



COMBINED USE OF ECOMIC[®], FITOMAS-E[®] AND MINERAL FERTILIZERS IN THE FORAGE PRODUCTION OF ANIMAL FOOD BASED IN TRITICALE (*X. Triticosecale* Wittmack), CV. INCA TT-7

Uso combinado de Ecomic[®], Fitomas-E[®] y fertilizantes minerales en la producción de forraje para la alimentación animal a base de Triticale (*x. Triticosecale* Wittmack), cv INCA TT-7

Rodolfo R. Plana Llerena[✉], Pedro J. González Cañizares and Francisco Soto Carreño

ABSTRACT. The study was aimed to decrease the application of mineral fertilizers in the production of triticale-based forage, with the combined use of EcoMic[®] (arbuscular mycorrhizal fungi) and Fitomas-E[®]. It was developed on a red Ferralitic leachate soil in the Station of Pastures and Forage Genetics Cattle Company (EPG) “Niña Bonita”, Artemisa province. The treatments were: 1. Absolute Control; 2. Application of mineral fertilizers 150, 92 and 120 kg ha⁻¹ of N, P₂O₅ and K₂O; 3. Application of mineral fertilizers 100, 54 and 70 kg ha⁻¹ of N, P₂O₅ and K₂O + EcoMic[®] (15 kg ha⁻¹) + Fitomas-E[®] 2 L ha⁻¹. Mycorrhizal variables, the percentage of N, P and K in the leaf tissue and crude protein percentage in forage were analyzed. The dry mass (forage) in kg ha⁻¹. The results showed that a decreased amount of mineral fertilizers did not reduce the quality of the fodder produced, since no significant differences among control treatments production and use of biofertilizer were recorded. Moreover higher percentage intensity and mycorrhizal colonization and the highest number of spores per g⁻¹ soil was recorded. The results showed the establishment of a mycorrhizal association among guest and host. It was recorded savings in production costs with the implementation of these, so it became more profitable to produce forage.

Key words: inorganic fertilizers, biofertilizers, vesicular arbuscular mycorrhizae

RESUMEN. El trabajo tuvo como objetivo la disminución de aplicación de fertilizantes minerales en la producción de forraje a base de triticale, mediante el uso combinado del EcoMic[®] (hongos micorrízicos arbusculares) y Fitomas-E[®]. Se desarrolló en un suelo Ferralítico Rojo Lixiviado en la Estación de Pastos y Forrajes de la Empresa Pecuaria Genética (EPG) “Niña Bonita”, provincia Artemisa. Los tratamientos fueron: 1. Testigo absoluto; 2. Aplicación de fertilizantes minerales 150, 92 y 120 kg ha⁻¹ de N, P₂O₅ y K₂O; 3. Aplicación de fertilizantes minerales 100, 54 y 70 kg ha⁻¹ de N, P₂O₅ y K₂O + EcoMic[®] (15 kg ha⁻¹) + FitoMas-E[®] 2 L ha⁻¹. Se analizaron las variables micorrízicas; el porcentaje de N, P y K en el tejido foliar; el porcentaje de proteína bruta en forraje y la masa seca (forraje) en kg ha⁻¹. Los resultados mostraron la disminución de la cantidad de fertilizantes minerales aplicados sin disminuir la calidad del forraje producido, pues no se registraron diferencias significativas entre los tratamientos testigo de producción y uso de los biofertilizantes. Por otra parte se registró mayor porcentaje de colonización e intensidad micorrízica y mayor número de esporas por g⁻¹ de suelo. Se demostró el establecimiento de una asociación micorrízica entre huésped y hospedero. Se registró un ahorro en los costos de producción con la aplicación de estos, por lo que se hizo más rentable la producción de forraje.

Palabras clave: abonos inorgánicos, biofertilizantes, micorrizas arbusculares vesiculares

INTRODUCTION

The negative impact of climate change on soil fertility and excess mineral fertilization of crops have been seriously affected food security in developing

Instituto Nacional Ciencias Agrícolas (INCA), gaveta postal 1, San José de las Lajas, Mayabeque, Cuba, CP 32700.

✉ plana@inca.edu.cu

countries. Because of this, steps should be taken to ensure a change in the sustainability and improvement of food production systems for all species, more in line with agroecology (1).

The livestock development requires the production and use of feed resources. Undoubtedly, fodder production for animal feed is essential for sustainable growth, the contribution in fiber and protein that gives your diet, which makes this type of food something irreplaceable in the production of milk and meat (2), which in turn directly influences human consumption.

However, in the production of biomass as hay, silage or green forage for direct consumption by animals, removal of soil nutrients is considerable, so it is necessary to apply fertilizer to restore nutrients removed by the biomass (3).

