Scientific Paper

Effect of EcoMic® and Pectimorf® on the growth of *Leucaena leucocephala* cv. Cunningham seedlings

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Abstract

The study was conducted at the Pastures and Forages Research Station Indio Hatuey in order to evaluate the effect of the bioproducts EcoMic® and Pectimorf® on the growth in nursery of *Leucaena leucocephala* cv. Cunningham. The treatments were: 1) control, 2) inoculation with EcoMic®, 3) imbibition in Pectimorf®, 4) inoculation with EcoMic® plus imbibition in Pectimorf®. A completely randomized design was used, with three replications. The evaluated variables were: emergence from the seeds, seedling height, number of branches and root length. The emergence was higher (71,43 % of seedlings emerged 35 days after seeding) in the treatment that consisted in imbibing the seeds in Pectimorf® during 16 h. The control was higher and differed significantly from the others in the variable number of branches. Regarding height, the imbibition in Pectimorf and the control did not differ statistically, and the treatments inoculated with EcoMic® alone and its combination with Pectimorf showed lower growth; however, these last two ones had incidence on a higher root growth of the leucaena seedlings. The imbibition in Pectimorf® during 16 h caused higher emergence from the seeds, while the treatments inoculated with EcoMic® showed the highest root growth; this indicates that it is possible to reduce the nursery period in this species.

Keywords: height, inoculation, branches, imbibition, nurseries

Introduction

Leucaena leucocephala (Lam.) de Wit. (leucaena) is a forage legume that can be used as protein complement for the animals. Its association with natural pastures is a practice which has had high acceptance by farmers (Sánchez *et al*., 2011); however, its slow establishment hinders its adoption in livestock production systems.

The association among microorganisms of different microbiological nature is used to promote crop growth and reduce their vegetative cycle (Martínez-Viera and Dibut, 2012). Since several years ago results are reported about the beneficial effect exerted by mycorrhizal symbiosis on crop growth and productivity, mainly associated to a higher absorption of nutrients and water (Ley-Rivas *et al*., 2015; Ruiz-Sánchez *et al*., 2015), as well as to the improvement of plant accessibility to the nutrients that are found in less assimilable forms (Bárzana, 2014; Velasco *et al*., 2016).

Multiple experiences are reported worldwide about the benefits of arbuscular mycorrhizal fungi –AMF– (Chacón-Solís and Santamaria-Gaona, 2015; Pérez *et al*., 2015), and in Cuba very good results have also been obtained (more height, vigor and leaf area, yield increase, higher utilization of nutrients and decrease of fertilizers) with the inoculation of efficient AMF strains in different economically important crops (Alarcón *et al*., 2013; Cruz *et al*., 2014).

Regarding Pectimorf® (product obtained from a mixture of oligogalacturonides), all the reviewed results refer its capacity to influence different physiological processes that stimulate plant growth and development (Terry *et al*., 2014; Álvarez and Reynaldo, 2015; Nápoles-Vinent *et al*., 2016).

Nevertheless, in Cuba there are few published scientific reports about the effect of the inoculation of *Leucaena leucocephala* cv. Cunningham with AMF (Flores-Bello *et al*., 2008), while there are no references of the bioactive product Pectimorf®. For such reason, the objective of this study was to evaluate the effect of the bioproducts EcoMic® and Pectimorf® on the growth in nursery of *Leucaena leucocephala* cv. Cunningham.

Materials and Methods

The study was conducted at the Pastures and Forages Research Station Indio Hatuey –Matanzas province, Cuba–, geographically located at 22º 48" N, 79º 32' W and at 19,9 masl.

In the nursery, perforated black polyethylene bags were used, of 1 kg capacity, in which a sub-

strate composed by Ferralitic Red soil and earthworm humus in 3:1 ratio, was put. Seven months old *L. leucocephala* seeds were used, which were scarified with hot water during three minutes, according to the methodology proposed by González and Mendoza (1995).

As AMF strain *Glomus cubensis* was used, obtained from a certified mycorrhizal inoculant which is produced in the department of biofertilizers and plant nutrition of the National Institute of Agricultural Sciences (INCA, for its initials in Spanish) –Mayabeque province, Cuba–. The seeds were inoculated following the coating technique, in proportion of 10 % of their weight, according to the recommendation made in the Manual de instructivo técnico del EcoMic® (Handbook of technical instructions of EcoMic®) (INCA, 2003). The bioactive product Pectimorf was obtained in the plant physiology and biochemistry laboratory of INCA and it was applied in a concentration of 10 mg L^{-1}, through the method of seed immersion during 16 h.

A completely randomized design was used with four treatments and three replications: 1) control, 2) inoculation with EcoMic®, 3) imbibition in Pectimorf® during 16 h, 4) inoculation with EcoMic® plus imbibition in Pectimorf® during 16 h.

The following evaluations were made in 20 plants per treatment (from a total of 29):

- Emergence: The emerged seedlings were counted with a weekly frequency, until 35 days after seeding.
- Height: it was weekly evaluated from the soil level to the apex of the apical branch, with a ruler graduated in centimeters, until concluding the evaluation (when the seedlings reached a mean height of 30 cm).
- Number of branches: through visual counting, 30 days after planting and at the end of the evaluation.
- Root length: at the end of the evaluation the root length of 20 plants per treatment was measured, with a ruler graduated in centimeters.

