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Antifungal activity of six botanicals against root crop diseases

Manuel K. Palomar, Erlinda O. Landerito, Avelina P. Molato, Doreen S. Cayanong and Victoria G. Palermo

Department of Pest Management
Leyte State University, Visca, Baybay, Leyte

ABSTRACT

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Sequential extractions were done in selected botanicals to test their bioactivity against important fungal pathogens of root crops. Extracts considered as potential fungicide were ampalaya (*Momordica charantia*) crude ethanolic/acetonic extract to *Colletotrichum gleosporoides* and *Phytophthora colocasiae*, asyang (*Mikania cordata*) crude ethanolic/acetonic extract to *P. colocasiae*, ginger (*Zingeber officinale*) crude ethanolic/acetonic extract to *Sphaceloma batatas*, kamantigue (*Impatiens balsamina*) crude ethanolic/acetonic extract to *Sclerotium rolfsii*, *S. batatas* and *C. gleosporioides*, olasiman (*Portulaca oleracea*) crude ethanolic extract to *S. rolfsii* and *S. batatas*, and saluyot (*Corchorus olitorius*) crude ethanolic extract to *S. batatas* and *P. colocasiae*. Phytochemical screening revealed that secondary metabolites such as flavonoids, steroids and terpenoids were present in the plants while only saluyot contained tannins and polyphenolic compounds.Flavonoids caused complete inhibition of colony growth of *S. batatas*. For *S. rolfsii*, the following flavonoidal extracts were fungicidal: ampalaya (*M.charantia*) using ethanol and acetone, ginger (*Z. officinale*) using ethanol, and kamantigue (*I. balsamina*) or olasiman (*P. oleracea*) acetonic extract to *C. gleosporioides*. Excised leaves inoculated with *P. colocasiae* treated with asyang (*M. cordata*), olasiman (*P. oleracea*), and ginger (*Z. officinale*) ethanolic/acetonic extracts showed no infection after 6 days, which indicates superiority to other extracts and that of the control. Planting treatment of yam setts with ampalaya (*M. charantia*) ethanolic/acetonic extracts followed by regular spraying with the same extract up to 6 months after planting (MAP) showed the best protection against yam anthracnose with degree of protection better than Benlate. Furthermore, taro plants treated with olasiman (*P. oleracea*) ethanolic extract exhibited the highest percent disease control and least percent tuber surface infection by *S. rolfsii*.

Keywords: biofungicide botanicals, plant extracts, secondary metabolites, bioactive components, phytochemical screening, root crop diseases.

Correspondence: M.K. Palomar . *Present Address:* Dept. of Pest Management, Leyte State University, Baybay, Leyte 6521-A, Philippines. *Tel. No.* (053) 335-2626

INTRODUCTION

Farmers have generally ignored root crop diseases even if most of them can be effectively controlled using synthetic fungicides. Such decision has ecological basis because problems related to the use of pesticides have arisen such as the tremendous residual effect of chemicals on people and the environment. Besides, the use of fungicides is not economically feasible for small-scale root crop growers. Since root crop diseases are becoming major problems, effective alternative control strategies against them are needed.

Kamantigue (*Impatiens balsamina* L.); garlic vine (*Pseudocalymna alliaceum*) (Lan) Sandwitz; garlic (*Allium sativum* L.) and marigold (*Tagetes erecta*) have been reported as effective against *Helminthosporium oryzae*, the brown spot pathogen of rice (Lapis and Dumancas, 1979). On the other hand, *in vitro* bioassay conducted by Palomar *et al.* (1994) revealed that crude extracts of kamantigue and ampalaya exhibited fungitoxic activity to one or more root crop fungal diseases.

Secondary metabolites of some botanicals can be tapped as alternative source of new "biopesticides". They offer the advantage of specificity of biological action, which greatly reduces the likelihood of harmful bioaccumulation in the soil and ground water residues. Crude extracts from botanicals like asyang (*Mikania cordata*), ampalaya (*Momordica charantia*), ginger (*Zingiber officinale*), saluyot (*Corchurus olitorius*), olasiman (*Portulaca oleracea*) and kamantigue (*I. balsamina*) using water as solvent are effective in controlling major pathogens of root crops (Palomar *et al.*, 1994). The aqueous extract, however, should be used immediately after preparation because its potency diminishes upon storage. A huge volume of extracts is also needed if many plants will be treated. Hence, it is necessary to isolate the bioactive components of these plant extracts using more suitable solvents like acetone and ethanol for more adequate and effective application especially on large-scale basis.

This paper presents the isolated secondary metabolites from selected botanicals tested against fungal diseases of root crops under laboratory and screenhouse conditions.

MATERIALS AND METHODS

Preparation of Plant Extracts

Fresh and healthy leaves of each plant were washed thoroughly with tap water and air-dried. For ginger, fresh corms were used and after washing were peeled and pounded using mortar and pestle before soaking in a specified solvent.

Four-hundred-gram samples of each plant under study were finely chopped and sequentially extracted with different solvents of increasing polarity i.e. from acetone to ethyl alcohol. Enough solvent was added to cover the samples. After 24 hours of soaking, the extract was filtered through a Buchner funnel with gentle suction. The filtrates were concentrated at 30°C-40°C using a rotary evaporator. The residues obtained were air-dried to remove residual solvent before proceeding to the next solvent extraction. The concentrated bioactive extracts were stored in amber bottles at a temperature of 0° to 5°C before use.

Phytochemical Screening of Extracts

The bioactive fraction was tested qualitatively for the presence of secondary metabolites (alkaloids, flavonoids, saponins, polyphenols, tannins and anthraquinones) following the method of Guevarra and Recio (1985). Alkaloid components were qualitatively analyzed using Culvenor-Fitzgerald Laboratory method and confirmed with Mayer's and Dragendorff's reagent. The presence of flavonoid compounds was detected using the Bate-Smith and Metcalf test as well as the Wilstatter "cyanadin" test. Tannins and polyphenolic compound were screened using gelatin tests, and ferric chloride test. Froth Test, Capillary Method and Liebermman-Burchard test were used to establish the presence of saponins in the plants under study. The latter test likewise detected the presence of unsaturated steroids and triterpenes.

Preparation of Flavonoidal Extract for Antifungal Assay

Acetone or ethanol extract were evaporated to incipient dryness. The residue obtained was defatted using hexane until the extract became almost

colorless. The hexane extract was discarded and the residue that contained the flavonoidal components was dissolved in 80% alcohol. A portion of this alcoholic flavonoidal extract was qualitatively tested using color reaction test for flavonoids.

Antifungal Assay of Crude Extracts

In Vitro Assay

Agar plates were prepared by adding to each plate 1 mL of concentrated bioactive extracts and pouring approximately 10 mL of potato dextrose agar (PDA) pre-cooled to 40°C. The plates were carefully rotated to ensure even mixing. After the mixture had solidified, a single sclerotial body of *S. rolfsii* was placed at the center of each plate whereas 5-mm dia. discs of mycelia were used for *L. theobromae*, *C. gleoesporoides* and *P. colocasiae*. Plates containing PDA alone and thiophanate methyl (Saprol EC at recommended rate) served as control. The plates were incubated at room temperature and measurement of mycelial growth was taken 72 hours after plating. For *P. colocasiae* incubation was extended to 6 days because of its slow mycelial growth.

On the other hand, for *Sphaceloma batatas*, 10-mL sweetpotato decoction was dispensed in test tubes and sterilized for 15 min psi. After the medium cooled, a single colony of one-week-old *S. batatas* culture was especially transferred and then 1-mL extract was added to each designated test tube. One mL of Benlate (RR) and Saprol EC (RR) suspension were used instead of the extract for control treatments. Number of colonies was noted after one week of incubation.

In Vivo Assay

Taro Leaf as Host. The petioles of detached taro leaf were wrapped with moistened cotton and individually placed in big petri dishes lined with moistened filter paper. Meanwhile, mycelial discs of *P. colocasiae* were dipped in different concentrated bioactive extracts for one week. Inoculation of pre-treated mycelial discs was employed by gently rubbing them onto both sides of the leaf surface. Treated and untreated control treatments were included as

checks. Assessment of percent disease severity was also done.

Taro Plant as Host. Taro suckers were sprayed with designated bioactive extracts such as ampalaya, kamantigue, and olasiman ethanolic and ginger acetonic at 1:1 dilution (vl.:vl.) before planting in polyethylene bags containing sterilized soil. Monthly drenching of extracts around the plants was done to test plants up to 5 months. Saprol EC (fungicide) and untreated control served as checks.

Five months after planting, the test plants were inoculated with 10 sclerotial bodies per plant. The inoculum was placed equidistantly around the base of each plant and lightly covered with soil particles to prevent dessication. Plants were harvested after seven months. Harvested tubers were cleansed of soil debris, weighed and brought to the laboratory for storage. After three weeks of storage, percent tuber surface infection was taken. The tubers were halved longitudinally and percentage infection was noted accordingly.

