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Preparation of a Slow Release Biofertilizer From a Polymeric Urea-Formaldehyde Matrix (PUFM)

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Abstract: Fertilizers industry faces the challenge of improving the efficiency of its products either by optimizing the fertilizers in use or by developing new types of them. During the last decade, controlled and slow release technologies have become more important. These technologies aim to increase the efficiency of the applied substance by increasing its action over time and avoiding losses of all kinds (leaching, volatilization). The main purpose of the current study was to obtain a slow release biofertilizer by incorporating microalgae into a polymeric urea-formaldehyde matrix (PUFM). The quantitative analysis of macronutrients and micronutrients in the microalgae was determined using different techniques including titration, UV and Atomic Adsorption Spectroscopy. The matrix and the formulation obtained (PUFM + CHLO) were also characterized by Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). The "*in vitro*" study showed a typical slow release behavior of nitrogen (N), phosphorus (P) and potassium (K) macronutrients. It was also shown that (PUFM+CHLO) formulation has the slowest macronutrients release time with a maximum release of 28%, 26% y 46% for (N-P-K) macronutrients respectively during a period of 30 days. The *"in vivo"* study exposed the benefits of the biofertilizer formulation (PUFM + CHLO) from conventional commercial fertilizer (CF) (NPK-14-5-12). Due to the presence of nutrients of natural origin in microalgae, (PUFM + CHLO) shows ecological effects which could also developing sustainable agriculture systems.

Keywords: Slow-release fertilizer; microalgae; polymeric matrix; nutrients

1 Introduction

Fertilizers are bioactive agents mainly composed of nitrogen, phosphorus and potassium. Their application to crops improve production in quality and quantity [1]. Depending on the method of application and climatic conditions, around 90% of the applied fertilizer amount never reaches its objective. The above have contributed to a severe environmental contamination including degradation of soils, water sources, eutrophication of maritime ecosystems, development of photochemical smog, increase in the global concentration of the powerful greenhouse gas nitrous oxide, as well as acidification of soils and accumulation of heavy metals in plants [2-4]. In agreement with the disadvantages previously exposed, it is necessary developing more efficient fertilizers including environmental friendly characteristics [5-8].

The industry of fertilizers must increase the efficiency of its products, improving or developing new types of fertilizers to avoid or reduce environmental pollution. It is considered that an ideal fertilizer

should have at least three characteristics: a high percentage of recovery and production; a minimum negative impact on the environment and with a single application of the product should be enough to cover the nutritional requirements during the entire culture period [9-14]. According to these characteristics, Slow Release Fertilizers (SRF) have a relevance nowadays. The SRF improves the efficiency in terms of a better use of the nutrients, they are called smart fertilizers and considered as ideal fertilizers. The obtention of SRF constitutes a technology that meets the three characteristics of an ideal fertilizer. The use of SRF helps preventing soil degradation, can be used to reduce the amount of fertilizer that is applied and make the nutrients available for longer periods of time after application, since they avoid losses due to leaching and volatilization of the fertilizer [3,9-15].

In the SRF, nutrients are contained in a matrix, which prevents uncontrolled dissolution and dispersion of the fertilizer promoting the prolonged release of the nutrients. Currently, scientists are working on the design of controlled release fertilizer technologies in organic and inorganic matrix in response to the need of environmentally friendly fertilizers [4,9,10]. The principle is based on the coating of the nutrients by layers of biodegradable polymers that will allow their release in a controlled manner depending only on the temperature and humidity of the soil. A greater release will occur when these factors increase, which overlaps with the increase in the needs of the plants [10,16]. Additionally, the encapsulation of nutrients allows this type of fertilizer to be applied in a localized manner.

The urea-formaldehyde as the slow-release fertilizers, plays an important role, being the first product which the slow release of nitrogen in agriculture was studied [17]. Investigations developed by Gonzalez 2007 allowed to obtain a polymeric low molecular weight suspension based on urea-formaldehyde for the encapsulation of a conventional commercial fertilizer (CF) (NPK-14-5-12), converting it into a semipermeable material. This process does not present high complexity, and is a more economical method, due to its conditions of synthesis [18].