Livestock fed mainly with fodder, has been displaced to marginal areas (4). However, intensive forage production is costly with continued use of mineral fertilizers. In addition, to these excessively used, they are a source of contamination of soil and groundwater.

Today, triticale has productivity in grain like wheat in favorable environments and intensive production systems; but their performance excels in production marginal conditions: dry water shortage or temporary, especially problematic soils. Their genetic superiority in biomass production and its resistance to most foliar diseases make it very competitive as fodder grain or plant -in entirely compared to other forage crops (barley or oats). Moreover, triticale with less water use makes it more sustainable than forages such as alfalfa or rye (5).

In response to this direction, the production of fodder needs the application of mineral or organic fertilizer. Also the use of bio-fertilizers which will restore soil nutrients from the extracted and others, as in the case of arbuscular mycorrhizal fungi (AMF) which allow through symbiosis with plants, to transport the necessary nutrients for them and improve the chemical-physical and biological soil conditions.

Furthermore, in previous studies, it has been reported that effective management of mycorrhizal symbiosis, which contributes to reduce nutrient losses and, in fact, high doses of fertilizers that require grasses, may be a promising practice, in ecological terms, economic and environmental (6).

Moreover, there are other bio-fertilizers that are being biostimulants or natural, anti-stress bioregulators, partial substitutes for conventional fertilization, and conducive to the development of the rhizosphere (symbiotic microorganisms living in the roots). It fixes atmospheric nitrogen and mobilize other mineral nutrients; as in the case of FitoMas-E®, whose mechanism of action promotes the comprehensive improvement of soil-plant complex and increasing the vitality of the crop, which protects it from many of common conditions to the stressed systems (7).

For the above, this experiment was developed with the aim of reducing the dose of mineral fertilizers and achieve fodder high yields with the use of bio-fertilizers, EcoMic® and FitoMas-E®. It is based on arbuscular mycorrhizal fungi; one with the *Glomus Cubense* species recently reclassified (8) and the other is a new derivative of the Cuban sugar industry which acts as a vegetable biostimulant (natural) with marked anti stress influence, most of the botanical species that belong economic crops (7), without reducing production levels, nor the quality.

MATERIALS AND METHODS

The experiment was conducted in Genetic Livestock Production Enterprise (EPG) "Niña Bonita" in Cangrejas, Bauta, Artemisa province. On a soil that was classified as Nitisol Ferralic Lixic, Eutrico, Rhodic (9). Its characteristics (the arable horizon), complemented with the number of residents spores per gram of soil are reflected in Table I.

Rainfall in the EPG "Niña Bonita" was 1261 mm, which was 83,1 % during the rainy season and 16,9 % (November to April) occurred in the dry season with the development of the experiment in question. An average temperature of 21,77 °C was observed and the average relative humidity was 73,91 %. The meteorological variables that were reported during the experiment showed a similar behavior to historical averages in the area.

For the production of forage crop used as triticale (x. *Triticosecale Wittmack*), cv. INCA TT-7 (10), due to its good adaptation to the conditions of western and central Cuba and high agricultural yields in dry matter forage and grains. The standard used was planting 100 kg ha⁻¹ of seed chorrillo along the groove. de semilla a chorrillo

An experimental design of random blocks followed with three treatments and ten repetitions to achieve the objectives proposed in the study.

Table I. Chemical characteristics and content of spore number of arbuscular mycorrhizal fungi (AMF) resident in the soil of the experimental area

Location	pH	MO (%)	P ₂ O ₅ (mg 100 g ⁻¹)	Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	CCB	Number spores of AMF g ⁻¹ de suelo
Pastures Microstation	6,5	3,25	2,8	9,7	2,2	0,15	0,21	12,26	2,8

The experimental plots have a total area of 29,4 m² in a planting of 7 x 0.70 m (six rows). The calculation area was 14 m².

Treatment 1 (T1) consisted of absolute control without application of mineral fertilizers and biofertilizers. Treatment 2 (T2) was the application of mineral fertilizer in doses of 460 kg ha⁻¹ has the most balanced formula 14-20-26 187 kg ha⁻¹ of urea (46-0-0 formula), which represents an application of 150, 92 and 120 kg ha⁻¹ of N, P₂O₅ and K₂O. Finally the treatment three (T3) had mineral fertilizer application in doses of 270 kg ha⁻¹ of balanced formula 14-20-26 + 135 kg ha⁻¹ of urea (46-0-0 formula), which represents an application of 100, 54 and 70 kg ha⁻¹ of N, P₂O₅ and K₂O + EcoMic®10 kg ha⁻¹ + FitoMas-E® 2 L ha⁻¹.