Yam Plant as Host. The tubers (var. Kinampay) were sliced approximately 100 g each and treated with concentrated bioactive extracts (ethanol/acetone extracted)/fungicide prior to planting. Treated tubers were planted in polyethene pots containing sterilized soil. Concentrated bioactive extracts were sprayed to plants at 3 months after planting (MAP) at biweekly interval until 6 MAP. After the third spraying, inoculation of *C. gleoesporoides* was undertaken. Weekly assessment of disease severity was done up to one month before harvest. Yield data were also recorded.

RESULTS AND DISCUSSION

Phytochemical Screening and Chromatographic Separation

Phytochemical screening of the six botanical plants revealed that bioactive compounds present were mainly flavonoids, tannins and polyphenoic compounds (Table1). Secondary metabolites such as anthraquinones, cyanogenic glycosides and saponins bioactive compounds that are known to be present in some plants were not detected. Their concentration in the extract may be too low to be detected using qualitative tests. Fleischhacker (1996) reported that secondary metabolites derived from *Portulaca oleracea* included

Table 1. Secondary metabolites detected in selected botanicals

Plant Extracts	Bioactive Compounds					
	Anthra-quinones	Steroids and Terpenoids	Cyanogenic glycosides	Flavonoids	Saponins	Tannins and Polyphenols
Ampalaya	-	+	-	+	-	-
Asyang	-	+	-	+	-	-
Ginger	-	+	-	+	-	-
Kamantigue	-	+	-	+	-	-
Olasiman	-	+	-	+	-	+
Saluyot	-	+	-	+	-	-

Table 2. Thin layer chromatographic profile of secondary metabolites from ethanolic extracts of selected species using various solvent systems[a]

Plant Extracts	Family	Solvent System	Ratio	# of Spots/ Separation
Ampalaya (*M. charantia*)	Cucurbitaceae	ethyl acetate: dichloromethane	3:7	6
Asyang (*M. cordata*)	Asteraceae	butanol: isopropanol	6:4	7
Ginger (*Z. officinale*)	Zingerberaceae	ethyl acetate: dichloromethane	1:1	8
Kamantigue (*I. balsamina*)	Balsaminaceae	benzene: ethyel acetate	11:9	7
Olasiman (*P. oleracea*)	Portulacaceae	butanol		6
		benzene:acetic acid	1:1	5
Saluyot (*C.olitoris*)	Tiliaceae	ethanol:ethyl acetate	2:1:1	.9

a - only solvent systems that gave good separation of bioative components.

cinnamic acid derivatives, cathechol amines and benzoic acid derivatives, which are phenolic compounds. Flavonoids, the major compound present in all plants studied, have a C_6-C_3-C_6 chain and are known to have different biological activities which include growth regulation, antimicrobial action and repellency or interactiveness to feeding insects (Hartborne, 1984). *Impatiens balsamina* leaves contain anthraquinones. The antifungal compound isolated was 2, 4 methoxynapthoquinone from 95% ethanol extracts of dried aerial part (Yang *et al.*, 2001). Fresh rhizomes of *Zingiber officinale* when subjected to steam distillation, yielded ginger oil in which curcumene was found to be the major constituent. The compounds exhibited significant antifungal activity against *Rhizoctonia solani* (Agrwal *et al.*, 2001).

Several solvent systems for thin layer chromatography (TLC) were tried to determine the most efficient solvent that gave better separation of secondary metabolites. It was observed that ethyl acetate was the common component

of the solvent system that provided better separation of bioactive compounds (Table 2). Howèver, further bioassay-guided fractionation should be done to determine the antifungal compounds.

I n Vitro Antifungal Assay of Concentrated Bioactive Extracts

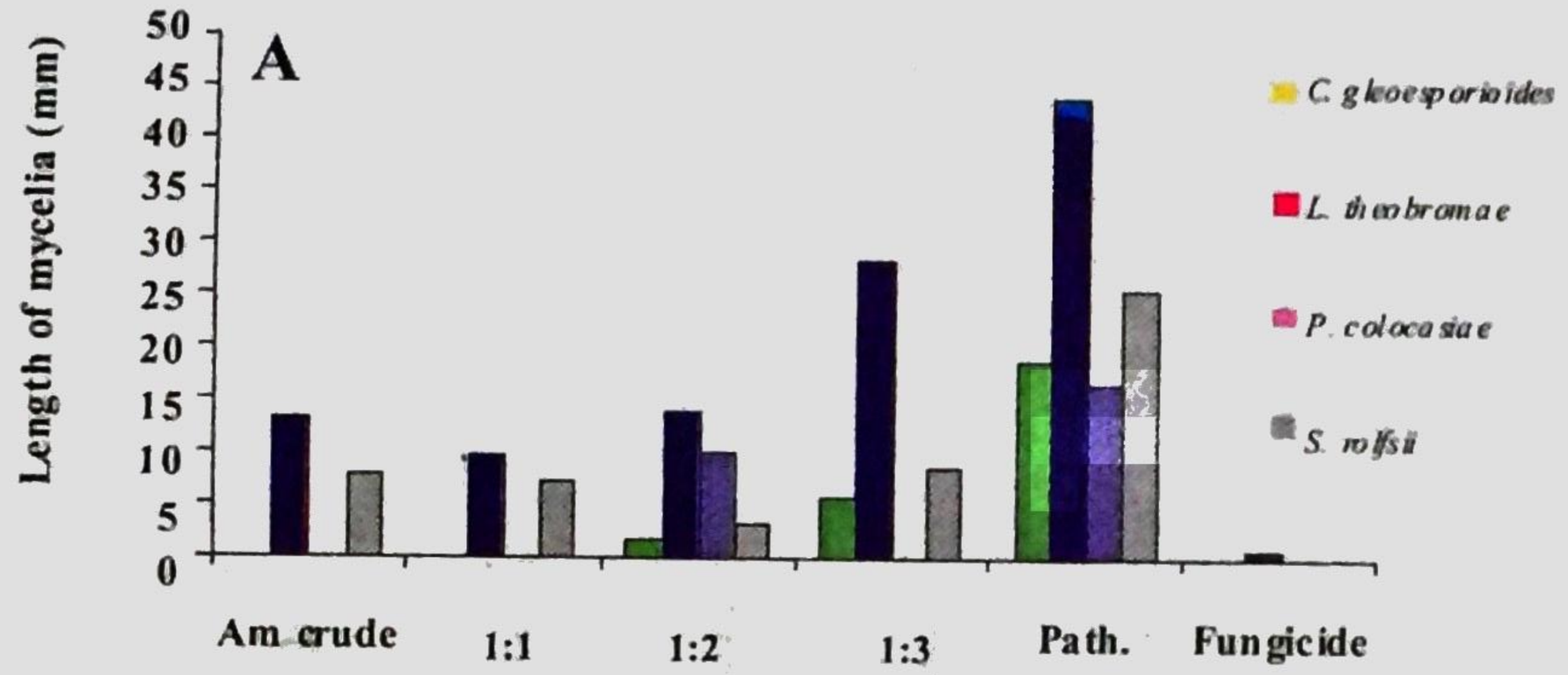
Sclerotium rolfsii. It was observed that crude kamantigue and olasiman ethanolic extracts were antifungal to *S. rolfsii*. Likewise, crude ampalaya and kamatigue ethanolic and at 1:1 dilution of extracts were fungistatic; Growth was inhibited to some extent which was even superior to the effect of Saprol EC at its manufacturer's recommended rate (Fig. 1a, b & c). Thus, it can be concluded that the above-mentioned extracts can be a potential alternative biofungicide against *S. rolfsii* infecting root crops both in the field and in storage.

