

INFLUENCE OF PARTIAL BIOFERTILIZATION WITH *AZOLLA FILICULOIDES* ON THE CULTIVATION OF TWO CULTIVARS OF GARLIC IN MONOCULTURE AND INTERCROPPING CONDITIONS

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Abstract. This study evaluated the effects of partial biofertilization with *Azolla filiculoides* Lam. on growth, photosynthetic rate, bulb quality, and yield in *Allium sativum* L. cultivars ‘Chinese’ and ‘Napuri’ in Punta de Bombón, Arequipa, Peru (2018-2019). The research involved two experiments: one under monoculture with partial biofertilization + 1/2NPK (PBM) and conventional NPK fertilization (CFM), and the other in intercropping with maize (*Zea mays*), using both partial biofertilization + 1/2NPK (PBI) and conventional fertilization (CFI). Results showed that partial biofertilization outperformed conventional fertilization in monoculture settings. The ‘Chinese’ cultivar exhibited superior root, leaf, and bulb growth under partial fertilization, whereas ‘Napuri’ excelled in bulb quality components like dry weight, diameter, and clove number. Photosynthesis peaked at 82 days after sowing (DAS) for ‘Chinese’ and 112 DAS for ‘Napuri’. In terms of yield, ‘Chinese’ outperformed ‘Napuri’ in both monoculture and intercropping, with the highest yield recorded in 2019 (12641 kg/ha for ‘Chinese’ and 9597 kg/ha for ‘Napuri’). These findings suggest that combining chemical and organic fertilizers, along with crop diversification, can sustain high yields while being environmentally friendly.

Keywords: agricultural sustainability, fertilizer reduction, growth, yield, *Allium sativum*

Introduction

Garlic (*Allium sativum* L.) holds significant importance for Perú, and its cultivation is viable throughout the year, ranging from sea level to 3000 m above sea level (masl). Peru boasts five cultivars of garlic, namely ‘Morado’, ‘Napuri’, ‘Massone’, ‘Barranquino’, and ‘Chinese.’ According to the Ministry of Agriculture and Irrigation (MINAGRI) report of 2020, Peru stands among the top 20 global garlic producers and holds the primary producer and exporter position of garlic in the Andean Community, with an annual production of approximately 113 thousand tons. Notably, the Arequipa region alone contributes 75% of the total garlic production in Peru (MIDAGRI, 2018).

The Arequipa region holds a prominent position in garlic production, owing to the high quality and yield of the crop that ranges from 10-20 t/ha. The months of September through December are of utmost importance for garlic production in this region, as reported by the Ministry of Agriculture and Irrigation (MINAGRI) in 2020. Within this region, the provinces of Arequipa and Islay serve as significant production points, situated at elevations of 2,300 and 9 masl, respectively. The Arequipa regional management report of 2018, indicates that the province of Arequipa has achieved a yield of 18.2 t/ha, while that of Islay was 11 t/ha. In the region of Arequipa, predominantly, cultivars ‘Chinese’ and ‘Napuri’ are planted. ‘Chinese’ garlic is favored

for its larger bulb size and higher yields, making it a popular choice among farmers. On the other hand, ‘Napuri’ garlic, although slightly smaller, boasts greater pungency, a characteristic highly desirable in culinary applications.

Despite being important for Peru’s economy, garlic cultivation in the Arequipa region is mainly conducted using monoculture systems. Moreover, due to its poor root system, garlic requires a significant amount of nitrogenous fertilizers, which can lead to soil degradation, contamination of groundwater, and reduced profitability, thereby increasing production costs. To address these issues, sustainable agriculture practices are essential, including the implementation of environmentally friendly management techniques that reduce the use of chemical fertilizers and agrototoxic substances, while improving the quality and yield of crops. Such practices align with the principles of sustainable agriculture (Altieri, 2009; Diacono and Montemurro, 2015; Bharali et al., 2017).

In contrast, intercropping has been shown to potentially result in higher yields compared to monoculture, as well as other benefits such as reducing pest damage, increasing the microbial diversity of the substrate, improving soil health, and using nutrients more effectively (Mohsen et al., 2021). The practice of intercropping is well-documented in South America and Peru (Benzing, 2001; Sauca and Urabayan, 2005; Liebman and Davis, 2009). Previous studies on intercropping with grasses and legumes have reported increased yields and profitability of the main crop (Ruiz-González and Victorino-Ramírez, 2015; Gómez et al., 2014; Chai et al., 2021). Another method is protective association, in which a protective crop is grown alongside the main crop to provide shade (Sauca and Urabayan, 2005). For the genus *Allium*, it has been observed that shading associated crops sown after the main crop favors growth and bulb formation (Brewster, 2008). Additionally, intercropping can create niches for pest control predators, reducing the need for agrochemicals (Sauca and Urabayan, 2005; Asociación ETC Andes, 2007; Chai et al., 2021; Li et al., 2023). Therefore, the objective of this research was to investigate the effects of partial biofertilization with *A. filiculoides*, under monoculture and intercropping conditions, on two garlic cultivars (‘Chinese’ and ‘Napuri’) during the years 2018 and 2019 in Punta de Bombón, Arequipa Region, Peru.

Material and methods

Experimental location

The experiment was conducted from 2018 to 2019 in the town of Punta de Bombón, Arequipa region, Peru (17°10’ S and 71°48’ W) at 9 masl (Fig. 1). The soil type was loam (38% sand, 26.4% clay and 35.6% silt) and represented a typical cropping soil of Punta de Bombón. Soil samples were collected at 0–0.3 m before the sowing, and at the end of the experiments. The samples were analyzed using standard methods (INIA, 2017) at the LAQ&S accredited Laboratory. The soil had a pH of 8.25, an electrical conductivity of 2.68 dS/m, CEC 19.8 mEq/100 g, and contained 2.87% organic matter, 0.83 mEq/100 g K, Na 0.54 mEq/100 g, Ca 15.32 mEq/100 g, Mg 3.11 mEq/100 g, usable P 11.68 ppm, and %N total 0.151. The soil was characterized as saline, poor in nitrogen and organic matter (INIA, 2017).

The site had a mean elevation of 9 m, and maximum and minimum temperature of 14.6°C and 21.6°C respectively, relative humidity of 83.5% and 0 mm precipitation for the year 2018. For the year 2019 the values were 16.7°C, 25.1°C, 76.8% and 0 mm

precipitation respectively. Meteorological data for the site were obtained from the Pampa Blanca Station in Punta de Bombón, which belongs to the National Meteorology and Hydrology Service (SENAMHI).



Figure 1. Experimental plots located in Punta de Bombon, Arequipa-Peru

Field experiments

The experiments were carried out in Punta de Bombon. The sowing dates were June 10, 2018, and May 20, 2019. The two garlic cultivars ('Chinese' and 'Napuri') were sown manually. The plot sizes were 100 m² with twelve furrows, each containing two 20-m-long rows. The plant density was 95 plants/m², and the crops were grown at a depth of 3-5 cm. The seeds were shelled from the bulb seven days before sowing, and the cloves had a size of 30-35 mm, which were used for planting. The irrigation system was based on gravity, and the crops were watered every 15 days, 2376 m³/ha were utilized for crop irrigation (Lipinski, 2015). The water quality was high in salts (RAS 5.18) with a classification of C4S2, characterized as saline (INIA, 2017).

Fertilization application was done in banding (Burba, 2003; Saluzzo, 2003). Two experiments were carried out for both cultivars. The first under monoculture conditions, the following treatments were applied: CFM: Conventional fertilization with N, P, K (522-276-180 Urea formaldehyde, dubbed UF); and partial biofertilization PBM: 1/2 N, P, K (261-138-90 UF) + 2 t/ha *Azolla*. The second, under intercropping conditions, CFI: N, P, K and PBI: 1/2 N, P, K + 2 t/ha *Azolla*, both treatments in intercropping with maize cv. PVM 580 (*opaco mal paso*) sown at 60 DDS of garlic. The maize was sown in furrows (12 furrows/plot), each containing four 20 m long rows, and 60 cm distance between plant and plant.

