# The Use of Indigenous *Trichoderma* in Controlling *Phytophthora palmivora* – An *In Vitro* Investigation

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## ABSTRACT

*Oil palm bud rot is a disease that destroys the young tissues of palms, and* Phytophthora palmivora *has been identified as the causal pathogen. The disease is devastating the South American oil palm industry. The pathogen is also known to be responsible for a number of serious diseases in cocoa, durian, jackfruit and coconut. A biosecurity threat is imminent in Malaysia due to the fact that* P. palmivora *is an indigenous pathogen affecting local commodity crops. Various research activities have been initiated to assess the potential threat imposed by the pathogen on oil palm. These include an investigation into one of the three components of biosecurity, i.e. developing long-term approaches for reducing and managing the effects of a potential outbreak. Four candidates of* Trichoderma virens *(PP9, PP29, T7b and T159c) that control* Ganoderma *effectively were selected for further investigation. An* in vitro *assessment subjected on 11 strains of* P. palmivora *to these* Trichoderma *isolates. Endophytic* T. virens *isolates T7b and T159c recorded good mycelial inhibition, ranging from 54%-77%, compared with the non-endophytic isolates of* T. virens *PP9 and PP29. The efficacy of extracellular metabolites of these four* T. virens *isolates was also tested on the two most aggressive strains representative of* P. palmivora *(P3 and P7). The study demonstrates the potential use of local* Trichoderma *isolates in controlling* P. palmivora*, and warrants further investigations to be conducted in the nursery and in the field.*

## ABSTRAK

*Reput umbut sawit adalah penyakit yang merosakkan tisu-tisu muda pokok sawit dan* Phytophthora palmivora *telah dikenalpasti sebagai patogen penyakit tersebut. Penyakit ini kini menghancurkan industri sawit di Amerika Selatan. Patogen ini juga menyebabkan beberapa penyakit serius pada koko, durian, nangka dan kelapa. Ancaman biosekuriti dikhuatiri di Malaysia kerana* P. palmivora *adalah patogen yang menjangkiti tanaman komoditi tempatan. Pelbagai aktiviti penyelidikan sedang dilakukan untuk menilai potensi ancaman oleh patogen ini terhadap sawit. Ini termasuk siasatan pada salah satu komponen biosekuriti, iaitu pendekatan jangka panjang untuk mengurangkan dan mengurus kesan wabak yang berpotensi. Empat agen kawalan* Trichoderma *(PP9, PP29, T7b dan T159c telah dikenalpasti dapat mengawal* Ganoderma *dengan berkesan dipilih untuk siasatan lanjut. Penilaian* in vitro *terhadap keberkesanan empat isolat tersebut dilakukan pada 11 isolat* P. palmivora*. Isolat* T. virens *endofitik T7b dan T159c mencatatkan perencatan miselium yang baik (antara 54% dan 77%) berbanding dengan isolat* T. virens *bukan endofitik PP9 dan PP29. Keberkesanan metabolit ekstraselular daripada empat isolat* T. virens *ini juga diuji pada dua strain* P. palmivora *yang paling agresif (P3 dan P7). Kajian ini menunjukkan potensi penggunaan isolat* Trichoderma *tempatan untuk mengawal* P. palmivora*, dan juga menunjukkan siasatan lanjut di tapak semaian dan ladang perlu dilakukan.*


**Keywords:** oil palm, bud rot, *Phytophthora palmivora*, biosecurity, *Trichoderma.*

## INTRODUCTION

Historically *Phytophthora*, or commonly known as the 'plant destroyer' caused a huge disease outbreak in Ireland referred to as the 'Irish Potato Famine' when it attacked potato farms and caused the death of over 1 million Irish people from starvation back in 1846-1851 (Henderson, 2005). The genus has more than 80 species that are notoriously known to be detrimental to plants. One of the most common tropical species is *Phytophthora palmivora*, and the species has been reported on more than 150 plant hosts. Among the important agricultural crops attacked by *P. palmivora* are cocoa and durian. In cocoa, for instance, the fungus causes black pod rot, decay of flower cushions and cherelles, stem canker, blight of shoots and chupons, dieback of bud-grafted and hand-pollinated hybrid seedlings, and root rot (Drenth and Guest, 2004). In durian, the fungus causes canker, root rot, patch canker, leaf blight and dieback of seedlings and trees, and fruit rot (Lim and Chan, 1986).