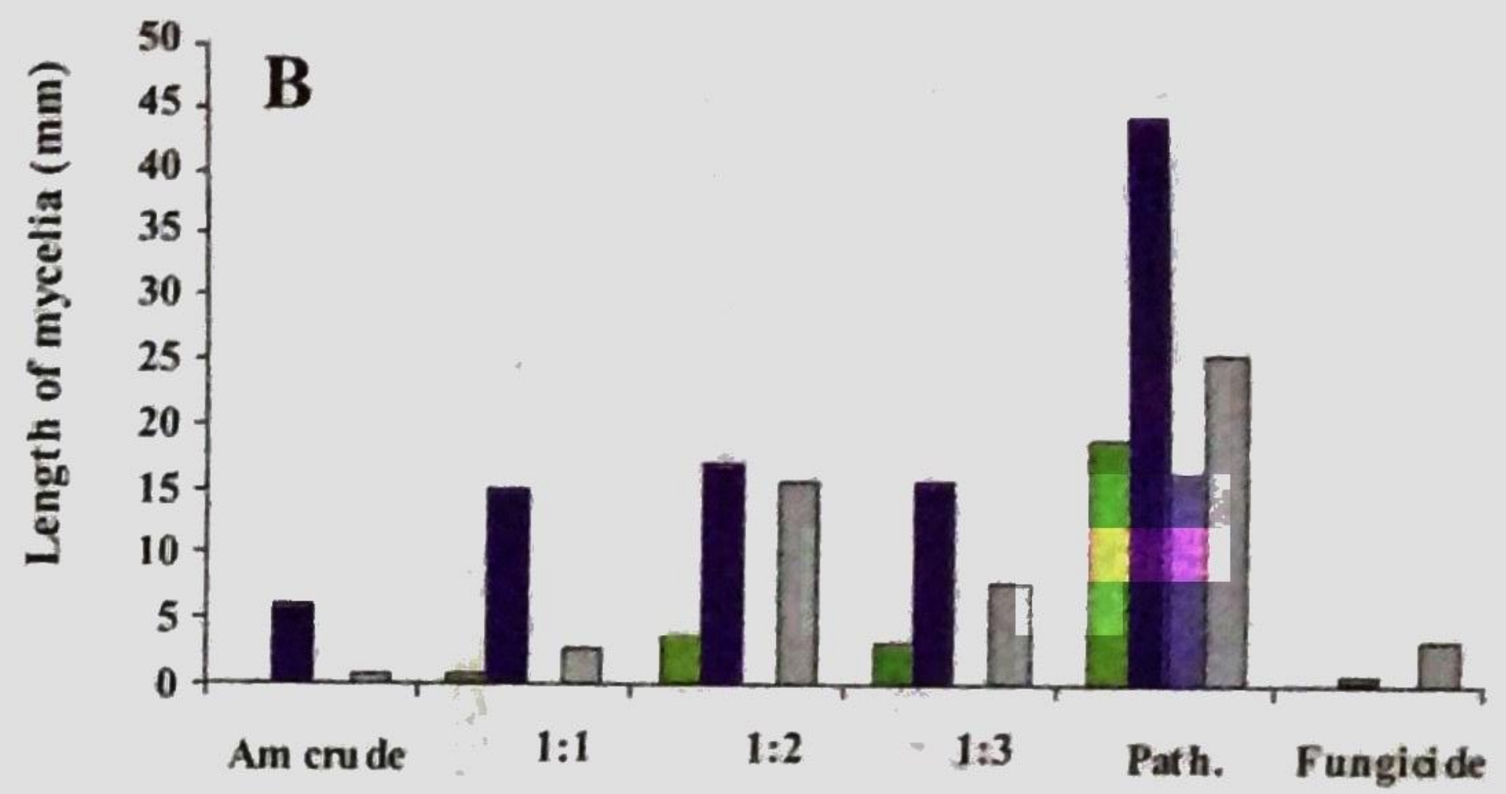
Fungistatic extracts, which inhibited the growth of the fungus to some degree, were amplaya ethanolic 1:1 (v/v) up to 1:3 dilutions, ampalaya acetonic 1:2, crude asyang acetonic and kamantigue acetonic at 1:2. The corresponding mycelial inhibition ranged from 85-96% which was only slightly lesser than when Benlate was used.

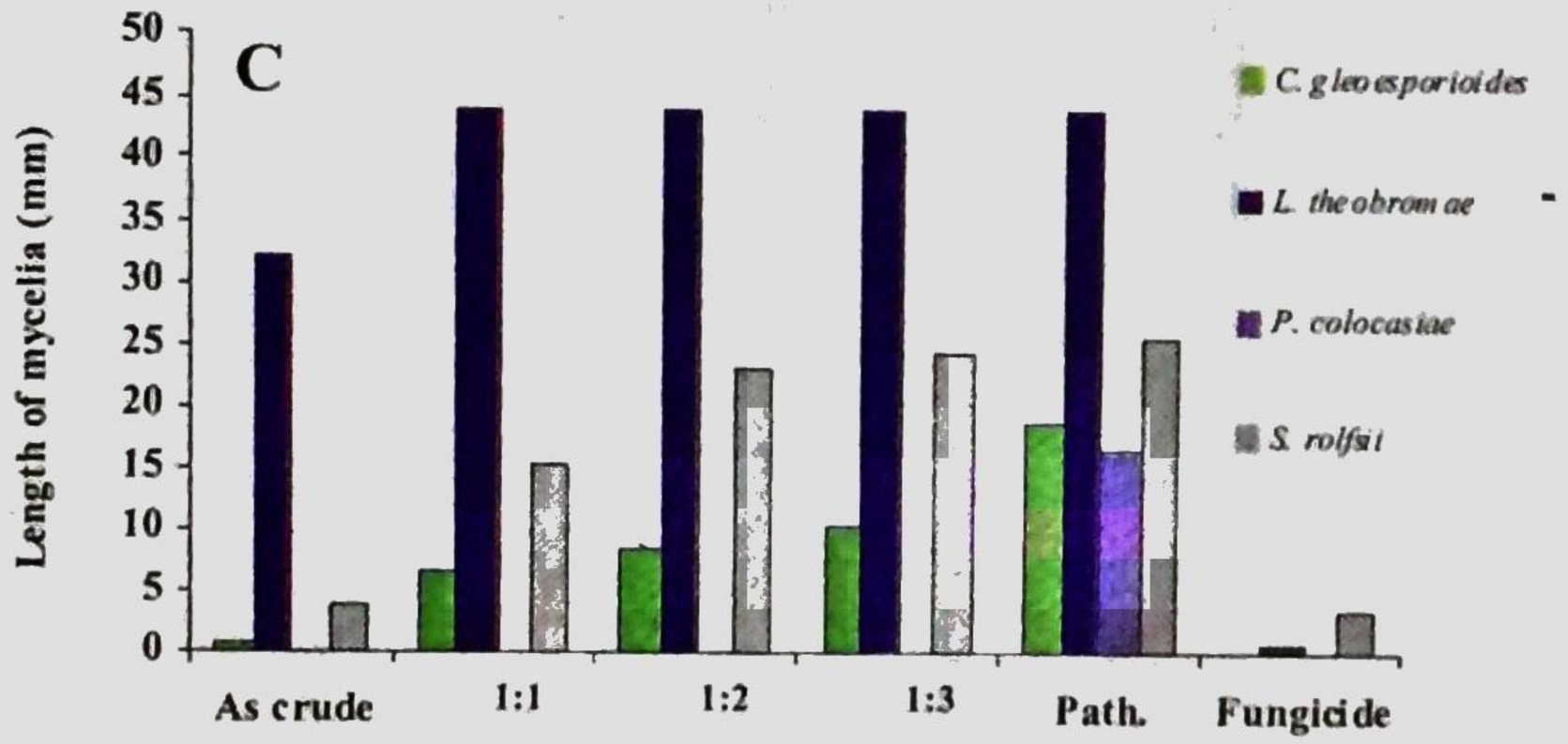
Lasiodiplodia theobromae. Concentrated bioactive extracts that possessed fungistatic activity for *L. theobromae* were crude kamantigue acetonic and crude ampalaya ethanolic extracts with corresponding 96.20 and 85.98% mycelial inhibition. The results were inferior to the effect of Benlate having a 98.86% inhibition of fungal growth. No fungicidal extracts were found against the fungus (Fig. 1a, b, & c).

Phytophthora colocasiae. Several-concentrated bioactive extracts showed fungicidal activity against *P. colocasiae*. These were extracts of ampalaya ethanolic and acetonic up to 1:1 dilution, ampalaya ethanolic 1:2 to 1:3 and acetonic 1:3 dilutions, crude asyang and acetonic up to 1:3 dilutions, crude ginger acetonic and 1:1, crude kamantigue ethanolic and acetonic up to1:3 dilutions, and crude saluyot ethanolic. The above-mentioned extracts/ dilution gave a 100% inhibition of fungal growth comparable to the effect of Saprol EC using the recommended rate.

Fungistatic extracts were from ampalaya acetonic 1:2, crude ginger ethanolic up to 1:2 dilutions, ginger acetonic 1:2 and 1:3, saluyot ethanolic 1:1 and crude saluyot acetonic and its 1:3 dilution. However, the results will be further verified to note possible degradation of sporangia as a result of extract







