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Original research article

Effect of a Bioproduct Based on *Tithonia diversifolia* and *Emilia coccinea* on the Growth and Protection of PIF Plantains in Nursery

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ABSTRACT

Plantain is a staple food that contributes to food security in sub-Saharan Africa. However, its production remains insufficient due to various constraints, including parasitic pressure from *Mycosphaerella fijiensis*. This study aims to evaluate the effect of a bioproduct based on *Tithonia diversifolia* and *Emilia coccinea* on the growth and protection of PIF plantains. Two bioproduct modalities (B1 and B2) were produced, and treated PIF plants were generated. Bioproduct stability, *in vitro* test against *Mycosphaerella fijiensis*, growth parameters, susceptibility to *Mycosphaerella fijiensis*, and biomarker accumulation on PIF plantain leaves were evaluated. Growth was monitored for six months, and the leaves aged three and six months were taken at different periods (BI, 3AI and 28AI). The results show that the bioproduct was very stable and should be stored at room temperature away from light. The *in vitro* confrontation test revealed a very significant ($P < 0.0001$) inhibitory effect of the bioproduct on *Mycosphaerella fijiensis* growth. It stimulated explant germination as well as production of a large number of seedlings with significant increase in diameter, height and leaf surface area by 78.05%, 47.42% and 246.90% respectively compared to untreated seedlings. In addition, seedlings of different ages treated with the bioproduct showed very low sensitivity to *Mycosphaerella fijiensis*, especially those aged six months. Bioproduct treatment also significantly improved the accumulation of defense biomarkers before and after inoculation, with a greater effect observed three days after inoculation. The use of the bioproduct B2 appears to be effective and eco-responsible, and could be an effective tool for improving plant quality.

Keywords: Biological product, *Mycosphaerella fijiensis*, PIF plantain, Growth and defense biomarkers.

RÉSUMÉ

Le plantain est un aliment de base qui contribue à la sécurité alimentaire en Afrique subsaharienne. Cependant, sa production reste insuffisante en raison de diverses contraintes, dont la pression parasitaire exercée par *Mycosphaerella fijiensis*. Cette étude vise à évaluer l'effet d'un bioproduit à base de *Tithonia diversifolia* et d'*Emilia coccinea* sur la croissance et la protection des plantains PIF. Deux modalités de bioproduits (B1 et B2) ont été produites et des plants PIF traités ont été générés. La stabilité du produit, le test *in vitro* contre *Mycosphaerella fijiensis*, les paramètres de croissance, la sensibilité à *Mycosphaerella fijiensis* et l'accumulation de biomarqueurs sur les feuilles de plantain PIF ont été évalués. La croissance a été suivie pendant six mois et les feuilles âgées de trois et six mois ont été prélevées à différentes périodes (BI, 3AI et 28AI). Les résultats montrent que le bioproduit est très stable et doit être stocké à température ambiante et à l'abri de la lumière. Le test de confrontation *in vitro* a révélé un effet inhibiteur très significatif ($P < 0,0001$) du bioproduit sur la croissance de *Mycosphaerella fijiensis*. Il a stimulé la germination des explants ainsi que la production d'un grand nombre de plantules avec une augmentation significative du diamètre, de la hauteur et de la surface foliaire de 78,05 %, 47,42 % et 246,90 % respectivement par rapport aux plantules non traitées. De plus, les semis de différents âges traités avec le bioproduit ont montré une très faible sensibilité à *Mycosphaerella fijiensis*, particulièrement ceux âgés de six mois. Le traitement au bioproduit a également amélioré de façon significative l'accumulation de biomarqueurs de défense avant et après le traitement au bioproduit. L'utilisation du bioproduit B2 semble être efficace, éco-responsable et pourrait être un outil efficace pour l'amélioration de la qualité des plantes.

Mots-clés : Produit biologique, *Mycosphaerella fijiensis*, plantain PIF, biomarqueurs de croissance et de défense.

1. INTRODUCTION

The banana tree is a monocotyledonous plant belonging to the Musaceae family. Originating from the hot and humid tropical forests of South-East Asia (Onautshu 2013), it is now cultivated in over 120 countries across tropical and intertropical zones covering more than 10 million hectares (Lassoudière 2007). Banana ranks first in global fruit production, with approximately 100 million tons produced annually (FAO 2024). It is rich in vitamins (A, B, and C) and mineral elements like magnesium, phosphorus, potassium, and calcium (Kwa *et al*. 2019). There are two main types: dessert bananas and cooking bananas, including plantains.

Global plantain production exceeds 30 million tons per year, with Cameroon producing around 5 million tons annually (FAO 2024). Plantains are a staple food in the intertropical forest zone, offering multiple processing forms (chips, fries, donuts, etc.) and modes of consumption (as both fruit and vegetables). Despite its potential, the banana-plantain tree remains a marginal crop (Ngo-Samnick 2011). Insufficient planting material, particularly high-quality material, hinders new plantation establishment.

The African Research Center on Banana and Plantains (CARBAP) developed a horticultural technique called "Plant from Stem Fragments" (PIF), enabling rapid multiplication (within 3-4 months) in nurseries, yielding 20 to 80 seedlings from a single sucker (Kwa *et al*. 2019). However, limitations include explant position, which affects plant vigor and low seedling yield due to contamination and acclimation (Ewané *et al*. 2019). In addition, persistent parasitic pressure in plantations reinforces the use of bad rejects, thus reducing the maximum operating duration (Ngo-Samnick 2011). All plant organs are sensitive to this parasitic pressure, leading to numerous diseases such as black Sigatoka.

Black Sigatoka is a foliar disease caused by the fungus *Mycosphaerella fijiensis* (*M. fijiensis*). It is the most destructive disease of the banana-plantain tree (Ewané *et al*. 2013). The plant is attacked at all stages of its growth, both in the nursery and in the field. Furthermore, to eliminate pathogens and boost production, chemical products such as fertilizers and pesticides are most often used. However, the repeated use of chemical inputs in agriculture has harmful effects on the environment and human health and often leads to the presence of pesticide residues in foods (UNCTAD 2016).

Therefore, using natural products for sustainable organic agriculture offers a promising alternative with reduced environmental and health impacts, while preserving natural biodiversity (Meshuneke *et al*. 2020). *Tithonia diversifolia* and *Emilia coccinea,* herbaceous plants from the Asteraceae family, are rich in mineral elements (N, P, K) and secondary metabolites likely to induce the synthesis of biomarkers involved in plant growth and defense.

Previous research has demonstrated the dual effect of clam shells and *T. diversifolia* in promoting growth and protecting PIF plantain plants against *M. fijiensis* in nurseries (Ewané *et al*. 2020a, Meshuneke *et al*. 2020, Tatsegouock *et al*. 2020). Moreover, the stimulatory effects of *Tithonia diversifolia* were recently highlighted as an excellent biostimulant, promoting both growth and protection of plantain vivoplants through biofertilization, biostimulation, and activation of plant defense mechanisms (Ewané *et al*. 2024). Also, the efficacy of aqueous extracts of *Emilia coccinea* has been clearly demonstrated in crop protection, particularly due to their significant activity against phytopathogenic pests (Martin *et al*. 2018). Thus, using a bioproduct based on *Tithonia diversifolia* and *Emilia coccinea* in nursery treatment could stimulate “PIF” banana plantain seedling growth and protection against biotic stress. Our study evaluates the effect of a bioproduct based on *T. diversifolia* and *E. coccinea* on PIF banana plantain seedling growth and susceptibility to *M. fijiensis*.

