

ASSESSING THE EFFICACY OF QUANTUM ION IN MANAGING COCOA BLACK POD DISEASE: *IN VITRO* AND *IN VIVO* STUDIES

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ABSTRACT – Cocoa black pod disease caused by *Phytophthora palmivora* poses a severe challenge to cocoa production, leading to significant yield losses and affecting the livelihoods of farmers. This aggressive pathogen infects cocoa pods, resulting in characteristic dark lesions, rapid decay, and eventual destruction of the pods, which compromises both quality and quantity of cocoa yields. Effective management strategies are urgently needed to mitigate its impact. This study evaluates the antifungal efficacy of Quantum Ion, a copper-based ionic solution, against *P. palmivora* through both *in vitro* and *in vivo* experiments. *In vitro* assays assessed mycelial inhibition on PDA amended with 1–200 ppm of Quantum Ion, while *in vivo* tests measured lesion suppression on cocoa pods treated with 20–2,000 ppm. The highest inhibition *in vitro* was observed at 200 ppm (100%), while *in vivo*, 2,000 ppm achieved 86.69% suppression. Lower concentrations were ineffective or promoted disease. These findings suggest that Quantum Ion could be an effective tool for integrated black pod disease management, pending further field validation.

Keywords: Black pod, *Phytophthora palmivora*, quantum ion, *in vitro*, *in vivo*

INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a vital commodity crop grown across tropical regions, providing a primary source of income for millions of smallholder farmers worldwide (Aneani *et al.*, 2012). However, global cocoa production is significantly hampered by black pod disease, predominantly caused by the oomycete *Phytophthora palmivora*. This pathogen is among the most destructive in cocoa cultivation, capable of causing pod losses of up to 30–40% annually, and in severe cases, exceeding 80% (Guest, 2007). Infected pods develop dark lesions that expand rapidly, resulting in pod rot, premature mummification, and a decline in both bean quality and yield (Merga, 2022).

Current disease management strategies rely heavily on fungicide application, phytosanitary practices, and resistant planting materials. Although chemical fungicides can suppress black pod outbreaks, their long-term use poses environmental and health concerns, and the development of resistant pathogen strains remains a significant threat (Jibat & Alo, 2023). As a result, there is increasing interest in developing alternative, eco-friendly solutions for disease control in cocoa systems.

One promising avenue involves the use of ion-based antimicrobial products such as Quantum Ion (copper based), which have shown potential in managing a range of plant pathogens due to their oxidative and membrane-disrupting properties (Hiser *et al.*, 2009). Despite its reported efficacy in other crops, the use of Quantum Ion in cocoa disease management, particularly against *P. palmivora*, remains largely unstudied.

This study aims to assess the efficacy of Quantum Ion in controlling *P. palmivora* through both *in vitro* and *in vivo* approaches. The *in vitro* component focuses on the inhibition of mycelial growth on Potato Dextrose Agar (PDA) amended with various concentrations of Quantum Ion, while the *in vivo* component examines its protective effect on artificially inoculated cocoa pods. The results are intended to inform integrated disease management strategies that align with sustainable agricultural practices and reduce dependence on conventional fungicides.

MATERIALS AND METHODS

Pathogen Isolation

Cocoa pods exhibiting typical symptoms of black pod disease, including brownish lesions, were randomly collected from a cocoa field at the Cocoa Research and Development Centre (CRDC) in Jengka, Pahang, Malaysia. The infected pods were placed in paper envelopes and transported to the laboratory for further analysis. Fragments measuring approximately 5×5 mm were excised from the margins of the lesions. These tissue segments were surface sterilized using a 0.5% sodium hypochlorite solution for 30 seconds and subsequently rinsed three times with sterile distilled water. The sterilized pieces were then blotted dry on sterilized tissue paper.

Each dried tissue segment was aseptically placed onto Potato Dextrose Agar (PDA) plates. The plates were incubated at $27-30^{\circ}\text{C}$ for 24 to 48 hours to allow initial fungal growth. Emerging hyphae from the plated tissues were carefully transferred to fresh PDA plates to obtain pure cultures. The subcultured plates were further incubated for 7 days at room temperature ($25-28^{\circ}\text{C}$) to promote full colony development, following the method described by Latifah *et al.* (2018).

In Vitro Antifungal Assay

Quantum Ion (commercially sourced from Fusion Resonance Intellectual Sdn. Bhd., 300 ppm stock concentration) was tested at 1, 20, 40, 60, 100, and 200 ppm in PDA. Each concentration was prepared by incorporating the appropriate volume of Quantum Ion into molten Potato Dextrose Agar (PDA) before pouring the medium into sterile Petri dishes. After solidification, a 5 mm agar disc from an actively growing 7-day-old *P. palmivora* culture was centrally inoculated on each plate. Control plates containing unamended PDA served as the negative control. Each treatment, including the control, was replicated five times.

