Allelopathic effect of botanical extracts on selected fungal pathogens and test plants

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ABSTRACT

The alellopathic effects of aqeous extracts of botanicals namely: *Cassia alata L., Mikania cordata* B.L. Robinson, *Dioscorea hispida* D. daemona Roxb., *Tinospora rumphii* L., *Stachytarpheta jamaicensis* (L) Vahl, *Piper betle* L., and *Peperomia pellucida* HBK. against selected fungal pathogens and test plants were studied.

Growth response of the test fungi to the extracts include inhibition of spore germination, and mycelial growth, and reduction in the number and size of lesion.

On the other hand, the allelopathic extracts caused inhibited germination of seed/tubers and reduction in root and shoot growth of the different test plants.

Keywords: Allelopathy, botanicals, herbicidal, fungicidal and growth inhibition

INTRODUCTION

Allelopathy refers to the biochemical interactions between all type of plants including microorganisms (Molisch, 1937). It includes both the inhibitory and stimulatory effects of the biochemical interactions among organisms.

In crop production especially with regards to weed management, the effect of allelopathy on yield reduction is always masked by the word competition. Generally, studies on weeds indicated that any reduction in yield is always accounted to crop-weed competition for resources needed for plant survival. Rice (1974) considers allelopathy as different from competition which involves removal of resources from the same pool. Mercado (1979) mentioned that allelopathic interactions per se may not be directly involved in competition but some weed scientists would consider allelopathy as an important mechanism which enables a species to inhibit the growth of another, resulting in greater accessibility to limited resources of the environment. One possible reason for the predominance of weed or any other plant could be because of allelopathy wherein any species of plant exudes chemical compounds affecting the growth and development of other organisms. The effect can be stimulatory but most of the time inhibitory to the growth of other organisms and therefore it can be exploited for pest control purposes.

This study was conducted with the following objectives: to determine the allelopathic effect of plant extracts on representative organisms (i.e.

fungi and plants); to determine the most promising plant extracts; and to determine the most sensitive test organisms.

MATERIALS AND METHODS

Survey collection and maintenance of plants

Survey and identification of plants with pesticidal potential in Mt. Pangasugan were done by referring to available literatures (Morallo-Rejesus, 1987; Noriel, 1989, Franje, 1984 and Lapiz and Dumancas, 1978). The identified plant species that were decided to be used for the different tests were collected from the said mountain and vicinity, placed in plastic bags and then brought to the laboratory for extraction.

Isolation and maintenance of test organisms

Fungal pathogens

The following plant pathogenic fungi namely: *Pyricularia oryzae* Cav., *Sclerotium rolfsii* Sacc. and *Rhizoctonia solani* Kuhn., *Colletotrichum gleosporoides* Pens, *Helminthosporium maydis* Nisik and Miyaki, *Helminthosporium oryzae* Breda de Haan, *Curvularia lunata* (Walker) Boedijn, and *Cercospora batatae* Zimmer were isolated following the standard tissue cultured method of isolation. The pathogens were cultured and maintained in potato dextrose agar (PDA). One (1) week-old culture of the different test fungal isolates were used in any of the fungicidal bioassay.

Weed/crops

Cucumber (*Cucumis sativum* L.), corn (*Zea mays* L.), rice (*Oryza sativa* L.) and purple nutsedge (*Cyperus rotundus* L.) were used as test plants to determine the allelopathic/herbicidal effects of the botanical extracts.

Extraction of the different plants with pesticidal potential

Among the different plants with pesticidal potential only 7 species (Table 1) were used in the bioassay. These plant species can be easily grown and produce greater biomass faster.

The leaves, stems or roots of the different plants were washed with running water to remove the dirt. They were chopped into pieces before liquifying them using a blender or osterizer. The leaves and stems were wrung and

Table 1. Pesticidal plants and the corresponding parts used during bioassay

	Pesticidal Plant	Plant Parts Used	Family
1.	*Cassia alata* L.	leaves	Papilionaceae
2.	*Dioscorea hispida* D. daemona Roxb.	tuber	Discoreaceae
3.	*Mikania cordata* B.L. Robinson	leaves and stems	Asteraceae
4.	*Peperomia pellucida* HBK	leaves and stems	Piperaceae
5.	*Piper betle* L.	leaves	Piperaceae
6.	*Stachytarpheta jamaicensis* (L.) Vahl.	leaves and stems	Verbenaceae
7.	*Tinosphora rumphii* L.	stems	Menispermaceae

squeezed manually to get the extracts. The extracts were strained using cheesecloth/nylon sheer into a sterile beaker (250 ml) and covered with aluminum foil.

For fungicidal bioassay, the different plant extracts were prepared aseptically. The plants were surface sterilized using 70% ethyl alcohol before extraction was made. Sterile distilled water was used as solvent. The extracts were placed in sterile container.

Bioassay

Fungicidal activity

Laboratory test

Spore germination. A drop of the different plant extracts at 50%, 35% and 15% concentrations by volume were smeared on the glass slides and air-dried for 5 to 10 minutes. Then, a drop of the spores of *C. gleosporoides* was introduced to the smeared glass slides and incubated for 24 hours in petri dishes lined with moistened filter paper. Thereafter, observation on spore germination was made under light compound microscope.

Mycelial growth response. Mycelial discs of *C. batatae*, *C. lunata* and *C. gleosporoides* were placed separately on the center of petri dishes with plated 9 ml Potato Dextrose Agar (PDA). Four (4) filter paper discs, each dipped in different plant extracts were placed in more or less equal distance from each other in each petri dish containing the different test organisms. After 24 hours incubation, the mycelial growth response of the different microorganisms on the plant extracts was determined based on the following rating:

- \- = no inhibition of the extract on test organism;
- \+ = slight inhibition of the extract on test organism;
- ++ = moderate inhibition of the extract on test organism; and
- +++ = complete inhibition of the extract on test organism.

Mycelial growth inhibition. One hundred percent (100%) concentration of the crude plant extracts were used in the bioassay test for fungicidal activity. One (1) ml of each plant extract was evenly incorporated separately to 10 ml melted PDA (previously maintained in water bath at 45°C) in sterile petri plates. Plated extract-PDA combinations were allowed to solidify before planting the test fungal pathogen namely: *S. rolfsii, R. solani, P. oryzae* and *C. gleosporoides*. The test fungal pathogens previously grown in plated PDA were subdivided equally by punching using sterile 6-mm diameter cork borer. A disk was planted at the center of the prepared extract-PDA combination. Control treatments using sterile water and fungicide Maneb (Mancoseb) were included for comparison. The fungal growth was taken after 24, 48 and 72 hours of incubation. Each treatment was replicated 4 times.