2. MATERIAL AND METHODS

2.1. Material

Banana-plantain shoots of the French variety were obtained from the Agricultural Research Institute for Development (IRAD) station in Nkolbisson, Yaoundé VII subdivision.

Tithonia diversifolia and *Emilia coccinea* used in the bioproduct formulation were collected around the Higher Teacher Training College of Yaoundé, Yaoundé III subdivision.

The *Mycosphaerella fijiensis* fungal strain was provided by the Phytopathology unit of the African Banana and Plantain Research Center (CARBAP), Njombé-Penja station, Littoral region.

White sawdust, fine sand, and black earth were collected in Yaoundé III subdivision. White sawdust served as a substrate for the germination of plantain explants, while a mixture of fine sand (1/3) and black earth (2/3) was used as a substrate for PIF plants under shade.

The chemical materials used were Terazeb (Mancozeb 80 WP), a broad-spectrum contact fungicide, and a chemical fertilizer N.P.K (20.10.10) as a positive control for plant protection and growth, respectively.

2.2. Experimental design

This study was conducted at the Plant Physiology and Biochemistry Laboratory of the Higher Teacher Training College of Yaoundé, located in an agro-ecological zone with bimodal rainfall. Explant germination was carried out in a semi-controlled greenhouse, and PIF plant acclimatization occurred under shade. The study consisted of two watering frequencies, each with four (4) treatments, in both greenhouse and shade conditions.

Two watering frequencies:

- once a week (WF1)
- twice a week (WF2)

Four treatments (two bioproduct modalities and two controls):

- B1: Bioproduct based on *T. diversifolia*
- B2: Bioproduct based on *T. diversifolia* and *E. coccinea*
- Ctrl+: Chemical fertilizer (for growth) or fungicide (for protection)
- Ctrl−: Water only

Each treatment, according to watering frequencies, was considered as an independent experimental unit. The PIF plants were generated from plantain explants, acclimatized under shade, and treated according to the watering schedule.

2.3. Bioproduct production and pH measurement

The bioproduct was produced using the protocol described by Tatsegouock *et al*. (2020). Briefly, leaves and stems of *T. diversifolia* and *E. coccinea* were weighed, placed in digesters adapted with aerators, and fermented for 20 days. The product was filtered, packaged, and two bioproduct modalities were obtained:

B1: bioproduct based on *T. diversifolia* (100%).

B2: bioproduct based on *T. diversifolia* (75%) and *E. coccinea* (25%).

pH measurements were taken monthly for the first two months, and again after four and eight months.

2.4. *In vitro* confrontation test between the bioproduct and the pathogenic strains *Mycosphaerella fijiensis*

The *in vitro* confrontation test was carried out using B1 and B2 bioproducts. The effect on *M. fijiensis* growth was assessed by culturing the pathogen on PDA medium enriched with 5% bioproduct. The control media consisted of autoclaved PDA and distilled water (5%), and autoclaved PDA and Terazeb (5%), for the negative and positive controls, respectively. A 5 mm^2 mycelial disc from the fungal strain was placed in the center of each Petri dish. Plates were incubated at room temperature under white light. Mycelial growth was evaluated every 3 days for 15 days using the following formula:

Mycelial growth (mm^2) = Mean radial growth at time T - Initial mean radial growth

2.5. Evaluation of bioproduct effect on plantain PIF seedling growth

Growth promotion was evaluated over 35 days and the treatments were applied through regular watering (once or twice a week). Explants were watered with B1, B2, chemical fertilizer, or water, once and twice a week, for approximately five weeks. The plants resulting from germination were weaned according to Meshuneke *et al*. (2020).

Growth was monitored during the weaning phase and for 50 days afterward, with the following parameters were recorded: germination percentage, plant height, stem diameter, and total leaf surface area.

2.6. Evaluation of bioproduct effect on PIF seedling susceptibility to *M. fijiensis*

Susceptibility to *M. fijiensis* was evaluated by inoculating three and six-month-old plant leaves with 50 µL of *M. fijiensis* spore suspension (10^6 spores/mL). Seven days after the appearance of the first symptoms, the plants were treated with the different modalities. Necrotic leaf surface was measured every three days for a period of 28 days, according to Ewané *et al.* (2013). Leaves were sampled at:

T0: (BI= Before Inoculation);

T1: (72 Hours AI= After Inoculation)

T2: (28 Days AI).

Terazeb (fungicide) served as positive control.

2.7. Evaluation of bioproduct effect on biomarker accumulation in PIF plantain plant leaves

The bioproduct effect on biomarker accumulation was evaluated in banana leaves. Total soluble proteins were extracted using a modified protocol of Pirovani *et al.* (2008) and quantified using the Bradford method (1976). Total phenol extraction was done using the protocol of El Hadrami (1997) and quantified by the method of Marigo *et al.* (1973). Enzymatic activities of polyphenol oxidase (PPO) and peroxidase (POX) were measured using the protocols of Van Kammenn and Broumer (1994) and Baaziz *et al.* (1995), respectively

Soluble sugars were extracted from the leaves of 6-month-old plants using a modified protocol of Babu *et al.* (2002) and quantified via absorbance at 620 nm (Dubois *et al.* 1956).

2.8. Statistical Analysis

Observations of the effects of the *T. diversifolia* and *E. coccinea*-based bioproduct on plantain seedling growth in nursery, *in vitro* confrontation tests, susceptibility to *M. fijiensis,* and biomarker accumulation were all statistically analyzed. The different variables (germination percentage, seedling height, stem diameter, and leaf surface area, necrotic leaf area, total proteins, total phenols, PPO, POX and total sugars) were subjected to a three-way mixed analysis of variance (ANOVA) using XLSTAT software 2022. Each plant was treated as an experimental unit, and period, watering frequency, age and sampling time were taken as factors. Tukey's test at 5% probability threshold was used to compare the means of the different treatments.

3. RESULTS

3.1. Variation in pH of bioproduct modalities over time

The bioproduct pH measurement over a period of eight months (240 days) showed similar variation across all modalities. A highly significant difference was observed between the modalities over time. During the first two months, pH increased, then decreased steadily until the eighth month of storage in all modalities (Figure 1). However, the pH values remained between 4.6 and 5.3.

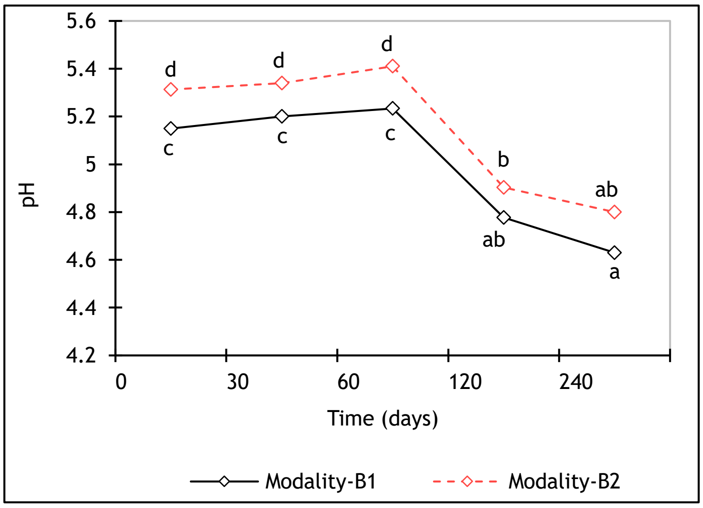


Figure 1. pH measurement of the bioproduct modalities (B1, B2) over time. Each point represents the average mean of three replicates with the standard deviation for each modality. The letters a, b, c and d represent the significantly different statistical groups obtained, Tukey's test at 5% probability threshold.