The EcoMic®, biofertilizer based on arbuscular mycorrhizal fungi (AMF) was used in the experiment. It was applied by the technique of coating seeds when planting, 10 % of the seed weight spores at 30 g of solid substrate⁻¹ (11).

FitoMas-E®, natural biostimulant growth biostimulante natural de crecimiento, composed of high-energy biochemicals, mainly amino acids, bioactive oligosaccharides, with marked influence anti stress was applied in doses of 2.0 L ha⁻¹, dissolved in water for foliar sprinkler^A. The phase of the culture where FitoMas- E® was sprinkled, was Z.29 Zadocks scale (12), 31 days after germination.

The variables analyzed in the experimental work were:

- ♦ Biomass (kg ha⁻¹) from the dry mass (DM), (13). For this fresh mass (FM) of the aerial part of the plants that were in the area calculation weighed each plot with a scale of 0,5 kg precision. A sample of 20 plants was taken per treatment to each replica, which took a stove air circulation at 60 °C until a constant mass to determine the percentage of dry mass (DM) using this formula:

$$DM (\%) = \frac{DM \text{ sample (g)}}{\text{fresh sample mass (g)}} \times 100$$

DM yield was estimated from the yield of MV and percentage of DM, using the following formula:

$$DM (\text{kg ha}^{-1}) = \left[\frac{FM (\text{kg plot}^{-1}) \times DM (\%)}{100} \right] \times f^B$$

being:

f = 0,48 (factor to convert DM performance of kg plot⁻¹ kg ha⁻¹ in the plots of 14 m² of experiment).

- ♦ The percentage foliar nutrient concentrations; nitrogen (N), phosphorus (P) and potassium (K) were determined according to the methodologies described in the Manual of Laboratory of the National Institute of Agricultural Sciences (INCA) (14).
- ♦ The calculation of the percentage of net protein (crude) was performed using the formula:

$$\% \text{ Protein} = \% \text{ Nitrogen} \times 5,27$$

(conversion factor for wheat) (15)

MYCORRHIZAL VARIABLES

Ten samples of rhizosphere soil were taken which were homogenized and rootlets after washed were extracted. When they were dried, also they were stained by the staining technique roots (16). Besides the frequency and intensity of colonization was determined by the methodology intercepts (17).

The amount of mycorrhizal spores taking a sample of 50 g of rhizosphere soil was determined, and the methodology described for extraction followed by species (18).

Index Fertilization Efficiency Scheme which was calculated using the formula (19) was used to calculate the share of biofertilizers and mineral nutrition fertilization triticales:

$$IEEF (\%) = \left[\frac{\text{Yield DM (t ha}^{-1}) \text{ treatment inoculated and fertilized} - \text{DM yield (t ha}^{-1}) \text{ Witness}}{\text{DM yield (t ha}^{-1}) \text{ of control}} \right] \times 100$$

^A Instituto de Suelos. *Registro Central de Fertilizantes. Listado Oficial de Fertilizantes Autorizados*. Ed. Impresiones MINAG, 2010, Cuba, 30 p.

^B González, C. P. J. *Manejo efectivo de las asociaciones micorrízicas vía inoculación y suministro de nutrientes para pastos del género Brachiaria*. Ph.D. Tesis, Instituto Nacional de Ciencias Agrícolas, 2013, Mayabeque, Cuba, 80 p.

Data were analyzed by SPSS 11.5 statistical processor (IBM) for Windows (20). All experimental data met the assumptions of normality and homogeneity of variance, so we proceeded to run the ANOVA model for simple classification. The Docimo d'ocima Duncan's multiple range was used (with a significance of $p < 0.05$) for discriminating means (21).

ECONOMIC ANALYSIS

The economic analysis was based on the experimental results, taking into account the comparison of costs of mineral fertilizers and the bio-fertilizers applied in treatments in cuestión^c, shown in Table II, as well as the cost of farming associated with work performed (22).

