INNOVARE JOURNAL OF AGRICULTURAL SCIENCE

Vol 4, Issue 2, 2016

ISSN - 2321-6832

Short Communication

# EFFECT OF IRRIGATION AND HYBRIDS ON MICROBIAL POPULATION DYNAMICSIN THE RIZHOSPHERE OF OIL PALM (*ELAEIS GUINEENSIS JACQ.*)*

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***Received: 17 December 2015, Revised and Accepted: 23 February 2016***

**ABSTRACT**

**Objective:** This research was carried out to evaluate the effect of irrigation and hybrids on the population dynamics of microorganisms associated with the rhizosphere of oil palm (*Elaeis guineensis Jacq.*).

**Methods:** Plots were installed under irrigated and non-irrigated conditions using three hybrids (Dura × Pisifera) of oil palm. A split-plot design was used with three replications. Samples of soil were extracted and processed to determine the populations of functional groups of heterotrophic bacteria, actinomycetes, fungi, phosphorus (P) solubilizing microorganisms, and vesicular-arbuscular (VA) mycorrhizae. Samples were taken during the wet (June) and the dry season (August).

**Results:** No significant differences were detected for bacterial populations (heterotrophic and actinomycetes) for irrigation and hybrids. Populations recorded for heterotrophic bacteria when water stress accounted for 28 centibars (cbs) were $7.43 \times 10^6$ colony forming units per g of dry soil (CFU/g) in irrigated plots, versus $5.86 \times 10^6$ CFU/g in non-irrigated plots. Fungal populations were $4.49 \times 10^3$ CFU/g in irrigated plots, while in non-irrigated plots were $1.81 \times 10^4$ CFU/g. Mycorrhizae levels ranged between 13.6% and 21.9% in irrigated and non-irrigated plots, respectively. The populations of P-solubilizing microorganisms ranged from $6.64 \times 10^5$ CFU/g in irrigated plots to $2.91 \times 10^4$ CFU/g in plots without irrigation.

**Conclusions:** Under the conditions of this study, in the oil palm rhizosphere, there were not significant differences between the bacterial populations (heterotrophs and actinomycetes) for irrigation and hybrids. Under the same conditions, significant differences were found among fungal populations, P-solubilizing microorganisms, and VA mycorrhizae, in the irrigation factor. The major bacterial populations were recorded in the rainy season 7 cbs; meanwhile, the higher fungal populations were recorded in the dry season (28 cbs). Hybrids not exert a major influence on microbial populations under the conditions of this study. With some of the microorganisms isolated in this study, starting tests are needed to identify potential bio-fertilizers, and/or bio-enhancers of soil properties.

**Keywords:** Vesicular arbuscular mycorrhizae, Phosphorus-solubilizing microorganisms, Oil palm, Heterotrophs, Irrigation.

## INTRODUCTION

Oil palm (*Elaeis guineensis* J.) is one of the most important crops in Ecuador with an area of 207.285 ha. It is also a crop of high socio-economic impact, and major source for production of clean and renewable biofuels. The main objective of the oil palm growers in Ecuador is increase yield; for this purpose, they apply good management practices for higher profitability [1]. However, there is a considerable reduction of yield due rapid change and impairment of the physical, chemical, and biological properties of soils. The latter has been largely unexplored; nevertheless, it is considered fundamental to study the soil biological community to solve the problem of exhaustion of soils and low production of plantations [2].

Oil palm demands large amounts of water, from germination to harvest of the last bunch at the end of the productive cycle. In Ecuador rainfall is irregular, therefore, it is necessary to keep the soil humidity in optimal status. Considering the soil as a major water reservoir and the depletion of this reserve for crop consumption and it will be required artificial recharge by irrigation [3].

On the other hand, oil palm extracts significant amounts of macro and micronutrients, for the production of an enormous quantity of biomass. Therefore, the exploitation of plant growing promoting rhizobacteria (PGPR's) or mycorrhizae could contribute to the nutrient acquisition and nutrient recycle.