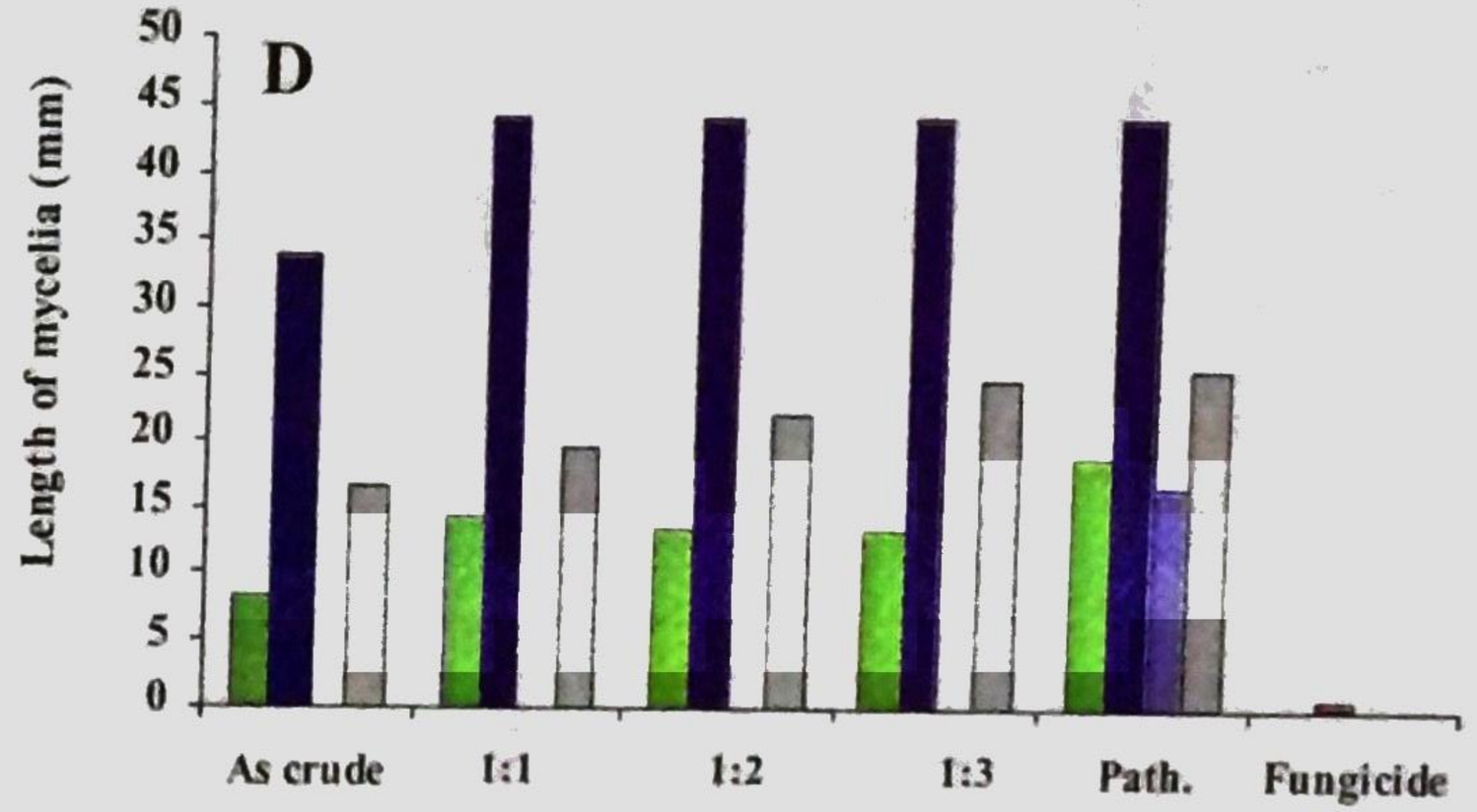
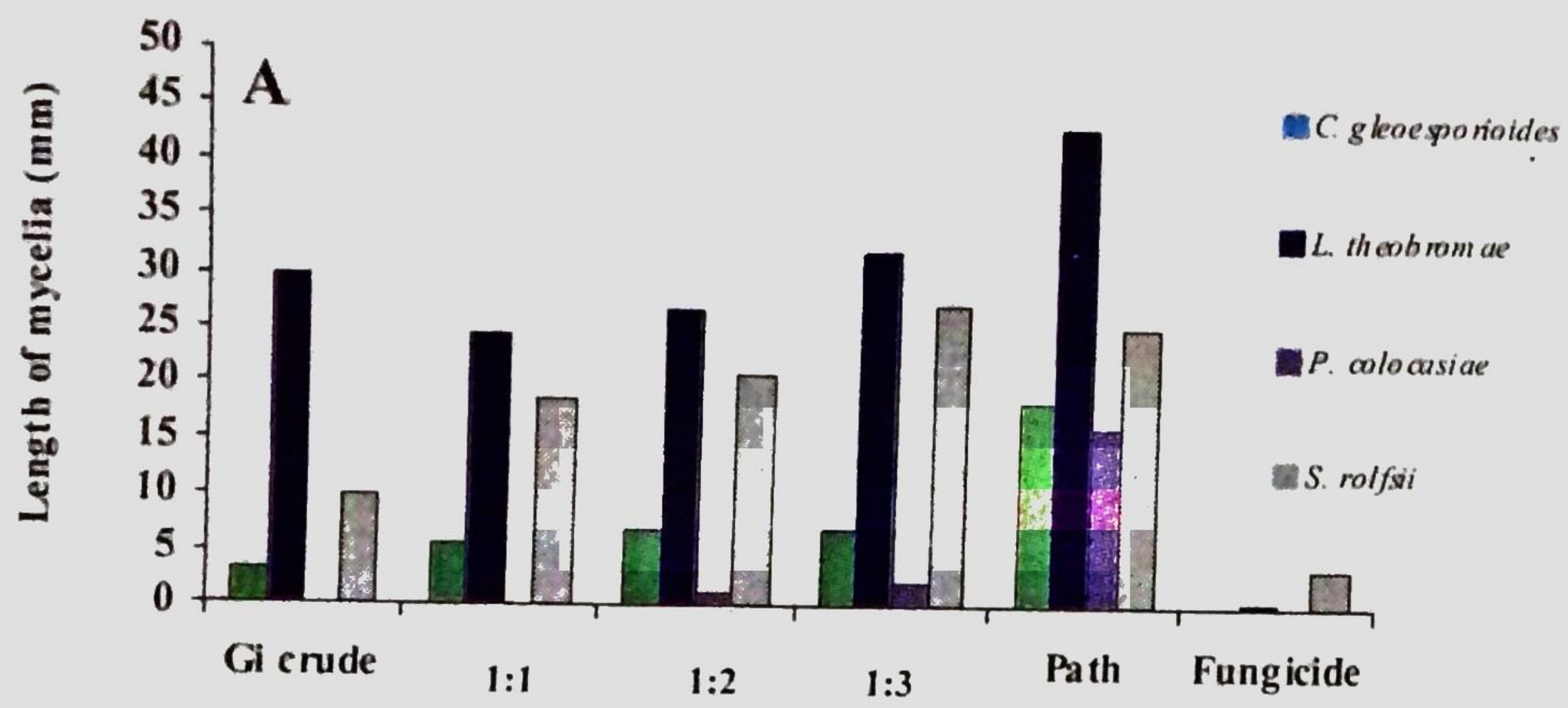
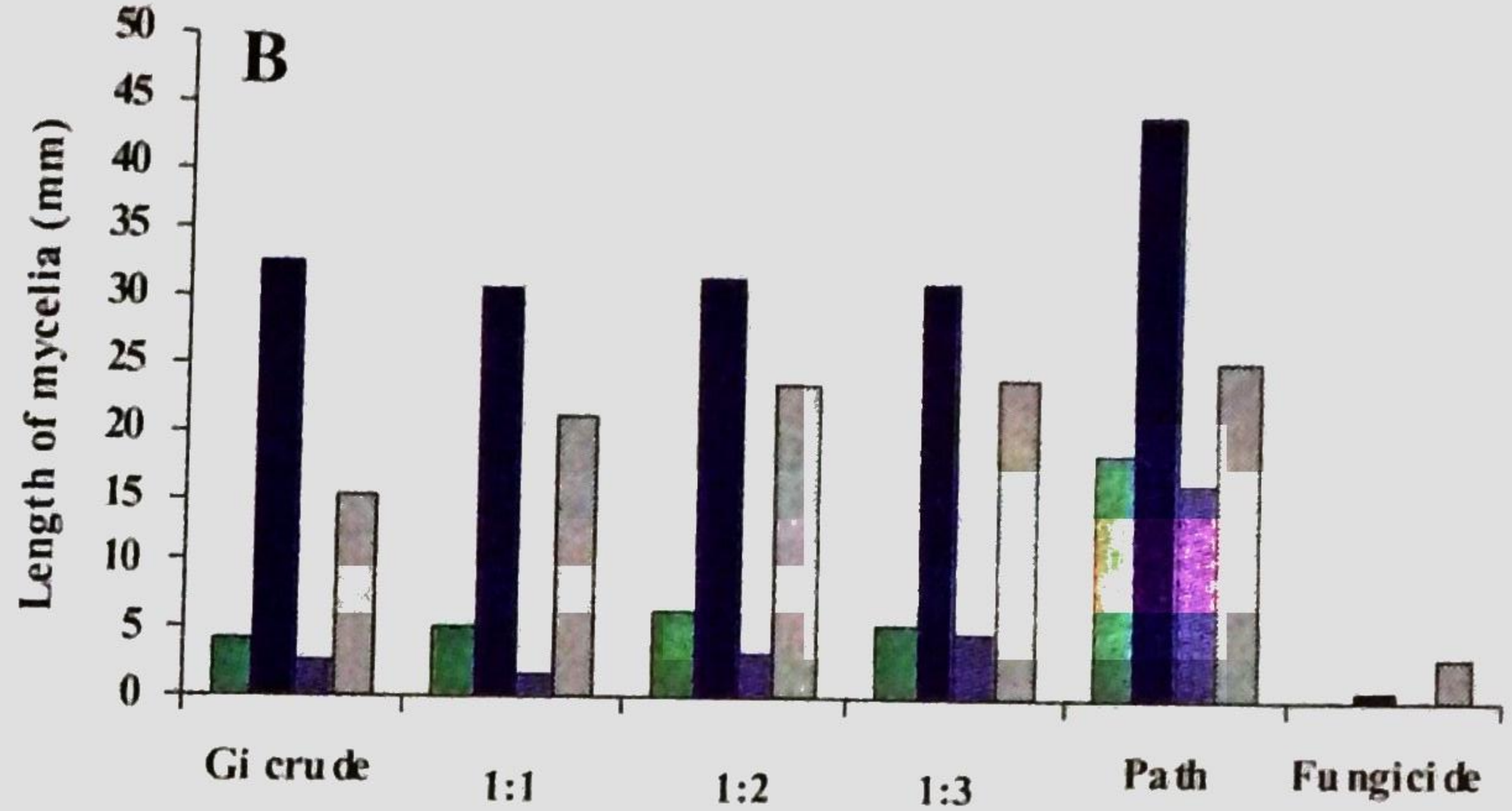
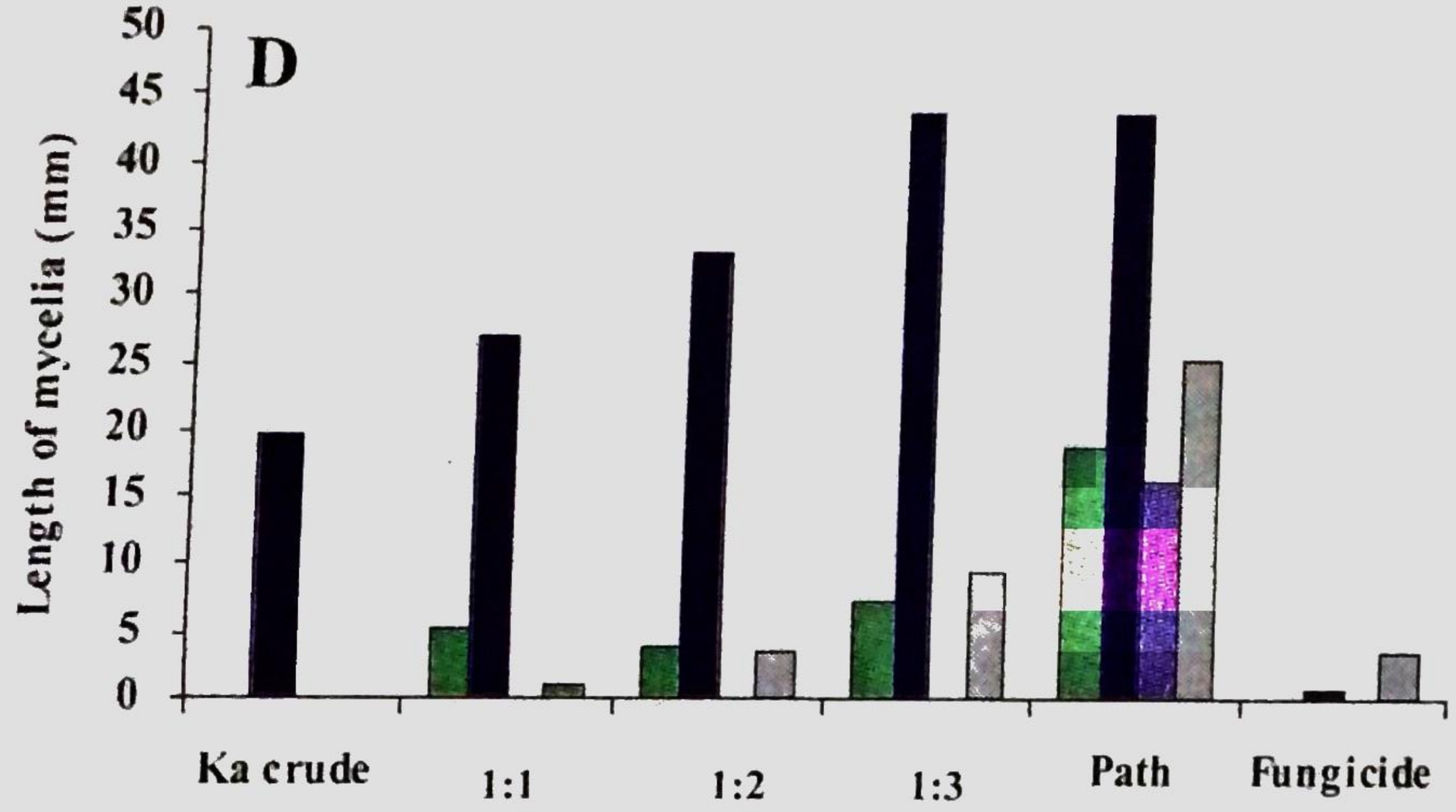