3.2. *In vitro* confrontation test between the bioproducts (B1 and B2) and *M. fijiensis*

The bioproducts B1 and B2 have a highly significant influence (*P*< 0.0001) on the mycelial growth of *M. fijiensis*, with a coefficient of determination R^2 equal to 100% (Table 1), and the most influential variable being the modality. The analysis of variance (ANOVA) of mycelial growth kinetics in the presence of the bioproducts showed a significant effect (*P*< 0.0001) of the modality and time variables, as well as their interaction (Table 1).

Table 1. Analysis of variance (ANOVA) of the effect of the bioproducts (B1 and B2) on the mycelial growth of the phytopathogenic strain *Mycosphaerella fijiensis* over a 15-day measurement period.

		Mycelial growth R^2= 100%	
Source	df	F	*P*
Modality	2	9216.733	**<0.0001**
Time (day)	5	1873.271	**<0.0001**
Modality*Time	10	1873.271	**<0.0001**

Values in bold correspond to tests for which the null hypothesis is rejected at a significance level of α =0.05. *df* is the degree of freedom, *F* is the F test value, and *P* is the probability.

A significant difference (*P*< 0.0001) was observed between the plant pathogenic strains over time, with the *Mycosphaerella fijiensis* strains exposed to the bioproducts B1 and B2, and the positive control showing no mycelial growth, in contrast to the negative control (Figure 2).

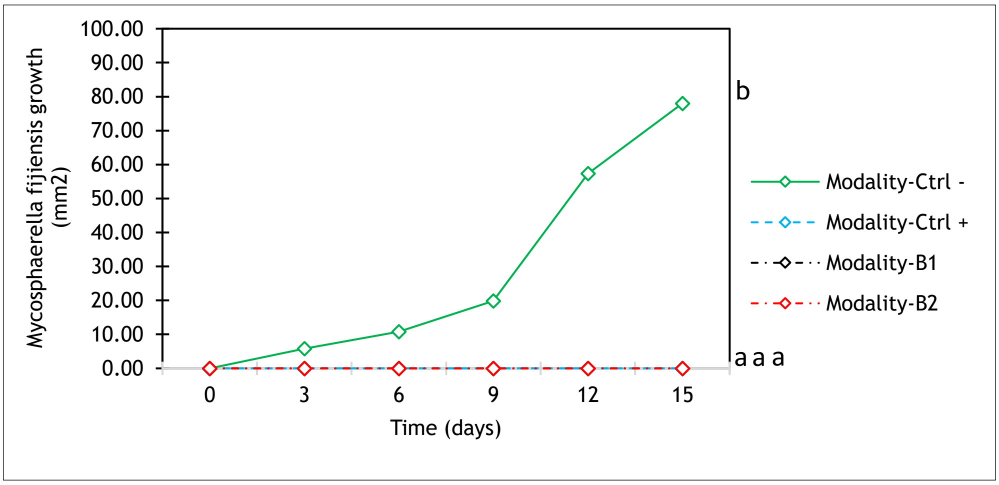


Figure 2. Influence of bioproduct B1 and B2 on the mycelial growth of *Mycosphaerella fijiensis* in the presence of controls (Ctrl+ and Ctrl-) on PDA medium over time. Each point represents the average mean of three replicates with the standard deviation for each modality. The letters a and b represent the significantly different statistical groups obtained, Tukey's test at 5% probability threshold.

Indeed, throughout the experimental period, mycelial growth inhibition was observed with both bioproducts (B1 and B2), as well as in the positive control (Ctrl+). Conversely, in the negative control (Ctrl-), mycelial growth remained exponential and continuous.

Thus, mycelial growth was continuous in the negative control (Ctrl-), whereas it was completely suppressed in the B1, B2 and Ctrl+ treatments (Figs. 2, 3). For the *M. fijiensis* mycelial growth, two distinct statistical groups were obtained: one for B1, B2 and Ctrl+, and another for the Ctrl-.

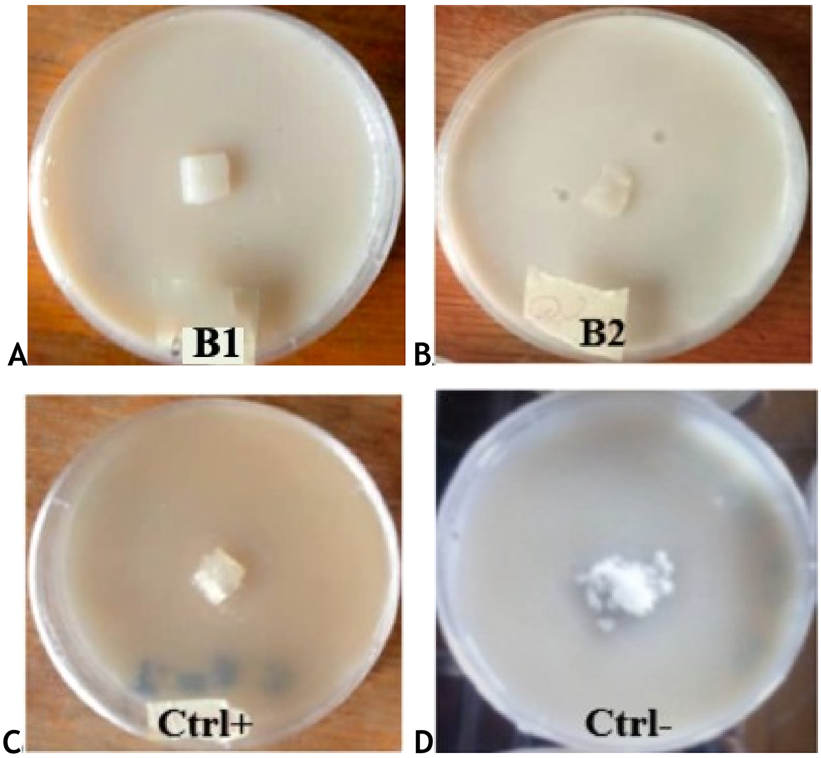


Figure 3. Mycelial growth of *Mycosphaerella fijiensis* on PDA medium: bioproduct (B1) (A), bioproduct (B2) (B), positive control (Ctrl+) (C) and negative control (Ctrl-) (D) over a period of 15 days.

3.3. Effect of bioproducts on the growth of plantain seedlings

Maintaining relative humidity through regular watering promoted the emergence of young shoots, which became visible from the 2nd week after sowing. The bioproduct had a highly significant effect (*P*< 0.0001) on the germination of PIF plantain seedlings compared to the controls (Figure 4), with modality B1 seedlings showing higher germination rates. Eyecup formation on the explants began on the 14th day after sowing across all treatments, regardless of watering frequency, with significant differences emerging from the 21st day onward (Figure 4A). The frequency of watering did not significantly affect germination of explants (Figure 4B). Full germination (100%) was achieved by day 35 after weaning for all treatments.

