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Efficacy of mangosteen peel extract and phosphonic acid on durian root rot caused by *Phytophthora palmivora in vitro*

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Abstract Phytophthora root rot caused by *Phytophthora palmivora* poses a notable challenge to durian farming in Thailand. Results indicated that all treatments containing mangosteen peel extract combined with phosphonic acid exhibited 100±0.00% inhibition of mycelial growth, outperforming the purified mangosteen peel extract alone. In contrast, the mangosteen peel extract alone resulted in mycelial growth inhibition of only 52.41±5.27%-97.50±0.60%. In conclusion, the study provided valuable insights into utilizing mangosteen peel waste for controlling durian root rot disease, reducing reliance on chemical fungicides in crop protection, minimizing environmental pollution, and adding value to agricultural waste.

Keywords: Durian root rot disease, Mangosteen peel extract, Phosphonic acid, *Phytophthora palmivora*

Introduction

The durian (*Durio zibethinus*), known as the "King of Thai Fruits", is a tropical fruit tree. It produces climacteric fruit and is an economically significant crop, highly favoured not only in Thailand but also in other countries (Wiangsamut and Wiangsamut, 2022; Wiangsamut and Wiangsamut, 2023). A significant portion (80%) of the durian produced in Thailand is designated for export purposes (Chomchalow *et al*., 2008). However, there have been obstacles in durian cultivation as a result of the root rot disease caused by *Phytophthora* sp. The pathogens responsible for durian diseases have led to crop losses, with control expenses estimated to exceed 20% of production costs (Suksiri *et al*.,

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2018). Symptoms of the disease manifest as root rot, leaf blight, stem or bark blight, and fruit rot. *Phytophthora* can infect durian trees at all stages of growth (Tongon and Soytong, 2021).

Phytophthora sp. is a genus of destructive plant-pathogenic oomycetes and soilborne pathogens that result in economic losses in agriculture and ecosystem damage on a global scale (Oerke, 2006). *P. palmivora* is distinguished within this genus by its extensive range of hosts, encompassing citrus, tomato, pineapple, tobacco, rubber, and olive trees (Misman *et al*., 2022). The motile zoospores produced by *Phytophthora* sp. are capable of swimming in water and infecting plant tissues, thereby causing disease. The capacity of the pathogen to endure prolonged periods in water and soil presents a notable obstacle to its control (Erwin and Ribeiro, 1996). In countries like Thailand, Malaysia, Vietnam, and others, *Phytophthora* sp., specifically *P. palmivora* poses a major threat to durian cultivation, resulting in root rot, fruit rot, or late blight throughout all growth stages (Lim and Luders, 1998). The proliferation of this devastating disease in durian trees is facilitated by tropical hot-humid conditions, heavy clay soil, and poor drainage. High humidity or even a small amount of water can make it easier for the flagellated zoospores to swim and infect the hosts (Stamps *et al*., 1990). The emergence of fungicide-resistant *Phytophthora* sp. in different countries, including Thailand, has caused alarm. For example, metalaxyl resistance in *P. palmivora* has been linked to durian disease (Kongtragoul *et al*., 2021). Phosphonic acid is currently under consideration as a potential substitute chemical for fungicide use in managing *Phytophthora* species.

Phosphonic acid, which is chemically classified as a fungicide, is associated with chemical groups such as potassium phosphate. Its functional group is characterized by a phosphorus atom bonded to three oxygen atoms (Demmer *et al*., 2011; Sevrain *et al*., 2017). Its effectiveness in controlling fungal diseases caused by oomycetes is attributed to its ability to translocate in phloem, allowing the chemical to move from leaf tissues to the crowns and roots (Ouimette and Coffey, 1990). In contrast, there is a potential risk of resistance to similar applications of metalaxyl in the future. Therefore, the development of fungicide chemicals or their combination with other natural extracts is crucial for crop protection. A byproduct of the medicinal plant is mangosteen peel which has long been utilized for treating skin infections, wounds, and diarrhea in Southeast Asia (Nakatani *et al.*, 2002).