Table II. Indicators of basic information for the calculating costs of the mineral fertilizer and bioproduct application

Indicator	Unit	CUP	References
Cost of labor for mineral fertilization (machinery, fuel and labor)	CUP ha ⁻¹	\$ 38,40	19
Balanced fertilizer price (Formula: 14-20-26)	CUP t ⁻¹	\$ 395,35	20
Price of fertilizer Urea. Formula: (46-0-0)	CUP t ⁻¹	\$ 334,50	20
Price of the mycorrhizal inoculant EcoMic [®]	CUP kg ⁻¹	\$ 2,50	12
Price of Fitomas-E [®]	CUP L ⁻¹	\$ 1,45	12

RESULTS AND DISCUSSION

Table III shows the results of the variables studied in experimental work. For the percentage of macronutrients contained in the feed, the results show that was 1,86 % for N; 0,237 % to 1,901 % phosphorus and potassium for treatment 3 (T3) and 1,81 % N; 0,237 % to 1,89 % phosphorus and potassium for treatment 2 (T2) with 150, 92 and 120 kg ha⁻¹ of N, P₂O₅ and K₂O without being different among them. In the absolute control treatment (without application of mineral fertilizers and bioproducts), significantly lower concentrations of N, P and K from the rest of the treatments were observed.

The results achieved with treatment 3, then allow considerably reduce the mineral fertilizer that is made in EPG "Niña Bonita", showing no significant differences in treatment 2, where mineral fertilizers applied in high doses.

The above stated is explained by the effect of an effective inoculation of AMF, as it allowed improving the concentrations of N, P and K in the aboveground biomass of forage, which demonstrates the importance of bio-fertilizers based arbuscular mycorrhizal fungi in making and assimilation of nutrients by plants (23).

Moreover, it can be considered that the use of natural growth stimulant (leaf application) influenced positively with their contribution macronutrients in the results of this treatment, which coincides with other authors (6), who expressed respect that, FitoMas-E[®] allows to increase the nutritional content of crops and increase agricultural yields thereof.

It can also be seen (Table III) that the application of bioproducts did not negatively influence the nutritional quality of forage.

In this regard, various authors (24, 25) indicate that the use of bioproducts in forage production does not diminish the nutritional quality of them and their use can significantly reduce application of mineral fertilizers, preventing pollution and damage to the ecosystem causing use in high doses.

As noted, the percentage of net protein (crude) shows that the treatment that most contributes to the quality of forage was the reduced with the implementation of AMF and FitoMas-E[®] fertilization, without differentiating significantly from the treatment of higher fertilization provided.

This result shows the possibility of reducing the dose of mineral fertilizers applied in the production of forage triticale based, with proper use of bio-fertilizers used in this study, without diminishing the quality of forage produced. In this sense, the results confirm the view on the importance of optimizing and streamlining the application of mineral fertilizers, with the application of biofertilizers, as the case of the AMF, expressed by different authors (26, 27).

The effect of the treatments on variables frequency and AMF colonization intensity and the number of spores g⁻¹ of soil is observed in Table III. Higher values significantly for T3 were recorded, compared with treatments 2 (T2) and absolute control (T1), who did not differ significantly among themselves, for these variables found in residents AMF.

^c MINAG (Ministerio de la Agricultura). *Precios de los fertilizantes y de la papa reconsumo*. Inst. Ministerio de la Agricultura, 2007, La Habana, Cuba, p. 1.

Table III. Effect of bioproducts FitoMas-E® and EcoMic® application in the experimental variables

Treatments	Foliar nutrient concentrations (%)			Crude Protein (%)	Mycorrhizal variables			Agricultural yield dry forage mass (kg ha ⁻¹)	Fertilizer Scheme Efficiency Index (%)
	N	P	K		Frecuency of colonization (%)	Colonization intensity (%)	# of spores 50 g ⁻¹ of soil		
1. Absolute control	1,319 b	0,192 b	1,583 b	7,27	31,92 b	2,031b	99,1 b	2710 b	
2. Application of 150, 92 y 120 kg ha ⁻¹ de N, P ₂ O ₅ y K ₂ O	1,81 a	0,237 a	1,893 a	9,22	30,68 b	1,972b	103,3 b	4335 a	59,96
3. Application of 100, 54 y 70 kg ha ⁻¹ de N, P ₂ O ₅ y K ₂ O + EcoMic® + FitoMas - E®	1,86 a	0,237 a	1,901 a	9,76	60,78 a	2,969a	292,3 a	4483 a	65,42
Es x	0,029**	0,004**	0,019***	0,316	1,305***	0,074*	14,66**	160,01	

Means with no common letter in the same column are significantly different at $P \leq 0.05$, according to Duncan 's test

These results demonstrate that application of AMF more FitoMas-E® and reduced dose of mineral fertilizers (T3), responds with increases in frequency and colonization intensity and spore content g⁻¹ of soil, against fertilization of high inputs. It allows greater absorption of nutrients by the crop and demonstrates the effective partnership between plant and AMF with positive increase thereof, with respect to treatments with high application of mineral fertilizers (T2) and the witness all (T1), which shows the activity of an efficient strain promoting greater plant-AMF relationship.

