Volume 1, Issue 1 — July — December-2015

ECORFAN

Journal- Republic of Nicaragua

ISSN-On line: 2414-8830

ECORFAN®

ECORFAN-Republic of Nicaragua

Indexing

Academic Google

ECORFAN Journal-Republic of Nicaragua, Volume 1, Issue 1, July-December 2015, is a journal edited semestral by ECORFAN. First Avenue Southwest, San Sebastian area, León, Republic of Nicaragua. P. C. 21000. WEB:www.ecorfan.org/republicofnicaragua/, journal@ecorfan.org. Editor in Chief: RAMOS-ESCAMILLA, María. ISSN-On line: 2414-8830. Responsible for the latest update of this number ECORFAN Computer Unit. ESCAMILLA-BOUCHÁN, Imelda, LUNA-SOTO, Vladimir, First Avenue Southwest, San Sebastian area, León, Republic of Nicaragua. C.P. 21000, last updated December 31, 2015.

Editorial Board

Arbitration Committee

Presentation

ECORFAN Journal-Republic of Nicaragua is a research journal that publishes articles in the areas of:

Agriculture, Forest Pathology, Sustainable Forest Management, Horticulture and Engineering and Integrated Water Use

In Pro-Research, Teaching and Training of human resources committed to Science. The content of the articles and reviews that appear in each issue are those of the authors and does not necessarily the opinion of the editor in chief.

In Number 1st presented an article Callus Genesis in *Lupinus montanus* HBK from explants cultivated in vitro *by RAMÍREZ-GONZÁLEZ, Gabriel, RODRÍGUEZ-DE-LA-O., José Luis, ZAPATA-MONTES, Nery Javier, ÁLVAREZ-MOCTEZUMA, José Guadalupe with adscription in the Universidad Autónoma Chapingo, in the next Section an article* Characterization of material pyrolytic biomass agricultural *by LUGO-VALENZUELA, Homero, VÁZQUEZ-PEÑA, Mario A., PRADO-HERNÁNDEZ, Jorge V. and VELÁZQUEZ-LÓPEZ Noé with adscription in the Universidad Autónoma Chapingo, in the next Section an article:* Collembola indicators of soil fertility *by CALYECAC-CORTERO, Humberta Gloria, MIRANDA-RANGEL, Andrés and JIMÉNEZ-MORALES, Margarita with adscription in the Universidad Autónoma Chapingo, in the next Section an article* Chemical compounds of essential oil of *Tagetes* species of Ecuador *by* ZAPATA-MALDONADO, Christian Iván, SERRATO-CRUZ, Miguel Ángel, IBARRA, Emmanuel and NARANJO-PUENTE, Blanca *with adscription in the Universidad Autónoma Chapingo, Mexico and Universidad de las Fuerzas Armadas ESPE, Ecuador, in the next Section an article:* Rove diversity (coleoptera: Staphylinidae) in six coffee agroecosystems of Central Valley of Costa Rica *by CALVO-ARAYA, José Alonso & GONZALEZ-HERRERA, Allan, with adscription in Universidad Nacional de Costa Rica, in the next Section an article* Diagnosis of mycotoxigenic fungi instored grain corn *by* MAIDANA-OJEDA, Marco, ACOSTA-RAMOS, Marcelo, ARÁMBULA-VILLA, Gerónimo and CABRERA-MARÍA, Graciela *with adscription in Universidad Autónoma Chapingo, Instituto Politécnico Nacional and Universidad Nacional del Nordeste, in the next Section an article Evaluation of production explant or boneless blackberry Rubus Glaucus* Benth *in vegetative multiplication phase in a system of temporary immersion by ZAPATA-MALDONADO, Christian Iván, LANDAZURI-ABARCA, Pablo Anibal and TAIPE, Marco Arturo, with adscription in the Universidad de las Fuerzas Armadas, Ecuador.*

Content

Article **ECORFAN Journal**
December 2015 Vol.1 No.1 1-6

Callus Genesis in *Lupinus montanus* HBK from explants cultivated in vitro

RAMÍREZ-GONZÁLEZ, Gabriel´*†, RODRÍGUEZ-DE-LA-O., José Luis´´, ZAPATA-MONTES, Nery Javier´´ and ÁLVAREZ-MOCTEZUMA, José Guadalupe´

´ Division of Forestry Science, Universidad Autónoma Chapingo, Mexico-Texcoco highway Km 38.5, Texcoco, CP 56230 State of Mexico.
´´Department of Plant Science, Universidad Autónoma Chapingo, Mexico-Texcoco highway Km 38.5, Texcoco, CP 56230 State of Mexico.

Received January 28, 2015; Accepted October 20, 2015

Abstract

Lupinus montanus HBK is a herbaceous species that is widely distributed in Mexico, primarily in the Sierra Madre Occidental and the Transversal Neovolcanic. It is characterized by synthesizing alkaloids whose fungicides, bactericides and hypoglycemic properties have been reported experimentally. However, obtaining these metabolites via extraction from cultivated plants involves investment costs that could turn it into a very profitable activity. In this research the main factors involved in the development of calluses that could be directed toward studying the synthesis and production of secondary metabolites or to micropropagation were evaluated via indirect organogenesis. The research results indicated that the factors evaluated (explant culture medium and incubation) and their interactions show differences in responses; under light conditions callus compact structure is obtained, while in dark friable calluses were obtained. The highest yields were obtained in biomass callus originated from Epicotyl in combinations of 2,4-D, BA and Kinetin in concentrations of the order of (4-6μM; 1-2μM and 2-2μM respectively) under dark conditions.

***Lupinus montanus*, Friable, necrosis, Auxin, cytokinin.**

Citation: RAMÍREZ-GONZÁLEZ, Gabriel, RODRÍGUEZ-DE-LA-O., José Luis, ZAPATA-MONTES, Nery Javier and ÁLVAREZ-MOCTEZUMA, José Guadalupe. Callus Genesis in *Lupinus montanus* HBK from explants cultivated in vitro. ECORFAN Journal-Republic of Nicaragua 2015, 1-1: 1-6

* Correspondence to Author (email: arzemir@gmail.com)
† Researcher contributing first author.

Introduction

Several studies point to the importance of in vitro for the initiation of cell suspension cultures for the synthesis of secondary metabolites callogenesis. Similarly, from calluses obtained in vitro can induce the formation of both plants (indirect organogenesis) and somatic embryos (direct embryogenesis) through which could obtain specific clones according own research interests (Martinez et al., 2010).

In woody plants such as *Lupinus montanus* herbaceous- HBK, induction and callus culture is somewhat more complicated compared to other plants. This complication is due primarily to the large amount of metabolic substances tissue released in response to oxidative stress to which it is subjected after being cut (Tang and Newton, 2004). While it is true that the use of some of these metabolites as lupanine alkaloid has been experimentally reported both medical area (Dove et al., 2011) and the agrochemical (Bermudez et al., 2009), it is also true that in the initial explant management stage, the combination of these with phenols and other exudates metabolites is undesirable.

The objective of this research was to evaluate the response to callus formation and their gain in biomass from three different types of explants (hypocotyl and epicotyl Cotyledon) grown in media added with different concentrations of auxin 2,4 dichlorophenoxyacetic acid (2,4-D), cytokinin 6-benzylaminopurine (BA) and 6-furfurylaminopurine (kinetin) and two types of incubation.

Material and methods

The research was conducted on the premises of the Laboratory of plant tissue culture in the Department of Plant Science at the Universidad Autónoma de Chapingo.

Plant material. As seedling explant source *Lupinus montanus* four weeks of age, obtained by germinating seeds in vitro in MS culture media (Murashigue and Skoog, 1962) were used. The seeds were obtained from plants collected in 2011 on the hill of Xipes (19 ° 00 '48' 'N, 97 ° 21' 20 " W), municipality of Libres, Puebla. The plants were identified by taxonomic keys and descriptions of Dunn (1979), subsequently they validated and placed in the National Herbarium of Mexico (MEXU) of the Institute of Biology of the Universidad Nacional Autónoma De México.

Obtaining explant. It was harvested at random a total of 10 seedlings after 4 weeks of growth. The regions used as explant source: hypocotyl, cotyledon and epicotyl, were sectioned in approximate size 3x3x3 mm, and then planted in MS (Murashigue and Skoog, 1962) in petri dishes under six different treatments with 24 replicates each. The treatments were the following T0 = control without plant growth regulators (RCV), T1 = 2,4-D (1.0 **μM**) + BA (0.5 **μM**), T2 = 2,4-D (1.0 **μM**) + kinetin (0.5 M), T3 = 2,4-D (2.0 **μM**) + BA (2.0 M) + kinetin (1.0μM), T4 = 2,4-D (4.0 **μM**) + BA (1.0 M) + kinetin (2.0μM) and T5 = 2,4-D (6.0 **μM**) + BA (2.0 M) + kinetin (2.0μM). In all cases the RCV were supplemented in MS 100%, supplemented with 0.40 mg L-1 thiamine, 100 mg L-1 myo-inositol, 3% sucrose and 7 g L-1 agar, and 100 mg L-1 of activated charcoal at a pH of 5.7 ± 0.1.

ISSN-On line: 2414-8830

Subsequently, 12 repetitions of each treatment were incubated under conditions of constant darkness at 25 ± 1 ° C, while the remaining 12 for each treatment period were incubated under light / dark (16: 8) at an average temperature of 25 ± 1 ° C, both the incubation period was four weeks to recording variables evaluated.

Callogenic response was evaluated with a qualitative scale of 0 to 1, where 0 = no answer, 1 = callus formation. For the variable level of necrosis, the scale was 0 to 3: where 0 = (<10%), 1 = (> 10 <30%), 2 = (> 30 <60%), 3 (> 60%) . The color and structure of the callus were determined by observations cream or green compact or friable, respectively. Furthermore, for quantitative evaluation variable (biomass) is randomly selected callus six samples of each treatment and the increase in fresh weight was obtained in each case.

Statistical analysis. The results of qualitative variables were analyzed under a completely randomized design (DCA) by Kruskal-Wallis test ($P \leq 0.05$). While results in increased biomass were evaluated in a factorial arrangement DCA, using the model:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + (AB)_{ij} + (AC)_{ik} + (BC)_{jk} + (ABC)_{ijk} + \xi_{ijkl}$$

Where:
Y_{ijkl} = Response biomass increase
μ = General Media
A_i = Effect of the explant (hypocotyl, cotyledon and epicotyl)
B_j = Effect of treatment (2,4-D: BA: Kinetin)
C_k = Effect of incubation (photoperiod: permanent darkness)
$(AB)_{ij}$ = Explant interaction Treatment
$(AC)_{ik}$= Explant interaction: incubation
$(BC)_{jk}$= Interaction treatment: incubation
$(ABC)_{ijk}$= Explant interaction Treatment: incubation
ξijk = Experimental error

The data analysis of variance and Tukey ($P \leq 0.05$) were submitted in all cases the Minitab 17.0 statistical software was employed (2013).

Results and discussion

Callogeneic response. After four weeks it shows that the culture media presenting combinations of plant hormones induced callus formation in three different types of explant under both incubation procedures (photoperiod: permanent darkness), contrasting with that observed in the control treatment (T0) in which there was no response (Figure 1).

No statistical difference ($p = 0.0789$) for callus formation between treatments with combinations of 2,4-D auxin BA with respect to those with combinations of 2,4-D with Kinetin found. Similarly explant level was not statistically different ($p = 0.0876$) because from the three tissues callus formation was presented.

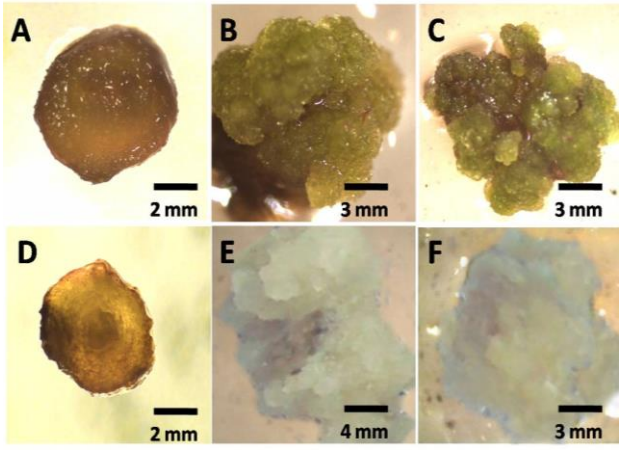


Figure 1 Callus formation under conditions of incubation photoperiod (16: 8). A) Hypocotyl (T0), B) epicotyl (T3), C) Cotyledon (T4); Callus formation under conditions of constant darkness. D) Epicotyl (T0), E) Epicotyl (T5), F) Hypocotyl (T3).

ISSN-On line: 2414-8830

These results agree with those reported by Montes et al., (2009), who managed to induce callus formation of Lupinus ascherbornii from cotyledon explants of root and hypocotyl, with combinations of 2,4-D and kinetin, in concentrations 1 mg L-1a 2 mg L- 1 (4 to 8 mM).

Degree of necrosis. The Kruskal Wallis noted that statistically there is no difference regarding the degree of necrosis of the explants incubated under conditions of photoperiod and permanent darkness (p-value = 0.105) treatments. In general, under dark conditions, the level of necrosis which had calluses formed from the three types of explant was less than 5% of all occurrences incubated in the different treatments. On the other hand, under conditions of light / dark (16: 8), the level of necrosis was close to 12% of all occurrences; 15 of them were for cotyledon, hypocotyl and 8 to 3 to Epicotyl. It should be noted that the degree of tissue necrosis presenting these corresponded in all cases to the category of level 1 (> 10 <30%).

Callus color and structure. It was observed that both the color and the structure of the callus were intimately associated with the incubation conditions (p-value = 0.000), regardless of the culture medium and the explant source. In terms of light / dark, chlorophyll present in the cells of the callus was expressed generating a uniform green pattern (Figure 2A) while under steady dark, chlorophyll was inhibited, as a result, the fabric the tissue acquired a creamy tonality (Figure 2B).

This fact agrees with the commonly observed in in vitro cultures of various species under both conditions, as said work Parsaeimehr et al., (2010) and Tavakkol et al; (2011), who when evaluating the effect of light and growth regulators on callus induction of herbaceous *Brassica napus* L., Ephedra observed the same tones in corns grown in light and dark.

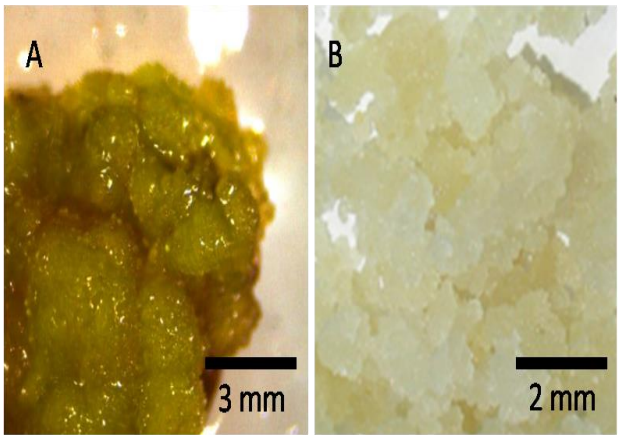


Figure 2 Color and structure calluses. A) Callus formed from hypocotyl explant under conditions of light / dark (T4). B) Callus formed from hypocotyl explant under conditions of permanent darkness (T4).

On the other hand, Figure 2A illustrates the compact structure of callus had grown photoperiod light / dark, while in the second case (Figure 2B), was friable structure type in all instances. This result is in line with those reported by Rajiv and Yadav (2006) on the influence of light on induction of friable calluses incubated at low intensities of light or dark, for various plant species.

Callus biomass. The variance analysis allowed observing the effects that the factors evaluated in increasing biomass. A level explants, there was statistically significant difference (p-value = 0.000) compared to the effect of the explant in biomass response, regardless of the medium or incubation condition which was subjected to (Figure 3A). The explant from which formed callus with the highest biomass was epicótilo (1.3307 ± 0.5151), whereas among the hypocotyl and cotyledon not point Tukey statistical difference (p-value = 0.0637).

ISSN-On line: 2414-8830

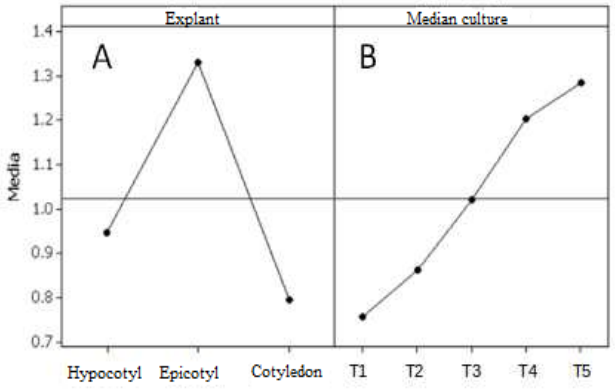


Figure 3 Charts major effects on biomass of corn. A) Effect of explant. B) Effect of culture medium.

A culture media level analysis of variance showed that there was a significantly different response for biomass response (p value = 0.000). In Figure 3B the positive effect of culture media with higher concentrations of 2,4-D, BA and kinetin in the callus biomass increase, regardless of the incubation was observed. Induced responses culture media T4 and T5 were significantly different and greater than those obtained with the other media. Similarly, the effect of incubation factor was statistically different (p-value = 0.000) to the response obtained in permanent darkness photoperiod.

Figure 4 shows the interaction between the various factors discussed in the experiment. The answer callus biomass was higher for the three types of explant cultures incubated under conditions of constant darkness (Figure 4A).

The interaction between culture media and incubation process, significant (p-value = 0.002). The average responses for media with higher content of 1 mM 2,4-D, BA and / or Kinetin (T3, T4, T5) were significantly different from those obtained with the media whose cytokinin concentrations were less than 1 PM (T1, T2).

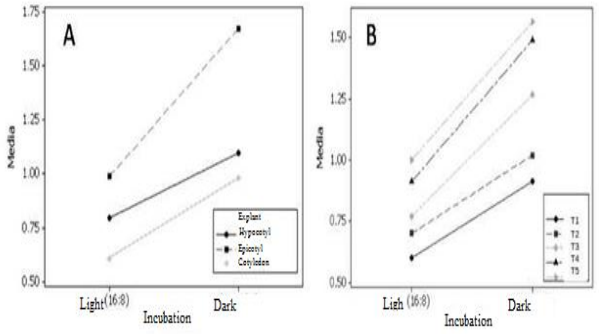


Figure 4 Interaction Charts. E) vs incubation Interaction explant. B) Interaction of culture medium vs incubation.

For any of the explants evaluated under any of the different culture media, the response in biomass is increased, going from a state of incubation in the light, to a phase of permanent darkness (Figure 4A).

The increase in biomass observed in the dark, could be associated to the fact that auxin not undergo degradation by photo-oxidation, allowing a greater extent are present in both the medium (exogenous) and the tissue (endogenous) further promoting cell elongation and consequently the further development of biomass compared to what happens in lighting conditions, as explained some research at the level of plant tissues (Taiz and Zeiger, 2010).

Conclusions

All combinations of different types used RCV induced callus formation from three different explants. Percentages of necrosis of the tissues were very low (5% in darkness photoperiod at 12%) and did not affect the survival of the tissue. Moreover, the calluses formed from any of the explants incubated both photoperiod and dark level differed coloration and structure being green and compact structure under conditions of light / dark, and cream and friable structure under conditions of constant darkness.

ISSN-On line: 2414-8830

Corns (both compact and friable) generated from epicotyl were those with higher gain in biomass, while cotyledon was the one that produced less biomass. With respect to the culture media, and increasing dose combination of 2,4-D, BA and kinetin potentiate a better response in biomass increase for the three types of explant.

Acknowledgmets

To co-authors for their time and dedication in reviewing and contribution to the development of this document. Dr. Jose Luis Rodriguez de la O, for knowledge, spaces and times provided for the realization of this research. The National Council of Science and Technology of Mexico through a scholarship to the first author through the National Quality Graduate Program.

References

Bermúdez, K., H.J. Martínez, R. Figueroa, L. Legal y M. Wink (2009). Activity of quinolizidine alkaloids from three Mexican *Lupinus* against the lepidopteran crop pest *Spodoptera frugiperda*. BioControl, 54: 459-46.

