

Antimycotic Inhibition of *Phytophthora colocasiae* (Taro Leaf Blight) Using Diverse Disease Management Paradigms

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Abstract. Cocoyam/taro production has been plagued with many constraints, especially taro leaf blight. This study aimed at providing useful control options for the management of taro leaf blight. Two in vitro sub-trials (using botanicals alone or chemicals+botanicals) were conducted. A completely randomized design with each treatment replicated thrice was used and analyzed at a 95% confidence level. *P. colocasiae* was effectively inhibited by Balsam, Thyme, Lantana, Eucalyptus, and Alligator-pepper up to 144 Hours After Inoculation (HAI) with a 144-HAI-moving average of 69.5%. At 48, 96, and 144 HAI, Eucalyptus 100% concentration, Thyme (50 and 100%), Lantana 100%, and Alligator-pepper (50 and 100%) were excellent control resources. Percentage inhibition of the pathogen by plant extracts ranged from 13.8-100%. Combinations of Mancozeb and MetCop (i.e. Metalaxyl+Copper(I)oxide) with botanicals (Balsam, Lantana, and Thyme) controlled *P. colocasiae*. Inhibition of radial growth of *P. colocasiae* ranged from 0-100% with a 168-HAI-moving average of 55.5%. Combining Thyme with Mancozeb or MetCop significantly inhibited *P. colocasiae* more compared to other treatments. Mancozeb+Balsam 50% and MetCop+Balsam 100%, followed by Mancozeb+Lantana 100%, MetCop+Lantana 100%, and Mancozeb+Balsam 100% were the best treatments. Mancozeb was better than MetCop. Chemical rates can be reduced by using botanicals since chemicals affect health.

Keywords: botanical, cocoyams, lesions, Peronosporales, tuber crop

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1. Introduction

Cocoyams (old-taro: *Colocasia esculenta* (L) Schott and new-tannia: *Xanthosoma sagittifolium* (L) Schott) are mainly cultivated in the tropics and sub-tropics (i.e.; Africa, Caribbean islands, Pacific islands, and South East Asia) [1]. Tabi et al. [1] reported that taro leaf blight is ubiquitous in Cameroon (central Africa region generally). Cocoyam production in Africa is centered in the West and Central Africa regions. Lum and Takor [2] reported a high

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incidence (77.9-96.5%) and severity (51.9 – 85.6%) of taro leaf blight in south West Cameroons. Taro leaf blight has been reported wherever cocoyams are cultivated.

The development of control measures against these pathogens is strongly recommended [3]. For instance, Fontem et al. [4] reported that at 7 and 10 days after exposure to crude extracts of weed species (*Mitracarpus villosus* (Sw.) DC. and *Ageratum conyzoides* L.) and Mancozeb (a.i. Mancozeb), this blight pathogen was significantly inhibited compared to the untreated control *in vitro*. Taro leaf blight is currently managed using Ridomil (composed using Metalaxyl (4%) and Mancozeb (64%)) [5]. Some of these chemical pesticides are phytotoxic and contain harmful chemical residues which bring about environmental pollution and may damage human health [6].

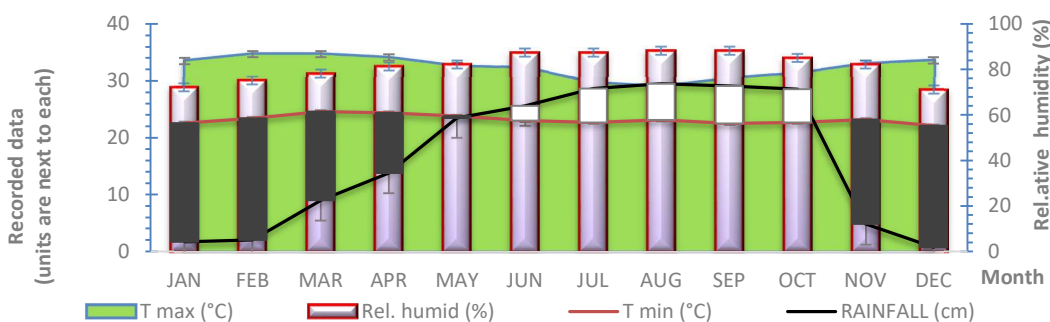
From the foregoing research, one can assume that investing in cocoyam production may be a profitable endeavor only if plant diseases are appropriately managed. The inadequacies stemming from disease management can hitherto hinder the enterprising farmers who are mostly resource-poor and barely subsist on their small farm holdings. Cocoyam is a food security crop for millions of people in the tropics and subtropics. It is produced as a source of nourishment, revenue, and raw materials in many countries. This study was conceived to tender some practical solutions (i.e. using chemical and botanical agents) to the management of this most tenacious disease problem of taro leaf blight in the global cocoyam production systems.