For both experiments nitrogen was applied in three parts: 196 UF at 22 days after sowing (DAS), 163 UF at 48 DAS, and 163 UF at 85 DAS, respectively. Phosphorus was applied in two parts, 138 UF at 22 DAS and the other 138 UF at 48 DAS. Potassium was applied in the background, 175 UF at 22 DAS.

The chemical composition of *Azolla* was N (4.16%), P (199.73 ppm), K (1.41 ppm), Ca (0.35%), Mg (0.61%), S (0.38%), Fe (258 ppm), Cu (25 ppm), Mn (533 ppm), Zn (49 ppm), Bo (84.56 ppm), C (37.07%), and the C/N ratio (8.91).

For post-emergence weed control, a combination of GOAL (a.i. Oxyfluorfen) at a rate of 50 cc per backpack with AGIL (a.i. Oxyfluorfen) at a rate of 15 cc per backpack was used. In addition, the fungicide AMISTAR (a.i. Azoxystrobin) was applied at a rate of 20 cc per backpack, and the insecticide VERTICAL (a.i. Tebuconazole) was applied at a rate of 200 cc per backpack. The nematicide DK-DATE (a.i. Oxamyl) was also applied at a rate of 200 cc per backpack. These treatments were alternated during crop growth. Weeding was manually performed four times throughout cultivation.

Response variables

Biomass

The research methodology employed in the study conducted by Di Benedetto and Tognetti (2016) was adhered to. In order to ensure a representative sample, five plants were selected at random for each treatment. Subsequently, every individual plant underwent a thorough washing process using distilled water, after which it was carefully dissected into distinct parts, namely roots, leaves, and bulbs.

The root sampling was conducted through careful excavation, approximately 10-12 cm around the root growth zone and reaching a depth of 20-30 cm, in accordance with the plant's phenological age (Fig. 2). Efforts were made to avoid damaging or breaking the roots during this process. Subsequently, the soil was meticulously removed to leave a smooth surface that facilitated root extraction. The root was delicately extracted, gently shaken to remove excess soil, and rinsed 2-3 times in water-filled containers. After allowing the water to drain, the roots were placed in properly labeled paper bags and transported to the laboratory for further processing.

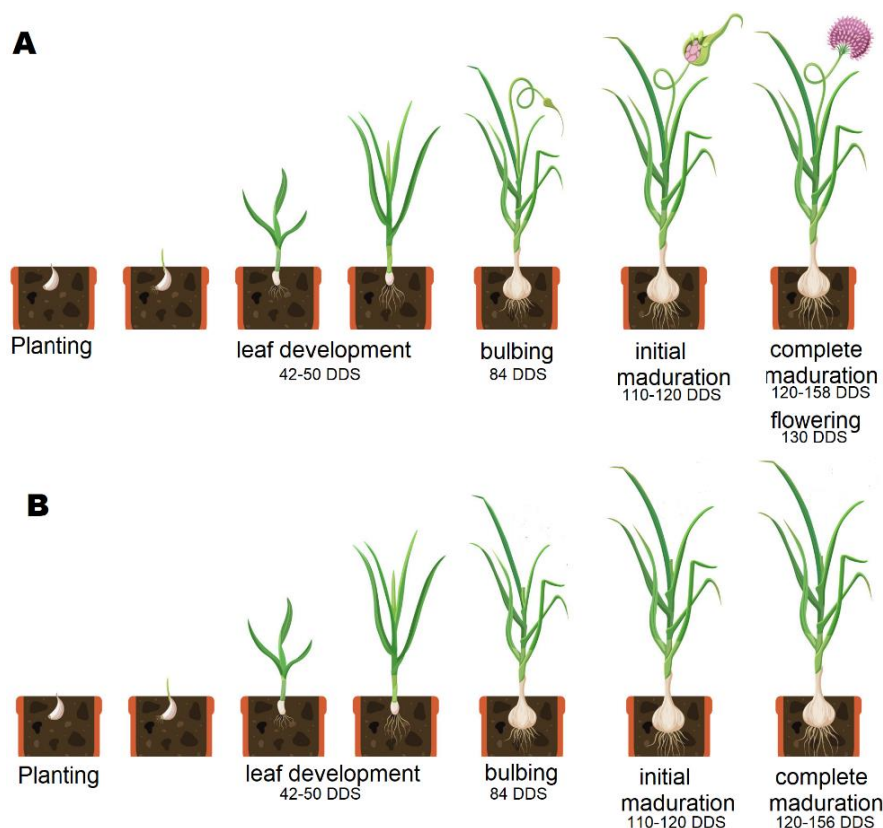


Figure 2. Phenological stages of the garlic used; (a) cv. 'Chinese'; (b) cv. 'Napuri'

The fresh weight of each respective component was meticulously measured and recorded. The measurements were taken at 47, 82, and 112 DAS for 2018 and at 28, 49, 83, 120, and 155 DAS for 2019 corresponding to the phenological stages shown in Figure 2.

Photosynthetic activity rate

The methodology described by Bharali et al. (2017) was followed to measure photosynthesis. An LI-6800 infrared gas analyzer (LI-COR, Inc. Lincoln, NE, USA) was used for the measurements. The measurements were taken at 27, 47, 82, and 112 DAS for 2018, and at 28, 49, 83, 120, and 155 DAS for 2019, between 11:00 AM and 12:00 PM.

The youngest mature leaf was selected as the focal point for measuring photosynthesis, adhering to the guidelines provided by Brewster (2008). A total of 30 plants were deliberately chosen as representatives for the photosynthesis measurements.

Yield and quality

In 2018 the experimental units were harvested at 155 DAS, and in 2019 between 156 and 158 DAS. The final yield (Kg/ha) was calculated using the equation 1.

$$\frac{\text{Kg}}{\text{ha}} = \frac{\text{Weight of total bulbs per treatment}}{\text{cultivation area}} \quad (\text{Eq.1})$$

The bulbs were categorized based on their equatorial diameter into two distinct groups: the “Extra” category, consisting of bulbs with a minimum diameter exceeding 45 mm, and the “Category I-II” group, encompassing bulbs with a minimum diameter below 30 mm. The evaluation of sample quality was conducted in accordance with the Quality Standards for Foreign Trade of garlic, initially established by CEPE in 1953 and subsequently modified by the Ministry of Agriculture of Spain in 1980 (CEPE, 1997). Essential parameters such as bulb weight, diameter, and the number of cloves were meticulously assessed as part of the quality evaluation process.

Statistical analyses

A factorial arrangement repeated measures analysis (ANOVA) was conducted to examine the effects of fertilization type and cultivar on both growth and photosynthetic rate. Following this, in cases where statistically significant differences were observed, the Duncan specificity test ($p < 0.05$) was employed to identify significant distinctions. Data analysis was carried out using the Statistica v.10.0 software package, while graphical representations were generated using the Sigma Plot v.11.0 program.

Results

The phenological cycle of the garlic crop in 2018 and 2019 had a duration of 151 days for cv. ‘Napuri’ and 158 days for cv. ‘Chino’ under monoculture and intercropping conditions, both crops reached full maturity. However, it is worth noting that the cv. ‘Napuri’ is a cultivar that does not produce flowers, regardless of the duration of cultivation

Under monoculture conditions

Biomass

Statistically significant differences ($p < 0.05$) (Table 1) were identified in the accumulation of root biomass between the two cultivars, as depicted in Figure 3A and B. These differences were observed when comparing conventional fertilization methods with partial biofertilization using *Azolla*. In 2018, the highest degree of root development was noted at 82 DAS for all plants. Notably, the cultivars ‘Chinese’ and ‘Napuri,’ which were biofertilized with *Azolla*, demonstrated notably greater root development (2.74 ± 0.68 g and 1.77 ± 0.44 g, respectively) in comparison to plants treated with conventional fertilization (0.85 ± 0.25 g and 0.51 ± 0.13 g, respectively). Furthermore, during 2019 the peak of root development occurred at 120 DAS. Within the cultivars, the biofertilized ‘Chinese’ variety exhibited the highest root biomass (1.61 ± 0.38 g), while the conventionally fertilized plants of the same cultivar displayed the lowest root development (0.48 ± 0.11 g).