Dove, E. R., T.A. Mori, G.T. Chew, A.E. Barden, R.J. Woodman, I. B. Puddey, S. Sipsas, and J.M. Hodgson. (2011). *Lupin* and soya reduce glycemia acutely in type 2 diabetes. British Journal of Nutrition, 106: 1045-1051.

Martínez, R., H.S. Azpiroz, J.L. Rodríguez, N.M. Cetina y M.A. Gutiérrez. (2010). La Biotecnología y su aplicación en los recursos forestales. In: Biotecnología Aplicada a los Recursos Forestales. Martínez, R.; Azpiroz, H.S.; Rojo, G.E.; Rodríguez, J.L. y Ramírez, B. (coords).1ª edición. Universidad Autónoma Indígena de México, Universidad Autónoma Chapingo. Colegio de Postgraduados. Campus Puebla. 1-36 pp.

Minitab Inc. (2013). Diseño de un experimento. Meet Minitab 16. 2-12pp.

Montes, E., Ma. Corona, G. Trejo, J. L. Rodríguez y K. Bermúdez. (2009). Evaluación de fitoreguladores para la inducción de callo de *Lupinus ascherbornii.* En: VII Simposio Internacional de Producción de Alcoholes y Levaduras. Acapulco, Guerrero, 2009. XIII Congreso Nacional de Biotecnología y Bioingeniería.

Murashige, T., y F. Skoog. (1962). A revised medium for rapid growth and bioassays with tobacco cultures. Physiology Plant. 15: 473–497.

Parsaeimehr, A., E. Sargsyan y K. Javidnia. (2010). Influence of plant growth regulators on callus induction, growth, chlorophyll, ephedrine and pseudoephedrine contents in *Ephedra procera.* Journal of Medicinal Plants Research Vol. 4(13), pp. 1308-1317.

Rajiv, T. y P.R. Yadav. (2006). Biotechnology Of Plant Tissues.Discovery Publishing House.1°.Edición (2006).Darya Ganj, New Delhí, India.74-87 pp.
Taiz, L. y Zeiger, E. (2010). Plant Physiology, Fifth Edition. Sinauer Associates. Sunderland, MA. 428- 453. pp.

Tang, W., y R. Newton. (2004). Increase of polyphenol oxidase and decrease of polyamines correlate with tissue browning in Virginia pine (*Pinus virginiana* Mill.).Plant Science 167: 621-628.

Tavakkol R., R. Angoshtari y S. Kalantari. (2011). Effects of light and different plant growth regulators on induction of callus growth in rapeseed (*Brassica napus* L.) genotypes. POJ 4(2):60-67

Article **ECORFAN Journal**
December 2015 Vol.1 No.1 7-11

Characterization of material pyrolytic biomass agricultural

LUGO-VALENZUELA, Homero*†, VÁZQUEZ-PEÑA, Mario A., PRADO-HERNÁNDEZ, Jorge V., VELÁZQUEZ-LÓPEZ Noé

Universidad Autónoma Chapingo. Department of Irrigation, Mexico-Texcoco highway, km 38.5, Chapingo, State of Mexico, C.P. 56230, Mexico.

Received March 14, 2015; Accepted October 21, 2015

Abstract

There are a lot of agricultural waste in agriculture. These are causes of pests in the soil due to its excess and further complicate agricultural work. A possible solution is to transform agricultural waste into organic additives, through a process of pyrolysis, these additions to the soil help to change their physical properties such as: porosity, apparent density and infiltration in the short term. Therefore, this work focused on quantities of materials pyrolytic encountered in corn, sorghum, chickpea, and cane, sawdust waste. The latter served as a witness. To achieve this, were collected 5 specimens of each material in the municipality of Guasave, Sinaloa, Mexico. The materials were ground and is sieved with a 2mm particle diameter. Subsequently implemented a bath of 50 ml of extract from orange peel and dried in the oven at 60 ° C. Finally the pyrolysis process was applied to 380 ° C for 35 minutes. The amount of pyrolytic material was used to determine the method of initial weight less final weight of processed materials. The results obtained were analyzed with the statistical method of mean differences. Treatments of corn and sorghum were which resulted with most material pyrolytic. The above give an added value to crop residues, which are usually sold at low cost to feed cattle.

Pyrolytic, Orange, biomass, organic residue extract.

Citation: LUGO-VALENZUELA, Homero, VÁZQUEZ-PEÑA, Mario A., PRADO-HERNÁNDEZ, Jorge V. and VELÁZQUEZ-LÓPEZ Noé. Characterization of material pyrolytic biomass agricultural. ECORFAN Journal-Republic of Nicaragua 2015, 1-1: 7-11

* Correspondence to Author (email: hlv_300@hotmail.com)
† Researcher contributing first author.

Introduction

Agriculture exist in a lot of product in the threshing corn plots (Figure 2), chickpea, sugarcane and sorghum (Arauzo, 2014) agricultural waste. These wastes are causing pests in the soil due to its excess and further complicate farming. However, this waste can be profitable and innovative if they become retaining moisture materials because they are inexpensive and are abundant in the state of Sinaloa (Jiménez, 2013). In the state of Sinaloa as in many regions of Mexico and the world it needs more efficient use of crop residues in organic agriculture. One possible solution is to transform agricultural waste into organic additives, by a pyrolysis process, these aggregates to the ground help to change their physical properties such as porosity, bulk density and infiltration in the short term (Ricardo, 2014). Pyrolytic production materials from agricultural biomass (Casini, 2014) have great economic potential and impact on the environment (Mora, 2014). Pyrolysis converts low value waste moisture adsorbents with a high utility value. Also, they can be used to retain macro and micro elements (Martinez, 2014) and as substrate in protected agriculture. Finally, you can export as activated filter (Liew, 2014) coal, which benefits the state's economy and the country. Pyrolysis, carbonization is based on agricultural waste, and is carried out at a temperature of 380 ° C for 35 minutes (Figure 1). To activate and accelerate the increase in porosity extract orange juice (Valencia, 2005) is added. According to Ekpete, pyrolytic material has applications such as micro and macro retainer chemicals in the water, water filter, odor eliminator and flavors in animal fat and others. The pyrolytic material can produce waste from cardboard, sawdust, raw timber and others (Penjumrasa, 2014). This work focused on knowing the quantities of pyrolytic materials found in waste corn, sorghum, chickpea, sugarcane and sawdust.

In Figure 1, the process shown in vitro pyrolysis.

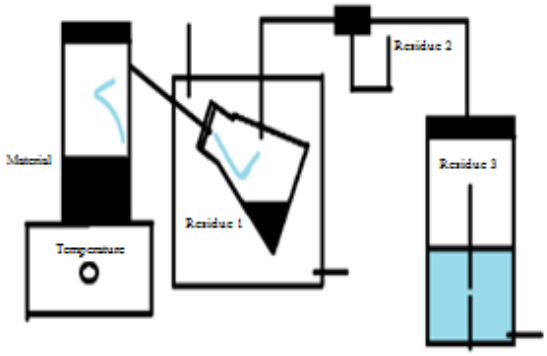


Figure 1 *pyrolytic process*

Materials and methods

In Figure 2, the threshing process is shown in an agricultural farm. As seen after threshing are organic residues.

Figure 2 *Process for obtaining threshing waste.*

Figure 3 *Collection of organic waste.*

ISSN-On line: 2414-8830

Figure 4 *Grinding process*

Figure 8 *Material processing (final)*

Figure 5 *Particle size*

Figure 9 *pyrolytic material*

Figure 6 *pyrolysis*

Figure 7 *Raw Material (initial)*

5 samples of 10 kg of agricultural waste were collected in the municipality of Guasave Sinaloa, Mexico (Figure 3). These residues were: corn, sorghum, chickpea, sugarcane and sawdust. The latter served as a control. Samples were then crushed with a power mill Aztec mark (Figure 4) and sieved with a diameter of 2mm particle (Figure 5). 100 g of each sample was weighed on an electronic scale (Brand Arda) (Figure 6). The samples were dried in an oven at a temperature of 60 degrees centigrade for 24 hours to 5 treatments., Then 50 ml bath of orange peel extract is applied and got the stove again, for final drying, finally the pyrolysis process, proposed by Varriano (2010), in physics laboratory of the Universidad Autónoma Chapingo, Texcoco, Edo Mexico, was applied in February of 2015.. To determine the amount of pyrolytic material initial weight method less final weight of the processed materials (Figure 7 and 8) was used.

ISSN-On line: 2414-8830

The results of pyrolytic material (Figure 9) obtained were analyzed using the statistical method of mean differences with an experimental design of 5 treatments with 5 repetitions. Data analysis was performed on MAT-LAB 2013.

Results

Table 1 shows that mean difference exists in the 5 treatments (maize (T-5), sorghum (T-4), chickpea (T-2), reed (T-3) and sawdust (T-1 is observed)), a method using pyrolysis at a temperature of 380 ° C and a time of 35 minutes.

	R-1 (gr)	R-2 (gr)	R-2 (gr)	R-4 (gr)	R-5 (gr)	Media (gr)
T-1	34	33.5	33.7	32.9	33.1	33.4
T-2	22.3	22.4	21.3	22.5	22.2	22.14
T-3	36	36.6	35.6	34.5	33.5	35.24
T-4	47.5	45.9	47.9	46.7	46.6	46.92
T-5	54.5	54.8	55.2	54.2	54.8	54.7

Table 1 *Experimental design*

In Figure 10 the averages of the 5 treatments content pyrolytic material obtained in the process pyrolisis observed. Treatments 4 and 5 were those that were most pyrolytic amount of material compared to T1 according to the pyrolysis process at a temperature of 380 ° C and 35 minutes with a particle size of 2 mm.

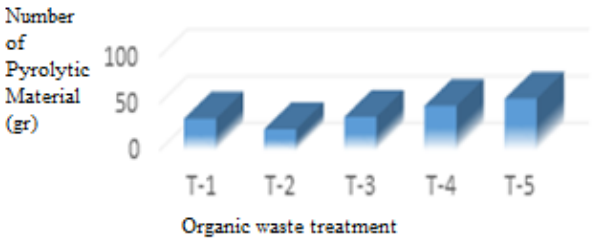


Figure 10 *Treatment of processed organic waste*

This is corroborated by the statistical test of mean difference where T-2, T-4 and T-5 treatments have a significant difference from the control (Figure 11).

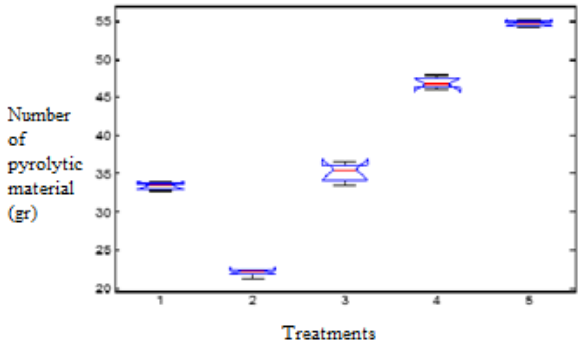


Figure 11 *Difference in treatment means*

Conclusions

According to the results of the calculations, it can be concluded that sorghum and maize are the materials from which most pyrolytic material can be obtained. This gives added value to crop residues, which are usually sold at low cost to feed livestock.

References

Rauzo, J. (2014). Introducción a las tecnologías de aprovechamiento de biomasa. *Thermochemical Processes Research Group (GPT)*, 23-27.

Calvo, G. (2012). Caracterización de residuos procedentes de los procesos de combustión de biomasa. viabilidad de uso como materiales de construcción. *Luco21Instituto de Ciencias de la Construcción Eduardo Torroja. IETcc-CSIC. Serrano Galvache, 4, 28033 Madrid*, 13-17.

Casini. (2014). Morphological changes evaluation by transmission electron microscopy (tem) of mesoporous catalyst employed in pirolisis of biomass. *Acta Microscopica Vol. 23, No.1*, 31-36.

ISSN-On line: 2414-8830

Ekpete. (2011). Preparation and Characterization of Activated Carbon derived from Fluted Pumpkin Stem Waste (Telfairia occidentalis Hook F). *Res.J.Chem.Sci Research Journal of Chemical Sciences*, 23-26.

Jiménez, M. (14 de septiembre de 2013). Produccion de biomasa. *Revista local*, págs. 12-16.

Liew, S. Q. (2014). Extraction and Characterization of Pectin from Passion Fruit Peels. *Agriculture and Agricultural Science Procedia 2*, 231 – 236.

Martínez, P. E. (2014). Biomasa residual vegetal: tecnologías de transformación y estado actual. *Innovaciencia 2 (1)*, 45-52.

Mora. (2014). Evaluación de la pirólisis térmica de aceite vegetal de desecho en un reactor bacht. *Revista EPN, VOL. 33, NO. 2*, 48-54.

Penjumrasa, P. (2014). Extraction and Characterization of Cellulose from Durian Rind. *Agriculture and Agricultural Science Procedia 2*, 237 – 243.

Ricardo, O. E. (2014). Evaluation of the Addition of Wood Ash to Control the pH of Substrates in Municipal Biowaste Composting. *Ingeniería Investigación y Tecnología, volumen XV (número 3)*, 469-478.

Valencia, M. (2005). Producción y caracterización de partículas y fibras cortas de β-sic obtenidas a partir de cáscara de arroz. *Revista EIA, ISSN 1794-1237 Número 4*, 81-93.
Varriano, N. (2010). Produccion sustentable de carbón activado a partir de caña de bambú. *Universidad Tecnológica Nacional Facultad Regional La Plata*, 12-23.

ISSN-On line: 2414-8830

Article **ECORFAN Journal**
December 2015 Vol.1 No.1 12-18

Collembola indicators of soil fertility

CALYECAC-CORTERO, Humberta Gloria´*†, MIRANDA-RANGEL, Andrés´ and JIMÉNEZ-MORALES, Margarita´´

´Laboratory of Micro and Mesofauna Area Soil Biology, Department of Agricultural, Universidad Autónoma Chapingo, km 38.5 highway Mexico-Texcoco. CP. 56230.
´´Master of Science in Plant Protection, Department of Agricultural Parasitology, Universidad Autónoma Chapingo.

Received May 2, 2015; Accepted November 16, 2015

Abstract

Pines, cedars and associations of the populations of forests are the most important types of trees in the study area. Fire is the main disturbance of the dynamics of these forests. This study aimed to analyze possible differences in chemical and biological properties of soil between six types of forest: A: burned and replanted forest with *Pinus* sp; B: Burned forest and not reforested; C: unburned forest and reforested; D: No burned and not replanted, young forest; E: Not burned and not reforested; the fire had occurred 11 years before the study. Soil properties were measured in the first 10 cm of depth that has more influence on forest recovery: Protein, humidity, dry matter and content of nitrogen (N). Burned and replanted forests had a higher protein concentration due to the type of forest and vegetation, which means that in 11 years it had regenerated by itself and the species with which this area was reforested it has prospered and the soil has good concentration of nutrients. The extreme values of proteins decrease the population of springtails. After a fire is desirable that the vegetation is restored as soon as possible to mitigate the potential loss of nutrients and promote the recovery of soil properties, which can be benefited by planting *Pinus* sp and associated with native species such as oak, fir and *Quercus*.

***Pinus*, colembolo natural forest, fire effects, postfire soil conditions.**

Citation: CALYECAC-CORTERO, Humberta Gloria, MIRANDA-RANGEL, Andrés and JIMÉNEZ-MORALES, Margarita. Collembola indicators of soil fertility. ECORFAN Journal-Republic of Nicaragua 2015, 1-1: 12-18

* Correspondence to Author (email: hcalyecac@yahoo.com)
† Researcher contributing first author.

Introduction

In the last decade, extensive forest fires have occurred in most continents, affecting a wide range of ecosystems (Williams & Bradstock, 2008) and the forests of the central area of Mexico have been no exception. The ecological consequences of these fires are site specific and highly associated with reproductive mode of the dominant species and the subsequent weather conditions the fire (Veblen et al., 2008). To help restore the affected ecosystems and fire management planning in these native forests, it is necessary to advance knowledge about the effect of the disturbance on the various components of the ecosystem and its resilience.

Within animal groups that live in the soil, the most important are the springtails and oribatid by their number, diversity, species abundance and activity mites. These groups are considered biogeographic and ecological indicators because of their great aptitude for speciation, estenotopía, short life and little power of dispersal of species adapted to the soil life and different soil types, and their eating habits, as degrading organic matter (Johnston, 2000; Palacios-Vargas, 2003).

Most springtails feed on fungal hyphae or decaying plant matter (Brown-Meneses et al., 2004). There are also some predatory species that feed on nematodes, rotifers and other springtails (Rusek, 1998; Palacios-Vargas et al; 2000). By its feeding they play an important role in the decomposition of organic matter and control the populations of bacteria and fungi (Palacios-Vargas et al., 2000).

Collembola are very valuable to soil structure, since most soils contain millions of its "pellets" that delay release of essential nutrients benefit the plant roots, as well as serving as a substrate for many microorganisms.

Collembola are prey to many insects, especially ants and beetles, as well as numerous predatory mites, which are a key element in the food chain (Palacios-Vargas et al., 2000). Its abundance, diversity of species and characteristics provides information on the environmental impact of ecosystems.

For areas of burned forests, there is little information regarding the use of soil fauna as bioindicator let alone using springtails, so it is necessary to study the relationship of soil components in burned forests

Objective

Relate soil nutrient status of a pine-oak forest, where a wildfire was presented with populations of springtails.

Relate the five types of variants pine oak forest species studied were developed in each of them.

Hypothesis

There is a direct relationship between the presence and size of populations of springtails and soil nutrient status.

Materials and methods

Study area. In the study area the prevailing climate is temperate humid summer long with average annual rainfall between 500 and 1,000 mm also have an annual average temperature of 15 ° c., with an average for most warmest months 10 ° C and for the coldest months can occur temperatures ranging from -3 to 18 ° C, have four distinct seasons; a relatively hot summer, autumn gradually lower temperatures with the passage of time, a cold winter and spring with higher temperatures gradually with the passage of time. No extreme weather events (Romero, 2009) is presented.

ISSN-On line: 2414-8830

The town lies at an altitude of 2.480 meters. The vegetation of the study area occurs in the low hills toward the high end, in the foothills of the Sierra Nevada, in the lower parts of predominantly agricultural land use temporary and the plains some areas with irrigated agriculture are located.

Collembola collection. The material studied corresponds to eight collections of litter per site six sampling sites pine and oak were established in the town of Tequexquinahuac, Texcoco, State of Mexico, these collections were made weekly from 2009 to 2010. In each study site were established at randomly 1 m^2, which constituted the sampling units, they were located in different zones of the study area with six types of forest. These experimental units were established in October 2009 to 2010 in the six sites, corresponding to a period of 11 years after the event the fire that occurred in 1998. Each of these six sites was considered as a treatment and as different sample points repetitions of each treatment.

Soil properties. In October 2009, 11 years fires have occurred sampling to analyze soil properties was made; in the same six sampling sites springtails. The four parameters or soil factors, with a fundamental character as soil descriptors were: protein, moisture, dry matter and nitrogen (N). To these they were sent for analysis in the laboratory of Animal Science at the Universidad Autónoma Chapingo.

Analysis of data

Data were analyzed for each location considering the six treatments (Forest types) with their respective repetitions (A: n = 6, B: n = 6, C: n = 6, D: n = 5, E: n = 5) by analysis of variance (ANOVA), assuming that the sampling error represents the experimental error.

Prior to the ANOVA assumptions of homogeneity of variance and normality were examined. Tukey-Kramer, which is a modification of the Tukey test for designs with the SAS System 2009 program used in each of the analysis and to determine from what treatments there were differences significant.

Results and discussions

The Braquistomelidae family had a larger population in the site C, followed by site D, after the Site, Site B and A respectively.

The largest population of the Isotomidae family appeared at the site C, followed by site D, E, B and A respectively.

Entomobridae families, and Sminturidae Onychiuridae occurred in similar proportions in the five sites and fewer regarding Braquistomelidae and Isotomidae families (Figure 1).