Figure 1a. Radial length of mycelia of four fungal pathogens treated with (A) ampalaya (Am) acetonic extract (B) ampalaya ethanolic extract (C) asyang (As) acetonic extract (D) and asyang ethanolic extract, at different concentrations (crude, 1:1, 1:2 and 1:3). Note: The controls include untreated pathogen alone (Path.) such as Phytophthora colocasiae (P. c.), Colletotrichum gleoesporioides (C. g.), Lasiodiplodia theobromae (L. t.) and Sclerotium rolfsii (S.r.). Benlate was used as fungicidal control of L.t. and C. g. while Saprol EC for S. r and P. c. No radial growth of mycelia indicates fungicidal effect of extracts.







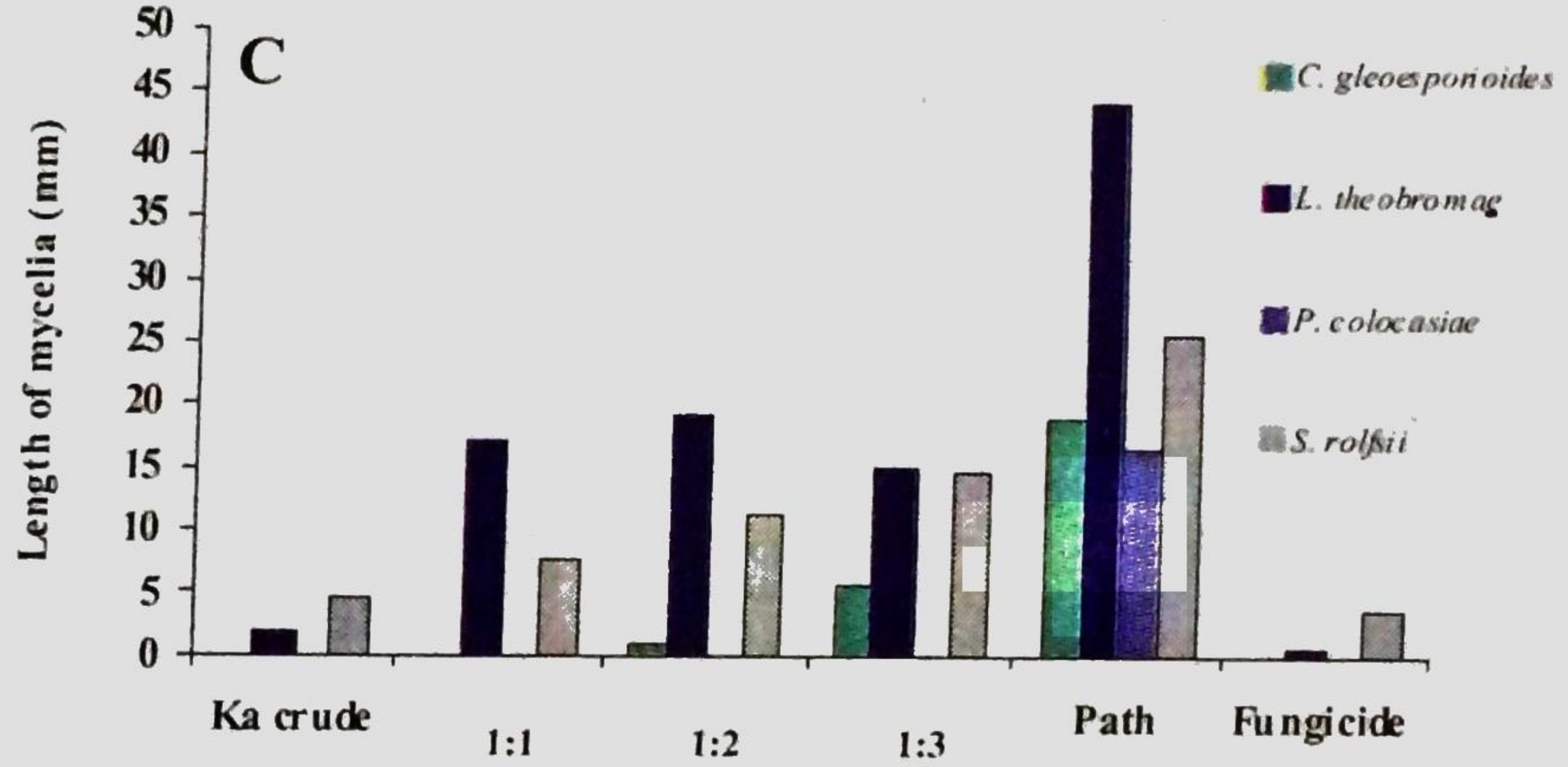
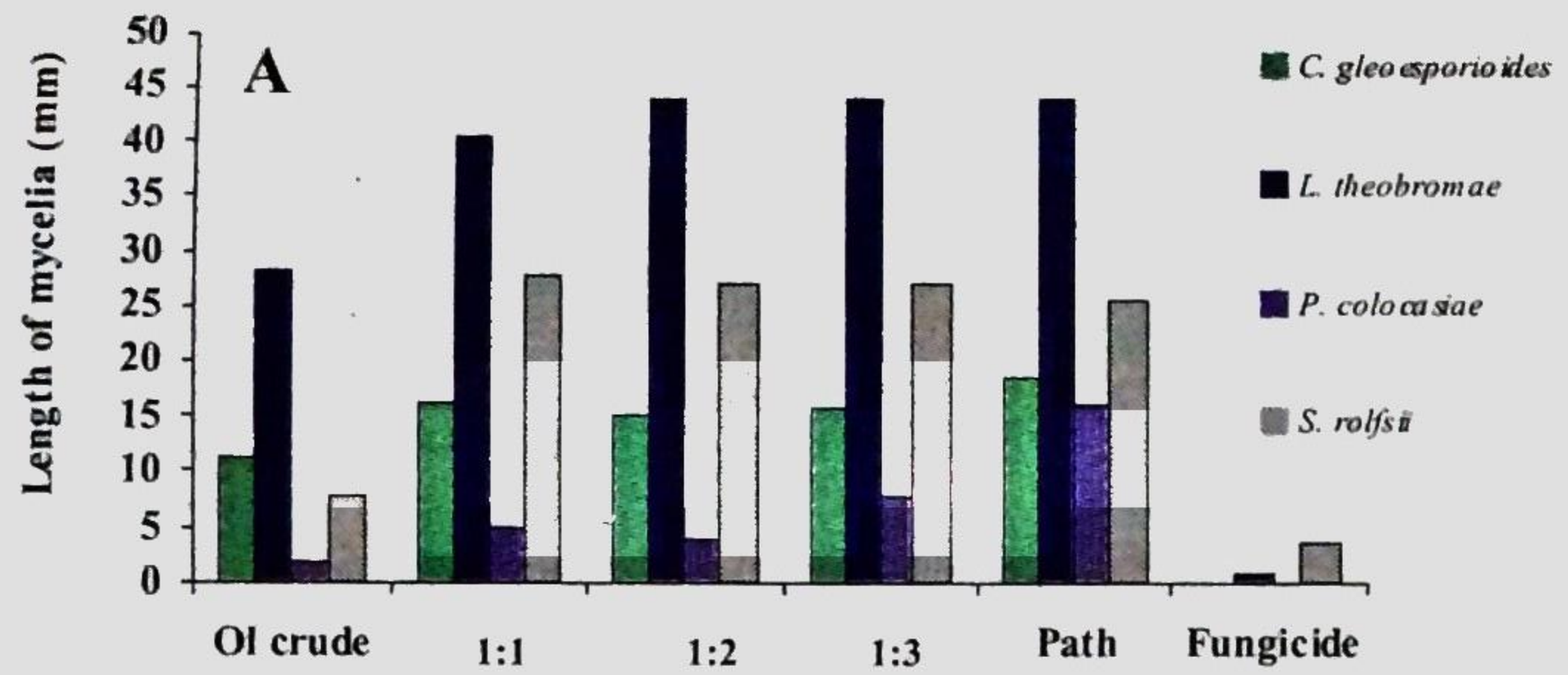
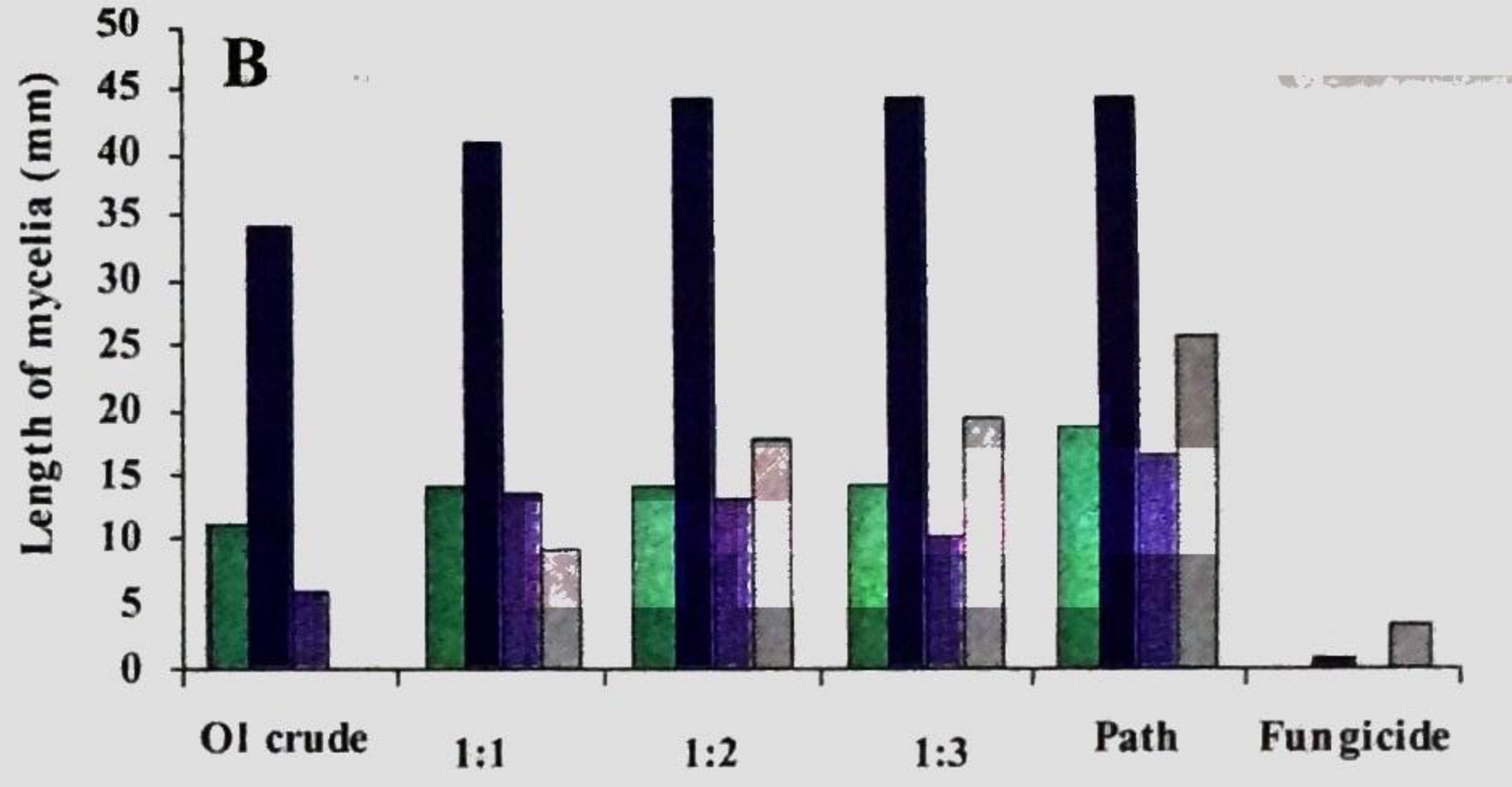
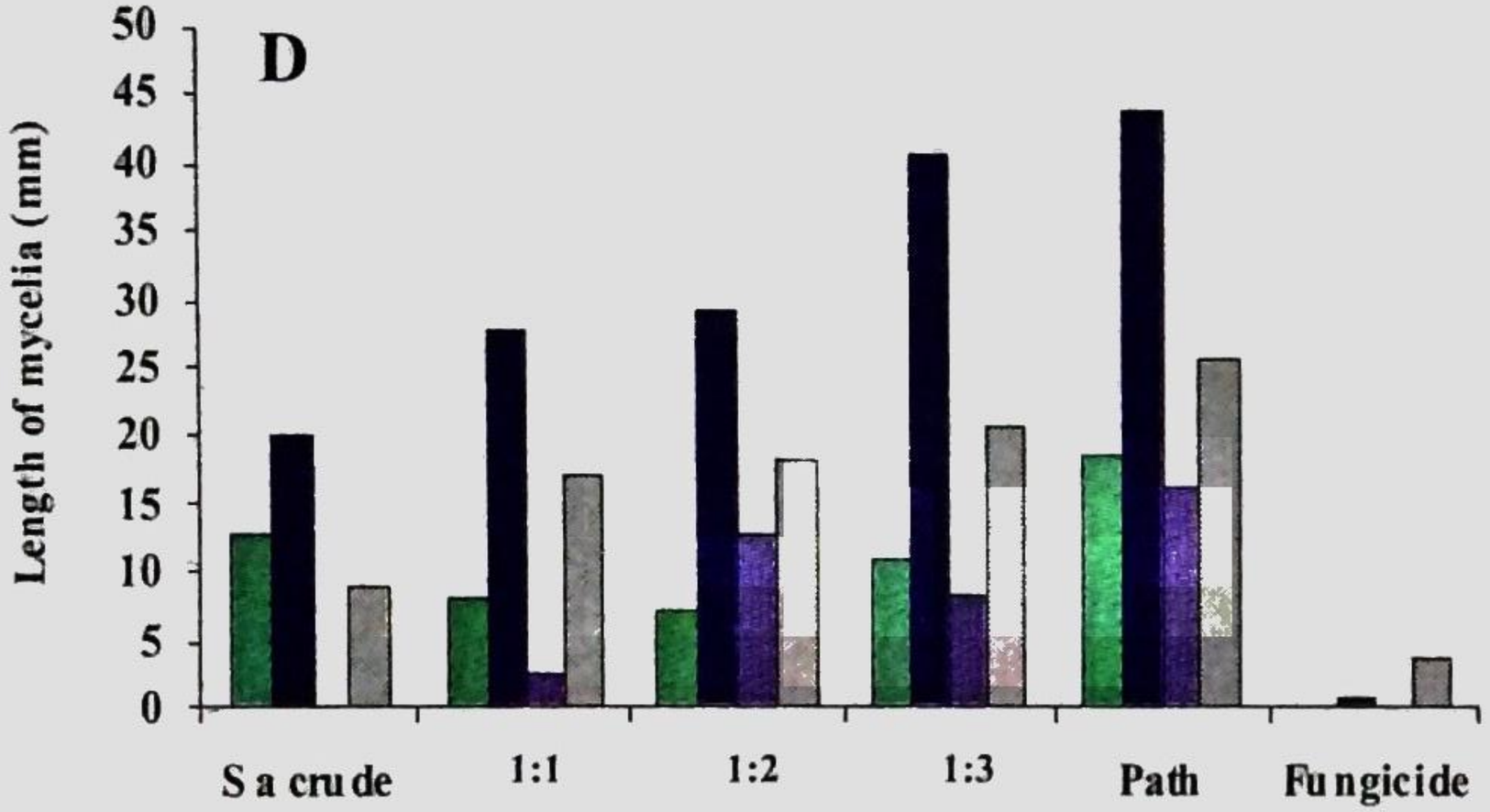