Microalgae are employed in agricultural systems as biofertilizers [19-22]. Recently, a consortium containing *Anabaena variabilis*, *Chlorella vulgaris* and *Azotobacter sp*, was found to improve germination and growth of rice plants and it was recommended as a biostimulator and a biofertilizer for crops also the growth of *Zea may* (maize) was improved with two strains of *Chlorella sp* [23-25]. This is because its high content of fiber, macro and micronutrients, amino acids, vitamins and plant phytohormones [26]. The incorporation of these nutrients of natural origin reinforce in the plants his resistance to diseases, to the environmental stress, activate his physiological functions, attaining crops healthier, with better nutrition and more vigorous [27-29].

In this project the main objective was to develop a biofertilizer by the incorporation of microalgae *Chlorella sp* (CHLO) and *Nannochloropsissp* (NANNO) in a polymeric urea-formaldehyde matrix (PUFM) using a microencapsulation method (extraction and evaporation of solvent). The biofertilizer obtained shows a slow release behavior in the *"in vitro"* study. The advantages of these biofertilizer respect to a conventional commercial fertilizer CF (NPK-14-5-12) were observed in the *"in vivo"* studies. The synthesized matrix (PUFM) in this paper aims to offer a method to obtaining coated materials with a low production cost that does not require a strict control of the reaction conditions. The obtained formulations (PUFM + CHLO) and (PUFM + NANNO) aim to feed and fortify the plants with their application. The presented procedure can be considered as an alternative to obtain universal formulations of biofertilizers and other materials could be considered. The use of biofertilizer, will replace the use of chemical inputs, thus favoring sustainable agriculture.

2 Materials and Methods

2.1 Determination of Microalgae Nutrients

2.1.1 Quantitative Analysis of Macronutrients (NPK)

These analyses were performed on the two varieties of microalgae studied. Atomic Adsorption Spectroscopy was used to determine potassium (K). The measurement was made at a wavelength of 766.5 nm, in a spectrometer of Atomic Absorption AA500 (PG Instruments) from Great Britain [30].

The determinations of the water-soluble phosphorus (P_2O_5) content were made by colorimetry at a wavelength of 450 nm in a UV/Visible spectrometer brand GENESYS 20 UV SPECTRONIC from the USA [31]. It was used a blank test solution and 1 or 2 cm quartz cuvettes of optical path.

The total nitrogen content (N_T) was determined by the Kjeldahl method with volumetric titration [32].

2.1.2 Quantitative Analysis of Micronutrients

Measurements were made on an AA Perkin-Elmer 2280by Atomic Adsorption Spectroscopy [33]. The experimental conditions are listed in Tab. 1.

Table 1: Experimental conditions fixed in the atomic adsorption spectroscopy measurement

Element	***Wavelength (nm)***
Zn	213.9
Cu	324.8
Ca	422.7
Mg	285.2
Na	589.6
Co	240.7
Fe	248.8
Power (kW)	1.2
Nebulizer flow (L/min)	1.2
Carrier gas	Acetylene
Auxiliary gas flow (L/min)	1.2
Observation height (mm)	1.5

2.2 Synthesis of PUFM and Microalgae Microencapsulation

The microencapsulation method of extraction and evaporation of solvent was used in the synthesis of the slow-release biofertilizers. The polycondensation reaction between urea and formaldehyde was carried out at room temperature. The stirring speed was 700 rpm for 40 min, until reaching a viscosity of 1.9 g/cm.s, which results in the formation of the matrix (PUFM). Then, is added "*in situ*" *Chlorella sp* (CHLO) or *Nannochloropsissp* (NANNO) microalgae the to be encapsulated, forming a stable polymer dispersion. The products obtained PUFM + CHLO and PUFM + NANNO were dried at room temperature. The matrix (PUFM) was synthesized using the same reaction conditions but without the addition of microalgae [18,34]. The experimental conditions set for the synthesis are shown in Tab. 2.

