Pertanika J. Trop. Agric. Sci. 48 (5): 1653 - 1676 (2025)

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Foliar Sprays of Biostimulants and PGRs Improve the Reproductive Parameters of Cacao Under Water Stress

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ABSTRACT

One of the essential factors influencing yield of crops is the availability of water. This study was conducted to determine the efficacy of biostimulants (oligocarrageenan and oligochitosan) and plant growth regulators (PGRs) such as cytokinin and paclobutrazol applied as foliar spray on the pod retention and yield of six-year-old grafted 'UF 18' cacao trees under a monocropped system during the dry season in Type III climate of the Philippines. Tap water (control), oligocarrageenan (150 ppm), oligochitosan (150 ppm), cytokinin (40 ppm), and paclobutrazol (500 ppm) were applied as a foliar spray onto cacao trees with young cherelles (BBCH 70-72) under irrigated and rainfed conditions. Among the foliar treatments, oligochitosan caused a higher flowering intensity after four months. Paclobutrazol reduced the incidence of cherelle wilt, while cytokinin improved the pod retention of cacao. The biostimulants and PGRs generally enhanced the leaf chlorophyll content of cacao, increased the mean seed fresh weight, produced higher percentages of full beans and lesser flat beans per tree, and had a better bean count than the control. The cacao trees treated with oligocarrageenan produced pods with heavier dry beans. Moreover, heavier dry bean weight per tree were recorded from cytokinin-, PBZ-, and oligocarrageenan-treated trees. Foliar sprays of biostimulants and PGRs resulted in a significant increase in yield per tree of cacao under water stress.

Keywords: Cytokinin, oligocarrageenan, oligochitosan, paclobutrazol, plant activators

ARTICLE INFO

Article history:
Received: 31 October 2024
Accepted: 13 February 2025
Published: 29 August 2025

DOI: https://doi.org/10.47836/pjtas.48.5.17

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INTRODUCTION

Cacao (*Theobroma cacao* L.) is one of the high-value crops cultivated in the Philippines but local production ranges only from 0.5 to 1.0 kg per tree per year or equal to about 500 to 1000 kg per hectare

e-ISSN: 2231-8542

(Department of Agriculture, 2017). The target yield per tree per year of cacao dried beans is set at 2.0 kg according to the 2016-2022 Philippine Cacao Roadmap (DA, 2017). However, it cannot be denied that the productivity of a cacao tree is limited by the prevailing environmental conditions.

Wood and Lass (2001) identified rainfall as the most important environmental factor impacting cocoa production. Water limitation or drought stress could have negative effects on growth, flowering, pod setting, pod retention (Adjaloo et al., 2012; Matias et al., 2024), and yield of cacao trees. Long dry spells with less than 100 mm of rain per month for more than three months can significantly impair cacao tree growth and yield (Lahive et al., 2019). Therefore, cacao production is favourable in regions where rainfall is evenly distributed throughout the year and have no dry season. According to Coronas (1920) classification, this is the Type IV climate of the Philippines. In regions with no distinct maximum rain periods and with one to three months of dry season which is the Type III climate of the country (Coronas, 1920), a higher yield of cacao could be attained if supplemental irrigation during the dry months is provided.

Water-limiting conditions during the dry season in Type III climate can be mitigated through the application of biostimulants and plant growth regulators (PGRs). Biostimulants are recognised in crop production since these substances enhance mineral nutrition uptake, provide protection, and improve the yield and quality of plants under abiotic conditions (Franzoni et al., 2022). In addition, biostimulant application is a sustainable agricultural practice to maintain crop yield under reduced fertiliser situations (Gupta, 2020). Biostimulants are categorised as microbial and non-microbial. Chitosan and seaweed extract (i.e., carrageenan) are examples of non-microbial biostimulants (Li et al., 2022), of great interest in the global crop industry, which are known to stimulate growth and provide protection to various crop species. Chitosan and carrageenan are long-chain carbohydrates derived from crustacean and fungal exoskeletons, and seaweed (algae) origins respectively. Irradiation of chitosan and carrageenan will result in the formation of shorter chain-oligosaccharides, and when they are bioactive, these are called oligosaccharins (oligochitosan and oligocarrageenan). Oligosaccharins act as a signal molecule that could activate several plant physiological processes leading to growth stimulation and plant defense (Albersheim & Darvill, 1985).

Plant growth regulators (PGRs) are organic compounds that could regulate the growth and development of plants. Paclobutrazol (PBZ) is one of the known plant growth regulators that enhances accumulation of carbohydrates and chlorophyll synthesis in plant leaves which supports the reproductive structures of cacao trees. On the other hand, cytokinin is a hormone that prevents chlorophyll degradation (Taiz & Zeiger, 2010), thus increasing crop yield even under abiotic stress condition.

Despite the growth regulatory effects of biostimulants and PGRs on several crops, there is no existing information on their effects on the pod retention and yield of cacao trees during water-limiting conditions.

As the cacao pods are developing, proteins and photoassimilates are being used up. In this study, it is hypothesised that foliar spraying of biostimulants and PGRs will influence cacao physiology, thereby enhancing pod retention and yield of tree during water-limiting conditions.

Hence, this study was conducted to determine the effects of foliar applications of biostimulants and PGRs on the pod retention and yield per tree of cacao during the dry season in Type III climate.

MATERIALS AND METHODS

This study was conducted from December 2022 to June 2023 in a six-year-old monocrop ‘UF 18’ cacao plantation in Bangcud, Malaybalay City, Bukidnon, Philippines (Latitude 7.991817^o, Longitude 125.131607°, at 326.71m of altitude). Cacao trees were planted at a spacing of 3x3 metres.

The experiment site belongs to the Type III climate where there is no distinct maximum rain period, and with a short dry season (Coronas, 1920) within a year. Weather data such as temperature and rainfall (Figure 1) were obtained from the weatherlink.com through the Mt. Kitanglad Agricultural Development Corporation situated in Lurugan, Valencia City, Bukidnon.