The results of this study corroborate those found by applying AMF in pasture production, where treatment inoculated with AMF exhibited higher percentages of mycorrhizal colonization, visual density and number of spores in the rhizosphere, that those not inoculated, which reflected the level occupation of the residents AMF (28).

By analyzing, the values obtained with high application of mineral fertilizers (T2) shows that no positive increase is not produced in the AMF activity by not registering differences, compared to absolute control. It is due to the application of high doses of fertilizers cannot be expected arbuscular mycorrhizal fungi efficient activity in biology rhizosphere of soil nor in other indicators, such as the stability of organic matter, microaggregates soil and improve its physical properties (29, 30).

The results demonstrate the desirability of reducing the mineral fertilization in high doses and can be replaced by biofertilizers as AMF and FitoMas-E®, which are harmless to the environment and ecosystems, as they allow, in turn, make the crops they were applied to achieve a higher nutritional quality (Table III).

In this sense when the AMF report, frequency and intensity of higher mycorrhizal colonization, provide more nutrients to cereals and these in turn, substances made to the AMF, primarily because of the symbiotic relationship established guest and host (31). In addition, efficient use of plant- arbuscular mycorrhizal fungi symbiosis may be a viable alternative in cereal production, reducing the mineral fertilization (32) and improve stability of the organic material, the structure and stability of soil aggregates (31, 32).

The results from the application of the treatments under study on the variables of agricultural forage yield (kg ha⁻¹ in dry biomass) and the Index of Efficiency

Scheme Fertilization in percent (IEEF) showed that the response to treatment 3 was higher, without significant differences with treatment 2. However, if they differ both absolute control treatment that is, the non-application of mineral fertilizers or biofertilizers.

These results indicate that may occur appreciable amounts of forage by decreasing the amount of mineral fertilizers when EcoMic® and FitoMas-E® are applied which are not aggressive to the environment or to the soil. A correspondence with the increased production of biomass (T3) which became effective mycorrhizal symbiosis, as frequency indices and mycorrhizal colonization intensity and number of spores produced so indicate (Table III), is also observed.

Similar results are related to the increase in agricultural yield fruit when inoculation with AMF is made to plants via symbiosis and the use of FitoMas-E® for its importance in the supply of nutrients in its capacity fitoestimulador growth and development plant (33).

In addition it is confirmed in this work, that the joint action of bio-fertilizers and rational use of mineral fertilizer it possible to obtain satisfactory results in agricultural production (33).

In addition, the results of studies in the cultivation of habichuela beans (*Phaseolus vulgaris* L. var. Verlili) showed the effectiveness of the products in growth, development and yield, where plants received the EcoMic® + FitoMas-E® (34) combination; ie are confirmed by this study, the positive results of the combined use of these bioproducts in different agricultural crops.

Another important aspect was the variable Index Efficiency Scheme Fertilization (IEEF), where a higher percentage of it is shown, with reduced levels of fertilization plus the application of arbuscular mycorrhizal fungi and FitoMas-E® (T3), without decreasing forage production in quantity or quality, about where the higher dose of mineral fertilizers (T2) is applied.

It should be noted that the results for this variable (% of IEEF) coincided with those obtained by decreasing the dose of mineral fertilizers with inoculation of AMF and application of FitoMas-E® in growing *Psidium guajava*. L cv, Enana Roja Cubana (33).

Moreover, these results confirm studies in the maize cultivation (35). When the AMF and FitoMas-E® were applied, improved the efficiency of crop fertilization scheme (IEEF), based on the knowledge of the contents nutritional soil and the use of these bioproducts, a proper management of fertilization is made, improving nutrition of plants grown and achieving a reduction in the dose of fertilizers used.

In Table IV, the economic impact is shown in the production of fodder with treatment 3, which was higher compared to the traditional production technology in the EPG "Niña Bonita" for the production of fodder, because there were savings 74,61 CUP ha⁻¹, by reducing fertilizer dosage without reducing yields. This meant a cost reduction of fertilization of 27,04 CUP to produce one ton of forage, so it can achieve a positive influence on the environmental and economic environment by reducing the application of mineral fertilizers (31).

In addition, controlling the excessive use of mineral fertilizers and promoting the use of biofertilizers, it promotes better crop development and the economy for producers in different crops is improved, such as cereals (36).