Table 2: Experimental conditions for the synthesis of slow release biofertilizer formulations

Formulation	***Urea (g)***	***Formaldehyde (g)***	***Microalgae(g)***
PUFM	24	34.5	-
PUFM + CHLO	24	34,5	5
PUFM + NANNO	24	34,5	5

2.3 Characterization of the Products and the PUFM Matrix

2.3.1 Fourier Transform Infrared Spectroscopy (FT-IR)

Infrared spectra were registered with a FT-IRTHERMO NICOLET Nexus-670. The sample (~ 5 mg) and KBr (~ 95 mg) were ground together in an agate mortar until the sample was dispersed. FTIR spectra were obtained in the wavenumber region between 500 cm^{-1} and 4000 cm^{-1}.

2.3.2 Scanning Electron Microscopy (SEM)

Morphology was analyzed by Scanning Electron Microscopy (SEM JEOL-5600 LV) at 20 kV Samples were coated with a layer of gold of approximately 20 nm using an EMS 550 sputter coating.

2.4 "In vitro" Studies of Macronutrients (NPK)

Nutrients release was monitored in an ideal medium (water). In three 500 mL Erlenmeyer flask with lid, 5 g of the PUFM loaded with the microalgae CHLO or NANNO. Conventional commercial fertilizer CF (NPK-14-5-12) was used as a witness or reference. Then, 500 mL of distilled water was added. The Erlenmeyer flask was placed in a shaker maintaining an agitation of 100 rpm during the entire test period (25-30 days). Aliquots of 5 mL of the aqueous phase were taken every 3 days. They were diluted with distilled water in a volumetric flask of 100 mL to determine the percentage of potassium oxide, total nitrogen and diphosphorus pentoxide in the solution. The aliquot volume extracted was replaced with distilled water each time the operation was performed to maintain "sink conditions" [10,46].

2.5 Statistical Studies of Release of K_2O of "in vitro" Studies

The data corresponding to the release of K_2O of each formulation obtained and the conventional commercial fertilizer, were compared by mean of analysis of variance using Statgraphics (Version 5 Plus). If the value of P is less than α (α = 0.05), it is considered statistically significant.

2.6 "In vivo" Studies

The tests were carried out at the Horticultural Research Institute "Liliana Dimitrova", in the cultivation of gladiolus (*gladiolus spp*. green variety). They were worked in a compacted red ferralitic soil with medium to high fertility. Two different treatments were evaluated, a conventional commercial fertilizer- CF (NPK-14-5-12) variant (T1) with two applications as recommended for this type of crop [35], as a witness. Second treatment was PUFM+ CHLO (T2) with one application during the vegetative cycle (60 days), at the rate of $40g/m^2$. During the development of the plantation, the following evaluations were made: number of campane (CN) by units, length of the spike (SL). (cm) and length of the floral stem. (FSL) (cm).

3 Results and Discussions

3.1 Characterization of Microalgae. Analysis of Macro and Microelements

Total nitrogen (N_T) takes values between 7 and 9%, the latter being the most relevant value, belonging to the *Chlorella sp* variety of microalgae that brings the higher amount of N_T (Tab. 3). The *Chlorella sp* can be expected considering its high content in proteins (55-67%) and aminoacids (19 of the 22 aminoacids, in which 8 are essential aminoacids) [36]. Nitrogen is an important element for plants in the formation of chlorophyll, nucleic acid and enzyme which directly influences the development of quality and yields of plants. In addition, *Chlorella sp* also presents a higher content of the macronutrients P_2O_5 and K_2O with respect to *Nannochloropsis sp*. This is very important since these macro nutrients directly participate in the vegetative development, fruit formation and agricultural yield.