2. Materials and Methods

2.1. Site of the Study

This inquiry was carried out at the Faculty of Agriculture Laboratory complex, Alex Ekwueme Federal University, Ndufu-Alike, Abakaliki (6.0690N, 8.1990E) in Ebonyi State. The State is in the derived savannah vegetation zone of Nigeria. This area is in the semi-humid tropics with lush vegetation cover and it experiences rainfall for 8-9 months a year. The mean relative humidity is usually above 70% for 9 months per annum. Weather factors play a major role in the occurrence of taro blight.

In Fig. 1, based on the configurations of the up/down bars, the cross-over points are in May-October; when the rainfall is fully established, coupled with low minimum temperatures this results in damp weather that may favor taro leaf blight disease outbreaks. The maximum daily temperature decreases until August, then it increases gradually, which may also aid the pathogen to thrive. As was stipulated by Misra et al. [7] this weather situation is the archetype for *P. colocasiae* to devastate and ravage cocoyam.



Sources: Climate-data.org. Timeanddate.com 2005-2015 base as compiled by this author (2022)

Figure 1. Climatic Pattern for Abakaliki (Ebonyi State) Showing the Monthly Rainfall, Monthly Mean Relative Humidity, Minimum and Maximum Monthly Temperatures

2.2. Isolation and Identification of the Fungi Utilized in the Trials

Infected taro leaves utilized for this study were aseptically collected from the Bamenda Highlands in Camerouns by a colleague and conveyed to our research laboratory. The fungi *P. colocasiae* was isolated using dehydrated commercial Potato Dextrose Agar (PDA) medium, which was autoclaved at 120°C and 15 psi for 15 min according to the manufacturer's (Lifesave Biotech) instructions. The isolated fungi were sub-cultured to obtain pure cultures which were used (during microscopy) to identify the fungi with the aid of literature on fungi morphology [8] – [9].

2.3. Trial 1: Effects of Plant Extracts on *P. colocasiae* in vitro

The experiment was carried out using Petri dishes. It was laid out in the laboratory using the completely randomized design (CRD) with 10 treatments, and each treatment was replicated three times. The treatment set included control, Lantana 50%, Balsam 50%, Eucalyptus 50%, Alligator Pepper 50%, Thyme 50%, Lantana 100%, Balsam 100%, Eucalyptus 100%, Alligator Pepper 100%, and Thyme 100%.

The plant resources were each weighed at 333.3 g leaves (Lantana, Balsam, and Eucalyptus leaves) or 166 g seeds/flowers (Alligator pepper and Thyme) per L of distilled water to make 100% concentrations. The binomial names of the plants used are lantana (*Lantana camara*), balsam (*Impatiens balsamina*), eucalyptus (*Eucalyptus globulus*), alligator pepper (*Aframomum melegueta*), and thyme (*Thymus vulgaris*).

The plant tissues were weighed, macerated (using a Warrington blender), and allowed to stand for seven hours to increase extraction. The extracts were strained through double-layer muslin clothing and passed through Whatman's No. 1 filter paper. This filtration improved the clarity of the filtrate before use. The data for this study were collected as shown below.

2.4. Trial 2: Effects of Synthetic Fungicides + Plant Extracts on the Radial Growth of *P. colocasiae*

The experiment was carried out using Petri dishes. It was laid out in the laboratory using the completely randomized design (CRD) with 12 treatments and each treatment was replicated three times. Each treatment was applied (into the Petri dishes) according to the generated layout.