A recent report revealed that bud rot disease of oil palm in four production areas of Colombia is caused by *P. palmivora* (Torres *et al*., 2010). Severe bud rot incidences were observed in Tumaco, Colombia, where more than 30 000 ha out of 35 000 ha (85%) of

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oil palm were destroyed by this pathogen between 2006 and 2009 (Martínez *et al.*, 2014). Although the pathogen is an indigenous species in Malaysia and Indonesia, no outbreaks of bud rot have been reported locally. On realising the possible devastation caused by the pathogen, Malaysian oil palm industry took immediate precaution. A series of experiments were conducted to assess the pathogenicity of local strains of *P. palmivora* on oil palm. There is no immediate effective control against the disease, and only the use of heritable resistance is a possible long-term control strategy for the disease. Other methodologies that include the use of polyamines, phosphonate ($H_3PO_3$) which is commonly branded as phosphate, and cultural practices such as plant surgery are being implemented as short-term measures in reducing the effects of the disease Berger *et al.*, 2015; Rocha *et al.*, 2005). There have been reports of promising results from the use of *Trichoderma* on *Phytophthora* species isolated from cocoa and rubber (Berger *et al.*, 2015; Hanada *et al.*, 2009). Previous work showed that four candidates of *Trichoderma virens* have proven to be effective against *Ganoderma* (Sundram 2013). Therefore, as a short-term measure for managing this disease, a study was initiated to investigate the potential use of local *Trichoderma* as a biocontrol agent against *P. palmivora*.

## MATERIALS & METHODOLOGY

### Dual Culture Assay

A dual culture test was carried out using isolates of *Trichoderma virens* (T7b, T159c and PP29) and of *T. asperellum* (PP9) as biocontrol agents to test against all available Malaysian *Phytopthora palmivora* isolates, PP1-PP5 and PP7-PP12. Mycelial agar discs of isolates of both biocontrol agent and pathogen on carrot media were placed in a parallel arrangement to investigate any antagonistic interactions. Three replicates were used in the experiment. The plates were incubated at 28ºC, and the data were collected seven days after inoculation. *Figure 1* illustrates the percentage inhibition of radial growth (PIRG) of the test isolates was being measured. The measurements were made using the following formula:

$$\text{Percentage inhibition of radial growth (PIRG), \%} = \frac{R1 - R2}{R1} \times 100\%$$

where

R1 = radial growth in control plate
R2 = radius towards the antagonist colony

### Poison Agar Assay

Antibiosis by two endophytic *Trichoderma* strains was studied as described by Sundram (2013). Both strains were cultured on separate fresh PDA plates for seven days. The cultures were then plated on PDA which then served as the starting culture for the potato dextrose broth (PDB) (Difco, France). Six 7-mm discs of fungi were cultured onto PDB in Erlenmeyer flasks at 28°C and at 150 rpm for 7 days. The culture filtrate was collected through filter paper (Filtres Fioroni 601) and by 0.22-$\mu$m syringe filter filtration. Subsequently, the efficacy of the culture filtrate was evaluated by dissolving concentrations of 20%, 40%, 60% and 80% in agar plates. A 6-mm agar disc of *G. boninense* was placed on PDA agar incorporated with different concentrations of the endophytic *Trichoderma* culture filtrates. The control agar was full strength PDA. PIRG was calculated based on the growth radius of *G. boninense*.