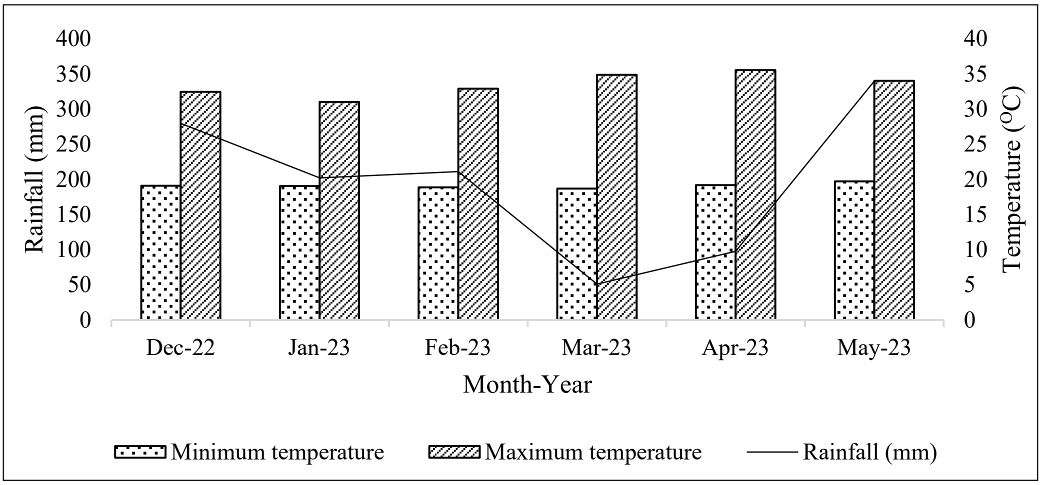

Figure 1. Minimum and maximum temperatures and amount of rainfall data

The soil bulk density of the area is 0.9 g/cm^3, 1.219 g/cm^3, and 1.030 g/cm^3 at depths of 0-30 cm, 30-60 cm, and 60-90 cm respectively. The data imply that the soil is favourable for root growth as well as tree growth of cacao. As a basic principle, the soil becomes compacted and unfavourable for plant growth if the soil bulk density is ≥2 g/cm^3.

To measure soil moisture, probes (gypsum blocks) were installed below the soil within the depths of 0.3 m, 0.6 m, and 0.9 m in both experiment set-ups (with irrigation and without irrigation). Soil moisture was measured at weekly intervals using a Delmhorst soil moisture tester (Figure 2). Weekly soil moisture data is expressed in centibars (Figure 3) using the conversion table specified in the DelmhorstTM KD-1 manual (http://www.moisturemetersdelmhorst.com/content/delmhorst/manual/KS-D1-Soil-moisture-meter-Manual-rev-04-11.pdf).

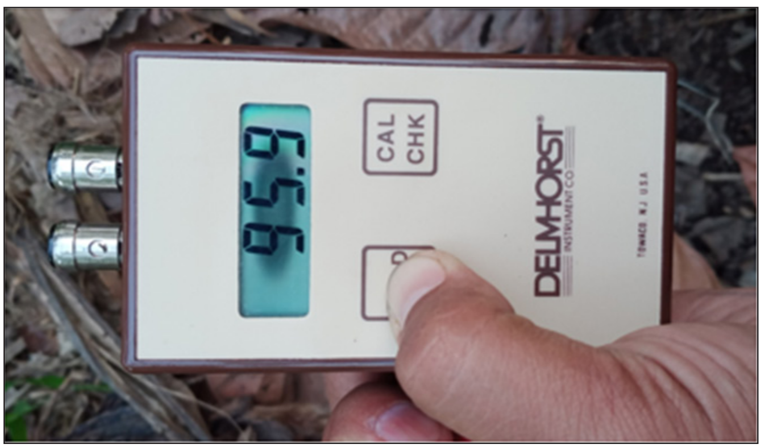

Figure 2. Measuring soil moisture using the Delmhorst™ KS-D1 (Towaco, N.J. USA) soil moisture metre

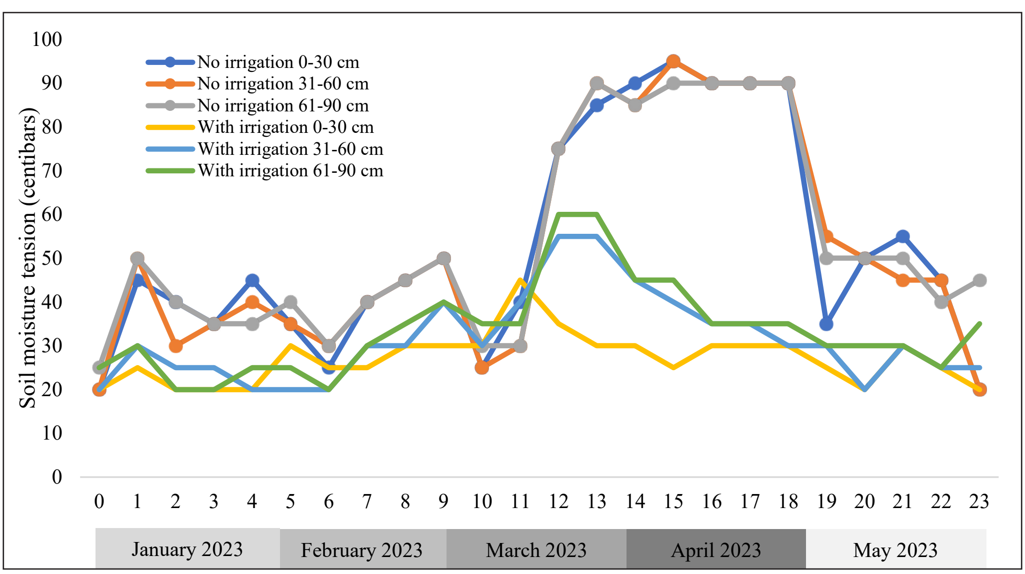

Figure 3. Soil moisture tension in experiments with irrigation and without irrigation

Six-year-old ‘UF 18’ cacao with uniform growth and cherelles ([BBCH 70-72] Azogue et al., 2023) set were selected as experimental trees. This study comprised two sets of experiments. Cacao trees in the first experiment set-up were not provided with irrigation throughout the experiment period. In the second experimental set-up, cacao trees were supplemented with irrigation based on the farmers’ practice (5 li/tree applied twice a week or at least once a week in the absence of rainfall). Each experiment set-up was arranged in a randomised complete block design (RCBD) with three replications. There were 15 experimental units per set-up.

Treatments consisted of 1) Tap water (negative control); 2) 150 ppm oligocarrageenan; 3) 150 ppm oligochitosan; 4) 40 ppm cytokinin; and 5) 500 ppm paclobutrazol. The source of oligocarrageenan, oligochitosan, cytokinin, and PBZ was VitalGro carrageenan® (5,500 ppm carrageenan byproducts as active ingredient), Chitosan oligosaccharide® soluble powder (99% a.i.), Stoller X-Cyte® (0.04% cytokinin as kinetin), and Greenfast® (25SC PBZ) respectively. Treatments were sprayed using a hand-operated shoulder sprayer (Lotus LTGT5000PSX) with an operating pressure of 0.3Mpa. The spraying volume was 5 L tree^{-1}. The pH of the water used to prepare the solutions was 7.1. Above ground parts of the trees including cherelles (BBCH 70-72) were sprayed with the treatment solution. These treatments were applied to plants in both sets of experiments (irrigated and rainfed).