Table 1. ANOVA table for root, bulb and leaf growth under monoculture conditions

ANOVA	Root growth		Bulb growth		Leaf growth	
Source of variation	2018	2019	2018	2019	2018	2019
Cultivar (CV)	*	*	*	*	*	*
Fertilization (F)	*	*	*	*	*	*
CV $\times$ F	N.S.	*	*	*	N.S.	*
R	*	*	*	*	*	*
R $\times$ CV	*	*	*	*	*	*
R $\times$ F	*	*	*	*	N.S.	*
R $\times$ CV $\times$ F	N.S.	*	N.S.	*	N.S.	*

*Significance, N.S.: No significance

The results indicate statistically significant differences ($p < 0.05$) (Table 1) in bulb biomass accumulation between cultivars and fertilization types, as depicted in Figure 3C and D. The peak of bulb development occurred at 112 DAS in 2018 and at 155 DAS in 2019. In 2018, the biofertilized cultivar ‘Napuri’ demonstrated the highest level of bulb development (2.34 ± 0.87 g) in comparison to the conventionally fertilized plants of the same cultivar, which exhibited the lowest biomass (0.99 ± 0.35 g). Moreover, during 2019, the overall bulb development was lower than that of 2018. Among the cultivars, the biofertilized ‘Napuri’ exhibited the highest bulb biomass (0.90 ± 0.26 g), while the biofertilized ‘Chinese’ cultivar displayed the lowest level of bulb development (0.63 ± 0.15 g).

Significant differences ($p < 0.05$) (Table 1) were observed in leaf biomass accumulation between cultivars and fertilization types (Fig. 3E, F). The highest biomass accumulation in 2018 was observed at 112 DAS, while in 2019, it was observed at 120 DAS, with a subsequent decline. Finally, in 2018, both cultivars (‘Chinese’ 2.53 ± 0.69 g and ‘Napuri’ 1.56 ± 0.27 g) exhibited the highest biomass accumulation when biofertilized, demonstrating statistically significant differences compared to conventionally fertilized plants.

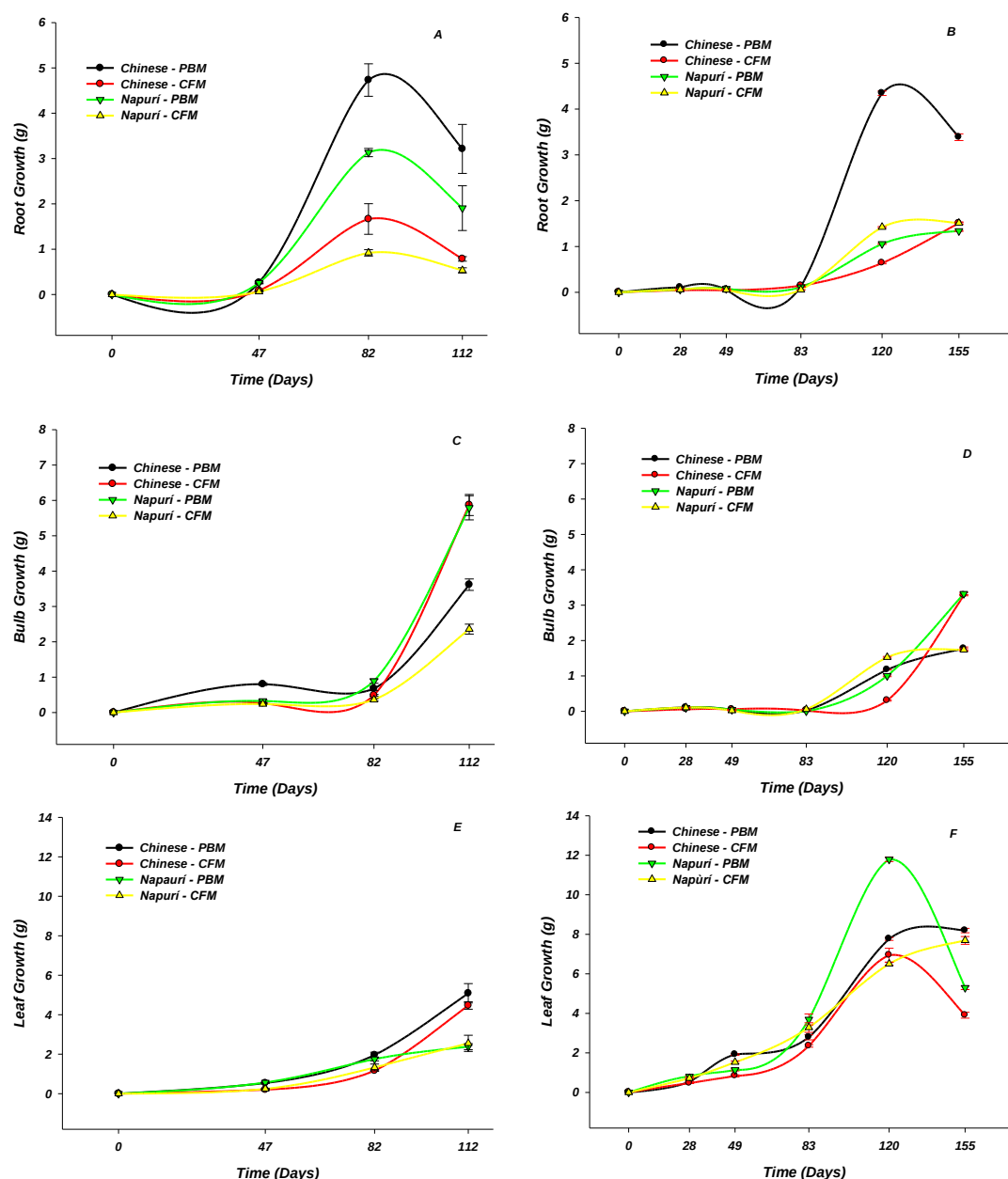


Figure 3. Growth of garlic cv. 'Chinese' and 'Napuri' under conventional fertilization (CFM) and partial biofertilization with *A. filiculoides* (PBM) during the 2018 (left) and 2019 (right) growing seasons. (A) and (B) Root growth. (C) and (D) Stem growth. (E) and (F) Leaf growth. The values correspond to the average $\pm$ the standard error

Photosynthetic activity rate

In 2018, no statistically significant differences were observed in the photosynthetic rate (Fig. 4A; Table 2). However, in 2019, the net photosynthetic rate of garlic plants was significantly influenced ($p < 0.05$) by both the cultivar and the type of fertilization (Fig. 4B; Table 2)

Among the cultivars, cv. 'Napuri' demonstrated the highest photosynthetic rate ($31.2 \pm 0.50 \mu\text{mol}/\text{m}^2/\text{s}^{-1}$) when subjected to conventional fertilization, while cv. 'Chinese' exhibited the lowest photosynthetic rate ($29.5 \pm 0.54 \mu\text{mol}/\text{m}^2/\text{s}^{-1}$) under the

same fertilization method. Additionally, the highest photosynthetic rates for all treatments were recorded during the complete maturation stage, which occurred at 155 DDS.

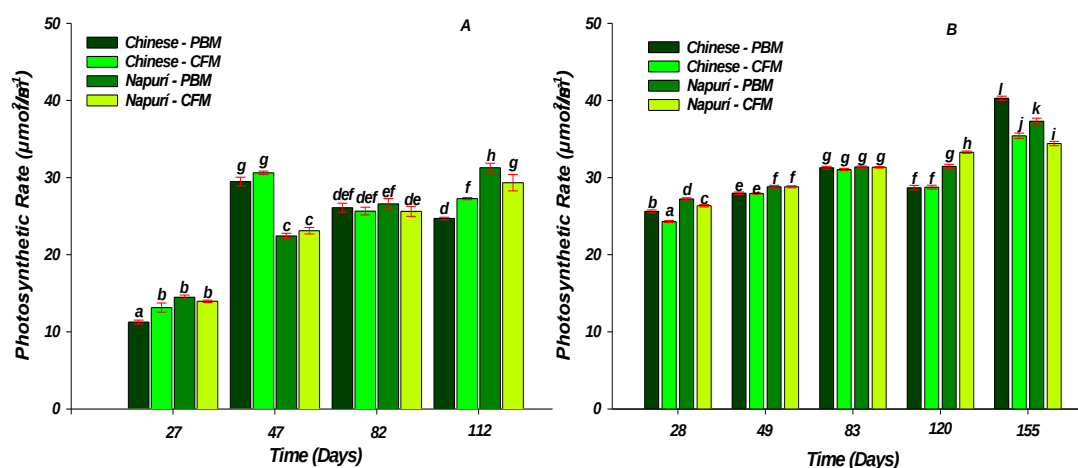


Figure 4. Photosynthetic rate of garlic cv. 'Chinese' and 'Napuri' under conventional fertilization (CFM) and partial biofertilization (PBM) with *A. filiculoides*. Photosynthetic rate during (A) the 2018 season and (B) 2019 season. The values correspond to the average $\pm$ the standard error. Values with the same letter do not present significant statistical differences

