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Short communication
EFFECT OF BIOENRAIZ® AS STIMULANT OF COFFEE PLANTS (*Coffea arabica* L.) GERMINATION AND THE DEVELOPMENT

Comunicación corta
Efecto del Bioenraiz® como estimulante de la germinación y el desarrollo de plántulas de cafeto (*Coffea arabica* L.)

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ABSTRACT. Biotechnological methods for obtaining of efficient products, from bacterias with beneficent properties, represent an alternative of interest for the development of economical important crops in the context of a sustainable agriculture. The effect of Bioenraiz® in germination and plants growth during the process of plants coffee obtaining of "Caturra rojo" (*Coffea arabica* L.) was studied. Three concentrations of the biopreparation: 200, 220 and 230 mL L^{-1} and a Control were evaluated. The application was carried out before the inoculation of the seeds through the imbibitions for 20 min and when the first pair of leaves was reached. The germination was evaluated 50 and 60 days after the inoculation. The height, diameter of shaft, pair of leaves and dry mass were evaluated to the seven months. Differences in the answer to the germination for both periods were evidenced, the best treatment was that of 200 mL L^{-1} of Bioenraiz®, where 79,4 and 94,5 %, respectively were obtained. Seeds treated with the biopreparation originated plants that showed significant differences with the control for the evaluated variables, effect that is attributed to the chemical composition of the biopreparation that is characterized by not causing adverse alterations in the ecological environment. The results contributed from the economic point of view, to the recovery of coffee in Cuba, achieving plantation material with natural products and the required quality.

Key words: coffee, plant growth regulators, auxins, germination

RESUMEN. El empleo de métodos biotecnológicos para la obtención de productos eficientes, a partir de bacterias con propiedades benéficas, representa una alternativa de interés para el desarrollo de cultivos de importancia económica en el contexto de una agricultura sostenible. Se estudió el efecto del Bioenraiz®, con propiedades auxínicas que estimulan la germinación y el crecimiento vegetal, durante el proceso de obtención de plántulas de cafeto del cultivar "Caturra rojo" (*Coffea arabica* L.). Los tratamientos consistieron en estudiar tres concentraciones del biopreparado: 200, 220 y 230 mL L^{-1} y un control. Se realizaron dos aplicaciones una antes de la siembra de las semillas a través de la imbibición por 20 min y cuando las plantas alcanzaron el primer par de hojas. Se evaluó la germinación a los 50 y 60 días después de la siembra. A los siete meses se evaluó la altura, diámetro del tallo, pares de hojas y masa seca. Se evidenciaron diferencias en la respuesta a la germinación para ambos períodos, el mejor tratamiento fue el de 200 mL L^{-1} de Bioenraiz®, obteniéndose 79,4 y 94,5 % a los 50 y 60 días después de la siembra, respectivamente. Las semillas tratadas con el biopreparado originaron plántulas que mostraron diferencias significativas y superaron los resultados mostrados por el control para las variables evaluadas, efecto favorable que se atribuye a la composición química del bioproducto. Se contribuye, desde el punto de vista económico, a la recuperación de la caficultura cubana, logrando material de plantación con productos naturales y la calidad requerida.

Palabras clave: café, reguladores del crecimiento vegetal, auxinas, germinación

INTRODUCTION

Coffee (*Coffea* spp.), is an internationally recognized crop; at present, more than 80 countries distributed in America, Africa, Asia, Australia and

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Europe grow coffee (1). In Cuba, this crop is a priority of the agricultural sector for being a beverage of habitual consumption by part of the population and because it is a export item that contribute to increase foreign currency income for the economic and social development of the country[A, B].

Most of the coffee consumed worldwide comes from the specie *Coffea arabica* L., the most cultivated one accounting for nearly 70 % of world's production (2, 3). It is attributed to the quality of the product, aromatic characteristics, and the low caffein level of its grains.

It should be noticed that since 2009, the country embarked upon the recovery of coffee production, but in spite of this, production figures fall short from meeting the domestic c demand and export goals. At the end of the 2012 season, only 7 100 tons were produced (4). Therefore, different strategies to improve new plots and develop the crop have been implemented as increased planted area and the production of enough quality planting material for growers, for which seedling production is essential (5).

The use of high quality physiological coffee seeds is considered as one of the main factors influencing production of more vigorous plants under field conditions equivalent to a higher productivity of the crop (6, 7). Keeping good quality coffee seeds during conservation is in fact one of the main concern of experts and growers (8), considering they quickly loose viability and of course, germination power (9).

