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EVALUACIÓN DE LA APORTACIÓN DE *AZOSPIRILLUM BRASILENSE* Y *RHIZOPHAGUS INTRARADICES* SOBRE LA CALIDAD DE PLÁNTULAS DE *CAPSICUM CHINENSE* JACQ.

TESIS

Que presenta:

Gregory Stephen Romain

Como requisito parcial para obtener el grado de:

Maestro en Ciencias en Horticultura Tropical

Director de tesis:

Dr. Carlos Juan Alvarado López

Conkal, Yucatán, México
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El comité de tesis del candidato a grado: **Gregory Stephen Romain**, constituido por los CC. **Dr. Carlos Juan Alvarado López**, **Dr. René Garruña Hernández** y **MC. Alfonzo Pérez Gutiérrez**, habiéndose reunido con el fin de evaluar el contenido teórico-metodológico y de verificar la estructura y formato de la tesis titulada: **Evaluación de la aportación de *Azospirillum brasilense* y *Rhizophagus intraradices* sobre la calidad de plántulas de *Capsicum chinense* jacq.**, que presenta como requisito parcial para obtener el grado de Maestro, según lo establece el Capítulo 2, inciso 2.13.3, de los Lineamientos para la Operación de los Estudios de Posgrado en el Sistema Nacional de Institutos Tecnológicos, dictaminaron su aprobación para que pueda ser presentada en el examen de grado correspondiente.

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DECLARATORIA DE PROPIEDAD

Declaro que la información contenida en las secciones de materiales y métodos, resultados y discusión de este documento, es producto del trabajo de investigación realizado durante mi estudio de posgrado y con base en los términos de la Ley Federal del Derecho de Autor y la Ley de la Propiedad Industrial le pertenece patrimonialmente al Instituto Tecnológico de Conkal. En virtud de lo manifestado reconozco que los productos intelectuales o desarrollos tecnológicos que se deriven de lo correspondiente a dicha información son propiedad de la citada institución educativa.

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RESUMEN

La aplicación de fertilizantes químicos ha alterado las propiedades fisicoquímicas y biológicas del suelo en muchos sistemas agroecológicos. En la actualidad, el costo del fertilizante está aumentando significativamente y, por lo tanto, la sostenibilidad agrícola parece estar en declive. El uso de biofertilizantes constituye un componente importante en la práctica agrícola sostenible por su papel en la fijación del nitrógeno atmosférico y la solubilización del fósforo. Los biofertilizantes también estimulan la síntesis de hormonas vegetales, lo que promueve una mejor absorción de nutrientes y una mayor tolerancia a la sequía y al estrés hídrico. Además, los efectos benéficos de los biofertilizantes sobre el crecimiento de las plantas también pueden atribuirse a mecanismos tales como; supresión de patógenos mediante la producción de antibióticos y sideróforos o actividad antagonista bacteriana y fúngica. Las investigaciones indican que, en comparación con aplicaciones individuales, las aplicaciones múltiples de microorganismos benéficos en algunos vegetales dan como resultado un aumento de las respuestas de las plantas. El uso de biofertilizantes en sistemas agrícolas sostenibles ha ido aumentando a lo largo de los años. Por lo tanto, el objetivo de este estudio fue evaluar la respuesta de plántulas de chile habanero variedad Mayapán inoculadas y co-inoculadas con *Rhizophagus intraradices* y *Azospirillum brasilense*. Las semillas fueron acondicionados individualmente y en combinación con *Azospirillum brasilense* y *Rhizophagus intraradices* durante 24h antes de ser sembradas en charolas. Las variables evaluadas fueron: tasa de emergencia, porcentaje de emergencia, altura, diámetro del tallo, volumen y longitud de las raíces, contenido de minerales, biomasa seca del vástago y de las raíces. Las variables morfológicas incluyeron: índice de calidad de las plántulas, índice de esbeltez y tasa de crecimiento relativo. Las semillas acondicionadas con *R. intraradices* + *A. brasilense* mostraron un aumento en la tasa de emergencia del 14% con respecto al control. Sin embargo, la altura y el diámetro del tallo, la longitud de la raíz y el brote, la biomasa seca de raíces y brotes de los tratamientos, así como la concentración de nutrientes en plántulas disminuyeron con respecto al control. Los resultados indican que la co-inoculación con *R. intraradices* y *A. brasilense* contribuyó a incrementar la tasa de emergencia. Por lo tanto, *R. intraradices* y *A. brasilense* pueden ser considerados y evaluados como una estrategia para mejorar la emergencia de las plántulas.

ABSTRACT

Application of chemical fertilizers has disturbed the physico-chemical and biological properties of soil in many agro-ecological systems. At present the cost of fertilizer is increasing significantly and thus agricultural sustainability appears to be on the decline. The use of biofertilizers constitute is an important component in sustainable agricultural practice for their role in atmospheric nitrogen fixation and phosphorous solubilization. Biofertilizers also stimulate the synthesis of plant hormones, which promotes better nutrient uptake and increased tolerance to drought and moisture stress. Additionally beneficial effects of biofertilizers on plant growth can also be attributed to mechanisms such as; suppression of pathogens by producing antibiotics and siderophores or bacterial and fungal antagonistic activity. Reports indicate that, compared to single applications, multiple applications of beneficial microorganisms on some vegetables result in increased plant responses. The use of biofertilizers in sustainable agricultural systems has been increasing over the years. Therefore, the objective of this study was to evaluate the response of Habanero pepper seedlings inoculated and co-inoculated with *Rhizophagus intraradices* and *Azospirillum brasilense*. The variables evaluated were: emergence rate, emergency percentage, height, stem diameter, root volume and length, mineral content, shoot and roots dry biomass. Morphological variables included: seedling quality index, slenderness index and relative growth rate. The data obtained were subjected to an analysis of variance and multiple means comparison test of (Duncan, $p \leq 0.05$). This was done with SPSS version 22. The seeds conditioned with *R. intraradices* + *A. brasilense* showed an increase in emergency rate of 14% with respect to the control. However, height, stem diameter, root and shoot length, roots and shoots dry biomass, as well as nutrient concentration in seedlings decreased with respect to the control. The results indicate that co-inoculation with *R. intraradices* and *A. brasilense* contributed to increase the emergency rate of habanero pepper. Therefore, *R. intraradices* and *A. brasilense* can be considered and evaluated as a strategy to improve the emergence of seedlings.

INTRODUCCIÓN

En México se encuentra una gran diversidad especies del género *Capsicum*. Sin embargo, sólo cinco especies se han domesticado: *C. annuum*, *C. chínense*, *C. pubescens*, *C. frutescens* y *C. baccatum* (Hernández-Verdugo *et al*., 1999). De ellos uno de los más importantes es el *C. chinense* lo cual por su origen se ha denominado "chile habanero de la Península de Yucatán" (DOF, 2012).

La producción de chile habanero es una actividad importante dentro de la producción agrícola Mexicana desde el punto de vista económico y social. Aproximadamente 80% de la producción se exporta como fruto fresco, principalmente a Estados Unidos, Japón, Canadá, Reino Unido, Corea del Sur, Italia y Alemania, el resto 20% se utiliza en la industria gastronómica (SAGARPA, 2012). En el 2015 se produjeron en México más de 8000 toneladas, con un rendimiento de 10.68 t ha^{-1} (SIAP, 2015).

Según Villa Castorena *et al*. (2014) existe una gran demanda para este producto en el mercado nacional e internacional. Para satisfacer este incremento se ha intensificado la producción y para ello se han empleado principalmente la tecnología convencional en los cultivos (Tamayo *et al*., 2014). Sin embargo, esta tecnología ha ocasionado numerosos efectos negativos como la contaminación del agua y el suelo, la pérdida de la fertilidad del suelo, el aumento de resistencia de las plagas, un incremento desmesurado en el precio de agroquímicos, intoxicaciones por agroquímicos entre otros.

Para contrarrestar este problema, el uso de los microorganismos benéficos del suelo es una alternativa económica y ecológicamente viable (Berg, 2009). Además de mejorar la disponibilidad de nutrientes a las plantas tienen otros beneficios significativos como: la producción de fitohormonas, antibióticos, antimicrobiales, y mejorar la adquisición de nutrientes etc. Los cuales influyen directa e indirectamente en el crecimiento y el rendimiento de los cultivos.

Bhattacharjee y Sharma (2012) reportaron que la inoculación con *Glomus fasciculatum* y *Rhizobium* incrementó el contenido de clorofila, nitrógeno y fósforo con respecto a las plantas no inoculadas. La inoculación combinada incrementó la clorofila, el nitrógeno y el fosforo (19%, 10% y 114%, respectivamente) en comparación con el testigo. Esto demuestra que los biofertilizantes inoculados en combinación tienen la capacidad de mejorar el estado nutricional de las plantas al incrementar la absorción de nutrientes. Por lo tanto, la inoculación combinada puede mejorar la productividad de las plantas.

Sivakumar y Thamizhiniyan (2012) realizaron un estudio para determinar el efecto de los hongos micorrizicos arbuculares (HMA) y *Azospirillum* sp. sobre el crecimiento, rendimiento y estado nutricional del tomate (*Lycopersicon esculentum*). Sus resultados demostraron que la mayor longitud de raíz, vástago, peso fresco, peso seco y rendimiento se obtuvo en el tratamiento de co-inoculación con HMA y *Azospirillum* sp. en varias etapas de su crecimiento. Estos resultados comprueban que los cultivos responden positivamente a la co-inoculación, mejoran la eficiencia del cultivo y aumentan el rendimiento del mismo.

Se ha confirmado que los bioinoculantes son capaces de promover el crecimiento y permiten incrementar el rendimiento en muchos cultivos, por tanto es importante estudiar la efectividad de los bioinoculantes como una alternativa sostenible para aumentar la productividad del cultivo de chile habanero.

Considerando que la inoculación y co-inoculación con *A. brasilense* y *R. intraradices* (bioinoculantes) puede mejorar el crecimiento, desarrollo y rendimiento de varias cultivos el objetivo de éste trabajo es evaluar el efecto de la inoculación e co-inoculación con *Rhizophagus intraradices* y *Azospirillum brasilense* (bioinoculantes) en la nutrición, crecimiento y rendimiento de chile habanero.

1.2 ANTECEDENTES

1.2.1 Hongos micorrizicos arbusculares y su mecanismo de colonización

Las interacciones en la rizosfera de plantas micorrizadas son particularmente relevante, según Cardon y Whitbeck (2007) funcionan como puente para el flujo de energía y materia entre plantas y suelos. Barea *et al.* (1997) sugieren que pueden mejorar el establecimiento del cultivo, incremento en la absorción de nutrientes poco móviles, proteger contra estrés ambiental y mejorar la estructura del suelo. Además, los hongos realizan importantes servicios relacionados con la dinámica del agua, el ciclo de los nutrientes, la descomposición de materia orgánica y la supresión de enfermedades (Bücking et al. 2012).

1.2.2 Azospirillum brasilense

Azospirillum brasilense pertenece a la subclase α-proteobacteria, de vida libre, conocidas como rizobacterias. Estas bacterias promueven el crecimiento de las plantas ya sea directa o indirectamente utilizando una amplia gama de mecanismos. Los efectos de su inoculación pueden ser acumulativos a través de mecanismos pequeños que operan consecutivamente creando un efecto final más grande sobre la planta y pueden variar según las especies de plantas, la cepa de *Azospirillum* y las condiciones ambientales (Bashan y de-Bashan, 2010).