Mangosteen (*Garcinia mangostana* L.) is commonly referred to as "the queen of fruits" and is a tropical fruit indigenous to Southeast Asia, specifically in countries like Thailand, Malaysia, and Indonesia, among others. The peel of the mangosteen fruit is rich in essential compounds such as phenolic compounds, flavonoids, xanthones, tannins, catechins, and anthocyanins, all of which possess

significant biological properties. These compounds demonstrate antioxidant properties and function as antimicrobial agents against fungi and bacteria (Plainsirichai *et al*., 2015; Suksamrarn *et al*., 2006; Chomnawang *et al*., 2007). Moreover, studies have indicated that mangosteen peel extract demonstrates inhibitory properties against a range of organisms including *Colletotrichum gleosporioides*, *Pestalotiopsis* sp., *Candida utilis* and *Saccharomyces cerevisiae, Xanthomonas oryzae* pv. *oryzae* (Ngamsaeng *et al*., 2006; Saepudin *et al*., 2019; Darapanit *et al*., 2021). Additionally, several research studies have emphasized the effectiveness of mangosteen peel extract in fighting fungal infections. For instance, Gopalakrishnan *et al*. (1997) found that xanthones extracted from mangosteen act as a natural antifungal agent, displaying activity against *Fusarium oxysporum vasinfectum*, *Alternaria tenius*, and *Dreshlera aryzae*. Chuebandit *et al*. (2012) conducted laboratory experiments to assess the efficacy of mangosteen peel extracts, specifically xanthone, in preventing and controlling fungal pathogens. The study revealed that xanthone at a concentration of 75% exhibited the highest effectiveness in inhibiting fungal growth in Lanzones fruits (or Longkong), with a suppression rate of 6.06% compared to the control group where fungal occurrence averaged at 19.05%. Interestingly, different concentrations of xanthone did not have a significant impact on the sweetness of Lanzones fruits. Furthermore, the utilization of mangosteen peel extract in combination with hexane at a concentration of 2000 ppm exhibited a remarkably significant impact on seed-borne fungi ($P<0.05$). Mangosteen peel extract with hexane at 1000 ppm showed no significant impact on seed germination based on seed germination tests ($P>0.05$). There were further experiments were conducted to evaluate the antifungal properties of mangosteen peel extract using three different solvents (hexane, ethyl acetate, and water) at concentrations of 1000, 2000, and 4000 ppm against *A. flavus* and *A. niger*. The results demonstrated that mangosteen peel extract with hexane at a concentration of 4000 ppm exhibited highly effective antifungal properties against both *A. flavus* and *A. niger* ($P<0.05$) (Monkhung and Duangkaew, 2021). Similarly, Wiangsamut *et al*. (2023) investigated the control of fruit rot in 'Monthong' durian (*Durio zibethinus*) caused by *Lasiodiplodia* sp. Treatment of inoculated 'Monthong' durian fruits with a 100% concentration of mangosteen peel fermented solution resulted in 56% of the fruits remaining undamaged, while the remaining 44% were damaged.

Therefore, based on the biochemical efficiency of mangosteen peel and phosphonic acid, the primary aim of this study was to examine the effect of purified mangosteen peel extract when mixed with phosphonic acid on the mycelial growth of root rot in durian caused by *Phytophthora plamivora* isolates Cl5-F11 and Cl5-F12 *in vitro*.

Materials and methods

Materials

The mangosteen peel was sourced from the Department of Food Innovation and Business at the Faculty of Agro-Industrial Technology, Rajamangala University of Technology Tawan-Ok, Chanthaburi Campus, Chanthaburi, Thailand. The sample was then taken to the laboratory and dried in a hot air oven at 65 °C for 2 days before the extraction process began.