Data Gathered

Leaf Chlorophyll Index

The leaf chlorophyll index was measured using the FieldScout CM 1000 (Spectrum Technologies Inc, 360 Thayer Court, Aurora, IL 60,504). Chlorophyll index was measured from the youngest fully-expanded sun leaf and shade leaf from four branches of each experimental tree. Shade leaves are those with larger leaf area and thinner. On the other hand, sun leaves have smaller leaf area and thicker (Taiz & Zeiger, 2010).

Cushions and Flowers

Cushions are former leaf axils where flowers are borne. Cushions and flowers were counted and recorded before treatment application. Subsequent counting and recording of these parameters per tree were done at monthly intervals.

Cherelle Wilt

The initial count of healthy cherelles (BBCH 70-72 stages) from each experimental tree was also counted and recorded. Cherelles (BBCH 70, BBCH 71, and BBCH 72) developed, after treatment application, were monitored and recorded at weekly intervals. Cherelle wilt incidence was monitored and recorded at weekly intervals. Existing cherelles (BBCH 70-72) before treatment application and those that developed after treatment application until 15 weeks were monitored until they reached the BBCH 75 (end of the cherelle wilt phase). Cherelle wilt incidence was then obtained using the formula below:

$$\text{Cherelle wilt (\%)} = \frac{\text{Number of cherelles wilted}}{\text{Number of cherelles developed}} \times 100$$

Pod Retention

Pod development was monitored (Figure 4) at weekly intervals until maturity and ripening. The total number of harvested cacao pods was counted manually. Pod retention per tree was obtained using the formula below:

$$\text{Pod retention (\%)} = \frac{\text{Number of pods harvested}}{\text{Initial number of cherelles set}} \times 100$$

Pod Weight

Cacao pods were harvested at the BBCH 81 stage. Individual pods harvested were weighed using a pre-calibrated weighing scale. The mean pod weight was computed using the formula:

$$\text{Pod weight (g)} = \frac{\text{Pod weight (g)}}{\text{Number of pods harvested}}$$

Fresh Weight of Seeds per Pod

Fresh seeds from harvested pods were collected and weighed. This parameter was computed using the formula:

$$\text{Fresh weight of seeds per pod (g)} = \frac{\text{Weight (g) of fresh seeds collected}}{\text{Number of pods harvested}}$$

Figure 4. Reproductive development of 'UF 18' cacao applied with different foliar treatments under irrigated and rainfed conditions (photos taken at different dates)

Number of Seeds per Pod

Fresh seeds from harvested pods were collected. This parameter was computed using the formula:

$$\frac{\text{Number of fresh seeds collected=Number of seeds per pod}}{\text{Number of pods harvested}}$$

Seed Fresh Weight

Fresh seeds from harvested pods were collected. This parameter was computed using the formula:

$$\text{Fresh weight per seed (g)} = \frac{\text{Weight of fresh seeds collected}}{\text{Number of seeds collected}}$$

Number of Full Beans per Tree

Seeds from harvested pods were collected and dried to 7% moisture. The total number of full beans collected from each tree was then recorded.

Weight of Full Beans per Tree

Seeds from harvested pods were collected and dried to 7% moisture. The weight of full beans collected from each tree was then recorded.

Number of Flat Beans per Tree

Flat beans are those seeds with cotyledons that are too thin (Wood & Lass, 2001). Seeds from harvested pods were collected and dried to 7% moisture. The total number of flat beans collected from each tree was then recorded.

Weight of Full and Flat Beans per Tree

Seeds from harvested pods were collected and dried to 7% moisture. The weight of flat beans collected from each tree was then recorded.

Full Beans per Pod

Full beans from harvested pods were collected. This parameter was expressed in percentage using the formula:

$$\text{Full beans per pod (\%)} = \frac{\text{Number or weight of full beans}}{\text{Full beans + flat beans}} \times 100$$

Flat Beans per Pod

Beans from healthy pods were collected. This parameter was expressed in percentage using the formula:

$$\text{Flat beans per pod (\%)} = \frac{\text{Number or weight of flat beans}}{\text{Full beans + flat beans}} \times 100$$

Dried Bean Weight

Dried beans were weighed and average dried bean weight was computed using the equation:

$$\text{Dried bean weight (g)} = \frac{\text{Weight of dried beans}}{\text{Number of dried beans}}$$

Bean Count

Bean count is the total number of cacao beans required per 100 grammes. Using the formula below, the bean count was computed:

$$\text{Bean count } (n\text{BEAN}) = \frac{n\text{WHOLE} \times 100}{m\text{WHOLE}}$$

Where nWHOLE is the number of whole beans, and mWHOLE is the mass of whole beans (g).

Dry Weight (DW) per Tree of Marketable Beans

The equation below was used to compute the DW for tree of marketable beans as influenced by the foliar treatments:

DW per tree of marketable beans = no. of pods retained × no. of full beans/pod × DW of full beans

Estimated Yield per Tree

The cherelles that reached BBCH 75 (Azogue et al., 2023) were considered in the computation of this parameter. The number of BBCH 75 pods that developed in the irrigated trees was considered as the pods retained for the wet season. On the other hand, the BBCH 75 pods that developed in the trees in the rainfed experiment was considered as the pods retained for the dry season. The following formula was used to compute for the:

Estimated yield per tree (dry season)	=	no. of pods retained (rainfed experiment) × no. of full beans/pod × DW of full beans;
Estimated yield per tree (wet season)	=	no. of pods retained (irrigated experiment) × no. of full beans/pod × DW of full beans; and
Estimated yield per tree per year	=	Yield per tree estimate (dry season) + Yield per tree estimate (wet season)

Statistical Analysis

Individual analyses of variance in randomised complete block design (RCBD) for each parameter was performed using the Statistical Analysis for Agricultural Research (STAR 2.0.1) software (http://bbi.irri.org/products). To examine the interaction between irrigation practice and foliar treatments for each parameter, combined analysis of variance was performed for parameter(s) in the individual analyses of variance that satisfies the assumptions of ANOVA for homogeneity of means. Significant differences between treatment means were separated using the Tukey's Honest Significant Difference (HSD) test at 0.05 level.