1.2.3 Mecanismos de promoción del crecimiento de las plantas por *Azospirillum brasilense*

Una de las principales propiedades de la relación planta-*Azospirillum* es la capacidad de producir fitohormonas. Las principales hormonas sintetizadas son: indoles, principalmente ácido indol-3-acético (IAA), giberelinas (AGs), ácido abscísico (ABA), poliamina cadaverina, citoquininas y etileno. Estas fitohormonas estimulan el crecimiento de las plantas que resulta en: mejor absorción de agua y minerales, aumento del metabolismo, alteración en la arquitectura de las raíces, lo que conduce a plantas más sanas y más grandes (Tsavkelova *et al*., 2006, Perrig 2007).

La fijación de nitrógeno era la forma original en la que se pensó que *Azospirillum* afectaba el crecimiento de las plantas (Okon *et al*., 1983), debido a que existe un aumento en la actividad nitrogenasa en las raíces inoculadas (Kennedy *et al*., 1997). Sin embargo muchos estudios muestran que la contribución de la fijación de nitrógeno por *Azospirillum* a la planta es mínima y en el mejor de los casos sólo incrementó 5% el N total en la planta (Bashan y Holguín, 1997). Otros estudios demostraron un aumento en el contenido de nitrógeno biológicamente fijado, específicamente en especies gramíneas por lo que se propusieron que el nivel de fijación de nitrógeno probablemente depende de la interacción bacteriana-cultivar (Saubidet y Barneix, 1998).

También se ha reportado que la inoculación de *Azospirillum* puede conducir a un mejor crecimiento de las raíces y un aumento en la densidad de los pelos radicales, lo que aumenta el aprovechamiento de agua y minerales. Por consiguiente, puede inducir aumento del volumen del sistema radicular, ayudando a las plantas a aprovechar mayores volúmenes de suelo, haciendo más eficiente la absorción de nutrientes (Morgenstern y Okon, 1987, Lin *et al*., 1983).

A. brasilense es capaz de mejorar la solubilización y movilización de minerales en general y fósforo en particular (Rodríguez *et al*., 2004). Adicionalmente Sarig, *et al*., (1990) propusieron que *Azospirillum* spp puede reducir los efectos estresantes ambientales, como la alta concentración de metales pesados, por la producción de sideróforos. Los sideróforos en condiciones de baja concentración de hierro exhiben actividad antimicrobiana contra diversas bacterias y hongos (Shah et al., 1992).

1.2.4 Sinergismo entre hongos micorrizico arbusculares y rhizobacterias en la agricultura

Los hongos micorrízicos arbusculares (MA) y rizóbacterias pueden interactuar sinérgicamente para estimular el crecimiento de las plantas a través de una serie de mecanismos que resultan en una mejor adquisición de nutrientes y un mayor crecimiento y rendimiento de los cultivos.

Una posible explicación de este fenómeno sugiere que ciertas rizobacterias promotoras de crecimiento vegetal (PGPR- por sus siglas en inglés) conocidas como "bacterias ayudantes de la micorrización" pueden estimular el crecimiento de MA que al asociarse con las raíces aumenta la capacidad para establecer interacciones simbióticas. El grupo más común de bacterias implicadas en estas interacciones son las bacterias Gram-positivas y las proteobacterias. Estos resultados sugieren que los PGPR seleccionados y los HMA podrían ser coinoculados para optimizar la formación y el funcionamiento de la simbiosis MA. (Linderman, 1997).
Además, Barea *et al.* (2002) propone que los hongos AM mejoran la capacidad de fijación de nitrógeno por las rizóbacterias debido a la mayor disponibilidad de fosfato para la fijación de nitrógeno a partir de una mayor absorción de fósforo. También se ha observado que ciertas especies de hongo MA son capaces de mejorar la descomposición de la materia orgánica y aumentar la captura de N por la planta.
En suelos con baja biodisponibilidad de fosfato, las bacterias pueden aumentar la disponibilidad de fosfatos solubles a través del proceso de mineralización (Smith y Read, 1997). Las bacterias solubilizantes de fosfato pueden liberar ion fosfato a partir de compuestos inorgánicos y orgánicos en el suelo, lo que aumenta la disponibilidad de iones de fosfato a hifas de HMA que luego pueden pasar a la planta. Aumenta en la absorción de fosfato también se ha vinculado a la absorción de otros micronutrientes esenciales del suelo por las hifas de hongo AM (Kucey et al., 1989).

1.2.5 Efecto de microorganismos en la germinación

Aparte de ser muy eficiente en la promoción del crecimiento y rendimiento de muchos cultivos, algunos autores han reportado que los bio-inoculantes pueden también estimular la germinación y emergencia de semillas vegetales (Kloepper *et al*., 1991).

Esto fue confirmado por Mishra *et al*. (2010) cuando reportaron que la aplicación de los PGPR provocó efectos estadísticamente significativos ($p<0.05$) sobre el porcentaje y la tasa de germinación bajo condiciones de salinidad. Esto demuestra que los PGPR ejercen efectos positivos en la germinación de semillas de manera natural.

Mangmang *et al*. (2015) evaluaron la eficacia de tres cepas de *A. brasilense* (Sp7, Sp7-S y Sp245) y dos métodos de inoculación de semillas (remojo y empapado) en la germinación de lechuga, tomate y pepino. Indicaron que la mayor velocidad de germinación ocurrió en tomate cuando se inoculó mediante el método de remoción. Las cepas Sp7-S y Sp245 produjeron mejor crecimiento de la raíz y vástago en tomate cuando se utilizó el método de remoción. Generalmente, los resultados muestran que el método de remoción fue comparativamente mejor en todas las especies. Se ha demostrado que la inoculación de semillas con microorganismos puede influenciar la germinación y emergencia de plántulas, por lo que puede disminuir el tiempo de permanencia en el vivero. Además a través de su inoculación se puede obtener plántulas de calidad que puede resultar en un mejor establecimiento en el campo.

1.3 HIPÓTESIS:

La inoculación con *Azospirillum brasilense* y *Rhizophagus intraradices* incrementa la tasa de emergencia, la calidad de plántula y optimiza la absorción de nitrógeno, fosforo y potasio.

1.4 OBJETIVOS:

1.4.1 General:

Evaluar la inoculación con *Rhizophagus intraradices* y *Azospirillum brasilense* (bioinoculantes) sobre la emergencia, parámetros de calidad y nutrición de plántulas de chile habanero.

1.4.2 Específicos:

1 Evaluar el efecto de la inoculación de *Azospirillum brasilense* y *Rhizophagus intraradices* sobre porcentaje, tasa de emergencia y crecimiento en plántulas de chile habanero.

2 Cuantificar el contenido foliar de nitrógeno, fósforo y potasio en las plántulas de chile habanero inoculadas con Azospirillum brasilense y Rhizophagus intraradices.

1.5 PROCEDIMIENTO EXPERIMENTAL

Esta investigación se realizó en el área de investigación del Instituto Tecnológico de Conkal. El material genético de chile habanero que se utilizó fue de la variedad Mayapán. El trabajo constó de cuatro tratamientos, los cuales fueron los siguientes: T1- *Azospirillum brasilense,* T2 - *Rhizophagus intraradices,* T3 - *Azospirillum brasilense* + *Rhizophagus intraradices,* T4 – sin inoculante como testigo.

Para el establecimiento del semillero se utilizaron charolas de 200 cavidades y se usó el sustrato comercial peatmoss (Sunshine) previamente esterilizado. Se utilizaron 200 semillas por cada tratamiento, para realizar la inoculación se imbibieron por 24h en 100ml de bioinoculantes a la concentración recomendado por el fabricante MicorrizaFer (*Rhizophagus intraradices*) 1:5 en agua y (*Azospirillum brasilense*) MaxiFer 1:6 en agua. En caso del testigo se utilizó solamente agua destilada. Después de la emergencia se tomaron 10 plantas por cada tratamiento para hacer análisis de laboratorio a los 20 y 60 días después de la siembra. Los variables evaluadas en esta fase fueron: porcentaje de germinación, velocidad de germinación, altura (cm), diámetro del tallo (mm), colonización, contenido de nutrientes, longitud radical (cm) y biomasa seca (g), índice de calidad de plántulas y tasa de crecimiento relativo. Los datos obtenidos se sometieron a un análisis de varianza y se realizó una prueba de comparación múltiple de medias.

1.6 LITERATURA CITADA

Barea, J.M., M. Gryndler, P. Lemenceau, H. Schüepp, and R. Azcón. 1997. Mycorrhiza/Bacteria interactions on plant growth promotion. In: Ogoshi, A., Kobayashi, L., Homma, Y., Kodama, F., Kondon, N., Akino, S., (eds) Plant growth promoting rhizobacteria, present status and future prospects. OECD, Paris. 150-158.

Barea, J.M., R. Azcon and C. Azcon-Aguilar. 2002. Mycorrhizosphere interactions to improve plant fitness and soil quality. Antonie Van Leeuwenhoek 81: 343–351.

Bashan, Y. and L.E. de-Bashan. 2010. Immobilized microalgae for removing pollutants: Review of practical aspects. Bioresource Technology 101**:** 1611-1627

Bashan, Y., and G. Holguin. 1997. *Azospirillum*–plant relationships: Environmental and physiological advances (1990–1996). Can. J. Microbiol. 43: 103–121.

Berg, G. 2009. Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Applied Microbiology and Biotechnology. 84(1): 11–18

Bhattacharjee, S. and G. D. Sharma. 2012. Effect of Dual Inoculation of Arbuscular Mycorrhiza and *Rhizobium* on the Chlorophyll, Nitrogen and Phosphorus Contents of Pigeon Pea (*Cajanus cajan* L.) Advances in Microbiology. 2: 561-564

Bücking, H., J. Abubaker, M. Govindarajulu, M.Tala, P.E. Pfeffer, G. Nagahashi, P. Lammers, Y. Shachar-Hill. 2008. Root Exudates Stimulate the Uptake and Metabolism of Organic Carbon in Germinating Spores of *Glomus intraradices*. New Phytologist; 180: 684-95.

Cardon, Z.G. and J.L. Whitbeck. 2007. The Rhizosphere. An ecological perspective. Elsevier Academic Press Burlington, MA. USA. 235.

Hernández, V. S., A. P. Dávila y K. Oyama. 1999. Síntesis del conocimiento taxonómico, origen y domesticación del género *Capsicum*. Boletín de la Sociedad Botánica de México 64: 65-84.

Kennedy, I. R., L. L. Pereg-Gerk, C. Wood, R. Deaker, K. Gilchrist, and S. Katupitiya. 1997. Biological nitrogen fixation in non-leguminous field crops: Facilitating the evolution between A*zospirillum* and wheat. Plant Soil 194: 65–79.

Kucey, R.M.N., H.H. Janzen, and M.E. Leggett. 1989. Microbiologically mediated increases in plant-available phosphorus. In Advances in Agronomy. Brady, N.C. (ed.). New York, USA: Academic Press. 199–228.

Lin, W., Y. Okon, and R. W. F. Hardy. 1983. Enhanced mineral uptake by Zea mays and Sorghum bicolor roots inoculated with *Azospirillum brasilense*. Appl. Environ. Microbiol. 45: 1775–1779.

Linderman, R.G. 1997. Vesicular-arbuscular mycorrhizal (VAM) fungi. In The Mycota. Caroll, G.C., and Tudzynski, P. (eds). Berlin, Germany: Springer-Verlag. 117–128.