Table IV. Economic evaluation of results

Treatments	Cost of fertilization (CUP ha ⁻¹)				Total	Saving (CUP)	Cost of fertilization / T forage (CUP)
	Complete formula 14-20-26	Urea 46-0-0	Ecomic®	Fitomas-E®			
Application of 150, 92 and 120 kg ha ⁻¹ of N, P ₂ O ₅ and K ₂ O	181,86	62,55	-	-	244,41	-	56,38
Application of 100, 54 and 70 kg ha ⁻¹ of N, P ₂ O ₅ and K ₂ O + EcoMic® + Fitomas - E®	106,74	45,16	2,90	15,00	169,80	74,61	37,92

CONCLUSIONS

- ◆ The application of arbuscular mycorrhizal fungi and FitoMas-E® product helps to reduce the amount of mineral fertilizer in forage production based on triticale, since there were no differences regarding treatment where standards fertilization where it was developed were applied the experiment.
- ◆ The cost of fertilizer per ton of forage produced was less when the AMF and FitoMas-E® were applied so it became more profitable forage production.
- ◆ Variables forage quality were similar and as a result of the AMF application, greater presence of these fungi was observed on the soil (where they were applied), reflecting greater biological activity in the rhizosphere of the crop, with a positive possible effect on the soil improvement.

BIBLIOGRAPHY

1. Dwivedi, S. L.; Sahrawat, K. L.; Rai, K. N.; Blair, M. W.; Andersson, M. S. y Pfeiffer, W. "Nutritionally Enhanced Staple Food Crops" [en línea]. En: ed. Janick J., *Plant Breeding Reviews*, Ed. John Wiley & Sons, Inc., Hoboken, NJ, USA, 26 de septiembre de 2012, pp. 169-291, ISBN 978-1-118-35856-6, [Consultado: 8 de junio de 2016], Disponible en: <<http://doi.wiley.com/10.1002/9781118358566.ch3>>.
2. Costa, K. A.; Severiano, E. C.; Simon, G. A. y Carrijo, M. S. "Extração de nutrientes do capim-marandusob doses e fontes de nitrogênio". *Revista Brasileira de Saúde e Produção Animal*, vol. 10, no. 4, 2009, pp. 801-812, ISSN 1519-9940.
3. Souza, R. F. de; Faquin, V.; Lima Sobrinho, R. R. y Oliveira, E. A. B. de. "Influência de esterco bovino e calcário sobre o efeito residual da adubação fosfatada para a *Brachiaria brizantha* cultivada após o feijoeiro". *Revista Brasileira de Ciência do Solo*, vol. 34, no. 1, febrero de 2010, pp. 143-150, ISSN 0100-0683, DOI 10.1590/S0100-06832010000100015.
4. Quero, C. A. R.; Enríquez, Q. J. F. y Miranda, J. L. "Evaluación de especies forrajeras en América tropical, avances o status quo". *Interciencia*, vol. 32, no. 8, 2007, pp. 566-571, ISSN 0378-1844.
5. Ammar, K. "Promoción y mejoramiento genético del triticale". *Revista EnIACe - CIMMYT. Agricultura de Conservación*, vol. 5, no. 16, 2013, pp. 27-29, ISSN en trámite.
6. Carneiro, R. F. V.; Martins, M. A.; Vásquez, H. M. y Detmann, E. "Doses de fósforo e inoculação micorrízica no cultivo de estilosantes em solo sob condições naturais". *Archivos de Zootecnia*, vol. 59, no. 227, septiembre de 2010, pp. 415-426, ISSN 0004-0592.
7. Montano, R.; Zuaznabar, R.; García, A.; Viñals, M. y Villar, J. "Fitomas E: Bionutriente derivado de la industria azucarera". *ICIDCA. Sobre los derivados de la caña de azúcar*, vol. 41, no. 3, 2007, pp. 14-21, ISSN 0138-6204.