Screenhouse test

Planting and maintenance of test plants. Rice (IR 50) and sweet corn were sown separately in 20 cm diameter pots containing garden soil. One corn seedling and 5 rice seedlings were grown in pots separately. They were maintained inside the screenhouse until the desired stage was attained.

Application of crude extracts. Thirty (30) ml of the crude extracts of the different plant species that were previously prepared were applied to 20-day old rice seedlings and to the fourth and fifth expanded leaves of corn using a plastic handsprayer. Plants were incubated in 30 x 60 cm plastic bag for 24 hours. The treatments were replicated 3 times with sterile water and Maneb as control treatments.

Preparation of inoculum and inoculation. One-week old cultures of *H. oryzae* and *H. maydis* were used in rice and corn, respectively. Preparation of inoculum was done aseptically. The mycellial suspension of *H. oryzae* was used due to the nonsporulation of the isolate. A standard inoculum concentration of 1.8×10^4 spores per ml for *H. maydis* was used. Twenty-four (24) hours after the application of crude extracts, the disease was inoculated to the corresponding test plants to test their protective effect. Inoculated plants were incubated in 30 x 60 cm plastic bags for another 24 hours. Plastic bags were removed the following day and observation of disease development was done after 5 and 11 days for rice, and 3 and 6 days for corn after inoculation.

Herbicidal activity

Seeds of cucumber, corn, rice and tubers of purple nutsedge were surfaced sterilized with 5% Calcium hypochlorite (Zonrox) for 3-5 minutes, rinsed with distilled water and then air-dried. Five (5) ml of each plant extracts was dispensed separately to petri dishes lined with filter paper. Concentrations of the plant extracts used were 50% and 100% with distilled water as diluent. Distilled water alone was used for the untreated control. Seeds/tubers of each test plant were sown in petri dishes containing the extracts. There were 3 replications per treatment with 20 seeds/tubers of the test plants per replicate.

Percent germination, root and shoot lengths and the corresponding growth inhibition were recorded one week after sowing/treatment. Percent growth inhibition was determined using the formula:

$$\% \text{ Growth inhibition} = \frac{\text{Control-Treated}}{\text{Control}} \times 100$$

Morphological observations were done to note down abnormalities due to the effects of different plant extracts.

EXPERIMENTAL DESIGN AND DATA ANALYSIS

The different laboratory and screenhouse tests were arranged using Completely Randomized Design (CRD) or Randomized Complete Block Design (RCBD) whichever was appropriate.

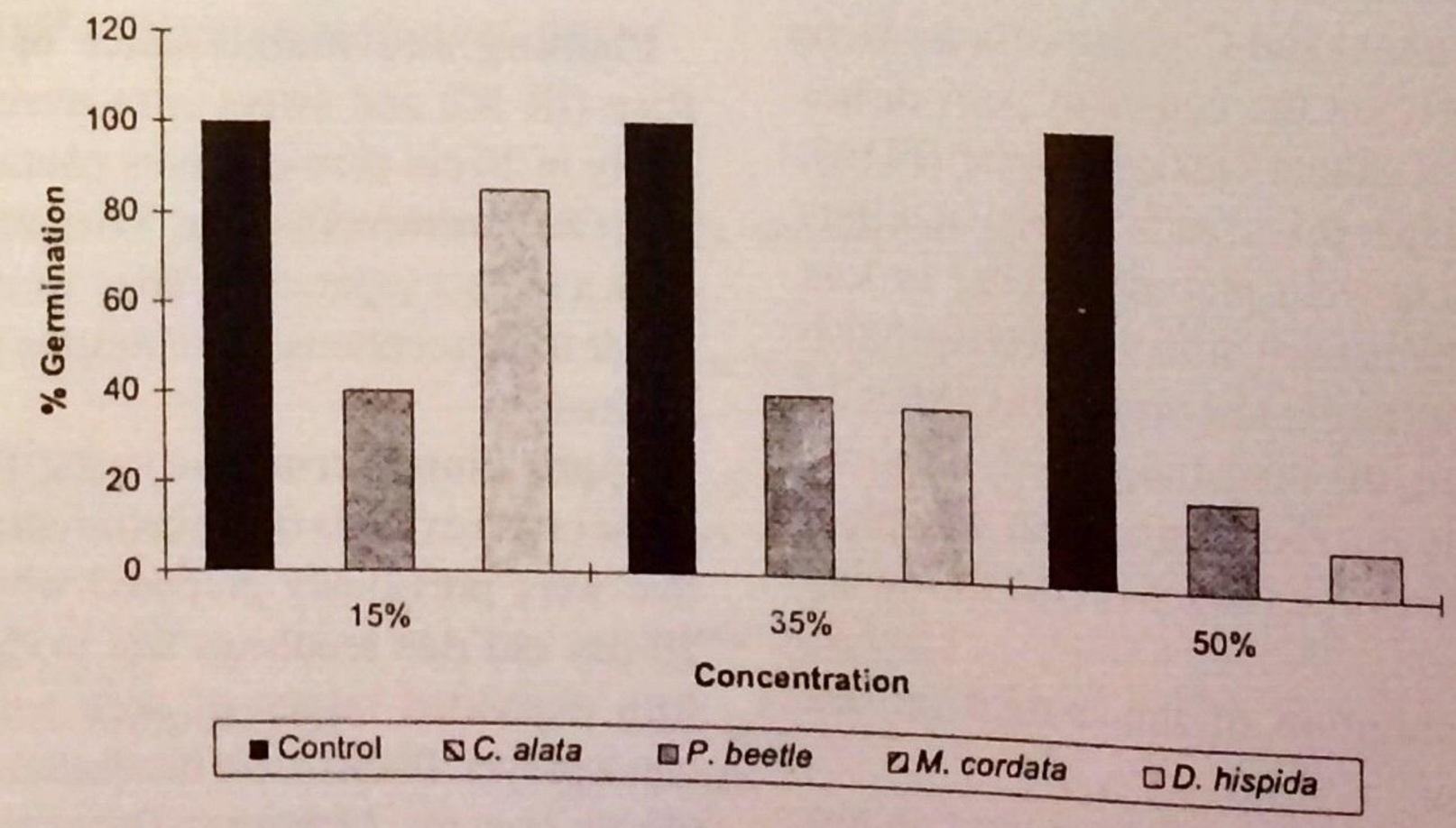

Figure 1. Percent spore germination of *C. gleosporoides* as affected by different plant extracts 24 hours after treatment.