Preparation of durian root rot pathogens

There were two species of *Phytophthora palmivora* isolates Cl5-F11 and Cl5-F12 acquired from the Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Thailand. These fungal species were cultured and transferred onto 9 cm sterilized petri dishes filled with potato dextrose agar (PDA), followed by an incubation period at a temperature of 28±2ºC for a duration of 7 days. After the incubation period, the fungal isolates were examined and transferred to PDA plates to investigate the effect of mangosteen peel extract combined with phosphonic acid on mycelial inhibition determination (Agrios, 2005).

Preparation of Mangosteen peel extract

Garcinia mangostana L. belongs to Clusiaceae was dried as previously described and then subjected to extraction using a 95% ethanol solvent at a ratio of 1:7 (dried mangosteen peel and ethanol). The obtained mixture was subsequently allowed to incubate for a duration of 48 hours. Subsequently, the mangosteen extract solution underwent filtration using a filter cloth. The filtrate was then subjected to evaporation using a rotary vacuum evaporator at a pressure of 45 mbar, 50 rpm at 45ºC for a duration of 10 hours (Pongpisutta *et al.,* 2011).

Effect of mangosteen peel extract mixed with phosphonic acid on mycelial inhibition determination

The experiment was laid out in a factorial in a completely randomized design (CRD) with six treatments, each replicated four times. The mangosteen peel extract (MPE) was prepared at different concentrations, specifically 10, 100, 1,000, and 10,000 ppm. The effect of combining mangosteen peel extract with phosphonic acid on inhibiting mycelial growth was assessed using the poisoned food technique, following the modified method of Cho *et al*. (2005). This

involved comparing the control group with the positive control—Alc. 60,000 ppm, where Alc. is an abbreviation of 95% ethyl alcohol. The phosphonic acid concentration was set at 50ml./20L. The experiment entailed inoculating the center of petri plates with a mycelial plug obtained from the edge of a 7-day-old fungal culture. The control group consisted of blank agar plug plates (PDA mixed with sterile distilled water). Four replicate plates of PDA crude extract per isolate were placed in an incubator at 28ºC, and the radial growth was measured daily for 7 and 10 days. The plant extracts' efficacy was measured, and the percentage of radial mycelial growth inhibition compared to the control was determined using the following formula:

$$\%\text{Inhibition} = (X\text{-}Y) \times 100/X$$

where X: diameter of a fungal colony grown on the negative control plate

Y: diameter of fungal colony grown on plates containing crude extracts or fungicides

Statistical analysis

The effect of varying concentrations of mangosteen peel extract and phosphonic acid on the growth of fungi on PDA media was evaluated using one-way analysis of variance (ANOVA), and any significant differences were identified through Duncan's multiple range test (DMRT) (Watts *et al*., 1989).

Results

The effect of mangosteen peel extract (MPE) was evaluated by preparing various concentration samples and studying their ability to inhibit the growth of *P. palmivora* isolates Cl5-F11 and Cl5-F12 in comparison with treatments mixed with phosphonic acid. Two species of *P. palmivora* (Cl5-F11 and Cl5-F12) were cultured, and the mycelial length was measured after 7 and 10 days of inoculation at 28ºC. On the 7th day of cultivation, following the application of purified mangosteen peel extract at 10 ppm, the inhibition of *P. palmivora* (Cl5-F11 and Cl5-F12) on mycelial growth exhibited effectiveness at 61.58±11.91% and 90.88±13.10 %, respectively (Table 1 and Figure 1). Upon elevating the level of mangosteen peel extract to 100 ppm, the inhibitory effects were found to increase to 68.40±9.54% and 98.61±2.78%, respectively. Further increasing the concentration to 1,000 ppm of mangosteen peel extract led to a sharp increase to 90.16±2.47% and 100±0.00% inhibition for *P. palmivora* (Cl5-F11 and Cl5-F12), respectively. However, this increase was not significantly different from the inhibitions observed at concentrations of Alc. 60,000 ppm, and 1,000 ppm of mangosteen peel extract. The results indicate a clear relationship between the

concentration of mangosteen peel extract and the percentage of mycelial growth inhibition, as illustrated in Table 1 and Figure 1.