For this reason, the constant quest for ways to favor seedling production and the productive growth of this crop is based on the articulation of conventional methods and modern techniques (10, 11, 12, 13), to guarantee the efficient conservation of germplasm to attain, among other goals, adequate germination levels of the seeds and the efficient development of seedlings during the production process of the planting material (5, 14).

In this regard, it is known that the production of efficient products from bacteria with beneficial properties is an alternative of great interest, (15, 16, 17), particularly for developing crops of economic importance in the context of a sustainable agriculture (18, 19, 20). These bioproducts effectively contribute to the survival and growth of crops since they reduce the negative effects of nutrition-associated stress, water relations, soil structure, soil pH, heavy metals, and pathogens (21, 22, 23, 24, 25).

Recent investigations aimed at Bioenraiz®, a bioproduct produced from a *Rhizobium* sp., isolate as a new plant growth bioregulator with scientific evidences that show its chemical composition based on auxins which play an important role in seed germination and the further development of the seedlings (26).

In recent years, research and basic studies aimed at making clear the biological role of this product on certain plant species have increased as well as to determine its relations with plant growth and development, its use to improve the quality of different processes. However, the effect of Bioenraiz®, when exogenously applied at different growth stages of the seedlings is still low so this article looks at evaluating the effect of this biopreparation in the production process of coffee seedlings (*C. arabica* cv. "Caturra rojo").

MATERIALS AND METHODS

Trials were done at the Plant Genetics and Breeding Department of the National Institute of Agricultural Sciences (INCA), from November 2011 till June 2012.

Seeds were sown on poly bags (14 x 22 cm), two seeds per bag of the cultivar "Caturra rojo" (*Coffea arabica* L.), in good phytosanitary shape. A Red Lixiviated Ferralitic Soil (27) was used, it was mixed with organic matter at the rate of 3:1 (v:v). The effect of Bioenraiz®, a product based on auxins and produced from a *Rhizobium* sp., strain isolated under natural conditions at the Labs of the Research Institute on Sugarcane Byproducts (ICIDCA) has been evaluated. The bioproduct used in this research does not cause adverse effects on the ecological environment.

A random block design with four treatments and three replicates was used. Each of them was made up of 30 bags. Treatments consisted in evaluating the effectiveness of different concentrations and application times of Bioenraiz®, for which a control (distilled water) and three concentrations of the bioproduct were used: Treatment 1-100 mL L^{-1}, Treatment 2-200 mL L^{-1} and Treatment 3-230 mL L^{-1}. The first application was done before sowing the seeds by dipping them in each of the solutions for 20 minutes; after the treatments, seeds were dried at the shadow and then were inoculated. The second application was done at the rate of 10 mL per plant when seedlings showed the first pair of leaves.

Germination was evaluated 50 and 60 days after sowing seeds. Seven months after sowing, seedling height (cm), stem diameter (cm), pairs of leaves, and dry mass (g), were evaluated in those seedlings sprayed when they had the first pair of leaves as well as in non- treated ones. Experimental data were statistically processed by the analysis of variance of double classification for $p<0,05$, and means were compared according to Duncan's Multiple Range Test with a previous transformation of data using the expression arcsen$\sqrt{\%}$ and SAS 9.0. software package.

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RESULTS AND DISCUSSION

When analyzing seed germination behavior 50 and 60 days after sowing, it was seen that Bioenraiz® had an important effect on such process (Figure 1) that can be attributed to the fact that growth regulators, either natural or synthetic, have biological effects on seed germination and the root system (28, 29, 30).

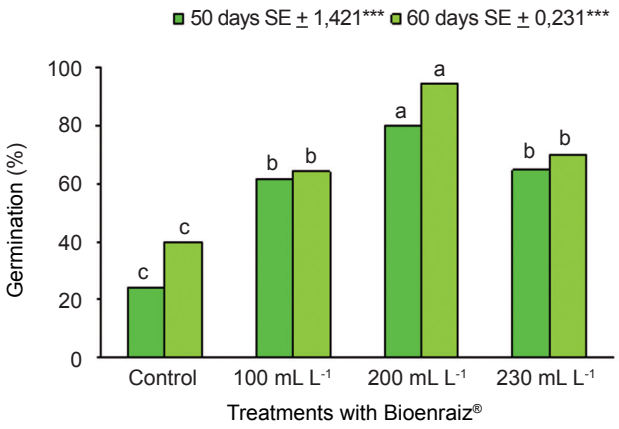

Figure 1. Response of *Coffea arabica* L. cv. "Caturra rojo" to the germination process with the use of Bioenraiz®, after 50 and 60 days of sowing seeds

The application of 200 mL L^{-1} of the biopreparation, allowed having the best results, values that significantly differ from the results of the rest of the treatments that ranged from 79,4 and 94,5 % of germination for 50 and 60 days respectively. It is worth saying that the response of a certain tissue or plant organ to auxins, complies with the concentration of this growth regulator and plant sensitivity to it.