The plates were incubated at $25 \pm 2^{\circ}\text{C}$, and colony diameters were measured daily over a 7-day period along two perpendicular axes. The percentage inhibition of diameter growth (PIDG) was calculated using the formula:

$$\text{PIDG (\%)} = \frac{D1-D2}{D1} \times 100$$

where:

D1= diameter growth of pathogen in control plate

D2= diameter growth of the pathogen in treatment plate

The inhibitory effect of Quantum Ion was categorized was scaled according to Sharfuddin and Mohanka (2012) as follows:

>75% = very high inhibition

61–75% = high inhibition

51–60% = moderate inhibition

<50% = low inhibition.

In Vivo Assay on Cocoa Pods

The *in vivo* efficacy of Quantum Ion was assessed on healthy, mature cocoa pods of clone KKM 5, selected for its susceptibility to black pod disease. The pods were first washed thoroughly under running tap water to remove surface debris. The stalk area of each pod was then swabbed with a cotton swab dipped in 70% alcohol and allowed to air dry under sterile conditions.

Each pod was then fully immersed in the respective Quantum Ion solution (20 ppm, 500 ppm, 1,000 ppm and 2,000 ppm) until the entire pod surface was evenly coated. After immersion, the pods were promptly air-dried under aseptic conditions prior to inoculation. Each treatment consist of six pods and untreated pods were served as control.

A 5 mm agar plug containing *P. palmivora* mycelium was placed on the treated pod surface. To maintain moisture and promote infection, the mycelial plug was covered with a piece of moistened cotton facial tissue (Figure 1A). The inoculated pods were then placed in plastic trays lined with wet paper towels (Figure 1B) and covered with transparent plastic bags (Figure 1C) and covered with transparent plastic bags to maintain high humidity (Figure 1C). The trays were kept at ambient room temperature for incubation.

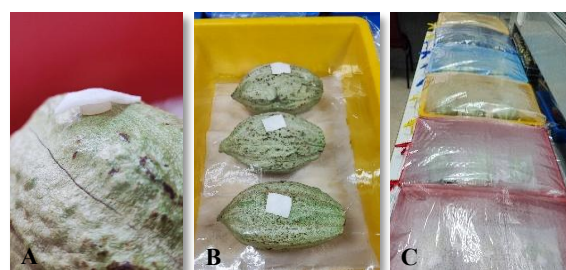


Figure 1: *In vivo* assay on cocoa pods. A: A wet cotton face was covered the mycelial plug to keep the moist. B: The inoculated pods were arranged in plastic trays lined with moist paper towels. C: The trays containing the pods were covered with transparent plastic bags.

Lesion diameters were measured daily for seven days. PIDG was calculated using the same formula as in the *in vitro* assay.

Data analysis

The data of PIDG were subjected to one-way analysis of variance (ANOVA) and the means were compared using Tukey's HSD test at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Pathogen Isolation

The isolation procedure successfully yielded fungal colonies from symptomatic cocoa pods. Within 24–48 hours of incubation on PDA, white, cottony mycelial growth was observed, which later developed into a light to dark grey colony with a somewhat fluffy appearance. Subculturing resulted in uniform, rapidly growing colonies characteristic of *P. palmivora*.

In Vitro Antifungal Activity of Quantum Ion

The antifungal activity of Quantum Ion against *P. palmivora* was evaluated *in vitro*, and the results demonstrated a clear concentration-dependent inhibition (Table 1; Figure 2). The highest concentrations, 200 ppm and 100 ppm, achieved complete (100.00%) and very high (94.29%) inhibition, respectively, with no statistically significant difference between them ($p > 0.05$). These concentrations entirely suppressed mycelial expansion on PDA, as observed both visually and through radial growth measurement. This is consistent with literature showing that copper ions and nanoparticles disrupt fungal growth via oxidative stress, membrane destabilization, and inhibition of spore germination (Malandrakis *et al.*, 2021; Chen *et al.*, 2022).

Table 1: Inhibition percentage of *P. palmivora* growth on PDA by different concentration of Quantum Ion after 7 days at 24°C under *in vitro* conditions.

Concentration (ppm)	PIDG (%)*
200	100.00 ^a
100	94.29 ^a
60	65.02 ^b
40	29.98 ^c
20	4.57 ^d
1	0.07 ^d

*Means with the same letter are not significantly different at $p < 0.05$ (Tukey's HSD).

Moderate inhibition (65.02%) was recorded at 60 ppm, while lower concentrations of 40 ppm and 20 ppm only achieved minimal suppression, with PIDG values of 29.98% and 4.57%, respectively. At 1 ppm, Quantum Ion exhibited negligible inhibition (0.07%), comparable to the control treatment. All differences among these treatment groups were statistically significant ($p < 0.05$), confirming the strong dose-dependent nature of Quantum Ion's inhibitory effect. This pattern supports the hypothesis that adequate ionic concentration is required to achieve antimicrobial thresholds needed to suppress pathogen development (Ibarra-Laclette *et al.*, 2022).