Table 1. Effect of various concentrations of mangosteen peel extract (MPE) on mycelial growth inhibition of *P. palmivora* C15-F11 and *P. palmivora* C15-F12

Isolate	Concentration (ppm)	% Mycelial growth inhibition	
		7 Days	10 Days
Cl5-F11	10	61.58±11.91^{b}	52.41±5.27^{d}
	100	68.40±9.54^{b}	73.01±7.38^{c}
	1,000	90.16±2.47^{a}	81.85±6.06^{b}
	10,000	94.43±2.67^{a}	97.50±0.60^{a}
	Alc. 60,000	100.00±0.00^{a}	45.49±6.95^{d}
	Control (Sterile distilled water)	0.00±0.00^{c}	0.00±0.00^{e}
C.V. (%)		9.90	9.26
Cl5-F12	10	90.88±13.10^{b}	88.09±8.46^{a}
	100	98.61±2.78^{ab}	95.17±5.70^{a}
	1,000	100.00±0.00^{a}	98.47±1.79^{a}
	10,000	100.00±0.00^{a}	100.00±0.00^{a}
	Alc. 60,000	100.00±0.00^{a}	55.31±27.94^{b}
	Control (Sterile distilled water)	0.00±0.00^{c}	0.00±0.00^{c}
C.V. (%)		6.43	15.96

Mean with different letters are statistically different ($\rho \leq 0.05$) according to Duncan's multiple range test.

During the 10-day cultivation period, it was observed that the percentage of mycelial growth inhibition for all treatments was lower compared to the 7-day cultivation period (Table 1 and Figure 1). Specifically, the mycelial growth inhibition percentage of Alc. 60,000 ppm was approximately one time lower for both species of *P. palmivora.* The low dose (10 ppm) of mangosteen peel extract showed ineffectiveness at 52.41±5.27%, while *P. palmivora* (Cl5-F12) exhibited a higher percentage of inhibition at 88.09±8.46%. At a concentration of 100 ppm, the application of mangosteen peel extract resulted in mycelial growth inhibition percentages of 73.01±7.38% and 95.17±5.70% for *P. palmivora* (Cl5-F11 and Cl5-F12), respectively. With 1,000 ppm mangosteen peel extract, the percentage

increased to 81.85 ±6.06% and 98.47±1.79%, respectively. The highest rates of growth inhibition were significantly ($\rho \leq 0.05$) exhibited at 10,000 ppm of extract with values of 97.50±0.60% of *P. palmivora* (Cl5-F11) and 100±0.00% of *P. palmivora* (Cl5-F12) as shown in Table 1 and Figure 1. In summary, the percentage of inhibition decreased with longer incubation times. Additionally, the effectiveness of mangosteen peel extract in managing *P. palmivora* Cl5-F12 surpassed that of *P. palmivora* Cl5-F11. Furthermore, all treatments of mangosteen peel extract demonstrated higher inhibition of durian root rot disease compared to the positive control (Alc. 60,000 ppm) after 10 days of incubation.