Treatments means were compared using the Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Allelopathic effect of botanical extracts on selected plant pathogenic fungi

Laboratory bioassay

The effect of the different plant extracts on spore germination of *C. gleosporoides* was determined. The four plant extracts used, namely: *C. alata, P. betle, M. cordata* and *D. hispida* significantly reduced the spore germination of *C. gleosporoides* (Figure 1). The spore germination of *C. glesporoides* was completely inhibited by *C. alata* and *M. cordata* extracts at all concentrations used. The extract of *C. alata* caused shrinkage of spores at 50% concentration. Other extract used caused either complete inhibition of spore germination accompanied by spore swelling or formation of shorter germ tube compared with the control (Figure 2). The extracts of *C. alata* and *M. cordata* were the most promising as far as inhibition of spore germination is concerned. It was noted that there was a corresponding reduction in spore germination of *C. gleosporoides* as the concentration of the different extracts was increased. *P. betle* extract was more effective than *D. hispida* even at 15% concentration indicating that it is more fungitoxic than the other. However, at 35% and 50% concentrations, the extracts of both *D. hispida* and *P. betle* exhibited almost the same inhibitory effects on spore germination. Results suggest that *D. hispida* extract was not effective at 15% concentration and a higher concentration of at least 35% was needed to cause a relatively lower spore germination. Johnson and Clark (1979) also observed that spore germination of *Bipolaris sorokiniana* was inhibited by water extracts from Guar root (?).

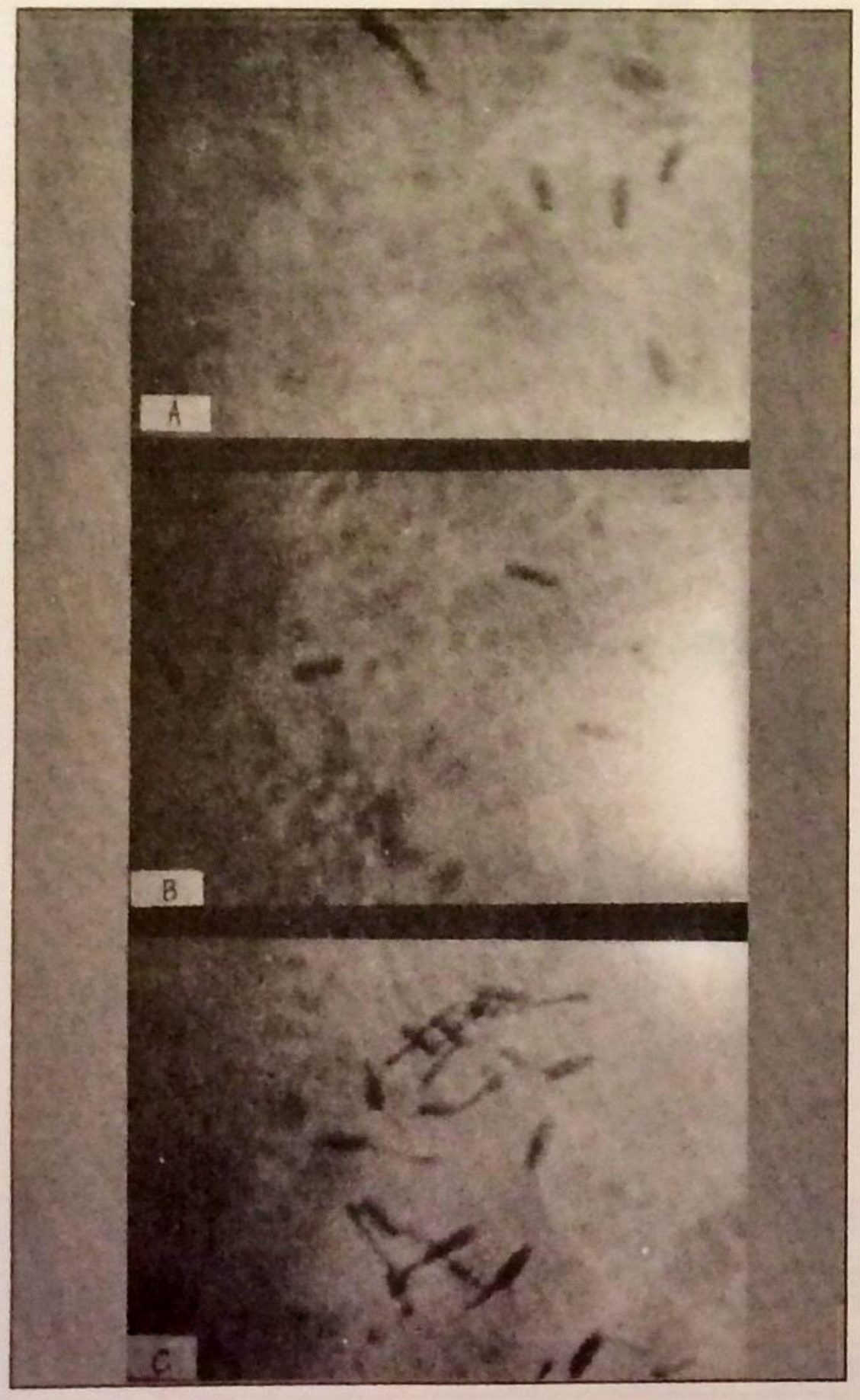

Figure 2. Spores of *Colletotrichum gleosporoides* showing the development of germ tube as affected by the extracts (a.) *M. cordata* (b) *C. alata* (c) Control.

Varied responses of three different species of fungi on the different plant extracts were also observed. Among the plant extracts, *D. hispida* caused complete inhibition of mycelial growth of the three tested plant pathogenic fungi (Table 2). This indicates that *D. hispida* contains fungitoxic compounds which could be more effective in controlling the mycelial growth of *C. batatae, C. lunata* and *C. gleosporoides*. The extract of *C. alata* caused slight growth inhibition of *C. batatae* and *C. gleosporoides*. On the other hand, *M. cordata* and *P. pellucida* extracts caused moderate inhibition of *C. gleosporoides*.

Complete inhibition (+++) means that myc-

Table 2. Mycelial growth response of three species of pathogenic fungi on different plant extracts at 50% concentraction.