The visual evidence (Figure 2) strongly corroborates the measured data, providing a clear depiction of the compound's efficacy at different concentrations. The inhibition trend suggests that Quantum Ion may interfere with key fungal processes such as cell wall integrity or sporangial germination at higher doses. However, the diminishing efficacy at sublethal concentrations indicates that precise dosing is crucial for optimal control.



Figure 2: Inhibitory effect of different concentration of Quantum Ion on mycelial growth of *P. palmivora*.

In Vivo Efficacy of Quantum Ion Against *P. palmivora*

The *in vivo* evaluation of Quantum Ion on cocoa pods infected with *P. palmivora* revealed a strong concentration-dependent inhibition of lesion development (Table 2; Figure 3). The highest concentration tested, 2,000 ppm, significantly reduced lesion size with a percentage inhibition of disease growth (PIDG) of 86.69%, indicating high efficacy in suppressing disease symptoms under pod inoculation conditions.

Table 2: Inhibition percentage of *P. palmivora* growth on cocoa pod by different concentration of Quantum Ion after 7 days at 24°C under *in vivo* conditions.

Concentration (ppm)	PIDG (%)*
2,000	86.69 ^a
1,000	26.04 ^b
500	36.47 ^{bc}
20	-15.18 ^c

*Means with the same letter are not significantly different at $p < 0.05$ (Tukey's HSD).

Visually, cocoa pods treated with 2,000 ppm had the smallest and least severe lesions, demonstrating clear suppression of *P. palmivora* infection (Figure 3). This aligns with the quantitative data and supports the

potential use of Quantum Ion as a protective agent under real host-pathogen interaction conditions.

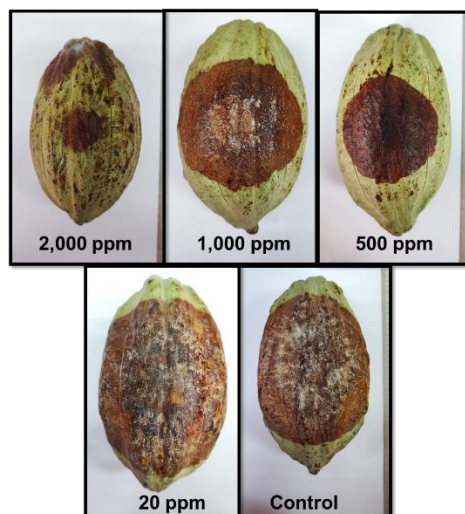


Figure 3: Inhibitory effect of different concentration of Quantum Ion to the lesion growth of *P. palmivora* on cocoa pod.

Interestingly, a lower concentration of 500 ppm resulted in a PIDG of 36.47%, while 1,000 ppm showed only 26.04%, a trend inconsistent with a linear dose-response. This variation might be attributed to localized differences in absorption or retention of Quantum Ion on pod surfaces or possibly due to biological variability in pod response to treatments, as differences in cuticle permeability and wax layer composition can influence ionic uptake and efficacy (Sadek *et al.*, 2022). It also suggests the possibility of non-monotonic dose-response effects, as observed in other ionic or nanomaterial-based plant treatments. Similar findings have been observed in copper nanoparticle studies, where low doses can activate plant antioxidative enzymes, but intermediate doses result in sub-lethal stress responses that neither inhibit the pathogen nor trigger full host resistance (Bushueva *et al.*, 2023); (Pandey *et al.*, 2022).

At 20 ppm, disease severity increased significantly, with a negative PIDG of -15.18%, indicating that lesion growth was more aggressive than in the untreated control. Figure 3 shows visibly larger and more necrotic lesions at this concentration, reinforcing the idea that sub-effective doses may fail to inhibit fungal growth, possibly by creating a more permissive environment conducive to pathogen growth.

Overall, both visual and quantitative data confirm that 2,000 ppm is the most effective concentration for managing black pod disease under *in vivo* conditions. These findings are promising for developing ionic-based alternatives to chemical fungicides, though further field trials are recommended to confirm consistency under variable environmental

conditions. Moreover, the observed dose-response trends could inform precision dosing guidelines for optimal disease control and guide formulation improvements for field application of Quantum Ion.

CONCLUSIONS

Quantum Ion showed effective inhibition of *P. palmivora* growth, with 200 ppm being the most effective concentration *in vitro*, while 2,000 ppm was required for significant disease suppression on cocoa pods *in vivo*. Lower concentrations, especially 20 ppm, were ineffective and sometimes promoted pathogen growth. These results highlight the importance of dose optimization, indicating Quantum Ion's potential as an alternative treatment for managing cocoa black pod disease, pending further field validation.

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