Mangmang, J.S., R. Deaker, y G. Rogers. 2015. Early seedling growth response of lettuce, tomato and cucumber to *Azospirillum brasilense* inoculated by soaking and drenching. Hort. Sci. Vol. 42(1): 37–46.

Mishra, M., U. Kumar, P. K. Mishra and V. Prakash. 2010. Efficiency of Plant Growth Promoting Rhizobacteria for the Enhancement of *Cicer arietinum* L. Growth and Germination under Salinity. Advances in Biological Research 4(2): 92-96.

Morgenstern, E., and Y. Okon. 1987. Promotion of plant growth and NO3 and Rb uptake in Sorghum bicolor X Sorghum sudanense inoculated with *Azospirillum brasilense*— Cd. Arid Soil Res. Rehabil. 1: 211–217.

Okon, Y., P. G. Heytler, and R. W. F. Hardy. 1983. N2 fixation by *Azospirillum brasilense* and its incorporation into host Setaria italica. Appl. Environ. Microbiol. 46: 694–697.

Rodriguez, H., T. Gonzalez, I. Goire, and Y. Bashan. 2004. Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium *Azospirillum* spp. Naturwissenschaften 91: 552–555.

SARGARPA. 2012. Delegación en el estado de Yucatán B058 Mérida, Yucatán.

Sarig, S., Y. Okon and A. Blum. 1990. Promotion of leaf area development and yield in Sorghum bicolor inoculated with *Azospirillum brasilense*. Symbiosis 9: 235–245.

Saubidet, M. I., and A. J. Barneix. 1998. Growth stimulation and nitrogen supply to wheat plants inoculated with *Azospirillum brasilense*. J. Plant Nutr. 21: 2565–2577.

Secretaria de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SIAP - SARGARPA). 2015. Márgenes de comercialización. Servicio de Información Agroalimentaria y Pesquera (SIAP). 2014. Chile habanero de la Península de Yucatán. Disponible en: http://www.siap.gob.mx. (Consulta: Mayo, 2016).

Shah, S., V. Karkhanis and A. Desai. 1992. Isolation and characterization of siderophore, with antimicrobial activity, from *Azospirillum lipoferum*. Curr. Microbiol. 25: 347–351.

Sivakumar, P.V. and P. Thamizhiniyan. 2012. Enhancement in growth and yield of tomato by using AM fungi and *Azospirillum*. International Journal of Environmental Biology. 2(3): 137-141.

Smith, S.E., and D.J. Read. 1997. Mycorrhizal Symbiosis. 2nd edition. Academic Press, San Diego, CA, USA. pp. 605.

Soria F., M., J. Tun S., A. Trejo R. y R. Terán S. 2002. Tecnología para Producción de Hortalizas a Cielo Abierto en la Península de Yucatán. SEP-DGETA. 3ª Edición. Conkal. Yucatán, México. 430 p.

Tamayo Manrique, J.M, E. Martínez, y E.G. Monforte Méndez. 2016. "Análisis comparativo de la sustentabilidad de dos unidades productivas de Chile Habanero convencionales y dos unidades productivas orgánicas en Yucatán, México", en Observatorio de la Economía Latinoamericana. Nº194. http://www.eumed.net/cursecon/ecolat/mx/2014/chile-habanero.html

Tsavkelova, E. A., S. Y. Klimova, T. A. Cherdyntseva, A. I. Netrusov. 2006. Microbial producers of plant growth stimulators and their practical use: A review Applied Biochemistry and Microbiology. 42(2): 117–126

Villa Castorena, M., E. A. Catalán, M. A. Inzunza, A. Román, H. Macías, y D. Cabrera. 2014. Producción hidropónica de chile habanero en invernadero. INIFAP. CENID-RASPA, SARGARPA. Torreón, Coah., México.pp. 56.

CHAPTER 2. Effects of bio-priming habanero pepper (*Capsicum chinense* Jacq.) seeds with *Rhizophagus intraradices* and *Azospirillum brasilense* on germination and growth of seedlings

2.1. RESUMEN

El uso de microorganismos benéficos influye positivamente en el desarrollo y rendimiento del cultivo. Por lo tanto, el objetivo de este estudio fue evaluar la respuesta de plántulas de chile habanero variedad Mayapán inoculadas y co-inoculadas con *Rhizophagus intraradices* y *Azospirillum brasilense*. Las semillas fueron acondicionados individualmente y en combinación con *Azospirillum brasilense* y *Rhizophagus intraradices* durante 24 h antes de ser sembradas en charolas. El experimento se realizó mediante un diseño completo al azar. Las variables evaluadas fueron: tasa de emergencia, porcentaje de emergencia, altura, diámetro del tallo, volumen y longitud de las raíces, contenido de minerales, biomasa seca del vástago y de las raíces. Las variables morfológicas incluyeron: índice de calidad de las plántulas, índice de esbeltez y tasa de crecimiento relativo. Los datos obtenidos fueron sometidos a un análisis de varianza y la prueba de comparación de medias múltiples (Duncan, $p \leq 0{,}05$) lo cual se realizó con SPSS versión 22. Las semillas acondicionadas con *R. intraradices* + *A. brasilense* mostraron un aumento en la tasa de emergencia del 14% con respecto al control. Sin embargo, la altura y el diámetro del tallo, la longitud de la raíz y el brote, la biomasa seca de raíces y brotes de los tratamientos, así como la concentración de nutrientes en plántulas disminuyeron con respecto al control. Los resultados indican que la co-inoculación con *R. intraradices* y *A. brasilense* contribuyó a incrementar la tasa de emergencia. Por lo tanto, *R. intraradices* y *A. brasilense* pueden ser considerados y evaluados como una estrategia para mejorar la emergencia de las plántulas.

Palabras claves: inoculación, acondicionamiento biológico, microorganismos benéficos

ABSTRACT

The application of synthetic fertilizers is a fundamental practice in conventional agriculture. However, in addition to the negative impacts on agroecosystems, this practice is very expensive, prompting research for alternatives. The use of beneficial microorganisms has a positive influence on the development and yield of various crops. Therefore, the objective of this study was to evaluate the response of habanero pepper seedlings Mayapán variety inoculated and co-inoculated with *Rhizophagus intraradices* and *Azospirillum brasilense*. The seeds were conditioned individually and in combination with *Azospirillum brasilense* and *Rhizophagus intraradices* for 24 h before sowed in trays. The experiment was performed using a completely randomized design. The variables evaluated were: emergence rate, emergency percentage, height, stem diameter, root volume and length, mineral content, shoot and roots dry biomass. Morphological variables included: seedling quality index, slenderness index and relative growth rate. The data obtained were subjected to an analysis of variance and multiple means comparison test of (Duncan, $p \leq 0.05$). This was done with SPSS version 22. The seeds conditioned with *R. intraradices* + *A. brasilense* showed an increase in emergency rate of 14% with respect to the control. However, height, stem diameter, root and shoot length, roots and shoots dry biomass, as well as nutrient concentration in seedlings decreased with respect to the control. The results indicate that co-inoculation with *R. intraradices* and *A. brasilense* contributed to increase the emergency rate of habanero pepper. Therefore, *R. intraradices* and *A. brasilense* can be considered and evaluated as a strategy to improve the emergence of seedlings.

Key words: inoculation, bioprime, beneficial microorganism.

2.2. INTRODUCTION

Habanero pepper production constitutes an important economic activity in Mexico, in particular the south eastern states (Campeche, Quintana Roo, Tabasco and Yucatan). Yucatan is one of the most important states with respect to the production of this vegetable in Mexico (López *et al*., 2006). González-Estrada (2006) reported that, there has been a significant increase in the demand for the habanero pepper globally. This demand has prompted a subsequent increase in its production. However, there are a number of factors which impede a high level of productivity of this commodity.

The availability of good quality seedlings at the moment of transplant is one of the greatest challenges for producers (Preciado *et al*. 2002). The principal factors which influence seedling quality as well as crop yield include: germination, fertilization, irrigation and pest and disease control (Guzman, 2003, Aloni *et al*. 1991). Nutrition stands out as one on the most important factors due to its effects on seedling morphology and physiology. Traditionally growers have employed synthetic fertilizers to supplement the nutritional requirements of seedlings. The challenge at this moment is the search for alternative strategies of mineral nutrition which satisfies crop nutritional needs to obtain adequate levels of crop yield and quality whilst at the same time minimize negative environmental impacts (Altieri, 1997).

Inoculation with plant growth promoting microorganisms (PGPM) has recently attracted much attention due to their positive effects in decreasing negative environmental impacts resulting from continued use of synthetic fertilizers and their positive effect on crop growth and yield (Castillo-Reyes *et al*., 2014).

Over the years various seed inoculation treatments have been developed and evaluated. One of them is bio-priming (Madhukeshwara and Sajjan, 2016). Bio-priming is a technique which integrates seed hydration and seed inoculation with beneficial organism for augmenting germination uniformity and velocity and thus final stands (Srivastava *et al*., 2009, Mangmang *et al*., 2015, Karthika and Vanangamudi, 2013). Several researchers have reported enhanced germination rates and superior seedling vigor through bio-priming with plant growth promoting microorganisms. This technique can also be used to protect seedlings against pathogens.

Madhukeshwara and Sajjan (2016) recorded significantly higher field emergence, and performance in maize hybrid GH-0727 after bio-priming with treatments of *Azospirillum brasilense.* Srivastava *et al*. (2010) in a study to control *Fusarium* wilt in tomato using microbiological agents identified significant increase in germination percentage (22–48%) and velocity (reduced germination time by 2.0–2.5 days) through application of *T. harzianum* and fluorescent *Pseudomonas* by seed bio-priming. Thus we want to answer the next question: Is seed bio-priming with native plant growth promoting microorganisms capable of improving seedling quality of habanero pepper?

Thus, the aim of this study was to determine the effects of plant growth promoting microorganisms *Azospirillum brasilense* and *Rhizophagus intraradices* on seedling emergence and quality of Habanero pepper.

2.3. METHODOLOGY

2.3.1. Experimental site

This study was conducted at the Instituto Tecnológico de Conkal (21° 04’ N and 89° 31’ W and 8 m above msl), Yucatán at 16.3 Km of the old Merida Motul highway in a gable roof-type greenhouse located, northeast of Mérida. The greenhouse consisted of a polyethelyne plastic roof (25% shade), and an anti-aphids mesh walls, The predominant climate type at the site is Awo (Xo) (i) g (Mardero *et al.*, 2012). The research was undertaken during the months of September to November 2016.

2.3.2. Preparation for sowing

Prior to sowing seeds were bio-primed for 24h (Garruña-Hernandez *et al*., 2014) at room conditions to allow sufficient time for imbibition and for microorganisms to bind to the seed coats. Seeds which had floated after half an hour were considered non-viable and therefore remove from the treatment and discarded (Murillo *et al*., 2000). The seeds from each treatment were then sieved and left to surface dry on filter paper at room conditions.

Prior to sowing, the substrate was sterilized in an Autoclave at 120 °C for 20 minutes. Seeds were then sown into 200 cavity polystyrene seed trays filled with the commercial substrate peat moss (Sunshine – Morelos, Mexico). After sowing, the seed trays were covered with a dark plastic sheet to maintain moisture until signs of seedling emergence.

2.3.3. Treatments and experimental design.

Habanero pepper (*Capsicum chinense* Jacq.) Mayapán variety formed the base material in this study. The inoculants comprised of two commercially available products namely: MaxiFert (*A. brasilense*) and MicorrizaFer (*R.intraradices*) (Biofabrica Siglo XXI, Mexico City, Mexico).

