



ORIGINAL PAPER

# Biostimulants and Paclobutrazol Foliar Sprays for the Alleviation of Black Pod Rot (*Phytophthora palmivora* Butler) Stress in Cacao (*Theobroma cacao* L.)

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## Abstract

Relying solely on synthetic fungicides to manage plant diseases poses environmental and human health concerns, suggesting the need to find alternative measures to reduce the impact of diseases on crop production. This study aimed to demonstrate the effectiveness of biostimulants and plant growth regulator (PGR) as foliar sprays for mitigating the *Phytophthora palmivora* Butler black pod rot (BPR) stress in *Theobroma cacao* L. Biostimulants (oligocarrageenan and oligochitosan), PGR (paclobutrazol), fosetyl-Al (positive control), and tap water (negative control) were applied as foliar sprays to BPR-infected cacao trees in monocrop and coconut–cacao intercrop planting systems. Laboratory or in vitro experiments were also conducted to determine the efficacy of biostimulants and paclobutrazol as protectants and eradicants of *P. palmivora* BPR. The results showed that the untreated intercropped cacao trees produced lighter pods. The positive effects of biostimulants and paclobutrazol on improving pod weight were observed only in the intercropping system but not in the monocrop system. The oligocarrageenan–treated cacao produced more and heavier fresh seeds, as well as heavier dried beans, resulting in a better bean count and pod index. Similarly, oligochitosan spray improved the bean production of a cacao pod in quantity and weight, resulting in a better pod index. Paclobutrazol improved the seed fresh weight and pod index. The in vitro experiments revealed that the efficacy of oligocarrageenan, oligochitosan, and paclobutrazol as protectants and eradicants of BPR was comparable to that of the fosetyl-Al. The results demonstrated the effectiveness of biostimulants and paclobutrazol for mitigating the BPR disease on cacao production.

**Keywords** Biotic stress · Disease tolerance · Elicitors · Oligosaccharins · Plant growth regulator

## 1 Introduction

Diseases are a major constraint in cacao (*Theobroma cacao* L.) production, limiting the yield potential of cultivars. Cacao diseases can cause yield losses of approximately 30–40% of the annual global yield (Ploetz 2016). The three most severe diseases, black pod rot (*Phytophthora* spp.), frosty pod rot (*Moniliophthora roreri*) and witches' broom (*Moniliophthora perniciosa*), are referred to as the 'trilogy

of crippling fungal diseases' in cacao (Evans 2007). In the Philippines, major cacao diseases include vascular streak dieback (VSD) caused by *Ceratobasidium theobromae* and black pod rot (BPR) caused by *Phytophthora palmivora* (Solpot 2020).

Black pod rot (BPR) caused by *Phytophthora palmivora* is globally important in cacao, resulting in 20–30% yield losses and up to 10% tree mortalities annually, with wetter cocoa-growing areas potentially facing total losses (Gates 2007). *Phytophthora* species infect all parts of the cacao plant from the seedling to mature stages (McMahon and Purwantara 2004). This pathogen can thrive in the soil beneath the cacao canopy. Black pod rot can be controlled through foliar spraying of synthetic fungicides, but environmental concerns arise (Leyte et al. 2017). It has been reported that relying solely on one synthetic fungicide in all cropping seasons can lead to fungal resistance (Parra and Ristaino 2001). Cacao breeding offers long-term solutions

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to this issue; however, traditional breeding methods are slow (Motamayor et al. 2008). The use of new techniques in crop breeding, such as molecular marker-assisted selection, also incurs a high financial cost. Therefore, alternative disease control methods should be explored.

Plants possess mechanisms that inhibit the spread of invading pathogens within the plant cells. One such example is the hypersensitive reaction (HR). Once HR is activated, plant tissues may acquire systemic acquired resistance (SAR) to a wide spectrum of diseases for an extended period. SAR can be artificially induced by spraying plants with substances that are known as plant activators. Biostimulants and plant growth regulators are examples of plant activators gaining popularity in agriculture because of their lower risk to humans and wildlife than fungicides or antibiotics and their long-lasting protective benefits.

Biostimulants are becoming more popular in crop production, as they provide plant defense under biotic stress conditions and are a cheaper alternative to plant disease control. Biostimulants are categorized as microbial or nonmicrobial (Rouphael and Colla 2020; Demirel et al. 2024). Chitosan and seaweed extracts (e.g., carrageenan) are examples of nonmicrobial biostimulants known to stimulate growth and provide protection to various crop species (du Jardin 2015). Chitosan and carrageenan are derived from crustaceans, fungal exoskeletons, and seaweeds (du Jardin 2015; Huq et al. 2022), respectively. The irradiation of chitosan and carrageenan results in the formation of shorter-chain oligosaccharides, known as oligosaccharins (oligochitosan; oligocarrageenan), which act as signaling molecules to elicit the production of metabolites such as phytoalexins and regulate metabolic pathways for plant defense against pathogens (Albersheim and Darvill 1985; Cote and Hahn 1994; Ochoa-Meza et al. 2021).

Oligochitosan and oligocarrageenan are effective in controlling plant pathogens. Oligochitosan has direct antimicrobial effects against plant pathogens and elicits the synthesis of secondary metabolites for plant defense (Riseh et al. 2022) which are supported by various reports. For example, Eysanti et al. (2019) reported that pathogenicity, disease incidence, and severity indices of *Phytophthora capsici* in chili plants are reduced in response to oligochitosan treatment. As an elicitor, oligochitosan induces phytoalexin (pisatin) synthesis which has antipathogenic action and a stronger activation of glucanase and chitinase in pea plants (Kendra et al. 1989). Also, markers of plant defense responses such as lipoxygenase (LOX), phenylalanine ammonia-lyase (PAL), and chitinase activities are induced by oligochitosan which makes the grapevine leaves resist *Botrytis cinerea* (Trotel-Aziz et al. 2006). In comparison, oligocarrageenan exhibits direct antimicrobial activities (Mani and Nagarathnam 2018) and modulates the activity of different plant defense signaling pathways, including jasmonate, salicylate,

and ethylene (Ghannam et al. 2013) which elicits the production of phytoalexins and pathogenesis related (PR) proteins (Hossain et al. 2024). In turn, systemic acquired resistance and/or induced systemic resistance are activated causing plants to become resilient against pathogens (Hossain et al. 2024). Oligocarrageenan also promotes the production of antioxidant enzymes in plants (Hossain et al. 2024) which regulate the reactive oxygen species (ROS), thus, preventing oxidative damage in plant cells and making them more tolerant to biotic stress (Krishnamurthy and Rathinasabapathi 2013).