The treatment set** included the following:

MaB100% = Mancozeb100% x Balsam100%,

MaL100% = Mancozeb100% x Lantana100%,

MaT100% = Mancozeb100% x Thyme100%,

MaB50% = Mancozeb50% x Balsam50%,

MaL50% = Mancozeb50% x Lantana50%,

MaT50% = Mancozeb50% x Thyme50%,

MeB100% = MetCop100% x Balsam100%,

MeL100% = MetCop100% x Lantana100%,

MeT100% = MetCop100% x Thyme100%,

MeB50% = MetCop50% x Balsam50%,

MeL50% = MetCop50% x Lantana50%,

MeT50% = MetCop50% x Thyme50%*.

*The percentage behind each combination treatment refers to the concentration of each agent that was utilized (i.e. 1:1 rate at that percentage for each agent thus MaB100% refers to Ma100% x B100%. ** The treatment set was confounded to avoid repeating some levels of the treatments when a full two-way table is used.

The plant extracts were prepared as discussed above. The chemical fungicides were prepared at the rate of 5 g/L of sterile distilled water. This was equivalent to the standard recommended field fungicide rate. The *in vitro* rates were drawn at the rates of 50 and 100 μ /L (per Petri dish) as required. These low rates of chemicals were chosen to frame future integrated management protocols of these diverse control practices; whereby chemical fungicides would be reduced.

The active ingredients of the plant extracts are as follows. Thyme has many active ingredients including mainly p-cymene (8.41%), γ -terpinene (30.90%), and thymol (47.59%). Balsam has many active ingredients including mainly benzyl cinnamate and Benzyl benzoate; Lantana has many active ingredients including mainly alkaloids, flavonoids, saponins, and tannins: valencene and Y-gurjunene. Alligqator pepper has many active ingredients including mainly Tannin, saponin, flavonoid, steroids, terpenoids, cardiac glycoside, alkaloids, and phenol. Eucalyptus has many active ingredients including mainly 1,8-cineole (63.1%), p-cymene (7.7%), α -pinene (7.3%), and α -limonene (6.9%)

Mancozeb™ 80% WP: Mancozeb is also called Manzate®, Dithane®, Penncozeb®, Fore®, and Roper®. It is a broad-spectrum contact fungicide whose chemical name is ethylene bisdithiocarbamate (EBDC). Tandem™ WP (henceforth called MetCop) is composed of Metalaxyl (12%) and Copper (1) Oxide (60%). Metalaxyl is a systemic fungicide used to control plant diseases and its chemical name is methyl N-(methoxyacetyl)-N-(2, 6-xylyl)-DL-alaninate. Copper was one of the earliest contact fungicides used in agriculture. The data collection procedures for this investigation are shown below.

2.5. Data Collection and Analysis (for both sub-trials)

The radius of the fungus colony was measured using a transparent ruler at 24-hour intervals starting from the end of day 1 to the last day when the trial was terminated. The data were used to calculate the percentage inhibition of the pathogen using equation (1)

$$PI = ((C - T) / C) \times 100\% \quad (1)$$

where: PI = Percentage inhibition of the growth of the fungus; C = Perpendicular* radius of the fungus colony in the control plate; T = Perpendicular radius of the fungus colony in the treated plate

*Perpendicular refers to the right angle measured through the centre of the Petri dish because other radii could be obtained especially the longest radius away from the source of the inhibition [9].

The data collected from all trials were subjected to analysis of variance (ANOVA) and the means were separated using the Duncan's multiple range test (DMRT) (as obtainable with GenStat Discovery 2nd Edition statistical package). Meanwhile, descriptive statistics (as available with IBM SPSS ((statistical package for social sciences) version 23) were used to illustrate the inhibition trends obtained after the application of the control agents against the pathogen.

3. Results and Discussion

3.1. Percentage inhibition of the radial growth of *P. colocasiae*

The results of the percentage inhibition of *P. colocasiae* as influenced by the application of botanicals are presented in Table 1. It shows that *P. colocasiae* was effectively inhibited by all the plant extracts (balsam, thyme, lantana, eucalyptus, and alligator pepper) in the early phase of the trial. It revealed that the plant extracts were able to keep the radial growth of *P. colocasiae* in check-up to 144 hours after inoculation (HAI). However, the level of containment varied with the plant extract. Throughout the trial (in both phases), Alligator pepper (50 and 100% concentrations), Thyme (both concentrations), Lantana 100%, and Eucalyptus 100% excelled in

the inhibition of *P. colocasiae*. Generally, the percentage inhibition of the pathogen by these plant extracts ranged from 13.8-100%.