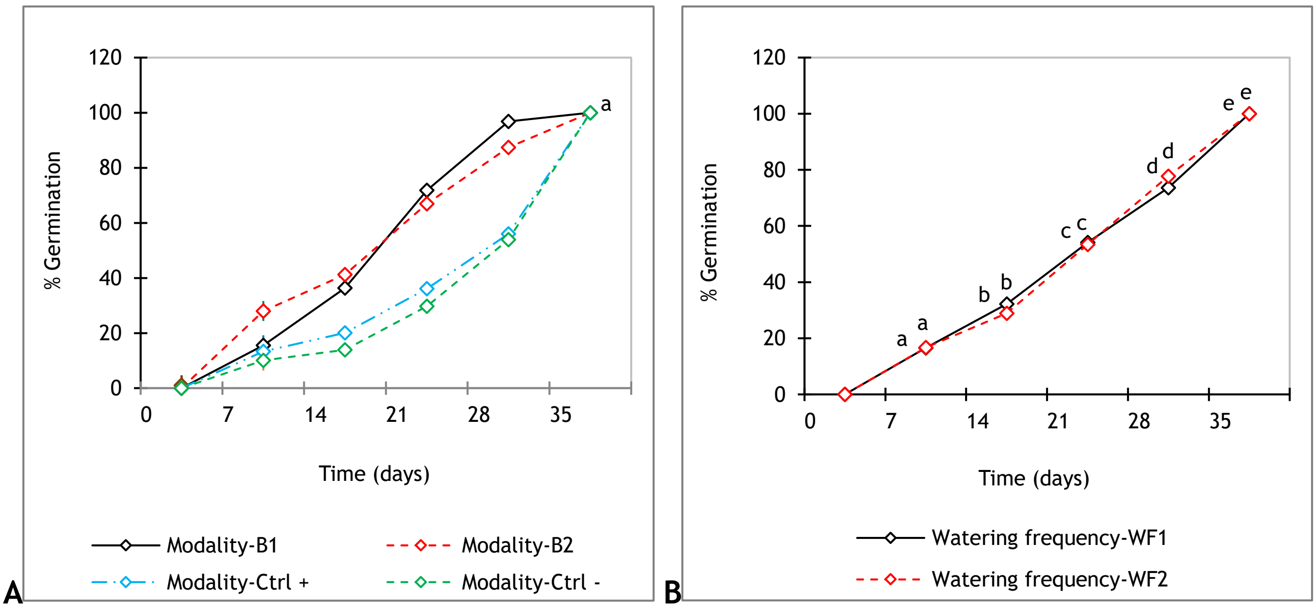


Figure 4. Germination percentage of plantain seeds treated with a bioproduct based on *Tithonia diversifolia* and *Emilia coccinea*. Interaction modality*time (A) and interaction watering frequency*time (B). Each point represents the average mean of three replicates with the standard deviation for each modality. The letters a, b, c, d and e represent the significantly different statistical groups obtained, Tukey's test at 5% probability threshold.

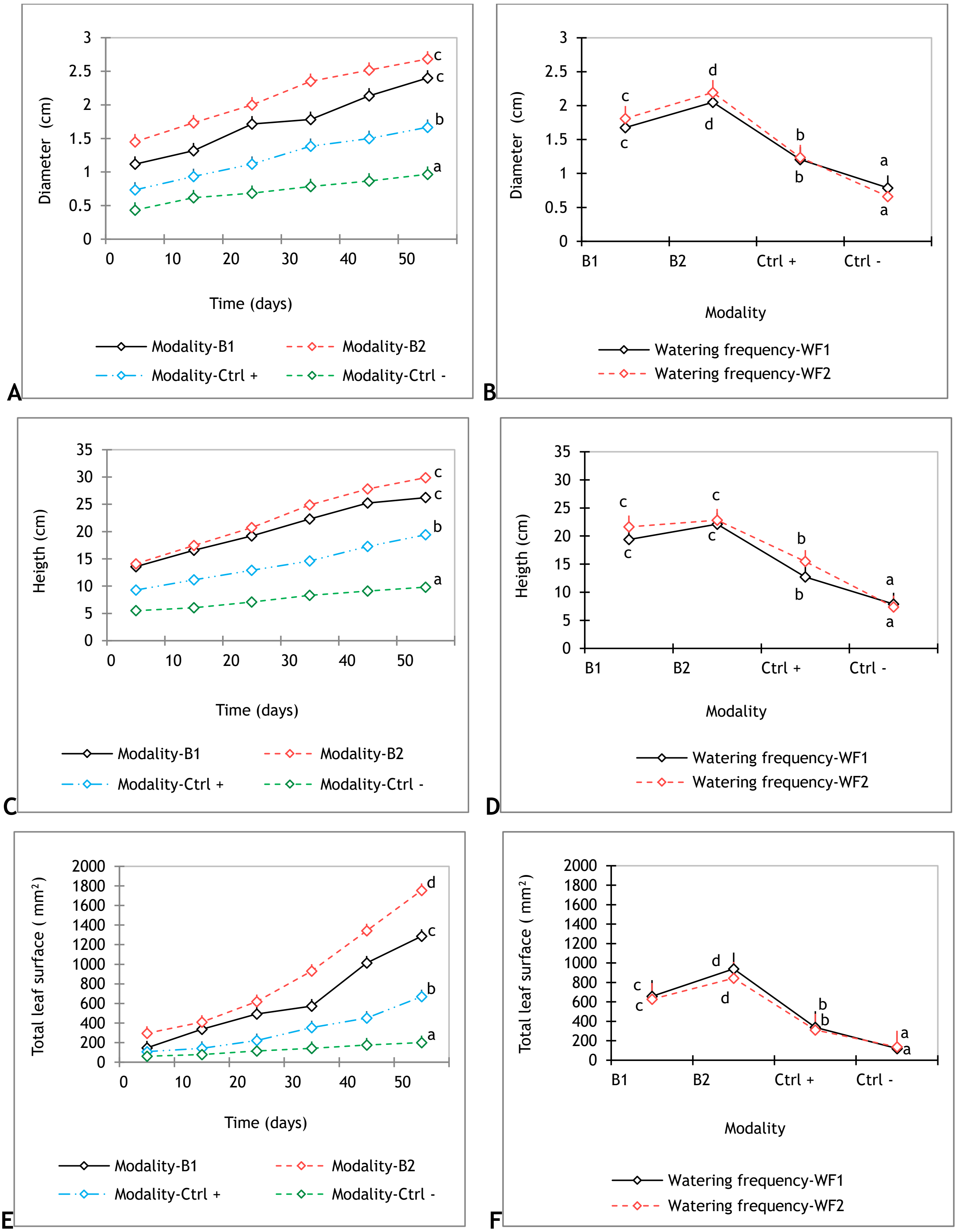


Figure 5. Influence of bioproduct based on *Tithonia diversifolia* and *Emilia coccinea* on the growth parameters of PIF plantain seedlings through interaction plots of time and modality, and interaction plots of modality and frequency respectively, for the height (A, B), the diameter (C, D) and the total leaf surface (E, F). Each point represents the average mean of three replicates with the standard deviation for each modality. The letters a, b, c and d represent the significantly different statistical groups obtained, Tukey's test at 5% probability threshold.

In addition, the bioproduct had a significant impact (*P*< 0.0001) regardless of the watering frequency, on the vegetative growth parameters of plantain PIF seedlings, particularly on the plant height, the stem diameter and the leaf surface area (Figure 5). Height, diameter and leaf area were much higher for modality B2 compared to controls, regardless of watering frequency (Figs. 5A, B, C, D and F). Watering twice a week (WF2) promoted greater seedling growth (WF1), except for leaf surface area, where WF1 had a greater effect (Figure 5F).