MicorrizaFer consisted of 30-35 spores per gram of product and was mixed at a ratio 1:5 (one part of the product to five parts of tap water) according to the manufactures recommendations. MaxiFer was a liquid product made up of 500 million colony forming units (CFU) per milliliter was mixed at a ratio of 1:6 (one part of the product to six parts of tap water) according to the manufacturer's recommendation.

The experiment comprised four treatments as follows:

- Treatment: (Control) – contained 200 primed seeds in a 100ml of distilled water.
- Treatment: contained 200 seeds bio-primed in a 100ml suspension of *R. intraradices.*
- Treatment: contained 200 seeds bio-primed in a 100ml suspension of *A. brasilense*.
- Treatment: contained 200 seeds bio-primed in a 100ml in a suspension of *R. intraradices* and *Azospirillum brasilense* in a ratio of 1:1.

The seed trays were divided into twenty repetitions, each consisting of 10 seedlings; making a total of 200 seeds per treatment. The experiment was laid out in a completely randomized design with four treatments. The trays were monitored daily for signs of emergence. Seedlings were considered emerged when there were visible appearance of the hypocotyl. Emergence data was taken at 24 h intervals. All the seedlings were kept under identical nursery condition up to 60 days.

2.3.4. Agronomic management

After the first signs of emergence the covers were removed and the seedling trays were placed in the greenhouse to continue the germination process. Seedlings were daily subjected to a light irrigation to reduce the risk of damping-off. Seedlings were fertilized with NPK (13-40-13) (Hakaphos Violeta - Munster, Germany) at the application rate of 2g L^{-1} of water and micronutrients (Fe, Zn, Cu, B, Mg and Mn) (SP1 Agristar, Jalisco Mexico) at the rate of 1 ml L^{-1} of water. Additionally, the pH was stabilized with an acidifier (Choice Agristar, Jalisco, Mexico) added to the irrigation water.

2.3.5. Sampling and measurement of variables

Sampling was realized at twenty (20) and sixty (60) days after sowing (DAS). During sampling, ten seedling were randomly selected from each treatment. Seedlings were selected using an online program (EPIDAT 4.2,). They were carefully uprooted without disturbing the root system and washed in running tap water. The excess water was dried out by placing them between folds of blotting paper.

The response variables were measured twice; at 20 days after sowing (DAS) and at 60 days after sowing. Shoot height measured from base of the stem to shoot tip with a measuring tape. Root length was measured from base of the stem to root tip with a ruler. Stem diameter was measured at the collar region with a digital Vernier caliper. To determine root and shoot biomass, seedlings samples were dried at 65°C for 72 h in separate paper bags in an oven (Teralab) and then weighed using an electronic scale (AHAUS Explorer Pro model EP214C).

2.3.6. Emergence percentage

On the appearance of the hypocotyl seedlings were recorded as emerged. This data was then used to determine emergence percentage (EP) using the following formula:

$$EP = \frac{n}{N} \times 100$$

n: number of seeds that were germinated (on the day D), N: total number of seed in each petri dishes (ISTA, 2013).

Emergence rates (ER) were calculated using the modified Timson index for germination rate (Khan and Ungar, 1984):

$$IT = \sum \frac{G}{t}$$

Where: *E* is the percentage of emerged seedlings at intervals of 24 h and *t* is the total emergence period. The Timson scale is used to calculate the percentage of emerged seedlings per 24 h interval. The results were expressed as final emergence percentage.

2.3.7. Mycorrhizal colonization evaluation

Mycorrhizal root infection was determined following the procedure developed by Rodríguez Yon *et al*. (2015). The technique utilizes blue washable Parker Quink for staining the roots. The 25 ml of the ink was mixed in 1000 ml of hydrochloric acid (1N) resulting in a 2.5% (V/V) solution.

Five seedlings were selected per treatment. Seedling roots were carefully washed in tap water to remove substrate residues. They were then placed in an oven at 70 °C for three days until a constant weight was obtained. The root segments were then clarified by heating them in a 10 % (m/v) solution of potassium hydroxide at 90 °C for 30 minutes and subsequently thoroughly rinsed to eliminate the potassium hydroxide solution. Thereafter the roots were placed in the ink solution and left to stand for 15 minutes at room temperature, followed by placement oven at 70°C for 15 minutes. After removing them from the oven, they were again thoroughly rinsed to remove excess ink from the roots. Finally to determine colonization percentage stained root fragments of about 1cm in length were mounted unto glass slides and subsequently observed for the presence of mycorrhizal structures using a microscope (model – Leica ICC50E). Each root segment was divided into five sections and the presence of mycorrhizal structures were recorded and quantified.

2.3.8. Seedlings quality index

Seedlings Quality Index was calculated using the formula presented by Dickson *et al*. 1960. Seedling quality index (SQI):

$$DQI = \frac{Seedling\ dry\ mass\ (g)}{\frac{Dry\ mass\ shoot\ (g)}{Dry\ mas\ root\ (g)} + \frac{Seedling\ height\ (cm)}{Stem\ diameter\ (mm)}}$$

2.3.9. Relative growth rate (RGR)

The relative growth rates of seedlings were determine utilizing the formula developed by Hunt *et al*. (2002).

Relative growth rate (RGR):

$$RGR = \frac{(LnW2 - LnW1)}{(T2 - T1)}$$

Where:

Ln – natural logarithm, W2 – total dry weight per plant at second sample date, W1 - total dry weight per plant at second sample date T2 - time in days at second sample date. T1 - time in days at second sample date

2.3.10. Nutrient analysis

Nutrient concentration was determined in seedlings 60 day after sowing, samples were dried at 65 °C for three days in an oven (model Teralab). Subsequently, samples were ground to pass through a 0.2 millimeter sieve. Then 10g of the ground sample were used to determined nutrient concentration in seedlings using a spectrophotometer (M4 TORNADO high performance Micro-XRF, Massachusetts, USA). Minerals determined included magnesium, calcium, phosphorus, sulphur, potassium, manganese, iron, copper and zinc. Nitrogen content was determined by the conventional micro- Kjeldahl method.

2.3.11. Data analysis

The experiments were conducted in a randomized complete block design, with each treatment replicated 10 times. The data were subjected to ANOVA and means were compared using Duncan's multiple range test least significant difference (LSD) significant test at $p<0.05$. The data were analyzed statistically by using SPSS software version 22.0.

2.4. RESULTS AND DISCUSSION

2.4.1. Effects of biopriming with *Rhizophagus intraradices* and *Azospirillum brasilense* on emergence of habanero pepper

Six days after sowing the highest emergence percentage (41.25%) was recorded by the coinoculated (*R. intraradices* + *A. brasilense*) treatment, whereas the lowest emergence percentage in the same time period was recorded by the singly inoculated *A. brasilense* treatment (8.13%) (Figure 1). The singly inoculated *R. intraradices* treatments and control registered similar emergence percentages throughout the experimental period. At day six the coinoculated seedlings and singly inoculated *R. intraradices* treatments recorded 15.62% and 5% emergence respectively higher than the control, whereas the singly inoculated *A. brasilense* treatment recorded 17.5% lower emergence percentage than the control. The coinoculated treatment maintained the highest emergence percentages throughout the entire germination period. Up to 9 DAS the individual *Azospirillum* treatment presented significantly lower emergence percentages with respect to the other treatments. From day 10 (DAS) and onwards, no significant difference were recorded among all treatments with respect to the control. The final emergence percentages for all treatments were about 90%. This demonstrates that the bio-inoculants did not negatively affect the final emergence percentages (Figure 1).

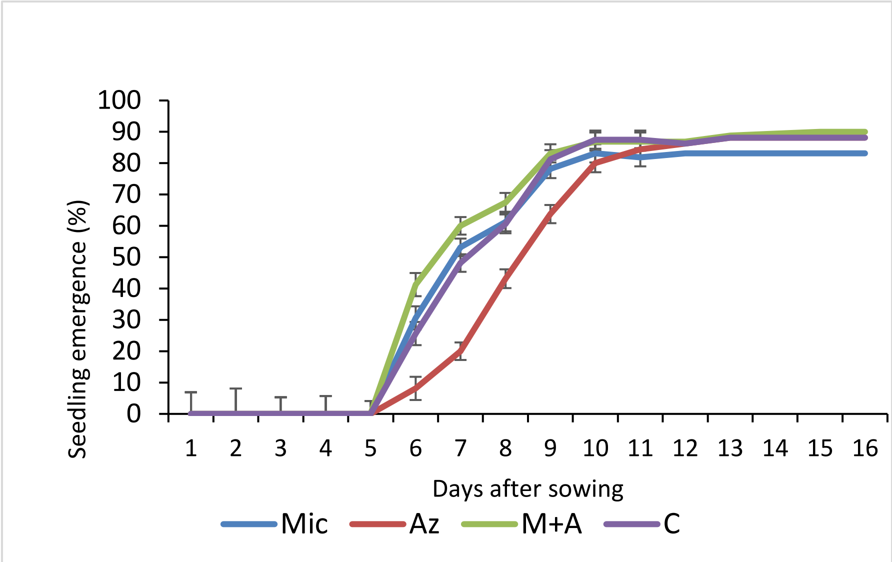

Figure 1. Effects of *R. intraradices* and *A. brasilense* inoculation on seedling emergence of habanero pepper. Mic (*R. intraradices*), Az (*A. brasilense*), M+A (*R. intraradices* + *A. brasilense*), C (control).

The highest emergence rate was registered by the co-inoculated treatment and was found to be significantly different form that of the singly inoculated *Azospirillum* treatment by 1.4 percent per day and only 0.4 percent higher per day than the control. Significant differences only existed between the co-inoculated treatment and the singly inoculated *Azospirillum* treatment while among the other treatment there were no significant differences (Figure 2).

Possible mechanisms for improved emergence rate include activation of water induced metabolic processes and production of phyto-hormones. Earlier experiments conducted by Garruña-Hernández *et al*. (2014) illustrated that the rate of emergence of habanero pepper seedlings can be enhanced by various priming agents such as KNO_3, PEG, ABA, and AG_3. The results obtained in our study for emergence rate were consistent with the findings of Garruña-Hernández *et al*. (2014).

Past studies (Madhukeshwara and Sajjan, 2016) have indicated that the inoculation with *Azospirillum* spp. increases the rate of emergence due to its capacity to produce plant growth promoting substances such as auxins, ethylene, GA_3 and cytokinins. However, this study provided contrary results with *A. brasilense* demonstrating lowest emergence rates (Figure 2).

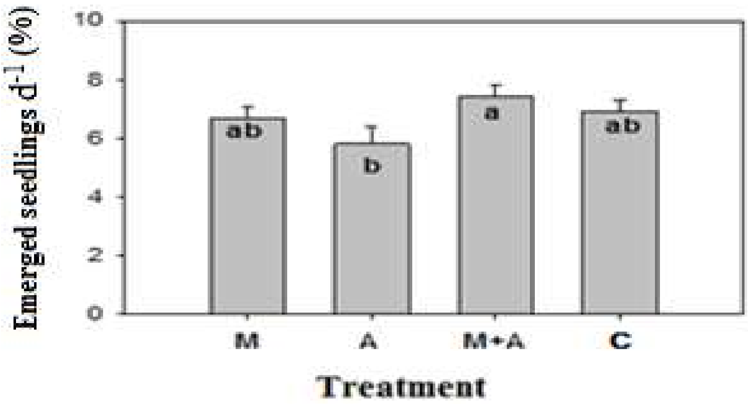

Figure 2. Effects of *R. intraradices* and *A. brasilense* inoculation on rate of seedling emergence of habanero pepper. Variable means with the same letter represent similar values based on Duncan Multiple Range Test ($p \leq 0.05$) n = 8. M (*R. intraradic*es), A (*A. brasilense*), M+A (*R. intraradices* + *A. brasilense*), C (control).