Table 3: Composition of macro and microelements of microalgae

Elements	*CHLO (%)*	*NANNO (%)*
Zn	2.7	5.8
Cu	3.7	6.2
Ca	3.2	1.2
Mg	2.2	1.8
Na	5.8	1.8
Co	2.7	4.1
Fe	1.3	2.01
N_T	9.70	7.13
P_2O_5	2.09	1.08
K_2O	1.10	0.92

The results of the microelements are also shown in Tab. 3. The presence of these microelements helps the plants in their resistance to pests and diseases. The microelements analyzed in the two varieties of microalgae such as: Cu, Ca, Na, Co, Zn, Fe and Mg, have considerable amounts that allow to naturally enrich the formulations of slow-release biofertilizers obtained and to nourish the crops where they will be applied. The microelements Fe, Mg and Zn intercede in the formation of chlorophyll and in the morphology of the cellular structure of plants. Co is essential for photosynthesis, it forms part of the enzymes responsible for the synthesis of proteins in plants [37,38]. This is the reason of our marked interest in incorporating them in the formulations of slow release biofertilizers. In addition, the incorporation of these microalgae may reduce the use of chemical fertilizers, contributing to a reduction in environmental pollution.

Microalgae also contain growth promoting substances called phytohormones, which together with macro and microelements, improve the availability of nutrients, during the vegetative cycle of the crop. This favors not only the higher productivity of crops, but the synthesis of nutritional and functional substances of interest for the care and improvement of consumer health [27,28]. Besides, the incorporation of these microalgae may reduce the use of chemical fertilizers, contributing to a reduction in environmental pollution among other benefits.

3.2 Synthesis of PUFM and Microalgae Microencapsulation

The urea-formaldehyde matrix was obtained by a non-linear polycondensation reaction, where the monomers have complementary functional groups that react in three successive steps, giving firstly terminal methylol groups and producing condensations of these to originate ethers groups, which eventually lead to branched and reticulated products. Fig. 1 presents the general reaction reported in the literature, based on which the synthesis of the (UF) matrix should proceed [18].

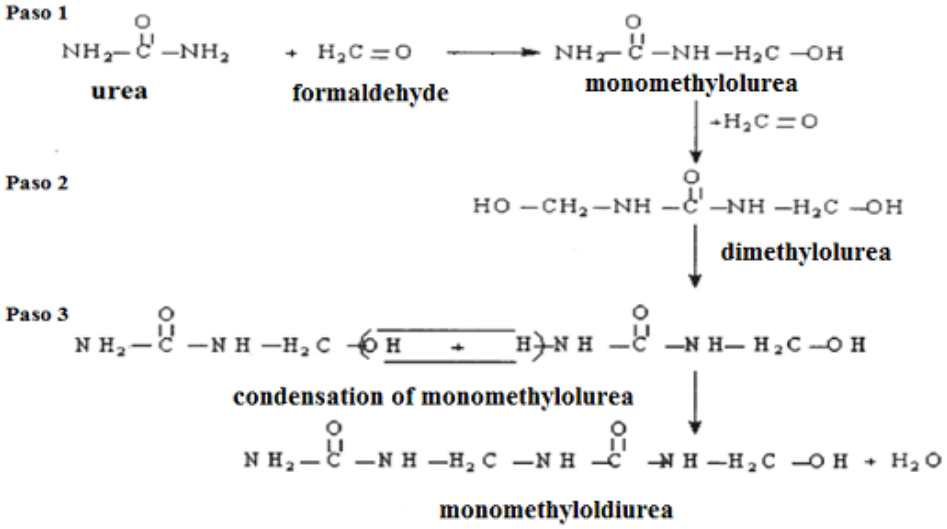

Figure 1: Scheme of the reported chemical reaction [39]

The development of a polymer of short or long chains depends on the conditions that are established for its synthesis, such as pH, temperature, reaction time, urea/formaldehyde ratio, the dilution and catalyst addition. In this work, the synthesis conditions allow obtaining a stable polymer suspension in which the level of crosslinking is low. In this way, the polymer maintains enough hydrophilicity to produce a stable dispersion and also allows to modulate the release of the occluded material. These characteristics are required for a matrix whose purpose is the encapsulation of products.