Fifty (50) days after weaning and acclimatization under shade, the average stem diameter of the plants treated with the bioproduct was 2.05 cm for WF2 and 1.86 cm for WF1 compared to 1.23 cm and 1.19 cm for the positive control at WF2, and 0.66 cm and 0.78 cm for the negative control respectively at WF2 and WF1, respectively (Figure 5B). Similarly, the average plant height in bioproduct-treated seedlings reached 22.23 cm for WF2 and 16.04 cm for WF1 compared to 15.48 cm and 12.71 cm for the positive controls and 7.38 cm and 7.42 cm for the negative controls, respectively (Figure 5D). The average leaf area of the plants treated with the bioproduct was 735.57 mm^2 for WF2 and 865.40 mm^2 for WF1, compared to 310.79 mm^2 and 330.96 mm^2 for the positive control, and 136.99 mm^2 and 134.70 mm^2 for the negative control, respectively (Figure 5F).

Overall, WF2 emerged as the most effective watering frequency for improving the agro-morphological parameters of PIF seedlings. It promoted strong leaf expansion and positively influenced both the stem diameter and plant height. The most influential factors in promoting growth were the treatment modality and time (Table 2).

Table 2. Analysis of variance (ANOVA) of a bioproduct based on *Tithonia diversifolia* and *Emilia coccinea* on the growth of PIF plantain seedlings [(height, diameter of pseudo-stem and total leaf surface area (TLS)].

		% Germination R^2= 99%		**Diameter (cm)** R^2= 96%		**Height (cm)** R^2= 99%		**Total leaf surface (mm^2)** R^2= 97%	
Variables	df	F	*P*	F	*P*	F	*P*	F	*P*
Watering Frequency (WF)	1	0.477	0.491	3.653	0.059	66.828	**<0.0001**	5.387	**0,022**
Modality	7	213.025	**<0.0001**	646.899	**<0.0001**	1772.206	**<0.0001**	526.984	**<0.0001**
Time (Day)	5	3975.113	**<0.0001**	162.213	**<0.0001**	447.229	**<0.0001**	304.708	**<0.0001**
WF*Modality	3	8.697	**<0.0001**	6.909	**0.000**	21.935	**<0.0001**	2.456	0,067
WF*Time	5	14.476	**<0.0001**	1.214	0,307	10.517	**<0.0001**	0.287	0,919
Modality*Time	15	75.587	**<0.0001**	6.123	**<0.0001**	23.918	**<0.0001**	40.668	**<0.0001**

Values in bold correspond to tests where the null hypothesis was rejected at a significance level of α= 0.05. *df* is the degree of freedom, *F* is the value of the F test, and *P* is the probability.

3.3. Effect of bioproduct on the induction of plantain seedling protection against *M. fijiensis*

The bioproduct had a highly significant influence (P< 0.0001) on the induction of protection of PIF plants against *M. fijiensis*, with a fairly lower necrotic leaf surface observed after inoculation (AI) compared to the controls, regardless of plant age and watering frequency (Figure 6). The coefficient of determination (R^2) was 100%, with the most influential variable being the treatment modality (Table 3).

The necrotic leaf surface area increased over time with a similar kinetics for both plant ages (3 and 6 months). The development of necrosis started on the 7th day in all modalities and increased exponentially in the control modality Ctrl- compared to all other treatment modalities. Regardless of age, the average necrotic leaf surface area was 42.5 mm^2 for modality B1 and 27.05 mm^2 for modality B2, 28 days after inoculation. On the same date for the control modalities, it was 70.05 mm^2 for the Ctrl+ modality and 432 mm^2 for the Ctrl- modality (Figs. 6A, B).

Watering frequency (WF) significantly influenced the development of necrosis on the leaves of plants regardless of age, with bi-weekly watering (WF2) significantly reducing susceptibility to *M. fijiensis*, except for the control modalities at six months (Figs. 6C, D). Generally, younger plants (3 months) exhibited more severe necrosis than older ones (6 months). Statistical analysis distinguished four groups based on necrotic surface area, each corresponding to a specific treatment modality (B1, B2, Ctrl+, Ctrl−). Among these, modality B2 conferred the highest level of protection, with a calculated protection rate of 90%.

Table 3. Analysis of variance (ANOVA) of a bioproduct based on *Tithonia diversifolia* and *Emilia coccinea* on the necrotic leaf surface of PIF plantain seedlings over time, for plants aged three and six months.

		Necrotic leaf surface (NLS) 3 months R^2= 100%		Necrotic leaf surface (NLS) 6 months R^2 = 100%	
Source	df	F	*P*	F	*P*
Watering frequency (WF)	1	152.620	**<0.0001**	128.040	**<0.0001**
Modality	3	26301.952	**<0.0001**	79948.396	**<0.0001**
Time (day)	8	3556.497	**<0.0001**	9589.273	**<0.0001**
WF*Modality	3	36.861	**<0.0001**	128.544	**<0.0001**
WF*Time	8	11.495	**<0.0001**	6.688	**<0.0001**
Modality*Time	24	1660.733	**<0.0001**	4929.669	**<0.0001**

Values in bold correspond to tests where the null hypothesis was rejected at a significance level of α= 0.05. *df* is the degree of freedom, *F* is the value of the F test, and *P* is the probability.