The present study was carried out to identify and contrast the magnitude of different soil microorganisms under three hybrids of oil palm in irrigated and non-irrigated conditions during the dry and wet season. The final objective of the project will be to generate bio-inoculants and/or bio-enhancers of soils that contribute to crop nutrition and crop protection in adverse seasons.

This research was carried out in the "Centro de Investigaciones de Palma Aceitera" situated in the province of Esmeraldas – Ecuador, at 264 m above the sea level, latitude 0°2'29"; longitude 79°24'54". The climate in this area is tropical with a temperature of 20-24°C, 626 hrs lux, relative humidity 87.8%, and annual precipitation of 2881 mm.

Intraspecific hybrids correspond to the crosses Dura × Pisifera of *E. guineensis*: Hybrid from ASD Costa Rica (Deli × Ghana), hybrid from "Instituto Nacional de Investigaciones Agropecuarias" (INIAP) (Deli × Yangambi) and hybrid from "Centre de Coopération Internationale en Recherche Agronomique pour le Développement" (CIRAD) (Deli × La Mé 2501).

Oil palms of 5 years after transplanting were used during this research. Factors in study were arranged in a split plot design with three replications, where irrigation received the main plots, and hybrids the subplots. The experimental area consisted of 18 plots with 30 oil palms each. The area of the main plots were 6230 $m^2$ (70 m × 89 m), and the subplot's area was 2047 $m^2$ (23m × 89m). The experimental unit consisted of eight palms.

Soil samples were collected using a disinfected drilling bore of 4 cm diameter in eight random locations at 1 m distance of the trunk of the palm. Samples were placed in Ziploc® bags and transported on ice. In

laboratory, a subsample of 200 g was separated and grinded to obtain quaternary roots for mycorrhizae analysis. A subsample of 30 g was sieved and separated for relative humidity analysis and serial dilutions.

To determine total microbial populations, 10 g of fresh sieved soil was mixed with 90 ml of distilled sterilized water. From the first dilution ($10^{-1}$), 1 ml was taken and mixed with 9 ml of water to form the 1:100 or $10^{-2}$ dilution. This procedure was successively repeated until the dilution $10^{-6}$. A value of 1 ml of the dilutions $10^{-5}$ and $10^{-6}$ was placed and 25 ml of specific media was poured onto the dilution. Immediately before agar solidification, the mixture media with dilution was homogenized with circular movements.

Specific media for heterotrophic bacteria was nutrient agar (beef extract 3, peptone 1.5, agar 15 g/L). For fungi, the media was rose bengal agar (D - glucose 10 g/L, peptone 5 g/L, KH2PO4 1 g/L, MgSO4 0.5 g/L, rose bengal [Sigma-Aldrich] 30 mg/L, streptomycin 35 mg/L, and agar 15 g/L). For phosphorus-solubilizing microorganisms, the media was agar Ramos Callao (yeast extract 2 g/L, D - Glucose 20 g/L, Ca3(PO4) 2-2 g/L, and agar – 22 g/L). For actinomycetes, the media was Casein Aga (starch soluble - 10 g/L, casein – 1 g/L, KH2PO4 – 1 g/L, K2HPO4-0.5 g/L, and agar – 10 g/L). Plates were incubated under 37°C for bacterial populations and 27°C for fungi.

The depigmentation and staining roots methodology for recording mycorrhizal colonization with the application of heat was used. Quaternary roots of oil palm were depigmented during 35 minutes in the autoclave at 1.4 atmospheres using 10% KOH. Roots were rinsed first with a solution of 10% H2O2 during 10 minutes, and later with tap water and acidified during 15 minutes with HCl 1 N. Roots were stained using a solution of 0.05% trypan blue in lacto glycerol, and then mounted in plaques for evaluation of the grade of colonization of the root.

Samples were taken during the wet season (January to June). Wet season is the part of the year when there was no need for irrigation, and dry season (July to December) is the part of the year with lower precipitation and water stress conditions. During the dry season an irrigation lamina of 150 mm was applied, based on evapotranspiration calculations with the help of a tensiometer and lysimeter. Data from colony forming units per g of soil (CFU/g) in this research were logarithmically transformed. Analysis of variance was performed using InfoStat [4].