For the development of our product was necessary to decrease the urea/formaldehyde molar ratio (UF) for the formation of the microencapsulation matrix like the reported matrix. This could be due to interactions between the microalgae and urea functional groups during the reaction, which will compete with the polycondensation reaction that gives rise to the matrix, causing andecrease in the urea necessary amount to form the reported polymer suspension [18]. Figs. 2 and 3 show the formulations obtained.

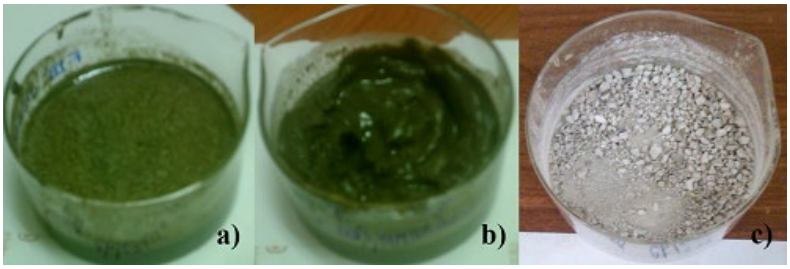

Figure 2: Formulation PUFM + CHLO, a) at the end of the synthesis, b) after two hours, c) once the solvent was completely evaporated

Figure 3: Formulation PUFM + NANNO, a) at the end of the synthesis, b) after two hours, c) once the solvent was completely evaporated

3.3 Physical-Chemical Characterization of the Product

3.3.1 Fourier Transform Infrared Spectroscopy (FT-IR.

The FT-IR spectra of *Chlorella sp* (CHLO), (PUFM), and (PUFM + CHLO) are presented in Fig. 4 respectively.

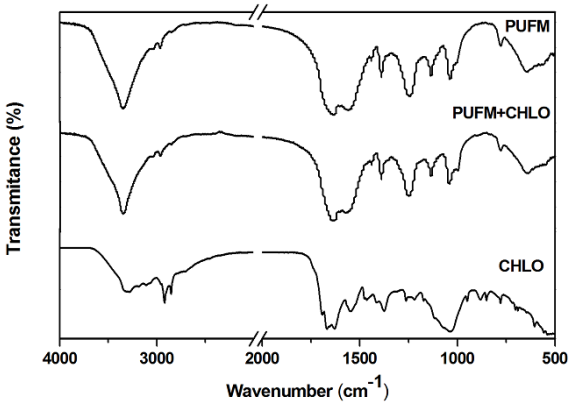

Figure 4: Infrared Spectra (FTIR) of the matrix PUFM, PUFM + CHLO formulation, *Chlorella sp* microalgae (CHLO)

For *Chlorella sp*, at 3277 cm^{-1}, the bands of -CH stretching of the primary and secondary amides are presented. The presence of lipids is observed at 2854-2921 cm^{-1}, while at 1628 cm^{-1}, carboxyl groups can be observed from the amine bending bands of the peptides that surround the proteins. This band is strongly marked in *Chlorella sp* by its high protein content. At 1060 cm^{-1} the bands of C-O, C-C, C-O-C corresponding to the polysaccharides present in the biomass of *Chlorella sp* [40-43]. In the case of PUFM, the bands of the NH and C=O stretching appear as expected [44] at 3330 and 1627 cm^{-1}, respectively. The bands observed in the spectrum corresponding to PUFM + CHLO fairly matched those in UF. This FTIR spectrum keeps some features of its components; however, it was difficult to detect any signal suggesting any CHLO- PUFM interaction. This result could be related to the fact that this system is a multicomponent material. Fig. 4 spectra also shows no displacement of their main bands (the bands of the NH and C=O stretching appear as expected [38] at 3330 and 1627 cm^{-1}, respectively). This behavior indicates the non-formation of new strong bonds. According to that, interactions between (PUMF) and (CHLO) could be considered weak as hydrogen bonds or Van der Waals interactions. The above helps the release of the microencapsulated active ingredient (CHLO) from the matrix (PUMF).

3.3.2 Scanning Electron Microscopy (SEM)

In Fig. 5 it is shown the morphology of the surfaces of the matrix (PUFM), the *Chlorella sp* microalgae (CHLO) and PUFM microparticles loaded with *Chlorella sp*, obtained by SEM analysis.