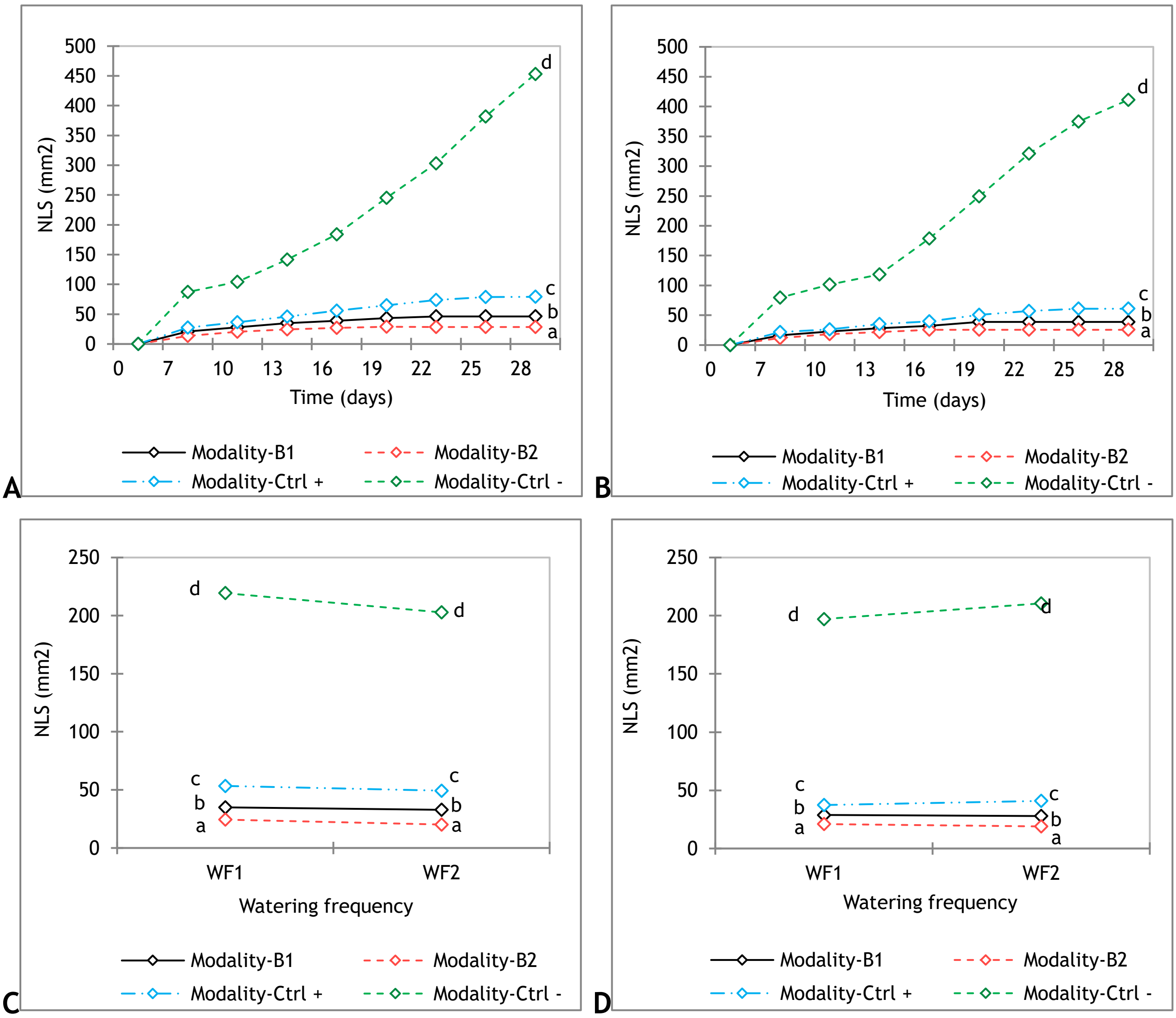


Figure 6. Influence of bioproduct based on *Tithonia diversifolia* and *Emilia coccinea* on the necrotic leaf surface (NLS) of PIF plantain seedlings in the course of time through interaction plot of watering frequency and modality for necrotic leaf surface, respectively for the plants aged three months (A, C) and six months (B, D). Each point represents the average mean of three replicates with the standard deviation for each modality. The letters a, b, c and d represent the significantly different statistical groups obtained, Tukey's test at 5% probability threshold.

3.4. Effect of the bioproduct on the accumulation of biomarkers in the leaves of PIF plantain plants

The different bioproduct modalities had a highly significant effect ($P < 0.0001$) on the accumulation of several biomarkers, notably total proteins, total phenols, total soluble sugars, and the enzymatic activities of peroxidases (POX) and polyphenol oxidases (PPO) in the leaves of treated plants, compared to controls. The coefficient of determination (R^2) was 100% (Table 4), with the most influential variable being the treatment modality. Modality B2 consistently resulted in higher levels of all these biomarkers, regardless of plant age (Figs. 7A, C, E, G).

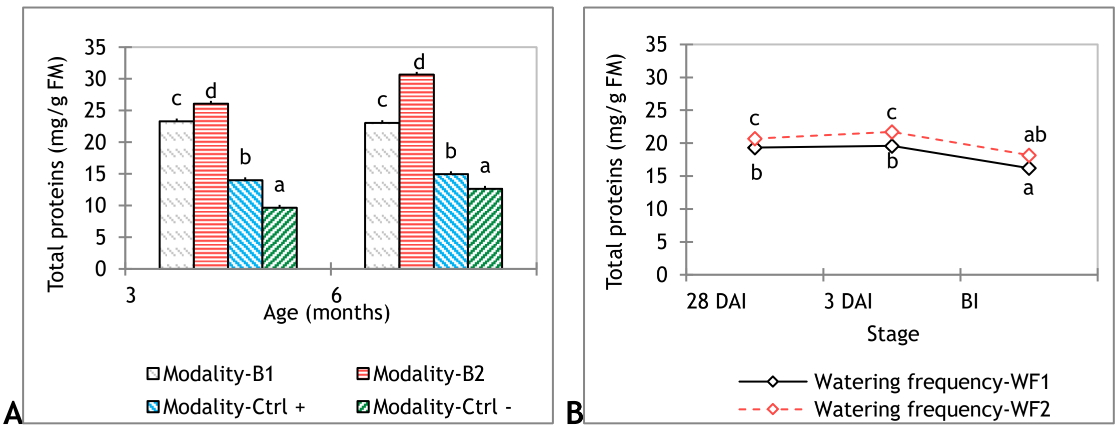
Table 4. Analysis of variance (ANOVA) of a bioproduct based on *Tithonia diversifolia* and *Emilia coccinea* on the accumulation of biomarkers (total proteins, total phenols, polyphenol-oxidase, peroxidase and total soluble sugars) in the roots of PIF plantain seedlings at different stages (before inoculation, three days post-inoculation and 28 days post-inoculation).

		Total proteins R^2= 99%		Total phenolics R^2= 99%		PPO R^2= 100%		POX R^2= 100%		Total soluble sugars R^2= 100%	
Variables	df	F	*P*	F	*P*	F	*P*	F	*P*		
Age (months)	1	166.020	**<0.0001**	39.959	**<0.0001**	29.644	**<0.0001**	94.184	**<0.0001**	-	-
Stage	2	176.632	**<0.0001**	425.060	**<0.0001**	281.111	**<0.0001**	551.173	**<0.0001**	1866.409	**<0.0001**
Watering Frequency (WF)	1	129.990	**<0.0001**	71.534	**<0.0001**	196.376	**<0.0001**	0.522	0.471	-	-
Modality	3	2421.603	**<0.0001**	5488.561	**<0.0001**	7664.910	**<0.0001**	11903.214	**<0.0001**	700.732	**<0.0001**
Age*WF	1	54.667	**<0.0001**	3.423	0.067	2.025	0.157	13.427	**0.000**	-	-
Age*Modality	3	44.803	**<0.0001**	4.835	**0.003**	4.495	**0.005**	1.610	0.191	-	-
Stage*Modality	6	26.746	**<0.0001**	54.593	**<0.0001**	108.004	**<0.0001**	2.587	0.079	30.242	**<0.0001**
WF*Modality	3	18.730	**<0.0001**	8.246	**<0.0001**	58.526	**<0.0001**	10.922	**<0.0001**	-	-

Values in bold correspond to tests where the null hypothesis is rejected at a significance level of α= 0.05. *df* is the degree of freedom, *F* is the value of the F test, and *P* is the probability.

Overall, the accumulation of total proteins and total phenols, and the enzymatic activities of peroxidases and polyphenol oxidases were greater in older seedlings (6 months) compared to young seedlings (3 months) (Figure 7). In both age groups, watering twice a week (WF2) significantly increased the contents of total proteins and total phenols, and the enzymatic activities of peroxidases and polyphenol oxidases (Figs. 7A, C, E, G), except for peroxidase activity in 3-month-old seedlings, which was slightly higher under once-a-week watering (WF1) (Figure 7E). Two statistically distinct groups were observed based on watering frequency.

Regardless of the sampling time (BI, 3AI and 28AI), the highest levels of total proteins, phenolic content, and polyphenol oxidases were recorded three days after inoculation (Figs. 7B, D, F, H) (3AI), whereas, for the peroxidase activity peaked which was higher at 28 days after inoculation (28AI) in seedlings (Figure 7F).