8. Rodríguez, Y.; Dalpé, Y.; Séguin, S.; Fernández, K.; Fernández, F. y Rivera, R. A. "*Glomus cubense* sp. nov., an arbuscular mycorrhizal fungus from Cuba". *Mycotaxon*, vol. 118, no. 1, 5 de enero de 2012, pp. 337-347, ISSN 0093-4666, 2154-8889, DOI 10.5248/118.337.
9. IUSS Working Group WRB. *World reference base for soil resources 2006. A framework for international classification, correlation and communication*. (ed. Micheli E.), (ser. World Soil Resources Reports, no. ser. 103), 2.ª ed., Ed. Food and Agriculture Organization of the United Nations, 2007, Rome, Italy, 128 p., ISBN 978-92-5-105511-3.
10. Plana, L. R.; González, C. P. J.; Álvarez, G. M. A.; Arzola, B. J.; Ramírez, P. J. F.; Marrero, C. Y. y Fundora, S. L. R. "INCA TT-7. Primer cultivar cubano de triticale (*X. Triticosecale* Wittmack)". *Cultivos Tropicales*, vol. 34, no. 3, 1 de julio de 2013, pp. 64-65, ISSN 0258-5936.
11. Fernández, F.; Gómez, R.; Vanegas, L.; Noval, B. M. y Martínez, M. A. *Producto inoculante micorrizógeno*. no. 22641, Inst. Oficina Nacional de Propiedad Industrial, 2000, Cuba.
12. Tottman, D. R.; Makepeace, R. J. y Broad, H. "An explanation of the decimal code for the growth stages of cereals, with illustrations". *Annals of Applied Biology*, vol. 93, no. 2, 1 de octubre de 1979, pp. 221-234, ISSN 1744-7348, DOI 10.1111/j.1744-7348.1979.tb06534.x.
13. de la Roza, D. M. B.; Martínez, F. A. y Argentería, G. A. "Determinación de materia seca en pastos y forrajes a partir de la temperatura de secado para análisis". *Pastos. Revista de la Sociedad Española para el Estudio de los Pastos*, vol. 32, no. 1, 2002, pp. 91-104, ISSN 0210-1270.
14. Paneque, P. V. M.; Calaña, N. J. M.; Calderón, V. M.; Borges, B. Y.; Hernández, G. T. C. y Caruncho, C. M. *Manual de técnicas analíticas para análisis de suelo, foliar, abonos orgánicos y fertilizantes químicos* [en línea]. Ed. Ediciones INCA, 2010, La Habana, Cuba, 157 p., ISBN 978-959-7023-51-7, [Consultado: 27 de enero de 2016], Disponible en: <<http://mst.ama.cu/578/>>.
15. Tkachuk, R. "Calculation of the nitrogen-to-protein conversions factor". En: Hulse J. H., Rachie K. O., y Billingsley L. W., *Nutritional standards and methods of evaluation for food legume breeders*, Ed. International Development Research Centre, Ottawa, 1977, pp. 78-82, ISBN 978-0-88936-137-9, OCLC: 5361557.
16. Phillips, J. M. y Hayman, D. S. "Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection". *Transactions of the British Mycological Society*, vol. 55, no. 1, 1 de agosto de 1970, pp. 158-168, ISSN 0007-1536, DOI 10.1016/S0007-1536(70)80110-3.
17. Trouvelot, A.; Kough, J. y Gianinazzi, P. V. "Mesure du Taux de mycorhization VA d'un Systeme Radiculaire. Recherche de Methodes d' Estimation ayant une Signification Fonctionnelle". En: eds. Gianinazzi P. V. y Gianinazzi S., *1st European Symposium on Mycorrhizae*, Ed. Institut National de la Recherche Agronomique, Paris, 1986, pp. 217-222, ISBN 978-2-85340-774-8.
18. Gerdemann, J. W. y Nicolson, T. H. "Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting". *Transactions of the British Mycological Society*, vol. 46, no. 2, junio de 1963, pp. 235-244, ISSN 00071536, DOI 10.1016/S0007-1536(63)80079-0.