Table 2. ANOVA table for photosynthetic rate under monoculture conditions

ANOVA	Photosynthetic rate	
Source of variation	2018	2019
Cultivar (CV)	N.S.	*
Fertilization (F)	N.S.	*
CV $\times$ F	*	*
R	*	*
R $\times$ CV	*	*
R $\times$ F	N.S.	*
R $\times$ CV $\times$ F	*	*

*Significance, N.S.: No significance

Yield and quality

The findings from the assessments of yield, diameter, fresh weight of the bulbs, and the number of cloves per bulb, focusing on the category of garlic classified as extra (>45 mm), are presented in *Tables 3* and *4* and *Figure 5*. The data reveals that the yield of the 'Chinese' cultivar surpassed that of the 'Napuri' cultivar (*Table 3*). Furthermore, the implementation of partial biofertilization using *Azolla* resulted in elevated yields for all cultivars in both seasons (*Table 3*).

The fresh weight of the bulbs was found to be significantly influenced ($p < 0.05$) by both the cultivar and the type of fertilization, as depicted in *Figure 5A* and *B*. In 2018, the biofertilized cv. 'Napuri' exhibited the highest bulb weight (50.7 ± 2.0 g), while the biofertilized cv. 'Chinese' displayed the lowest bulb weight (39.2 ± 0.8 g). However, it is noteworthy that the cv. 'Chinese' did not exhibit significant differences between the two types of fertilization.

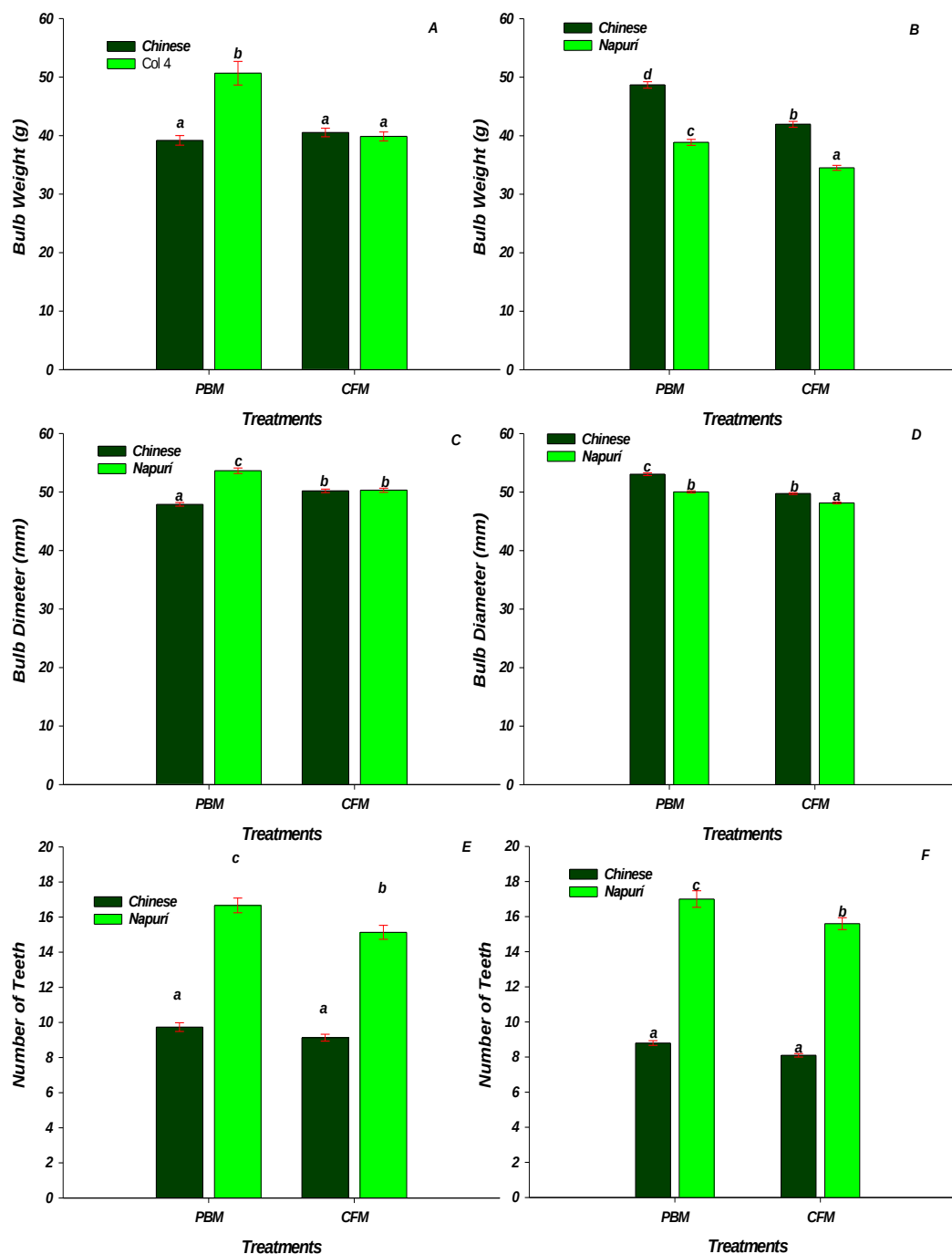


Figure 5. Quality of garlic bulbs cv. 'Chinese' and 'Napuri' during 2018 and 2019 seasons. (A) and (B) Fresh weight of the bulb. (C) and (D) Bulb diameter. (E) and (F) Number of cloves per bulb. The values correspond to the average $\pm$ the standard error. Values with the same letter do not present significant statistical differences

The diameter of the bulbs exhibited significant differences between the cultivars in 2018, as shown in Figure 5C. The biofertilized cv. 'Napuri' demonstrated the largest bulb diameter (53.6 ± 0.5 mm), while the cv. 'Chinese' exhibited the smallest diameter (47.9 ± 0.3 mm) when subjected to biofertilization. In 2019, the diameter of the bulbs was significantly influenced by both the cultivar and the type of fertilization, as

depicted in *Figure 5D*. The implementation of biofertilization resulted in an increased bulb diameter for both cultivars.

Table 3. Yield of garlic cv. ‘Chinese’ and ‘Napuri’ during the 2018 and 2019 seasons under conventional fertilization (IF) and partial biofertilization with *A. filiculoides*

Cultivar	Treatment	Bulb yield (t/he)	
		2018	2019
‘Chinese’	PBM	6605 ± 0.12 d	9749 ± 0.22 d
	CFM	4261 ± 0.92 c	7647 ± 0.73 b
‘Napuri’	PBM	3730 ± 0.77 b	8947 ± 0.69 c
	CFM	3221 ± 0.47 a	6382 ± 0.24 a
ANOVA		Bulb yield	
Source of variation		2018	2019
Weight (W)		*	*
Fertilization (F)		*	*
W × F		*	*

The values correspond to the average ± the standard error. Values with the same letter do not present significant statistical differences. ANOVA, *Significance, N.S.: No significance

The number of cloves exhibited a significant influence ($p < 0.05$) (*Table 4*) from both the cultivar and the type of fertilization, as illustrated in *Figure 5E* and *F*. In both 2018 and 2019, cv. ‘Napuri’ displayed a greater number of cloves compared to cv. ‘Chinese’. Notably, the biofertilized cv. ‘Napuri’ showcased the highest number of cloves (16.6 ± 0.4 cloves in 2018 and 17.0 ± 0.5 cloves in 2019), while the conventionally fertilized cultivar ‘Chinese’ demonstrated the lowest number of cloves (9.1 ± 0.2 cloves in 2018 and 8.1 ± 0.1 cloves in 2019).