Plant Extract	Mycelial Growth Response		
	Cercospora batatae	*Curvularia luna*	*Colletotrichum gleosporoides*
C. alata	+	-	+
P. betle	-	-	+++
M. cordata	-	-	++
P. pellucida	-	-	++
D. hispida	+++	+++	+++

elial growth of test fungi is 100% inhibited or no growth at all. On the other hand, moderate inhibition (++) means that the growth of the fungi is inhibited 50% and more while slight inhibition (+) signifies less than 50%.

On the other hand, among the three pathogenic fungi, *C. gleosporoides* positively responded to the different plant extracts indicating that it is more sensitive to the allelopathic effects of the extracts than to the other two species. The different extracts might contain allelopathic substances which are selectively fungitoxic and are capable of inhibiting the mycelial growth of *C. gleosporoides* but not with the other species.

Figures 3 to 5 show the different plant extracts exhibiting varying degrees of toxicity against the test fungi, namely: *C. gleosporoides, H. oryzae, P. oryzae, R. solani,* and *S. rolfsii.* The extracts of *M. cordata, C. alata* and *D. hispida* were the most fungitoxic in terms of inhibiting the mycelial growth of the pathogenic fungi even after 72 hours of incubation. Likewise, *P. oryzae* was the most inhibited by the extracts while *S. rolfsii* was the least. The effectivity of the extracts, however, was reduced as the incubation period was prolonged. Similar observations regarding the reduction in the effectivity of the botanical extracts was also noted by Noriel (1989) in *H. maydis* and Lapiz and Dumancas (1978) in *H. oryzae* using different species. The possible explanations for the reduction in the effectiveness of the extracts as speculated by Lapiz and Dumancas are as follows:

1. the active ingredient was volatile hence rapidly dissipated with time;
2. the active agent was not absorbed by the

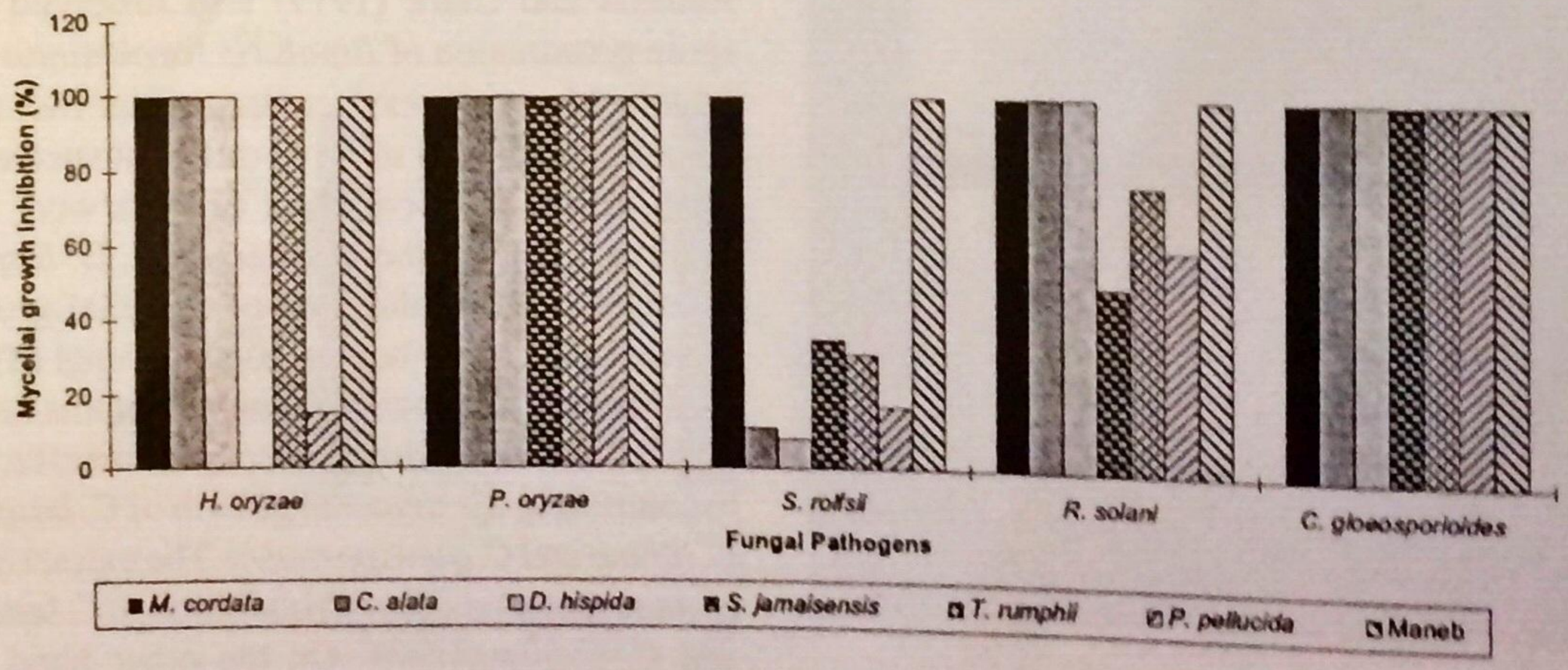

Figure 3. Mycelial growth inhibition (%) of plant pathogenic fungi as affected by plant extracts after 24 hours of incubation.

medium to give a long lasting effect to the test organism or did not diffuse in the medium;

3. degradation of the active compound due to exposure to atmosphere and light; and
4. the transformation of the active compound to inactive form by reaction with atmosphere.

Likewise, Franje (1984) observed that the fungicidal activity of some plant extracts against *Colletotrichum lindemuthianum* Bri. and Cav., *Xanthomonas phaseoli* var. *sojenci* Starr and Burh and *Cercospora cruenta* Sacc. was limited up to 1:50 dilution.