RESULTS

Effects of Biostimulants and PGRs on the Chlorophyll Index of Cacao Leaf Under Different Irrigation Practices

Cacao Sun Leaves

There was no significant interaction observed between irrigation practice and foliar treatment on the chlorophyll reading of cacao sun leaves from 1MAT to 4MAT. Significant effects of foliar treatments on the cacao sun leaf chlorophyll reading at 2MAT and 3MAT were observed only in the rainfed experiment (Figure 5) and not in the irrigated experiment. At 2MAT, the foliar spray of oligocarrageenan, and PBZ treatments resulted in higher chlorophyll index in cacao sun leaves compared to the control. At 3MAT, the chlorophyll index of the cacao sun leaves from trees applied with cytokinin and paclobutrazol was higher compared to other foliar treatments (Figure 5).

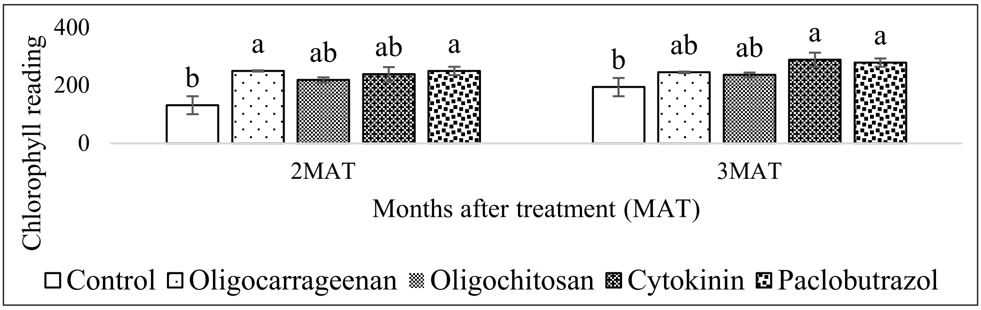

Figure 5. Effects of foliar treatments on the chlorophyll index of cacao sun leaves as measured by the FieldScout CM 1000 at 2MAT and 3MAT under rainfed condition
cv (%)= 27.43; *p*= .0272* (2MAT)
cv (%)= 18.52; *p*= .0450* (3MAT)
Treatment means with the same superscript within 2MAT and 3MAT respectively are not significant according to Tukey's HSD test (p ≤.05)

Cacao Shade Leaves

Significant interaction between irrigation practice and foliar treatment on the chlorophyll reading of cacao shade leaves from 1MAT to 4MAT was not observed. Significant effects of foliar treatments on shade leaf chlorophyll reading at 3MAT was observed only in the irrigated experiment (Figure 6). The chlorophyll index of the cacao shade leaves at 3MAT was higher in trees applied with biostimulants and PGRs treatments compared to the control (Figure 6).

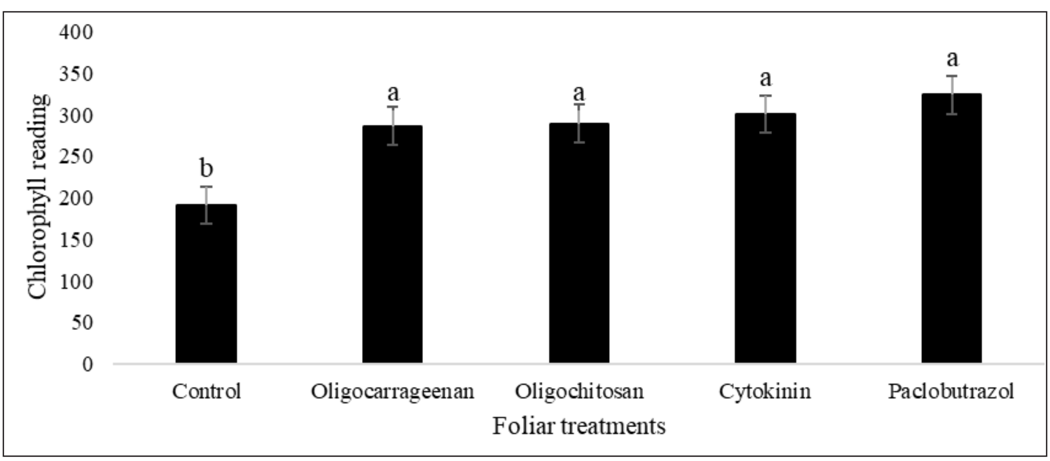

Figure 6. Effects of biostimulants and PGRs on the chlorophyll index of cacao shade leaves at 3 MAT based on the FieldScout CM 1000 under irrigated condition
cv (%)= 7.91; *p*= .0007**
Treatment means with the same superscript are not significant according to the Tukey's HSD test (p ≤.01)

Cacao Flowering in Response to Biostimulants and PGRs Under Different Irrigation Practices

Cushions

The percentage change in number of cushions in cacao was not significantly influenced by the interaction between irrigation practice and foliar treatments. At 4MAT, there was a significant (p= .0425) difference observed in the percentage change in number of cushions within cacao trees in response to foliar treatments under the irrigated experiment (Figure 7) but not in the rainfed condition. The highest percent change in number of cushions at 4MAT (370.24%) was notable on cacao trees applied with oligochitosan and was comparable to that of cacao trees applied with cytokinin (264.71%), paclobutrazol (277.72%), and the control (291.09). The oligocarrageenan treatment had resulted in lower percentage change in the number of cushions (228.78%) at 4MAT but was comparable to those of cytokinin, paclobutrazol, and control treatments under the irrigated experiment.

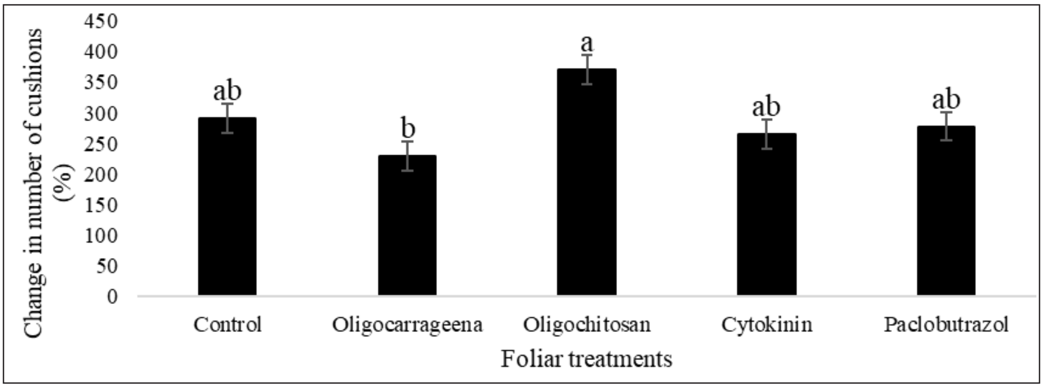

Figure 7. Percent change in number of cushions in cacao trees at 4MAT in response to foliar treatments under the irrigated condition
cv (%)= 13.38; p= 0.0192*
Treatment means with the same superscript are not significant according to the Tukey's HSD test ($p \leq .05$)

Flowers

The interaction between irrigation practice and foliar treatment was not significant on the percentage change in the number of flowers. The different foliar treatments, however, exhibited significant differences in terms of percentage change in the number of flowers formed at 4 MAT in the rainfed condition (Figure 8) and not observed in the irrigated experiment. Oligochitosan foliar spray had resulted in higher percentage change in the number of flowers but was comparable to those treated with oligocarrageenan, cytokinin, and paclobutrazol (Figure 8). The control trees exhibited lower percentage change in the number of flowers at 4MAT (Figure 8).