Table 4. ANOVA table for bulb weight, bulb diameter and number of cloves under monoculture conditions

ANOVA	Bulb weight		Bulb diameter		Number of cloves	
Source of variation	2018	2019	2018	2019	2018	2019
Cultivar (CV)	*	*	*	*	*	*
Fertilization (F)	*	*	N.S.	*	*	*
CV × F	N.S.	*	*	*	N.S.	N.S.

*Significance, N.S.: No significance

Under intercropping conditions

Biomass

Significant differences ($p < 0.05$) (*Table 5*) were observed in root biomass accumulation between cultivars and types of fertilization, as depicted in *Figure 6A* and *B*. In 2018, both cultivars exhibited greater biomass accumulation when subjected to biofertilization, with the highest value observed in the biofertilized cv. ‘Chinese’ (2.11 ± 0.59 g) and the lowest in the conventionally fertilized cv. ‘Napuri’ (0.67 ± 0.22 g).

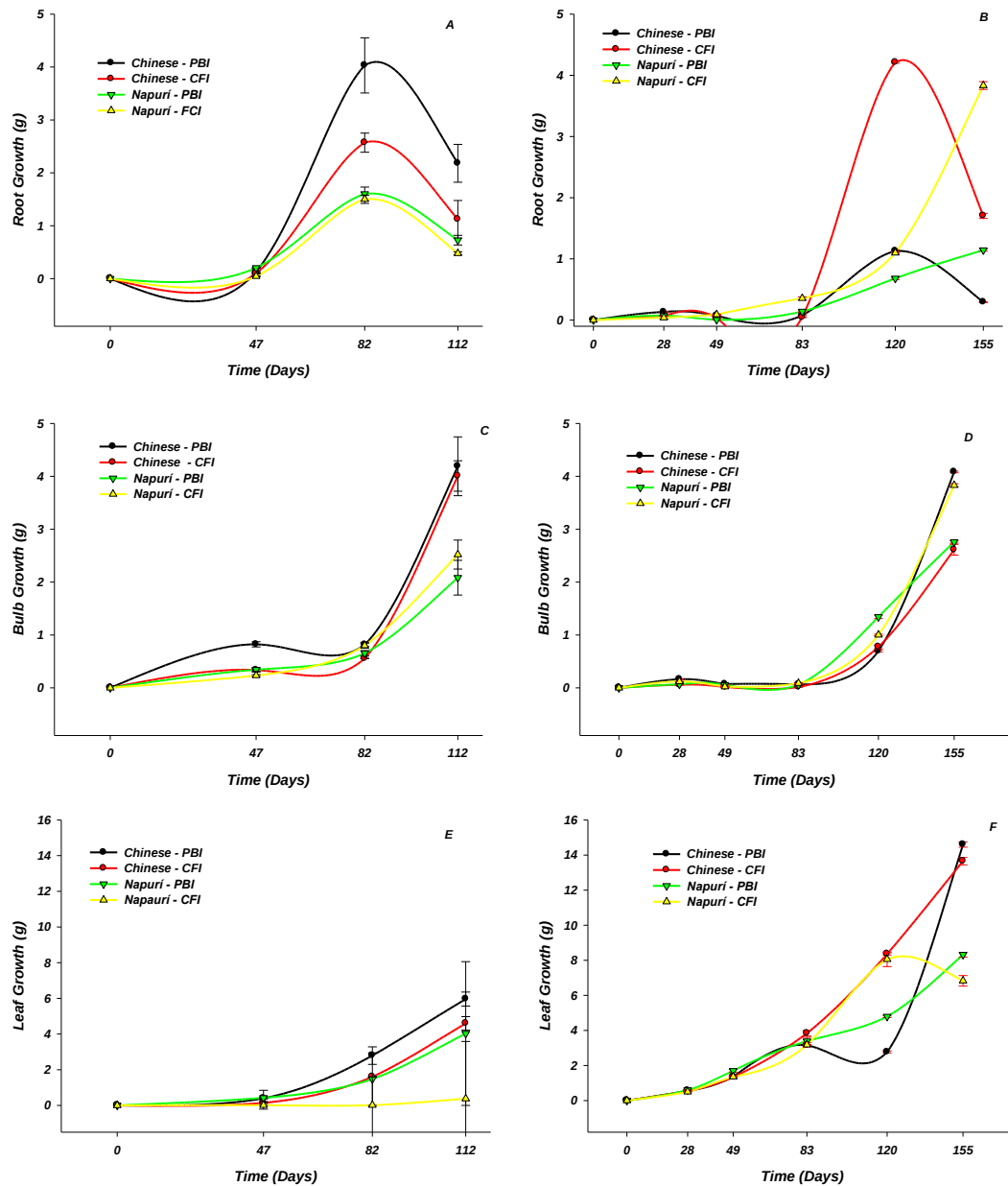


Figure 6. Growth of garlic cv. ‘Chinese’ and ‘Napuri’ intercropping with maize under conventional fertilization (CFI) and partial biofertilization with *A. filiculoides* (PBI) during 2018 (left) and 2019 (right) growing seasons. (A) and (B) Root growth. (C) and (D) Stem growth. (E) and (F) Leaf growth. The values correspond to the average $\pm$ the standard error

Significant differences ($p < 0.05$) (Table 5) were found in the biomass of bulbs between cultivars for the 2018 season, as shown in Figure 6C. However, no statistically significant differences were observed between the types of fertilization. The highest biomass accumulations were recorded in the biofertilized cv. ‘Chinese’ (1.94 ± 0.58 g), followed by the conventionally fertilized cv. ‘Chinese’ (1.63 ± 0.60 g). For the 2019 season, significant differences ($p < 0.05$) were found in both cultivars and types of fertilization, as displayed in Figure 6D. The highest biomass accumulation was obtained

with the biofertilized cv. ‘Napuri’ (0.90 ± 0.26 g), while the lowest value was observed in the biofertilized cv. ‘Chinese’ (0.63 ± 0.15 g).

Regarding leaf biomass, significant differences ($p < 0.05$) (Table 5) were identified between cultivars and types of fertilization, as illustrated in Figure 6E and F. In 2018, the highest biomass accumulation was observed in the biofertilized cv. ‘Chinese’ (3.05 ± 0.83 g). In 2019, both conventionally fertilized cultivars displayed a higher fresh weight than their biofertilized counterparts, with the conventionally fertilized cv. ‘Chinese’ achieving the highest value (0.55 ± 0.10 g).

Table 5. ANOVA table for root, bulb and leaf growth under intercropping conditions

ANOVA	Root growth		Bulb growth		Leaf growth	
Source of variation	2018	2019	2018	2019	2018	2019
Cultivar (CV)	*	*	*	*	*	*
Fertilization (F)	*	*	N.S.	*	*	*
CV $\times$ F	N.S.	*	N.S.	*	N.S.	*
R	*	*	*	*	*	*
R $\times$ CV	*	*	*	*	*	*
R $\times$ F	*	*	N.S.	*	N.S.	*
R $\times$ CV $\times$ F	*	*	N.S.	*	N.S.	*

*Significance, N.S.: No significance

Photosynthetic activity rate

During the 2018 season, significant differences ($p < 0.05$) (Table 6) were observed in the photosynthetic rate, which were influenced by the type of fertilization. In both cultivars, the highest photosynthetic rate was achieved when subjected to biofertilization, in comparison to their conventionally fertilized counterparts. Notably, the biofertilized cv. ‘Chinese’ exhibited the highest photosynthetic rate (24.09 ± 2.02 $\mu\text{mol}/\text{m}^2/\text{s}^{-1}$), while the conventionally fertilized cv. ‘Napuri’ displayed the lowest rate (20.50 ± 1.36 $\mu\text{mol}/\text{m}^2/\text{s}^{-1}$).

Table 6. ANOVA table for photosynthetic rate under intercropping conditions

ANOVA	Photosynthetic rate	
Source of variation	2018	2019
Cultivar (CV)	N.S.	*
Fertilization (F)	*	*
CV $\times$ F	N.S.	N.S.
R	*	*
R $\times$ CV	*	*
R $\times$ F	*	*
R $\times$ CV $\times$ F	*	N.S.