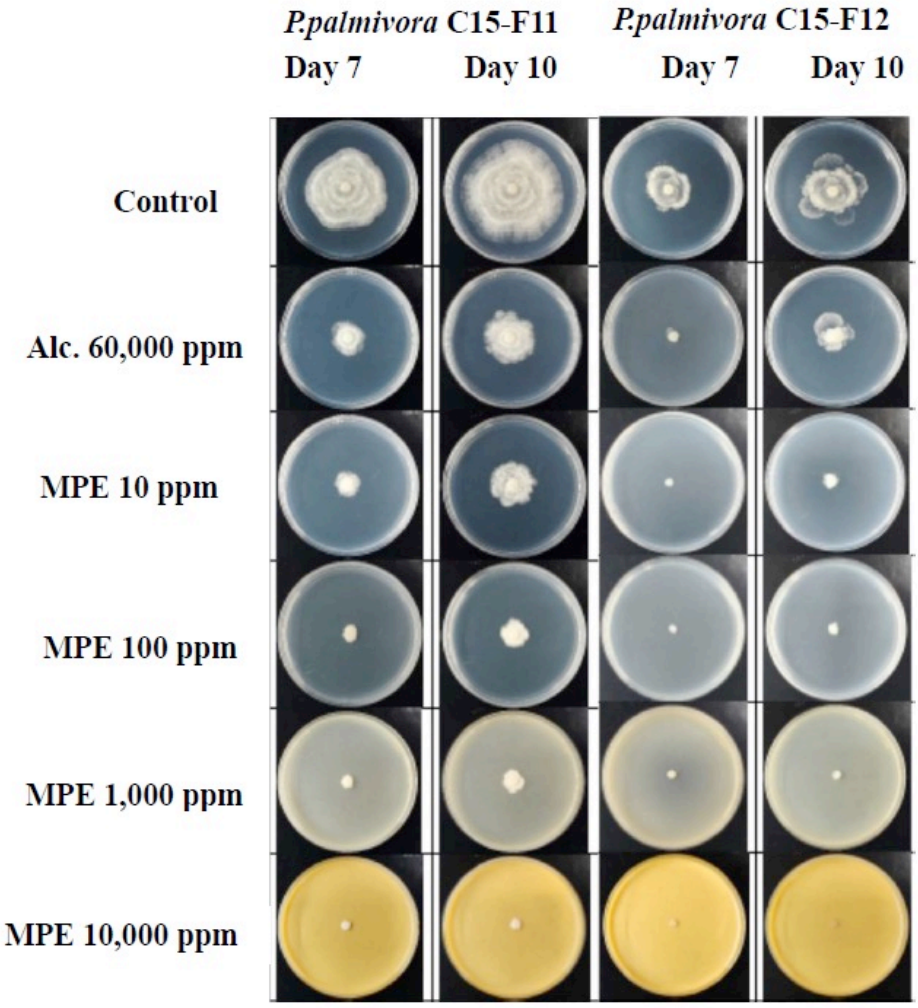

Figure 1. Effect of various concentrations of mangosteen peel extract (MPE) on mycelial growth of *P. palmivora* C15-F11 and *P. palmivora* C15-F12

The application of various concentrations of mangosteen peel extract mixed with phosphonic acid (10, 100, 1,000, and 10,000 ppm) at the concentration of 50 ml/20L showed effective control for both cultivars of durian root rot disease. The treatments inhibited *P. palmivora* C15-F11 and *P. palmivora* C15-F12 significantly ($\rho \leq 0.05$) higher than the positive control (Alc. 60,000 ppm). Interestingly, a low dose (10 ppm) of mangosteen peel extract mixed with phosphonic acid exhibited 100±0.00% inhibition to control *P. palmivora* (Cl5-F11 and Cl5-F12) more effectively than the purified mangosteen peel extract (Table 2 and Figure 2). Furthermore, the low concentration of mangosteen peel extract mixed with phosphonic acid completely inhibited the growth of two species of *P. palmivora* for cultivation 7 and 10 days as presented in Table 2 and Figure 2.