## RESULTS AND DISCUSSION

The use of pesticides, which include fungicides, to control crop diseases has triggered public concerns, especially with regard to food safety due to the presence of pesticide residues in food products, as well as the detrimental effects of fungicides, such as causing soil microbial degradation (Hanada *et al.*, 2009). Therefore, transformation of disease control strategy using potentially effective microorganisms is feasible to achieve the objective of sustainable, environmentally friendly and safer crop production. The dual culture assay to assess the mycoparasitic interaction of antagonistic *Trichoderma* on the pathogen *P. palmivora* isolates produced the following results. It can be concluded that isolates of *T. virens*, namely T7b, T159c, PP9 and PP29, were able to inhibit the growth of all the *P. palmivora* isolates with PIRG ranges of 52.69%-77.43%, 54.10%-71.55%, 30.00%-82.72% and 32.19%-61.23%, respectively (*Table 1*, *Figures 2* and *3*). Lower inhibition of the pathogen was probably due to slower growth of the particular pathogen isolate in the media, thus giving lower values of R1. Overall, the results indicated that T159c was the most effective *T. virens* isolate against Malaysian *P. palmivora* isolates based on its consistent inhibitory reaction. In addition, most *Trichoderma* isolates showed aggressive mycoparasitism characteristics due to their tendency to overlap, coiling and invade the *P. palmivora* isolates. The mycoparasitic approach *via Trichoderma*-based biological control has been extensively reported against various *Phytophthora* spp. (Berger *et al.*, 2015; Tondje *et al.*, 2007), proving the effectiveness of *Trichoderma* antagonism towards *P. palmivora*. *Figures 2* and *3* show the whole array of the dual culture assays carried out on all the isolates of *P. palmivora* using *T. virens* isolates T7b and T159c. This is a preliminary investigation to gauge the potential use of biocontrol agents on the threat posed by the local *P. palmivora* pathogen on oil palm. All 11 *P. palmivora* isolates selected for testing had different virulence capacity as reported by previous researchers (Intan-Nur *et al.*, 2017). It has been found that the Malaysian isolates of

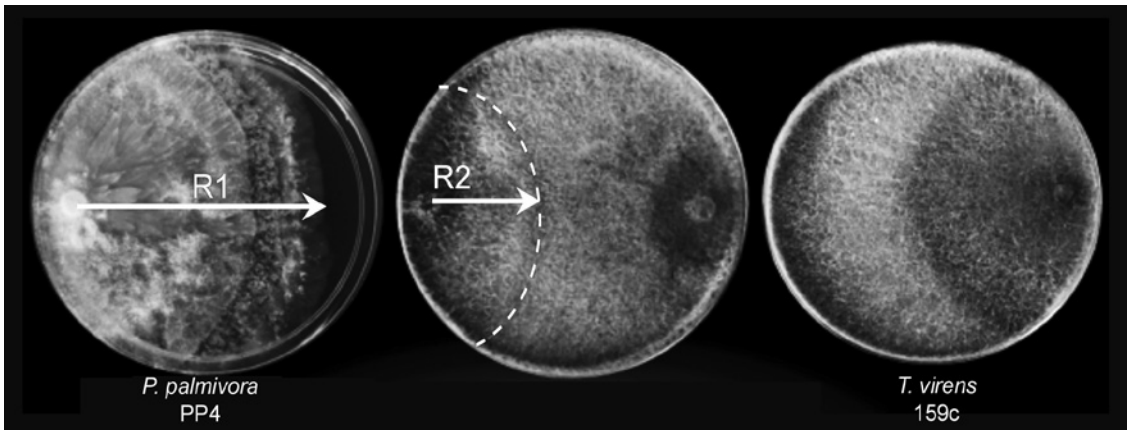


*Figure 1. Measurement of percentage inhibition of radial growth (PIRG) in dual culture assay of* Trichoderma virens *T159c against* Phytophthora palmivora.