The results observed for accumulated seedling emergence and seedling emergence rate can be attributed to the capacity of *Azospirillum* spp. to synthesize certain plant hormones such as auxins, gibberellins and cytokinins (Tien *et al*., 1979). Among the hormones synthesized, indole acetic acid (IAA) is of greatest significance (Thuler *et al.*, 2003). Ona *et al*. (2003) suggests that after depletion of the carbon source in the media, large amounts of IAA is released in cultures during the stationary phase of the bacteria in cultured medium, nevertheless *Azospirillum*-IAA biosynthesis occurs during all stages of culture growth (Malhotra and Srivastava, 2009). On the other hand Indole acetic acid concentration increases with the age of the culture until bacteria reaches the stationary phase. It can therefore be assumed that higher concentrations of *Azospirillum* spp. would synthesize higher quantities of IAA. Cassán *et al*. (2011) proposes that *Azospirillum brasilense* is capable of synthesizing IAA at concentration levels sufficient to produce morphological and physiological changes in young seedling tissues. Research has also demonstrated that increased concentrations of IAA is likely to interfere with the physiological processes of seedlings (Taiz y Zeiger, 2006).

Nonetheless, the increased emergence rate of the co-inoculated treatment over the individual inoculated *Azospirillum* treatment can be attributed to the lower levels of IAA in the co-inoculated treatment which enhances hypocotyl and epicotyl growth, whereas the higher levels of IAA in the individual *A. brasilense* treatment inhibited growth of the hypocotyl and epicotyl and thus lower emergence rates were recorded.

These results corroborate with investigations conducted by Karthika and K. Vanangamudi (2013) who documented decreased germination rates of maize bioprimed with various concentration of *Azospirillum* (10, 15 and 20%) within a 24 hour period. After 24hrs the lowest germination percentages reported in the treatments with the highest concentration of azospirillum.
On the other hand the results of this study disagrees with results obtained by Madhukeshwara and Sajjan (2016) who obtained higher field emergence (78.9%) at higher concentrations (20%) of *Azospirillum* spp. whereas the lowest field emergence (73.2%) were obtained with lower *Azospirillum* spp. concentration (10%). They proposed that secretions of gibberellic (GA3) by *Azospirillum brasilense* during the bio-priming process might have stimulated cell elongation of the hypocotyls and epicotyls thus resulting in increased growth of the same and thus a higher emergence. Therefore difference results in this study compared to ours could be due to the fact that this experiment was performed under field conditions where a number of different factors can influence seedling emergence including a plethora of competing micro-organisms thus reducing the efficacy of *Azospirillum* spp. to compete with other native soil microbes resulting in lower levels of IAA which could negatively affect seedling emergence.

Similarly Mariappan *et al*. (2014) in their research on the effects of biopriming *Pongamia pinnata* seeds with liquid biofertilizers (*Azospirillum* and Phosphobacterium) at various concentrations and periods of storage reported reduced germination percentage of the treatment which had a concentration of 1.5% *Azospirillum* compared to *Azospirillum* treatment of 0.5% concentration during treatment period of six months. These findings reveal the influence of *Azospirillum* concentration and biopriming duration on seedling quality parameters.

2.4.2. Assessment of mychorrhizal colonization of habanero pepper seedling roots

Observation of root fragments revealed successful mycorrhizal colonization to *R. intraradices* (AMF) either individually or in combination with *A. brasilense* (Figure 3). The structures observed are characteristic of arbuscular mycorrhizae fungi (hyphae, vesicles). Visible hyphae and vesicles observed on several segments of the root surface served as indicators of successful mychorrhizal colonization. The individual AMF treatment resulted in 56% colonization, whereas mychorrhization in the co-inoculated treatment was slightly higher at 59%. These results differed from those obtained by Constantino *et al.* (2008) who reported 57.3% mycorrhizal colonization when singly inoculated and 42 and 37% colonization when co-inoculated with *A. brasilense* and *A. chroococum* respectively in habanero pepper. Results of studies conducted by Kuppuajendran (2012) demonstrated that co-inoculation with rhizobacteria can improve mycorrhization of AM fungi which concurs with the results obtained in this study.

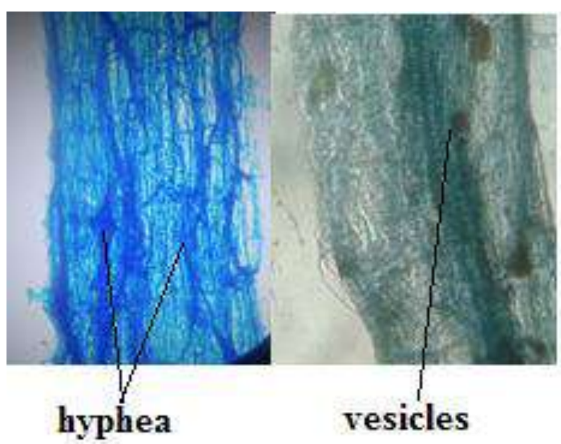

Figure 3. Different mycorrhizal structures observed in the roots of habanero pepper plants

2.4.4. Effects of biopriming on seedling height

Variance analysis of seedling height 20 days after sowing (DAS) revealed no significant difference among the treatments with respect to control. Nonetheless, significant differences existed between the singly inoculated *Azospirillum* treatment and the coinoculated treatment with respect to the singly inoculated mycorrhizae treatment. The singly inoculated treatment of *R. intraradices* and the coinoculated treatment recorded 40.18% and 0.74% less growth with respect to the control, whereas the singly inoculated *Azospirillum* treatment recorded 6.87% more growth than the control (Figure 4).

However, at 60 DAS there existed significant difference among the treatments with respect to the control. The coinoculated treatment presented the highest means (5.16 cm) whilst the singly inoculated mycorrhizae treatment presented the lowest means (4.01 cm). The singly inoculated treatments of mycorrhizae and *Azospirillum* presented significant differences with respect to the control, registering 16.41% and 2.4% less growth respectively, than the control. Whereas the coinoculated treatment recorded 7.55% higher growth than the control. Though, the coinoculated treatment presented the highest means it was not significantly different from the control (Figure 4).

The increase in height obtained at 60 DAS were 36.66, 57.25, 64.95 and 73.73% for *Azospirillum*, mycorrhizea, control and coininculated treatments respectively. Comparing the results obtained at 20 and 60 DAS it can be observed that the singly inoculated mycorrhizea maintained the same response pattern with respect to the control whereas the response of the singly inoculated *Azospirillum* treatment varied with respect to the control. At 20 DAS this treatment registered the highest means whereas at 60 DAS it was among the lowest (2.4% less than the Control). On the contrary the coinoculated treatment presented the highest means at 60 DAS (Figure 4).

In the case of the singly inoculated *Azospirillum* treatment results could be attributed to ethylene suppression of seedlings growth. High quantities of auxins produced by the singly inoculated *Azospirrillum* treatment may have provoked ethylene biosynthesis. IAA and auxins concentrations above optimal levels in a wide variety of plant tissues enhances the action of ACC synthase activity. ACC synthase transforms S-Adenosylmethionine (AdoMet), produced in the Yang cycle to 1-aminocyclopropane-1-carboxylic acid (ACC), which is then converted to ethylene by the action of ACC oxidase. High levels of ethylene inhibits subsequent growth of hypocotyl and retards stem elongation (Taiz y Zeiger, 2006).

On the other hand the increased height obtained for the co-inoculated seedlings at 60 DAS can be attributed to enhanced nutrition following co-inoculation with *A. brasilense* and *R. intraradices* which contributed to improved plant growth. Jha *et al*. (2012) suggests that during the seedling stage, the root system are not well developed and therefore the fungal symbiosis might play a vital role in nutrient uptake and thus enhances growth. This is achieved through the modification of root architecture by the fungal hyphae which increases the transference of minerals (nitrogen and phosphorous in particular), water and other important substances necessary for vegetative growth. The fungal mycelium is able to improve nutrient absorption efficiency (Barea *et al*., 1987).

Additionally, results suggest that co-inoculation with *R. intraradices* and *A. brasilense* increased the production of gibberellin (GA), and cytokinins, which resulted in greater cell elongation which may explain why this treatment recorded greatest seedling height at 60 DAS. These results concurs with research done by Cacciari *et al*. (1989) who demonstrated that the amounts of gibberellins and cytokinins produced by a mixed culture of *A. brasilense* and *Arthrobacter giacomelloi* were higher than those of the single cultures.

2.4.5. Effects of biopriming on stem diameter

The analysis of variance for stem diameter at 20 DAS revealed no significant difference among the treatments with respect to the control. Nevertheless, there existed significant difference only between the individual *Azospirillum* sp. and individual mychorrizea treatments. The highest values however were obtained by the individual mycorrhizea treatment. At 60 DAS there were no significant difference among the treatments, nevertheless the control presented the highest means while the individual *Azospirillum* sp. treatment presented the lowest mean. Among the inoculated treatments the coinoculated treatment showed superior girth at 60 DAS. However, the *Azospirillum* treatment at 60 DAS registered the greatest percentage increase in girth of 86.73%, whilst mychorrhizea, control and coinoculated recorded 38.58, 61.39 and 63.42% increase in girth respectively (Figure 4).

Results from this experiment coincides with experiments conducted by Hernández and Chailloux (2004) who investigated to effects of various combinations of HMA with several strains of rhizobacterias in tomato seedlings. Their results demonstrated that un-inoculated fertilized tomato seedlings were superior in girth compared to the inoculated ones. These results could be due to the parasitic stage which is experienced in the initial stages of the symbiosis in which there is an increased consumption of photosynthates by the inoculants and thus a reduction in growth is experienced by the host plants (Dodd *et al*. 1996).