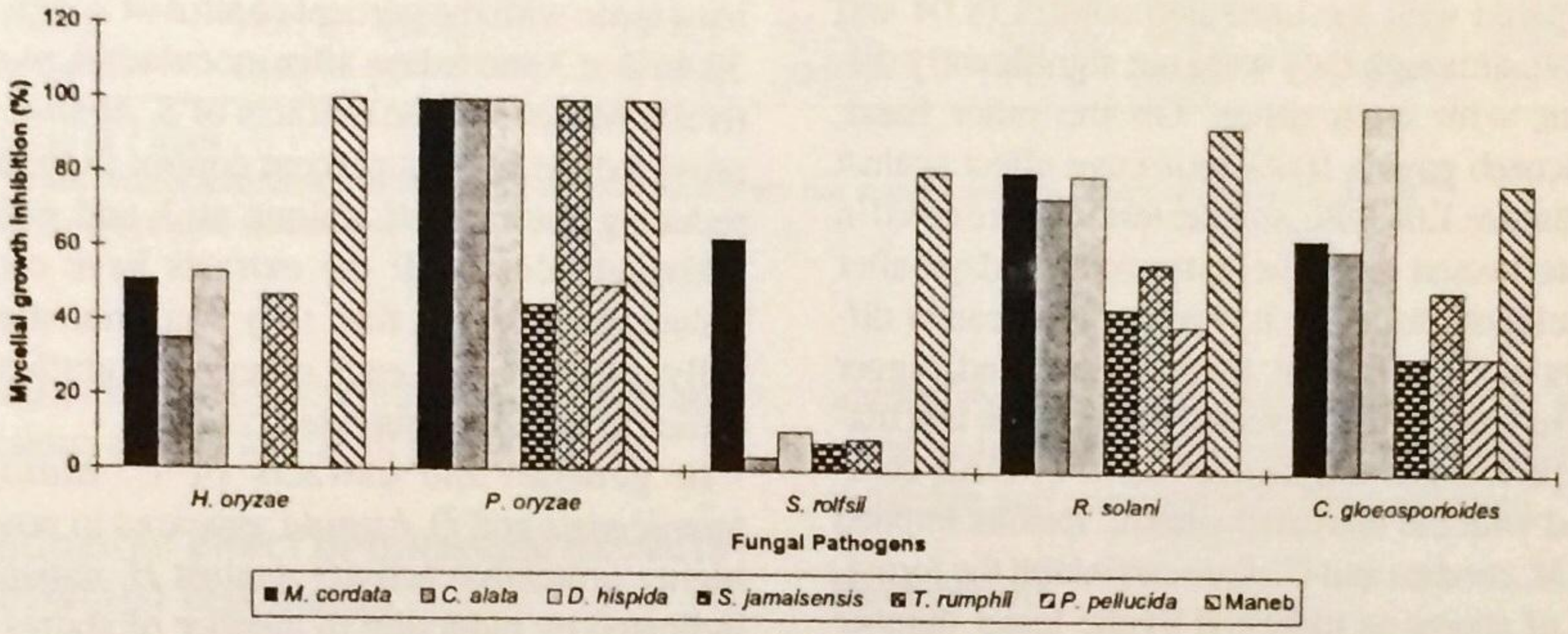

Figure 4. Mycelial growth inhibition (%) of plant pathogenic fungi as affected by plant extracts after 48 hours of incubation.

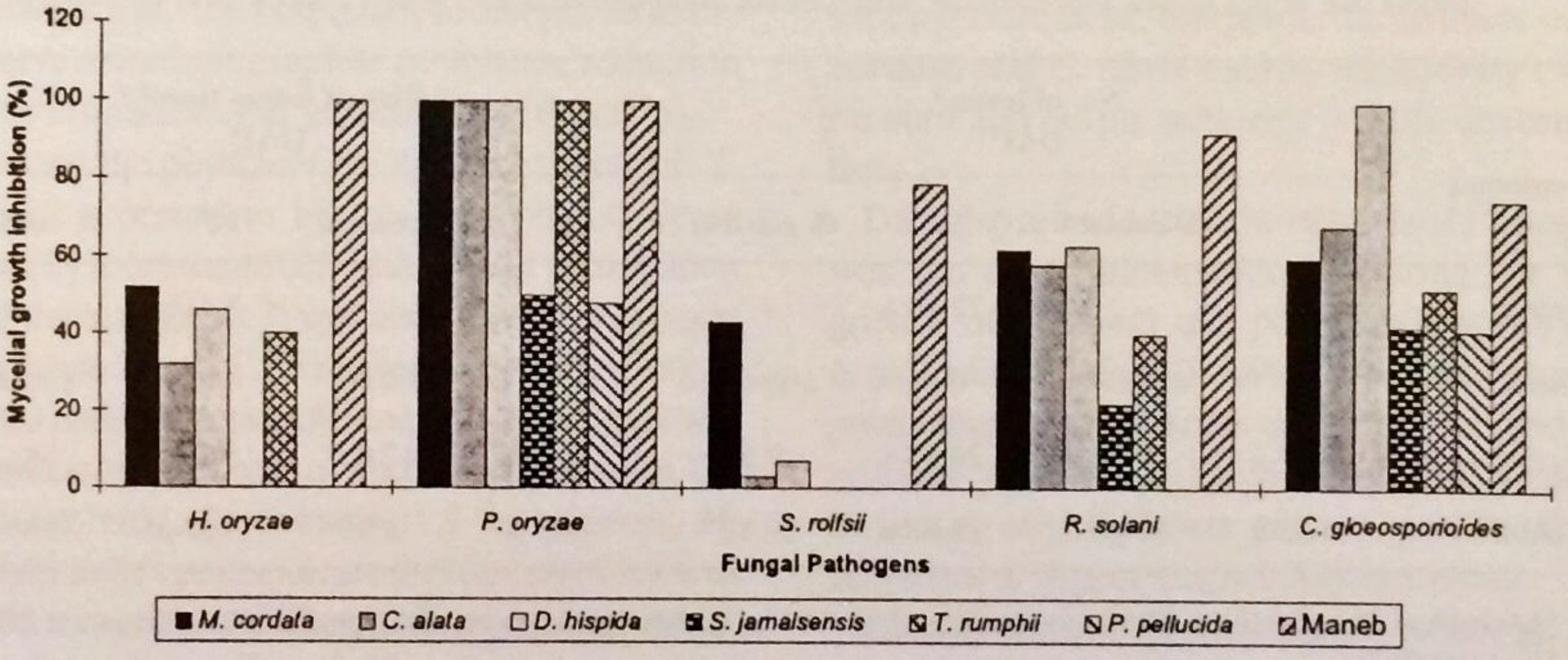

Figure 5. Mycelial growth inhibition (%) of plant pathogenic fungi as affected by plant extracts after 72 hours of incubation.

Screenhouse bioassay

Helminthosporium oryzae

The protective effect of *M. cordata* and *C. alata* extracts against *H. oryzae* on rice seedlings is shown in Table 3. Lesser number of lesions was observed in plants treated with *M. cordata* (6.44 and 20.60 and *C. alata* (4.55 and 16.00) extracts at 5 and 11 days after inoculation (DAI), compared with the untreated control (8.04 and 22.80), although they were not significantly different with each other. On the other hand, Mancozeb gave a 100% protective effect against the disease. Likewise, smaller lesions were noted in plants treated with the extracts at 5 days after inoculation, however, it was not significantly different with the control. On the other hand, bigger lesions were noted in treated plants as the inoculation period was lengthened (i.e. at 11 DAI) compared with the untreated control. Results implied that *M. cordata* and *C. alata* can inhibit the formation of spores as indicated by the lesser number formed. However, the extracts do not have much inhibitory effect on further development of lesion size as shown by bigger lesions at 11 DAI.