**TABLE 1. PERCENTAGE INHIBITION OF RADIAL GROWTH (PIRG) OF MALAYSIAN *Phytophthora palmivora* BY *Trichoderma***

| ***P. palmivora* isolate** | **Percentage inhibition of radial growth by *Trichoderma* (%)** | | | |
|---|---|---|---|---|
| | **T7b** | **T159c** | **PP9** | **PP29** |
| PP1 | 57.34 ± 3.23 | 54.10 ± 0.22 | **82.72 ± 3.29** | 60.35 ± 0.80 |
| PP2 | **77.43 ± 0.30** | 64.82 ± 0.23 | 54.86 ± 1.78 | 62.14 ± 5.64 |
| PP3 | 58.15 ± 2.82 | 60.63 ± 0.24 | 57.82 ± 3.00 | **61.23 ± 1.50** |
| PP4 | **66.47 ± 0.30** | **66.03 ± 0.05** | 57.45 ± 1.45 | 65.01 ± 0.29 |
| PP5 | **65.34 ± 0.27** | 64.87 ± 0.21 | 56.06 ± 0.59 | 59.94 ± 0.90 |
| PP7 | 63.97 ± 0.28 | **66.71 ± 0.05** | 57.33 ± 0.53 | 64.86 ± 0.29 |
| PP8 | 59.09 ± 1.80 | **67.41 ± 0.14** | 53.98 ± 4.10 | 63.59 ± 0.33 |
| PP9 | 62.23 ± 0.46 | **67.41 ± 0.13** | 59.32 ± 0.00 | 63.97 ± 0.14 |
| PP10 | 64.56 ± 0.75 | **71.55 ± 0.03** | 65.80 ± 1.07 | 66.76 ± 0.69 |
| PP11 | 64.70 ± 0.41 | **70.55 ± 0.09** | 30.00 ± 1.20 | 32.19 ± 0.66 |
| PP12 | 52.69 ± 5.24 | **61.33 ± 0.08** | 53.65 ± 6.33 | 53.58 ± 1.86 |

Note: Mean values in bold represent the highest PIRG on the tested *P. palmivora* strains.

*P. palmivora* have different spectra of virulence, with *P. palmivora* isolated from durian being more pathogenic than the isolates from cocoa (Intan-Nur *et al.*, 2017).

The dual culture assay demonstrates the physical mechanisms of *Trichoderma* while the poison agar assay demonstrates the antibiosis mechanism of the biocontrol agents. Antibiosis is a defence mechanism causing the suppression of pathogens using secondary metabolites exuded by the biocontrol agents and in this case *Trichoderma*. The poison agar test showed positive suppression of the representatives of *P. palmivora*, P3 and P7. Incorporation of *Trichoderma* metabolites at 40% to 80% showed increasing suppression of the pathogen by all the *Trichoderma* isolates. One example shown in *Figure 4* is suppression in poison agar which had been incorporated with 40% to 80% of the exudates of PP29. *T. virens* isolate T7b gave the most consistent suppression (of more than 80%) in the poison agar assay against P3 and P7 (*Figure 5*).

## CONCLUSION

The study was initiated to look into the possibility of biological control of the pathogen *P. palmivora* in the event of an outbreak. An environmentally friendly biocontrol agent, *Trichoderma* was selected. Effective suppression and inhibition of the pathogen was observed through dual culture and poison agar assays to assess the effectiveness of the antagonist in controlling *P. palmivora*. It was found that endophytic *Trichoderma* isolates T7b and T159c were the most promising antagonists in suppressing *P. palmivora's* growth. Further research