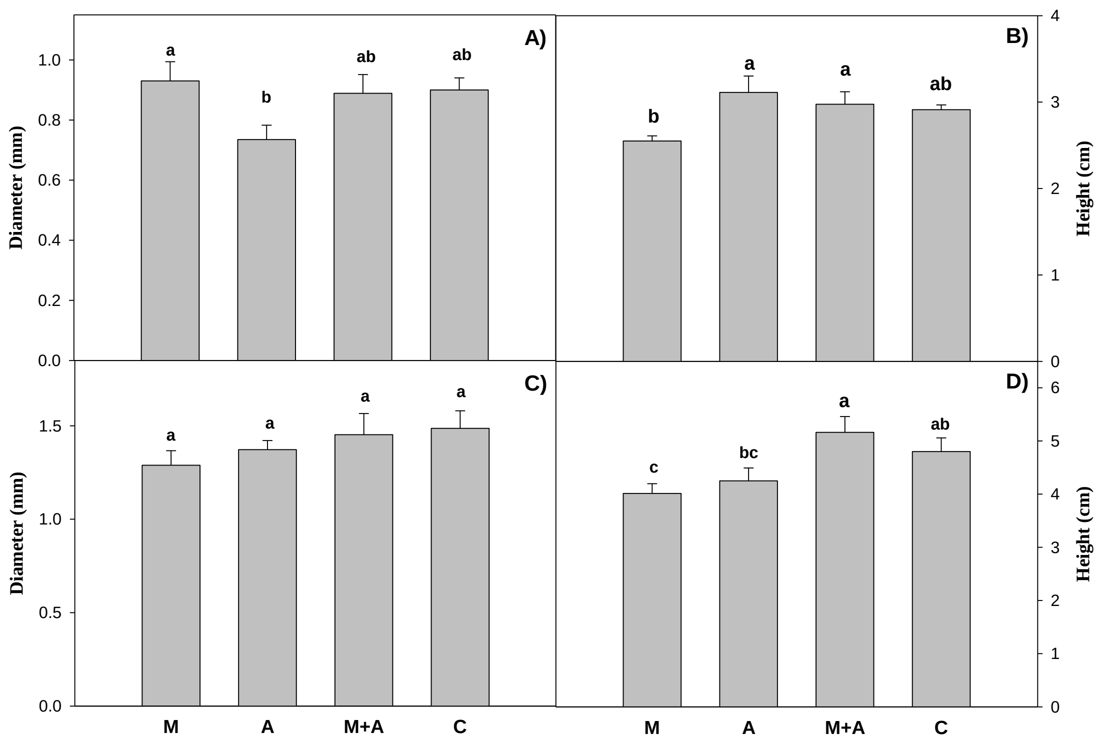

Figure 4. Effects of *R. intraradices* and *A. brasilense* inoculation on seedling height at 20 (B) and 60 (D) days after sowing and stem diameter of habanero pepper at 20 (A) and 60 (C) days after sowing. Bars with the same letter represent similar values based on Duncan Multiple Range test (p ≤ 0.05) n = 8. M (*R. intraradices*), Az (*A. brasilense*), M+A (*R. intraradices* + *A. brasilense*), C (Control).

2.4.5. Effects of biopriming on Root length

The analysis of variance for root length at 20 and 60 DAS. At 20 DAS, the longest roots were recorded by the control treatment which demonstrated significant difference with respect to the individual *Azospirillum* sp. treatment. The rest of the treatments recorded no significant difference with the control. At 60 DAS root length was significantly lower in the individual *Azospirillum* sp. and coinoculated treatments with respect to the control, whilst no significant difference was recorded with the individual mychorrhizea treatment with respect to the control (Table 1). There was an increase of 41.15, 41.63 and 48.5% in root length of the control, mycorrhizea and *Azospirillum* sp. treatments respectively, the co-inoculated treatment however was 17.95% less than the control. Except for the individual AMF inoculated treatment, the other inoculated treatments were less effective than the control in promoting root lengths in habanero pepper seedlings. The result for this variable concurs with experiments conducted by Juge *et al*. (2012), they suggested that the host plant directs huge quantities of photosynthates to support growth and maintenance of the microorganisms in the symbiosis, as a result shoot and root biomasses are significantly reduced as compared to the control.

2.4.6. Effects of biopriming on biomass production of habanero pepper seedlings

Highest shoot dry mass was recorded by the control at 20 and 60 DAS. However, from analysis of shoot biomass at 20 DAS it was observed that the individual inoculants of *R. intraradices* and *A. brasilense* were less effective in increasing shoot biomass than the control. While the co-inoculated treatment was on par with the control, nonetheless it was statistically equal to the individual inoculants. The individual *Azospirillum* sp, AMF and co-inoculated treatments presented 35, 34 and 14% less shoot biomass respectively at 20 DAS with respect to the control (Table 1).

At 60 DAS shoot biomass presented a similar pattern as previously observed, with the control recording maximum biomass and the individual *R. intraradices* treatment presenting the lowest. Significant differences were only observed between individual *R. intraradices* treatment with respect to the control and the co-inoculated treatment. Additionally, the individual *A. brasilense* treatment was statistically on par with the co-inoculated treatments and the control. In this time period the co-inoculated seedlings and individual treatments of *Azospirillum* and AMF registered 2.5, 43, and 23.73% less biomass production than the control.These results indicate that the co-inoculated treatment was more effective in shoot biomass accumulation with respect to the single inoculant treatments. It was also observed that the *Azospirillum* treatments produced the greatest percentage increase in shoot biomass (Table 1).

From the variance analysis of root dry mass at 20 DAS, significant differences were recorded with the individual *Azospirillum* sp. treatment registering the highest value of 0.0014 g/plant, nevertheless it was on par with the control. In the same manner the co-inoculated treatment was on par with both the AMF and the control. The least effective treatment was the individual AMF treatment. The individual AMF treatment, co-inoculated and control treatments at 20 DAS produced 57, 43 and 21% less root dry matter than the individual *Azospirillum* sp. treatment (Table 1).

On the contrary at 60 DAS no significant difference was recorded among the treatments however, the individual AMF treatment was numerically higher than the other treatments. Whilst at 60 DAS AMF, *Azospirillum* sp. and co-inoculated treatments produced 31, 22.31 and 14.87% less root dry matter respectively than the control. The greatest percentage increases in root biomass, 60 DAS were achieved by the individual and combined AMF treatments with the singly inoculated AMF treatment having the greatest percentage increase in root biomass. This increase in biomass may be attributed to the influence of AMF in lateral root development which may have contributed to the increased root biomass observed in the AMF treatments (Harrison, 2005). Such results may be due to AMF capacity to solubilize, absorb and translocate minerals such as phosphorus and nitrogen which play a key role in root development. Diniz *et al*. (2009) corroborated that root dry matter content was also found to be higher in sweet pepper seedlings inoculated with arbuscular mycorrhiza fungi. It has also been suggested by various researchers that the germinating spores release active symbiotic signal called Myc-factors which are not only symbiotic signals but also plant growth regulators that are able to positively modify root development (Fusconi, 2014).

With respect to total biomass at 20 DAS, the control presented the highest means however presented no significant differences with the individual *Azospirillum* sp. and co-inoculated treatments. Only individual AMF treatment registered significant difference with respect to the control. AMF, *Azospirillum* sp. and co-inoculated recorded 38, 37 and 15% less total biomass respectively with respect to the control. Similar results were obtained at 60 DAS, with significant difference only being recorded between the control and the AMF treatments. The highest biomass values were obtained by the control, whilst the co-inoculated, *Azospirillum* and AMF presented 4.67, 25.16 and 41.53% less biomass production respectively. The highest percentage increases were recorded by the *Azospirillum* treatments. This could be attributed to the synthesis of GA3 by *A. brasilense* and subsequent translocation to the aerial parts of the plant, consequently enhancing cell elongation, thus positively affecting plant biomass production (Madhukeshwara1 and Sajjan, 2016).

Although many studies reveal a positive effect of inoculants on seedling growth quite the opposite was observed in our study. These results can be attributed to the fact *R. intraradices* and *Azospirillum* sp. requires time and energy to establish the symbiosis (Dodd *et al*., 1996). The results provide further evidence that in the early stages of the symbiosis these microorganisms are more parasitic in nature, since they consumes large quantities photosynthates (16 moles of ATP to fix one mole of nitrogen) for their own metabolism thereby reducing the available quantities for biomass generation by the host plant and thus growth is slower (Urzúa, 2005), creating a situation in which the host plant must both satisfy its physiological requirements and provide energy to the symbiont microorganisms, whereas in the case of the control most of the energy produced is used for biomass production (Salinas-Ramírez *et al*., 2011).

Among the inoculated treatments the co-inoculated treatment recorded enhanced biomass production with respect to their individually inoculated counterparts. These results can be explained by the synergistic effects of both *A. brasilense* and *R. intraradices* in enhancing the performance of plants. Research suggests that absorption of nutrients in co-inoculated plants is more balanced, yielding better growth. Roots colonized by *R. intraradices* may use the extraradical mycelium to explore a greater volume of soil and translocate more efficiently greater quantities of nutrients to the plants, subsequently increasing biomass production (Linderman, 1992). Of equal importance is the activity of *Azospirillum*. *Azospirillum* spp. are known for the synthesis of auxin-type phytohormones that affect root morphology by increasing the number of lateral roots and root hairs thereby improving nutrient uptake from the soil which in turn enhances plant growth (Bashan *et al*., 2004). These results concurs with previous studies reported by Vafadar *et al*. (2013) that the strong synergistic relationships between PGPR and AMF significantly increased growth and development in inoculated plants.

Table 1. Effects of *R. intraradices* and *A. brasilense* inoculation on root length, shoot biomass, root biomass and total biomass of habanero pepper.

	Root length (cm)		Shoot dry mass (g/plant)		Root dry mass (g/plant)		Total biomass (g/plant)	
Treatment	**20 DAS**	**60 DAS**	**20 DAS**	**60 DAS**	**20 DAS**	**60 DAS**	**20 DAS**	**60 DAS**
R. intraradices	4.713 ab (0.14)	6.675 ab (0.51)	0.0043 b (0.0005)	0.0672 b (0.007)	0.0006 c (0.0007)	0.0242 a (0.003)	0.0049 b (0.0005)	0.0914 b (0.008)
A. brasilense	4.175 b (0.37)	6.200 b (0.18)	0.0042 b (0.0003)	0.0886 ab (0.009)	0.0014 a (0.0001)	0.0188 a (0.009)	0.0056 ab (0.0003)	0.1074 ab (0.009)
R. intraradices* + *A. brasilense	5.012 a (0.27)	6.175 b (0.26)	0.0056 ab (0.0008)	0.1162 a (0.015)	0.0008 bc (0.0001)	0.0167 a (0.002)	0.0064 ab (0.0009)	0.1329 a (0.014)
Control	5.225 a (0.12)	7.3750 a (0.26)	0.0065 a (0.001)	0.1193 a (0.019)	0.0011 ab (0.0002)	0.0206 a (0.005)	0.0076 a (0.001)	0.1399 a (0.005)

Variable means with the same letter represent similar values based on Duncan Multiple Range test ($p \leq 0.05$) n = 8 (SE) M (*R. intraradices*), Az (*A. brasilense*), M+A (*R. intraradices* + *A. brasilense*), C (control).0

2.4.7. Effects of biopriming on Relative growth rate of habanero pepper seedlings

The relative growth rates (RGR) for all treatments were relatively low with values ranging from 0.01 g (g/day) in the singly inoculated mycorrhizae treatment to 0.0158 g (g/day) in the singly inoculated *Azospirillum* treatment (Figure 5). This may be due to reduced daylight hours experience during the months of October – November during which the experiment was conducted resulting in low biomass production. Significant difference was only recorded between the singly inoculated mycorrhizae treatment and the singly inoculated *Azospirillum* treatments, with the later registering the highest values. This response could be attributed to the capacity of *Azospirillum* sp. to promote seedling development through the production of growth stimulating phyto-hormones (Carrillo-Castañeda *et al*., 2000). Similar results were reported by Canto-Martin *et al.* (2004) in their study of the effects of various levels of *Azospirrilum* concentration in the production of habanero pepper.