On the other hand, paclobutrazol was originally developed as a fungicide in the triazole family. Several studies cited in a review by Desta and Amare (2021) have shown that paclobutrazol is an effective systemic fungicide against fungal diseases. Jacobs and Berg (2000) reported that paclobutrazol at relatively high concentrations (25–100%) inhibits the mycelial growth of fungal pathogens that attack woody plants. Similarly, the mycelial growth of soil-borne pathogens (*Macrophomina phaseoli* and *Fusarium oxysporum* f. sp.) was inhibited by paclobutrazol at 40 and 50 ppm, respectively (Bolu and Cimen 2006). Paclobutrazol belongs to the triazole group and inhibits the cytochrome P-450-dependent 14 $\alpha$  demethylation step in ergosterol biosynthesis in some fungi (Burden et al. 1987).

Despite the potential of oligocarrageenan, oligochitosan, and paclobutrazol for alleviating the negative effects of diseases on plant growth, there is no available information on the mitigating effects of biostimulants (oligocarrageenan and oligochitosan) and paclobutrazol treatments on cacao yield parameters under black pod rot stress. Additionally, the efficacy of oligocarrageenan, oligochitosan, and paclobutrazol as protectants and eradicants of cacao BPR is currently unknown. Thus, this study aimed to demonstrate the effectiveness of biostimulants and plant growth regulator (PGR) foliar sprays for alleviating the negative impact of *P. palmivora* black pod rot (BPR) in cacao.

## 2 Materials and methods

### 2.1 Laboratory experiments

#### 2.1.1 Collection, isolation, and pathogenicity testing of *Phytophthora palmivora*

*Phytophthora palmivora* was isolated from cacao pod rot samples collected from the clonal garden of Central Mindanao University, Musuan, Maramag, Bukidnon, Philippines. Cacao pods showing symptoms of black pod rot were collected and processed at the CMU Plant Disease Clinic Laboratory. The samples were washed in running tap water, surface-sterilized in 10% commercial bleach for 5 min, rinsed

three times with sterilized distilled water, and blotted dry sections (approximately 2 mm<sup>2</sup>) from the edge of an actively growing lesions were excised.

V8 juice agar (V8JA) medium (3 g of calcium carbonate, 17 g of agar, 200 mL of V8 juice, and 1 L of sterilized distilled water) was prepared and supplemented with 4 mL of nystatin 200 mL<sup>-1</sup>. The cut sections were then planted in sterile petri plates with one drop of lactic acid to suppress bacterial growth before the V8 juice agar was poured. The seeded plates were sealed and incubated in an inverted position at room temperature until mycelial growth was observed. Briefly, the descriptions given by Erwin and Ribeiro (1996) was followed to identify the *P. palmivora*. A segment of fungal growth emerging from the seeded tissues was transferred to a V8JA culture plate for purification and maintenance of the isolates. A pure culture from the CMU Plant Disease Culture Laboratory was used for pathogenicity tests.

Pathogenicity tests were performed by pricking surface-sterilized cacao pods with a needle. A mycelial disc (19.64

mm<sup>2</sup>) from a pure culture of the pathogen was then placed on the pricked surface. An agar disc from the control plate was introduced onto the pricked surface. Treated and control pods were incubated inside plastic bags with a wetted gauze pad until symptoms appeared on the pricked surface.

Initially, the pathogen was cultured on V8 juice agar media. However, the pathogen growth in this medium was very slow (Fig. 1). Therefore, the pathogen was reisolated from the most severely infected pods and grown on carrot agar. Petri dishes containing cultured pathogens were then exposed to continuous light with modifications from the procedure of Schmitthenner and Bhat (1994) to allow sporangial development.

A pathogenicity test was conducted to confirm the virulence of oomycetes isolated from infected cacao pods and cultured. The inoculated pods exhibited brown pod rot (Fig. 2). A pod displaying advanced symptoms was selected for reisolation of the pathogen in the laboratory. Microscopic examination revealed sporangia of *P. palmivora* from infected cacao pods (Fig. 3).

**Fig. 1** Growth of *P. palmivora* in V8 juice and carrot juice agar media

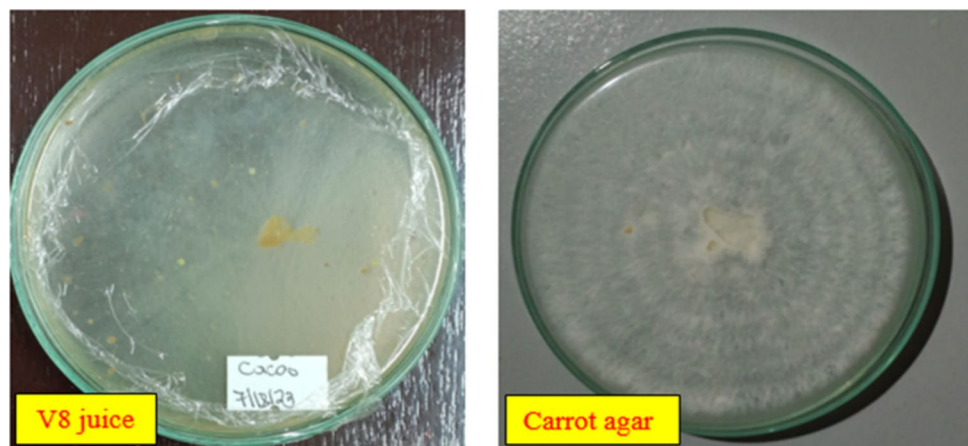


**Fig. 2** The result of the pathogenicity test shows the pod rot symptom caused by *P. palmivora*. The photo was taken 4 days post inoculation. The pod displaying advanced symptoms was brought to the Pest Disease Clinic of Central Mindanao University for re-isolation of the pathogen





**Fig. 3** Sporangia of *P. palmivora* (pointed by red arrows) exposed during the microscopic examination

### 2.1.2 Laboratory experimental design and treatment application

The experiment was conducted in a completely randomized design (CRD) with three replications. The treatments included distilled water (control) and various concentrations (50, 100, 200, 500, 1000, 2000, and 4000 ppm) of oligochitosan, oligocarrageenan, paclobutrazol, and Aliette®. Healthy ‘UF-18’ cacao pods (BBCH 79–80 according to Azogue et al. [2023] classification) were collected and surface sterilized.

