *Trends Plant Sci.* 9(6), 263-266.

- [13] Mouria, B., A. Ouazzani-Touhami and A. Douira. 2008. «Effet des diverses souches de *Trichoderma* sur la croissance d'une culture de tomate en serre et leur aptitude à coloniser les racines et les substrats». *Phytoprotection* 88(3), 103-110.
- [14] Akladios, S.A. and S.M. Abbas. 2012. «Application of *Trichoderma harziaunum* T22 as a biofertilizer supporting maize growth». *African Journal of Biotechnology* 11(35), 8672-8683.
- [15] Besnard, O. and P. Davet. 1993. «Mise en évidence de souches de *Trichoderma* spp. à la fois antagonistes de *Pythium ultimum* et stimulatrices de la croissance des plantes». *Agronomie* 13, 413-421.
- [16] Zaidi, S., M.M. Senoussi, A. Oufroukh and W. Harrat. 2018. «The Effect of in Vitro and in Vivo *Trichoderma* sp. (TR2) on the Reduction of Infection of the Tomato Variety (Elgon) contaminated with *Fusarium oxysporum* f. sp. *lycopersici*». *World journal of environmental biosciences*, 7(4), 1-8.
- [17] Ousley, M.A., J.M. Lynch and J.M. Whipps. 1993. «Effect of *Trichoderma* on plant growth: A balance between inhibition and growth promotion». *Microbial Ecol.* 26, 277-285.
- [18] Hibar, K., M. Daami-Remadi, H. Khiareddine and M. El Mahjoub. 2005. «Effet inhibiteur *in vitro* et *in vivo* du *Trichoderma harzianum* sur *Fusarium oxysporum* f. sp. *radicis-lycopersici*». *Biotechnol., Agron. Soc. Environ.* 9(3), 163-171.
- [19] Ouedraogo, E. and E. Hien. 2015. «Effet d'un compost enrichi par des spores du clone *Trichoderma harzianum* (Rifaï) sur le rendement du niébé et du maïs sous abris au Burkina Faso». *International journal of biological and chemical sciences* 9(3),1330-1340.
- [20] Kaya, C., M. Ashraf, O. Sonmez, S. Aydemir, A.L. Tuna and M.A. Cullu. 2009. «The influence of arbuscular mycorrhizal colonisation on key growth parameters and fruit yield of pepper plants grown at high salinity». *Scientia Hort.* 121(1), 1-6.
- [21] Harman, G.E., C.R. Howell, A. Viterbo, I. Chet and M. Lorito. 2004. «*Trichoderma* species opportunistic, a virulent plant symbiont». *Nature Reviews Microbiology* 2, 43-56.
- [22] Ezzat, M.F., M.H. El-Katatny, Y.M.M. Moustafa and M.M.M. Idres. 2012. «Single and double inoculation with *Azospirillum brasilense*/ *Trichoderma harzianum*: effects on seedling growth and/or yield of wheat (*Triticum vulgare*) and corn (*Zea mays*)». Conf. *Minia international conference for agriculture and irrigation in the nil basin countries*. March 26-29.
- [23] Kamaruzzaman, M., M.M. Rahman, M.S. Islam and M.U. Ahmad. 2016. «Efficacy of four selective *Trichoderma* isolates as plant growth promoters in two peanut varieties». *International Journal of Biological Research* 4(2), 152-156.
- [24] Caron, J. 2002. «Le pouvoir antagoniste de *Trichoderma*». Conf. Journées horticoles régionales, St-Rémi 5 décembre.
- [25] A. Hamdia and N. Kalaivani. 2014. «Evaluating the efficacy of *Trichoderma* spp. and *Bacillus subtilis* as biocontrol agents against *Magnaporthe grisea* in rice». *Australian journal of crop science* 8(9), 1324-1335.