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Survey and evaluation of promising mycoherbicides for the control of asyang [*Mikania cordata* (Burm.F.) B. L. Robinson]

Lualhati M. Noriel, Yolanda C. Mangaoang, Rodolfo A. Paningbatan, Elizabeth P. Parac, and Rowena V. Salva

Department of Pest Management, College of Agriculture, Leyte State University, Baybay, Leyte 6521-A, Philippines

ABSTRACT

The study surveyed and collected diseased specimens of asyang [*Mikania cordata (Burm*. F.) B. L. Robinson] from various places of Leyte and Southern Leyte; isolated and identified the promising fungal pathogens (technically known as mycoherbicide) for the control of asyang; and determined the host range of the promising mycoherbicides.

Seventeen fungal isolates were found pathogenic to asyang with 4-9 days incubation period. The isolates from the towns of Capoocan (Cap), Matalom (Mat-2) and Tomas Oppus (TO) consistently caused significantly higher number of lesions per square leaf centimeter of leaf surface causing early death of leaves than the other isolates. Based on lesion and conidial characteristics, Cap isolate was identified as *Cercospora mikaniacola* Stevens while Mat-2 and TO isolates as *Curvularia pallescens* Ellis. Moreover, the promising isolates were also found non-pathogenic to different host plants tested.

Keywords: asyang, *Mikania cordata*, biological weed control, *Cercospora mikaniacola*, *Curvularia pallescens*, mycoherbicides

Correspondence: L. M. Noriel *Address*: Department of Pest Management, College of Agriculture, Leyte State University, Baybay, Leyte, 6521-A Philippines. *Tel. No*. (053) 335 2626.

INTRODUCTION

Mikania cordata [(Burm F.)B. L. Robinson] locally known as "asyang" in the Philippines is a creeping vine or climbing woody perennial vine belonging to Family *Asteraceae*. Its nodes found along the stems and branches easily form roots when these come in contact with the soil. It also produces seeds which can easily be disseminated by wind. The twining stems and entangled branches of asyang bear many leaves which form a mat that completely covers the crops and gradually smother them (Mercado, 1991).

In the Philippines, asyang is a very aggressive weed which is observed frequently in young secondary regrowth areas, along roadsides, fallow lands and in tree plantations and fruit tree orchards. It is a dominant weed species along hedgerows and in legume-planted fallow areas after rice specifically in Matalom, Leyte. It was also observed heavily infesting trees at the closed-canopy area of Mt. Pangasugan in Baybay, Leyte which is the major reason why this study was conceptualized.

Asyang can be effectively controlled by herbicide. However, their use is slowly minimized nowadays because of economic reason and the adverse effects on the environment and non-target organisms including man. Moreover, continuous herbicide application can cause development of weed resistance to herbicides. Asyang can also be controlled by manual and mechanical means but these methods prove laborious especially when dealing with large scale weed control.

Biological control of weeds using pathogenic fungi technically known as mycoherbicides offers opportunities for overcoming the above-mentioned inadequacies. Mycoherbicides are highly specific disease-inducing fungi isolated from diseased weeds and sprayed on fields to control specific target weed without harm to the crop or any non-target species in the environment. Watson (1992) has indicated that biological weed control, particularly the augmentation of the indigenous fungal pathogens has great potential impact to reduce chemical inputs and to prove viable, and more economical and effective control of weeds like asyang.

Two commercial mycoherbicides are available in the United States namely, COLLEGO and DEVINE. COLLEGO specifically controls northern jointvetch (*Aeschynomene virginica* L.) in rice and soybean. This consists of dried, living spores (conidia) of fungus *Colletotrichum gloesporioides* (Penz.) Sacc. f. sp. *aeschynomene* formulated as wettable powder. On the other hand, DEVINE is made up of spores of soil-borne fungus, *Phytophthora*

palmivora (Butler). It is used for the control of strangler vine (*Morremia odorata* [H. and A.] Lind. L.) in citrus. Biomal is also registered in Canada as a mycoherbicide for the control of round-leafed mallow in field crops (Quimby and Walker, 1982).