*Significance, N.S.: No significance

Significant differences ($p < 0.05$) (Table 6) were observed in the net photosynthetic rate of garlic plants during the 2019 season, influenced by the cultivar and type of

fertilization (Fig. 7). The biofertilized cultivars exhibited higher rates of photosynthesis compared to their conventionally fertilized counterparts. Among the cultivars, biofertilized cv. ‘Napuri’ demonstrated the highest net photosynthetic rate ($32.34 \pm 0.89 \mu\text{mol m}^{-2} \text{s}^{-1}$), while conventionally fertilized cv. ‘Chinese’ had the lowest rate ($30.90 \pm 0.53 \mu\text{mol m}^{-2} \text{s}^{-1}$). Additionally, the highest photosynthetic rates for all treatments were recorded during the complete maturation stage, which occurred at 155 DDS.

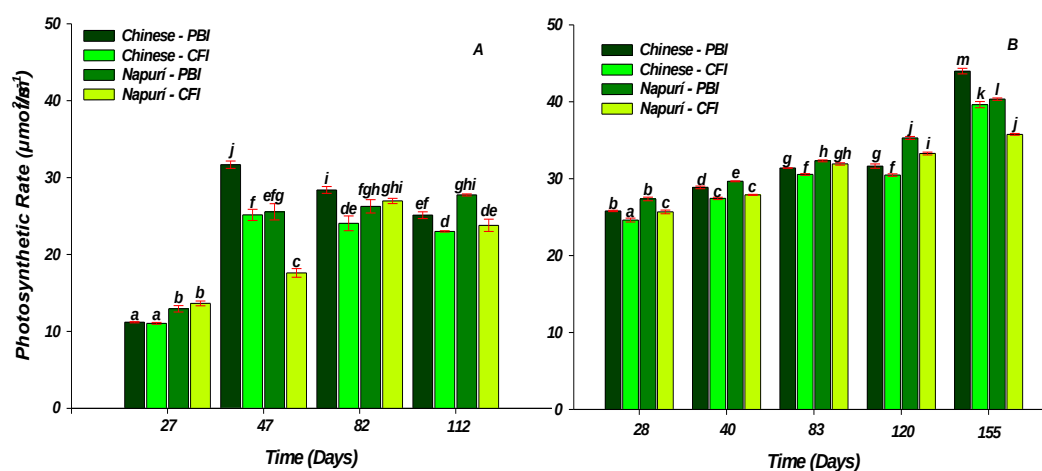


Figure 7. Photosynthetic rate of garlic cv. ‘Chinese’ and ‘Napuri’ in intercropping with maize. Under conventional fertilization (CFI) and partial biofertilization (PBI) with *A. filiculoides*. Photosynthetic rate during (A) the 2018 season and (B) 2019 season. The values correspond to the average $\pm$ the standard error. Values with the same letter do not present significant statistical differences

Yield and quality

The results of the evaluations pertaining to the yield, quality, and fresh weight (Tables 7 and 8; Fig. 8) of the garlic classified in the extra category (>45 mm) are presented. It is evident that the yields of the cultivar ‘Chinese’ were superior to those of the cultivar ‘Napuri’ (Table 7). However, it is noteworthy that crops intercropped with maize and subjected to partial biofertilization using *Azolla* displayed higher yields compared to their conventionally fertilized counterparts in both seasons (Table 7).

The weight of the bulbs exhibited significant differences ($p < 0.05$) (Table 8) influenced by both the cultivar and the type of fertilization (Fig. 8A, B). In the 2018 season, it was observed that both cultivars displayed higher bulb weights when subjected to biofertilization compared to their conventionally fertilized counterparts. Particularly, the biofertilized cv. ‘Napuri’ attained the highest weight (44.2 ± 1.0 g), while the conventionally fertilized cv. ‘Chinese’ exhibited the smallest weight (36.8 ± 0.8 g). In the subsequent season of 2019, it was observed that the biofertilized cv. ‘Chinese’ achieved the highest bulb weight (60.4 ± 0.6 g), whereas the biofertilized cv. ‘Napuri’ displayed the lowest weight (36.7 ± 0.3 g).

The diameter of the bulbs displayed significant differences ($p < 0.05$) (Table 8) influenced by both the cultivar and the type of fertilization (Fig. 8C, D). In the 2018 season, it was observed that both biofertilized cultivars exhibited larger bulb diameters compared to their conventionally fertilized counterparts. Notably, the biofertilized cv.

‘Napuri’ showcased the largest diameter (51.6 ± 0.4 mm), while the conventionally fertilized cv. ‘Chinese’ displayed the smallest diameter (49.0 ± 0.3 mm). Similarly, in the subsequent season of 2019, the biofertilized cv. ‘Chinese’ manifested a larger diameter (57.8 ± 0.3 mm), while the conventionally fertilized cv. ‘Napuri’ exhibited the smallest diameter (50.3 ± 0.3 mm).

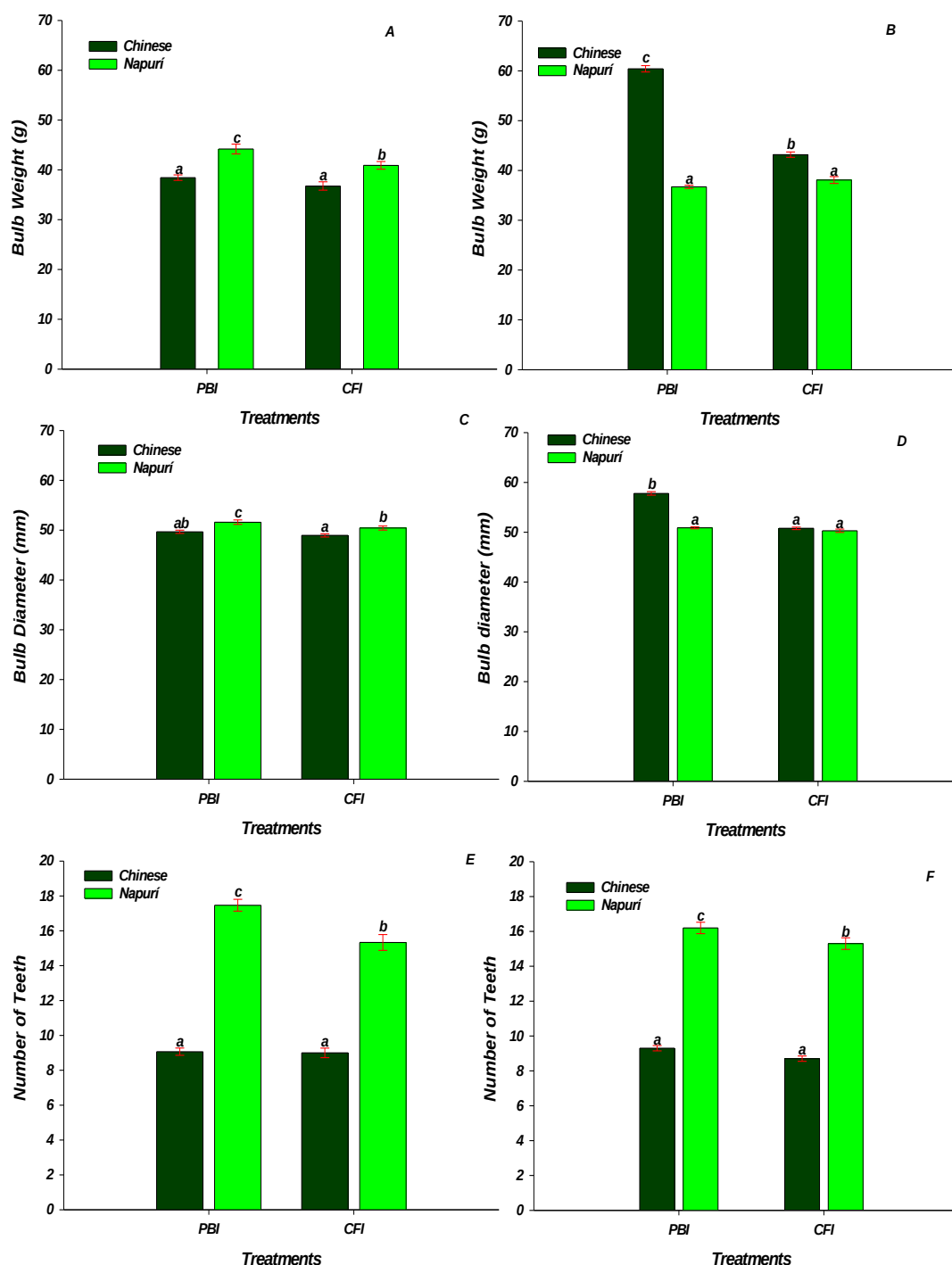


Figure 8. Quality of garlic bulbs cv. ‘Chinese’ and ‘Napuri’ during the 2018 and 2019 seasons were grown in intercropping with maize. (A) and (B) Fresh weight of the bulb. (C) and (D) Bulb diameter. (E and (F) Number of cloves per bulb. The values correspond to the average $\pm$ the standard error. Values with the same letter do not present significant statistical differences