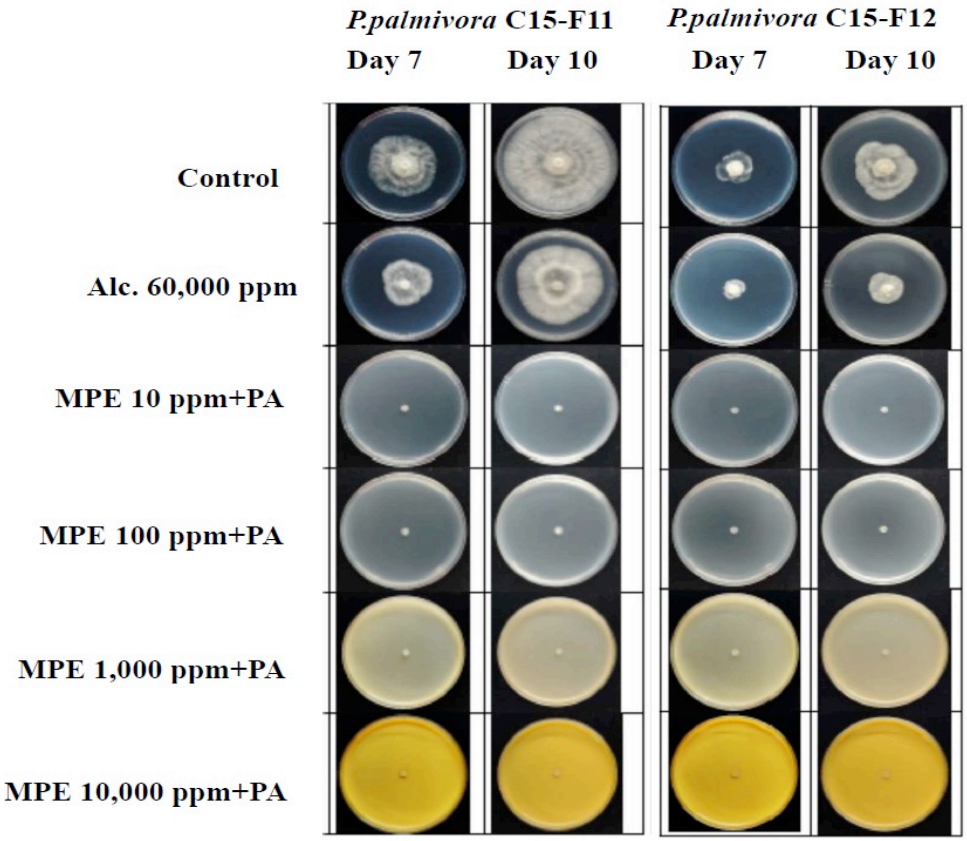

Figure 2. Effect of various concentrations of mangosteen peel extract (MPE) mixed with phosphonic acid (PA) on mycelial growth of *P. palmivora* C15-F11 and *P. palmivora* C15-F12

Table 2. Effect of various concentrations of mangosteen peel extract mixed with phosphonic acid on mycelial growth inhibition of *P. palmivora* C15-F11 and *P. palmivora* C15-F12

Isolate	Concentration (ppm)	% Mycelial growth inhibition	
		7 Days	10 Days
Cl5-F11	10+Phosphonic acid	100.00±0.00 [a]	100.00±0.00[a]
	100+Phosphonic acid	100.00±0.00[a]	100.00±0.00[a]
	1,000+Phosphonic acid	100.00±0.00 [a]	100.00±0.00[a]
	10,000+Phosphonic acid	100.00±0.00 [a]	100.00±0.00[a]
	Alc. 60,000	45.11±39.75[b]	33.89±39.37[b]
	Control (Sterile distilled water)	0.00±0.00 [c]	0.00±0.00[c]
C.V. (%)		21.87	22.22
Cl5-F12	10+Phosphonic acid	100.00±0.00[a]	100.00±0.00[a]
	100+Phosphonic acid	100.00±0.00[a]	100.00±0.00[a]
	1,000+Phosphonic acid	100.00±0.00[a]	100.00±0.00[a]
	10,000+Phosphonic acid	100.00±0.00[a]	100.00±0.00[a]
	Alc. 60,000	73.41±18.44[b]	49.94±11.62[b]
	Control (Sterile distilled water)	0.00±0.00[c]	0.00±0.00[c]
C.V. (%)		9.54	6.32

Mean with different letters are statistically different ($\rho \leq 0.05$) according to Duncan's multiple range test.