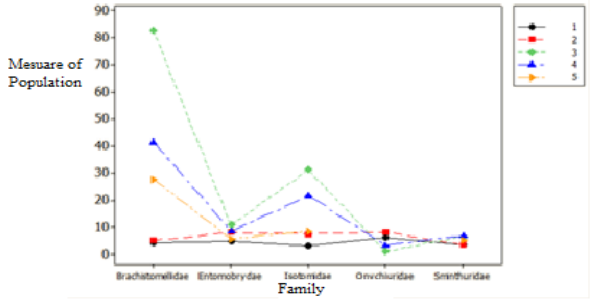


Figure 1 Population of springtails per family in the five study sites

* 1: Site A; 2: Site B; 3: Site C; 4: Site D; 5: Site E.

The greater abundance of Isotomidae, is attributed to their species, that have been recorded as highly adaptable to disturbance caused by agricultural and forestry practices, and as mentioned Mendoza et al. (1999), they can grow in soils with low or high organic matter content (Figure 2).

ISSN-On line: 2414-8830

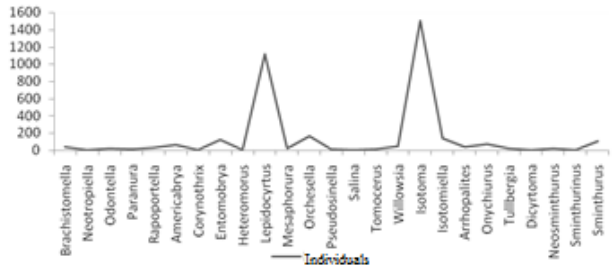


Figure 2 Population density of springtails per family present at the study sites

Statistical analysis

In the ANOVA results for populations of Collembola was no significant difference among the six types of forests pvalue = 0.0002.

In the mean comparison Tukey-Kramer high values of population of Collembola was observed in forest type C: unburned forest and reforested, although there was no significant difference with the D: Forest unburned and not replanted, young forest; where if there was significant difference from previous treatments but not to each other were: A: Burned forest and reforested; B: Burnt forest and not reforested and E: No, not reforested burned, old forest. They corroborate with Figure 1.

The ANOVA for Protein significant difference between sampling sites pvalue <0.0001 and mean comparison Tukey-Kramer found that the best treatment was the forest B: Burnt forest and reforested; although there was no significant difference with the forest C: unburned forest and reforested; where if there was significant difference from previous treatments was the D: Forest unburned and not replanted, young forest, and treatments had lower concentration of nitrogen were E: Forest unburned and not replanted, old forest and A: Forest burned and reforested

Site A (burned and reforested with pine forest) showed a decrease in proteins that are composed of nitrogen and hydrogen, this decrease is due to the tree species with which they replanted the site because in the pine needles a decrease is observed the activity of the soil microfauna that gives rise to a lower rate of litter decomposition and therefore better protein production. The cause lies in the chemical-nutritive pine litter, poorer in calcium and rich in nitrogen and resin compounds as inhibitors, waxes and lignin as indicated Schlatter and Otero (1995) features.

For forest type B (Burnt forest and not reforested) also presented a poor population of springtails but a high amount of nitrogen and hydrogen, elements that make up the protein (reflected in the Tukey-Kramer tests because they were the same results), this relationship is because in soils with excess nitrogen is a significant decrease in populations of springtails as shown Kopeszki (1997) who observed a decrease in the growth and abundance of populations of Collembola, due to the presence of acids (SO4), heavy metals and excess nitrogen fertilizers in soils. As a result, the rate of decomposition, kolMO was lower.

For forest type C: unburned forest and reforested and D: Do not burned and not replanted, young forest; had the largest population of springtails and also had a lot of protein (nitrogen, hydrogen) moderate, these forests have similar features because both were not burned; C was reforested, and D was not reforested but a young forest which can be deduced that have greater diversity of species which favors the presence of springtails in the soil. The growth rate of the population of springtails serves as a biomarker of health or soil quality as indicated Kopeski (1997). Overall the response of different ecosystems to changes in nitrogen vary with the type of vegetation, soil fertility and the ability to replenish (Wan et al., 2001).

ISSN-On line: 2414-8830

Fire generates different conditions on the ground, which may have implications for the emergence of seedlings (Kennard and Gholz 2001) as in *Pinus* sp. (Pauses et al., 2003). (Urretavizcaya 2005, 2006) indicates that regeneration in the short term is not controlled only by changes in soil fertility, but by a combination of factors such as the availability of seeds and changes in soil temperature patterns in the areas burned, associated with changes in tree and shrub cover after the fire (Urretavizcaya et al., 2006), factors that match the Kitzberger et al; reported. (2005).

The results of this work can contribute to recommendations regarding the further handling of a fire in the forests of the region Tequexquinahuac or regions with similar climatic conditions and vegetation in this area. It is widely known that the greatest risks of erosion after fire occur in the rainy season immediately after the disturbance (Goh and Phillips, 1991; DeBano, 2000; Robichaud et al., 2003; Neary et al., 2005; Cerda and Doerr, 2005). It is also known that timber harvesting may increase the risk of erosion and nutrient loss (Beschta et al., 1995, 2004, 2000 McIver and Starr, Page-Dumroese et al., 2006). Ensure that the post fire vegetation is established to mitigate potential losses of nutrients and promote the recovery of soil properties prior to the extraction of wood can be burned along with restoration planning for planting, a measure to implement in the management of these areas

Conclusions

Forests C and D have a higher population of springtails, which means that this type of organisms are highly sensitive to disturbances such as fires and changing vegetation when these forests are replanted.

The B and C sites had the highest concentration of protein due to the type of forest and vegetation, which means that it was regenerated in 11 years and the species with which this area prospered reforested and the soil has good concentration of nutrients.

The high concentration of proteins containing nitrogen and hydrogen reduce the rate of reproduction of Collembola and therefore their populations.

Pines by its high concentrations of inhibitors such as resin, waxes and lignin decreases decomposition rate by Collembola litter and hence protein production.

Populations with high density of Collembola was found that occurs in ground conditions with moderate protein concentration. The forests where disturbances such as fire and reforestation, did not showed the highest population of collembola.

References

Bidwell, R.G.S. (1990). Fisiología vegetal. Primera edición en español. Editorial AGT Editor S.A., México. 784 p.

CONAFOR. 2014. Reporte semanal de resultados de incendios forestales 2014. Centro Nacional de Control de Incendios Forestales 2014. pp.21.

INEGI. 2009. Prontuario de información geográfica municipal de los Estados Unidos Mexicanos. Texcoco, México. Clave geoestadística 15099. pp.9.

Hopkin, S.P. 1997. Biology of springtails (Insecta: Collembola). Oxford University Press, Oxford, 333 p.

ISSN-On line: 2414-8830

Kopeszki, H. 1997. An active bioindication method for the diagnosis of soil properties using Collembola. Podobiologia,41:159-166.

Kennard, D.K., H.L. Gholz. 2001. Effects of high- and low-intensity fires on soil properties and plant growth in a Bolivian dry forest. *Plant and Soil* 234: 119-129.

Luque, V. G. Estructura y propiedades de las proteínas. 163 p.

Moya, J. R. 2002. Variación estacional del perfil nutritivo y digestibilidad in sítu de materia seca, proteína cruda y fibra detergente neutro, del follaje de ocho especies arbustivas del noreste de México. Universidad Autónoma de Nuevo León. Facultad de Ciencias Biológicas. División de Estudios de Postgrado. 160 p.

Romero, L. A. 2009. Manifestación de impacto ambiental. Modalidad particular. Construcción de una unidad habitacional ecológica en el Predio las Cruces, Tequexquináhuac, Texcoco, Estado de México. Primera Etapa: Cambio de Uso del Suelo. Universidad Autónoma Chapingo. pp. 228.

Schlatter, J.E, Otero, L. 1995. Efecto de Pinus radiata sobre las características químico-nutritivas del suelo mineral superficial. BOSQUE 16. p. 29-46.

Wan S., D. Hui, Y. Luo. 2001. Fire effects on nitrogen pools and dynamics in terrestial ecosystems: a meta-analysis. *Ecology* 82(11): 1349-1365.

Certini, G. 2005. Effects of fire on properties of forest soils: a review. *Oecologia* 143: 1-10.

Connell, J.H., R.O. Slatyer. 1977. Mechanisms of succession in natural communities and their role in community stability and organization. *American Naturalist* 111: 1119-1144.

DeBano, L.F. 1990. The effect of fire on soil properties. Symposium on Management and Productivity of Western-Montane Forest Soil, Boise, Idaho. April 10-12.

DeBano, L.F. 2000. The role of fire and soil heating on water repellency in wildland environments: a review.*Journal of Hydrology* 231-232: 195-206.

DeBano, L.F., D.G. Neary, P.F. Ffolliott. 1998. Fire's effects on ecosystems. New York, USA. John Wiley. 333 p.

Goh, K.M., M.J. Phillips. 1991. Effects of clearfell logging and clearfell logging and burning of a *Nothofagus* forest on soil nutrient dynamics in South Island, New Zealand-changes in forest floor organic matter and nutrient status.*New Zealand Journal of Botany* 29: 367-384.

Kennard, D.K., H.L. Gholz. 2001. Effects of high- and low-intensity fires on soil properties and plant growth in a Bolivian dry forest. *Plant and Soil* 234: 119-129.

Kitzberger, T. 1994. Fire regime variation along a northern Patagonian forest-steppe-ecotone: stand a landscape response. Tesis de Doctorado. Department of Geography, University of Colorado, Boulder, Colorado. 203 p.

Kitzberger, T., E. Raffaele, K. Heinemann, M.J. Mazzarino. 2005. Effects of fire severity in a north Patagonian subalpine forest. *Journal of Vegetation Science* 16: 5-12.

ISSN-On line: 2414-8830

Neary, D.G., C.C. Klopatek, L.F. DeBano, P.F. Ffolliott. 1999. Fire effects on belowground sustainability: a review and synthesis. *Forest Ecology and Management* 122: 51-71.

Neary, D.G., K.C. Ryan, L.F. DeBano, J.D. Landsberg, JK Bronw. 2005. Introduction. *In* Neary DG, KC Ryan, LF DeBano eds. Wildland fire in ecosystems. Effects of fire on soil and water. Gen. Tech. Rep. RMRS-GTR-42-vol.4 (revised 2008). USDA, Forests Service, Rocky Mountain Research Station. p. 1-17.

Robichaud, P., L. Macdonald, J. Freeouf, D. Neary, D. Martin, L. Ashmun. 2003. Postfire Rehabilitation of the Hayman Fire. USDA Forest Service Gen. Tech. Rep. RMRS-GTR-114. 293 p.

Sparks, D.L., A.L. Page, P.A. Helmke, R.H. Loeppert, P.N. Soltanpour, M.A. Tabatabai, C.T. Johnston, M.E. Sumner. 1996. Methods of Soil Analysis. Part 3. Chemical Methods. SSSA Book Series Nr. 5. Madison, Wisconsin, USA. SSSA, ASA. p. 437-474.

Urretavizcaya, M.F. 2005. Cambios ambientales y restauración ecológica postincendio en bosques de *Austrocedrus chilensis*. Tesis de doctorado. Bariloche, Argentina. Universidad Nacional del Comahue. 205 p.

Urretavizcaya, M.F. 2006. Restauración postfuego de bosques de ciprés de la cordillera: comportamiento de distintos plantines a 4 y 5 años de su plantación. *In* Segunda Reunión Patagónica y Tercera Nacional sobre Manejo y Ecología del Fuego – Eco-Fuego 2006. Esquel, abril de 2006. CIEFAP-UNPat-PNMF-DGByP.

Urretavizcaya, M.F., G.E. Defossé. 2004. Soil seed bank of *Austrocedrus chilensis* (D. Don) Pic. Serm. et Bizarri related to different degrees of fire disturbance in two sites of southern Patagonia, Argentina. *Forest Ecology and Management* 187: 361-372.

Urretavizcaya, M.F., G.E. Defossé, H.E. Gonda. 2006. Short-term effects of fire on plant cover and soil conditions in two *Austrocedrus chilensis* (cypress) forests in Patagonia, Argentina. *Annals of Forest Science* 63: 63-71.

Wan, S., D. Hui., Y. Luo. 2001. Fire effects on nitrogen pools and dynamics in terrestial ecosystems: a meta-analysis. *Ecology* 82(11): 1349-1365.

ISSN-On line: 2414-8830

Article **ECORFAN Journal**
December 2015 Vol.1 No.1 19-26

Chemical compounds of essential oil of *Tagetes* species of Ecuador

ZAPATA-MALDONADO, Christian Iván´*†, SERRATO-CRUZ, Miguel Ángel´, IBARRA, Emmanuel´ and NARANJO-PUENTE, Blanca´´

Institute of Horticulture. Universidad Autónoma Chapingo. Km 38.5 Highway México-Texcoco. 56230. Chapingo, State of Mexico
Laboratory of Phytochemistry. Universidad de las Fuerzas Armadas ESPE.Av. Grl. Rumiñahui. Route of the Volcanoes. Sangolquí-Ecuador

Received April 14, 2015; Accepted November 5, 2015

Abstract

It has limited information on the chemical composition of *Tagetes* species distributed in Ecuador, which limits its use as a biopesticide, for that reason, to advance the characterization of this plant genetic resource, in this work the oil composition described essential obtained by hydrodistillation of five species in situ plants. Using a procedure GC / MS the following major compounds were determined: anethole (66,997%), estragole (31,685 &) and anis aldehyde (1.495%) in *T. filifolia*; trans-tagetone (52.76%), 4-ethyl-4-methyl-1-hexene (25.56%), verbenone (3.32%), 1-verbenone (3%), β-ocimene (8.62%), β-linalool (1.19%) and cis-tagetone (6.21%) in *T. terniflora*; trans-tagetone (33.97%), 4-ethyl-4-methyl-1-hexene (13.85%), cariofilene (3.18%), β-ocimene 16.96%), trans-ocimene (3.73%), cis-tagetone (9.1 %), 1-verbenone (11.69%) and verbenone (16.57%) in *T. minuta*; trans-tagetone (17.89%), trans-ocimene (3.73%), 1-verbenone (13.89%), verbenoana (24.34%), epoxy pinane 2.3 (0.44%) and 4-ethyl-4-methyl-1- hexene (39.69%) in *T. zypaquirensis*; and trans-tagetone (30.91% 9, trans-ocimene (25.66%) and valeric acid (43.43%) in *T. multiflora*.

***Tagetes*, Ecuador, essential oil, chemical composition.**

Citation: ZAPATA-MALDONADO, Christian Iván, SERRATO-CRUZ, Miguel Ángel, IBARRA, Emmanuel and NARANJO-PUENTE, Blanca. Chemical compounds of essential oil of *Tagetes* species of Ecuador. ECORFAN Journal-Republic of Nicaragua 2015, 1-1: 19-26

* Correspondence to Author (email: cchhee.18@gmail.com)
† Researcher contributing first author.

Introduction

The genus *Tagetes* (Compositae) appeared on the American continent 65 million years ago(Turner, 1997). So far they have referred 53 species distributed in the Americas from the southwestern United States to southern Argentina (Plant List, 2013).

Refer some species of *Tagetes* (Asteraceae) took advantage of Mesoamerica medicinal, ornamental and ceremonial purposes in ancient times (Kaplan, 1960; Neher, 1968; Estrada, 1986; cited by Serrato, 1999) In the Florentine Codex. In countries like Argentina, Chile, Peru, Bolivia and Colombia, the *Tagetes* even be used as a medicinal plant or food preparation and flavor (*T. minuta* and *T. terniflora*) and flavor (Ulloa, 2006). In Ecuador, *Tagetes filifolia* and *T. terniflora* are used as food additives (De la Torre et al., 2008), also used to dye clothing such as *T. minuta* (brown) and *T. filifolia* (yellow color); and anise (*T. filifolia*) an alcoholic beverage flavor is prepared and given to the chicha and humita (Bertero et al., 2009).

Today we seek further applications of these plants for their chemical properties. Specifically we are working with the essential oil and the aqueous portion of the distillation to leverage their chemical compounds as bactericides (*T. patula*) (Wan et al., 2015), antitripanosomal (T. caracasana, *T. zyparquirensis* and *T. heterocarpa*) (Escobar et al., 2009); Biopesticides (*T. caracasana* and *T. zypaquirensis*) (Barrientos et al., 2012) for its biological effect against fungi and nematodes (Tariq et al., 2010).

The low production costs of essential oil of this species (Serrato, 2003) and its organic origin, represent an important economic and ecological option compared to synthetic insecticides products, which, besides being a source of environmental pollution and damage to human health, are partly due to unprofitability of agricultural production systems.

There are few studies on endemic species *Tagetes* Ecuador in chemical composition. Therefore, this paper aims to provide knowledge about the chemical composition of species distributed in Ecuador, in addition to well protect the natural resources of this country.

For Ecuador eight species are recognized: *T. terniflora, T. filifolia, T. verticillata, T. minuta, T. multiflora, T. zypaquirensis* and *T. dianthiflora* (Tropicos, 2015) they are found in the Andes, between the 2000-3500 meters, while introduced species and domesticated and *T. erecta* and *T. patula* covering an altitudinal range of 0-3000 meters (Tropicos, 2015).

Among the chemical compounds of *Tagetes* they are: thiophenes, phenols, flavonoids, coumarins and terpenes, which have biological activity nematicide, insecticide and acaricide (Nava et al, 2012.).

Several environmental factors are involved in the chemical composition of the essential oil, for example, nutrition, climate issues, water availability, amount and intensity of light and soil type; in mint, monoterpenes metabolism is influenced by environmental factors. (Burbott and Loomis, 1967). *T. filifolia* in different geographic areas of Argentina where it is distributed naturally variation of chemical compounds is observed in the essential oil (Maestri et al., 1991).

ISSN-On line: 2414-8830

Whereas no background on the profile of essential oils Ecuador species and that the distribution of these species correspond to different geographic locations, the objective of this study was to identify the chemical constituents in the essential oil of plants and five species populations obtained in situ.

Methodology

Collection of biological material

To perform the essential oil extraction plant fresh tissue it was collected in five species of flowering *Tagetes* Ecuador.

In the case of *T. filifolia*, the samples are located in the provinces of Canar, Loja, Pichincha; *T. terniflora*, in the provinces of Canar, Chimborazo, Pichincha, Tungurahua; *T. minuta* in the province of Pichincha; *T. multiflora* in the provinces of Azuay, Canar, Chimborazo, Pichincha and *T. zypaquirensis* in the provinces of Bolivar, Canar, Carchi, Chimborazo, Cotopaxi, Imbabura, Pichincha (Table 1).

Tagetes species	altitude meters	coordinates
filifolia	3090	N 0'17'24,2"
		O 78'21'17,2"
filifolia	2497	S 00'15'42,1"
		O 78'22'59,9"
filifolia	2461	S 1'4'11,1"
		O 78'1'49"
minuta	2656	S 0'5'59,8"
		O 78'26'44,3"
minuta	2500	N 00'00'44,0"
		O 78'25'52,2"
minuta	2445	S 0'6'7,1"
		O 78'18'23,4"
multiflora	2445	S 0'6'7,1"
		O 78'18'23,4"
multiflora	2010	S 0`1`29,02`` O 78`20`5,3``
multiflora	1935	S 0`4`11.8`` O 78`22`23.89``
terniflora	2656	S 0'5'59,8"
		O 78'26'44,3"
terniflora	2600	S 1'25'57,4"
		O 78'30'52,7"
terniflora	2078	S 4´0,7´22,15`` O 70`12`7,51``
terniflora	2010	S 0`1`29,02`` O 78`20`5,3``
zypaquirensis	3154	S 0'41'56"
		O 78' 5'2,8"
zypaquirensis	3090	N 0'17'24,2"
		O 78'21'17,2"
zypaquirensis	2490	N 0'16'46,7"
		O 78'14'48,3"
zypaquirensis	2078	S 4´0,7´22,15`` O 70`12`7,51``

Table 1 Coordinates of biological material collected

Extraction of essential oil

The fresh tissue was cut into pieces of 3 cm and led to distillation in a Clevenger (Günther, 1948) series-connected computer, using round flasks of 1 L capacity; distillation of essential oils by hydrodistillation was a process that lasted for 45-60 min. Each species and population separately distilled. Once the extraction is completed, the amber oil was stored in base plates and cooling were carried.