It is important to point out that the other two concentrations of Bioenraiz® resulted in lower germination values that exceeded the control's in both evaluation dates. It seems that concentrations of Bioenraiz® below or above 200 mL L^{-1}, were less effective to promote germination in the cultivar under study, so a decrease of this indicator was observed. It might be due to the endogenous levels of auxinic compounds, since in general, the response to exogenous application practices complies with the influence of differente factors like the free or combined availability at the intracell level (31).

Moreover, there was an abnormal growth in some seedlings from seeds treated with the highest concentration of the biopreparation as well as marked inhibition of the formation process of true leaves. This behavior could be attributed to the fact that Bioenraiz® is mostly composed of indol acetic acid (IAA), indol butiric acid (IBA) and indol propionic acid (IPA), all of them with auxinic properties. Plant growth regulators work at low concentrations in plant physiological processes and when the required levels are not applied, physiological disorsers may be induced causing significant morphological changes (32).

Auxin availability in plant tissues can be regulated through its synthesis rate, transport speed among organs and different deactivation mechanisms like the combination with other molecules as sugars and aminoacids (33). Some studies show the presence of auxinic combinations found in seeds and seedlings (34), being these combinations reversible, unlike the degradation that an irreversible process means.

All these facts confirm the link between metabolism and the hormonal action mechanism of plants directly related to all development stages and specially to the initial seed germination stage and seedling formation (35).

Different trials have shown the production of substances with regulating effect on plant growth as those from bacteria and its impact on different processes associated to plant growth and development (17, 18, 19, 36). In this regard, the need of developing studies that facilitate the selection and use of adequate rates of these products, as per the objectives of the process to be induced, are needed, since they are responses that greatly comply with the specie and the physiological status of the plant.

On the other hand, the results shown by Bioenraiz® had a significant effect on the development of coffee seedlings treated with concentrations from 200 and 230 mL L^{-1} with significant differences compared to the control in variables like seedling height, stem diameter, number of pairs of leaves and foliar dry mass (Tables I and II).

It is good to note that when the biopreparation was applied as a pre-germinating treatment, concentrations of 200 and 230 mL L^{-1} induced values without significant differences for the evaluated variables, except foliar dry mass; though it is an indicator of interest because it reflects the biological yield of the plant. Such an effect could be due to the favorable role of auxins in adequate concentrations which in turns, depends on the synthesis and application level, transportation and degradation, all of which influence the formation of root hairs and the number and elongation of lateral roots. This development makes water and nutrients uptake easier by the plant as well as a higher interaction with the environment of the rhizosphere (29). It has been stated that the positive actions of auxins on these interelated processes bring about a reduction of cell wall pressure while inducing specific enzymes synthesis which leads to increased plasticity of the cell wall and a better germination.

This behavior is related to the response to biostimulation reported for other coffee cultivars when rhizospheric microorganisms or products derived from them have been used in seedling production. Mention could be made on Azotobacter in clones of *Coffea canephora* P., which showed favorable results in the nursery stage (37).

Table I. Development of coffee seedlings (*Coffea arabica* L. cv 'Caturra rojo') seven months after planting with the application of Bioenraiz® during germination

Treatments	Height (cm)	Stem diameter (cm)	Number of pairs of leaves	Foliar dry mass (g)
Control	11,0 b	0,16 c	4,1 c	1,3 c
Bioenraiz® 100 mL L^{-1}	13,5 b	0,27 b	5,0 b	1,5 c
Bioenraiz® 200 mL L^{-1}	17,8 a	0,34 a	6,0 a	3,1 a
Bioenraiz® 230 mL L^{-1}	17,9 a	0,35 a	5,5 ab	2,6 b
Standard Error ±	0,801***	0,013**	0,123**	0,138***

Table II. Development of coffee seedlings (*Coffea arabica* L. cv 'Caturra rojo') seven months after planting with the application of Bioenraiz® after the emission of the first pair of leaves

Treatments	Height (cm)	Stem diameter (cm)	Number of pairs of leaves	Foliar dry mass (g)
Control	11,7 c	0,19 c	4,2 c	1,6 c
Bioenraiz® 100 mL L^{-1}	17,9 b	0,34 ab	6,1 b	2,9 b
Bioenraiz® 200 mL L^{-1}	20,9 a	0,38 a	7,5 a	4,6 a
Bioenraiz® 230 mL L^{-1}	11,9 c	0,29 b	4,3 c	1,8 c
Standard Error ±	0,902**	0,015**	0,120**	0,154***

Likewise, when *Azotobacter chroococcum* was used in *Coffea arabica* L, seedlings, there was a better behavior when the application was done at transplanting with the first pair of leaves.