The results of the percentage inhibition of *P. colocasiae* due to the combined application of synthetic fungicides and botanicals are presented in Table 2. It revealed the potential of inhibiting *P. colocasiae* with combination rates of synthetic fungicides and biopesticides. The stage shows excellent performance from all the combinations with only Mancozeb+Balsam (both at 50% concentration) and Mancozeb+Lantana (at both 50% and 100% concentrations) showing low levels of inhibition of the pathogen (13.6-37.5%, 11.0-54.2% and 11.7-35.4% inhibition respectively).

The combinations were still excellent materials for inhibiting *P. colocasiae in vitro* even up to 168 HAI. The inhibition was quite impressive although the same control agents that started up poorly in phase one, showed lower levels of inhibition in phase 2. Generally, the level of inhibition ranged from 0.0-100% reduction of the radial growth of this pathogen.

3.2. Separation of the Means of the Treatments

The separation of the means of a representative sample of the data was carried out using Duncan's Multiple Range Test (DMRT) ($P \leq 0.05$) after subjecting the data that were collected to the Analysis of Variance (ANOVA) procedure. The effects of plant extracts on *P. colocasiae*; showing the placement of plant extracts based on overall performance according to their general ranking are presented in Table 1. After carrying out preliminary tests on the data the researcher decided to present only the data collected at 48-hour intervals to reduce drudgery and befuddlement of the readers. The analysed data presented were quite representative of the overall data from the trial.

At 48 HAI, all the treatments were significantly different ($P \leq 0.05$) from the control with the best treatments being Thyme 100% and Alligator pepper 100% followed by Alligator pepper 50%. At 96 HAI, the trend persisted but the best treatments were now Eucalyptus 100%, Thyme 100%, Lantana 100%, and both levels of Alligator pepper. All treated plots were significantly different ($P \leq 0.05$) compared to the control. At 144 HAI the trend was similar to those obtained during the first intervals presented. To sum, up these observations, the researcher determined the overall performance of the plant extracts by studying the ranking carefully and this produced the placement of the plant extracts in Table 1 according to their performance lot. The best rates were placed in lot 1 and the least inhibitory lot is the control.

The case of combined pesticide treatments was handled likewise. The effects of the chemical fungicides + botanicals on the radial growth of *P. colocasiae* are presented in Table 2. Only 48-hour intervals were utilized for this exercise to reduce bulk and increase the efficiency of the presentation. The results revealed that combining Thyme with Mancozeb or MetCop (at either

50% or 100% concentration) significantly ($P \leq 0.05$) inhibited *P. colocasiae* more than the other combinations throughout.

At 24 HAI, all these combination treatments were statistically at par, but significantly different ($P \leq 0.05$) compared to the control. Getting to 72 HAI, the best treatments were no longer only combinations of Thyme, but also Mancozeb+Balsam (at 50% concentration) and MetCop+Balsam (at 100% concentration). This group was closely followed by Mancozeb+Lantana (at 100% concentration), MetCop+Lantana (at 100% concentration), and Mancozeb+Balsam (at 100% concentration).

At 120 and 168 HAIs, the new best-performing combinations lost their potency but the combinations with Thyme maintained theirs. The main effects between Mancozeb and MetCop or between Thyme, Balsam, and Lantana were significantly different. More emphasis is being laid on combining the control agents because of the need to reduce the number of chemical pesticides being used. Some chemical pesticides have damaging effects on human health and the environment. Based on the ranking of the means, the treatments were placed in lots starting from the best treatments as shown in Table 2. Thus on a general note, the combination treatments were all significantly different from the control during this trial.

Table 1. Percentage Inhibition, Uninfected Area, selected Mean Ranking of *P. colocasiae* with Botanical