Rainfall for the planting cycle 2007-2008 showed a drastic reduction during July to November (dry season); on the other hand, the wet season was present during months of January to June (Table 1). In addition, there was residual humidity during the months of June and July, it was evident in the values marked by the tensiometers of lysimeters. During the planting year, 2007-2008 rainfall presented a unimodal system. In average, temperature was higher in the wet season versus the dry season.

Sampling made in the wet season presented conditions of saturated soils 7 centibars (cbs). The rainfall the day before sampling was 5.6 mm.

**Table 1: Summary of rainfall and average temperature in the experimental site**

| Locality | Season | Temperature (°C)[1] | | Rainfall (mm)[2] | |
|---|---|---|---|---|---|
| | | 2007 | 2008 | 2007 | 2008 |
| CIPAL (Esmeraldas-Ecuador) | Dry (July-November) | 22.0 | 21.0 | 249.0 | 400.0 |
| | Wet (January-June) | 24.0 | 26.0 | 2751.0 | 2679.0 |

Source: National Institute of Climatology of Ecuador (INHAMI). CIPAL: Centro de Investigaciones de Palma Aceitera

During the dry season, there was not rainfall, and the tensiometer recorded 28 cbs which indicates a high force of retention of water; therefore, a lamina of irrigation of 150 mm during the dry season had to be incorporated to meet the needs of the crop.

According to the results of this research, microbial populations recorded in the environments studied were different for P-solubilizing organisms, fungi, and mycorrhizae. We cannot detect statistical differences for bacteria (heterotrophic and actinomycetes) (Table 2). A general ANOVA for five functional groups is shown. Bacterial populations (heterotrophic and actinomycetes) recorded in the different experimental units, either with one or another hybrid, were not statistically different; nor statistical differences between blocks and the interaction irrigation × hybrid were found (Table 2).

Heterotrophic bacterial populations detected belong to genus *Arthrobacter*, *Pseudomonas* and *Bacillus*, according to the morphological and basic chemo-taxonomic configuration (gram, catalase, and oxidase). In the dry season, there were not statistical differences between irrigation, hybrids, interactions, and blocks. However, heterotrophic bacterial populations during wet season were higher than dry season. This behavior was consistent also in the irrigated plots versus the non-irrigated plots (Table 3).

During the wet season, the largest heterotrophic bacterial populations were detected in both plots with irrigation and non-irrigated plots. These same plots, in the second evaluation, were less populated, probably due to the influence that began to exert the lack of moisture in the population dynamics. In the wet season, the highest population corresponded to the hybrid CIRAD, while the lowest was obtained with the hybrid ASD; whereas, in the dry season, the situation was the opposite, that is, the highest population was detected in the hybrid ASD, and less in the hybrid CIRAD. The trend data between the wet and dry season remains at INIAP and CIRAD hybrids with higher population data at the first assessment (wet season); while the trend was reversed in the hybrid ASD, presenting the highest populations of heterotrophic bacteria in the dry season (Table 3).

Similarly to what happened with heterotrophic bacteria, populations of actinomycetes did not differ statistically. There was a tendency to lower values on irrigated versus non-irrigated plots. In addition, by relating the data of obtained in the wet season versus the dry season, there is a marked inhibitory effect of water stress in actinomycetes (Table 3). Populations of actinomycetes registered on hybrids showed slight variations in the evaluations of the wet and dry seasons. The hybrid with a greater proportion of these bacteria in the wet season was ASD; while in the second evaluation, the highest population was recorded in the hybrid INIAP. Table 3 shows a downward trend for the three hybrids comparing the data from the wet versus the dry season.

Phosphorus-solubilizing microorganisms were detected due the formation of a clear halo around the colonies. Likewise bacteria isolated from the rhizosphere of oil palms, there was no statistical difference for irrigation, hybrids, and the interaction during the wet season.