### 2.1.3 Efficacy of biostimulants and PGR as protectants

The pods were placed under running tap water to remove any surface dirt. These were then surface sterilized before proper inoculation. Test solutions of various concentrations were sprayed onto the surface using an atomizer and allowed to dry completely. Pathogens from the agar disc cultures were collected using a 5 mm hollow punch (Lotus LTHT900HPX) and introduced at the point of inoculation (Fig. 4). The inoculated pods were then individually placed inside a sterilized plastic bag with a wet cotton ball. The experimental setup was concluded 9 days after treatment application.

### 2.1.4 Efficacy of biostimulants and PGR as eradicants

The setup was similar to that described above, with the only difference being that the pathogens from the agar disc were introduced at three inoculation points (base, middle, and tip of the pods). Control pods were introduced to agar discs that were free of pathogens. All inoculated pods were placed individually in a sterilized plastic bag for two days to allow the pathogen to establish at the inoculation points. After incubation, the pods were sprayed with different

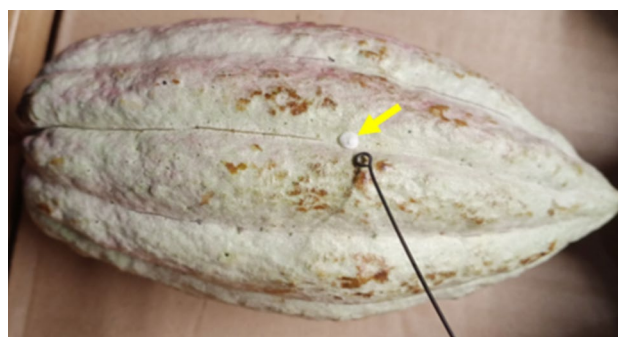


**Fig. 4** *Phytophthora palmivora* was introduced on top of the pricked surface of the cacao pods using an agar disc culture (as indicated by the yellow arrow)

concentrations of the biostimulants and PGR. The disease severity rating of the pods was assessed seven days after the treatment application.

### 2.1.5 Data gathered in the laboratory experiment

**Percent disease incidence** The number of cacao pods showing BPR symptoms was counted and recorded at the end of the experiment. The percentage of disease incidence was calculated using the following equation.

$$\text{Disease incidence(\%)} = \frac{\text{Number of pods infected with BPR}}{\Sigma \text{ pods}} \times 100$$

**Disease severity** The severity of BPR symptoms exhibited by cacao pods in response to the varying concentrations of different foliar treatments, at the end of the experiment, were assessed using the disease severity rating table (Table 1).

## 2.2 Field experiments

### 2.2.1 Time and place of the study

Two field experiments were conducted simultaneously in monocrop and intercrop cacao plantations from December

**Table 1** Disease severity rating used in the laboratory experiment

| Rating | Severity rating   | Lesion diameter (cm) |
|--------|-------------------|----------------------|
| 1      | Very low          | 0.0–3.0              |
| 2      | Low               | 3.1–6.0              |
| 3      | Moderately low    | 6.1–9.0              |
| 4      | Moderately severe | 9.1–12.0             |
| 5      | Severe            | 12.1–15.0            |
| 6      | Highly severe     | > 15.0               |

2022 to May 2023. Six-year-old grafted ‘UF 18’ cacao trees with developing pods and cherelles were selected.

In the first experiment, cacao trees were grown as monocrops under high-density planting (1,111 plants per hectare) in Bangcud, Malaybalay City, Bukidnon (latitude: 7.991817°, longitude: 125.131607°, altitude: 326.71 m). The cacao trees in the second experiments were intercropped with approximately 40-year-old coconut palms (about 15 m tall) located in V3M7+47R National Coconut Project (latitude: 7.884229°, longitude: 125.062657°, altitude: 335.62 m) at Central Mindanao University.

In the intercropping system, coconut palms were planted at a spacing of 9×9 m, with two rows of cacao trees planted between the coconut palms at a distance of 3×3 m. The population of cacao trees per hectare in this planting system is approximately 667.

### 2.2.2 Soil characteristics, field experiment setup and foliar treatment applications

The soil texture of the experimental areas is clay loam. Soil moisture content in both experimental setups was maintained at  $\geq 75\%$  by providing supplemental irrigation. The average soil pH in the intercrop and monocrop sites were 5.63 and 5.64, respectively.

The incidence of BPR was recorded in each experiment before foliar treatment. At the monocropped site,  $4.13 \pm 1.19$  pods per tree were infected with BPR, whereas at the intercropped site, the number was  $6.07 \pm 1.10$  pods. Only trees with BPR-infected cacao pods and those bearing pods at BBCH75 to BBCH78 (Azogue et al. 2023) were chosen as experimental trees for this study. The BPR-infected pods were removed from the trees before foliar application.