In the Philippines particularly in ViSCA, Baybay, Leyte, biological weed control studies have been reported by Po (1996) specifically on the fungus *Helminthosporium solani* infecting itchgrass [*Rottboella cochichinensis* (Lour.) W.D. Clayton]. The fungus was able to reduce the vegetative and reproductive capacity of inoculated itchgrass compared with the uninoculated weed. Furthermore, Valleser (20050 reported the promising effects of *Bipolaris* sp. for the control of itchgrass. At IRRI, *Alternaria* sp. is found effective for the control of *Sphenoclea zeynalica* Gaerth, a broadleaf lowland rice weed (Mabbayad and Watson, 1993).

This study was done to conduct, survey and collect diseased asyang in selected places in Leyte and Southern Leyte; isolate and identify the promising fungal isolates (mycoherbicide) for the control of asyang and determine the host range of the promising mycoherbicides.

MATERIALS AND METHODS

Survey, collection and isolation of fungal pathogens causing disease of asyang

Different places of Leyte and Southern Leyte were surveyed for naturally occuring fungal disease of asyang. Infected plants were collected, placed in paper envelopes, labeled and brought to the laboratory for preliminary diagnosis of the disease. Pathogens were isolated using the tissue planting method and maintained in potato dextrose agar (PDA) culture medium by serially transferring to new slants at 2-4 weeks intervals.

Pathogenicity test of the fungal isolates

Twenty (20) cm asyang cutting were planted in 10-cm diameter plastic pots for pathogenicity test of the different fungal isolates. Five replicate pots were provided per isolate.

Each fungal isolate was mass produced in flat bottles containing 30 ml PDA. Spore suspension was prepared from 2-week old fungal culture by

adding 30 ml sterile water and 2 drops of Tween 80 per bottle. Spores per ml were counted and adjusted to a concentration of 10^5/ml using a haemacytometer. Inoculation was done late in the afternoon by handspraying the young leaves of one month old asyang with 15 ml of the prepared spore suspension per pot. The inoculated leaves were labeled and covered with plastic bag overnight and maintained thereafter outside the screenhouse. The incubation period, i.e. the number of days from inoculation to appearance of symptoms was determined. The characteristics of the lesion, and lesion number in 1 cm square area of sample leaf were noted. An index paper with a hole at the center equivalent to 1 cm square was used to determine the area. The percent disease severity of asyang inoculated with promising isolates was also noted starting at one week after inoculation up to 4 weeks. This was obtained from visual estimate of infected area as a percentage of the total area of leaf.

Identification of the promising mycoherbicides

Microscopic examination of diseased asyang leaves infected by the promising isolates identified as the Cap (Capoocan) and Mat-2 (Matalom) and TO (Tomas Oppus) isolates yielded abundant conidia and conidiosphores. The causal fungi were isolated into pure culture and examined under the microscope. Fungal colony growth and color *in vitro* were determined after 2 weeks of growth. Size, shape, color and number of septations of 50 randomly selected conidia of Cap and Mat-2 isolates (TO isolate was found similar to Mat-2 isolate) were determined. The "Keys to Species of Philippine Phytopathogenic Fungi" compiled by Quimio (1975) was used in keying out the genus and species of *Curvularia* sp. "A Monograph of the Fungus Genus Cercospora" by Chupp (1953) was used. The identification of the two promising isolates was confirmed by a Mycology expert of the University of the Philippines, Los Baños, Laguna.

Host range test

Different species of fruit and forest seedlings were tested to determine the host range of the most promising pathogenic isolates (Cap, Mat-2, TO) from asyang. These were obtained from the Department of Horticulture (Pomology) and at *GTZ* nursery in ViSCA. Likewise, additional crops such as cereals, ornamentals, rootcrops and vegetable crops were also included in the host-range test (Appendix Table 2). Two-week old fungal culture of the

promising isolates was used and 10^5 spores/ml concentration was inoculated by spraying into the new shoots of asyang and the seedlings of the different host plants with 15 ml/pot of the prepared spore suspension. The inoculated seedlings were placed inside plastic chamber maintained at high relative humidity for two days. These were watered daily for the survival of the pathogens. The appearance of symptom was observed until 2 weeks after inoculation. The test was conducted in three trials.