Figure 1b. Radial length of mycelia of four fungal pathogens treated with (A) ginger (Gi) acetonic extract (B) ginger ethanolic extract (C) kamantigue (Ka) acetonic extract (D) and kamantigue ethanolic extract, at different concentrations (crude, 1:1, 1:2 and 1:3). Note: The controls include untreated pathogen alone (Path.) such as Phytophthora colocasiae (P. c.), Colletotrichum gleoesporioides (C. g.), Lasiodiplodia theobromae (L. t.) and Sclerotium rolfsii (S.r.). Benlate was used as fungicidal control of L.t. and C. g. while Saprol EC for S. r. and P. c. No radial growth of mycelia indicates fungicidal effect of extracts.







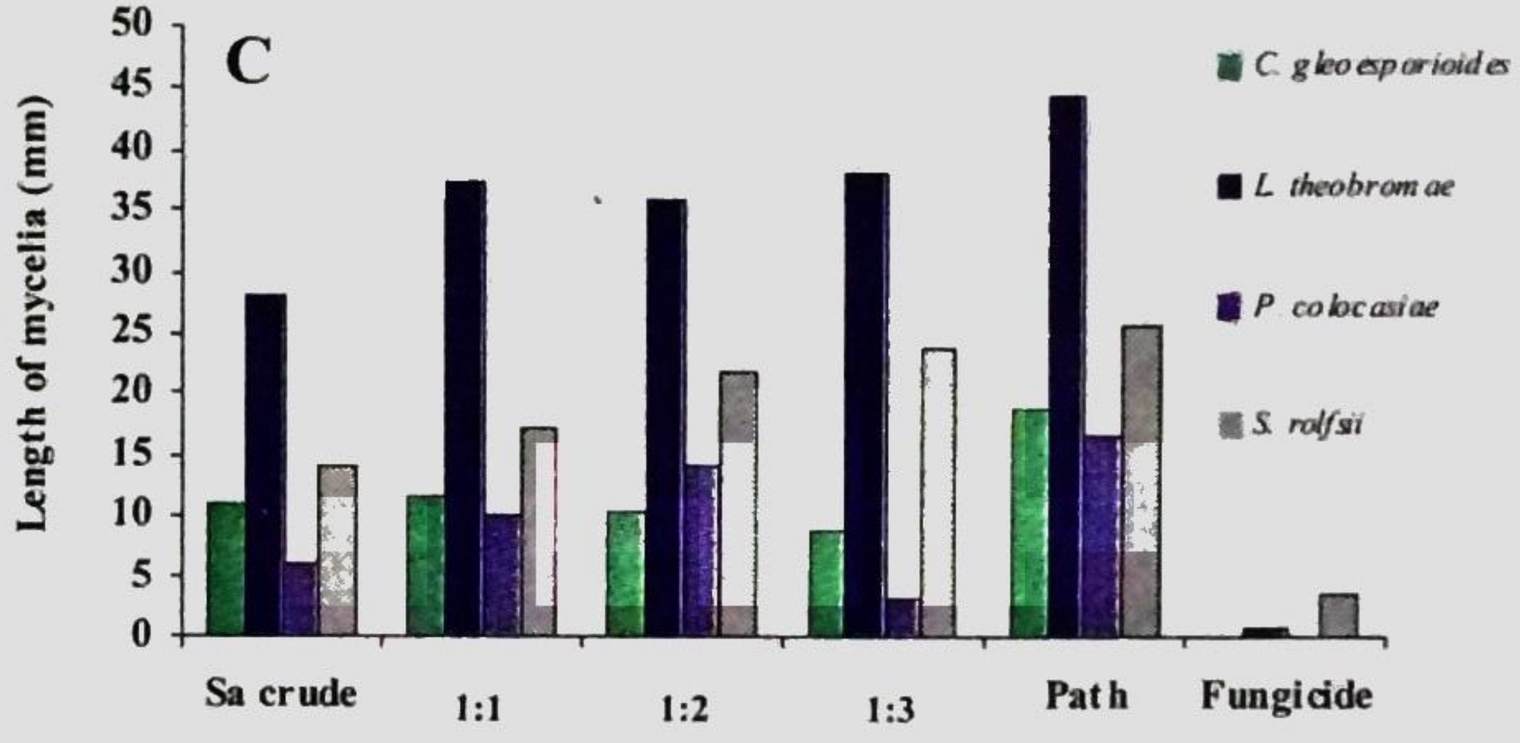


Figure 1c. Radial length of mycelia of four fungal pathogens treated with (A) olasiman (Ol) acetonic extract (B) olasiman ethanolic extract (C) saluyot (Sa) acetonic extract (D) and saluyot ethanolic extract, at different concentrations (crude, 1:1, 1:2 and 1:3). Note: The controls include untreated pathogen alone (Path.) such as Phytophthora colocasiae (P. c.), Colletotrichum gleoesporioides (C. g.), Lasiodiplodia theobromae (L. t.) and Sclerotium rolfsii (S.r.). Benlate was used as fungicidal control of L.t. and C. g. while Saprol EC for S. r. and P. c. No radial growth of mycelia indicates fungicidal effect of extracts.

absorption.

Results revealed that inoculated leaf with *P. colocasiae* treated with asyang, olasiman, ginger ethanol and ginger acetonic extract showed no infection or blighting symptom after 6 days of observation (Table 3) which indicates their potential to control infection caused by *P. colocasiae* in taro. Leaves inoculated with the pathogen dipped in olasiman acetonic and kamantigue ethanolic extracts also showed no infection until 5 days followed by ampalaya acetonic extract that exhibited infection after 3 days of incubation. Other extracts such as kamantigue acetonic and saluyot ethanolic showed 10%, ampalaya ethanolic (15%), saluyot acetonic (50%) and asyang acetonic (75%) disease infections, which were lower compared to the control tretament using distilled water. Most of these extracts were even better (except asyang acetonic) compared to Saprol EC (40% infection).

Sphaceloma batatas. Results of the single colony assay of *S. batatas* on liquid medium indicated the following possess fungicidal activity: crude ginger ethanolic extract, crude ginger acetonic and up to 1:2 dilution, crude kamantigue ethanolic and acetonic extracts, crude olasiman ethanolic and crude saluyot ethanolic extracts. A corresponding 100% inhibition of colony growth that was also superior to the effect of Benlate (RR) was observed. Fungicidal screening revealed that Benlate was better than Saprol EC in controlling the scab pathogen. Fungistatic extracts that approximately gave 75% inhibition of colony growth were crude ampalaya ethanolic, crude asyang ethanolic, ginger ethanolic at 1:1 dilution, ginger acetonic at 1:3 dilution, saluyot ethanolic at 1:1 and crude saluyot acetonic extracts (Table 4). Results indicated that the aforementioned extracts were potential alternative sources for the control of stem and foliage scab disease of sweetpotato.