Table 7. Yield of garlic cv. ‘Chinese’ and ‘Napuri’ during the 2018 and 2019 seasons, grown in intercrop with maize, under conventional fertilization (CFI) and partial biofertilization with *A. filiculoides* (PBI) conditions

Cultivar	Treatment	Bulb yield (t/he)	
		2018	2019
‘Chinese’	PBI	8352 ± 0.15 c	12641 ± 0.20 d
	CFI	4652 ± 0.88 b	8997 ± 0.11 b
‘Napuri’	PBI	3770 ± 0.79 a	9597 ± 0.28 c
	CFI	3616 ± 0.75 a	8365 ± 0.55 a
ANOVA		Bulb yield	
Source of variation		2018	2019
Weight (W)		*	*
Fertilization (F)		*	*
W × F		N.S.	*

The values correspond to the average ± the standard error. Values with the same letter do not present significant statistical differences. ANOVA, *Significance, N.S.: No significance

The number of cloves per bulb was significantly affected ($p < 0.05$) (Table 8) by both the cultivar and the type of biofertilization (Fig. 8E, F). In both 2018 and 2019, the cv. ‘Napuri’ had a higher number of cloves compared to the cv. ‘Chinese’. Finally, the biofertilized cv. ‘Napuri’ had the highest number of cloves in both years (18.0 ± 0.3 cloves and 16.2 ± 0.3 cloves, respectively).

Table 8. ANOVA table for bulb weight, bulb diameter and number of cloves under intercropping conditions

ANOVA	Bulb weight		Bulb diameter		Number of cloves	
Source of variation	2018	2019	2018	2019	2018	2019
Cultivar (CV)	*	*	*	*	*	*
Fertilization (F)	*	*	*	*	*	*
CV × F	N.S.	*	N.S.	*	*	N.S.

*Significance, N.S.: No significance

Discussion

The findings of the study demonstrate that both the genotype and type of fertilization exerted influence over the growth, photosynthetic rate, quality, and yield of garlic. Specifically, the utilization of partial biofertilization yielded positive outcomes in terms of root and leaf development throughout the two growing seasons under monoculture conditions, in contrast to plants that were fertilized with inorganic methods. This outcome aligns with the findings reported by Islah (2010), whereby organic fertilization displayed a significant enhancement in the aboveground portion of garlic plants in comparison to the control group. Conversely, Hosna and Shadia (2015) observed no increase in leaf count among biofertilized garlic plants relative to the control group, albeit a significant elevation in chlorophyll content was noted.

Within this investigation, the cultivar ‘Napuri’ exhibited the highest photosynthetic rate, irrespective of whether it under conventional or biofertilization methods, a phenomenon potentially attributed to the fact that while both cultivars reach complete maturation at 155 DDS, cv. ‘Napuri’ is a non-flowering cultivar (Fig. 2). This characteristic of cv. ‘Napuri’ may influence the absence of solute translocation, thereby avoiding chlorophyll degradation, unlike the cv. ‘Chino’, which undergoes flowering. However, it became evident that the photosynthetic rate does not directly influence bulb development. Instead, growth is potentially more influenced by nutrient absorption than by photosynthesis. This fact could be attributed to the improvement of soil texture and the establishment of a robust root system resulting from the use of organic fertilizers, as indicated by previous studies conducted by Islah (2010) and Albano et al. (2023).

The yield of garlic crops is significantly influenced by the genotype, as evidenced by the data presented in Tables 3 and 7. Specifically, the cultivar ‘Chinese’ consistently achieved a higher yield compared to the cultivar ‘Napuri’ across both evaluated seasons. This consistent outcome suggests that the observed yield disparity could potentially be attributed to the higher fiber content present in the ‘Chinese’ cultivar. A parallel response was noted in onion cultivation, wherein the variation in fiber content among cultivars was linked to their inherent genetic variability, as reported by Petrovic et al. (2020).

Nevertheless, noteworthy disparities in yield between seasons were observed, suggesting the influence of various factors such as planting date and the prevalence of pests (including thrips, nematodes, and rust) that adversely impacted the growth and development of the crops, particularly during the 2018 season. However, despite these challenges, the application of biofertilization exhibited a positive effect on both cultivars throughout the two seasons, irrespective of whether they were grown under monoculture conditions or intercropped with maize.

The most substantial enhancements in yield were witnessed in monoculture settings, where the partially biofertilized cv. ‘Chinese’ (PBM) exhibited a remarkable increase of 55.1% compared to the control (CFM). This outcome aligns with the findings reported by Islah (2010), who conducted research on cv. ‘Chinese’ and observed yield increases of 12.3% and 13.8% during two seasons when biofertilizers were employed as compared to the control group receiving recommended chemical fertilizers. Similarly, Hosna and Shadia (2015) documented a significant surge in the yield of garlic bulbs from cv. ‘Chinese’ when biofertilizers were employed in comparison to the control group. These discoveries underscore the potential viability of biofertilizers as a strategic approach for enhancing garlic crop yield, particularly when faced with adverse conditions similar to those encountered in the 2018 season.

The quality of garlic holds significant importance in both its commercialization and consumption, with crucial determinants including bulb weight and diameter. Through careful observation in this study, it has been noted that garlic genotypes exert a substantial influence on these parameters. Specifically, the cultivar ‘Napuri’ exhibited the most considerable bulb diameter and weight, along with the highest number of cloves (Figs. 5 and 8).

Moreover, notable advancements were observed when the garlic underwent partial biofertilization, resulting in a substantial improvement in the aforementioned parameters. Supporting this finding, Islah (2010) documented a significant enhancement in both bulb diameter and fresh weight of ‘Chinese’ garlic cultivar when subjected to biofertilization in comparison to conventional fertilization methods. Similarly, Hosna and Shadia (2015) reported a noteworthy increase in bulb diameter, fresh weight, and dry weight of ‘Chinese’

garlic cultivar upon biofertilization, when contrasted with the control group. Consistently, Assefa et al. (2015) also confirmed a comparable response, demonstrating that the amalgamation of organic and inorganic fertilizers led to a significant rise in the number of cloves per garlic bulb across two growing seasons, in contrast to the control group.

In recent years, there has been a notable surge in scientific investigations pertaining to the utilization of *Azolla* as a biofertilizer. *Azolla* exhibits the capacity to supply crucial macro and micronutrients, vitamins, and growth regulators, while also demonstrating environmentally friendly attributes (Maham et al., 2019).

Within the context of rice cultivation, comprehensive evaluations have been conducted to assess the impact of *Azolla*, both in its pure form and when combined with urea. The results of these studies indicate a significantly higher yield (14%) when *Azolla* was employed in conjunction with urea, as opposed to the utilization of urea alone (8%). This substantial yield increase can be attributed to *Azolla*'s ability to enhance nitrogen (N) use efficiency and overall crop yield. Moreover, economic analyses have revealed that the implementation of *Azolla* treatments yields either comparable or higher net economic benefits in comparison to the exclusive use of urea. Consequently, these findings strongly imply that substituting 25% of urea-N with *Azolla* biofertilizer represents a financially advantageous approach for farmers (Yao et al., 2018).