Determination of chemical compounds

The chemical composition of the essential oil was analyzed by gas chromatography-mass spectrometry (GC-MS) on a Polaris Q Finnnigan Trace GC Ultra equipped with Polaris Q mass detector, electron impact (70 eV). One diphenyl dimethyl polysiloxane 5MX RTX-column (5:95) of 30 x 0.25 mm D x 0.25 microns was used. The injector and detector were set at 250 and 300 ° C. The oven temperature started at 70 ° C, 1 min remained well and was programmed to reach 250 ° C at a rate of 20 ° C.min-1.

ISSN-On line: 2414-8830

Helium as carrier gas was used at a flow rate of 1ml min-1. Diluted samples (1/100 in methylene v / v chloride) 1 uL injected manually, Split (scan) mode and three replicates of each species were made.

For a description of the major results only compounds relative percentages corresponded to more than 1% were considered.

Quantitative data were obtained from a percentage of peak area.

Identification of components was done by comparing retention indices and mass spectra relative to the NIST database system GC-MS and spectral data published by Allured Publishing Corp., Carol Stream, Illinois, (Adams, 2001).

Results and discussion

The retention times of the majority in the essential oil molecules species from Ecuador showed 4.4 to 10.6. The major species were composed as follows: anethole (66.9%), estragole (31.6%) and anise aldehyde (1.4%) in *T. filifolia*; trans-tagetone (52.7%), 4-ethyl-4-methyl-1-hexene (25.5%), verbenone (3.3%), 1-verbenone (3%), β-ocimene (8.6%), β-linalool (1.1%) and cis-tagetone (6.2%) in *T. terniflora*; trans-tagetone (33.9%), 4-ethyl-4-methyl-1-hexene (13.8%), caryophyllene (3.1%), β-ocimene 16.9%), trans-ocimene (3.7%), cis-tagetone (9.1 %), 1-verbenone (11.69%) and verbenone (16.5%) in *T. minuta*; trans-tagetone (17.8%), trans-ocimene (3.7%), 1-verbenone (13.8%), verbenoana (24.3%), 2.3 epoxy pinene (0.4%) and 4-ethyl-4-methyl-1- hexene (39.6%) in *T. zypaquirensis*; and trans-tagetone (30.9%), trans-ocimene (25.6%) and valeric (43.4%) acid in *T. multiflora*. Secondary metabolites in *T. filifolia* correspond to phenylpropanoids, while in other species it is terpenes.

The results are described for the first *Tagetes* species of Ecuador, and give an idea of intra- and inter-specific variability that exists in the type of chemical and the concentration of the same partners phyto geographic area they inhabit such species.

Previously it reported some species composition: dill and estragole in *Tagetes* filifolia (. Maestri et al, 1991); tagetone, dihydrotagetone, ocimene and ocimenone in *T. terniflora*; tagetone and dihydrotagetone in *T. minuta* (Chamorro et al., 2008) in *T. zypaquirensis* dihydrotagetone; and tagetone, ocimenone and tagetenona in *T. multiflora*.

The next action research would be the assessment of the performance of oils by piloting as they have limited data of biomass production and oil extraction on an industrial level *Tagetes* (Saavedra et al., 2003) to determine the economic viability of this biotechnology (Serrato, Barajas & Diaz, 2007).

Specifically on the variability of essential oils (Figures 1, 2, 3 and 4) several trends: a) the relative abundance of molecules among populations of the same species differs depending on geographical origin, for example, in *T. filifolia* found in higher percentage anethole and estragole in both populations and *T. zypaquirensis* find molecules as trans-tagetone, verbenone, 1-verbenone, trans ocimene, pinane 2.3 epoxy and 4-ethyl-4-methyl-1- hexane (Figures 1 and 4); b) molecules that occur in some people, not produced elsewhere and *T. minuta* (trans-tagetone (64.48%), trans-ocimene (11.2%) and cariofilene (9.56%)) and *T. terniflora* (verbenone (8.94%), 1-verbenone (9.07%) and 0.23% cariofilene)) (Figures 2 and 3).

ISSN-On line: 2414-8830

These results match with those obtained by other authors (Giorgi et al., 2005; Badoni et al, 2009;. Sarvari, 2009; Mahzooni et al, 2012; Mahzooni-Kachapi et al., 2014), who emphasize in different species the content of essential oil interacts with the geographic location, confirming that between individuals of the same species, chemical components, in some cases vary in concentration and some others disappear and others appear as changes in altitude that developed. In *T. filifolia* variability compounds in the essential oil is a function of the different phyto geographical areas of Argentina (Maestri, Zygadlo, Grosso, Abburra, & Guzman, 1991).

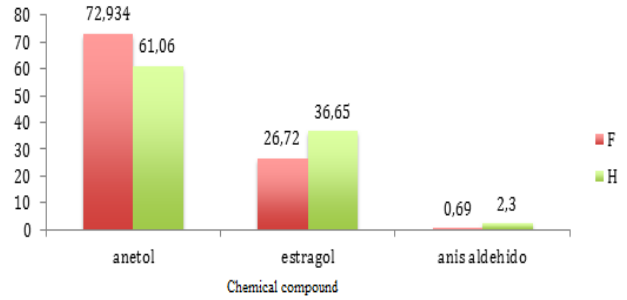


Figure 1 Chemical composition of *Tagetes filifolia*. Populations (F and H)

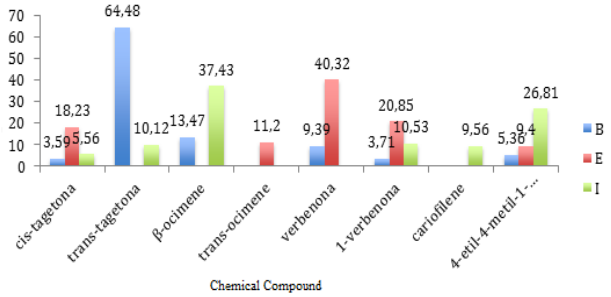


Figure 2 Chemical composition of *Tagetes minuta*. People (B, E and I)

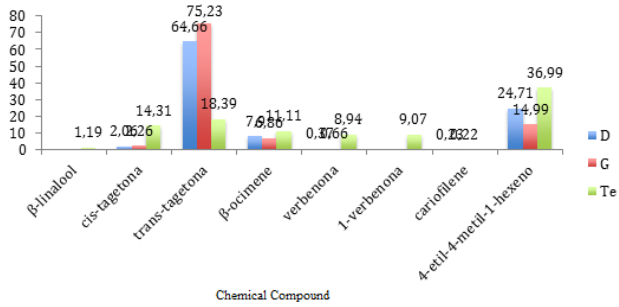


Figure 3 Chemical composition of *Tagetes terniflora*. Towns (D, G and I)

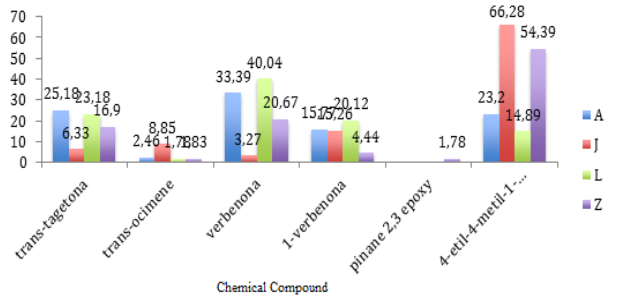


Figure 4 Chemical composition of *Tagetes zipaquirensis*. Populations (A, Y, L and Z)

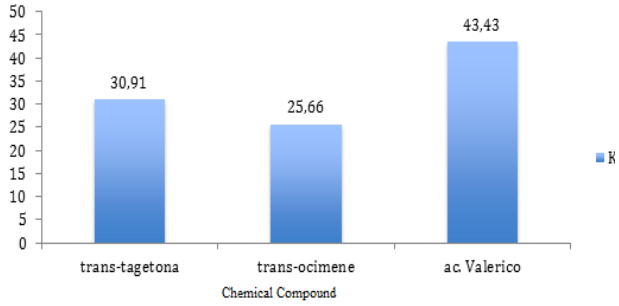


Figure 5 Chemical composition of *Tagetes multiflora*. Populations (K)

Conclusions

In the essential oil of the five species of **Tagetes** Ecuador phenylpropanoids and terpenes found in relative abundance the variability depending on geographical origin.

ISSN-On line: 2414-8830

References

Calmasur, O., Aslan, I., & Sahin, F. (2006). Insecticidal and acaricidal effect of three Lamiaceae plant essential oils against Tetranychus urticae Koch and Bemicia tabaci genn. *Indus. Crops Prod. , 23* (2), 140-146.

Chamorro, E., Ballerini, G., Sequeira, A., Zalazar, M., & Velasco, G. (2008). Chemical composition of essential oil from *tagetes minuta L.* leaves and flowers. *Journal of the Argentina Chemical Society , 96* (1-2), 80-86.

Laribi, B., Berraieb, I., Kouci, K., Sahli, A., Mougou, A., & Marzouk, B. (2009). Water deficit effects on carawar (Carum carvi L.) growth, essential oil, and fatty acid composition. *Ind. Crop Prod. , 31*, 34-42.

Li, Y., Craker, L., & Potter, T. (1996). Effect of light level on esential oil of sage (Salvia officinalis) and thyme (Thymus vulgaris). *Acta Horticulturae , 426*, 419-426.

List, T. P. (2013). *The Plant List*, 1.1. Recuperado el Septiembre de 2015, de A working list of all plant species: http://www.theplantlist.org/

Crepet, W., & Stuessy, T. (1978). A reinvestigation of the fossil viguiera croniquisii (Compositae). *Brittonia , 30* (4), 483-491.

Cretasico Superior. (2015 йил 25-Marzo). Retrieved 2015 йил 15-05 from Wikipedia, The Free Encyclopedi: http://es.wikipedia.org/wiki/Cret%C3%A1cico_Superior

Cubillo, D., Sanabria, G., & Hilie, L. (1999). Evaluación de la repelencia y mortalidad causada por insecticidas comerciales y extractos vegetales sobre Bemicia tabaci. *Manejo integral de plagas , 53*, 65-72.

Barrientos, J. C., Reina, M. L., & Chacón, M. I. (2012). Potencial económico de cuatro especies aromáticas promisorias para producir aceites esenciales en Colombia. *Revista Colombiana de Ciencias Hortícolas , 6* (2), 225-237.

Bertero, D., Mas, M. T., Verdú, A., & Trillo , C. (2009). Plantas andinas y sus usos tradicionales. *Revista Ciencia Hoy , 19* (112).

Bettaieb, I., Knoiua, S., Hamrouni, I., Limam, F., & Marzouk, B. (2011). Water decicient impact on Fatty acid and essential oil composition and antioxidant activities of cumin (Cuminum cyminum). *Agric Food Chem , 59* (1), 328-334.

Bourgou, S., Bettaieb, I., Saidani, M., & Marzouk, B. (2011). Fatty acid, essential oil, and phenolics modifications of black cumin fruit under NaCl stress conditions. *Agric. Food Chem , 59*, 328-334.

Burbott, A., & Loomis, W. (1967). *Effects of light and temperature on the monoterpenes of peppermint.* Plant Physiology.

De la Torre, L., Navarrete, H., Muriel, P., Macía, M., & Balslev, H. (2008). *Enciclopedia de las Plantas Utiles del Ecuador.* (Herbario QCA, & Herbario AAU, Edits.) Quito, Ecuador: Aarhus.

Domínguez, X. A. (1973). *Métodos de investigación fitoquímica.* México: LIMUSA.

Ermat, P., Reza, M., Toorchi, M., & Dabbagh, A. (2013). The influence of light intensities and nitrogen on growth of Hypericum perforatum L. *Int. of Agriculture , 3* (4), 775-781.

ISSN-On line: 2414-8830

Escobar, P., Viviana, L., Milena, S., Durán, C., & Stashenko, E. (2009). Composición química y actividad anti- tripanosomal de aceites esenciales obtenidos de *Tagetes* (Fam. Asteraceae), recolectados en Colombia. *Centro de Investigación de Enfermedades Tropicales* , 280-286.

Fait, A., Iversen, B., Tiramani, M., Visentin, S., & He, F. (2004). Serie Proteccion de la salud de los trabajadores. *Prevencion de los riesgos para la salud derivados del uso de plaguicidas en la agricultura (1)* . (I. F. Tuula Solasaari-Pekki, Ed., & I. C. Safety, Compiler) Italia.

Giorgi, A., Bononi, M., Tateo, F., & Cocucci, M. (2005). Yarrow (Achillea millefolium L.) growth at different altitudes in Central Italian Alps: Biomass yield, oil, content and quality. *Joural of Herbs, Spices and Medicinal Plants , 11* (3), 47-58.

Hui Hua Wan , Bei Zhou Song, Guang Bo Tang, Jie Zhang, & Yun Cong Yao . (2015). What are the effects of aromatic plants and meteorological factors on Pseudococcus comstocki and its predators in pear orchards? . *Agroforest Syst* .

Humberto Cortés, N., & Sandoval Reyes, F. (2011 йил Noviembre). *Ventajas y desventajas de los insecticidas químicos y naturales* . (U. V.-F. Químicas, Ed.) Poza Rica de Hgo, Veracruz, Mexico.

Keller, P., & Luettge, U. (2005). Phtosynthetic light use by three bromeliads originating from shaded sites (Ananas ananassoides, Ananas comosus) and exposed sities (Pitcairnia pruinosa) in the medium Orinoco basin. *Bio Plant , 49*, 73-79.

Kumar, R., Sharma, S., & Pathania, V. (2013). Effect of shading and plant density on growth, yield and oil composition of clary sage (Salvia scarlares L.) in North Western Himalaya. . *The Journal of Essential Oil Research , 25* (1), 23-32.

Maestri, D., Zygadlo, J., Grosso, N., Abburra, R., & Guzmán, C. (1991). Anales de Biologia. *Composición química del aceite esencail de poblaciones argentinas de Tagetes filifolia Lag. (Compositae) , 17* (6), 133-137.

Mahzooni, S., Mahdavi, M., Akbarzadeh, M., Roozbeh , N., & Rezazadeh, F. (2012). Comparison of chemical compositions of essence in Stachys lavandulifolia in three habitats in Mazandaran province. *Congres of Medicinal Plants Yasouj* , 262.

Mahzooni, S., Mahdavi, M., Jouri, M., Akbrazadeh, M., & Roozbeh, N. (2014). The effects of altitude on cheical compositions and function of essentia oils in Stachy lavandulifolia Vahl. *Int. J. Med. Arom. Plants , 4* (2), 107-116.

Mossi, A., Pauletti, G., Rota, L., Echeverrigaray, S., Barros, I., Oliveira, J., y otros. (2012). Effect of diferent liming levels on the biomass production and essential oil extraction yield of Cunila galioides Benth. *74* (4), 787-793.

Nava Pérez, E., Garcia Guitiérrez, C., Camacho Báez, J. R., & Vázquez Montoya, L. (2012). Bioplaguicidas: una opción para el control biológico de plagas. *Ra Ximhai* , 17-29.

Patzelt, E. (1985). *Flora del Ecuador.* (B. C. Ecuador, Ed.) Quito, Ecuador: Imprefepp.

ISSN-On line: 2414-8830

Pérez, M., Navas, J., Pascual, M., & Castillo, P. (2003). Nematicidal activity of essential oils and amendements from Asteraceae against rott knot nematodes. *Plant Pathol , 52*, 395-401.

Sánchez Martín, M. J., & Sánchez Camazano, M. (1984). Instituto de Recursos Naturales y Agrobiología. *Los plaguicidas, adsorción y evolución en el suelo , primera*. (C. S. Científica, Ed.) Salamanca, España.

Saaverda N., Cosme W., Viturro C., Molina A., Molina S., (2003). Ensayo Piloto de Extracción de Volátiles de *Tagetes terniflora* HBk. http://www.efn.unc.edu.ar/otros/bibliocentro/index_archivos731-Tagetes.pdf.

Serrato Cruz, M. A. (1999). Variabilidad genética del Cempoalxóchitl. México: Colegio de Postgraduados.

Serrato, M., Díaz, F., & Barajas, J. (2005). Seasonal influence on phenology and essential oil content of *Tagetes filifolia* Lag. . *Annalen der Meteorologie , 41* (1), 82-85.

Serrato, M., Reyes, B., Ortega, L., Domingo, L., Gómez, N., López, E., y otros. (2003). Anisillo (*Tagetes filifolia Lag*.): recurso genético mexicano para controlar mosquita blanca (Bemicia sp. y Trialeurodes sp.) . *Revista del Jardín Boánico Nacional , 24* (1-2), 67-70.

Tariq, R., Naqvi, S., Choudhary, M., & Abbas, A. (2010). Importance and implementation of essential oil of Pakistanian Acorus calamus Linn., as a biopesticide. (J. Bot, Ed.) *42*, 2043-2050.

Terblanche, F., & Coronelius, G. (2000). A literature survey of the antifugal activity of essential oil constituents. *Essential Oil Bearning Plants , 3* (3), 139-156.

Tropicos. (2015). *Missouri Botanical Garden. Catalogue of vascular plants of Ecuador*. From http://tropicos.org/Name/40009619?projectid=2

Turner, B. L. (1997). Fossil history and geography. *Biology and Chemestry of the Compositae* , 21-40. London: Academic Press.

Turner, B. (1996). The Comps of Mexico-A systematic account of the family Asteraceae. *Phytol Memoirs , 6* (10), 1-93.

Ulloa, C. (2006). Aromas y sabores andinos. (M. Moraes, L. Kvist, B. Ollgard, & H. Balslev, Edits.) *Bótanica Económica de los Andes Centrales* .

Xu, L. W., Chen, L., Huan-yang, Q., & Yan-Ping, S. (2012). *Phytochemicals and their biological activities of plants of Tagetes.* (C. Herb, Ed.) Retrieved 2015 йил Mayo from http://www.google.com.mx/search?q=chinese+herbal+medicine+tagetes+2012&hl=es-MX&gbv=2&rlz=1W1ADFA_esMX447&oq=chinese+herbal+medicine+tagetes+2012&gs_l=heirloomserp.12...5648.11390.0.12794.13.12.0.0.0.0.921.921.61.1.0...0.0...1ac.1.15.heirloom-serp.fNhu-m

ISSN-On line: 2414-8830

Article **ECORFAN Journal**
December 2015 Vol.1 No.1 27-36

Rove diversity (coleoptera: Staphylinidae) in six coffee agroecosystems of Central Valley of Costa Rica

CALVO-ARAYA, José Alonso´*† & GONZALEZ-HERRERA, Allan´´

´Universidad Nacional de Costa Rica. School of Agricultural Sciences, Entomology Laboratory. Heredia Costa Rica.
´´Universidad Nacional de Costa Rica. School of Agricultural Sciences, Laboratory of Entomology. Heredia Costa Rica.

Received January 21, 2015; Accepted October 23, 2015

Abstract

The family Staphylinidae is second family belonging to the Order Coleóptera, with most (47 744) described species. The rove beetles are insect ecologically significant, carrying out different roles in agroecosystems; in many cases as predators of agricultural pest. Between March of 2007 and October of 2008, samples of these coleopterans were carried out in six coffee agroecosystems belonging to Central Valley of Costa Rica. The sampling consisted in the Quadrat Sampling Method, in this method a wood quadrat (0,5 x 0,5 m) was placed around coffee plants, where litter was collected and subsequent it was sieved and placed into Berless funnels to insect extract. Analyses of the components of biodiversity for the species, were carried out, calculating biological diversity index (Shannon-Wiener), species evenness, Margalef richness, Jaccard similarity index, non-metric multi-dimensional scaling (MDS) and analysis of similarities (ANOSIM) as well.

A total of 1406 specimens were captured. Of these, 382 (27,17%) were collected in Palmares, 355 (25,25%) in Tres Ríos Sombra, 212 (15,08%) in Barreal Sol, 183 (13,02%) in Barreal Sombra, 161 (11,45%) in Tres Ríos Sol and 113 (8,04%) in Naranjo.