RESULTS AND DISCUSSION

Isolation, identification and pathogenicity of fungal pathogens infecting asyang

Seventeen fungal isolates from various places in Leyte were found pathogenic to asyang with an average range of 4-9 days incubation period (Table 1). Of the seventeen isolates screened against asyang, three were found most pathogenic namely: Cap, Mat-2 and TO. These three isolates consistently caused higher number of lesions per square centimeter of leaf area that significantly differ from other isolates (Table 2).

The identity of the three most pathogenic isolates (Cap, Mat-2, and TO) was determined based on the lesions, colony growth and characteristics of conidia. The Cap isolate identified as *Cercospora mikaniacola* Stevens formed 2 mm circular, gray lesion with white center. The colony growth *in vitro* appeared grayish white. On the other hand, the isolates from Mat-2 and TO were found to be similar and identified as *Curvularia pallescens* Ellis. The lesions formed were circular to irregular in shape with gray to black coloration and brown border. The lesion size is bigger than that of Cap isolate (approx. 5 mm or more). *In vitro* culture of *C. pallescens* exhibited black colony growth.

Conidial characteristics of the isolates e.g. conidial shape, septation, color and size (Fig. 1) observed from infected leaves and from pure culture revealed that the Cap isolate is typical of *Cercospora mikaniacola* and the Mat-2 and TO isolates are characteristic of *Curvularia pallescens*.

The Cap isolate revealed black colonies with conidiophores which are simple and arising in clusters and cylindrical obclavate, subhyaline conidia, with long obconically truncate base, 6-7 septa, and conidial measurement of

Table 1. Incubation period (days) of the 17 fungal isolates after inoculation on *Mikania cordata* plants [1/]

Fungal Isolates[2/]	Incubation Period (day)[3/] TRIAL		
	1	2	3
Mah	7.0c	7.8def	8.0b
May blk.	6.0d	6.0h	6.0d
Cap	6.0d	7.2fg	7.0c
Maa blk.	8.0b	8.0cde	8.0b
Mal blk.	4.0f	4.4i	4.0f
TO	8.0b	8.0cde	8.0b
Maa com	5.0e	5.0i	5.0e
Mat com	6.0d	7.0g	7.0e
Mal com	7.0c	8.4a-d	8.0b
SL	4.0f	5.0i	4.2f
Ab	8.0b	7.4efg	8.8a
Hin	8.0b	8.6abc	8.0b
Hil	9.0a	8.8ab	8.6ab
VB	9.0a	8.0cde	8.2ab
B	9.0a	8.6abc	8.6ab
Mer	9.0a	9.0a	8.8a
Mat-2	8.0b	8.2bcd	8.2ab

[1/] Average of 5 replications

[2/] Legend:

Mah- Mahaplag, Leyte
May blk. - Maybog (Black),Leyte
Cap - Capoocan
Maa blk - Maasin (Black), So.Leyte
Mal blk. - Malitbog (Black)
TO - Tomas Oppus
Maa com - Maasin (Common)
Mat - Matalom (Common)
Mal - Malitbog (Common)
SL - Sri Lanka
Ab - Abuyog
Hin - Hindang
Hil - Hilongos
VB - Visca, Baybay
B - Baybay
Mer - Merida
Mat-2 - Matalom (2)

[3/] In a column, means followed by common letters are not significantly different at 5% level by DMRT.

Table 2. Number of leaf lesions per square cm of leaf area due to infection of the fungal isolates one month after inoculation on *M. cordata* plants (three trials) [1/]

Fungal Isolates[2/]	Lesion Number[3/] TRIAL		
	1	2	3
Mah	2.4f	3.4cde	3.6ab
May blk.	4.4de	4.0cde	4.bc
Cap	10.8a	12.2a	8.4c
Maa blk.	4.8cd	3.6cde	3.0c
Mal blk.	3.4def	3.2cde	3.8bc
TO	8.8b	8.4b	9.0a
Maa com	2.4f	3.2cde	3.2c
Mat com	6.2c	4.0cde	3.8bc
Mal com	3.6def	4.2cde	3.4c
SL	4.4de	4.4c	4.0bc
Ab	5.0cd	4.0cde	3.4c
Hin	2.2f	2.4e	3.5c
Hil	2.8ef	2.6de	2.8c
VB	2.4f	3.2cde	3.2c
B	2.6f	3.4cde	2.6c
Mer	3.5def	3.0cde	4.0bc
Mat-2	7.8b	9.2b	5.4b