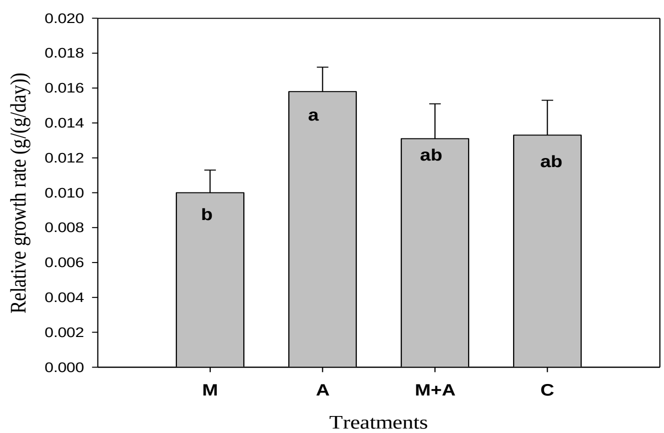

Figure 5. Relative growth rate of habanero pepper seedlings inoculated and co-inoculated with *R. intraradices* and *A. brasilense*. Bars with the same letter indicate similar values based on Duncan Multiple Range test ($p \leq 0.05$) n = 8. SE, M (*R. intraradices*), A (*A. brasilense*), M+A (*R. intraradices* + *A. brasilense*), C (control).

2.4.8. Effects of biopriming on seedling morphology parameters of habanero pepper

2.4.8.1. Seedling quality index

As a result of the low growth rate experienced, seedling quality index demonstrated no significant differences d among the treatments (Figure 6). The control registered the highest value (0.0204) whilst the singly inoculated treatment recorded the lowest (0.0129). Likewise no significant difference was observed among the treatments (Figure 8).

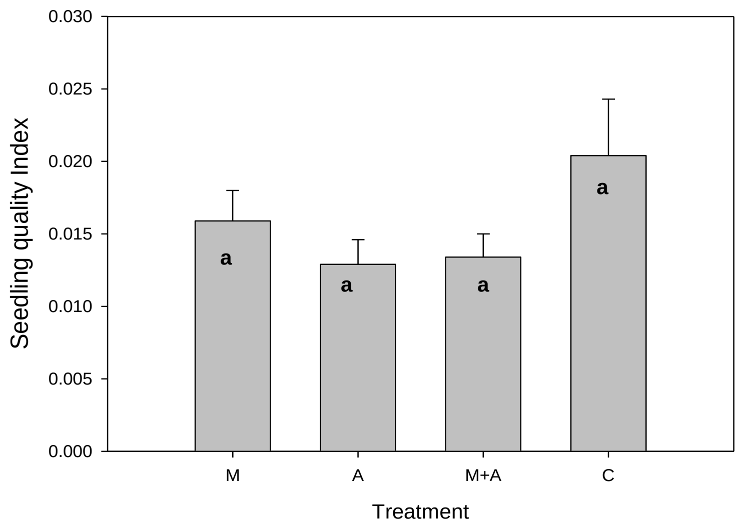

Figure 6. Seedling quality index of habanero pepper seedlings inoculated and co-inoculated with *R. intraradices* and *A. brasilense*. Bars with the same letter indicate similar values based on Duncan Multiple Range test ($p \leq 0.05$) n = 8. SE, M (*R. intraradices*), A (*A. brasilense*), M+A (*R. intraradices* + *A. brasilense*), C (control).

2.4.8.2. Robustness index

Robustness index is the ratio between seedling height (cm) and collar diameter (mm) it is an indicator of the seedlings capacity to resist adverse field conditions at the moment of transplant. No significant differences were observed among the treatments. However, the co-inoculated treatment registered the highest value of 3.6152 followed by the individual the control (3.519), and the singly inoculated *Azospirillum* treatment (3.1574). The singly inoculated mycorrhizae treatment had the lowest value of 3.3093 (Figure 7). It is preferable that this value be under 6; a lower number suggests a better plant quality.

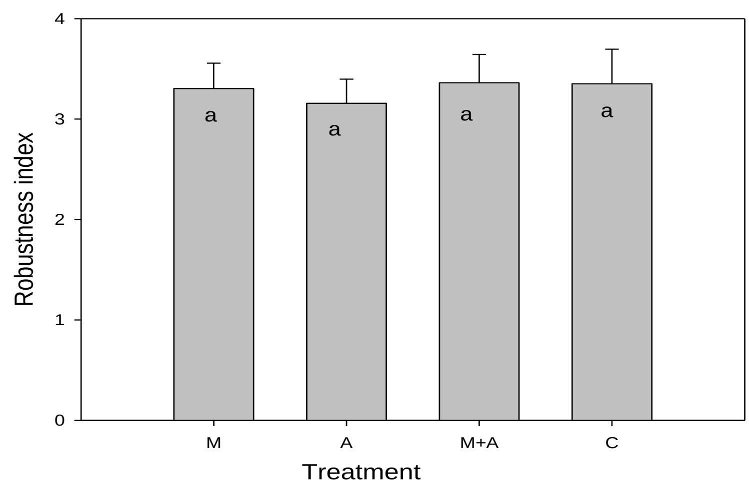

Figure 7. Robustness index of habanero pepper seedlings inoculated and co-inoculated with *R. intraradices* and *A. brasilense*. Bars with the same letter indicate similar values based on Duncan Multiple Range test ($p \leq 0.05$) n = 8. SE, M (*R. intraradices*), A (*A. brasilense*), M+A (*R. intraradices* + *A. brasilense*), C (control).

2.4.9. Nutrient concentration in seedling tissue

The application of microbial inoculants had significant effects on the seedling nutrient concentration. The values for leaf nitrogen content ranged from 3.58 to 3.63 %, for phosphorous from 0.30 to 0.46 %, for potassium from 16.06.58 to 20.68 %, for calcium from 5.19 to 6.91%, for sulphur from 0.14 to 0.197 and for copper from 0.0067 to 0.023 (Table 2).

The concentration of sulphur and copper was highest in the singly inoculated *R. intraradices* treatment however, their concentrations were statistically equal with *A. brasilense* and the control. The singly inoculated treatment of *A. brasilense* recorded highest result for phosphorous and calcium concentration in plant tissue but was on par with the singly *R. intraradices* treatment and the control these results could be attributed to increased nutrient mobilization (Murty and Ladha, 1988). The control presented highest concentration of potassium. The combined inoculation of *A. brasilense* and *R. intraradices* recorded lowest nutrient concentrations among all treatments (Table 2).

In the present study, stem diameter, shoot biomass, total dry matter and root length were highest in the control. These results may be attributed to better root growth and development which resulted in higher nutrient concentration in the seedling tissue. Potassium is essential for many plant processes including activation of enzymes plant growth and development. It has been reported that potassium activates at least 60 different enzymes involved in plant growth (Salisbury and Ross, 1992). Other functions of potassium in the plant includes: water use efficiency, regulation of the photosynthetic rate through ATP production, water and nutrient transport and the synthesis of protein. Results of this study differ from those obtained by Kuppurajendran (2012), who reported that nutrient concentration in plant tissue was higher in *Erythrina indica* L. seedling inoculated with *Azospirillum* with respect to the control, and was statistically on a par with triple inoculation of *Azospirillum* + Phosphobacterium + AMF.

These results could also be related to increase copper (Cu) concentration in the control in comparison to the other treatments. Copper is an essential nutrient involved in many physiological processes for normal growth and development (Yruela, 2005). It is intricately linked in the electron transport of photosystem I (PSI) (Shikanai *et al*., 2003) as it is involved in plastocyanin formation. It also essential in the activities of photosystem II (PSII) activity. Copper is also essential in the formation of the thylakoid membrane (Droppa *et al*. (1987). Therefore as a consequence of this higher concentration in the control the photosynthetic process could have been more efficient in the control and thus resulted in a greater assimilation of photosynthates in theses seedlings.

Nutrient concentration of the roots colonized by *R. intraradices* had no significant difference with the control, they were either equal or superior in certain minerals such as N, P, Ca, S, and Cu (Table 2). This enhanced nutrition could be attributed to the capacity of *R. intraradices* to use extraradical mycelium to explore greater volumes of the substrate thus absorbing and translocating minerals from the substrate to plant more efficiently, resulting in improved nutrition (Lindermann, 1992). The results from our experiment concurs with results obtained by Constantino *et al*. (2008) who studied various methods of inoculation using PGPR and AMF. They reported that the seedling colonized by *R. intraradices* treaments had greater nutrient than the other inoculant treatments.

Table 2. Effects of *r. intraradices* and *a. brasilense* inoculation and co-inoculation on nutrient concentration (%) in habanero pepper (*c. chinense*) seedlings.

Treatments	**N%**	**P (%)**	**K (%)**	**Ca (%)**	**S (%)**	**Cu (%)**
R. intraradices	3.63	0.45 a (0.015)	18.77 ab (0.901)	6.15 ab (0.31)	0.197 a (0.012)	0.023 a (0.003)
A. brasilense	3.61	0.46 a (0.019)	19.81 ab (0.708)	6.91 a (0.11)	0.193 ab (0.014)	0.017 a (0.003)
R. intraradices* + *A. brasilense	3.58	0.30 b (0.043)	16.06 b (1.911)	5.19 b (0.51)	0.14 b (0.015)	0.0067 b (0.003)
Control	3.63	0.42 a (0.012)	20.68 a (0.573)	5.39 b (0.17)	0.197 a (0.003)	0.020 a

Variable means with the same letter represent similar values based on Duncan Multiple Range test ($p \leq 0.05$) n = 10. (SE). *R. intraradices*, *A. brasilense*, *R. intraradices + A. brasilense*, control.

2.5. CONCLUSIONS

Overall, in this study the treatments applied to the seeds did not provide expressive increases in the variables studied, when compared to the control. This may have been as a result of the inoculation method, duration, doses or a combination of these factors used in the experiment. Many promising results have been achieved by seed inoculation but however, many studies are still providing inconsistent results. Therefore, further research is required with this technique in terms of inoculation techniques, doses, crop responses to bio-inoculants strains and synergies among inoculants etc. so that such technology could yield better results.

The performance of the co-inoculated treatment (*A. brasilense* and *R. intraradices*) was comparable to that of the control in many of the evaluated growth variables and had no significant effects on the morphological variables. Thus from our research we can conclude the following:

1. Co-inoculation was the most effective in terms of seedling emergence resulting in a 15.62 % increase in emergence rate with respect to the control, six days after the sowing.
2. Growth variables of the co-inoculated treatment were statistically equal to the control and superior to the individual inoculants.
3. Inoculation did not affect the relative growth rate (RGR) and seedling quality index (SQI).
4. *R. intraradices* inoculated seedling were either equal or superior in mineral concentration than the control
5. Co-inoculation resulted in seedlings with the lowest concentration of minerals among the treatments.

The use of bio-inoculants has demonstrated great potential for increasing seedling emergence and productivity. Though their use is still not very common, results achieved in other studies have shown that the incorporation of such treatments in seeds is a promising technique for improving seedling emergence therefore, we recommend therefore further research into this technology. Therefore the use of bio-inoculants should be considered as a viable alternative in sustainable seedlings production.

2.7. REFERENCES

Aloni, B., T. Pashkar, L. Karni. 1991. Nitrogen supply influences carbohydrate partitioning of pepper seedlings and transplant development. Amer. Soc. Hort. Sci. 116:995-999.

Altieri, M. A. 1997. Agroecología. Bases científicas para una agricultura sustentable. Asociación Cubana de Agricultura Orgánica (ACAO)-CLADES, Habana La Habana: pp. 325

Antoun, H. and D. Prèvost. 2005. Ecology of Plant Growth Promoting Rhizobacteria. In: PGPR: Biocontrol and Biofertilization, Z.A. Siddiqui (Ed.), Springer, Dordrecht, the Netherlands pp. 1–38

Barea, J.M., C. Azcón-Aguilar, R. Azcón. 1987. Vesicular arbuscular mycorrhiza improve both symbiotic N2 fixation and N uptake from soil as assessed with a 15N technique under field conditions. New Phytol. 106:717–725

Bashan, Y., G. Holguin, and L. E. de Bashan. 2004. *Azospirillum*-plant relationships: physiological, molecular, agricultural, and environmental advances (1997‑2003). Can. J. Microbiol. 50:521‑577.