Helminthosporium maydis

Table 4 shows the protective effect of plant extracts against *H. maydis* on corn. The different plant extracts provided varying degrees of protective effect against the leaf spot disease. The extracts of *C. alata* was the most effective as shown by lesser number of lesions produced and the highest percent control at 3 and 6 DAI. On the other hand, the extracts of *P. pellucida* was the least toxic with the percent control of 3.20% and 37.46% at 3 and 6 days after inoculation, respectively. Moreover, the extracts of *S. jamaicensis* provided the highest percent control in terms of reducing the size of lesions at 3 and 6 DAI. Although almost all the extracts have caused reduction of lesion size, they were not statistically different with each other but significantly different with the untreated control.

In general, the extracts of *C. alata, S. jamaicensis* and *D. hispida* appeared to possess higher protective activity against *H. maydis* as indicated by reduction in number of spores and size of lesions. Noriel and Robles (1990) reported that extracts of *P. oleracea* possessed protective and therapeutic activities against *H.*

Table 3. Number and size of lesions (mm²) caused by *Helminthosporium oryzae* in rice seedlings as affected by protective effect of *Mikania cordata* and *Cassia alata* extracts at 5 and 11 days after inoculation.[1]

Treatment	No. of lesion[2] DAI[4]				Size of lesion (mm²)[3] DAI[4]			
	5	% control	11	% control	5	% control	11	% control
Control	8.04 a	-	22.80 a	-	2.01 a	-	3.41b	-
Maneb	0 b	100.00	0 b	100.00	0 b	100.00	0 c	100.00
M. cordata	6.44 a	19.90	20.60 a	9.64	1.75 a	12.97	3.75 a	-34
C. alata	4.55 a	43.40	16.00 a	29.82	1.83 a	8.95	3.56 ab	-15

[1]Average of 3 replications. Means in a column followed by a common letter are not significantly different at 5% level (DMRT).
[2]Average of 5 leaves per plant per replicate.
[3]Average of ten lesions per replicate.
[4]Days after inoculation.

Table 4. Number of lesions (per 10cm² leaf area) and mean size of a lesion (mm) caused by *H. maydis* in corn seedling as affected by protective effect of different plant extracts.[1]

Treatment	Number of lesions/(Percent Control)[2]				Size of lesion/(Percent Control)			
	3 DAI[3]		6 DAI		3 DAI[3]		6 DAI	
Control	54.67 a	-	62.17 a	-	4.93 a	-	13.03 a	-
Maneb	0 d	(100.00)	0 e	(100.00)	0 e	(100.00)	0 c	(100.00)
M. cordata	33.17 b	(39.32)	37.50 be	(39.68)	4.05 bv	(17.84)	9.77 b	(25.01)
C. alata	17.17 c	(68.59)	23.67 d	(61.92)	3.40 cd	(31.03)	10.18 b	(21.87)
D. hispida	26.33 bv	(51.83)	32.17 bcd	(48.25)	3.90 cd	(20.89)	9.73 b	(25.32)
P. pellucida	33.67 b	(38.41)	39.00 b	(37.26)	4.73 ab	(4.05)	11.03 b	(15.34)
S. jamaicensis	24.67 bc	(54.87)	29.83 cd	(52.01)	3.23 d	(34.48)	9.68 b	(25.70)

[1]Mean of 2 trials
[2]Means within a column followed by a common letter are not significantly different at 5% level (DMRT)
[3]DAI - Days after inoculation

maydis as indicated by the reduction in the number of lesions as well as size of lesion in corn.

Allelopathic effect of botanical extracts on selected test plants

Germination

The germination of all test plants such as rice, corn, cucumber and purple nutsedge was significantly affected by plant extracts regardless of concentration. The responses of test plants to the extracts included complete inhibition, reduction and/or stimulation of germination (Figure 5).

Among the pesticidal plants, the extracts of *T. rumphii* appeared to be the most phytotoxic as shown by more than 50% inhibition of germination of all the test plants. It was also noted that extracts of *D. hispida* and *C. alata* were also promising as natural herbicides as indicated by complete growth inhibition of test plants of 100% concentration. On the other hand, the extracts of *S. jamaicensis, M. cordata* and *P. pellucida* are the least phytotoxic as shown by lesser inhibitory action on the germination of test plants (Figure 6).

All test plants showed sensitivity to the pesticidal extracts at 100% concentration. However, among the test plants, rice and cucumber were most sensitive to the extracts while purple nutsedge and corn were the least affected.

Root growth

Figure 6 presents the root growth of selected test plants as influenced by botanical extracts. Generally, the response of test plants to the extracts at 100% concentration included complete root growth inhibition and/or reduction in root measurement. However, the extracts of *M. cordata* and *C. alata* caused stimulatory effect on corn and purple nutsedge at 50% concentration.

Data also revealed that the extracts of *T. rumphii* was the most allelopathic inhibiting the root growth of different test plants followed by *D. hispida* and *C. alata*. *S. jamaicensis* also showed promising results while *P. pellucida* and *M. cordata* appeared to be the least phytotoxic as shown by slightly lower percent growth inhibition even at higher extract concentrations.