[1/] Average of 5 replications

[2/] Legend:

Mah- Mahaplag, Leyte
May blk. - Maybog (Black),Leyte
Cap - Capoocan
Maa blk - Maasin (Black), So.Leyte
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Ab - Abuyog
Hin - Hindang
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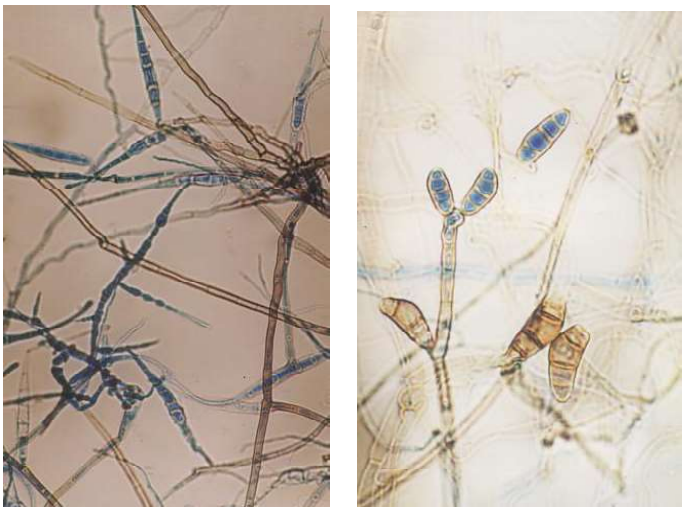
VB - Visca, Baybay
B - Baybay
Mer - Merida
Mat-2 - Matalom (2)

[3/] In a column, means followed by common letters are not significantly different at 5% level by DMRT.



A B

Figure 1. The conidia and conidiophore of *Cercospora mikaniacola* Stevens (A)and *Curvularia pallescens* Ellis (B) isolated from asyang in Capoocan, and Matalom and Tomas Oppus, Leyte, respectively.

A

B

Figure 2. Photograph of the asyang plants taken 6 weeks after inoculation of fungal isolates. A - Cap isolate (*Cercospora mikaniacola*). B -TO isolate (*Curvularia pallescens*). Take note of the dead leaves of asyang inoculated with Cap isolate 6 weeks after inoculation (WAI).

Table 3. Percent disease severity[1/] on *Mikania cordata* plants taken starting one week after inoculation of the most pathogenic isolates[2/]

Fungal Isolates	Disease Severity %[3/] Week after inoculation			
	1	2	3	4
Cap	54.0a	72.0a	88.0a	100.0a
Mat-2	46.0a	58.0b	78.0a	90.0a
TO	44.0a	58.0b	76.0a	92.0a

[1/] Visual estimates based on leaf area infected with respect to the total leaf area
[2/] Average of 5 replications.
[3/] In a column, means followed by common letters are not significantly different at 5% level by DMRT

55 μm and 4.72 μm wide. On the other hand, the Mat-2 isolate produced gray colonies, simple brown conidiophores, 4-5 celled pale brown conidia with scarcely protuberant hilum. smooth-walled and median cell slightly curved. Conidia measured 25.24 μm long and 10.43 μm wide.

Regarding disease severity, results showed that the Cap isolate (C. *mikanicola*) was more pathogenic and aggressive than the Mat-2 and TO isolates (*C. pallescens*) causing significantly more severe leaf spots at 2 weeks after inoculation. Six weeks after inoculation the leaves of Cap-inoculated plants eventually died (Table 3 and Fig. 2).

Host range studies

Host range studies conducted in three trials using different species of fruit and forest tree seedlings, cereals, rootcrops, ornamentals and vegetable crops showed that promising mycoherbicides namely, *C. mikaniacola* and *C. pallescens* were not pathogenic to any of the test plants. Only asyang was found positive to infection. The host specificity of the two fungal isolates holds promise for their use as potential mycoherbicides for the control of asyang.

CONCLUSION

The study has revealed the potential of using mycoherbicides for effective control of asyang. The fungal isolates from Capoocan and Matalom (and Tomas Oppus) are found to be the most promising mycoherbicides which are identified as *Cercospora mikaniacola* and *Curvularia pallescens*, respectively. Both fungal pathogens are also found host specific to asyang only.

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