Cacciari, I., D. Lippi, T. Pietrosanti, and W. Pietrosanti. 1989. Phytohormone-like substances produced by single and mixed diazotrophic cultures of *Azospirillum* and *Arthrobacter*, Plant Soil. 115:151–153.

Canto Martín, J.C., S. Medina Peralta, D. Morales Avelino. 2004. Efecto de la inoculación con *Azospirillum* sp. en plantas de Chile habanero (*Capsicum chinense* jacquin). Tropical and Subtropical Agroecosystems. 4(1): 21-27.

Carrillo-Castañeda, G., J. Juárez, D. Ruiz y R. Müller. 2000. Aumento del Rendimiento de Tomate (*Lycopersicon esculentum* Mill) cuando la raíz se desarrolla colonizada por microorganismos. Biotecnología Aplicada.17: 171-176.

Cassána, F., D. Perriga, V. Sgroya, O. Masciarellia, C. Pennab and V. Lunaa. 2011. *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109, inoculated singly or in combination, promote seed germination and early seedling growth in corn (*Zea mays* L.) and soybean (Glycine max L.). European Journal of Soil Biology. 47(3): 214.

Castillo-Reyes, F., J. D. Sánchez Chaparro, S. E. Rangel-Estrada y Canul-Ku, J. 2014. Efecto de microorganismos en la promoción de la germinación de semillas de la cactácea *echinocactus platyacanthus* link & otto. Interciencia. 39: 863-867.

Constantino, M., R. Gómez-Álvarez, J. D. Álvarez-Solís, V. Geissen, E. Huerta, and E. Barba. 2008. Journal of Agriculture and Rural Development in the Tropics and Subtropics.109 (2): 169‑180.

Dickson, A., A.L. Leaf, J.F. Hosner. 1960. Quality Appraisal of White Spruce and White Pine Seedling Stock in Nurseries. Forest chronicle. 36: 10-13.

Diniz, K. A., P. A. Silva, J. A. Oliveira, and J. R. E. Evangelista. 2009. Sweet pepper seed responses to inoculation with microorganisms and coating with micronutrients, amino acids and plant growth regulators. Sci. Agric. 66 (3): 293-297.

Dodd, J. C.; S. Rosendahl, M. Govannetti, A. Broome, L. Lanfranco, y C. Walker. 1996. Inter and intraspecific variations within the morphologically-similar arbuscular mycorrhizal fungi *Glomus mosseae* and *Glomus coronatum*. New Phytologist. 133: 113-122.

DOF. 2012. NORMA Oficial Mexicana NOM-189-SCFI. 2012. Chile Habanero de la Península de Yucatán (*Capsicum Chinense* Jacq.)-Especificaciones y métodos de prueba.

Droppa, M., J. Masojidek, Z. Rózsa, A. Wolak, L.I. Horváth, T. Farkas, G., Horváth. 1987. Characteristics of Cu deficiency-induced inhibition of photosynthetic electron transport in spinach chloroplasts. Biochim. Biophys. Acta 891:75-84

Fusconi, A. 2014. Regulation of root morphogenesis in arbuscular mycorrhizae: what role do fungal exudates, phosphate, sugars and hormones play in lateral root formation? Annals of Botany 113: 19-33.

Garruña-Hernández, R., Latournerie-Moreno, L., Ayala-Garay, O., Santamaría, J. M., Pinzón-López, L. 2014. Acondicionamiento pre-siembra: una opción para incrementar la germinación de semillas de chile habanero (*capsicum chinense jacq*.). Agrociencia 48: 413-423.

González-Estrada, T. 2006. Habanero growing systems. Fiery Foods. 42: 21-23.

Guzmán M, and A. Sánchez. 2003. Influence of nitrate and calcium increments on development, growth and early yield in sweet pepper plants. Acta. Hort. 609: 207-211.

Harrison, M. J. 2005. Signaling in the arbuscular mycorrhizal symbiosis. Annual Review of Microbiology 59: 19–42.

Hernández, M.I. y M. Chailloux. 2004. Las micorrizas arbusculares y las bacterias rizosféricas como alternativa a la nutrición mineral del tomate. Cultivos Tropicales. 25 (2): 5-12

Hunt, R., D. R. Causton, B. Shipley, and A. P. Askew. 2002. A modern tool for classical plant growth analysis. Ann. Bot. 90: 485-488.

International Seed Testing Association, ISTA. 2013. Hand book for seedling evaluation. 3rd edn. Bassersdorf, Switzerland.

Jha, A., A. Kumar, R. K. Saxena, M. Kamalvanshi, and N. Chakravarty. 2012. Effect of Arbuscular Mycorrhizal Inoculations on Seedling Growth and Biomass Productivity of Two Bamboo Species. Indian J Microbiol. 52(2): 281–285.

Juge, C., D. Prévost, A. Bertrand, M. Bipfubusa, and F. P. Chalifour. 2012. Growth and biochemical responses of soybean to double and triple microbial associations with *Bradyrhizobium, Azospirillum* and arbuscular mychorrhizea. Applied soil ecology. 61: 147-157.

Karthika C., and K. Vanangamudi. 2013. Biopriming of maize hybrid COH(M) 5 seed with liquid biofertilizers for enhanced germination and vigour. African Journal of Agricultural Research. 8(25): 3310-3317.

Khan, A. A. and N. E Tolbert. 1966. Inhibition of lettuce seed germination and root elongation by derivatives of auxin and reversal by derivatives of cycocel. Physiol. Plant. 19: 81–86.

Khan, M. A., and I. A. Ungar. 1984. Effects of salinity and temperature on the germination and growth of *Atriplex triangularis* Willd. Am. J. Bot. 71: 481-489.

Linderman, R. G. 1992.Vesicular-arbuscular mycorrhizae and soil microbial interactions; in: Micorrhizae in sustainable agriculture. Am. Soc. Agron. 54: 45-70.

Madhukeshwara, B.P. and A. S. Sajjan. 2016. Influence of Bio-Priming on Field Performance and Yield in Maize Hybrid International Journal of Agricultural Research and Crop Sciences. 1 (1): 16-19.

Malhotra, M. and S. Srivastava. 2009. Stress-responsive indole-3-acetic acid biosynthesis by *Azospirillum brasilense* SM and its ability to modulate plant growth. Eur. J. Soil Biol. 45: 73–80.

Mangmang, J.S., R. Deaker and G. Rogers. 2015. Early seedling growth response of lettuce, tomato and cucumber to *Azospirillum brasilense* inoculated by soaking and drenching. Hort. Sci. (Prague) Vol. 42 (1): 37–46.

Mardero, S., E. Nickl, B. Schmook, L. Scheiner, J. Rogan, Z. Christman, y D. Lawrence. 2012. Sequías en el sur de la península de Yucatán: análisis de la variabilidad anual y estacional de la precipitación. Investigaciones geográficas, Boletin del Instituto de Geografía-Unam 78: 19-33.

Mariappan, N., P. Srimathi and L., Sundaramoorthi. 2014. Effect of liquid biofertilizers on enhancement of germination in stored seeds of *Pongamia pinnata*. Journal of Agricultural and Crop Research 2 (11): 218-221

Murillo-Amador, B., E. Troyo-Diéguez, H. G. Jones, F. Ayala-Chairez, C. L. Tinoco-Ojanguren, and A. López-Cortés. 2000. Screening and classification of cowpea genotypes for salt tolerance during germination. J. Exp. Bot. 67: 71-84

Murty, M. G. and J. K. Ladha. 1988. Influence of *Azospirillum* inoculation on the mineral uptake and growth of rice under hydroponic conditions. Plant Soil. 108: 281‑285;

Ona, O., I. Smets, P. Gysegom, K. Bernaerts, J. V. Impe, E. Prinsen and J. Vanderleyden. 2003. The effect of pH on indole-3- acetic acid (IAA) biosynthesis of *Azospirillum brasilense* sp7. Symbiosis 35: 199–208.

Preciado R, P., A. Lara-Herrera, C. M. Á. Segura, P. E. O. Rueda, V. J. A. Orozco, C. P. Yescas y T J. A. Montemayor. 2002. Amonio y fosfato en el crecimiento de plántulas de chile jalapeño. Terra Latinoamericana. 26 (1) Sociedad Mexicana de la Ciencia del Suelo, A.C. Sociedad Mexicana de la Ciencia del Suelo, A.C. Chapingo, México.

Rajendran, K. 2012. Effects of bioinoculants on seedling growth, biochemical changes and nutrient uptake of *Erythrina indica* L.in semi-arid region of south India. J. Biomet Biostat. 3(2): 134-139.

Rodríguez, Yon, Y., Pérez, L. A., A. M. Carmona Pérez, Y. M., Medina G, L. R., S. K. Fernández y A. M. Echevarría. 2015. Alternativa de la técnica de tinción para determinar la colonización micorrízica. Cultivos Tropicales. 36(2): 18-21.

Salinas-Ramírez, N., J. A. Escalante-Estrada, M. T. Rodríguez-González, and E. Sosa-Montes. 2011. Yield and nutritional quality of snap bean in terms of biofertilization. Tropical and Subtropical Agroecosystems. 13: 347 – 355

Salisbury, F.B. and C.W. Ross, 1992. Plant Physiology. Wadsworth Publishing Company, Belmont - California. 4th Edition, Wadsworth Publishing, Belmont, 357-381.

Shikanai, T., P. Müller-Moulé, Y. Munekage, K. K. Niyogi, M. Pilon. 2003. PPA1, a P-type ATPase of Arabidopsis, functions in copper transport in chloroplasts. Plant Cell. 15:1333-1346.

Sivritepe, H. O. and A.M. Dourado. 1995. The effect of priming treatments on the viability and accumulation of chromosomal damage in aged pea seeds. Ann. Bot. 75: 165-171.

Srivastava, R., A. Khalid, U. S. Singh and A.K., Sharma. 2010. Evaluation of arbuscular mycorrhizal fungus, flourescent *Pseudomonas* and *Trichoderma harzianum* formulation against *Fusarium Oxysporum* f.sp. l*ycopersici* for the management of tomato wilt. Elservier. Biological control. 53(1): 24-31

Taiz, L. y E. Zeiger. 2006. Plant physiology 4th edition. Sinauer Associates, Inc.

Thuler, D. S., E. I. S. Floh, W. Handro, and H. R. Barbosa. 2003. Plant growth regulators and amino acids released by *Azospirillum* sp in chemically defined media. Lett. Appl. Microbiol. 37: 174–178.

Tien, T. M., M. H. Gaskins and Hubell, D. H. 1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (Pennisetum americanum L). Appl. Environ. Microbiol. 37: 1016–1024.

Urzúa, H. 2005. Beneficios de la Fijación Simbiótica de Nitrógeno en Chile. Cien. Inv. Agr. 32(2): 133-150.

Vafadar, F., R. Amooaghaie, and M. Otroshy. 2014. Effects of plant-growth-promoting rhizobacteria and arbuscular mycorrhizal fungus on plant growth, stevioside, NPK, and chlorophyll content of *Stevia rebaudiana*. Journal of Plant Interactions. 9(1):128-136

Yruela, I. 2005. Copper in plants. Braz. J. Plant Physiol. 17 (1): 145-156.