Discussion

The results showed that using *in vitro* mangosteen peel extract to control *P. palmivora* (Cl5-F11 and Cl5-F12) indicated that the pure mangosteen extract

displayed the highest inhibition of *P. palmivora* colony, exceeding 80% at concentrations of 1,000 and 10,000 ppm compared to the control. These results align with previous studies, where researchers observed that mangosteen pericarp crude extract effectively controlled *P. palmivora* by completely inhibiting mycelial growth. The inhibitory effects on the growth of *Colletotrichum gloeosporioides* were observed when mangosteen peels were extracted at concentrations of 100, 1,000, and 10,000 ppm (Prasothong *et al*., 2011; Koohapitagtam and Thongnim, 2015) . Moreover, Pongpisutta *et al*. (2011) evaluated the activity of mangosteen pericarp extract obtained through maceration with 95% ethyl alcohol on mycelial growth of the *C. gloeosporioides,* the causal agent of mango anthracnose. The antifungal properties may have stemmed from the bioactive compounds found in mangosteen peel, such as α-mangostin, β-mangostin, γ-mangostin, along with various other compounds like terpenes, anthocyanins, tannins, flavonoids, and polyphenols. *Garcinia mangostana* extracts and xanthones have been found to have a range of positive effects, including antioxidant, antitumor, anti-allergic, anti-inflammatory, antibacterial, antifungal, and antiviral properties (Pedraza-Chevierri *et al*., 2008; Ye *et al*., 2020). Studies have shown that the extract from mangosteen peel has notable antifungal effects against pathogenic fungi.

The combination of mangosteen peel extract and phosphonic acid has proven to be highly effective in controlling durian root rot disease in both cultivars. Even when present in low concentrations, the mixture combination demonstrated inhibition of the proliferation of two species of *P. palmivora* after 7 and 10 days of incubation. Phosphonic acid in the form of phosphonate anion has been shown to directly act on fungi, leading to a reduction in growth. Grant *et al*. (1990) found that the decrease in growth is associated with a quick reduction in the overall adenylate pool. Moreover, Pegg *et al*. (1990) showed that trunk injections of partially neutralized phosphonic acid solutions are effective in rehabilitating and safeguarding avocado trees infected by *P. cinnamomic*. They also found that pre-harvest spray of potassium phosphonate or post-plant sprays of potassium phosphonate effectively controlled root and heart rot of pineapple. Boer and Greenhalgh (1990) discovered that phosphonic acid effectively protected subterranean clover plants (cv. Woogenellup) from severe tap-root rot caused by *P. clandestina* in irrigated pastures, as well as several ornamental plant species grown in pasteurized potting mixes from root rot caused either by *P. cinnamomi* or *P. cryptogea*. Garbelotto *et al*. (2009) observed that injecting phosphonic acid was effective in controlling the growth of pathogens on saplings of California coast live oak trees infected with *P. ramorum*, the

fungus responsible for sudden oak death (SOD). The treatment with phosphonic acid was successful in reducing hyphal growth *in vitro*. In addition, the combination of mangosteen peel extract and phosphonic acid showed a significant inhibition of mycelial growth and long-lasting protection.

In conclusion, the results of the study suggest that utilizing a combination of mangosteen peel extract and phosphonic acid demonstrated increased effectiveness against *P. palmivora* in vitro when compared to solely using mangosteen peel extract. Furthermore, the result of this will be utilized in durian fields moving forward based on specific outcomes. This investigation represents a novel contribution towards utilizing mangosteen peel waste for managing durian root rot disease caused by *P. palmivora*, thereby reducing the reliance on chemical agents for fungal disease control in agriculture, promoting environmental protection, minimizing waste pollution, and enhancing the value of agricultural by-products.

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