Treatments	Percentage inhibition (%) at						Area (%)**	Selected mean ranking at		
	24 h	48 h	72 h	96 h	120 h	144 h		48 h	96 h	144 h
Aligator pepper 100%	100	100	83.1	72.2	68.2	78.4	95.6	0.0a	0.83a	1.67a
Thyme 100%	78.67	68.8	55.9	51.1	50.3	66.4	91.2	0.07ab	0.93a	2.00a
Aligator peppr 50%	80.0	68.8	53.8	47.8	45.9	63.4	86.0	0.50bc	1.57a-c	2.83bc
Eucalyptus 100%	100	58.3	53.8	50.0	58.6	69.8	94.8	0.67c	1.50ab	2.33ab
Lantana 100%	100	52.1	46.2	61.1	65	60.8	90.8	0.77c	1.17a	3.03bc
Eucalyptus 50%	56.0	37.5	30.8	33.3	36.9	57.3	92.0	1.0c	2.00b-d	3.30c
Thyme 50%	86.22	79.2	64.3	57.0	54.8	69.4	90.9	0.73c	2.00b-d	4.50de
Balsam 100%	100	56.3	23.1	18.9	42.7	46.1	72.0	0.70c	2.43de	4.17d
Lantana 50%	68.0	47.9	21.5	24.4	29.9	35.3	52.0	0.83c	2.27c-e	5.00e
Balsam 50%	72.0	50.0	13.8	14.4	17.2	25.9	46.0	0.80c	2.57de	5.73f
Control	-	-	-	-	-	-	-	1.60d	3.00e	7.73g
mean x	84.09	61.9	44.6	43	46.9	57.3	81.1	0.6	1.7	3.3
SED								0.25	0.33	0.52

The percentage behind each combination refers to the concentration of each component of the combined rate. ** Uninfected area 144 h (%)

Means followed by the same letter(s) in a column are statistically similar based on DMRT ($P \leq 0.05$)

Table 2. Effects of fungicides+botanicals on *P. colocasiae*; Showing Placement of Plant Extract based on Overall Performance as Shown by the Ranking of the Treatment Means and Percentage Inhibition

Treatments	Percentage inhibition (%) at							area (%)**	Selected mean (cm) separation at			
	24 h	48 h	72 h	96 h	120 h	144 h	168 h		24 h	72 h	120 h	168 h
Mancozeb+Thyme 100%	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0a	0.0a	0.0a	0.0a
MetCop + Thyme 100%	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0a	0.07a	0.50ab	1.67ab
Mancozeb + Thyme 50%	100.0	75.0	81.5	88.6	75.2	78.0	77.3	94.0	0.0a	0.0a	0.97abc	1.57ab
MetCop +Thyme 50%	100.0	95.8	81.5	79.0	82.8	84.5	83.0	95.2	0.0a	0.40ab	0.90a-c	1.50ab
Mancozeb+Balsam 50%	100.0	37.5	6.2	0.0	1.3	7.3	18.6	14.0	0.0a	0.47ab	1.60b-d	2.47b
MetCop + Balsam 100%	100.0	95.8	96.9	100.0	90.4	90.5	81.1	87.9	0.0a	0.60ab	2.00c-e	3.50bc
Mancozeb+Balsam 100%	100.0	58.3	44.6	47.6	52.2	61.2	60.2	80.8	0.0a	1.07bc	3.20e	4.73c
MetCop +Lantana 100%	100.0	100.0	100.0	88.6	81.5	83.2	82.2	88.0	0.0a	1.20b-d	2.50de	3.50bc
Mancozeb+Lantana 100%	100.0	35.4	3.1	5.7	2.5	15.9	11.7	10.4	0.0a	1.27b-e	4.67f	7.83d
MetCop+Lantana 50%	100.0	37.5	23.1	38.1	44.6	46.1	39.4	58.0	0.0a	1.67c-f	2.90e	5.33c
MetCop +Balsam 50%	100.0	93.8	50.8	50.5	38.9	49.6	46.2	53.6	0.0a	2.03d-f	5.17f	7.17d
Mancozeb + Lantana 50%	100.0	54.2	41.5	14.3	10.8	11.6	11.0	10.8	0.0a	2.10ef	5.10f	7.77d
Control	-	-	-	-	-	-	-	-	0.8b	2.17f	5.23f	8.80d
mean	100.0	71.2	57.2	55.7	52.8	57.1	55.5	63.0	0.1	1.0	2.6	4.3
SED									0.59	0.39	0.58	0.51

* The percentage behind each combination refers to the concentration of each component of the combined rate. ** Uninfected area 168 h (%)

Means followed by the same letter(s) in a column are statistically similar based on DMRT ($P \leq 0.05$)

In this study, the application of plant extracts (Eucalyptus, Alligator pepper, Thyme, Balsam, and Lantana) successfully inhibited the taro leaf blight inducer. This affirmed the findings of Lum and Takor [2] who successfully utilized aqueous leaf extracts of goatweed (*Ageratum conyzoides* L.) and Mancozan in-vitro to inhibit *P. colocasiae*. They reported that farmers were not aware of any pesticidal plants that can be used to manage taro leaf blight.