Laboratory Assay of Flavonoidal Components

Flavonoidal components isolated from six botanical extracts showed 100% disease control for *S. batatas*, a very promising indication that flavonoids from these plant species possessed fungicidal activity. No colony growth was observed after one week. The results obtained were superior to the effect of synthetic fungicide (Benlate) used as positive check. The 100% inhibition of colony growth indicates the great potential of these plants for the control of *S. batatas*. Likewise, for *S. rolfsii* the following flavonoidal extracts possessed

Table 3. Percent disease observed on excised taro leaves inoculated with *Phytophthora colocasiae* pre-treated with different plant extracts

Plant Extracts	Days Interval			
	1	2	3	6
Ampalaya				
Acetone	0.0	0.0	2.5	5.0
Ethanol	10.0	10.0	12.5	15.0
Asyang				
Acetone	10.0	22.5	37.5	75.0
Ethanol	0.0	0.0	0.0	0.0
Ginger				
Acetone	0.0	0.0	0.0	0.0
Ethanol	0.0	0.0	0.0	0.0
Kamantigue				
Acetone	7.5	10.0	10.0	10.0
Ethanol	0.0	0.0	0.0	3.0
Olasiman				
Acetone	0.0	0.0	0.0	2.0
Ethanol	0.0	0.0	0.0	0.0
Saluyot				
Acetone	0.0	0.5	1.5	5.0
Ethanol	2.5	5.0	10.0	10.0
Control:				
Distilled Water (inoc.)	15.0	25.0	92.5	100.0
Distilled Water (Uninoc.)	0.0	0.0	0.0	0.0
Saprol	10.0	10.0	10.0	40.0

Table 4. Rating and percent inhibition of colony growth (PIC) of *Sphaceloma batatas* using single colony assay after one week of treatment [a]

Plant Extract/ Concentration	Solvent			
	Ethanol	PIC	Acetone	PIC
Ampalaya				
Crude	few	75	few	75
1:1	high	0	high	0
1:2	high	0	high	0
1:3	high	0	high	0
Asyang				
Crude	few	75	few	75
1:1	high	0	high	0
1:2	high	0	high	0
1:3	high	0	high	0
Ginger				
Crude	no growth	100	no growth	100
1:1	few	75	no growth	100
1:2	high	0	no growth	100
1:3	high	0	few	75
Kamantigue				
Crude	no growth	100	no growth	100
1:1	high	0	high	0
1:2	high	0	high	0
1:3	high	0	high	0
Olasiman				
Crude	no growth	100	high	0
1:1	high	0	high	0
1:2	high	0	high	0
1:3	high	0	high	0
Saluyot				
Crude	no growth	100	few	75
1:1	few	75	moderate	50
1:2	moderate	50	high	0
1:3	moderate	50	high	0
Control:				
Benlate (RR)	few	75		
Sweetpotato	high	0		
Decoction alone				
alone				

Table 5. Effect of flavonoidal extract (1:1 vol/vol ratio) on the radial length of mycelia of fungal pathogens of root crops

Flavonoidal Extract	Radial Length of Mycelia (mm)			
	C. gleosporioides	*L.theobromae*	*P. colocasiae*	*S. rolfsii*
Ampalaya				
Acetone	0.33	8.00	0.00	0.00
Ethanol	0.66	3.67	0.00	0.00
Asyang				
Acetone	3.00	35.00	0.00	0.00
Ethanol	0.33	16.33	0.00	5.00
Ginger				
Acetone	0.60	9.67	0.00	15.00
Ethanol	1.33	13.00	0.00	0.00
Kamantigue				
Acetone	0.50	10.33	0.00	0.00
Ethanol	2.50	13.33	0.00	16.00
Olasiman				
Acetone	0.00	24.33	0.00	0.00
Ethanol	3.83	9.00	0.00	10.00
Saluyot				
Acetone	4.67	29.67	0.00	8.50
Ethanol	3.83	9.00	0.00	10.50
Control:				
PDA alone	14.33	44.00	19.67	32.50

Table 6. Disease severity rating of *Colletotrichum gleosporioides* in yam treated with botanical extracts under screenhouse condition

Treatments	Average Monthly Disease Severity Rating [1]				
	4th	5th	6th	7th	Mean
Kamantigue					
Ethanol	5.50	6.63	7.87	9.00	7.25
Acetone	4.07	5.00	8.47	8.60	6.54
Ampalaya					
Ethanol	3.00	3.13	3.53	6.07	3.93
Acetone	3.00	3.26	3.40	6.77	4.11
Asyang					
Acetone	6.72	6.78	7.11	8.61	7.31
Benlate (RR)	7.00	6.47	8.47	8.47	7.60
Control (Untreated)	7.67	7.27	7.93	8.87	7.94

Rating Scale
1 - Less than 1% leaf area infected (LAI)
3 - 1-15% LAI
5 - 16-25% LAI
7 - 26-50% LAI
9 - >50% LAI

fungicidal activity: ampalaya ethanolic and acetonic extracts, ginger ethanolic, and kamantigue or olasiman acetonic extracts (Table 5). It gave a corresponding 100% inhibition of mycelial growth that was also superior to the effect of Saprol EC. For tannins and polyphenolic compounds,there was no significant effect on the growth and development of *S. rolfsii*.

On the other hand, flavonoidal extracts of ampalaya using ethanol and acetone possessed fungistatic activity against *L. theobromae*. Likewise, olasiman flavonoidal acetonic extract was fungicidal to *C. gleosporioides* while the rest were fungistatic namely: ampalaya, asyang, ginger, kamantigue, olasiman ethanol and saluyot extracts.

Table 7. Effect of different extracts in pot experiments on rotting of taro tubers caused by *Sclerotium rolfsii* three weeks after harvest

Extracts (1:1 dilution v/v)	% Tuber Surface Infection	Percent Disease Control
Olasiman (Ethanol)	22.58	71.19
Ampalaya (Ethanol)	80.13	-2.24
Kamantigue (Acetone)	44.98	42.61
Kamantigue (Ethanol)	77.50	1.11
Ginger (Acetone)	54.63	30.29
Control (*S. rolfsii* alone)	78.37	0
Saprol EC (RR)	49.22	37.19

All the flavonoidal extracts found in the six botanical plants were fungicidal to *P. colocasiae*.

In Vivo Screening of Promising Secondary Metabolites/Extracts

Yam Anthracnose. Yam plants pre-treated with ampalaya ethanolic and acetonic extracts were rated resistant against the disease (Table 6). Other plant extracts and Benlate treated plants showed susceptible reaction similar to the control plants. This shows that ampalaya ethanolic and acetonic extracts have the potential to be used as an alternative to fungicide specifically Benlate against yam anthracnose.

Taro Tuber Rot. Olasiman ethanolic extracts exhibited the highest percent disease control and least percent tuber surface infection, followed by kamantigue acetonic and ginger acetonic extract. In addition, olasiman extract surpassed the activity of Saprol EC (fungicide) 34.05%. Other extracts exhibited almost no control (kamantigue ethanolic) or even negative percent disease control (ampalaya ethanolic) (Table 7).

CONCLUSION

Phytochemical screening indicated that all six botanical plants possessed flavonoids, steroids and terpenoids while only olasiman contained tannins and polyphenolic compounds. Flavonoids were fungicidal to *Sphaceloma batatas*,

Phytophthora colocasiae and *Sclerotium rolfsii*. Tannins and polyphenolic had no effect on the growth and development of *S. rolfsii*.

Among the solvent systems employed tried only ethyl acetate proved to be the common component that provided better separation of secondary metabolites present in the concentrated bioactive extract. Concentrated bioactive extracts of ampalaya, ginger, kamantigue and saluyot contains at least 6, 7, 8, and 9 major compounds, respectively.

Inoculated excised leaves with *P. colocasiae* employed with asyang, olasiman, ginger ethanol or acetonic showed no infection or blighting symptom after 6 days of observation which indicates superiority from other potential extracts tested in vitro to control infection caused by *P. colocasiae* in taro. These extracts also performed better than Saprol in inhibiting infection. Meanwhile, screenhouse assay showed that yam plants pre-treated with ampalaya ethanolic/acetonic extracts were resistant against anthracnose disease due to *C. gleoesporioides*. This indicates that these extracts have the potential to be used instead of Benlate.

IMPLICATIONS AND RECOMMENDATION

Bioassay-guided fractionation, purification and identification of active principle present in the six botanical plants should be undertaken because they are very important in explaining the action manifested by the extracts with fungicidal activity. Identification of the specific chemical compound is also necessary if mass production of the "biofungicide" is an option to be considered. It is also recommended that shelf-life studies of the concentrated bioactive extracts should be done to determine the duration of their effectiveness when stored under room condition or when refrigerated. Furthermore, the purified extracts should be tested to evaluate their validity under farmers' field conditions.

The study confirms the idea that the same biofungicidal materials like flavonoids maybe isolated from different botanical plants. Therefore, flavonoids maybe extracted from a combination of plant species or from other sources and are expected to produce a similar effect against target pathogens.

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