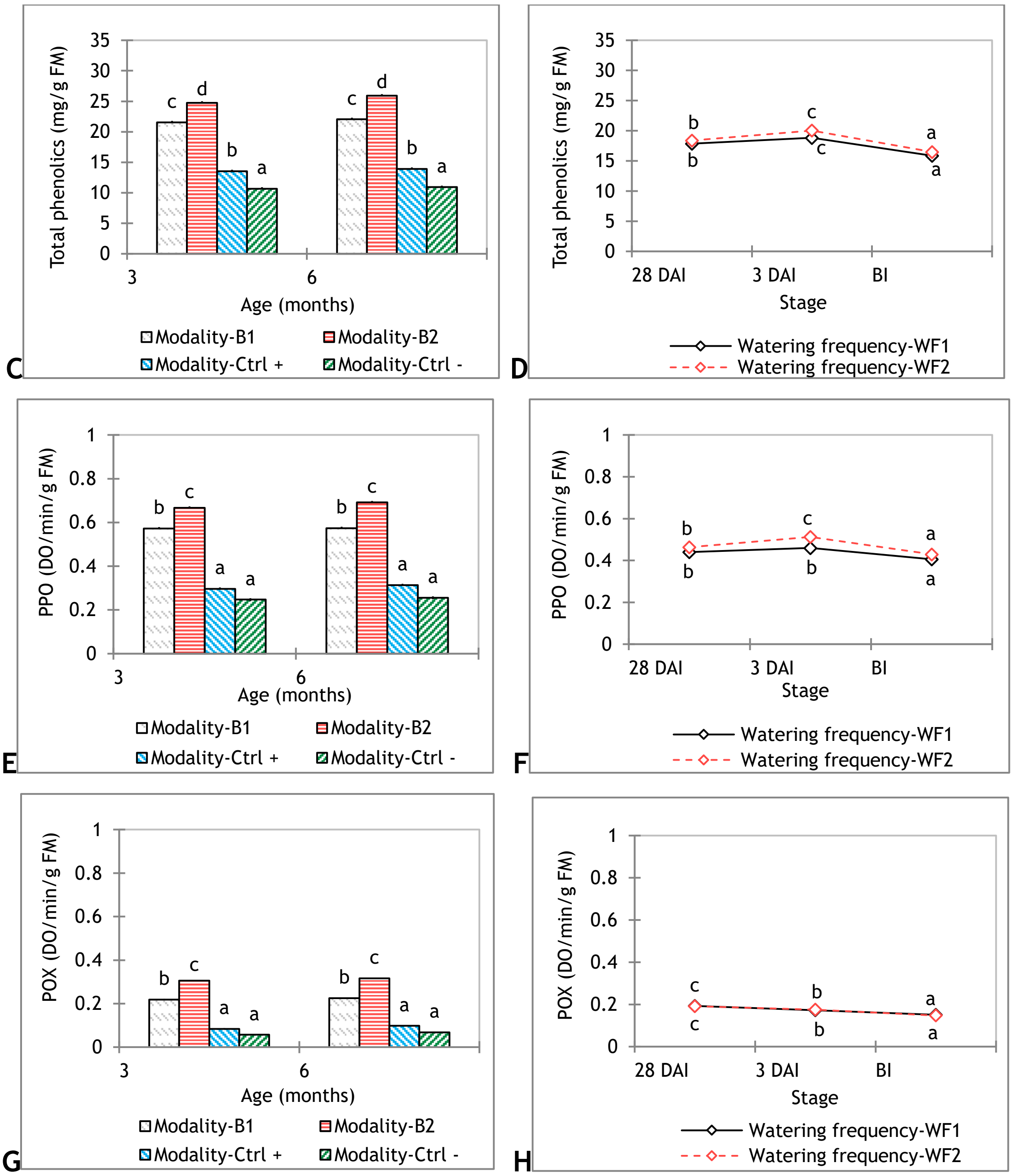


Figure7. Effects of bioproduct based on *Tithonia diversifolia* and *Emilia coccinea* on the accumulation of biomarkers (total proteins, total phenolics, peroxidase and polyphenol-oxidase) in the leaves of PIF plantain seedlings before, three days, and 28 days after inoculation. Each point represents the average mean of three replicates with the standard deviation for each modality. The letters a, b, c and d represent the significantly different statistical groups obtained, Tukey's test at 5% probability threshold.

The bioproduct also had a strong effect on the accumulation of total sugars in the leaves of six-month-old PIF plants treated bi-weekly ($P< 0.0001$). The total sugar content was significantly higher in the bioproduct modalities (B1 and B2) compared to the control modalities (Ctrl+ and Ctrl-). Among the two treatments, modality B2 induced a greater sugar accumulation (Figure 8A). A significant difference was observed between pre-inoculation (BI) and 28 days post-inoculation periods (28AI). The total sugar contents were much higher before inoculation for all modalities (Figure 8B). Three distinct statistical groups were obtained for the accumulation of total sugars, including one for the bioproduct modalities B1 and B2, one for the Ctrl- and one for the Ctrl+.

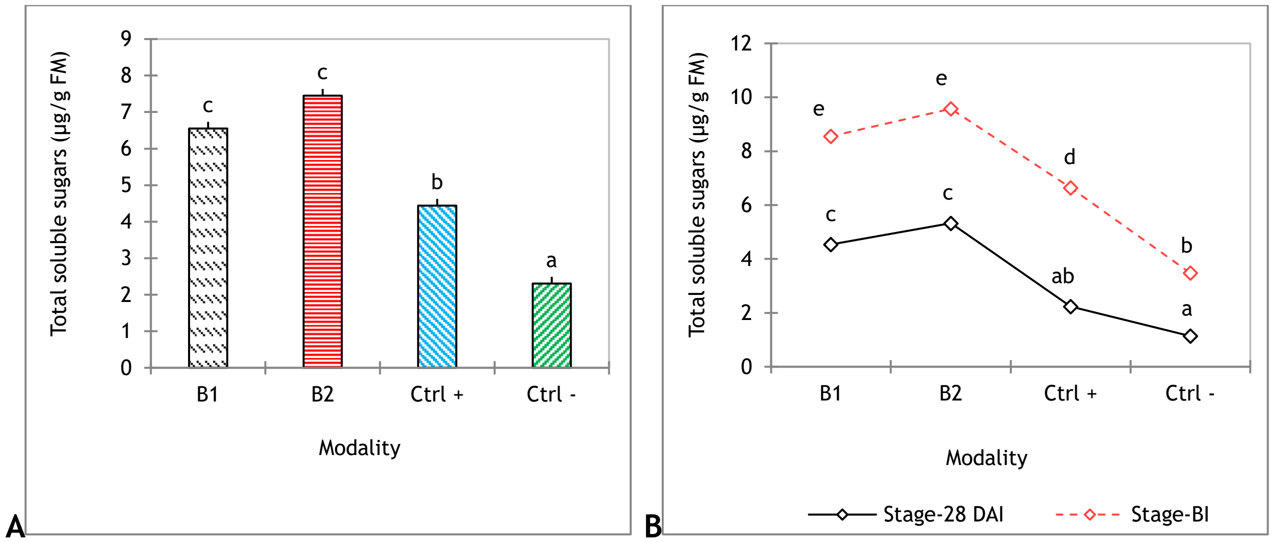


Figure 8. Effects of bioproduct based on *Tithonia diversifolia* and *Emilia coccinea* on the accumulation of total soluble sugars in the leaves of PIF plantain seedlings before inoculation (BI) and 28 day after inoculation (DAI). Each point represents the average mean of three replicates with the standard deviation for each modality. The letters a, b, c, d and e represent the significantly different statistical groups obtained, Tukey's test at 5% probability threshold.