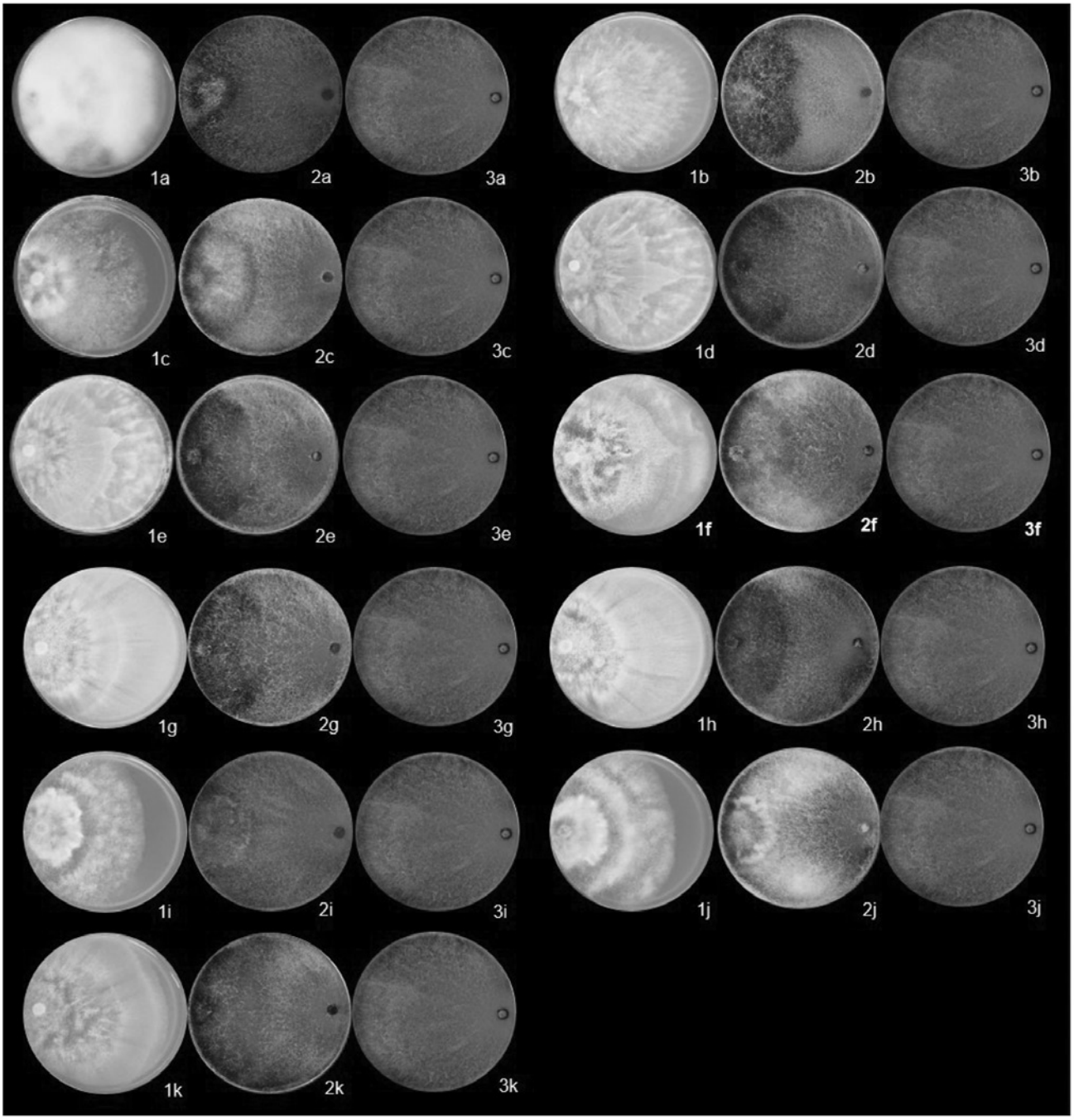


*Figure 2. Mycoparasitism of* Trichoderma virens *T159c on* Phytophthora palmivora *(PP1 – PP12). Series a1-3 correspond to strain PP1, series b1-3 correspond to strain PP2, series c1-3 correspond to strain PP3, series d1-3 correspond to strain PP4, series e1-3 correspond to strain PP5, and series f1-3 correspond to strain PP7. Series g1-3 correspond to strain PP8, series h1-3 correspond to strain PP9, series i1-3 correspond to strain PP10, series j1-3 correspond to strain PP11, and series k1-3 correspond to strain PP12. Series 1a-1k = controls of* P. palmivora*; series 2a-2k = interaction between the antagonist and* P. palmivora*; series 3a-3k = controls of antagonist strain* T. virens *T159c.*

using nursery and field screening procedures on highly infective hosts of *P. palmivora* is crucial to examine the efficacy of the promising isolates of T7b and T159c. Although Malaysia's biosecurity plan identifies important pathways for the prevention of accidental introduction of exotic pests and diseases, it is still crucial to carry out research on the long-term measures for control. This will safeguard the future of the oil palm industry in this country.