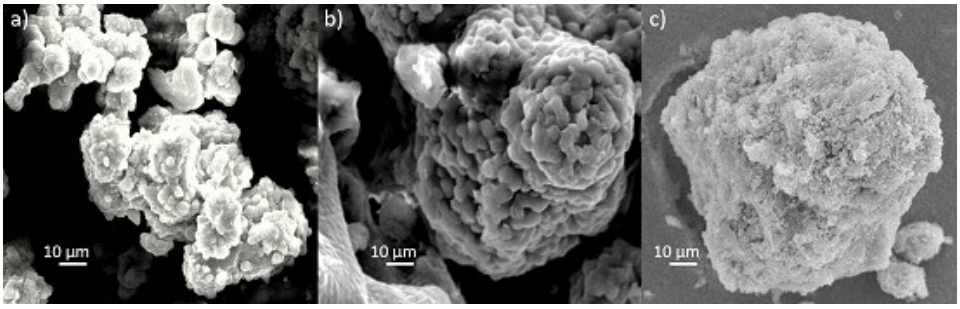

Figure 5: Scanning electron micrographs (SEM) of the: a) matrix PUFM, b) *Chlorella sp* microalgae, c) obtained formulation PUFM + CHLO

In all the samples, pellets with hemispherical tendency, irregular and heterogeneous are observed, with the aggregate formation. In the formulation PUFM + CHLO obtained it is observed that the agglomerates have approximately smaller particle sizes of the order of 4 µm (micrometric). This morphology could contribute to the exit of the nutrients to the dissolution medium. All of which is corroborated in the*"in vitro"* study.

3.4 "In vitro" studies

Fig. 6 shows the release profiles of the macronutrients (NPK) for two formulations obtained: PUFM + CHLO, PUFM + NANNO and the conventional commercial fertilizer CF (NPK 14-5-11).

From Fig. 6 it can be seen the release percentage of each macronutrient in the formulations obtained PUFM + NANNO and PUFM + CHLO. Potassium (46-50%) and Nitrogen (28-48%) have higher percentages of releases than Phosphorus (26-32%). A reason could be the solubility in water of the macronutrients, being potassium, the most soluble macronutrient followed by nitrogen and phosphorus. In the case of conventional commercial fertilizer (NPK-14-5-12), without encapsulating, it releases approximately a 20-30% from the first day the macronutrients. This accelerated release is precisely the fundamental cause of the environmental contamination of fertilizers.

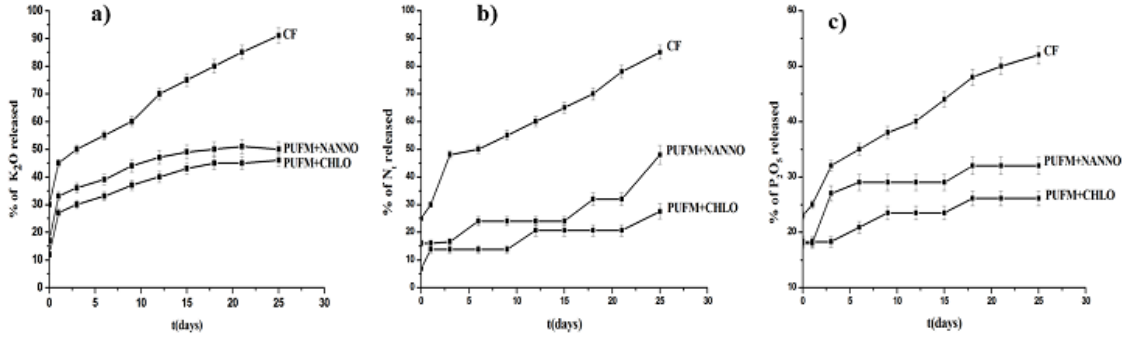

Figure 6: Comparison in PUFM + CHL, PUFM + NANNO, CF (NPK 14-5-11) of the a) % of K_2O release, b) % of N_t and c) % of P_2O_5