Nitrogen plays a pivotal role in facilitating robust vegetative growth and optimal expansion of leaves in *Allium* crops, while also exerting influence on bulb size (Diriba-Shiferaw, 2016; Yao et al., 2018). Furthermore, research has demonstrated that the application of organic matter sources enhances the total nitrogen content and improves its absorption (Islah, 2010). In the present study, the enhanced development of leaves and bulbs can be attributed to the positive interaction between *Azolla* and microbes, which leads to increased nitrogen absorption and reduced nitrogen loss, thereby enhancing its availability (Yao et al., 2018).

Additionally, *Azolla* serves as a source of micronutrients such as boron (B) and zinc (Zn) (Table A1), both of which are vital for bulb development and yield. Zinc plays a crucial role in auxin metabolism and other enzymatic reactions that promote leaf elongation. Moreover, it exhibits a synergistic effect with nitrogen, stimulating plant growth and augmenting the percentage of dry matter, total soluble solids, and bulb acidity (Zaki et al., 2014; Assefa et al., 2015; Diriba-Shiferaw, 2016).

The growth responses, photosynthesis, fresh weight, bulb quality, and yield exhibited remarkable similarity under both monoculture and intercropping conditions, with the partial biofertilization treatments utilizing *Azolla* demonstrating the highest values. Although there are no significant statistical differences (data not shown) between crops under monoculture or intercropping conditions all treatments displayed higher yield values in intercropping setups compared to monoculture conditions, which can be attributed to intercropping's capacity to enhance nitrogen use efficiency and improve soil properties (Li et al., 2023).

Despite corn being a nitrogen-consuming crop, the growth of garlic thrived in the intercropping system. This phenomenon can be attributed to the advantages derived from intercropping two species with differing canopies and growth periods, resulting in improved light capture, enhanced ground cover, and reduced proportion of light reaching the ground. Consequently, this protective effect exerted on the smaller crop contributes to its favorable growth outcomes (Chai et al., 2021; Li et al., 2021). Hence, it is advisable to conduct further research using the most effective biofertilization treatments in both monoculture and intercropping systems, employing a larger number of replicates to adequately demonstrate these effects.

Finally, different trends were observed in various soil parameters (*Table A2*). pH values generally decreased in 2018 but showed a slight increase across all treatments in 2019. Electrical conductivity also decreased in both years, suggesting potential changes in soil composition. Organic matter (OM) levels decreased compared to the initial value, although treatments with biofertilizer showed an increase compared to conventional and intercropping treatments. Nitrogen (N) levels decreased overall, with higher decreases seen in biofertilizer treatments in 2018 and slightly higher decreases in conventional and intercropping treatments in 2019. Conversely, phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) levels tended to increase in all biofertilizer treatments. Regarding cation exchange capacity (CEC), levels of Ca, Mg, and Na increased, while K levels showed a decreasing trend across all treatments. These trends suggest the influence of biofertilizer treatments on soil composition and quality throughout the study period.

Conclusions

Based on the provided text, we can draw the following three conclusions:

Partial biofertilization utilizing *Azolla* showed positive effects on the growth, photosynthetic rate, quality, and yield of garlic. The cv. 'Chinese' exhibited the most significant response to biofertilization, resulting in a substantial increase in yield compared to the control group. This highlights the potential viability of biofertilizers, particularly *Azolla*, in enhancing garlic crop production.

The genotype of garlic and the type of fertilization employed significantly influence the growth, bulb quality, and yield of garlic. Different garlic cultivars exhibited variations in their response to biofertilization, with the cv. 'Napuri' showing larger bulb diameter and weight, as well as a greater number of cloves. This highlights the importance of selecting appropriate genotypes and utilizing suitable fertilization methods to optimize garlic production.

Intercropping garlic with maize can lead to improved growth and yield compared to monoculture conditions. The intercropping system offers advantages such as enhanced light capture, better ground cover, and reduced light reaching the ground, which contribute to favorable growth outcomes for garlic. This suggests that intercropping can be a beneficial approach for garlic cultivation, even when grown alongside nitrogen-consuming crops like maize.

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APPENDIX

Table A1. Chemical analysis of *Azolla filiculoides*

Element	Unit	Value
N	%	4.16
P	ppm o mg/kg	199.73
K	%	1.41
Ca	%	0.35
Mg	%	0.61
Fe	ppm o mg/Kg	258
Cu	ppm o mg/Kg	25
Mn	ppm o mg/Kg	533
Zn	ppm o mg/Kg	49
B	ppm o mg/Kg	84.56
S	%	0.38

Table A2. Physicochemical characterization of agricultural soil prepared at the beginning and end of the experiment

Punta de Bombón			Physical analysis				Chemical analysis					Available elements		Exchangeable cation exchange capacity				CEC	PES
			Sand %	Clay %	Silt %	Clase textural	CO3 Ca %	pH	E.C. mS/cm	O.M %	N %	P ppm	K ppm	Ca ⁺⁺ meq/100 g	Mg ⁺⁺ meq/100 g	K ⁺ meq/100 g	Na ⁺ meq/100 g	meq/100 g	%
Beginning 2018			38	26.4	35.6	Loamy	4.72	8.25	2.68	2.87	0.151	11.68	360	15.32	3.11	0.83	0.54	19.8	2.73
End 2018	Chino	PBM	28.4	30.2	43.5	Loamy	3.62	7.45	1.03	1.32	0.079	92.14	370	17.92	3.84	0.39	0.69	21.6	3.20
		CFM	24.8	31.2	41.6	Loamy	3.51	7.63	1.06	1.63	0.059	95.72	420	17.91	3.51	0.65	0.71	21.4	3.24
	Napuri	PBM	28.6	29.2	44	Loamy	3.62	7.52	1.05	1.33	0.089	92.58	380	17.62	3.36	0.58	0.73	25.8	2.91
		CFM	29.8	30.7	37	Loamy	3.61	7.45	1.03	1.31	0.073	92.75	392	17.93	3.52	0.81	0.68	20.6	3.19
	Chino	PBI	29.8	28.5	41.6	Loamy	3.7	7.61	1.05	1.35	0.071	93.89	380	17.98	3.45	0.43	0.77	22.9	3.08
		CFI	26.7	29.2	36	Loamy	3.72	7.11	1.04	1.41	0.069	89.85	380	17.85	3.31	0.75	0.65	22.18	3.11
	Napuri	PBI	30.8	28.8	41	Loamy	3.61	7.35	1.06	1.16	0.071	91.07	395	16.93	3.61	0.62	0.65	23.3	2.98
		CFI	28.2	30.4	40	Loamy	3.6	7.44	1.04	1.51	0.072	89.32	410	17.81	3.83	0.61	0.69	22.8	3.1

Beginning 2019			32.4	25.6	42	Loamy	3.96	8.07	1.57	0.98	0.055	23.63	480	16.78	3.38	1.19	0.45	21.8	1.97
End 2019	Chino	PBM	24.8	27.2	48	Loamy	4.69	8.59	0.43	1.88	0.097	29.74	540	13.96	2.84	1.39	0.41	18.6	2.20
		CFM	34.8	27.2	38	Loamy	3.96	8.66	0.46	1.79	0.095	25.72	420	12.44	2.51	1.05	0.4	16.4	2.44
	Napuri	PBM	38.8	27.2	34	Loamy	3.74	8.52	0.5	1.95	0.099	52.58	420	11.53	2.36	0.98	0.43	15.3	2.81
		CFM	34.8	27.2	38	Loamy	4.56	8.48	0.63	2.66	0.137	32.7	420	12.58	2.5	0.94	0.58	16.6	3.49
	Chino	PBI	30.8	25.2	44	Loamy	4.55	8.6	0.52	2.27	0.115	83.89	480	13.35	2.75	1.23	0.57	17.9	3.18
		CFI	36.8	27.2	36	Loamy	3.72	8.01	0.79	2.32	0.12	69.85	480	11.99	2.36	1.25	0.5	16.1	3.11
	Napuri	PBI	30.8	27.2	42	Loamy	5.06	8.54	0.68	2.16	0.112	31.04	480	12.95	2.6	1.2	0.55	17.3	3.18
		CFI	34.8	25.2	40	Loamy	4.27	8.54	0.48	3.05	0.155	37.32	420	11.87	2.38	1.06	0.49	15.8	3.10