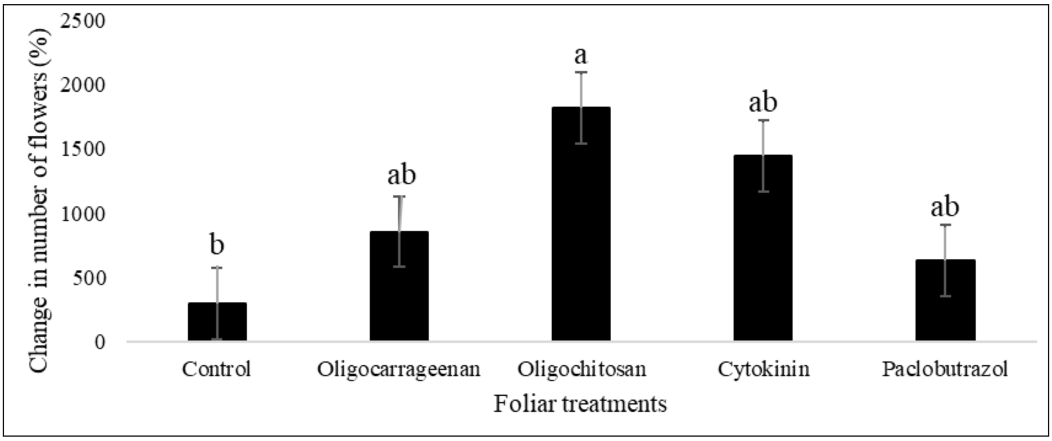

Figure 8. Effects of foliar treatments on the percentage change in the number of flowers in cacao trees at 4MAT under the rainfed condition
cv (%)= 49.51; *p*= .0325*
Treatment means with the same superscript are not significant according to the Tukey's HSD test ($p \leq .05$)

Effects of Biostimulants and PGRs on Cherelle Wilt Incidence and Pod Retention under Different Irrigation Practices

The cherelle wilt and pod retention were not influenced by the interaction between the irrigation practice and foliar treatment. The effects of biostimulants and PGRs on cherelle wilt incidence and pod retention were only significant under the irrigated condition and not observed in the rainfed experiment (Table 1). High incidence of cherelle wilt (68.50%) was observed from the control trees. In contrast, lower incidence of cherelle wilt (37.52%) was noted from trees treated with paclobutrazol. The cherelle wilt incidence also seems to have decreased considerably with cytokinin treatment but the effect was statistically comparable with the control (Table 1).

Table 1
Cherelle wilt incidence and pod retention of cacao trees in response to biostimulants and PGRs under the irrigated experiment

Foliar treatment	Cherelle wilt incidence (%)	Pod retention (%)
Control	68.50 ± 4.48^{a}	50.80 ± 21.08^{b}
Oligocarrageenan	56.47 ± 16.98^{ab}	55.03 ± 23.22^{ab}
Oligochitosan	54.96 ± 14.58^{ab}	47.32 ± 25.56^{b}
Cytokinin	43.98 ± 0.84^{ab}	85.03 ± 24.22^{a}
Paclobutrazol	37.52 ± 9.48^{c}	70.46 ± 24.47^{ab}
cv (%)	19.08	18.67
Pr (>F)	.0372*	.0148*

Treatment means within column with same superscript are not significant according to the Tukey's HSD test ($p \leq .05$)

The highest pod retention (85.03%) under the irrigated experiment was recorded in cytokinin-treated cacao trees. Lower pod retention was observed in cacao trees in the oligochitosan and control treatments with 47.32, and 50.80 % respectively (Table 1).

Effects of Biostimulants and PGRs on the Seed Fresh Weight of Cacao Under Different Irrigation Practices

Significant (*p*= .0280) interaction between the irrigation practice and foliar treatment on seed fresh weight of cacao was observed (Figure 9). Under the rainfed condition, lighter fresh seed weight was recorded from cacao in the control. Under the irrigated condition, the fresh seed weight of cacao in the control did not vary with the cacao applied with different foliar treatments (Figure 9).

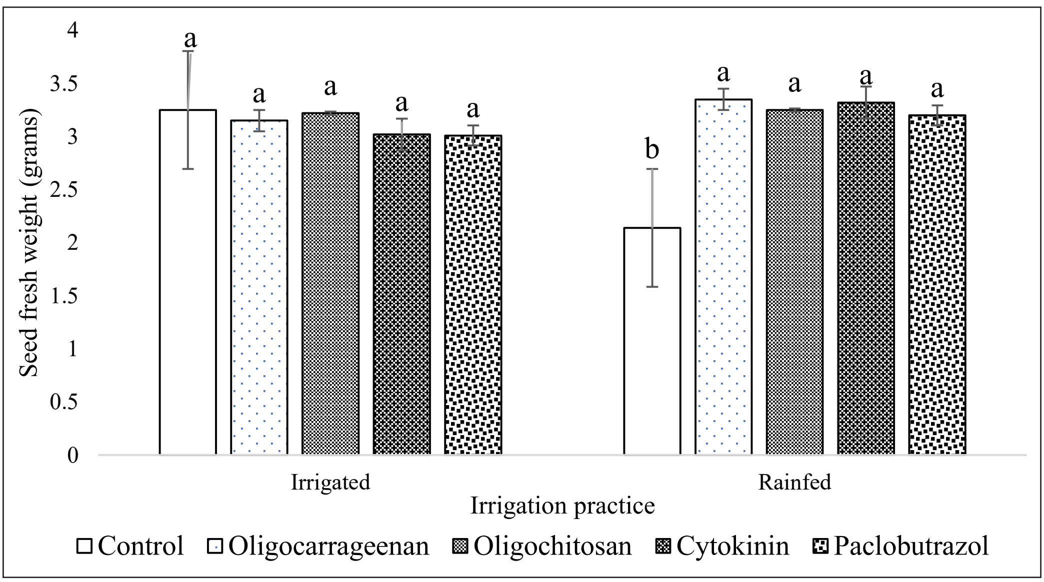

Figure 9. Interaction effect between the irrigation practice and foliar treatment on the mean seed fresh weight of cacao
cv (%)= 12.25; *p*= .0280*
Treatment means with the same superscript within same irrigation practice are not significant according to the Tukey's HSD test ($p \leq .05$)