Among the test plants, purple nutsedge and corn were the least sensitive to the extracts especially at lower concentrations while cucumber and rice were more sensitive as indicated by

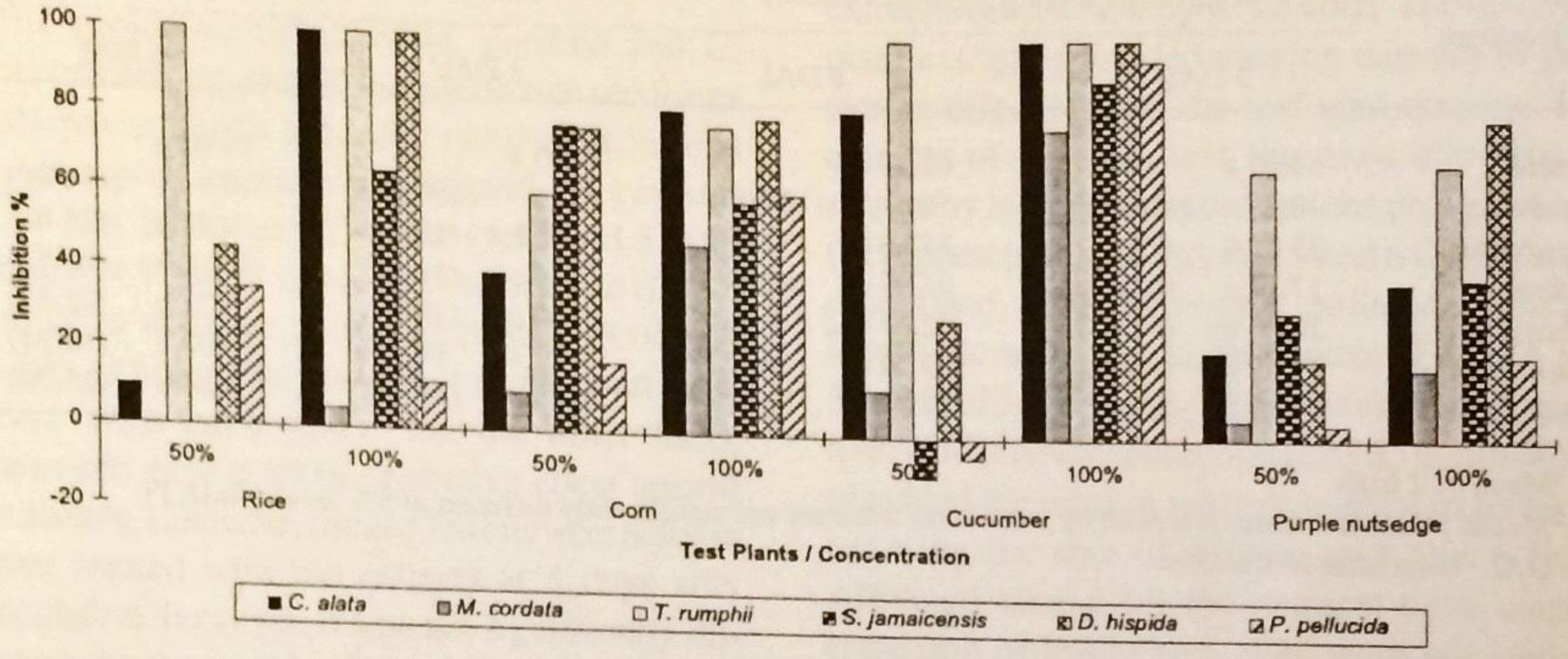

Figure 6. Germination inhibition of test plants as affected by different plant extracts 7 days after treatment.

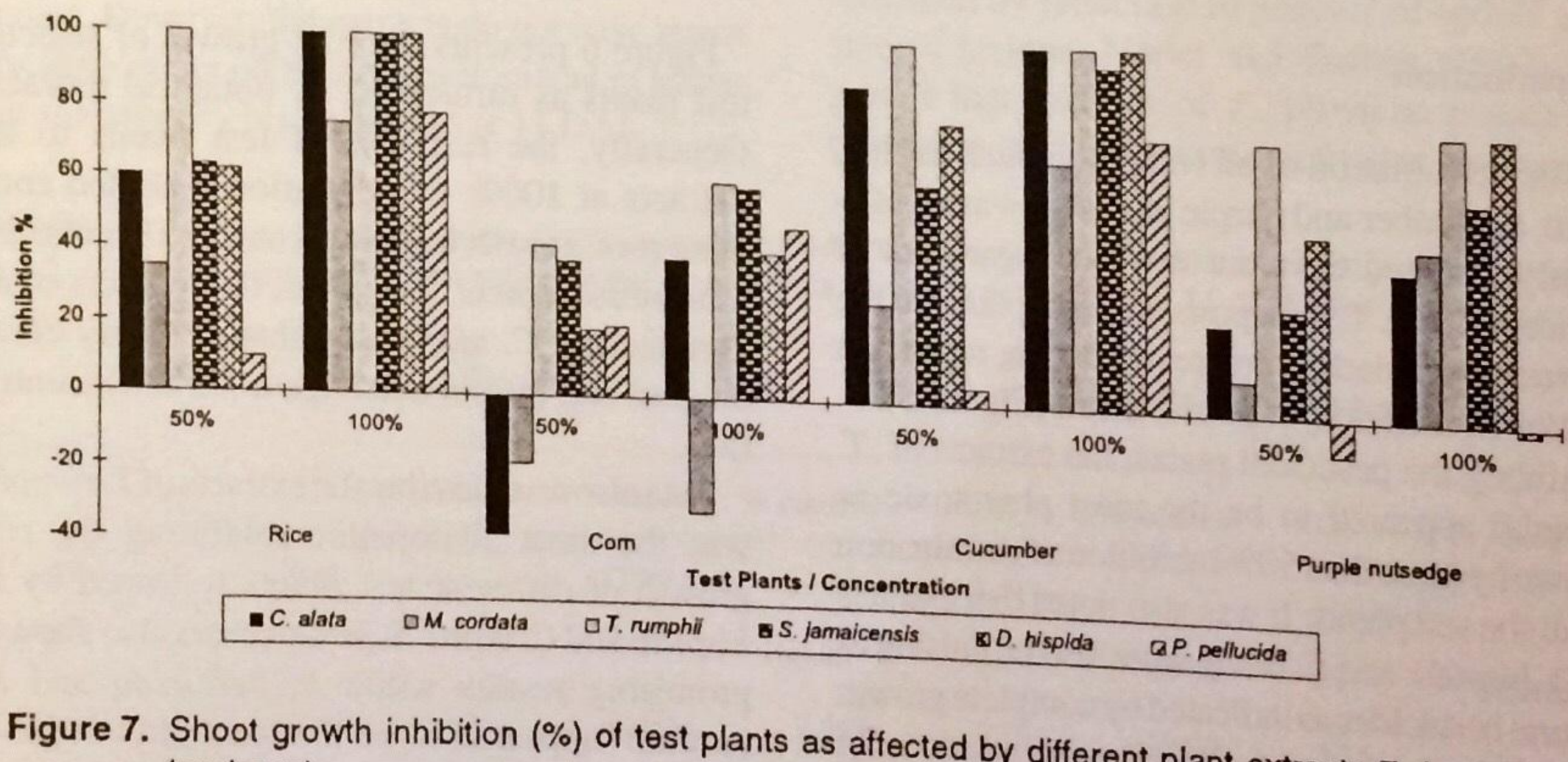

Figure 7. Shoot growth inhibition (%) of test plants as affected by different plant extracts 7 days after treatment.

higher percent root growth inhibition. Noriel (1989) observed that there was a direct proportional relationship between the degree of growth inhibition and extract concentration. Moreover, she noted that generally, root tissues were more sensitive to the extracts than shoots and germination of test plants.