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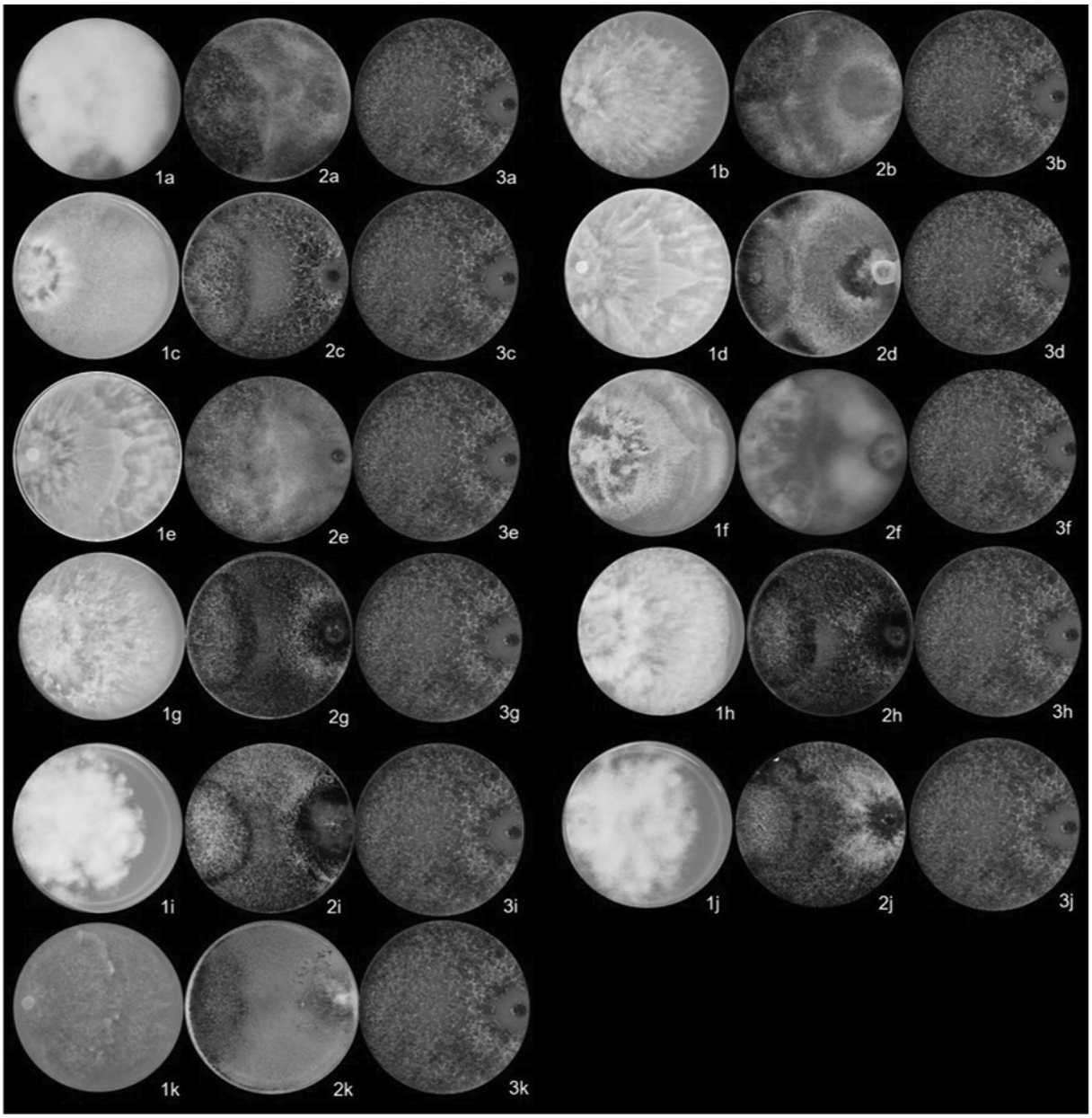