Effects of Biostimulants and PGRs on the Production of Full Beans and Flat Beans Under Different Irrigation Practices

The interaction between irrigation practice and foliar treatment on the production of full beans and flat beans of a cacao tree was not significant (Table 2). Significant differences on the full beans and flat beans production of a cacao tree were however exhibited by those foliar treatments. As shown in Table 2, control trees produced lesser full beans and more flat

Table 2
Full beans and flat beans production of a cacao tree in response to biostimulants and PGRs under different irrigation practices

Foliar treatment (Fol)	Irrigation practice (IP)	Full beans (%) based on weight		Flat beans (%) based on weight		Full beans (%) based on number		Flat beans (%) based on number	
		$\bar{x}_1$	$\bar{x}_2$	$\bar{x}_1$	$\bar{x}_2$	$\bar{x}_1$	$\bar{x}_2$	$\bar{x}_1$	$\bar{x}_2$
Control	Irrigated	94.84±1.3	$94.54±2.5^b$	5.16±1.3	$5.46±2.5^a$	85.26±1.0	$83.87±6.9^b$	14.74±1.0	$16.13±6.9^a$
Control	Rainfed	94.24±3.5		5.76±3.5		82.48±8.8		17.52±8.8	
Oligocarrageenan	Irrigated	98.95±1.1	$98.82±2.6^a$	1.05±1.1	$1.18±2.6^b$	97.11±2.4	$94.04±6.8^a$	2.89±2.4	$5.96±6.8^b$
Oligocarrageenan	Rainfed	98.69±0.8		1.31±0.8		90.97±7.5		9.03±7.5	
Oligochitosan	Irrigated	99.19±0.5	$98.71±2.6^a$	0.81±0.5	$1.29±2.6^b$	97.01±2.4	$96.04±6.8^a$	2.99±2.4	$3.96±6.8^b$
Oligochitosan	Rainfed	98.23±1.9		1.77±1.9		95.07±5.2		4.93±5.2	
Cytokinin	Irrigated	97.88±1.0	$98.29±2.2^a$	2.12±1.0	$1.71±2.2^b$	94.84±1.7	$94.95±6.6^a$	5.16±1.7	$5.05±6.6^b$
Cytokinin	Rainfed	98.70±0.5		1.30±0.5		95.06±1.7		4.94±1.7	
Paclobutrazol	Irrigated	97.54±1.6	$97.38±2.2^a$	2.46±1.6	$2.62±2.2^b$	90.31±4.3	$90.23±6.4^a$	9.69±4.3	$9.77±6.4^b$
Paclobutrazol	Rainfed	97.23±1.3		2.77±1.3		90.16±2.7		9.84±2.7	
cv (%)		1.65	1.65	65.78	65.78	5.17	5.17	58.09	58.09
Pr (>F)	IP	$.6547^{ns}$	-	$.6547^{ns}$	-	$.1686^{ns}$	-	$.1686^{ns}$	-
	Fol	.0016**	.0016**	.0016**	.0016**	.0026**	.0026**	.0026**	.0026**
	IP:Fol	$.9008^{ns}$	-	$.9008^{ns}$	-	$.7829^{ns}$	-	$.7829^{ns}$	-

$\bar{x}_{1-M}$eans of foliar treatment in irrigated and rainfed experiments, and irrigation practice for the same foliar treatment are not significant according to Tukey's HSD test
$\bar{x}_2$- Superscript represent the significance between foliar treatment means within the column according to the Tukey's HSD test
ns- not significant
**- significant at $p \leq .01$

beans. This situation was reversed by the foliar spray of biostimulants (oligocarrageenan and oligochitosan) and PGRs (cytokinin and PBZ).

Effects of Biostimulants and PGRs on the Dried Bean Weight of Cacao under Different Irrigation Practices

There was no significant interaction between irrigation practice and foliar treatments on the dried bean weight of cacao. The dried bean, however, was significantly (p= .0287) influenced by the foliar treatment (Figure 10). The cacao in the control treatment produced lighter dried beans (1.35 g). Foliar spray of oligocarrageenan resulted in heavier dried beans (1.50 g) of cacao as shown in Figure 10.

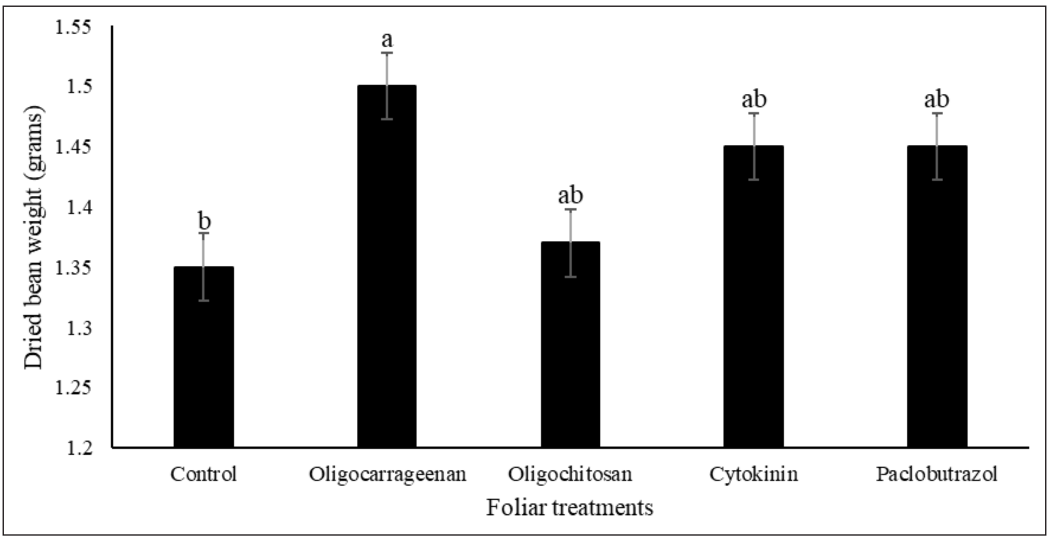

Figure 10. Average dried bean weight of cacao in response to foliar treatments
cv (%)= 5.85; p= .0287*
Treatment means with the same superscript are not significant according to the Tukey's HSD test ($p \leq .05$)

Effects of Biostimulants and PGRs on the Bean Count of Cacao under Different Irrigation Practices

There was no significant interaction observed between irrigation practice and foliar treatment on the bean count of cacao. Rather, the bean count of cacao was significantly (p= .0378) influenced by the foliar treatment (Figure 11). Highest bean count, meaning lighter beans, was recorded in the control trees with 74.42. In contrast, the oligocarrageenan foliar spray resulted in lowest or better bean count (67.50).