According to the ANOVA summary (Table 2), a statistical difference was detected for the irrigation factor in the dry season. A general trend was detected in the factors of study, where the higher population estimates were obtained in the dry versus the wet season, which allows to suggest that there is an effect of water stress for the dry season. Hybrids did not influence the populations of phosphorus-solubilizing microorganisms. Arithmetic differences between them not reached statistically significant levels; however, in the hybrid ASD the population was higher than the population of INIAP and CIRAD (Table 4). For the dry season higher values were registered ASD, followed by the hybrid INIAP, and finally the hybrid CIRAD (Table 4). P-solubilizing microorganisms registered were higher in the dry season, probably due to the greater amount of fungi of the genus *Penicillium* in the plates with Agar Ramos Callao growth medium.

**Table 2: Square means of heterotrophic bacteria, actinomycetes, P-solubilizing microorganisms, total fungi and % VA-mycorrhizae**

| Source of variance | df | Mean Squares | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Heterotrophic Bacteria | | Actinomycetes | | P-solubilizing microorganisms | | Fungi | | Mycorrhizae | |
| | | Wet | Dry | Wet | Dry | Wet | Dry | Wet | Dry | Wet | Dry |
| Block | 2 | $0.12^{ns}$ | $0.01^{ns}$ | $0.17^{ns}$ | $0.03^{ns}$ | $0.19^{ns}$ | $0.01^{ns}$ | $0.03^{ns}$ | $0.01^{ns}$ | $0.25^{ns}$ | $0.34^{ns}$ |
| Irrigation (I) | 1 | $0.01^{ns}$ | $0.04^{ns}$ | $0.10^{ns}$ | $0.01^{ns}$ | $0.01^{ns}$ | 0.51* | $0.06^{ns}$ | 1.74* | 0.88* | 0.75* |
| Hybrids (H) | 2 | $0.14^{ns}$ | $0.01^{ns}$ | $0.09^{ns}$ | $0.01^{ns}$ | $0.02^{ns}$ | $0.02^{ns}$ | $0.14^{ns}$ | 0.56* | $0.31^{ns}$ | $0.06^{ns}$ |
| I × H | 2 | $0.04^{ns}$ | $0.01^{ns}$ | $0.07^{ns}$ | $0.03^{ns}$ | $0.04^{ns}$ | $0.02^{ns}$ | $0.39^{ns}$ | $0.32^{ns}$ | 0.97* | $0.65^{ns}$ |
| Grand Mean CFU/g** | | $8.7\times10^{6}$ | $6.6\times10^{6}$ | $6.7\times10^{6}$ | $3.0\times10^{6}$ | $2.0\times10^{5}$ | $1.1\times10^{4}$ | $1.1\times10^{4}$ | $1.2\times10^{4}$ | 12.55% | 17.51% |
| Coefficient of variance (a) (%) | | 5.7 | 2.2 | 4.3 | 2.4 | 8.2 | 4.1 | 11.4 | 5.8 | 13.5 | 11.2 |
| Coefficient of variance (b) (%) | | 4.8 | 1.9 | 3 | 2.1 | 6.1 | 3.9 | 18.2 | 8.2 | 9.6 | 29.5 |

*Statistical differences (α=0.05) according to Fisher test. **Data of CFU/g were transformed using $Log_{(10)}$ × before running ANOVA. VA: Vesicular arbuscular, CFU: Colony forming units

**Table 3: Effect of irrigation and hybrids on microbial population dynamics of heterotrophic and actinomycetes in the rhizosphere of oil palm**

| Effect | Log CFU/g | | | |
|---|---|---|---|---|
| | Heterotrophic | | Actinomycetes | |
| | Wet | Dry | Wet | Dry |
| Irrigation | | | | |
| Irrigated | 6.91±1.34* | 6.87±1.12 | 6.73±1.25 | 6.45±1.13 |
| Non-irrigated | 6.96±1.34 | 6.77±1.12 | 6.90±1.25 | 6.50±1.13 |
| Hybrids | | | | |
| ASD | 6.72±1.37 | 6.84±1.13 | 6.89±1.21 | 6.48±1.14 |
| INIAP | 6.87±1.37 | 6.8±1.13 | 6.73±1.21 | 6.51±1.14 |
| CIRAD | 7.13±1.37 | 6.81±1.13 | 6.85±1.21 | 6.44±1.14 |