*Figure 3. Mycoparasitism of* Trichoderma virens *T7b on* Phytophthora palmivora *(PP1 – PP12). Series a1-3 correspond to strain PP1, series b1-3 correspond to strain PP2, series c1-3 correspond to strain PP3, series d1-3 correspond to strain PP4, series e1-3 correspond to strain PP5, and series f1-3 correspond to strain PP7. Series g1-3 correspond to strain PP8, series h1-3 correspond to strain PP9, series i1-3 correspond to strain PP10, series j1-3 correspond to strain PP11, and series k1-3 correspond to strain PP12. Series 1a-1k = controls of* P. palmivora*; series 2a-2k = interaction between the antagonist and* P. palmivora*; series 3a-3k = controls of antagonist strain* T. virens *T7b.*

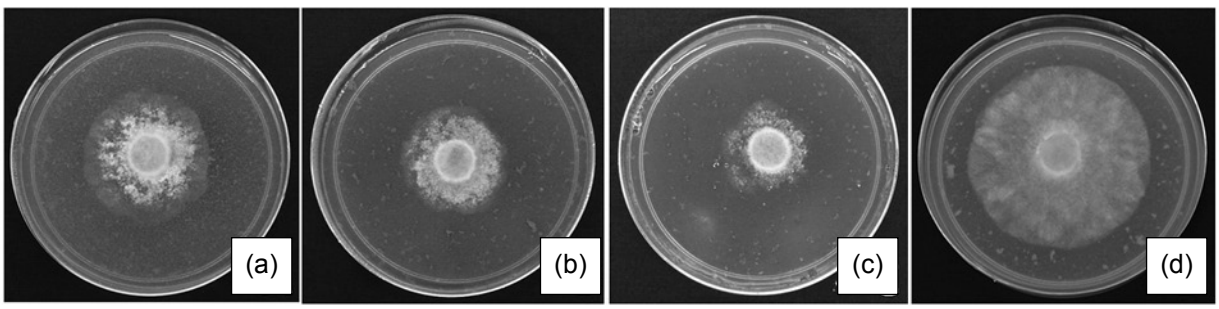


*Figure 4. Poison agar assay at (a) 40%, (b) 60% and (c) 80% incorporation of secondary metabolites secreted by* Trichoderma virens *PP29 tested against* Phytophthora palmivora *PP7. (d) Control PP7 on carrot agar.*

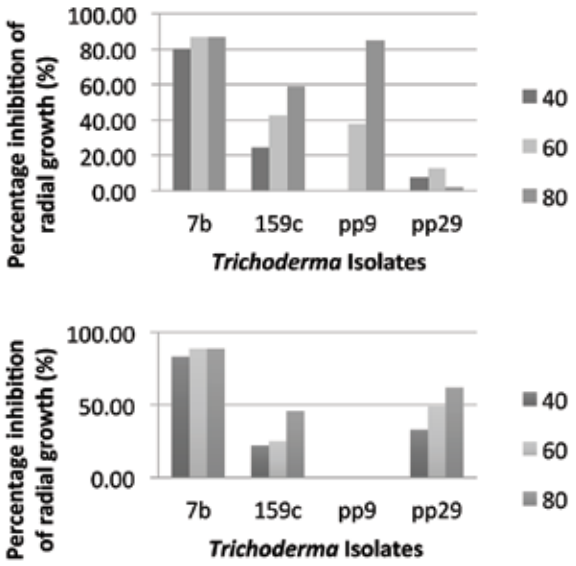


*Figure 5. Poison agar assay subjecting P3 and P7 isolates of* Phytophthora palmivora *to incorporation of metabolites from the respective* Trichoderma *isolates at concentrations of 40%, 60% and 80%.*

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