Iwuagwu et al. [10] utilized ethanol, methanol, and petroleum ether plant extracts and synthetic fungicides to control *A. niger* on cocoyams (old cocoyam and new cocoyam). Plant extracts of lemon grass (*Cymbopogon citratus*) leaves and stalks, fruits of pepper-fruit (*Dennettia tripetala*) and Apron plus significantly inhibited *A. niger* (at the rate of 49.0, 39.5, and 75.0% respectively). The higher concentrations of agents produced higher inhibition. These findings concur with the findings of this study.

While Wavare et al. [11] reported that aqueous extracts of flowers of marigold (*Tagetes erecta*) exhibited potential antifungal activity against *Sclerotium rolfsii*. Another report by Hussain et al. [12] revealed that *Eucalyptus camaldulensis*, *Acacia nilotica*, *Azadirachta indica*, *Crotalaria juncea*, *Ocimum basilicum*, and *Prosopis juliflora* inhibited *Aspergillus flavus*, *Aspergillus niger*, *Fusarium solani*, *Macrophomina phaseolina* and *Rhizoctonia solani* by suppressing

mycelial growth. These studies confirmed the current findings on the use of plant extracts to control plant pathogenic fungi.

Ndifon and Lum [13] reported that all the plant extracts (including *Eucalyptus globulus*, *Andrographis paniculata*, *Melaleuca cajuputi*, and *Euphorbia hirta*) inhibited the growth of *Aspergillus niger* significantly compared to the control and the level of inhibition was higher at 100% concentration than at 50% concentration of the botanicals. These findings agree with the findings herein that *Eucalyptus* spp. and other plant extracts can control fungi plant disease agents.

Confirmations of the usefulness of eucalyptus and lantana were found in the work of Chavan et al. [14]. They reported that rhizome rot (*Pythium aphanidermatum*) was significantly inhibited by all the botanicals compared to the untreated control. Average mycelial growth inhibition ranged from 21.65% (*Eucalyptus globulus*) to 86.33% (*A. indica*).

Sanasam et al. [15] reported that plant extracts of garlic and turmeric (67.7%) inhibited *Sclerotium rolfsii*. Research on the control of *P. Colocasiae* is still in its infancy. In a related study, Ndifon et al. [16] revealed that ginger and garlic seed and soil dressing significantly controlled *Fusarium* wilt in *Solanum aethiopicum* cultivation. Sneha et al. [17] reported that garlic and ginger inhibited the pathogenic *S. rolfsii*. These findings reveal that plant extracts sourced locally can be used to control various fungi spp.

Tabi et al. [1] in the West Cameroons reported that there was no disease incidence during the dry season, but 18.2, 27.3, 27.3, and 100.0% disease incidence were recorded for Callomil plus (72 WP), Mancoxyl plus (720 WP) and 1:1 ratio Callomil plus+Mancoxyl plus and control during rainy season respectively. The disease severity was 75% in control but lower in treated plots. Manju et al. [18] utilized foliar sprays of Fungiforce™ (i.e. contact and systemic fungicide from Copper oxide (600 g) and Metalaxyl (120 g)) at different concentrations to effectively control taro leaf blight. They reported no significant differences between the 4 cultivars in the different field sites. Misra et al. [7] reported that copper and metalaxyl fungicides are very effective against taro leaf blight. These findings concur with the findings attained in this trial using MetCop.

4. Conclusion and Recommendation

This study revealed that Lantana, Balsam, Eucalyptus, Alligator-pepper, and Thyme were effective against the growth of *Phytophthora colocasiae* in vitro. Combining Lantana, Balsam, and Thyme with either Mancozeb or MetCop (i.e. Metalaxyl + Copper (I) oxide) was effective as well in reducing the radial growth of the pathogen. The efficacy of Thyme in combination with synthetic fungicides was most impressive. These control agents are recommended for the

control of *P. colocasiae* while more research on these agents continues. The researchers should aim at reducing chemical fungicides through the integration of these control paradigms as the research progresses.

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