The study consisted of two sets of experiments, with treatments in each setup replicated three times, totaling 15 experimental units. Each experimental unit included two trees. The treatments included 1) tap water (control); 2) 150 ppm oligocarrageenan; 3) 150 ppm oligochitosan; 4) 500 ppm paclobutrazol; and 5) 3400 ppm Aliette® WP fungicide (80% fosetyl-Al, Bayer CropScience, St. Louis, MO, USA; as a positive control). The sources of oligocarrageenan, oligochitosan, and paclobutrazol were VitalGro carrageenan® [5,500 ppm carrageenan byproducts as the active ingredient (a.i.)], chitosan oligosaccharide® soluble powder (99% a.i.), and Greenfast® (25SC PBZ), respectively. The treatments were applied using a hand-operated shoulder sprayer (Lotus LTGT5000PSX) with an operating pressure of 0.3 Mpa and a spray volume of 5 L tree<sup>-1</sup>. The pH of the water used in the solutions was 7.1. The aboveground parts of the trees were sprayed with the treatment solution, which was applied to the plants in both sets of experiments (monocrop and intercrop).

### 2.2.3 Observations and measurements

Before treatment, diseased pods were removed from the trees. Observations and measurements were taken for all pods (BBCH 75 to BBCH 78) remaining on each tree, which served as the baseline count for healthy pods. Pod development was monitored until BBCH 81 (Azogue et al. 2023), as shown in Fig. 5. The experiment was continued until all initial pods (BBCH 75 to BBCH 78) from all the experimental trees had ripened and were harvested for data collection on yield and pulp juice quality.

### 2.2.4 Data gathered in the field experiment

The pods displaying BPR symptoms were counted and recorded weekly. Data on the pod weight, fresh weight of the seeds per pod, number of seeds per pod, and average fresh weight of the seeds were collected immediately after harvest. The dried bean weight, bean count and pod index parameters were collected after the seeds were dried to a moisture content of 7%.

## 2.3 Statistical analysis

Analysis of variance was performed using Statistical Analysis for Agricultural Research (STAR 2.0.1) software (<http://bbi.irri.org/products>). The treatment means were compared using Tukey’s Honest Significant Different (HSD) test at the 5% level of significance.



Fig. 5 UF 18 cacao pod at BBCH 81 stage

### 3 Results

#### 3.1 Biostimulants and paclobutrazol as protectants and eradicants of *P. palmivora* BPR

##### 3.1.1 Biostimulants and paclobutrazol as protectants

The lesions on the cacao pods in the control treatment were visible as early as 3 days post inoculation. By 9 days post inoculation, the *P. palmivora* BPR disease had continued to spread on the surface of the cacao pods in the control treatment, reaching a disease severity rating of 5, which was classified as a severe disease (Table 2). Compared with the control, the most effective concentrations of oligocarrageenan, oligochitosan, and paclobutrazol as

protectants for preventing *P. palmivora* BPR disease were 100–4000, 100/1000–4000, and 100–2000 ppm, respectively (Table 2). These treatment concentrations significantly reduced the severity of BPR disease to very low levels. These treatments and concentrations were comparable to those of the Aliette® fungicide at concentrations ranging from 50–2000 ppm. Other concentrations of oligocarrageenan, oligochitosan, paclobutrazol, and Aliette also resulted in some reduction in infection, but the results revealed only a low severity of disease, with no significant variation from the control treatment.

In terms of the percentage of cacao pods exhibiting BPR symptoms, oligocarrageenan and paclobutrazol at concentrations of 1000–4000 and 200–2000 ppm, respectively completely suppressed the occurrence of BPR symptoms in all cacao pod samples. The oligochitosan (1000

**Table 2** Efficacies of biostimulants and paclobutrazol as protectants for cacao pods against BPR

| Treatment                 | <sup>1/</sup> Diameter of lesion (cm) | <sup>1/</sup> BPR infected pods (%) | Disease severity rating |                |
|---------------------------|---------------------------------------|-------------------------------------|-------------------------|----------------|
|                           |                                       |                                     | Rating                  | Description    |
| Control                   | 17.17 <sup>a</sup>                    | 100.00 <sup>a</sup>                 | 5                       | Severe         |
| 50 ppm Oligocarrageenan   | 2.33 <sup>b</sup>                     | 33.33 <sup>b</sup>                  | 2                       | Low            |
| 100 ppm Oligocarrageenan  | 1.25 <sup>b</sup>                     | 33.33 <sup>b</sup>                  | 1                       | Very low       |
| 200 ppm Oligocarrageenan  | 0.28 <sup>b</sup>                     | 33.33 <sup>b</sup>                  | 1                       | Very low       |
| 500 ppm Oligocarrageenan  | 0.67 <sup>b</sup>                     | 33.33 <sup>b</sup>                  | 1                       | Very low       |
| 1000 ppm Oligocarrageenan | 0.00 <sup>b</sup>                     | 0.00 <sup>b</sup>                   | 1                       | Very low       |
| 2000 ppm Oligocarrageenan | 0.00 <sup>b</sup>                     | 0.00 <sup>b</sup>                   | 1                       | Very low       |
| 4000 ppm Oligocarrageenan | 0.00 <sup>b</sup>                     | 0.00 <sup>b</sup>                   | 1                       | Very low       |
| 50 ppm Oligochitosan      | 8.08 <sup>ab</sup>                    | 66.67 <sup>a</sup>                  | 3                       | Moderately low |
| 100 ppm Oligochitosan     | 0.67 <sup>b</sup>                     | 33.33 <sup>b</sup>                  | 1                       | Very low       |
| 200 ppm Oligochitosan     | 2.58 <sup>b</sup>                     | 33.33 <sup>b</sup>                  | 2                       | Low            |
| 500 ppm Oligochitosan     | 1.33 <sup>ab</sup>                    | 50.00 <sup>ab</sup>                 | 1                       | Very low       |
| 1000 ppm Oligochitosan    | 0.05 <sup>b</sup>                     | 16.67 <sup>b</sup>                  | 1                       | Very low       |
| 2000 ppm Oligochitosan    | 0.83 <sup>b</sup>                     | 33.33 <sup>b</sup>                  | 1                       | Very low       |
| 4000 ppm Oligochitosan    | 0.67 <sup>b</sup>                     | 16.67 <sup>b</sup>                  | 1                       | Very low       |
| 50 ppm Paclobutrazol      | 9.80 <sup>ab</sup>                    | 66.67 <sup>a</sup>                  | 3                       | Moderately low |
| 100 ppm Paclobutrazol     | 0.17 <sup>b</sup>                     | 16.67 <sup>b</sup>                  | 1                       | Very low       |
| 200 ppm Paclobutrazol     | 0.00 <sup>b</sup>                     | 0.00 <sup>b</sup>                   | 1                       | Very low       |
| 500 ppm Paclobutrazol     | 0.00 <sup>b</sup>                     | 0.00 <sup>b</sup>                   | 1                       | Very low       |
| 1000 ppm Paclobutrazol    | 0.00 <sup>b</sup>                     | 0.00 <sup>b</sup>                   | 1                       | Very low       |
| 2000 ppm Paclobutrazol    | 0.00 <sup>b</sup>                     | 0.00 <sup>b</sup>                   | 1                       | Very low       |
| *4000 ppm Paclobutrazol   | 12.33 <sup>ab</sup>                   | 100.00 <sup>a</sup>                 | 3                       | Moderately low |
| 50 ppm Aliette            | 0.50 <sup>b</sup>                     | 33.33 <sup>b</sup>                  | 1                       | Very low       |
| 100 ppm Aliette           | 0.33 <sup>b</sup>                     | 33.33 <sup>b</sup>                  | 1                       | Very low       |
| 200 ppm Aliette           | 0.42 <sup>b</sup>                     | 16.67 <sup>b</sup>                  | 1                       | Very low       |
| 500 ppm Aliette           | 1.17 <sup>ab</sup>                    | 66.67 <sup>a</sup>                  | 1                       | Very low       |
| 1000 ppm Aliette          | 1.25 <sup>ab</sup>                    | 66.67 <sup>a</sup>                  | 1                       | Very low       |
| 2000 ppm Aliette          | 0.33 <sup>b</sup>                     | 16.67 <sup>b</sup>                  | 1                       | Very low       |
| *4000 ppm Aliette         | 9.42 <sup>ab</sup>                    | 100.00 <sup>a</sup>                 | 3                       | Moderately low |