Similarly, when a bacterial biopreparation from Burkholderia cepacia Palleroni and Holme was used during growth and development of coffee seedlings of *Coffea canephora* P. cv. Robusta, favorable results were reached for indicators like the pair of leaves, seedling height and foliar dry mass (38). Other positive responses have been reported in crops like rice (*Oryza sativa*) and corn (*Zea mays*) with the use of bioproducts from other bacteria species stimulating plant growth (19, 36).

The results with the lowest concentration of Bioenraiz® applied during germination, showed values not different from the control's for indicators like seedling height and foliar dry mass; however, this concentration induced a greater stem diameter and more pairs of leaves for the cultivar under study as compared to the control (Table I).

The foliar spray of the biopreparation at the rate of 10 mL per plant when seedlings reached the first pair of leaves, favorably influenced their morphological development (Table II), that is, values of growth indicators exceeded not only the control, but also those reached when Bioenraiz® was applied during germination.

In this case, the treatment with 200 mL L^{-1} resulted the most effective for all indicators evaluated. Figure 2 shows the favorable status of preceding seed plants treated with 200 mL L^{-1} of foliar-sprayed Bioenraiz®, when they showed the first pair of leaves. Seedlings characterized for showing an adequate vigour and brilliant green leaves. The biopreparation based on auxinic components exogenously applied to the leaves can penetrate through the sieve tubes after being absorbed and transported to vascular parenchima (39).

Figure 2. Seedlings of *Coffea arabica* L. cv. "Caturra rojo" treated with Bioenraiz®, by dipping seeds into 200 mL L^{-1} and foliar spray after the emission of the first pair of leaves

Tabla II shows a certain depressive effect for evaluated variables when Bioenraiz® was applied after the emission of the first pair of leaves by using the highest concentration of the biopreparation (230 mL L^{-1}). It confirms that increased concentration of this bioproduct can inhibit some features of metabolism or certain stages of the growth and development processes of this crop. The quantity of auxinic compounds in leaves depends on the age of these tissues though it is said that young tissues are more efficient (33). In this case, for stem diameter lower values than those attained with 200 mL L^{-1} for the control, were recorded.

In this regard, previous studies confirm the potential of different bacterial genera (36, 37), and beneficial effects of using biopreparations from such bacteria temselves (19, 38), in different physical and physiological status of microorganisms; and using different application ways on morphophysiological indicators of coffee plants (6, 14, 37, 38). There is a higher growth and survival percentage of treated plants. Such effects are attributed to the contribution of growth biostimulants as auxins, cytokinins, gibberellins, aminoacids and vitamits that allow the acceleration of plant development (18).

These behavior indicate a favorable response of the plant material to biostimulation and the effectiveness of treatments with biopreparations of bacterial origin, depending on their concentration, specie, cultivar or genotype being studied and the application way.

As the indiscriminate use of chemicals is one of the main causes of great ecological disorders in agro-ecosystems, the interest for microbiology has gained momentum in recent years with the use of products of biological-origin in agriculture to increase plant productivity and replace chemicals that contaminate the environment (23, 40). The results of this research are important for the possible economic and environmental impact in addition to consider the characteristics and favorable effects of Bioenraiz®.

In general, this alternative could contribute to producing optimun quality coffee planting material on a profitable basis, with a significant reduction of chemicals and the consequent saving of inputs, improvement of soils and protection of the environment. It is a feasible practice to be included in the integrated management of coffee plots.

CONCLUSIONS

- The application of 200 mL L^{-1} of Bioenraiz®, promoted seed germination in coffee plants (*Coffea arabica* L. cultivar "Caturra rojo") 50 days after sowing the seeds with 79,4 % and 60 days after with 94,5 %, percentages that exceeded the rest of the treatments.
- Seed plants treated with Bioenraiz®, after seven months, reached statistical values for the evaluated variables that exceeded the control's, so this practice could be recommended for massive seedling production since it guarantees the survival at transplanting.

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