19. Siqueira, J. O. y Franco, A. A. *Biotechnologia do solo: fundamentos e perspectivas* [en línea]. Ed. MEC: ABEAS - Lavras: ESAL: FAEPE, 1988, Brasilia, Brasil, 235 p., [Consultado: 8 de junio de 2016], Disponible en: <<https://books.google.com/cu/books?id=9eZoygAACAAJ>>.
20. IBM Corporation. *IBM SPSS Statistics* [en línea]. versión 11.5, [Windows], Multiplataforma, Ed. IBM Corporation, 2003, U.S, Disponible en: <<http://www.ibm.com>>.
21. Duncan, D. B. "Multiple Range and Multiple F Tests". *Biometrics*, vol. 11, no. 1, 1 de marzo de 1955, pp. 1-42, ISSN 0006-341X, DOI 10.2307/3001478.
22. Cino, D. M.; Padilla, C. y Sardiñas, Y. "Propuesta de fichas de costo de nuevos cultivos forrajeros". *Revista ACPA*, vol. 26, no. 2, 2007, pp. 48-49, ISSN 0138-6247.
23. Hodge, A.; Helgason, T. y Fitter, A. H. "Nutritional ecology of arbuscular mycorrhizal fungi". *Fungal Ecology*, vol. 3, no. 4, noviembre de 2010, pp. 267-273, ISSN 17545048, DOI 10.1016/j.funeco.2010.02.002.
24. Maguire, R. O.; Kleinman, P. J. A. y Beegle, D. B. "Novel Manure Management Technologies in No-Till and Forage Systems: Introduction to the Special Series". *Journal of Environment Quality*, vol. 40, no. 2, 2011, p. 287, ISSN 1537-2537, DOI 10.2134/jeq2010.0396.
25. Bonfante, P. y Genre, A. "Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis". *Nature Communications*, vol. 1, no. 4, 27 de julio de 2010, pp. 1-11, ISSN 2041-1723, DOI 10.1038/ncomms1046.
26. Mujica Pérez, Y. "Inoculación de hongos micorrízicos arbusculares (HMA) por dos vías diferentes en el cultivo del tomate (*Solanum lycopersicum* L.)". *Cultivos Tropicales*, vol. 33, no. 4, diciembre de 2012, pp. 71-76, ISSN 0258-5936.
27. Helgason, B. L.; Walley, F. L. y Germida, J. J. "No-till soil management increases microbial biomass and alters community profiles in soil aggregates". *Applied Soil Ecology*, vol. 46, no. 3, noviembre de 2010, pp. 390-397, ISSN 0929-1393, DOI 10.1016/j.apsoil.2010.10.002.
28. González, P. J.; Rivera, R.; Arzola, J.; Morgan, O. y Ramírez, J. F. "Efecto de la inoculación de la cepa de hongo micorrízico arbuscular *Glomus hoi-like* en la respuesta de *Brachiaria HÍBRIDO* cv. MULATO II (CIAT 36087) a la fertilización orgánica y nitrogenada". *Cultivos Tropicales*, vol. 32, no. 4, diciembre de 2011, pp. 05-12, ISSN 0258-5936.
29. Raviv, M. "The use of mycorrhiza in organically-grown crops under semi arid conditions: a review of benefits, constraints and future challenges". *Symbiosis*, vol. 52, no. 2-3, diciembre de 2010, pp. 65-74, ISSN 0334-5114, 1878-7665, DOI 10.1007/s13199-010-0089-8.
30. Miransari, M. "Arbuscular mycorrhizal fungi and nitrogen uptake". *Archives of Microbiology*, vol. 193, no. 2, febrero de 2011, pp. 77-81, ISSN 0302-8933, 1432-072X, DOI 10.1007/s00203-010-0657-6.
31. Gutjahr, C.; Casieri, L. y Paszkowski, U. "*Glomus intraradices* induces changes in root system architecture of rice independently of common symbiosis signaling". *New Phytologist*, vol. 182, no. 4, 1 de junio de 2009, pp. 829-837, ISSN 1469-8137, DOI 10.1111/j.1469-8137.2009.02839.x.
32. Hirel, B.; Tétu, T.; Lea, P. J. y Dubois, F. "Improving Nitrogen Use Efficiency in Crops for Sustainable Agriculture". *Sustainability*, vol. 3, no. 12, 7 de septiembre de 2011, pp. 1452-1485, ISSN 2071-1050, DOI 10.3390/su3091452.
33. Ramos, H. L.; Reyna, G. Y.; Lescaille, A. J.; Telo, C. L.; Arozarena, D. N. J.; Ramírez, P. M. y Martín, A. G. M. "Hongos micorrízicos arbusculares, *Azotobacter chroococcum*, *Bacillus megatherium* y FitoMas-E: una alternativa eficaz para la reducción del consumo de fertilizantes minerales en *Psidium guajava*, L. var. Enana Roja cubana". *Cultivos Tropicales*, vol. 34, no. 1, marzo de 2013, pp. 05-10, ISSN 0258-5936.
34. Terry, A. E.; Ruiz, P. J.; Tejeda, P. T. y Díaz, de A. M. M. "Respuesta del cultivo de la habichuela (*Phaseolus vulgaris* L. var. Verilili.) a la aplicación de diferentes bioproductos". *Cultivos Tropicales*, vol. 34, no. 3, septiembre de 2013, pp. 05-10, ISSN 0258-5936.
35. Calderón, P. A. A.; Marrero, C. Y. J.; Martín, C. J. V. y Mayo, I. "La fertilidad de los suelos y su importancia en el empleo de bioproductos en la provincia de Sancti Spiritus". *Cultivos Tropicales*, vol. 34, no. 2, junio de 2013, pp. 16-23, ISSN 0258-5936.
36. Corbera, G. J. y Nápoles, G. M. C. "Efecto de la inoculación conjunta *Bradyrhizobium elkanii*-hongos MA y la aplicación de un bioestimulador del crecimiento vegetal en soya (*Glycine max* (L.) Merrill), cultivar INCASOY-27". *Cultivos Tropicales*, vol. 34, no. 2, junio de 2013, pp. 05-11, ISSN 0258-5936.

Received: September 30th, 2014

Accepted: February 2nd, 2015