<sup>1/</sup>Treatment means within a column followed by the same letter as superscripts are not significantly different at 5% Tukey's HSD test

\*Treatment concentration causing phytotoxicity symptoms to cacao pods

and 4000 ppm), paclobutrazol (100 ppm), and Aliette (200 and 2000 ppm) treatments were also effective, with only 16.67% of the cacao pods showing BPR symptoms. Additionally, oligocarrageenan (50–500 ppm), oligochitosan (100–200 ppm; 2000 ppm), and Aliette (50–100 ppm) reduced the percentage of BPR-infected cacao pods to only 33.33%, which was comparable to the previously mentioned treatments and concentrations.

Overall, the most effective treatments and concentrations used as protectants for BPR were oligocarrageenan (100–4000 ppm), oligochitosan (100 and 1000–4000 ppm), paclobutrazol (100–2000 ppm), and Aliette (50–2000 ppm) as shown in Table 2.

### 3.1.2 Biostimulants and paclobutrazol as eradicants

Cacao pods inoculated with the *P. palmivora* pathogen in agar discs showed BPR symptoms two days after inoculation. The infection begins underneath the agar discs with the pathogen. Table 3 shows the effects of biostimulants (oligocarrageenan and oligochitosan) and PGR (paclobutrazol) at different concentrations as eradicants or curative treatments for cacao pods already infected with *P. palmivora*.

The most effective concentrations of oligocarrageenan, oligochitosan, and paclobutrazol as curative treatments or eradicants to prevent the spread of *P. palmivora* BPR disease infections were 50–200/1000–4000, 50–4000, and 500–2000 ppm, respectively (Table 3). These treatments and concentrations reduce the severity of BPR disease to very low levels. The efficacies of these treatments and concentrations were comparable to those of Aliette at concentrations ranging from 50–100 and 500 ppm.

## 3.2 Effects of biostimulants and paclobutrazol on pod and bean traits at harvest in monocropped and intercropped systems under BPR stress

### 3.2.1 Pod weight

A significant interaction effect ( $p=0.0489$ ) was detected between foliar treatment and the planting system on the pod weight of cacao at harvest. This suggests that the impact of foliar treatment on pod weight is influenced by the planting system; conversely, the effect of the planting system on pod weight is influenced by foliar treatment (Fig. 6). The results indicated that lighter pods were found only on the control trees under intercropping systems, in contrast to other trees grown as either monocrops or intercrops with various foliar treatments applied.