4. DISCUSSION

The effect of the bioproduct based on *Tithonia diversifolia* and *Emilia coccinea* was demonstrated by the stimulation of growth and protection of PIF plantain plants in nurseries. The results obtained show a marked positive effect of repeated applications of the bioproduct in promoting the growth of PIF plants, inducing resistance to *Mycosphaerella fijiensis* through reduced susceptibility, and enhancing the accumulation of biomarkers. These results are in line with previous studies that demonstrated the effect of natural products on the promotion of growth in several plants (Kasango *et al*. 2013, Bilong *et al*. 2017, Téné *et al*. 2017). In addition, these biofertilizing, bioprotective and biostimulating actions of natural products have also been demonstrated in the banana-plantain tree in nursery (Tatsegouock *et al*. 2020, Meshuneke *et al*. 2020).

The minor variation in pH observed across all modalities of the bioproduct revealed its stability over time. The pH range observed also suggests the absence of contamination by additional microflora, supporting its compliance with storage standards (Najat 2016). This stability could be attributed to the storage conditions, opaque containers, room temperature and airtight sealing, which likely prevented oxidation of the bioactive compounds and microbial contamination from air exposure.

The production of PIF banana-plantain seedlings showed distinct morphological differences between treated and control seedlings. The growth dynamics during both germination and vegetative phases differed significantly, with treated seeds exhibiting enhanced development. Indeed, treatment with the bioproducts significantly increased all agro-morphological parameters (percentage of germination, diameter and height of the pseudostem, and leaf surface area). Our results are consistent with earlier reports on promoting the growth of PIF banana-plantain seedlings (Tatsegouock *et al*. 2020).

The enhanced germination speed and vegetative growth parameters of the banana-plantain may be attributed to the presence of nutrients contained in the bioproduct, such as nitrogen, phosphorus, potassium and magnesium, well known for their physiological roles in promoting growth and improving soil properties (Purbajanti *et al*. 2019, Kulcheski *et al*. 2015). In addition, the metabolites contained in the bioproduct, such as proteins and phenolic compounds, also play a physiological role in plants (Sharma *et al*. 2019).

The bioproduct significantly reduced the susceptibility of banana-plantain PIF seedlings to *M. fijiensis*. The antifungal and repellent properties of *T. diversifolia* and *E. coccinea* have been previously documented against various insect pests and phytopathogens (Martin *et al*. 2018, Umar *et al*. 2015). Regardless of plant age, treated plants exhibited stronger resistance than controls. The reduced susceptibility to *M. fijiensis* observed in six-month-old plants may be due to cellular modifications such as cell wall reinforcement or production of antimicrobial compounds. At this developmental stage, plants are more capable of perceiving both biotic and abiotic stress in nature, and deploying sophisticated and effective defense responses. Moreover, the lower disease susceptibility observed in treated seedlings is likely due to induced resistance against *M. fijiensis*. Many studies have also reported that *T. diversifolia* and *E. coccinea* contribute to the protection of plants by modifying the microbial population and stimulating defense mechanisms (Kaho *et al.* 2011, Martin *et al.* 2018). In addition, the application of the bioproduct on the PIF plantain plants could constitute a protective biofilm on the plant surface, preventing pathogen entry via cuticles or stomata.

The accumulation of defense biomarkers was significantly higher in plants treated with the bioproducts compared to controls. The proportions of these biomarkers in the leaves of PIF seedlings were much higher post-inoculation, as stress induces the accumulation of these resistance-related biomarkers. The involvement of defense biomarkers has been demonstrated in the defense mechanisms of banana tissues (Dhakshinamoorthy *et al.* 2014, Ewané *et al.* 2020b) as well as in the tissues of other plants (Pusztahelyi *et al.* 2015, 2018).

The bioproduct based on *Tithonia diversifolia* and *Emilia coccinea* may also act as an elicitor, triggering systemic-induced resistance of plants through the accumulation of defense molecules before a possible infestation (Thakker *et al.* 2013). However, resistance levels against black Sigatoka were lower than those reported by Tatsegouock *et al.* (2020) with fermented *T. diversifolia* extracts. The induction of systemic resistance by the bioproduct could be due to its composition and capacity to stimulate defense mechanisms and increase the synthesis of plant defense metabolites such as phenolic compounds, PR proteins and defense enzymes. Further investigation is needed to elucidate the precise defense mechanisms involved.

The bioproduct enhanced the accumulation of proteins, phenolic compounds, as well as the enzymatic activities of peroxidases and polyphenol oxidases, irrespective of the age of the seedlings and the frequency of watering. These biomarkers accumulate abundantly at twice-weekly watering (WF2) compared to those of once a week watering (WF1) and are much more important in seedlings aged six (06) months, probably due to greater cumulative exposure and developmental stage (Mayer Harel 1979, Aloni *et al.* 2004). However, the accumulation of biomarkers peaked three (03) days post-inoculation, possibly reflecting an early defense response prior to symptom expression, probably because after inoculation, the perception of the presence of a pathogen by the plant is translated by the immediate establishment of defense mechanisms even before the first symptoms appear. Thus, the plant frees itself from pathogenic attacks through preformed molecules and the synthesis of new defense molecules (Pusztahelyi *et al.* 2015, Andersen *et al.* 2018). At the 28th day post-inoculation (28 PI), the biomarker levels declined slightly, likely because the initial response had already mobilized key defense biomarkers.

The low levels of total sugars present in the leaves of PIF banana plantain plants treated with the bioproduct after inoculation could be due to the stimulating properties of the constituents of *T. diversifolia* and *E. coccinea* (Kandungu *et al.* 2013, Bosch 2019). These results align with previous work which showed that cultivars of more resistant peppers had low sugar content compared to sensitive cultivars (Young ▫ Hwang 1991). The significant accumulation of sugars in the plant before inoculation is likely due to their involvement in the growth process, while the low content revealed after infection seems to be linked to their involvement in defense processes. This could also be due to the use of sugars as an energy source (primary metabolites) and/or elicitors in the synthesis of secondary metabolites (Mann *et al.* 1994). Indeed, in stressful situations, the plant uses these forms of carbohydrate reserves to better resist biotic threats. These results underscore the multifunctional role of sugars in banana plant growth and defense. For instance, studies carried out on rice demonstrated the induction of resistance to *Magnaporthe oryzae* thanks to treatments formulated with sucrose (Ariza *et al.* 2007).

5. CONCLUSION

This study highlights the fertilizing and pesticidal potential of a bioproduct based on *Tithonia diversifolia* and *Emilia coccinea* for plantain cultivation. Regardless of the watering frequency, the bioproduct not only promoted the growth of plantain seedlings but also conferred protection against biotic stress. This suggests that the bioproduct based on *T. diversifolia* and *E. coccinea* could be used as a biofertilizer and biopesticide for the production of healthy, vigorous, and disease-tolerant PIF banana-plantain seedlings. Its application could play a key role in establishing sustainable plantations, while preserving environmental resources. In essence, this bioproduct based on *Tithonia diversifolia* and *Emilia coccinea* aligns with the concept of producing “more with less”. However, further research is warranted to identify the nature and concentration of its bioactive components and assess their broader applicability to other crops.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE. Not applicable.

AVAILABILITY OF DATA AND MATERIALS. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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CONFLICTS OF INTEREST. The authors declare that there is no conflict of interest regarding the publication of this article.

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