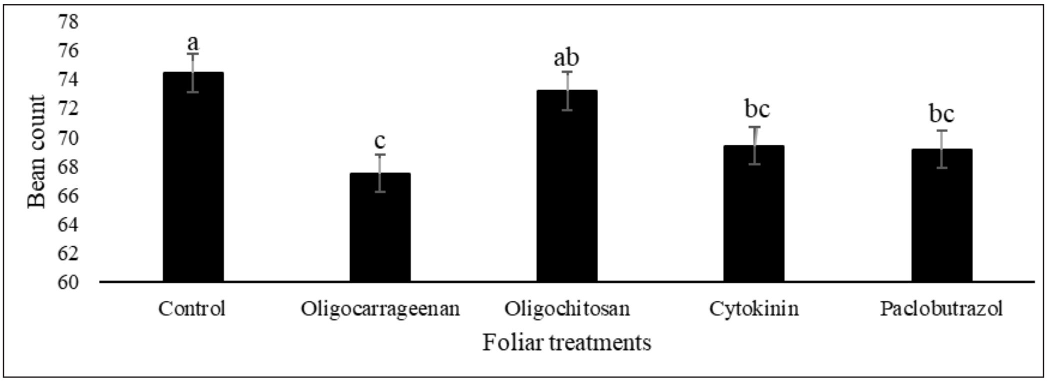

Figure 11. Bean count of cacao in response to foliar treatments
cv (%)= 5.60; *p*= .0378*
Treatment means with the same superscript are not significant according to the Tukey's HSD test (p ≤.05)

Dry Weight per Tree of Marketable Beans in Response to Biostimulants and PGRs

The dry weight (DW) per tree of marketable beans considered here was only for one month (May) harvest. The irrigation practice during the dry season did not affect the efficacy of biostimulants and PGRs on the dried weight of marketable beans in cacao. Foliar treatments, however, influenced the DW per tree of 'UF 18' cacao. Heavier DW per tree of marketable beans was achieved through the foliar spray of cytokinin, paclobutrazol, and oligocarrageenan (Figure 12). This is comparable to the DW per tree of marketable beans of oligochitosan-treated trees. The control trees had the lowest yield per tree but was comparable with those of oligochitosan-treated trees (Figure 12).

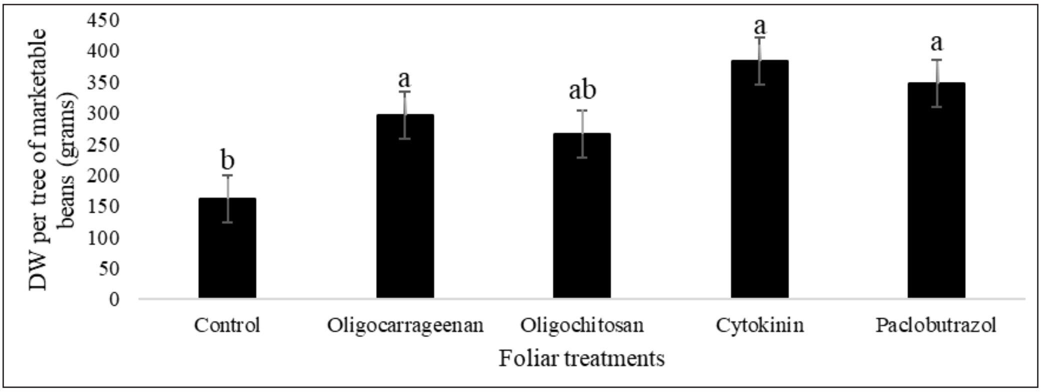

Figure 12. Dry weight per tree of marketable beans
cv (%)= 33.87; *p*= .0151*
Treatment means with the same superscript are not significant according to the Tukey's HSD test (p ≤.05)

Yield per Tree per Year of Cacao in Response to Biostimulants and PGRs under Bukidnon, Philippines

The estimated yield per tree per year of cacao is shown in Table 3. During the wet season (irrigated), the yield per tree estimate of the control trees is doubled through the cytokinin foliar spray. However, foliar spray of biostimulants and PGRs during the dry season (rainfed) did not cause a significant yield difference compared to the control. The foliar spray of cytokinin, paclobutrazol, and oligocarrageenan, however, showed to be promising in producing higher yield per tree per year with 2.6226, 2.3614, and 1.9834 kg respectively compared to the control with only 1.1120 kg. Likewise, oligochitosan resulted in a slight increase of yield per tree per year versus the control.

Table 3
Estimated yield per tree per year of cacao in response to biostimulants and PGRs under Bukidnon, Philippines condition

Foliar treatment	Yield per tree per season (kg)		Yield per tree per year (kg)	Yield difference versus the control (kg)
	Dry (rainfed)	Wet (irrigated)		
Control	0.3746±0.231	0.7374±0.132^{b}	1.1120±0.080^{c}	-
Oligocarrageenan	1.1485±0.120	0.8349±0.189^{ab}	1.9834±0.628^{ab}	0.8714
Oligochitosan	0.9753±0.773	0.8108±0.136^{ab}	1.7861±0.471^{b}	0.6741
Cytokinin	1.0016±0.169	1.6210±0.302^{a}	2.6226±0.004^{a}	1.5106
Paclobutrazol	1.0574±0.511	1.3040±0.413^{ab}	2.3614±0.258^{ab}	1.2494
cv (%)	38.19	28.70	17.93	-
P (>F)	.1424^{ns}	.0290*	.0065**	-

^{ns}- not significant; *- significant at p ≤.05; **- significant at p ≤.01
Treatment means with the same superscript are not significant according to the Tukey's HSD test

DISCUSSION

Cacao trees can be cultivated as a monocrop and an intercrop to existing perennial crops such as coconut and banana. But the latter is ideal especially during the dry season as it creates microclimate that minimise evapotranspiration to retain more cacao pods per tree. However, not all local farmers venturing cacao production employs intercropping. Supplemental irrigation during the dry season may be an alternative for a monocrop cacao plantation, however, this practice is costly and labourious. Thus, biostimulants and plant growth regulators (PGRs) that will increase pod retention and yield per tree in monocropped cacao plantations during water-limiting condition or during the dry season in the Type III climate of the country is a potential solution, hence this study.