Shoot growth

Application of plant extracts at sowing markedly affected the shoot growth of rice, corn cucumber and purple nutsedge. The extract of *T. rumphii* was again the most inhibitory. Likewise, *D. hispida* was also promising as well as *C. alata* except on corn which caused stimulatory effect on shoot growth together with the extract of *M. cordata*. Among the test plants, corn was the least affected by the extracts followed by purple nutsedge as shown by slightly lower growth inhibition compared with the other test plants (Figure 8).

The responses of test plants to the different extracts included failure of shoot to emerge and/or slower rate of shoot growth as indicated by shorter measurement of the shoot, and stimulation of growth in some cases.

RECOMMENDATION

This study needs further evaluation especially in the field before any conclusion can be drawn. It is only after verification that any recommendation can be made as to what are the botanical extracts which are effective against plant diseases and target weeds. With regards to herbicidal activity, post emergence activity of the plant extracts against different test plants should also be taken into consideration in addition to the preemergence effect as shown in this study.

However, future study should concentrate on the most promising botanical extracts as indicated in the results of this study.

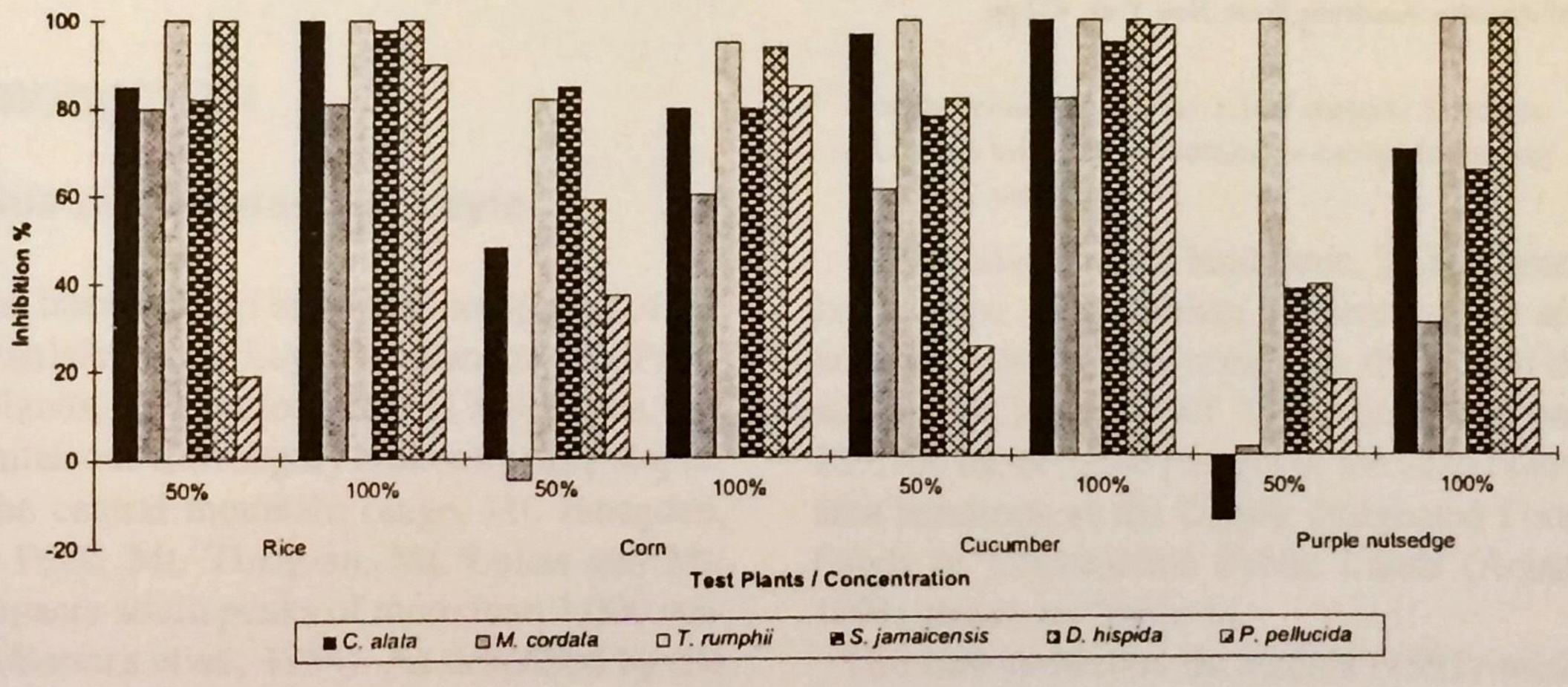

Figure 8. Shoot growth inhibition (%) of test plants as affected by different plant extracts 7 days after treatment.

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REFERENCES

FRAHNJE, N. (1984)
Evaluation of medicinal plant extracts as protectant and therapeutant against legume pathogens. Ph.D. Dissert., University of the Philippines, Los Baños, College, Laguna, Philippines. 142 pp.

JOHNSON, D.A. and C.E. CLARK (1979)
Effect of guar and guar extracts on common root of winter wheat ans spore germination of *Bipolaris sorokiniana*. Plant Dis. Reptr. 60 (10): 811-815.

LAPIZ, D. and E. DUMANCAS (1978)
Fungicidal activity of crude plant extracts against *Helminthosporium oryzae*. Philipp. Phytopathol. 14:23-27.

NORIEL, L.M. (1989)
Endogenous pesticides from ten common weed species in the Philippines. Ph.D. Dissert., University of the Philippines, Los Baños, College, Laguna, Philippines, 135 pp.

NORIEL, L.M. and R.P. ROBLES (1990)
Fungicidal activity of *Portulaca oleraceae*Nisik and Miyaki in corn (*Zea mayz* L.). Philipp. J. Weed Sci. 17:26-32.

MOLISCH, H. (1937)
"Der Einfluss einer Pflanze auf die andere - Allelopathie." Fisher, Jena.

MORALLO-REJESUS, B. (1987)
Botanical pest control research in the Philippines. Philipp. Entomol. 7 (1):1-30

RICE, E.L. (1984)
Allelopathy. Academic Press. New York. 422 pp.