Variance analysis was made and the means were compared through Duncan's test (Duncan, 1955) for 5 % of significance, after verifying that they fulfilled the normal distribution and variance homogeneity adjustment. The statistical program SPSS was used, in its version 10.0 for Windows XP.

Results and Discussion

Figure 1 shows the percentage of emerged seedlings in each of the treatments, 35 days after seeding.

The treatment that consisted in putting the seeds in hot water during 3 min. and then imbibing them in Pectimorf® for 16 h recorded the highest percentage of emerged seedlings (71,43 %) 35 days after seeding. It is possible that this performance responds to a sudden decrease of the leucaena seed hardness, which has been reported by other authors (González and Navarro, 2001; González and Mendoza, 2008).

In addition, it is stated that the bioactive product can stimulate the biochemical processes that originate seed germination, propitiating its acceleration (Izquierdo *et al*., 2009), and the oligogalacturonides that compose it can regulate the processes related to growth and development (Messiaen and Van Cutsem, 1994).

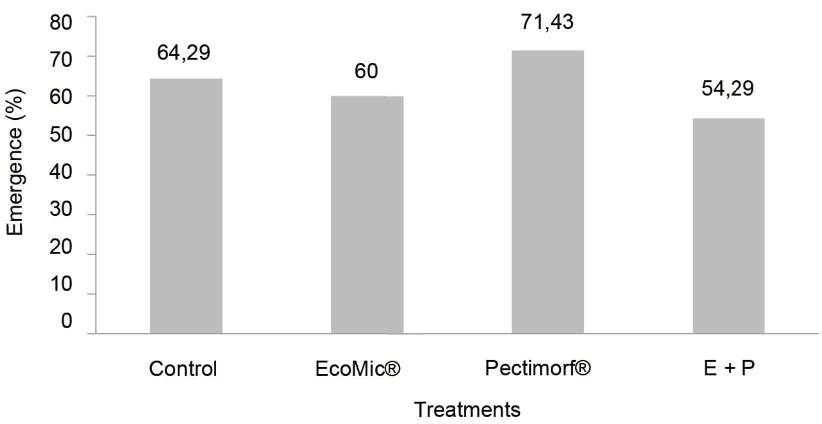

Figure 1. Percentage of emerged seedlings in each treatment.

The treatments inoculated with EcoMic® and the combination EcoMic® plus Pectimorf® showed the lowest germination values of the seeds from tree species; although a significant potential is indicated in the vigor increase of other species, such as rice, radish, mulberry and sorghum (Pentón *et al*., 2011; Terry *et al*., 2014). Regarding EcoMic, Noda and Castañeda (2012) found a positive response of the inoculation of mycorrhizal fungi in the emergence of *Jatropha curcas* seeds, which differs from the results of this study.

Likewise, when analyzing the effect of the bioproducts on seedling height (fig. 2), it was observed that the treatments inoculated with EcoMic® and its combination with Pectimorf® showed lower growth and differed statistically from the control and from the treatment inoculated with Pectimorf®, and the last ones did not differ between them.

This could have been due to the fact that during the first stage of establishment the AMF acts parasitically and demands higher flow of photosynthates, with regards to the benefits it provides for the plant (Pérez-Ortega, 2010). In addition, the nutrient availability in the system determines the efficiency of the mycorrhizal symbiosis, so that a high availability decreases the presence of mycorrhizal structures inside the roots. On the other hand, the number of AMF spores increases significantly with the rise in the number of plant species present (Pentón *et al*., 2013), which does not occur under nursery conditions.

Regarding the number of branches (fig. 3), the control treatment was higher and differed significantly from the others, while the treatments inoculated with EcoMic® (alone and in its combination with Pectimorf®) showed the lowest values for this variable. They varied between 9 branches plant^{-1} when the EcoMic® eas used alone and combined with Pectimorf®, to 12 branches plant^{-1} in the control. This coincides with the reports by several authors (Wencomo, 2004; Medina *et al*.,

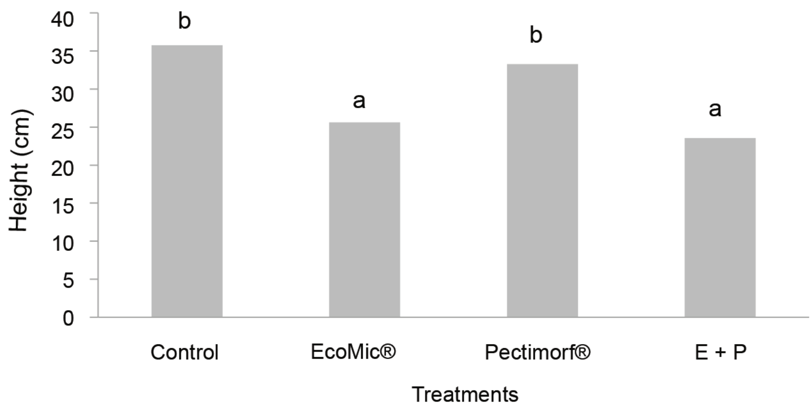

Figure 2. Effect of the bioproducts on seedling height.