Ten species determined and five undetermined belonging to seven subfamilies were caught. According to the biological diversity analysis, Palmares shows the highest richness of rove beetles in the sampled places.

Staphylinidae, agroecosystems, quadrat, biodiversity.

Citation: CALVO-ARAYA, José Alonso & GONZALEZ-HERRERA, Allan. Rove diversity (coleoptera: Staphylinidae) in six coffee agroecosystems of Central Valley of Costa Rica. ECORFAN Journal-Republic of Nicaragua 2015, 1-1: 27-36

* Correspondence to Author (email: josealonsocalvoaraya@gmail.com)
† Researcher contributing first author.

Introduction

The coffee industry is an activity of great economic importance in Costa Rica. Current market trends and requirements regarding the production and marketing of coffee in the world has seen the need for a more conservationist, friendly agriculture with the environment and with less use of external inputs, resulting in a coffee of highest quality and differentiated. Which is why, in recent years sustainable agriculture, has appeared as a way to maintain the productivity of agro-ecosystems, preventing the degradation of natural resources. So from an ecological perspective, there is a need to develop incentives for farmers to adopt alternative production systems that generate fewer negative effects on the environment.

Invertebrates in the fields play an important role as partners to determine the biodiversity of agro-ecosystems (Fuller et al 1995;. McCracken and Bignal 1998). The soil invertebrate fauna, including many groups of predators such as ground beetles and rove beetles, which play an important role in the natural control of pests. Increasing natural predation of pests through a more conservation farming, resulting in a reduction in pesticide use (Marasas et al., 2001).

Introducing shade trees in coffee system has a number of advantages such as improved aeration, drainage and soil fertility. According to Rice and Ward (1996) coffee cultivation associated with shade trees can act as shelter for some biodiversity and migratory and resident birds, insects, reptiles, mammals, among others.

Also Moguel and Toledo (2004), point to the importance of conserving the diversity being between 84 and 184 species of birds and 609 species of arthropod to in shade coffee.

Also, the incorporation of trees in coffee plantations may favor several groups of insects, which can achieve high levels of diversity in shaded plantations, among which highlights the Coleoptera (Nestel et al. 1993) order. In this group stands the family Staphylinidae, which is the second largest family of beetles with known number of species 47.744 (Bouchard et al. 2009). This family is distributed worldwide and is found in virtually all types of ecosystems. About half of rove beetles are found in mulch, becoming one of the most common and ecologically important insects of the soil fauna (Bohac 1999).

Staphylinidae most specific are known as predatory mites feeding on, collémbolos, nematodes and small insects and larvae; it can be used as potential drivers of some herbivorous insects (Bohac 1999; Bouchard et al. 2009). So far few studies have been developed in Costa Rica, to assess biodiversity in agro staphylinids coffee. So from the perspective of conservation agriculture, knowledge of biodiversity in the coffee agro staphylinids and functionality necessary; since some genres they might be acting as agents of biological control of pests, especially the coffee berry borer (*Hypothenemus hampei* (Ferrari).) Also staphylinids can be used as bio-indicators of environmental state and particularly of human influence agroecosystems (Bohac 1999). The objectives of this research is the identification of genres Staphylinidae species, collected in six agro coffee in the Central Valley of Costa Rica, to analyze the biodiversity present in each of the sampled sites and evaluate the possible effect of shade trees in coffee plantations staphylinids on biodiversity.

ISSN-On line: 2414-8830

Materials and methods

Location

The research was conducted in six different coffee agroecosystems belonging to the Central Valley of Costa Rica (Table 2). Within which they were handled entirely conventional systems and other managed alternately with different types of shade trees (Table 1).

Locality	Management	Altitude (m)	geographical coordinates	Average annual precipitation (mm)	Species shadow
three Rivers	Sun	1432	"lat 9,54836ºN", "long 83,58850ºW"	2 690,95	no
three Rivers	Shadow	1385	"lat 9,55072ºN", "long 83,59214ºW"	2 690,95	Eucalyptus deglupta, Inga sp.
Barreal	Sun	1010	"lat 9,58070ºN", "long 84,08574ºW"	1 543,35	no
Barreal	Shadow	1019	"lat 9,58010ºN", "long 84,08558ºW"	1 543,35	Eucalyptus deglupta
Palmares	Shadow	970	"lat 10,03120ºN", "long 84,25014ºW"	2 647,4	Erythrina sp., Inga sp., Gliricidia sepium, Dracaena fragrans (L) Ker-Gawl, Yucca Baker guatemalensis 1872.
Orange tree	Sun	1035	"lat 10,05381ºN", "long 84,22793ºW"	2 647,4	no

Table 1 Location of management systems and shade species used

For better understanding of the data in the following tables, the following abbreviations are used: TrSol, TrSombra, BSOL, BSombra, PSombra, nsol mean Tres Rios Sol, Three Rivers Shadow, Sun Barreal Barreal Shadow, Palmares and Naranjo, respectively.

Sampling

The samples were taken monthly from March 2007 to October 2008, so that each system was sampled a total of 20 times.

In each of the sites described above 10 different points samples they were taken. The first point was set at 100 meters from the entrance of each plot, the rest of the points are taken at random at a distance of 100-200 m between them. For sampling a combination of established methods used by Martin 1977; Bestelmeyer et al. 2000 for the study and sampling of arthropods inhabitants of litter and humus, which consisted of placing a wooden quadrant 0.5 x 0.5 m on the ground and so the coffee plants surround individually. All litter and litter located within the quadrant were collected and deposited in plastic for transport to the laboratory of Entomology at the National University of Costa Rica where they were sieved through a sieve (pore size 3 mm) bags. The resulting material was about 300 g deposited Berlese funnels for three days to extract staphylinids, which were collected in bottles of alcohol (75%).

Taxonomic identification

The specimens taken from each sample were retained and labeled. The species identification was performed at the Center of Studies in Zoology from the University of Guadalajara in Jalisco, Mexico.

Analysis of data

Shannon-Wiener index (H ') of biological diversity were calculated acording to the equation: where pi is the proportion of each species individually in the system, as well as Simpson biodiversity index according to the equation ni is calculated individuals in the species i and n is the total number of individuals.. And equity of Pielou species (J '= H' / H 'max) where H' max = ln (n). And species richness (Margalef) by the equation d = (S-1) / log N, where S = total number of species and N = total number of individuals.

ISSN-On line: 2414-8830

Jaccard similarity index, which is estimated based on absence-presence of species are also calculated. According to CJ equation j = (a + b - j), where j = Number of species in common between the two sites, a = No species in the first place and b = No. species at the second site. In a complementary way a multidimensional scaling (MDS) and an analysis of similarity of a road (ANOSIM) to detect differences in the structure of communities was held staphylinids.

To counteract the effect of the abundance of zeros one dummy variable is added. In the pretreatment of a square root data for processing data so that the most abundant and rare species had no influence on the analysis it is applied.

For the above software FIRST 6 (Plymouth Routines in Multivariate Ecological Research) Version 6.1.13 and PERMANOVA + Version 1.0.3 (Clarke and Gorley 2006) was used.

Results and discussion

Species composition

1406 individuals, from ten to five indeterminate certain species and seven subfamilies (Table 1) were collected. Indeterminate species could classify only to the level of subfamily. The subfamily with the highest number of species was Staphylininae 6, unlike most of the rest, which presented only two species and in the case of Paederinae, Pselaphinae and Scydmaeninae, which were only represented by a single species (Table 2).

Subfamilies with more individuals were Paederinae with 432 (30.73%), followed by Osoriinae with 333 (23.68%) and Staphylininae 261 (18.56).

Similarly Garcia et al. (2001); Garcia and Chacón de Ulloa (2005) reported 78 species grouped in eight subfamilies, of which Staphylininae Paederinae and were the most abundant and most species richness in dry forests in Colombia. Also, Jiménez Sánchez et al. (2009), an analysis of temporal variation of the diversity of rove beetles in tropical deciduous forest in Morelos, Mexico reported the second highest number of individuals within the Paederinae subfamily.

Gender	subfamily	Frequency	%
Thinocharis sp.	Paederinae	432	30,73
Aneucamptus sp.	Osoriinae	330	23,47
Lissohypnus sp.	Staphylininae	144	10,24
Coproporus sp.	Tachyporinae	123	8,75
Apocellus sp.	Oxytelinae	99	7,04
Indeterminada (Scy)	Scydmaeninae	60	4,27
Paederomimus sp.	Staphylininae	58	4,13
Indeterminada (Pse)	Pselaphinae	56	3,98
Carpelimus sp.	Oxytelinae	41	2,92
Indeterminada (St1)	Staphylininae	35	2,49
Indeterminada (St2)	Staphylininae	18	1,28
Indeterminada (St3)	Staphylininae	4	0,28
Nacaeus sp.	Osoriinae	3	0,21
Philonthus sp.	Staphylininae	2	0,14
Bryoporus sp.	Tachyporinae	1	0,07
Total		1406	100

Table 2 Total number of species, families and individuals Staphylinidae found in coffee systems studied

The distribution of the individuals species was heterogeneous, where *Thinocharis* sp. was the one who had the highest number of individuals with a total of 432 (30.73%), followed by *Aneucamptus* sp. 330 (23.47%). *Lissohypnus species* sp., *Coproporus* sp. and *Apocellus* sp., showed similar abundance between them. On the other hand species with fewer individuals they accounted for an undetermined species (St3), *Nacaeus* sp., *Philonthus* sp. and *Bryoporus* sp. each with less than 1% of all individuals.

When comparing the number of species identified for each of the systems, it shows that three of them (BSOL, BSombra and PSombra) had twelve species, followed by TrSol eleven species. On the other side they presented nsol TrSombra and only ten species each.

ISSN-On line: 2414-8830

A total of nine common species, *Apocellus* sp., *Carpelimus* sp., *Coproporus* sp., Indeterminate (PSE) Undetermined (SCY), *Lissohypnus* sp., Paederomimus sp. occurred in the six study sites which were *Aneucamptus* sp.. and *Thinocharis* sp. (Table 3). Moreover, the species *Philonthus* sp. and *Bryoporus* sp. they were present only in TrSol and BSombra respectively. For other systems no exclusive species was found.

The appearance of the species *Philonthus* sp. in TrSol, may be due to environmental conditions present in the system as a management system under the sun, the temperature of the soil surface and stubble this is usually higher compared to a shadow system. According to Hofmann and Mason (2006), a species belonging to the same genus as is the case *Philonthus cognatus* is an inhabitant of soils with high temperatures and high degree of compaction.

	agroecosystem											
	TrSol		TrSombra		BSol		BSombra		PSombra		NSol	
Gender	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Thinocharis sp.	65	40,37	241	67,89	21	9,91	16	8,74	73	19,11	16	14,16
Aneucamptus sp.	26	16,15	31	8,73	93	43,87	87	47,54	76	19,90	17	15,04
Lissohypnus sp.	15	9,32	17	4,79	17	8,02	37	20,22	39	10,21	19	16,81
Coproporus sp.	3	1,86	7	1,97	30	14,15	18	9,84	63	16,49	2	1,77
Apocellus sp.	13	8,07	10	2,82	15	7.08	4	2,19	31	8,12	26	23,01
Indeterminate (SCY)	9	5,59	11	3,10	12	5,66	13	7,10	7	1,83	8	7,08
Paederomimus sp.	19	11,80	18	5,07	7	3,30	1	0,55	5	1,31	8	7,08
Indeterminate (PSE)	7	4,35	7	1,97	6	2,83	1	0,55	23	6,02	12	10,62
Carpelimus sp.	1	0,62	12	3,38	5	2,36	3	1,64	16	4,19	4	3,54
Indeterminate (St1)	0	0	0	0	1	0,47	0	0	34	8,90	0	0
Indeterminate (ST2)	1	0,62	0	0	2	0,94	0	0	14	3,66	1	0,88
Indeterminate (St3)	0	0	0	0	3	1,42	1	0,55	0	0	0	0
Nacaeus sp.	0	0	1	0,28	0	0	1	0,55	1	0,26	0	0
Philonthus sp.	2	1,24	0	0	0	0	0	0	0	0	0	0
Bryoporus sp.	0	0	0	0	0	0	1	0,55	0	0	0	0

Table 3 absolute and percentage numbers Staphylinidae species found in the six coffee systems. Heredia, Costa Rica. 2009

The structure of dominance and roles staphylinids species varied depending on the type of management and the geographic location of the plantation. The most abundant species in the system and TrSombra TrSol was *Thinocharis* sp. While in the BSOL, BSombra PSombra systems and the most abundant species was *Aneucamptus* sp. Furthermore *Apocellus* sp. It was the dominant species in nsol (Table 3). These species showed a clear dominance in each system evaluated with respect to other species found within the system, in this case, it is possible that these species are mostly using available resources, compared to the rest which gives them a competitive advantage to increase their populations in the systems evaluated. Opposite situation seen in the case of PSol, where the effect of higher plant diversity of shade trees is reflected in better balance between species of rove beetles showing no clearly dominant species in the system, where the diversity of resources available within the system allows community structure more stable staphylinids. This related to other factors such as type of operation, characteristics, in situ that may have influenced the dominance and abundance of rove beetles.

	agroecosystem					
	TrSol	TrSombra	BSol	BSombra	PSombra	NSol
No. species	11	10	12	12	12	10
No. individuals	161	355	212	183	382	113
% Of individuals	11,45	25,25	15,08	13,02	27,17	8,04
Margalef richness	6,36	4,75	6,73	7,62	5,17	7,44
Equity Pielou	0,83	0,78	0,86	0,77	0,90	0,91
Shannon-Wiener	2,00	1,8	2,14	1,92	2,25	2,09

Biodiversity staphylinids

Systems with greater number of individuals were PSombra staphylinids (382) followed by TrSombra (355) and third BSOL (212). According to Shannon-Wiener index (Table 4), Pal shows the highest value of species diversity with a value of 2.25, followed by BSOL and nsol (2.14 and 2.09 respectively).

ISSN-On line: 2414-8830

The highest values of richness (Margalef index), which had BSombra (7.62) and nsol (7.44), presenting these two sites more functional relationship between number of species compared to the total number of individuals. The sites with greater equity corresponded to nsol (0.91) and PSombra (0.90), there being a higher proportion in terms of rove beetles species in those in the communities; It is all species in these similar sites in the number of individuals. Two of the remaining systems (BSOL and TrSol) presented intermediate values of equity analysis values of 0.86 and 0.83, respectively.

The most diverse agricultural ecosystem according to Shannon-Wiener index is PSombra, which significantly exceeded the other systems evaluated. These results may be related to a greater diversity of shade trees in this system, accompanied by high precipitation regimes since according to Buse and Good (1993), the increasing diversity of habitats, it may result in increased diversity of rove beetles.

The greatest diversity in PSombra, indicates that most of rove beetles prefer sites with high moisture content and a greater diversity of plant species and litter, which provides favorable environmental conditions and greater resources available for settlement in that system. These families of beetles are commonly found in nature, particularly in terrestrial habitats with wet conditions. (Bouchard et al. 2009).

Biodiversity values show that the resources provided by the PSombra system favors different species of rove beetles occupy these habitats and abundant display them. According to Frank and Thomas (2008) these insects prefer damp places, living on leaves, forest soils and other places with high organic matter content.

As well as several adults and larvae of rove beetles are associated with flowers. Therefore greater diversity and species composition in the coffee brings different micro habitats and different resources for the arrival of rove beetles and other organisms and insects, which could be directly related to the eating habits of this family of beetles. Also this could be due to other factors not analyzed in this research as altitude, physical and chemical soil characteristics, degree of erosion, farming practices, temperature, topography, vegetation of the area, wind), among other biotic and abiotic factors that could impact directly or indirectly on the abundance and diversity of staphylinids in coffee agroecosystems evaluated. About Jiménez Sánchez et al. (2009) mentions that at a local level, environmental variables such as altitude, slope, sunlight and water retention capacity of the soil, explaining changes in the structure and composition of vegetation. All these biotic and abiotic factors create a mosaic of microhabitats with differences in structure and composition of flora and fauna. In Mexico it has been reported that local, Staphylinidae fauna is distributed very unevenly due to variations in humidity, temperature and habitat disturbance (Jiménez-Sánchez et al. 2009).

On the other hand, the presence of high biodiversity in unshaded (BSOL and nsol) sites might be related to habitat preferences of some species.

Species similarity among sites sampled

Comparing species composition between systems, the index used shows that the most similar systems together were the TrSol and agro nsol worth 90.91 (Table 5). These were followed by BSOL and PSombra (84.62), and BSombra TrSombra, TrSombra and PSombra, BSOL and nsol, PSombra and values nsol 83.33 each.

ISSN-On line: 2414-8830

	Systems					
Systems	TrSol	TrSombra	BSol	BSombra	PSombra	NSol
TrSol	---------					
TrSombra	75,00	----------				
BSol	76,92	69,23	---------			
BSombra	64,29	83,33	71,43	----------		
PSombra	76,92	83,33	84,62	71,43	----------	
NSol	90,91	81,82	83,33	69,23	83,33	---------

Table 5 Indices of similarity (Jaccard) species of Staphylinidae in agroecosystems coffee in Costa Rica

By contrast the most dissimilar sites correspond to TrSol and BSombra (64.29), and BSOL TrSombra (69.23), and nsol BSombra (69.23) systems. There was a middle group with values between 71.43 and 76.92. Which indicates that there is a difference in the composition of communities in coffee agroforestry systems staphylinids (with and without shadow), because in most cases the sites were for similar systems under a single management, with it is the If TrSol and nsol?

The chart shows that EMD sites showed different communities staphylinids. Four of agro-ecosystems were grouped into two subgroups formed by TrSol and nsol, PSombra and Bsol. Showing these grouping similarities between peer groups. Conversely completely different sites respects others were BSombra and TrSombra (Fig. 1).

The sites showed differences in the composition of communities of rove beetles (Fig. 1), each site species selectivity.

The *Bryoporus* sp., Scy, and *Nacaeus* sp species. They were the most influential in the community of species in TrSombra staphylinids. Also *Apocellus* sp., PSE, St1, St2, St3 and *Coproporus* sp. They were shown to be most abundant in local conditions and Bsol PSombra. Species like Lissohypnus sp., *Carpelimus* sp. and *Aneucamptus* sp. They showed preference for BSombra and Psombra.

For *Philonthus* sp. and *Paederomimus* sp. They showed a slight dominance under the ecological conditions of TrSol and nsol

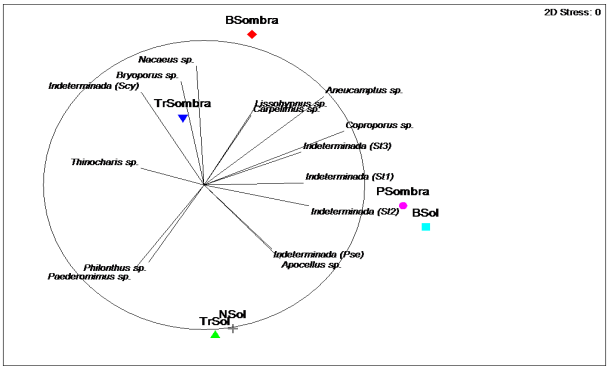


Figure 1 EMD in coffee systems studied. Heredia. Costa Rica

The results of ANOSIM one-way (Table 6) revealed that there were significant differences in the communities of staphylinids in coffee agro Central Valley Costa Rica analyzed (global R: 0.036 p <0.01).; In the individual analysis by pairs of sites only TrSol, BSOL groupings BSOL, BSombra; BSombra, nsol, showed no significant differences between them

Treatments	R Value	P Value
Pairwise		
TrSol, TrSombra	0,034	0,01
TrSol, BSol	0,022	0,02
TrSol,BSombra	0,024	0,01
TrSol, PSombra	0,022	0,01
TrSol, NSol	0,013	0,01
TrSombra, BSol	0,082	0,01
TrSombra, BSombra	0,097	0,01
TrSombra, PSombra	0,04	0,01
TrSombra, NSol	0,091	0,01
BSol, BSombra	0,002	15
BSol, PSombra	0,017	0,01
BSol, NSol	0,021	0,01
BSombra, PSombra	0,03	0,01
BSombra, NSol	0,015	0,02
PSombra, NSol	0,04	0,01

* Based on the similarity index Bray-Curtis.
Number of permutations 9999, overall R: 0.036 p <0.01%

Table 6 Analysis of similarity of a track (ANOSIM) *

ISSN-On line: 2414-8830

The data observed in the species similarity index, EMD and ratified in ANOSIM can see that there is a difference in the composition of communities in coffee staphylinids agroforestry systems analyzed. These results reflect the distinctive character of the fauna of rove about litter on the floors of coffee, proving that the different elements associated with the production systems, as in the case of vegetation associated with coffee, affects staphylinids communities in Costa Rica.