With these "*in vitro*" studies, it is proven that the proposed synthesis methodology is valid to microencapsulate microalgae and obtain a slow release biofertilizer. It was observed in all cases that the percent total nitrogen (N_T), phosphorus (P_2O_5) and potassium (K_2O) released is lower for the two obtained formulations PUFM + CHLO and PUFM + NANNO showing a slow release behavior in comparison with conventional commercial fertilizer CF (NPK-14-5-12). This could be explain considering the matrix PUFM as a physical barrier that prevents the immediate release of these nutrients to the solution, demonstrating the effectiveness of the coating obtained.

The PUFM + CHLO sample was the one that showed a better slow release behavior. After 15 days, the content of the nutrients released reaches a plateau, keeping the release until the 30 day. In these types of coated biofertilizers, the nutrients are available to the plant during a longer period, which causes their slower assimilation and avoids the possible losses, giving the plant more time to assimilate them. All of this causes a decrease in the number of applications of nutrients in the field. It represents a positive effect on the cost (less number of applications) and also environmental benefits.

3.5 Statistical Studies of Release of % K_2O of "in vitro" Studies

The comparison of variances (σ^2) was performed to the percentage of potassium released (K_2O) obtained from the studies "*in vitro*", the conventional commercial fertilizer CF (NPK-14-5-12) and PUFM + CHLO and PUFM + NANNO. The results showed homogeneity of the variances (σ^2) for 95% confidence. Potassium (K_2O) was selected for this study because it is the most soluble macronutrient contained in the formulations obtained.

Considering the previous results we proceeded to the determination and comparison of the means of the samples. In Table 4 tabulates the means (X) corresponding to the obtained formulations PUFM + CHLO and PUFM + NANNO and the conventional commercial fertilizer CF (NPK-14-5-12), in relation with the percentage of potassium (K_2O) released in the study "*in vitro*".

Table 4: Mean values of percentage of potassium (K_2O) released in the study "*in vitro*"

Formulations	**% of K_2O released**
	$X \pm \Delta X$
PUFM + CHLO	38 a ± 5
PUFM + NANNO	44 a ± 6
CF (NPK-14-5-12)	68 b ± 9

where $X \pm \Delta X$ is the means and confidence intervals. Different letters in the same column denote significant differences at the 95% confidence level.

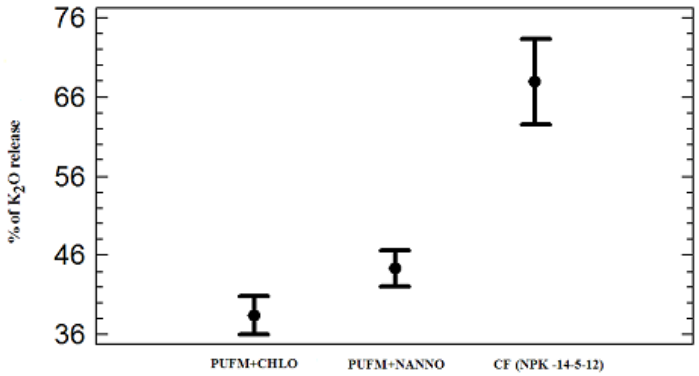

Figure 7: Graphic of means for % nutrient K_2O release of the formulations PUFM + NANNO and PUFM + CHLO, CF (NPK-14-5-12)

Fig. 7 shows that the % macronutrient K_2O release of the formulations (PUFM + NANNO and PUFM + CHLO are statistically significant differences at the 95% confidence level regarding commercial fertilizer CF (NPK-14-5-12). The formulation PUFM + CHLO releases the nutrient K_2O slower than PUFM + NANNO. The formulation PUFM + CHLO retains more nutrients for a longer period of time. It can produce a slower assimilation of the nutrients for the plant, decreasing the possible losses of solubility, the number of applications of the product and a positive economic and environmental effect, according to [45].