**Table 3** Efficacies of biostimulants and paclobutrazol as eradicants of cacao BPR

| Treatment                 | <sup>1/</sup> Diameter of lesion (cm) | Disease severity rating |                |
|---------------------------|---------------------------------------|-------------------------|----------------|
|                           |                                       | Rating                  | Description    |
| Control                   | 14.58 <sup>a</sup>                    | 5                       | Severe         |
| 50 ppm Oligocarrageenan   | 0.50 <sup>b</sup>                     | 1                       | Very low       |
| 100 ppm Oligocarrageenan  | 0.58 <sup>b</sup>                     | 1                       | Very low       |
| 200 ppm Oligocarrageenan  | 0.67 <sup>b</sup>                     | 1                       | Very low       |
| 500 ppm Oligocarrageenan  | 3.67 <sup>ab</sup>                    | 2                       | Low            |
| 1000 ppm Oligocarrageenan | 0.67 <sup>b</sup>                     | 1                       | Very low       |
| 2000 ppm Oligocarrageenan | 0.83 <sup>b</sup>                     | 1                       | Very low       |
| 4000 ppm Oligocarrageenan | 1.08 <sup>b</sup>                     | 1                       | Very low       |
| 50 ppm Oligochitosan      | 1.00 <sup>ab</sup>                    | 1                       | Very low       |
| 100 ppm Oligochitosan     | 0.50 <sup>b</sup>                     | 1                       | Very low       |
| 200 ppm Oligochitosan     | 0.50 <sup>b</sup>                     | 1                       | Very low       |
| 500 ppm Oligochitosan     | 0.58 <sup>b</sup>                     | 1                       | Very low       |
| 1000 ppm Oligochitosan    | 0.50 <sup>b</sup>                     | 1                       | Very low       |
| 2000 ppm Oligochitosan    | 0.58 <sup>b</sup>                     | 1                       | Very low       |
| 4000 ppm Oligochitosan    | 0.50 <sup>b</sup>                     | 1                       | Very low       |
| 50 ppm Paclobutrazol      | 15.00 <sup>a</sup>                    | 5                       | Severe         |
| 100 ppm Paclobutrazol     | 4.92 <sup>ab</sup>                    | 2                       | Low            |
| 200 ppm Paclobutrazol     | 7.70 <sup>ab</sup>                    | 3                       | Moderately low |
| 500 ppm Paclobutrazol     | 0.75 <sup>b</sup>                     | 1                       | Very low       |
| 1000 ppm Paclobutrazol    | 0.92 <sup>b</sup>                     | 1                       | Very low       |
| 2000 ppm Paclobutrazol    | 4.83 <sup>b</sup>                     | 2                       | Low            |
| *4000 ppm Paclobutrazol   | 9.00 <sup>ab</sup>                    | 3                       | Moderately low |
| 50 ppm Aliette            | 0.92 <sup>b</sup>                     | 1                       | Very low       |
| 100 ppm Aliette           | 0.75 <sup>b</sup>                     | 1                       | Very low       |
| 200 ppm Aliette           | 4.17 <sup>ab</sup>                    | 2                       | Low            |
| 500 ppm Aliette           | 0.58 <sup>b</sup>                     | 1                       | Very low       |
| 1000 ppm Aliette          | 4.75 <sup>b</sup>                     | 2                       | Low            |
| 2000 ppm Aliette          | 4.25 <sup>b</sup>                     | 2                       | Low            |
| *4000 ppm Aliette         | 1.00 <sup>b</sup>                     | 1                       | Very low       |

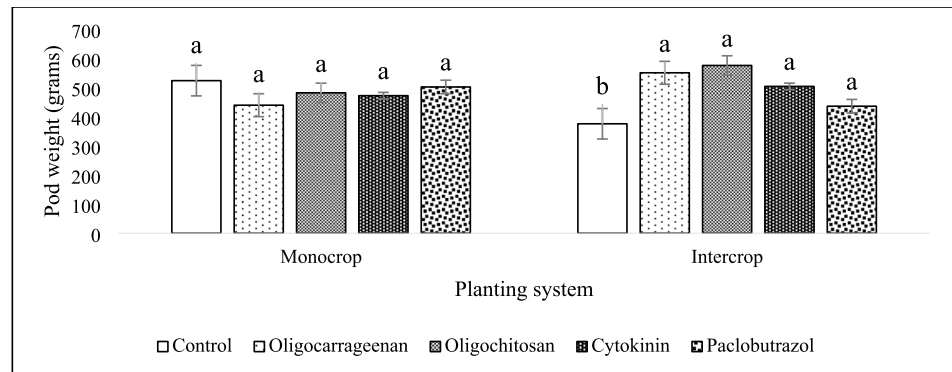
<sup>1/</sup>Treatment means within a column followed by the same letter as superscripts are not significantly different at 5% Tukey's HSD test

\*Treatment concentration causing phytotoxicity symptoms to cacao pods

### 3.2.2 Number of seeds per pod

No significant interaction effect was detected between the foliar treatment and the planting system on the number of seeds per cacao pod at harvest (Table 4,  $p=0.3654$ ). The effect of the planting system on the number of seeds per pod was also not significant ( $p=0.6574$ ). However, the effect of foliar treatment on the number of seeds produced inside the cacao pods were significant ( $p=0.0249$ ), as shown in Table 4. Pods treated with oligocarrageenan and oligochitosan produced more seeds than those in the control treatment, which resulted in a lower number of seeds per cacao pod.

**Fig. 6** Interaction between planting system and foliar treatment on the pod weight of cacao., cv (%)=15.92;  $p=0.0489$ , Treatment means with the same superscript are not significantly different according to Tukey's HSD test ( $p \leq .01$ )



cv (%)= 15.92;  $p= 0.0489$

Treatment means with the same superscript are not significantly different according to Tukey's HSD test ( $p \leq .01$ ).

**Table 4** Number of seeds per pod and fresh weight of seeds per cacao pod in response to biostimulants and paclobutrazol under BPR stress

| Foliar treatment (Fol) | No. of seeds/pod    | Fresh weight of seeds/pod |
|------------------------|---------------------|---------------------------|
| Control                | 24.44 <sup>b</sup>  | 82.01 <sup>b</sup>        |
| Oligocarrageenan       | 31.03 <sup>a</sup>  | 114.85 <sup>a</sup>       |
| Oligochitosan          | 31.27 <sup>a</sup>  | 102.22 <sup>a</sup>       |
| Paclobutrazol          | 29.77 <sup>ab</sup> | 102.27 <sup>a</sup>       |
| Fosetyl-Al             | 29.96 <sup>ab</sup> | 100.54 <sup>a</sup>       |
| cv (%)                 | 12.07               | 12.93                     |
| Pr(> F)                | 0.0249*             | 0.0088**                  |

Treatment means within a column with the same superscripts are not statistically significant according to Tukey's HSD test ( $p \leq 0.05$ )

### 3.2.3 Fresh weight of seeds

No significant interaction effect ( $p=0.2956$ ) was detected between the foliar treatment and the planting system on the fresh weight of the cacao seeds per pod (Table 4). Additionally, the fresh weights of cacao seeds from different foliar treatments were similar in both monocropped and intercropped planting systems. However, foliar treatment had a significant effect ( $p=0.0088$ ) on the fresh weight of seeds in a cacao pod (Table 4). Compared with the control, trees treated with biostimulants, paclobutrazol, or fosetyl-Al produced cacao pods whose fresh weight was heavier.

### 3.2.4 Dried bean weight

No significant interaction effect was found between foliar treatment and the planting system on dry bean weight. Similarly, the planting system did not significantly influence the weight of dried beans. Only the foliar treatment had a significant effect ( $p=0.0409$ ) on dry bean weight (Table 5). Compared with the control, the oligocarrageenan and fosetyl-Al treatments resulted in a greater dry bean weight for cacao, with 5.30% and 6.82% increases, respectively.