Understanding the different abiotic and biotic factors affecting the abundance, distribution and biodiversity in general staphylinids, it could be applied to biological control tactics within a system of integrated pest management. Since it has been shown in other crops staphylinids different species act as predators of various pests. For example Collins et al. (2002) showed that staphylinids can reduce aphid populations in cereals.

As for these living species of rove beetles on leaves, in this case the coffee mulch analyzed for their habit some identified here might be preying on larvae of the coffee berry borer, since this insect pest often develops part of its life cycle in the grains left on the ground. According to Varley and Gradwell (1971), *Philonthus decorus* (Gravenhorst, 1802) is a predator which feeds on moth pupae winter (*Operophtera brumata* (Lepidoptera: Geometridae) on the ground.

Therefore eating habits and behavior of some staphylinids identified here is to seek food in places on litter moisture and therefore compete to some degree with the development life cycle of the coffee berry borer on grain left on the floor in coffee systems, which sometimes come to suppress populations of mites and insect pests in different crops (Frank and Thomas 2008).

In general, the importance of staphylinids in relation to the competition with the coffee berry borer is unknown, which is required to conduct research to understand and quantify the significance of this family of beetles on the dynamics of these insect pest populations.

Conclusions

Regarding habits Staphylinidae in coffee systems very little is known in Costa Rica, so this study is try to measure the biodiversity of species belonging to this family and their possible use as biological control agents in these systems. Overall in this study significant differences in the sampled sites were obtained, reflecting the type of management in coffee affects the population dynamics of rove beetles in the soil. In this sense it is necessary to conduct future studies focused on the ecology of rove beetles, identifying detail abiotic and biotic factors which influence their distribution and abundance in coffee and determining which species act as predators of the coffee berry borer.

Acknowledgements

This research was carried out thanks to the economic contribution of CONICIT. We thank Dr. J. L. Navarrete-Heredia University of Guadalajara, Jalisco, Mexico, for his help in the identification of the species.

References

Bestelmeyer, B.T.; Agosti, D; Alonso, L.E; Brandão, C.R.F; Brown, W.L; Delabie, J.H.C; Silvestre, R. 2000. Field Techniques for the Study of Ground-Dwelling Ants: An overview, Description, and Evaluation. pp.122-144. In: Agosti, D; Majer, J.D; Alonso, L.E; Schultz,T.R. (Eds.). Ants. Standard Methods for Measuring and Monitoring Biodiversity . Smithsonian Institution Press. Washington and London.

Bohac, J. 1999. Staphylinid beetles as bioindicators. Agriculture, Ecosystems and Environment 74 (1999) 357–372

Bouchard, P; Grebennikov, V.V; Smith, A. B.T; Douglas, H. 2009. Biodiversity of Coleoptera. In Foottit, R.G; Adler, P.H. eds. Insect Biodiversity: Science and Society. Blackwell Publishing Ltd. p. 265-301.

Buse, A; Good, J. E. G. 1993. The effects of conifer forest design and management on abundance and diversity of rove beetles (Coleoptera: staphylinidae): implications for conservation. *Biological Conservation* 1993, 64, 67-76.

Clarke K. R.; R. N. Gorley. PRIMER v6: User Manual/Tutorial, PRIMER-E, Plymouth, UK, 2006.

Collins, K.L; Boatman, N.D; Wilcox, A; Holland, J.M; Chaney, K. 2002. Influence of beetle banks on cereal aphid predation in winter wheat. Agric. Ecosyst. Environ. 93, 337–350.

Frank, H.J; Thomas, M.C. 2008. Rove Beetles (Coleoptera: Staphylinidae). pp. 3218-3224. In Encyclopedia of Entomology. Second edition. Ed.Capinera, J.L. Springer Springer Verlag.

Fuller, R.J; Gregory, R.D; Gibbons, D.W; Marchant, J.H; Wilson, J.D; Baillie, S.R; Carter, N. 1995. Population declines and range contractions among farmland birds in Britain. *Conservation Biology*, 9, 1425–1441.

García, R.; Armbrecht, I.; Ulloa-Chacón, P. 2001. Staphylinidae (Coleoptera): composición y mirmecofilia en bosques secos relictuales de Colombia. Staphylinidae (Coleoptera). Composition and myrmecophilia in dry relictual forests of Colombia. Folia Entomológica Mexicana 40: 1-20.

García, R.; Chacón de Ulloa, P. 2005. Estafilínidos (Coleoptera: Staphylinidae) en fragmentos de bosque seco del Valle geográfico del río Cauca. Revista Colombiana de Entomología. 31: 45-50.

Hofmann, T.A; Mason, C.F. 2006. Importance of management on the distribution and abundance of Staphylinidae (Insecta: Coleoptera) on coastal grazing marshes. Agriculture, Ecosystems and Environment 114 (2006) 397–406.

Jiménez- Sánchez, E; Zaragoza-Caballero, S; Noguera, A.F. 2009. Variación temporal de la diversidad de estafilínidos (Coleoptera) nocturnos en un bosque tropical caducifolio de México. Revista Mexicana de Biodiversidad 80: 157-168, 2009.

Martin, J.E.H. 1977. Collecting, preparing and preserving insects, mites, and spiders. The Insects and Arachnids of Canada. Part 1. Agriculture Canada, Ottawa, 182 pp

Marasas, M; Sarandon, S; Cicchino, A. 2001. Changes in soil arthropod functional group in a wheat crop under conventional and no tillage systems in Argentina. Applied Soil Ecology 18 (2001) 61–68

Moguel, P. y V.M. Toledo. 2004. Conservar produciendo: biodiversidad, café orgánico y jardines productivos. *Biodiversitas* 55: 2-7 pp. CONABIO.

Nestel, D., F. Dickschen, and M. A. Altieri. Diversity patterns of soil macro-Coleoptera in Mexican shaded and unshaded coffee agroecosystems: an indication of habitat perturbation. Biodivers. Conserv. 2:1993. 70–78.

Rice, R; Ward, J. 1996. Coffee, conservation, and commerce in the Western Washington DC. Smithsonian Migratory Bird Center. 47 p.

Varley, G.C; Gradwell, G.R. (1971) The use of models and life tables in assessing the role of natural enemies. In Biological Control, (ed. C.B. Huffaker), pp. 93–112. Plenum Press, New York.

ISSN-On line: 2414-8830

Article **ECORFAN Journal**
December 2015 Vol.1 No.1 37-47

Diagnosis of mycotoxigenic fungi instored grain corn

MAIDANA-OJEDA, Marco´*†, ACOSTA-RAMOS, Marcelo´, ARÁMBULA-VILLA, Gerónimo´´ and CABRERA-MARÍA, Graciela´´´

´Universidad Autónoma Chapingo.Mexico-Texcoco highway 36.5 km. Chapingo, Texcoco, State of Mexico, Mexico.
´´Instituto Politécnico Nacional. Center for Research and Advanced Studies -Santiago de Querétaro, Qro. México.
´´´Universidad Nacional del Nordeste. 868 May 25, Corrientes, Argentina.

Received March 14, 2015; Accepted October 27, 2015

Abstract

Fungi in stored grains can cause harmful effects on the health of consumers due to the mycotoxins that they produce. aflatoxins are the most toxic and carcinogenic fungal metabolites that are frequently more found in nature. During 2014/2015 an investigation was conducted to analyze the interaction of storage conditions with the incidence of mycotoxigenic fungi and aflatoxin concentration in stored grain corn. 27 samples of stored corn grain from the main producers states of Mexico were taken, and the conditions in which they were stored were registered. Fungi and aflatoxins incidence were quantified in laboratory, and the interaction effect of seed conditions with those parameters. High incidence of *Fusarium* and *Aspergillu*s species were found, with 32 and 8% respectively, but with low levels of Aflatoxins. Also, we found influence between the levels of aflatoxins with the storage time and *Aspergillus* incidence. A high influence of grain moisture and volumetric weight with *Aspergillus* incidence was observed. Also we found an influence with the level of aflatoxins, storage time and *Aspergillus* incidence.

***Aspergillus*, aflatoxin, storage condition.**

Citation: MAIDANA-OJEDA, Marco, ACOSTA-RAMOS, Marcelo, ARÁMBULA-VILLA, Gerónimo and CABRERA-MARÍA, Graciela. Diagnosis of mycotoxigenic fungi instored grain corn. ECORFAN Journal-Republic of Nicaragua 2015, 1-1: 37-47

* Correspondence to Author (email: marcomo-1987@hotmail.com)
† Researcher contributing first author.

Introduction

Currently corn, along with wheat and rice are globally 75% of the produced grains. They provide 56% of carbohydrates and 50% protein food in the world, and in addition to providing nutrients for animals elements is a basic raw material for the processing industry (Suleiman et al., 2013;. Simopoulos, 2002 ; Morris and López-Pereira, 2000; FAO, 1993; Stoskopf, 1985; Malavolta et al., 1974).. Stored grain corn, depending on storage conditions and health of the culture from which they come, can be attacked by differentiated fungi pathogens into two groups. Field and store (Suleiman et al., 2013; Miller, 2008; Jouany, 2007; Carrillo, 2003; Arbeláez Torres, 1978; Christensen and Kaufmann, 1969). In Mexico, the most important fungal genera that infect grain storage are *Aspergillus*, *Fusarium* and *Penicillium* (Crhistensen and Lopez, 1962; Arrúa Alvarenga et al., 2012;. Garcia and Martinez, 2010; Hernández et al., 2007; Gallardo et al., 2006;. Bucio et al., 2005), they also are reported worldwide (Stumpf et al., 2013;. Maširević et al., 2012;. Marín et al., 2012;. Ghiasian et al., 2004;. Orsia et al., 2000). These fungi can cause harmful effects on the health of consumers by generating mycotoxins (Binder, 2007; Cabanes, 2000; Pitt, 2000; Martinez and Benavides Moreno Ocampo, 1988). 300 fungal toxins (.; Binder, 2007; Carrillo, 2003 Arroyo-Manzanares et al., 2014) are known. Aflatoxin is problem for many products, but when it comes to grains, is mainly a problem in maize (Miller, 1995).

The aflaxotinas B1, B2, G1 and G2 are the most toxic and carcinogenic fungal metabolites that are naturally more frequently, and the most important producers of *Aspergillus* species are the Flavi section, including *A. flavus, A. parasiticus* and other species (Richard, 2007; Bennett and Klich, 2003; Brasel Hussein, 2001; Widstrom, 1996; Miller, 1995).

Mexican Official Standard (NOM-188-SSA1-2002) sets a limit of 20 ppm aflatoxins in cereal grains for human consumption (Ministry of Health, 2002).

While aflatoxin limits tolerated in globally food vary from 0 to 35 ppm, more frequently from 4 to 20 ppm (FAO, 2004). The concentrations of mycotoxins are a function of fungi in stored grains developed and competition between them, which is determined the humidity, storage time, water activity, temperature, pH, substrate composition and presence of pests (Sanchis et al., 2007;. Shapira and Paster 2004). In stored grains with high humidity (>14%) and temperature (>20 ° C), and / or drying can be potentially contaminated inadequate (Ominski et al., 1994). Contamination of grains with mold and fungi is recognized as one of the most important problems in tropical countries around the world (Kaaya and Kyamuhangire, 2006), where the only viable solution to mycotoxins in the fork is to prevent growth fungal (Tefera, 2012; Carrillo, 2003).

This calls for the study of the interactions between these factors, allowing a better understanding of the influence of storage conditions on the content of mycotoxins in stored corn kernels, and therefore set design management strategies that reduce mycotoxin levels, prevention of fungal growth on stored grains.

The objective of this research was to analyze the interaction of storage conditions and the incidence of fungal mycotoxigenic and Introduction

ISSN-On line: 2414-8830

Materials and methods

The works were carried out in the Laboratory of pathogenic fungi of expertise in plant protection Universidad Autónoma Chapingo, located at km 38.5 of the highway Mexico - Texcoco, Chapingo, State of Mexico, and the laboratory of organic material of CINVESTAV Queretaro, located in the Real Fractionation Juriquilla, Santiago de Queretaro, Queretaro State.

The implementation period of the study was between the months of July 2014 to September 2015. corn samples were taken of grain stores of the major producing states (Table 1). They were performed in accordance with the provisions of the Official Mexican Standard (NOM-188-SSA1-2002) that sets the limits of aflatoxins in cereal grains.

State	Samples
Chiapas	6
Guanajuato	3
México	5
Morelos	2
Oaxaca	2
Puebla	4
Sinaloa	3
Tabasco	1
Veracruz	1
Total	**27**

During and immediately after sampling, the principal conditions in the grains were determined (humidity, type of storage, damaged grains, volumetric weight, storage time and genetic material) were determined.

12 seeds of each sample were used, which were initially disinfected on its surface, for 2 minutes, sodium hypochlorite at 2% rinsed with sterile distilled wáter and dried with sterile paper.

Later they were placed in petri dishes (3 seeds per Petri dish) containing medium potato-dextrose agar culture and incubated about 7 days at 27 (± 2) ° C (Hernandez et al., 2007; Carrillo, 2003; Quiñones Martinez, 2011). Isolations and purifications were performed by culturing hyphae tip of each strain of the fungus for subsequent identification (Morales et al., 2007; Ruiz Castañeda, 2001; French and Teddy, 1980).

The morphological identification of fungi was performed by microscopic observation of assemblies from developed isolates in Petri dishes on PDA culture medium and AA with carnation, with the technique of tape (transparent adhesive), (Arenas, 2003).

The observed and descriptions structures were compared with descriptive identification keys (Sampson et al., 2014;. Leslie and Summerell, 2006; Carrillo, 2003; Moreno-Martinez and Benavides Ocampo, 1988; Nelson et al., 1981; Booth, 1971) .

The biological and morphological characteristics considered during identification were: growth rate, appearance of pigmentation and aerial mycelium colony level in culture, presence or absence, arrangement, size, shape and color of micro and mesoconidias; size, shape, color and number of cells macroconidia, presence or absence of chlamydospores (Fusarium strains), size and shape of the bladder and head and presence or absence of metulae in *Aspergillus* strains.

For molecular identification as isolation frequency was considered and the eight most common strains were selected.

ISSN-On line: 2414-8830

For the extraction of fungal DNA, the protocol described by the company Macrogen DNeasy Plant Mini Kit Kit, Quiagen Brand, consisting of the following is followed:

Each fungus purified and incubated for 7 days in culture medium PDA, transferred 50 to 100 mg of mycelium, a microcentrifuge tube (Eppendorf), 400 uL of Buffer AP1 and 4 mL of RNase was added, stirred at vortex.

Samples were incubated in a water bath for 10 min at 65 ° C, stirring by inversion 2 or 3 times; They were added 130 mL of Buffer AP2 5 min and kept on ice. The mixture was placed in the QIAshredder mini spin column and centrifuged for 2 min at 14,000 rpm.

The liquid passed through the column to a new microcentrifuge tube, to which were added 1.5 volumes of buffer AP3 / E transferred. This solution was placed in a DNeasy Mini spin column, centrifuged for 1 min at 8,000 rpm, it was transferred to a new tube, and added 500 uL of buffer AW; He centrifuged again for 1 min at 8,000 rpm, and 500 uL of buffer AW was added, and then centrifuged for 2 min at 14,000 rpm, for the purpose of washing the DNA; finally, the DNeasy Mini spin column to a new tube was transferred, they were put 100 mL of buffer AE DNeasy for elution, incubated for 5 min at room temperature and centrifuged for 1 min at 8,000 rpm (Qiagen, 2012).

Once the DNA of the 8 strains obtained, the product was sent to Laboratories Macrogen in Korea, for the amplification of two universal regions corresponding to the ITS region and the region of the genes coding for the factor elongation.

Aspergillus strains for amplification of the ITS region was made and the universal primers ITS-4 (5'-TCCTCCGCTTATTGATATGC-3 ') and ITS-5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') rRNA genes were used, the subunit 18S, 5.8S and 28S, which amplify an internal intergenic spacer (ITS) and generate a product of varying size between 550 and 585 base pairs (bp) approximately (Samson et al., 2014).

Meanwhile, for strains with morphological characteristics corresponding to Fusarium, genes encoding elongation factor EF1 and EF2 GGAAGTACCAGTGATCATGTT ATGGGTAAGGAGGACAAGAC which generate a product of 700 bp (O'Donnell et al., 1998).

Quantification of aflatoxin was performed using affinity chromatography based on monoclonal antibodies, which detect aflatoxins B1, B2, G1 and G2 in grains (Aflatest / VICAM), validated by AOAC (1995). For the same the following procedure was followed: 50 grams of sample were ground and 5 g of free iodine salt, then they were mixed in a solution of methanol / distilled water 80:20 in a blender for 1 minute were added.

It later went through filter paper into a beaker; 10 mL of the obtained solution was diluted in 40 mL of distilled water. Subsequently, 2 mL of the extract filtered and the affinity column is spent per Aflatest at a rate of 1-2 drops.seg^{-1}, just as the affinity column Aflatest was washed 2 times by passing distilled water at a rate of 1- 2 drops.seg^{-1} 5mL.

ISSN-On line: 2414-8830

Aflatoxins were eluted from the affinity column by passing through the same 1 mL of HPLC grade methanol at a rate of 1-2 drops.seg-^{1}, collecting the resulting in a 10 mL tube; He was added 1 mL Aflatest developer, and placed for 30 seconds in a fluorometer VICAM Series 4 E, previously calibrated.

The data were sorted into a spreadsheet (Excel), and then multiple linear regression tests were performed using SAS version 9.3 software, including the frequency of isolates aflatoxinasy levels recorded storage conditions during sampling.

It was considered as a criterion variable for the repressor enter and remain in the model must have less than 0.05 type I error and where it was necessary, the intercept was not included in the model.

Results and discussion

General characteristics of the samples

Samples of corn kernels studied came from different types of establishments, either producer or commercial premises local storage, being the most part, from producing.

Storage forms varied according to the type of establishment where they came from. Those samples obtained from producers generally were stored in drums of 100 or 200 liters, or in bags, and less frequently in trojas or cozcomates. In the storage premises commonly performed in sacks, while in storage facilities was took place on grain silos with more advanced technology.

The reduced availability of technology in the grain storage by small farmers causing a greater amount of loss mainly in tropical and subtropical regions (IICA, 2012; Munkvold, 2003a; Jouany, 2007).

Mostly grains were analyzed native and hybrid varieties Asgrow7573, and lower frequencies and other hybrids like Cronos DK2060.

The stored grain moisture ranged from a range of 14.4 and 22.2%, with an average of 13.24% (Table 2). In 70% of grain moisture content was 13% or less, which according Munkvold (2003b) is recommended for storage of corn.

The storage time was also very variable (from 4-42 months), with 60% of samples stored for less than a year, with longer storage facilities producers destine their production to consumption.

Characteristics	Average	Minimum	Maximum
Humidity	13.24	10.40	22.20
Storage time (months)	11.81	4.00	42.00
Damage to the seed (%)	19.81	1.00	84.00
Volumetric weight (kg.hL-1)	72.24	46.90	80.70

Table 2 Characteristics of the samples studied grain corn. Year 2014/2015

The damage observed in the seeds were mechanical damage during harvest or insect damage, and were around 20%. Both storage time, as the level of damage having grains are determining factors in the incidence of mycotoxigenic fungi, and therefore of mycotoxins in the kernels (Christensen and Kaufmann, 1969; Binder, 2007) by.