*Results are expressed as means±SD. SD: Standard deviation, CFU: Colony forming units, INIAP: Instituto Nacional de Investigaciones Agropecuarias, CIRAD: Centre de Coopération Internationale en Recherche Agronomique pour le Développement

The analysis of the interactions of the factors under study indicates that the highest population of microorganisms for the wet season was on the interaction irrigated × INIAP, while in the dry was on the interaction non-irrigated × ASD. No significant differences were detected in the wet season for total fungi; while for the dry season, it was possible to find significant differences ($p<0.05$). In both evaluations, the largest populations of fungi were detected in non-irrigated plots, probably due to a cumulative effect of water stress which allowed further development of fungi.

Among data from the wet and dry season, a similar behavior is observed for irrigated versus non-irrigated plots; while in irrigated plots a decrease of CFU/g was recorded, in non-irrigated plots CFU/g increased (Table 4). There was not effect of the hybrids in the fungi populations. In the hybrids ASD and INIAP CFU/g decreased; meanwhile for the hybrid CIRAD CFU/g increased in the dry season (Table 4).

For colonization of mycorrhizae, statistical effect for the interaction irrigation × hybrids. This interaction was ordered; therefore, increments of the endophyte were found for the non-irrigated plots versus the irrigated plots for the three hybrids. For the main factors, effect of irrigation was detected in the dry and wet season (Table 2). Higher levels of colonization were found in the non-irrigated versus irrigated plots (Table 4). There was a tendency for hybrids in the colonization of vesicular arbuscular (VA) mycorrhizae with higher levels of colonization in the dry season, therefore, there is an effect of the dry season in the colonization for all hybrids.

The microbial population in the rhizosphere varied according to the kingdom and microbial group. Microbes that occurred in greater proportion were heterotrophic bacteria, and actinomycetes, in their respective order. The estimates of population were based on the recovery of colonies that developed in the culture media, the number of colonies corresponded to 1-5% of the total population of microorganisms of soils [5].

For heterotrophic bacteria, the most frequent genus isolated during this research were *Arthrobacter*, *Pseudomonas*, and *Bacillus* which are part of the group of PGPR. Those are characteristic of the rhizosphere and typically produce hormones (auxins, gibberellins, cytokinins, ethylene, etc.) that increase both the length and number of roots of crops, and dry weight of the plant material. Although there were not statistical differences among bacterial populations, there is a cumulative effect of irrigation on these populations that can be higher over time.

According to the morphological traits and basic chemo-taxonomic configuration, the predominant genus for the actinomycetes was *Streptomyces* spp., with compact colonies of cottony consistency and earthy smell [5,6]. The analysis of the factors under study indicate that populations of actinomycetes were lower for all factors and levels on samples collected during the dry season (evaluation in August), which allows us to infer that their population is affected at the time of water stress [5].

For P-solubilizing microorganisms, the grand mean of the experiment was $2.02 \times 10^{5}$ CFU/g. The populations obtained in this study differ from those reported in the country by Morales [7], who recorded an average of $1.08 \times 10^{6}$ CFU/g in the rhizosphere.

The population difference is probably due to both the method of assessment used, as well as climate and soil cultivation history [8]. In this regard, it is considered that the number of inorganic phosphate solubilizing microorganisms varies widely from one soil to another [9]. Statistical difference was detected for the irrigation factor in the dry season. Probably the difference in the number of microorganisms in irrigated plots versus the non-irrigated plots was due to moisture allowed microbes continue their metabolic activity rather than a possible break due to the drought.

*Aspergillus* and *Penicillium* are considered as predominant in the experimental plots; however, other genus found in this study were: *Trichoderma* spp., *Rhizopus* spp., *Gliocladium* spp., *Geotrichum* spp., *Cladosporium* spp., *Cunningamella* spp., and *Mucor* spp.; data consistent with Farrow [10] who in tropical soils of Brazil found as frequently isolated genera *Penicillium* spp., *Aspergillus* spp., *Fusarium* spp., *Trichoderma* spp., *Chaetomium* spp. and *Cunningamella* spp.