3.6 Studies "in vivo" of Formulation PUFM + CHLO

Fig. 8 shows the results of "*in vivo*" study for PUFM + CHLO. In the case of agronomic studies, the formulation PUFM + CHLO was selected because it has the slowest macronutrients release in the "*in vitro*" studies. A small increase in the values of the agronomic parameters (length of the spike (SL) by cm and length of the floral stem (FSL) by cm) is observed for the application of the treatment PUFM + CHLO regarding the conventional commercial fertilizer (CF) (NPK-14-5-12) in the cultivation of the gladiolus. For the parameter number of campane (CN) by units, the value obtained for the PUFM + CHLO is slightly lower than the (CF) (NPK-14-5-12). Despite these results, is very important to take into account that formulation PUFM + CHLO was applied only once during the vegetative cycle (60 days), while the (CF) (NPK-14-5-12) was applied twice during this period.

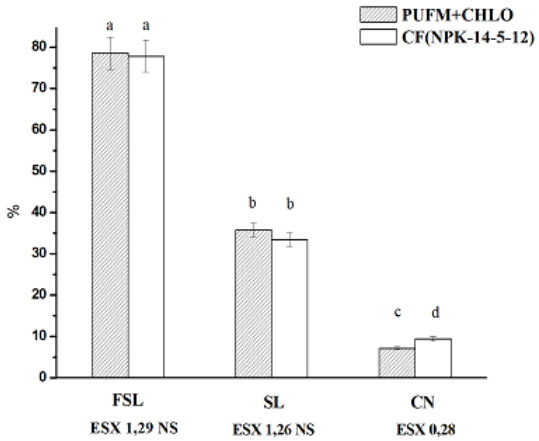

Figure 8: Agronomic evaluation of PUFM+ CHLO, CF (NPK 14-5-12). Error bars display 95% confidence intervals. Different letters display significant differences between treatments at the 95% confidence level

The slow release formulations obtained offer some benefits regarding (CF) (NPK-14-5-12). The PUFM + CHLO supplies nutrients slowly to plants, increased the efficiency of these. All this results in a decrease in the number of applications with a possible positive economic result, by reducing the labour costs necessary for the application of the product.

With the application of PUFM + CHLO, possible losses due to leaching, volatilization and the excess of chemical products that pollute the environment are avoided. Besides, with this synthesis is possible to take advantage of a natural origin product (microalgae) that offers ecological benefits compared to conventional chemical fertilizers.

These results demonstrate the efficiency of the use of PUFM + CHLO, requiring only one application during the crop cycle. Unlike the treatment (CF) (NPK-14-5-12) that needs to be applied twice for the cycle of the cultivation of the gladiolus. The formulation PUFM + CHLO could be an alternative to the use of chemical fertilizers NPK and for environmental sustainability.

4 Conclusions

Quantitative analysis of macro and micronutrients were performed to *Chlorella sp* (CHLO) and *Nannochloropsis sp* (NANNO) microalgae varieties, which showed a high content of nutrients of natural origin to be used as biofertilizer. The microencapsulation and evaporation of solvent method used for the synthesis of PUFM + CHLO and PUFM + NANO formulations, which was a suitable technique for obtaining a slow release biofertilizer. Weak interactions between PUFM matrix and microalgae CHLO and high heterogeneity and irregularity of the surface in the formulation PUFM+CHLO contribute to the release nutrients to the environment. Both formulations, PUFM+CHLO and PUFM + NANNO, release nutrients more slowly than that with CF (NPK-14-5-12). There are statistically significant differences between the release of K_2O from our formulations and that with CF (NPK-14-5-12). The PUFM + CHLO formulation has a slower release behavior. In the studies carried out in the cultivation of gladiolus, there are no appreciable differences between the agronomic parameters evaluated for the treatments PUFM+CHLO and CF (NPK-14-5-12). Nevertheless the formulation PUFM + CHLO contributes to improve the quality of the crops, with a smaller number of applications in the field and environmental benefits. This study showed that with a single application of PUFM + CHLO, agronomic parameters similar to CF (NPK-14-5-12) were obtained, which is of considerable environmental relevance.

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