For the volumetric weight average was 72.24 kg.hL-1, with a range that varied from 46.9 to 80.7 kg / hl, values very close to those reported by Peña Betancourt et al., (2013), who analyzed 15 samples from from different states of Mexico, Creole and hybrid grain corn varieties intended for consumption, they found that the volumetric weight fluctuated between 49 and 80 kg.hL-1.

ISSN-On line: 2414-8830

Frequency of isolation of fungi

The genera most frequently (incidence) were *Fusarium* and *Aspergillus*, with 32 and 8% incidence respectively.

These two genres together with *Penicillium* (1.1%) are the most common fungi in stored grain worldwide (Stumpf et al., 2013;. Maširević et al., 2012;. Marín et al., 2012;. Arrúa Alvarenga et al., 2012. ; Garcia and Martinez, 2010; Hernández et al., 2007; Gallardo et al., 2006; Bucio et al., 2005;. Ghiasian et al., 2004;. Orsia et al., 2000;. Crhistensen and Lopez, 1962).

Other genera of fungi were isolated *Cladosporium* (2.4%), *Rhizopus* (1.1%) and *Trichoderma* (1.4%) (Figure 1).

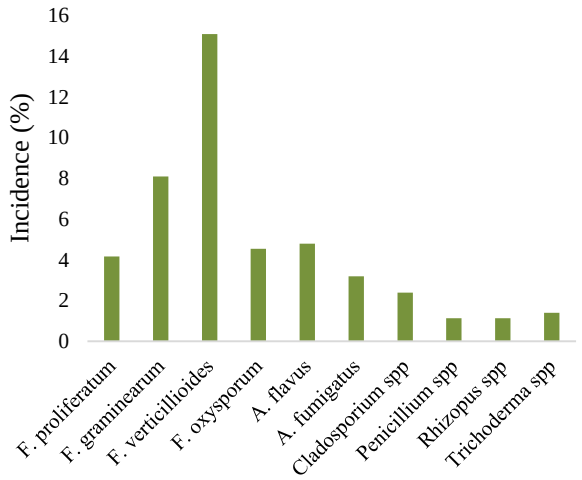


Figure 1 Frequency of isolation of fungi (%) in stored corn kernels from different states of Mexico

4 species of *Fusarium* (*F. verticillioides* 15.1%, 4.5% *F. oxysporum*, *F. proliferatum* 4.2% and 8.1% *F. graminearum*), and two species of *Aspergillus* (*A. flavus* and *A. fumigatus* 4.8% were identified 3.2%).

When multi-linear regression with a probability of type I error of 0.05 between the moisture content in the grains, the storage time, damage and volumetric seed weight, the incidence of *Aspergillus*, could generate a model, corresponding to the best fit to the following equation:

Incidence of *Aspergillus* = 2.11 (% moisture content in grain) - 0.27 (volumetric weight).

Some factors such as damage to grains either mechanically or by insects, facilitating fungal infection (Setamouet al., 1997), were part of the model when the probability of type I 0.1 error is allowed, but to reduce it 0.05 was no longer in the best-fit model, thus indicating that, although it has influenced the level of incidence of *Aspergillus*, the moisture content in the grains and the volumetric weight, had greater influence.

The grain moisture was the most influential factor in the incidence of *Aspergillus* spp. inthe evaluated samples, which coincides with Ominski et al., (1994), and together with temperature, strongly influenced the fungal invasion in stored grain.

The influence of volumetric weight is that associated with increased grain hardness (Correa et al., 2002), thus generating a greater difficulty to be invaded by microorganisms. Which partially explains the low incidence of these in stored grain.

However, none models separately acting factors, and the degree of fungal invasion depends on an interaction between them (Christensen and Kaufmann, 1969; Binder, 2007).

ISSN-On line: 2414-8830

Aflatoxin levels

Aflatoxin levels detected in the samples had a variation of between 0 and 68 ppm, with an average of 9.74 ppm (Figure 2). 93% of the samples had an aflatoxin concentration below the level allowed by the Official Mexican Norm (NOM-188-SSA1-2002), which sets the limits of aflatoxins in cereal grains for human and animal consumption 20 ppm.

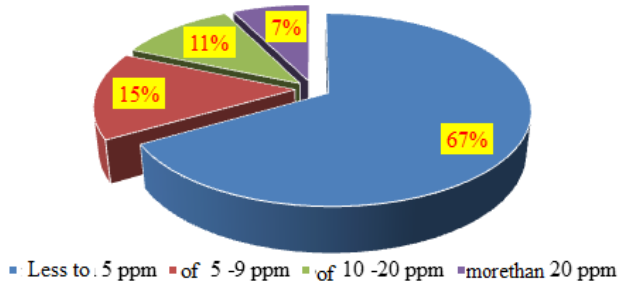


Figure 2 Levels of mycotoxins in samples stored corn kernels from different states of Mexico over the years 2014/2015

The mycotoxin levels above 20 ppm were found to a lesser extent, with a total of 7%. The best fit for this variable was the following model: aflatoxin levels (ppm) = 0.49 (storage time) + 0.68 (% incidence of *Aspergillus*).

According to background, Hell et al., (2000), in a study conducted in the countries of the east coast of Africa, found as the most influential factors in the accumulation of aflatoxins storage time, damage to the seed and use of local products or plants (excerpts) as protectants during storage.

But the degree of fungal invasion is well documented as one of the main factors causing postharvest mycotoxin content (Chulze, 2010; Bennett and Klich 2003; Swanson, 1987).

Conclusions

Fungi with the highest incidence in samples of corn grains were *F. verticillioides*, *F. oxysporum*, *F. proliferatum*, *F. graminearum*, *A. flavus* and *A. fumigatus*. As the moisture content in the grains and the volumetric weight of these factors most influence on the incidence of *Aspergillus* in the samples studied.

In 81% of the samples they were detected aflatoxin, although in lower concentrations to 20 ppm in 93% of them. Only 7% of the samples was out of the norm with concentrations greater than 20 ppmC and maximum of 49 ppm. The most influential factors in the levels of aflatoxins in grains were: storage time and the incidence of *Aspergillus* spp.

References

AOAC (Association of Analytical communities). 1995. Official Methods of Analysis of AOAC International In 16th edition AOAC International, Arlington, VA. Ed. Cunniff, P. pp. 20–21. Chapter 49.

Arbeláez Torres, G. 1978. Los problemas de las aflatoxinas en sorgo (*Sorghum bicolor*). In: El cultivo del sorgo. Instituto Colombiano Agropecuario, Bogotá, CL. no 26: 132-161.

Arenas, R. 2003. Micología médica ilustrada. 2nda Edición. McGraw Hill. México DF. 352p.

Arroyo-Manzanares, N; Huertas-Pérez, JF; Gámiz-Gracia,L; García-Campaña, M. 2014. Control de micotoxinas en alimentos. Revista Boletín GRASEQA no 7: 16-31.

ISSN-On line: 2414-8830

Arrúa -Alvarenga, AA; Quezada Viay, MY; Vázquez Badillo, ME; Flores Olivas, A. 2012. Incidencia de hongos potencialmente toxigénicos en maíz (*Zea mays* L.) de diferentes orígenes geográficos en México. Fitosanidad, 16(1):49-50.

Bennett, JW; Klich, M. 2003. Mycotoxins. Clinical Microbiology. Review 16: 497-516.

Binder,EM. 2007. Managing the risk of mycotoxins in modern feed production. Animal Feed Science and Technology, 133:149–166

Booth, C. 1971. The genus *Fusarium*. Commonwealth Mycological Institute, Kew Surrey. 237p.

Bucio, CM; Martínez, O; Morales, R. 2005. Contaminación con hongos en maíz recién cosechado en el estado de Guanajuato, durante el año 2003. Memorias del VII Congreso Nacional de Ciencia de los Alimentos, Gto., México, 2005.

Cabañes, F. 2000. Micotoxinas emergentes. Introducción. RevIberoamMicol; no 17:61-62.

Carrillo L. 2003. Los hongos de los alimentos y forrajes. Universidad Nacional de Salta. Salta, AR. 128 p.

Castañeda Ruiz, RF. 2001. Parámetros empleados para la identificación de los hongos conidiales. Pasado y presente en la taxonomía de los Hifomicetes. Rev. Protección Veg. 162(3): 92-101.

Christensen, CM; Kaufmann, HH. 1969. Grain Storage: The Role of Fungi in Quality Loss. U of Minnesota Press, Fungi in agriculture. 153 p.

Christensen, CM; López, LC. 1962. Daños que causan en México los hongos de granos almacenados. INIA, MX. Folleto técnico. no 44:31p.

Chulze, SN. 2010. Strategies to reduce mycotoxin levels in maize during storage: a review. Food Additives and Contaminants 27(5): 651-657.

Correa, CE; Shaver, RD; Pereira, M. 2002. Relationship between corn and ruminal in situ starch degradability. J. Dairy Sci.85:3008-3012.

FAO (Organización de las Naciones Unidas para la Agricultura y la Alimentación). 2004. Reglamentos a nivel mundial para las micotoxinas en los alimentos y en las raciones en el año 2003. FAO, Roma, IT. 45 p.

FAO (Organización de las Naciones Unidas para la Alimentación y la Agricultura) 1993. El maíz en la nutrición humana. (En línea). Consultado 02 ago 2014. Disponible en http://www.fao.org/docrep/t0395s/T0395S02.htm

French, E.R; Teddy, T.H. 1980. Métodos de investigación fitopatológica. Bib. Orton IICA/CATIE, San José, CR. 289 p.

Gallardo, E; Ibarra, G; Sánchez, R; Cuamea, G; Molina, D; Parra, N; Rosas, E; Cortez, M. 2006. Micobiota de maíz (*Zea mays* L.) recién cosechado y producción de fumonisina B1 por cepas de *Fusarium verticillioides* (Sacc.) NIRENB. Rev. Mex. de Fitopatología 24 (1): 27-34

ISSN-On line: 2414-8830

García, G; Martínez, R. 2010. Especies de *Fusarium* en granos de maíz recién cosechado y desgranado en el campo en la región de Ciudad Serdán, Puebla. Rev. Mex. Biodiv. 81(1): 15-20

Ghiasian, SA; Kord-Bacheh, P; Rezayat, SM; Maghsood, AH;Taherkhani, H. 2004. Micoflora of Iranian maize harvested in the main production areas in 2000. Micopathología. no 158:113-121.

Hell, K; Cardwella, KF; Setamoub, M; Poehling, HM. 2000. The influence of storage practices on aflatoxina, contamination in maize in four agroecological zones of Benin, West Africa. Journal of Stored Products Research 36: 365-382.

Hernández, S; Reyes, M; García, J; Mayek, N; Reyes, C. 2007. Incidencia de hongos potencialmente toxígenos en maíz (*Zea mays* L.) almacenado y cultivado en el norte de Tamaulipas, México, Rev. Mex. De Fitopatología 25 (2):127-133.

Hussein, HS; Brasel, JM. 2001. Toxicity, metabolism, and impact of mycotoxins on humans and animals. Toxicology 167:101–134.

IICA (Inter American Institute for Cooperation on Agriculture). 2012. Post Harvest Losses in Latin America and the Caribbean: Challenges and Opportunities for Collaboration (en linea). Consultado 09 ago 2015. Disponible en http://www.iica.int/Esp/Programas/SeguridadAlimentaria/Documentos%20Seguridad%20Alimentaria/IICA%20Post%20Harvest%20Loss%20ReportFinal.pdf

Jouany, JP. 2007. Methods for preventing, decontaminating and minimizing the toxicity of mycotoxins in feeds. Animal Feed Science and Technology, 137: 342–362

Kaaya, AN;Kyamuhangire, W. 2006. The effect of storage time and agro-ecological zone on mould incidence and aflatoxin contamination of maize from traders in Uganda", International Journal of Food Microbiology 110(3):217–223.

Leslie-F. J; Summerell-A, B. 2006. The *Fusarium* Laboratory Manual. Blackwell Publishing 369 p.

Malavolta, E; Haag, H.P; Mello, FAF. 1974. Nutrição mineral e adubação de plantas cultivadas. Sao Paulo, BR, CompañíaEditoralPaulista. 727 p.

Marín, S.; Ramos, A.J; Cano-Sancho, G; Sanchis, V. 2012. Reduction of mycotoxins and toxigenic fungi in the Mediterranean basin maize chain. PhytopathologiaMediterránea 51(1):93−118.

Maširević, S; Medić-Pap, S; Birvalski, S. 2012. Mycoflora of maize seed. Research Journal of Agricultural Science, 44 (2): 58-62.

Miller, D. 1995. Fungi and Mycotoxins in Grain: Implications for Stored Product Research. Review. J. stored Prod. Res. 31(1):l-16

Miller, D. 2008. Mycotoxins in small grains and maize: Old problems, new challenges, Food Additives & Contaminants: Part A, 25:2, 219-230.

Morales, R.I; Yañez, M.J; Silva, H.V; García, SG; Guzmán, DA. 2007. Biodiversity of *Fusarium* species in Mexico associated with ear rot in maize, and their identification using a phylogenetic approach. Mycopathologia 163(1):31-9.

ISSN-On line: 2414-8830

Moreno Martínez, E; Benavides Ocampo, C. 1988. Manual para la identificación de hongos en granos y sus derivados. UNAM, D.F. México. 109 p.

Morris, ML; López-Pereira, MA. 2000. Impactos del mejoramiento de maíz en América Latina, 1966-1997. México, D.F. CIMMYT. 45 p.

Munkvold, GP. 2003a. Cultural and genetic approaches to managing mycotoxins in maize. Annual Review of Phytopathology 41:99-116

Munkvold, GP. 2003b. Epidemiology of *Fusarium* Diseases and their Mycotoxins in Maize Ears. European Journal of Plant Pathology 109: 705–713.

Nelson, PE; Tousson, TA; Cook, R. 1981. F*usarium*: Disease, Biology and Taxonomy. Pennsylvania University Park, USA and London, UK: Pennsylvania State University Press.

O'Donnell, K; Kistler, C; Cigelnik, E; Ploetz, RC. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. Proceedings of the National Academy of Sciences of the United States of America 95: 2044–2049.

Ominski, KH; Marquardt, RR; Sinah, RN; Abramson, D. 1994. Ecological aspects of growth and mycotoxin production by storage fungi. In: Miller, J.D., Trenholm, H.L. (Eds.), Mycotoxins in Grain-compounds other than Aflatoxin. Eagan Press, St. Paul, pp. 287–312.

Orsia, RB; Corrëa, B; Possib, CR; Schammass, AE; Nogueira, JR; Dias, SMC; Malozzic, MAB. 2000. Mycoflora and occurrence of fumonisins in freshly harvested and stored hybrid maize. Journal of Stored Products Research no 36: 75-87.

Peña Betancourt, S.D; Carmona Medero, M.A; Valladares Carranza, B. 2013. Comparación de calidad física, contenido de fenoles y aflatoxinas en maíces híbridos y nativos. Revista Mexicana de Ciencias Agrícolas 4 (5):779-788

Pitt, J.I. 2000. Toxigenic fungi and mycotoxins. British Medical Bulletin 56(1): 184-192.

Quiagen. 2012. Manual del kit QIAamp® DSP DNA Blood Mini. Versión 2. Alemania.

Quiñones Martínez M. 2011. Manual de Prácticas de Eumicota. Universidad Autónoma de Ciudad Juárez. Ciudad Juárez, Chihuahua.

Richard, J.L. 2007. Some major mycotoxins and their mycotoxicoses—An overview. International Journal of Food Microbiology 119:3–10.

Samson, RA; Visagie, CM; Houbraken, J.; -B. Hong, S; Hubka, V; Klaassen, CH.W; Perrone, G; Seifert, K.A; Susca, A; Tanney, J.B; Varga, J;Kocsub, S; Szigeti, G; Yaguchi, T; Frisvad, J.C. 2014. Phylogeny, identification and nomenclature of the genus *Aspergillus*. Studies in mycology 78: 141–173.

Sanchis, V; Marín, S; Ramos, AJ. 2007. Factores determinantes en la producción de micotoxinas. En: Soriano del Castillo JM, eds. Micotoxinas en alimentos. España: Díaz de Santos, 63-68.

Secretaría de Salud. 2002. Norma oficial mexicana NOM-188-SSA1-2002, productos y servicios. Control de aflatoxinas en cereales para consumo humano y animal. Especificaciones Sanitarias. Diario oficial. Ciudad de México, MX. 15 de octubre 2002. 41 p.

ISSN-On line: 2414-8830

Setamou, M; Cardwell, KF; Schulthess, F; Hell, K. 1997. *Aspergillus flavus* infection and aflatoxin contamination of preharvest maize in Benin. Plant Dis. 81:1323–1327.

Shapira, R; Paster, N. 2004. Control of mycotoxins in storage and techniques for their decontamination. In: Mycotoxins in Food. Detection and Control. Eds. Magan, N; Olsen, M. CRC Press, WoodheadPublishin Limited, England. pp. 190–223.

Simopoulos, AP. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother 56: 365–379.

Stoskopf, NC. 1985. Cereal Grain Crops. Reston, Reston Publishing Company. Technology &Engineering - 516 p.

Stumpf, R; Dos Santos, J; Gomes, LB; Silva, CN; Tessmann, DJ; Ferreira, FD; Machinski, Junior, M; Del Ponte, EM. 2013. *Fusarium* species and fumonisins associated with maize kernels produced in Rio Grande do Sul State for the 2008/09 and 2009/10 growing seasons. Brazilian Journal of Microbiology 44 (1): 89-95 Suleiman, R.A; Rosentrater, K.A; Bern, C.J. 2013. Effects of deterioration parameters on storage of maize: A Review. Journal of Natural Sciences Research, 3(9):146-165.

Swanson BG. 1987. Mycotoxins on fruits and vegetables. Acta Horticulturae 207:49-61.

Tefera, T. 2012. Post-harvest losses in African maize in the face of increasing food storage. Food Sec. 4:267–277

Widstrom, N.W. 1996. The aflatoxin problem with corn grain. Advances in Agronomy 56: 219-280.

ISSN-On line: 2414-8830

Article **ECORFAN Journal**
December 2015 Vol.1 No.1 48-54

Evaluation of production explant or boneless blackberry *Rubus Glaucus* Benth in vegetative multiplication phase in a system of temporary immersion

ZAPATA-MALDONADO, Christian Iván´*†, LANDAZURI-ABARCA, Pablo Anibal and TAIPE, Marco Arturo

´Universidad de las Fuerzas Armadas ESPE. Laboratory in vitro plant tissue culture AGROBIOTECH. Av. Grl. Rumiñahui. Route of the Volcanoes. Sangolquí-Ecuador.

Received January 7, 2015; Accepted September 22, 2015

Abstract

In vitro cultivation of mulberry boneless Castile was performed using the temporary immersion system (SIT) to evaluate the dependence of the production of *Rubus glaucus* Benth in vegetative phase with respect to time of the cycle SIT were 12 , 15 , and 18 hours; and with respect to introducing culture medium which came the initial explants for SIT . As a result it was found that the best treatments for a good development of new shoots that will be obtained from the multiplication phase in the SIT are: cycle time of 12 hours and medium solid introduction; in addition to make the project economic analysis determined that multiplying the arrears boneless SIT increases in production compared to traditional propagation and consequent lower production costs , obtaining a profit ratio of 5 : 1

In vitro cultivation, mulberry boneless Castile, temporary immersion system, production costs.

Citation: ZAPATA-MALDONADO, Christian Iván, LANDAZURI-ABARCA, Pablo Anibal and TAIPE, Marco Arturo. Evaluation of production explant or boneless blackberry *Rubus Glaucus* Benth in vegetative multiplication phase in a system of temporary immersion. ECORFAN Journal-Republic of Nicaragua 2015, 1-1: 48-54

* Correspondence to Author (email: cchhee.18@gmail.com)
† Researcher contributing first author.

Introduction

In Ecuador, the demand for arrears of Castile boneless *Rubus glaucus* Benth to plus 3%, because this plant is related to a greater number of branches producing and tillering between 15% to 20% higher than the traditional default with spine (Sigarroa & Garcia, 2011). Ecuador is growing in the agricultural field; They are constantly developing new commercial productions, thanks to its growing conditions (Ramirez, 2009). The blackberry has productivity problems in Ecuador, by the use of traditional breeding. According to Monteiro (2004), the blackberry is propagated by vegetative methods such as cuttings and layering; these types of processes facilitate the spread of pests and diseases that affect the quality and quantity of production and consequently increase the economic loss to the producer; while by the in vitro method, a large number of outbreaks would be obtained from small amounts of tissue (Pati, Sharma, Sood, & Ahuja, 2004).