**Table 5** Dried bean weight, bean count, and pod index of cacao in response to biostimulants and paclobutrazol under BPR stress

| Foliar treatment (Fol) | Dried bean weight (g) | Bean count          | Pod index |
|------------------------|-----------------------|---------------------|-----------|
| Control                | 1.32 <sup>b</sup>     | 75.85 <sup>a</sup>  | 36.60     |
| Oligocarrageenan       | 1.39 <sup>a</sup>     | 72.31 <sup>b</sup>  | 26.96     |
| Oligochitosan          | 1.37 <sup>ab</sup>    | 73.09 <sup>ab</sup> | 27.80     |
| Paclobutrazol          | 1.37 <sup>ab</sup>    | 73.13 <sup>ab</sup> | 29.34     |
| Fosetyl-Al             | 1.41 <sup>a</sup>     | 71.04 <sup>b</sup>  | 28.44     |
| cv (%)                 | 3.23                  | 3.30                | -         |
| Pr(> F)                | 0.0409                | 0.0407              | -         |

Treatment means within a column with the same superscripts are not statistically significant according to Tukey's HSD test ( $p \leq 0.05$ )

### 3.2.5 Bean count

The bean count refers to the number of beans required to produce 100 g of dried beans (Bureau of Agriculture and Fisheries Standards 2008). Therefore, a lower bean count indicates heavier beans. In the present study, no significant interaction effect was detected between the foliar treatment and planting system on the bean count of cacao. Similarly, the planting system did not affect bean count. However, foliar treatments had significant effects on the cacao bean count (Table 5). The highest bean count was recorded from the control trees, whereas the oligocarrageenan and fosetyl-Al treatments resulted in a lower or improved bean count (Table 5).

### 3.2.6 Pod index

The pod index is the number of pods needed to obtain 1 kg of dried beans, with a lower pod index being more favorable. In this study, the pod indices from cacao trees subjected to different foliar treatments in monocrop and intercrop planting systems were statistically similar (Table 5). However, it is important to note that the pod index is not a growth

parameter but rather an indicator. In practical terms, a difference of 10 is considered significant, and the range of the values derived from this study fall between 25– and 38–. This observation is interesting, as it challenges the previous belief that the pod index is a characteristic specific to a variety. For the UF-18 clone used in this study, the pod index is 20 (<https://nsic.buplant.da.gov.ph/counter.php?link=downloadables/nsic-plantation.xlsx>).

### 3.3 Effects of biostimulants and paclobutrazol on BPR incidence in monocropped and intercropped cacao

#### 3.3.1 Black pod rot

In this study, no significant interaction effect was detected between the planting system and foliar treatment on the incidence of BPR in cacao. The effects of both the planting system and foliar treatment were also found to be insignificant.

Based on the symptoms observed, the pathogen responsible for the pod rot was determined to be not only *P. palmivora* but also *Lasiodiplodinium* sp. pod rot. *Lasiodiplodinium* sp. is a higher form of fungus that cannot be controlled by fosetyl-Al, which is the recommended fungicide for *P. palmivora*. The color of the lesions caused by *Lasiodiplodinium* sp. in cacao pods was darker (blackish) than the brownish color of the lesions caused by *P. palmivora*, as shown in Fig. 7.

This was confirmed by the Plant Disease Clinic of Central Mindanao University, where the mycelial growth from cacao pod samples isolated in the laboratory was found to be black. *Lasiodiplodinium* sp. causes cacao pods to rot completely within a week, in contrast to *P. palmivora*, resulting in a varying number of disease-infected cacao pods within a tree.

## 4 Discussion

Cacao cultivation in the Philippines today employs a low external input strategy, as most farmers are resource poor. The dominant farming system is agroforestry system such as planting under coconuts, accommodating an average of 600 cacao trees per hectare. This cropping system, however, is prone to black pod rot disease caused by *P. palmivora* (Niether et al. 2020). Thus, natural biostimulants and plant growth regulator that can overcome biotic stress are most appropriate, hence this study.

The popular biostimulants tested in this study are carrageenan fragments or oligosaccharides (oligocarrageenan) produced by irradiating the carrageenan from red seaweed (<https://vitalgro.site/aboutus/>) and chitosan oligosaccharides (oligochitosan). Oligocarrageenan has previously been shown to increase yield and confer crop protection properties in rice (Abad et al. 2018), whereas oligochitosan is effective in several crops (Barka et al. 2004; Esyanti et al. 2019; Chaves-Gomez et al. 2020). Paclobutrazol, a PGR, has both growth regulatory and fungicidal properties and was originally developed as a fungicide (Cimen et al. 2004; Blaadow et al. 2006), but it has also been shown to increase the number of flower cushions in cacao (Nieves et al. 2024). Fosetyl-Al served as a positive control, being a long-used industry standard. The effects of these biostimulants and paclobutrazol on cacao yield parameters under biotic stress, and management of black pod rot disease were examined.

In this study, significant interaction effects between the planting system and foliar treatments were demonstrated based on pod weight. Lighter cacao pods were recorded from control trees in the intercropped planting system under BPR stress conditions than from trees with different

**Fig. 7** Cacao black pod rot symptoms (pointed by an arrow) caused by *Lasiodiplodinium* sp. (a) and *P. palmivora* (b)



a. *Lasiodiplodinium* sp. cacao pod rot



b. *P. palmivora* cacao pod rot

foliar treatments in monocrop or intercrop systems. This could be due to the favorable microclimatic conditions in the intercropped planting system (Mensah et al. 2023) for the black pod rot pathogen. In addition to the reduction in light reaching cacao tree canopies due to shade (Mensah et al. 2023) from coconuts, *P. palmivora* infects all parts of trees (McMahon and Purwantara 2004), affecting photosynthetic performance and resulting in reduced pod weight. The results revealed that foliar sprays of biostimulants and paclobutrazol reversed this situation, leading to greater pod weight in the intercropped planting system than in the control.