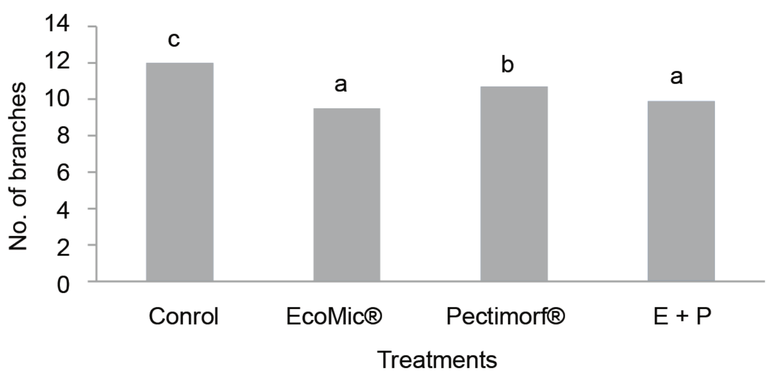

Figure 3. Effect of the bioproducts EcoMic® and Pectimorf® on branch emission.

2011) when studying the growth of this species in nursery; nevertheless, such values are considered low compared with the ones reported by Medina and García (2010) in studies that preceded this research, based on the use of alkaline substrates (22-27 branches).

Root length is shown in figure 4. It must be emphasized that, although the inoculation with EcoMic® did not have a remarkable effect on emergence and height, it influenced a higher root development of the leucaena seedlings (17,33 cm), and although it did not differ statistically from the combination EcoMic®/ Pectimorf® (14,6 cm) significant differences were observed when comparing it with the control and with the imbibition in Pectimorf® (9,53 and 8,00 cm, respectively), which did not differ from each other; this indicates that the inoculation with EcoMic stimulated higher growth of the root system.

Similar results were found by Flores-Bello *et al*. (2008) in leucaena plants inoculated with *Glomus etunicatum* and *Glomus intraradices*; besides, they coincide with the reports by several authors in other crops of interest (Alonso-Contreras *et al*., 2013; Pentón *et al*., 2014; Ruiz-Sánchez *et al*., 2015; Ruiz-Sánchez *et al*., 2016).

This result could have been due to the established symbiotic relationship, which benefits fungi with the supply of carbon sources from the plant, within which specific signals of mycorrhization are induced, which influence root growth. As mentioned before, the establishment of the fungus facilitates the flow of photosynthates from the aerial part to the root zone; the AMF uses part of those photosynthates to produce metabolic energy, and this way it ensures its maintenance and growth; the other part is mobilized in the form of sugars and lipids of intra- and extra-radical fungal mass (Pérez-Ortega, 2010).

If it is taken into consideration that the root constitutes a reserve organ for regrowth, besides its anchoring and nutrition functions, the highest root growth that was reached in the seedlings during the nursery stage should influence later a higher exploration of the rhizosphere and the extraction of nutrients from the deepest soil layers, important aspects to be considered to face adverse conditions when transplant to the field takes place.

According to the results, it is concluded that there was a positive effect of the imbibition in Pectimorf® for the variable emergence from the seeds, unlike EcoMic®. On the other hand, the imbibition of the seeds in the two bioproducts did not significantly stimulate height; while the root length of the seedlings showed the highest values with the use of EcoMic®, which indicates that it is possible to reduce the nursery period of this species.

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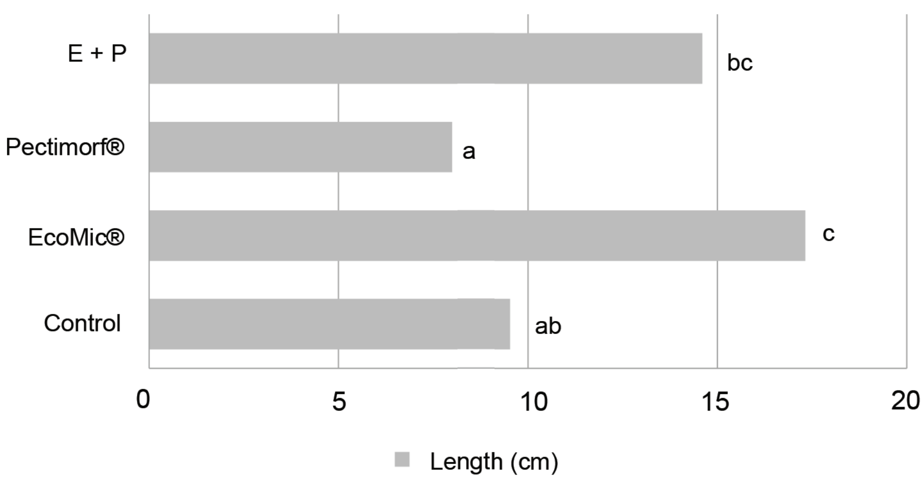

Figure 4. Effect of the bioproducts EcoMic® and Pectimorf® on root length.

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