The biostimulants tested in this study are the oligocarrageenan and oligochitosan, as these two are gaining popularity nowadays. The oligocarrageenan and oligochitosan biostimulants have been reported to reduce the negative impact of limited water conditions in various crops by stimulating some plant physiological processes that improved plant growth and yield (Moenne & Gonzalez, 2021; Thye et al., 2022; Bongalos et al., 2019; Vicena et al., 2024). On the other hand, the PGRs used in this study are cytokinin and PBZ. Cytokinin is a phytohormone which prevents degradation of leaf chlorophyll especially during abiotic stress conditions. Also, PBZ application has been reported to increase cytokinin levels according to several studies cited by Desta and Amare (2021). Foliar applications of these PGRs could improve nutrient uptake as well as photosynthesis of cacao during the dry season.

Chlorophyll is the green pigment in the chloroplast responsible for capturing photosynthetically active radiation (Mandal & Dutta, 2020). Thus, plants with higher leaf chlorophyll content presumably have better photosynthetic efficiency. Generally, the foliar applications of biostimulants and PGRs increased the chlorophyll index of cacao leaves in this study. This finding is supported by numerous reports that oligocarrageenan and oligochitosan increased the chlorophyll content of leaves which allow plants to synthesise more photoassimilates (Abad, Dean et al., 2018; Chen et al., 2023; Hossain et al., 2024; Khalil & Eldin, 2021; Li et al., 2021; Naeem et al., 2014; Safikhan et al., 2018; Shukla et al., 2016; Thye et al., 2022) that are needed especially by the developing fruits. Similarly, cytokinin and PBZ are PGRs that enhance chlorophyll biosynthesis in plant leaves as well as preventing chlorophyll degradation (Desta & Amare, 2021; Fathy et al., 2022; Fletcher et al., 1982; Liu et al., 2022; Solichatun et al., 2021; Tesfahun, 2018; Xu & Huang, 2017) which supports the result of this study.

It has been reported that oligochitosan enhances the flowering intensity of plants (Ahmed et al., 2016; Sultana et al., 2017) which conforms to the result of this study. In any fruit crop production, however, fruit setting is more important than just flowers. Once cacao flowers are successfully pollinated, these will become cherelles. The young cherelles (<BBCH 75) are susceptible to wilting especially when there is a limited supply of photoassimilates within a tree. In this study, the incidence of cherelle wilt was reduced through the foliar spray of paclobutrazol. Moreover, cytokinin resulted in higher retention of cacao pods which corroborates to the report of Kumara et al. (2023) that cytokinin enhances fruit retention in mango trees.

The aim of tailoring horticultural technology in fruit and plantation crops production is to improve yield and quality. This study reveals that the average weight of fresh cacao bean was lighter in experiment set-up without supplemental irrigation during the dry season. This condition was reversed with the foliar spray of biostimulants and PGRs. Among foliar treatments, oligocarrageenan resulted in the production of heavier dried beans. Moreover, foliar spray of biostimulants and PGRs resulted in better bean count, with more full beans, and lesser flat beans than the control.

Cacao dried beans are the raw material in making chocolates. In the Philippines, the target yield per tree per year of cacao is set at 2 kg. However, average production ranges only from 0.5 to 1.0 kg per tree per year (DA, 2017) despite the use of high-yielding grafted cacao clones. In this study, the estimated yield per tree per year of cacao not applied with biostimulants or PGRs is at 0.82 kg. The foliar spray of oligocarrageenan, oligochitosan, cytokinin, and paclobutrazol exhibited as a promising solution to improve the yield per tree per year of 'UF 18' cacao by ~0.8714, ~0.6741, ~1.5106, and ~1.2494 kg of dried beans, respectively under Bukidnon, Philippines condition.

Unlike fertilisers and phytohormones, biostimulants do not directly supply nutrients and hormonal needs of crops (Gupta et al., 2023). Instead, biostimulants stimulate plant physiological processes that enhance nutrient uptake and accumulation of dry matter and eventually improve crop yield (Jardin, 2015) especially under abiotic stress conditions (Li et al., 2022). Numerous reports corroborate the findings of this present study that oligocarrageenan foliar spray improves crop yield. For example, oligocarrageenan had improved the yield of peanut (Abad, Aurigue et al., 2018), mungbean (Gatan et al., 2019), and coffee (San et al., 2020) which supports the findings of this present study. On the other hand, PGRs are classic compounds used to improve crop yield under abiotic stress such as water-limiting conditions. These compounds directly regulate the plant's endogenous hormone level that led to an accumulation of more photoassimilates and thereby increasing dry matter and yield of crops (Sosnowski et al., 2023). Among the phytohormones, cytokinin treatment is proven to improve yield of crops as this hormone is responsible for maintaining leaf chlorophyll, enriching plant nutrients, cell division, and have a strong impact on crop yield (Mandal et al., 2022; Prasad, 2022; Sharma et al., 2022). These recognised effects of cytokinin in plants might have also occurred in this present study, thus improving cacao yield performance especially during the dry season in Bukidnon, Philippines. In comparison, paclobutrazol has been well-studied and known to improve the yield of mango (Yeshitela et al., 2004) and cacao (Nieves et al., 2024) trees. Several studies cited in the review of Desta and Amare (2021) have shown that the paclobutrazol application resulted in increased levels of cytokinin and regulates the sink-source relationships of trees, thus this PGR also improves the yield of cacao in this present study.

CONCLUSION AND RECOMMENDATION

This is the first report that the biostimulants (oligocarrageenan and oligochitosan) are bioactive in cacao and positively influenced the reproductive characters of cacao under water stress. Likewise, PGRs (cytokinin and PBZ) also improved the reproductive characters of cacao during water stress. Among the foliar treatments, oligochitosan exhibited potential to increase cacao flowering intensity. Cherelle wilt incidence is reduced by paclobutrazol treatment, while cytokinin increased the pod retention of cacao. Generally, the biostimulants and PGRs foliar spray improved most of the yield parameters of cacao measured in this study during the dry season in Bukidnon, Philippines. Among the biostimulants and PGRs tested, oligocarrageenan, cytokinin, and paclobutrazol were noteworthy in the production of heavier dry weight per tree of marketable beans. The foliar spray of biostimulants and PGRs resulted in significant increase versus the control in terms of yield per tree per year of cacao. Thus, foliar sprays of these biostimulants and PGRs are recommended to boost cacao production during the dry season.

ACKNOWLEDGMENT

The senior author extends his infinite gratitude to the Department of Science and Technology's Accelerated Science and Technology Human Resource Development Programme and Central Mindanao University for the PhD scholarship grant.

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