Many unidentified species had thermophilic features and specialized reproductive structures (spores) allowing them to withstand adverse conditions of temperature [11]. However, the presence of spores is an important feature for Gram-positive bacteria such as *Bacillus*

**Table 4: Effect of irrigation and hybrids on microbial population dynamics of P-solubilizing microorganisms, fungi, VA-mycorrhizae in the rhizosphere of oil palm**

| Effect | P-solubilizing microorganisms | | Fungi | | Mycorrhizae | |
|---|---|---|---|---|---|---|
| | Log CFU/g | | Log CFU/g | | % endophyte | |
| | Wet | Dry | Wet | Dry | Wet | Dry |
| Irrigation | | | | | | |
| Irrigated | 5.27±1.39* | 5.82±1.19[a] | 4.01±1.39 | 3.65±1.18[b] | 10.95±0.27[b] | 13.06±0.28[b] |
| Non-irrigated | 5.34±1.39 | 5.46±1.19[b] | 4.07±1.39 | 4.26±1.18[a] | 14.15±0.27[a] | 21.95±0.28[a] |
| Hybrids | | | | | | |
| ASD | 5.43±1.35 | 5.72±1.02 | 3.97±1.91 | 3.90±1.34[b] | 14.53±0.24 | 17.88±0.85 |
| INIAP | 5.20±1.35 | 5.71±1.02 | 4.00±1.91 | 3.88±1.34[b] | 11.86±0.24 | 17.23±0.85 |
| CIRAD | 5.24±1.35 | 5.60±1.02 | 4.13±1.91 | 4.28±1.34[a] | 11.25±0.24 | 17.42±0.85 |

Letters denote statistical differences (α=0.05) LSD Fisher. *Results are expressed as means±SD. SD: Standard deviation, CFU: Colony forming units, INIAP: Instituto Nacional de Investigaciones Agropecuarias, CIRAD: Centre de Coopération Internationale en Recherche Agronomique pour le Développement

which has predominance on tropical soils. In a similar study, Aciego *et al*. [12] determined that in the beginning of the rainy season the average population of fungi for their experiment was 1.1 × 104 CFU/g. These values are within a range similar to those obtained in this study. The data generated in this study are consistent with the criteria presented by Olalde and Aguilera [13], who indicate that in land dedicated to agriculture populations of 4.6 × 104 CFU/g can be found. Taylor and Parkinson [14] indicate that under conditions of water stress in soils there is a predominance of species belonging to the genus *Penicillium*, species that soon disappear with increasing water content in the soil. The predominant fungal specie found in this study was *Penicillium* in the Agar Ramos Callao and the rose bengal media. However, *Fusarium* sp., *Gliocladium* sp., Cylindrocarpum sp., increase their population in the rhizosphere under higher humidity conditions. There is necessary to point out that species of *Penicillium* are easily detached from the sporangia, therefore, there is a high quantity of spores available to grow and overpopulate the plate, impeding the growth of other fungal species.

For VA-mycorrhizae, the highest population levels were recorded in the dry season, not only in irrigated plots, but also in non-irrigated plots. This behavior allows to assume that probably due to the effect of moisture, fungus tends to develop more becoming infective and it could be the reason for most intense levels of colonization. Gonzáles [15] reported that the arbuscular mycorrhizal fungus helps to maintain adequate water status during periods of drought; therefore, the fungus - root symbiosis presents a favorable development in the plots with water stress; hence this is probably another reason to have higher levels of endophyte in the dry season. *Glomus* sp., and *Acaulospora* sp., were the most recorded genus in the present study. These genus have also been identified by Morales [7] in the province of Manabí in the Pacific coast of Ecuador, and Duicela *et al*. [16] in the Northern Zone and Center of the Ecuadorian highlands.

Our results could support other studies for the selection of microorganisms that improve the mineral nutrition and protection to adverse conditions.

**ACKNOWLEDGMENTS**

We thank Rommel Vargas and Rocio Morales, for their invaluable help in this research.

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