Regardless of the planting system, the effects of foliar treatments on cacao under biotic stress included a reduction in the number of seeds developed per pod and lighter seeds, resulting in an inferior bean count and a poorer pod index. Foliar sprays of biostimulants, specifically oligocarrageenan, improved all these parameters. Similar results were observed with oligochitosan treatments, except for the dry bean weight, which was negligible in practical terms. Compared with the control, both oligocarrageenan and oligochitosan improved the pod index of cacao. Paclobutrazol also had stimulatory effects on several parameters, such as heavier seed fresh weight and a better pod index. As expected, compared with the control, the positive control (fosetyl-Al) improved the seed fresh weight, dry bean weight, and pod index of cacao.

Under field conditions, the biostimulants and paclobutrazol reduced the negative impact of BPR stress on yield parameters leading to a better pod index. These biostimulants stimulate physiological processes to help plants endure biotic stress conditions, increasing crop yield (Abad et al. 2004; Moenne and Gonzalez 2021; Hossain et al. 2024). Paclobutrazol also had considerable ameliorating effects on the adverse impact of biotic stress, especially BPR disease, in cacao. Paclobutrazol was originally developed as a fungicide under the triazole fungicide (Cimen et al. 2004; Blaedow et al. 2006; Güneş et al. 2024) thus, it has also resulted in better protection of cacao under BPR stress conditions.

The BPR incidence in the field, however, was not reduced by the biostimulants and paclobutrazol sprays. This is most probably due to the presence as well in the field of *Lasiodiplodinium* sp.; a higher form of fungus. Even the fosetyl-Al treatment did not reduce BPR incidence in the field. However, under controlled or in vitro conditions, this study demonstrated that oligocarrageenan, oligochitosan, and paclobutrazol are effective in controlling cacao BPR disease. The lowest effective concentration of oligocarrageenan, oligochitosan, paclobutrazol as a protectant is 100 ppm. As an eradicant, it only requires 50 ppm for oligochitosan and oligocarrageenan, whereas 500 ppm was the lowest effective concentration for paclobutrazol. The foliar application of oligocarrageenan, oligochitosan, and paclobutrazol

plausibly serves as a chemical barrier or protection against the pathogen and as a curative control of the disease, making them potential alternatives to fosetyl-Al for controlling BPR disease. Their effectiveness to control BPR disease in cacao was comparable to the results from the recommended commercial fungicide (fosetyl-Al). Thus, the foliar sprays of biostimulants and paclobutrazol, is a cheaper and safer alternative to fosetyl-Al in managing BPR in cacao.

The effectiveness of biostimulants as protectants and eradicants of BPR is supported by numerous reports that oligocarrageenan and oligochitosan are elicitors of metabolites with antipathogenic properties (Vera et al. 2011; Trouvelot et al. 2014; Shukla et al. 2016; Mousavi et al. 2017, 2018; Salah et al. 2018). For example, plant defense pathways, including the jasmonate, salicylate, and ethylene signaling pathways (Shukla et al. 2016; Hossain et al. 2024), which activate pathogenesis-related proteins such as PR1, PR2, PR5, and PR3 as well as plant defensin (PDF 1.2), can be activated through oligocarrageenan (Hossain et al. 2024) treatment. Oligocarrageenan has also been reported to inhibit the mycelial growth of fungi by increasing the permeability of their plasma membrane (Mani and Nagarathnam 2018). Compared with oligocarrageenan, oligochitosan has direct antimicrobial effects against pathogens, such as hyphal distortion and retraction (Muzzarelli et al. 2001), damage to the plasma membrane, electrostatic interactions with DNA and RNA, and release onto the microbial surface (Riseh et al. 2022). Oligochitosan also stimulates the production of phenolic compounds and the accumulation of proteins (Tayo et al. 2017), which could help reduce the severity of plant diseases. On the other hand, paclobutrazol is currently registered as a plant growth regulator, but it was originally developed as a fungicide in the triazole family. Several studies referenced in the review by Desta and Amare (2021) demonstrated that paclobutrazol is an effective systemic fungicide against fungal diseases, supporting the findings of this study.

## 5 Conclusion and Recommendation

The effectiveness of oligocarrageenan, oligochitosan and paclobutrazol foliar sprays in mitigating the adverse effects of black pod rot disease in cacao was confirmed in this study. Under field conditions, the oligocarrageenan-treated cacao trees produced heavier pods, and more/heavier seeds (fresh and dry) that improved the bean count and pod index under BPR-stress condition. In comparison, oligochitosan ameliorated the impact of biotic stress on cacao as evidenced by heavier pod weight, more/heavier seeds per pod, and better pod index. The paclobutrazol-treated cacao trees produced heavier pods and heavier seeds per pod which improved the pod index under BPR stress. The potential of foliar sprays

of oligocarrageenan and oligochitosan as a crop protection measure for the control of *P. palmivora* BPR disease was also demonstrated under in vitro conditions where they were shown to act as a protectant and eradicator. The lowest effective concentration of oligocarrageenan, oligochitosan, and paclobutrazol as a protectant against BPR disease was 100 ppm. As an eradicator, the lowest effective concentration of oligocarrageenan, oligochitosan, and paclobutrazol for the BPR disease was 50, 50, and 500 ppm, respectively.

Foliar applications of oligocarrageenan, oligochitosan, and paclobutrazol should be used for BPR disease control after field sanitation. Continued use and field sanitation, are important, along with evaluating the effectiveness of oligocarrageenan, oligochitosan, and paclobutrazol against BPR disease.

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Dr. Edna A. Aguilar and Dr. Eureka Teresa M. Ocampo reviewed the field experiment design, reviewed critically the manuscript with significant inputs, and approved the final version of the manuscript.

Dr. Calixto M. Protacio reviewed the field experiment design, supervise the conduct of the research, reviewed and revised critically the manuscript, and approved the final version of the manuscript.

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**Data availability** Data are contained within the article.

## Declarations

**Competing interests** The authors have no relevant financial or non-financial interests to disclose.

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