Being limited production boneless arrears of Castile for the reasons mentioned, it is looking for new strategies to increase the multiplication of *Rubus glaucus* Benth, and for this one of the techniques of vegetative propagation in vitro culture is used. Temporary Immersion System (TIS), which apart from cheaper production costs and overcome the difficulties of static cultures, they exhibit the ability to automate some stages of cultivation in vitro, allow ease of scale and increase the rates of multiplication is used , development and productivity of materials spread (Pérez, Ponce, Jiménez, & Agramonte, 1998).

Materials and methods

The mother plant blackberry castile boneless was collected in Sangolquí-Ecuador, located in the S 0`18`16.37``y O 78`27`06.92`` coordinates, at an altitude of 2.472 meters.

Collection of plant material

The sampled plants were selected in situ choosing explants with the best features: more leaves, free of disease; besides plants with an adequate number of buds and similar physiological conditions (Pierik, 1990).

Disinfection of plant material

twig washings vigorously with a solution of commercial detergent detergent, then immersed in a solution of Benlate® 2 g Tetracycline (5 mg), liquid soap (100 ml), iodine (5 ml) and citric acid (0.1 g), all volumetric 4 liters of distilled water for 45 minutes.

After disinfection, it was rinsed with distilled water three times for one minute each wash. The braces are cut into pieces of 10 cm, leaving the center of each cut one to two buds.The plant material was transferred to a laminar flow chamber, and placed in an 8% solution of commercial bleach for ten minutes, washed with distilled water three times for ten minutes.

Establishment of in vitro culture

Preparation of culture medium

Murashigue salts and 50% Skoog macro- and micronutrients were used additionally placed 15 gL -1 sucrose, 0.5 mL BAP [1500 mL L-1] and 0.1 mL AIA [1500 mL L-1]; was adjusted pH 5.6 culture medium, then 4 g of agar was added. 20 ml of medium was dispensed into pre-sterilized containers and then the containers were autoclaved at a pressure of 15 PSI at 121 ° C for 30 minutes.

ISSN-On line: 2414-8830

Planting in vitro explant

After disinfection, the explants were seeded in the culture media for establishing and transferred to growth room where they remained for a period of 20 days at constant light conditions (16 hours light and 8 hours dark), the average temperature 23 ° C.

Temporary immersion system

Preparation of liquid culture medium for multiplication

Murashigue salts and 50% Skoog macro- and micronutrients were used additionally placed 30 gL -1 sucrose, 1.3 ml BAP [1500 mL L-1] and 0.3 ml AIA [1500 mL L-1]; the pH of the culture medium was adjusted to 5.6. Means at a pressure of 15 PSI at 121 ° C were autoclaved for 30 minutes.

Planting in vitro explant

Temporary immersion system, formed by two glass flasks of 850 ml capacity, one for the growth of the explants and the other as a reservoir of culture medium was used. These bottles were connected together by a silicone hose 6 mm diameter.

The culture medium was circulated from a vial to another depending on the opening or closing according to the programmable timer to determine the frequency and duration of immersion. At the entrance of bottles hydrophobic filters (0.20 um,) was placed to ensure sterility air. The air pressure was regulated by a pressure gauge.

The explants used in the course of SIT were taken from the establishment phase, and those explants that had similar characteristics were considered: length (3 cm) and stem diameter (2 mm), and were free of contamination.

Apical cut to plant in containers corresponding to the explants bioreactors. After seeding, the cultures were taken and remained in the growth chamber for a month in constant light conditions (16 hours light and 8 hours darkness) and average temperature of 23 ° C. after a month, we proceeded to the collection of the data.

Programming SIT

We worked with the constant immersion time two minutes, varying only the frequency and means of introducing the initial explants bioreactor.

Results and discussion

Selection of the optimal dose of BAP or BAP + PBZ in liquid culture medium partial immersion for the multiplication of blackberry *Rubus glaucus* Benth boneless.

Standardizing the culture medium for multiplication was performed by applying HIDE (paclobutrazol) at four different concentrations to homogenize the shoot growth.

Trat.	No. Outbreaks		Bud length (mm)		Bud diameter (mm)	
	Media		Media		Media	
T1	3.4	c	33.7	d	1.05	b
T2	3.3	b	3.9	c	1.10	c
T3	3.3	b	2.2	b	1.20	d
T4	0	a	0	a	0	a

Means with common letter are not significantly different ($p > 0.05$)

Table 1 Duncan Test. Variables analyzed in the partial immersion assay

In the variable number of outbreaks, the T1 (1.3 mg.L-1 BAP) had a higher number of sprouts (3.4 buds per explant); in the variable length of the outbreak, T1 (1.3 mg.L-1 BAP) had a longer length of shoots (33.7 mm).

ISSN-On line: 2414-8830

And for the variable diameter of the outbreak, have the T3 (1.3 mg.L-1 BAP; HIDE 1.25 mg.L-1) has the value of the average diameter higher outbreak (1.2 ± 0.01 mm) with respect to the other treatments (Table 1).

Positive results were obtained in treatments T2 (BAP: 1.3 mg.L-1; HIDE: 0.75 mg.L-1) and T3 (BAP: 1.3 mg.L-1; HIDE: 1.25 mg.L-1), the which showed homogeneous outbreaks, but decrease the number and length thereof; also thickened stem diameter, and these results are similar to those described by Rodriguez Aranguren & Farres (2005) in his research that works with concentrations HIDE 0.125 mg.L-1. This result was similar to that found by Avilán, Soto, Escalante, Rodriguez, & Ruiz (2003), who noted the effect of paclobutrazol in curtailing the size of outbreaks.

Using data shoot length depending on the concentration of HIDE, a polynomial regression, which was plotted (Figure 1) and the equation thereof was calculated was performed with reference to 35mm as ideal length for working in multiplication of delay in SIT. As a result the following equation is obtained: y = 1.786 + 0.006x2-0.253x, with R2 = 0.973. Where x is the length of the outbreak is expected to achieve (35 mm) and y is the concentration of HIDE to be found.

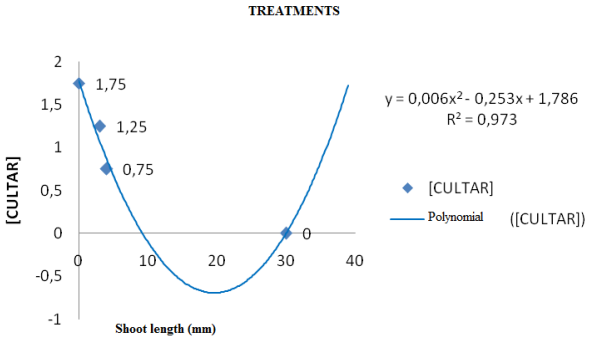


Figure 1 Linear regression polynomial concentration CULTURE.

Thus it was determined that the concentration of HIDE Ideally y = 0.281 mg.L-1. With this concentration, it can be obtained theoretically homogeneous shoots and bud expected length.

In addition, the T1 (1.3 mg.L-1 BAP) as the most suitable treatment to continue the multiplication phase delay in the Temporary Immersion System since according to Figure 1 shows is considered as low HIDE concentration, shoot length increases.

Evaluation of three days of temporary immersion cycle of production, for boneless explants blackberry *Rubus glaucus* Benth, in the multiplication phase of the SIT.

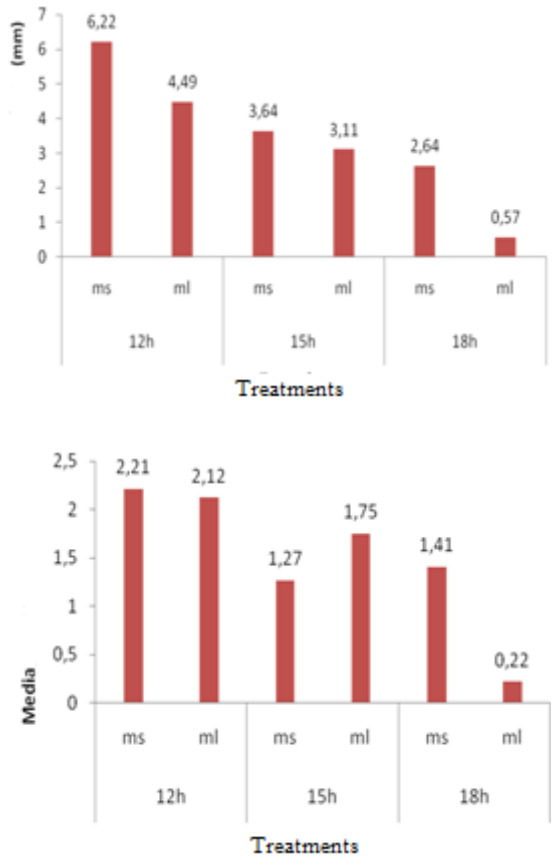


Figure 2 Mean (cycle-time interaction through introduction) of the variables fresh weight, shoot length, number of leaves per shoot and production rate

ISSN-On line: 2414-8830

The value of the highest average for the fresh weight was in the TR1 (12 hours, solid medium) (1 ± 0.04g); for shoot length was obtained TR1 (12 hours, solid medium) (6.22 ± 0.12 mm); for the variable number of leaves per shoot was the TR1 (12 hours, solid medium) (5.07 ±. 0,17 cm and the production rate corresponds to TR1 (12 hours, solid medium) (2.21 ± 0.23) compared to other treatments.

According Castro & Gonzales (2002), to achieve development in in vitro tissue culture, it is necessary to provide plants with essential nutrients sufficient, so they are not a limiting factor for multiplication in the plant growth factor. Therefore, the T5 (18 hours, liquid medium) and T6 (18 hours, solid medium) treatments reported no multiplication; According Escaletes and DOSBA, (1993), the phenolization is characterized by a change in the color of the tissue from green to brown, and this happens not to have a willingness bioregulators; as well as in the present work, where explants changed color from green to brown by the phenolization and also for dehydration they suffered when there are very long frequencies.

The results showed significant differences between treatments with the same cycle time but with the initial explants from different culture media introduction. Thus, T1, T3 and T5, despite being with T2, T4 and T6 respectively in the same cycle time, had higher production index (greater stem length, the greater number of leaves, fresh weight) per stem initial explants solid culture medium introduction.

These results also reflect according Debergh and Maene (1981) and Robert Smith (1990) and Preil and Hempfling (2002), Bautista, et al., (2004).

Who claim that the explants that are in liquid culture medium the surface of the explant come into direct contact with the environment which allows the most efficient uptake and release of nutrients which can accumulate in the tissue area that disperse quickly in liquid culture medium in the solid toxic metabolites.

It can also be attributed to a higher absorption of growth regulators in the culture medium changes from solid to liquid (Jambhale, 2000). Research has shown that in the liquid medium, the availability of water, minerals, and growth regulators, is greater when compared with semisolid and solid media culture, which promotes faster growth (Debergh, Harbaoui , & Lemeur, 1981).

In addition, the peculiarity that randomly explants, regardless of treatment had shoot formation took two forms: the shoots formed from an origin point, and throughout the explant, this can be attributed to a lack light distribution or arrangement in which the explants are inside the bioreactor by the agitation produced by the medium when in the immersion time, since the direction in which the sprouts are grown from explants are conditioned by the auxin production, and this tends to accumulate in the plant part that is shaded; therefore if the plant does not have shade, auxin drop from the apical part of the explant to the base, promoting the formation of buds from the same point, otherwise, they will go along the explant (Squeo & Cardemil, 2006).

Cost analysis

The comparative analysis of costs between multiplication of delay in SIT and conventional form showed that the increase in the SIT has lowered cost and more useful than conventional multiplication.

ISSN-On line: 2414-8830

In the SIT, in the best treatment production plants 170 in a month was obtained with PVP 7.48 USD per plant and a return of 62 months, while conventionally multiplying an output of 35 plants in one month is obtained with a PVP 32.05 USD per plant and a return of 296 months. Thus it is said that the production rate of blackberry *Rubus glaucus* Benth is 5-1 between SIT and multiplication in the conventional manner respectively.

Conclusions

For multiplication of delay in SIT, the T1 (1.3 mg.L-1 BAP 0 mg.L-1 HIDE) gave highest number of outbreaks (4 shoots per explant) and longer (35mm) to T2 (3 shoots per explant, shoot length 30mm), T3 (3 shoots per explant, shoot length 20mm) and T4. Paclobutrazol at concentrations of 0.75 mg.L-1 (T2) and 1.25 mg.L-1 (T3), homogenizes the length of outbreaks of blackberry *Rubus glaucus* Benth boneless without inhibiting their growth and development altogether but decreases number of shoots and length.

The treatment showed higher production of blackberry *Rubus glaucus* Benth boneless SIT in bioreactors are working 12 hours cycle time and their means of explants derived from solid introduction.

1 (SIT Multiplication: PVP = 7.48; conventional Multiplication: PVP = 32.05) with the use of SIT producing mulberry *Rubus glaucus* Benth because 5 is increased relative to the conventional method which reduces production costs and increases earnings.

References

ABT, A. E. (18 de 02 de 2010). Produccion de mora se incrementa. *Diario el Hoy* .

Avilán, L., Soto, E., Escalante, H., Rodríguez, M., & Ruíz, J. (2003). Manejo de altas densidades de población en mango. *Revista Digital del Centro Nacional de Investigación Agropecuarias de Venezuela* .

Castro, D., & Gonzales, J. (2002). Eucalyptus (Eucalyptus gradis Hill ex Maiden) micropropagation in a temporary immersion system. *Agricultura sostenible* , 68-78. Colombia.

CIRAD, C. d. (2003). Recipiente con Inmersión temporal para cultivo de tejidos de plantas. *Las principales ventajas del sistema Rita* .

Debergh, P., Harbaoui, Y., & Lemeur, R. (1981). Mass propagation of flobe artichoke (Cynara scolymus): evaluation of different hypotheses to overcome vitrifi-cation with special reference to water potencial. *Physiol Plant* , 53:181-187.

Jambhale, N. P. (2000). Efecto de la cantidad de subcultivos en la mutlipilicacion in vitro de cuatro clones de banano. *INFOMUSA , 10* (1).

Pati, P., Sharma, M., Sood, A., & Ahuja, P. (Marzo de 2004). Direct shoot regeneration from leaft explants of Rosa Damascena Mill. *BioOne 2010 US/Canada , 40 (2)* , 192-195.

Paucar, M. (2011). *Organogénesis directa in vitro a partir de explantes de hojas de mora (Rubus glaucus* Benth*).* Escuela Politécnica del Ejército, Departamento de ciencias de la vida, Sangolquí.

Pierik, R. (1990). Cultivo in vitro de las plantas superiores. *L.M.S., Ayerbe, Trad. Mundi-Prensa*.

ISSN-On line: 2414-8830

Rodríguez, K., Aranguren, M., & Farrés, E. (2005). Efecto del paclobutrazol en el desarrollo vegetativo e inicio de la floracion en dos cultivares de mango (Mangifera indica L.). *Instituto de Investigación en Fruticultura Tropical* . Cuba.

Sigarroa, A., & García, C. (2011). Establecimiento y multiplicacion in vitro de mora de castilla (*Rubus Glaucus* Benth) variendad sin espina, mediante ápices meristemáticos.

Squeo, F. A., & Cardemil, L. (2006). Hormonas y Reguladores del Crecimiento: Auxinas, Giberelinas y Citocininas. En *Fisiología Vegetal*. La Serena, Chile: Universidad de La Serena.

Stewart, C. (2008). Plant Biotechnology and Genetics: Principles, Techniques, and Applications. *University of Tennessee*.

ISSN-On line: 2414-8830

Instructions for authors

A. Submission of papers to the areas of analysis and modeling problems of the:

- Agriculture
- Forest Pathology
- Sustainable Forest Management
- Horticulture
- Engineering and Integrated Water Use

B. The edition of the paper should meet the following characteristics:

-Written in English. It is mandatory to submit the title and abstract as well as keywords. Indicating the institution of affiliation of each author, email and full postal address and identify the researcher and the first author is responsible for communication to the editor

-Print text in Times New Roman #12 (shares-Bold) and italic (subtitles-Bold) # 12 (text) and #9 (in quotes foot notes), justified in Word format. With margins 2 cm by 2 cm left-right and 2 cm by 2 cm Top-Bottom. With 2-column format.

-Use Calibre Math typography (in equations), with subsequent numbering and alignment right: Example;

$$r_i = \frac{p_i - p_j}{p_j} = \frac{p_i}{p_j} - 1 \quad (1)$$

-Start with an introduction that explains the issue and end with a concluding section.

- Items are reviewed by members of the Editorial Committee and two anonymous. The ruling is final in all cases. After notification of the acceptance or rejection of a job, final acceptance will be subject to compliance with changes in style, form and content that the publisher has informed the authors. The authors are responsible for the content of the work and the correct use of the references cited in them. The journal reserves the right to make editorial changes required to adapt the text to our editorial policy.

C. Items can be prepared by self or sponsored by educational institutions and business. The manuscript assessment process will comprise no more than twenty days from the date of receipt.

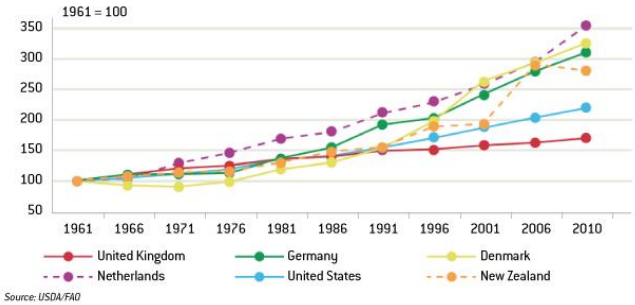
D. The identification of authorship should appear in a first page only removable in order to ensure that the selection process is anonymous.

E. Charts, graphs and figures support must meet the following:

-Should be self-explanatory (without resorting to text for understanding), not including abbreviations, clearly indicating the title and reference source with reference down with left alignment number 9 with bold typography.

-All materials will support gray scale and maximum size of 8 cm wide by 23 cm tall or less size, and contain all content editable.

- Tables, grpahs and figures should be simple and present relevant information. Prototype;



Graph 1 Agriculture Factor Productivity

F. References are included at the end of the document, all its components will be separated by a comma and must the following order:

- Articles: Babcock, B. A. (2015). Geographical indications, property rights, and value-added agriculture. Iowa Ag Review, 9(4), 1.

- Books: Ohkawa, K., Johnston, B. F., & Kaneda, H. (2015). Agriculture and economic growth: Japan's experience. Princeton University Press.

- WEB Resources: www.agriculture.gov.sk.ca, see: (August, 25-2015)

The list of references should correspond to the citations in the document.

G. The notes to footnotes, which should be used and only to provide essential information.

H. Upon acceptance of the article in its final version, the magazine tests sent to the author for review. ECORFAN-Republic of Nicaragua only accept the correction of types and errors or omissions from the process of editing the journal fully reserving copyright and dissemination of content. Not acceptable deletions, substitutions or additions which alter the formation of the article. The author will have a maximum of 10 calendar days for the review. Otherwise, it is considered that the author (s) is (are) in accordance with the changes made.

I.Append formats Originality and Authorization, identifying the article, author (s) and the signature, so it is understood that this article is not running for simultaneous publication in other journals or publishing organs.

Leon, Republic of Nicaragua ____, ____ 20_____

Originality Format

I understand and agree that the results are final dictamination so authors must sign before starting the peer review process to claim originality of the next work.

_____

Article

_____

Signature

_____

Name

Leon, Republic of Nicaragua ____, ____ 20_____

Authorization Form

I understand and accept that the results of evaluation are inappealable. If my article is accepted for publication, I authorize ECORFAN to reproduce it in electronic data bases, reprints, anthologies or any other media in order to reach a wider audience.

_____

Article

_____

Signature

_____

Name

ECORFAN Journal-Republic of Nicaragua

ISSN-On line: 2414-8830