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# Initiating the use of fungi for biocontrol of weeds in Malaysia

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**Abstract.** This paper is an account of the progress, problems and future directions in the use of indigenous fungi as biocontrol agents of weeds in Malaysia. Three weed species are highlighted. *Eichhornia crassipes*, the major weed problem of rice-field irrigation and drainage canals is mainly diseased by *Myrothecium roridum*. The broad host-range of this fungus precludes its use as a mycoherbicide. In plant-house studies, however, *M. roridum* was found to increase the efficacy of the introduced weevil, *Neochetina eichhorniae*. The weevil on its own has thus far failed to establish effectively and to suppress the weed population. The integration of these two agents is discussed. More recently we isolated an *Alternaria* sp. similar to *A. eichhorniae*. Results on the preliminary assessment of this isolate are presented. *Sphenoclea zeylanica* is an important broad-leaf weed in rice fields. An isolate of *Colletotrichum gloeosporioides*, which is pathogenic on this weed, was recently obtained. Spore concentrations of $2x10^6$/ml can cause complete kill within a week to 10 days. Disease severity is affected by both plant age and dew period. Limited host-range testing indicates the host specificity of the isolate. *Mikania micrantha* is a serious weed of plantation crops. Most stands of this weed are infected by *Cercospora mikaniicola*. Particular problems with this pathogen are poor sporulation in culture and slow disease development. The effects of nutrients on spore germination and their relationship to disease severity are discussed.

## Introduction

Weeds are a constant threat to agricultural production all over the world. Severe yield losses coupled with other factors such as reduced farm labour forces have led to ever-increasing usage of chemical herbicides. Reflecting the world-wide scenario, herbicide consumption in Malaysia spiralled from RM30 million in 1970 to RM210 million in 1992 (Lee and Teoh 1994). Awareness of the need to escape this pesticide treadmill has prompted increased research into alternative weed control methods, in particular biological control. In Malaysia, a much needed impetus for biological weed control was realized with the establishment, in 1986, of a National Co-ordinating Committee on Biological Control (Mohamed *et al.* 1992). The main thrust of the research activities sponsored by this committee has been the utilization of exotic herbivorous insects. The use of fungi has lacked support and has thus far been restricted to individual laboratories and on a relatively small scale (Caunter 1994). However, a start has been made and in this paper we present an overview of progress, focusing on three weed species.

The initial step was to search the published literature host-pathogen records for weeds. The primary source relevant to Malaysia is the check-lists of hosts and diseases by Singh (1973). For the weed species mentioned in this paper, the host-pathogen records are given in Table 1. No claim was made, however, for confirmation of pathogenicity following Koch's postulates. The pathogens listed, therefore, would more appropriately be classified as associated with apparently-diseased plant parts of the relevant weed species. A search of published materials in journals and various conference and symposium proceedings revealed a similar lack of studies on fungal diseases of weeds. The earliest publication that records a specific attempt to identify and assess the potential of fungi as biological weed control agents in Malaysia is that of Caunter (1982) on waterhyacinth, *Eichhornia crassipes* (Mart.) Solms.

## Waterhyacinth

Waterhyacinth was the first weed species in Malaysia investigated in the context of the use of fungi for its control. Preliminary disease surveys were conducted in

**Table 1.** List of recorded pathogens on three weed species in Malaysia (from Singh 1973).

| Weed Species | Pathogens |
|---|---|
| *Mikania micrantha* | *Cercospora* sp. |
| | *Cercospora mikaniicola* |
| *Echinochloa colona* | *Pyricularia oryzae* |
| *Imperata cylindrica* | *Didymaria* sp. |
| | *Dinemasporium* sp. |
| | *Ascochyta* sp. |
| | *Circinoconis paradoxa* |
| | *Lophodermium* sp. |
| | *Pussinia rufipes* |
| | *Colletotrichum graminicola* |

1981-1982 resulting in the identification of three fungal pathogens (Caunter 1982). Of these, the prevalent pathogen and also the most destructive was *Myrothecium roridum*. As previously reported, both Indian (Ponnappa 1970) and Thai isolates (Rakvidhyasastra *et al.* 1978; Puripanpinyo 1984) of *M. roridum* were not host specific. The Malaysian isolate was tested on thirteen plant species and sporulation on diseased lesions was only observed on two other species besides waterhyacinth (Caunter and Mohamed 1990a).

Surveys continued, but no new pathogens were encountered until 1994 when an isolate of *Alternaria* was obtained. In culture (potato dextrose agar), a red pigment is produced and lesions on infected leaves are surrounded by a yellow halo. The isolate thus appears to conform to the description of *A. eichhorniae* first reported from India by Nag Raj and Ponnappa (1970). Confirmation of the identity of this isolate is, however, problematic. The isolate differs from *A. eichhorniae* in that the conidia have no, or only a few longitudinal septa. It appears to be similar to *A. longissima* but its conidia are generally smaller. Nevertheless, the isolate is clearly pathogenic on waterhyacinth, developing lesions within four days of inoculation. Leaves of both old and young plants were equally susceptible. Elongated lesions also developed on inoculated petioles. Sporulation of the isolate is inconsistent and we are attempting to improve this.

In view of the current lack of any pathogen that has the potential to be developed into a mycoherbicide for waterhyacinth control, we have focused our efforts on studying the integration of *M. roridum* with herbivorous insects. The weevil, *Neochetina eichhorniae*, which was released in southern Thailand was fortuitously introduced into Malaysia. The weevil on its own, however, was ineffective in controlling the weed. In greenhouse trials, plants inoculated with *M. roridum* at levels reflecting field infections and which were subsequently exposed to the weevil were significantly reduced in growth, as compared with plants exposed to the weevil alone (Caunter and Mohamed 1990b). We have not, however, been able to test this effect in the field as the weevil population has steadily declined. Two other insect species have recently been introduced into Malaysia (Anwar *et al.* 1994). These are a second species of *Neochetina*, viz., *N. bruchi*, and a moth, *Sameodes albiguttalis*. Initial releases of *N. bruchi* have already been made, whereas approval is still pending from the Department of Agriculture for the release of the moth. Future studies will therefore focus on the integration between fungi and various insect species.

### *Sphenoclea zeylanica*

*Sphenoclea zeylanica* Gaertn. is a troublesome weed in rice fields in the Kerian, Seberang Perai and Muda rice-growing areas of Malaysia. Although once easily controlled by 2,4-D, resistance to this herbicide has developed (Itoh 1991). As there were no previous records of diseases of this weed, we conducted a survey in the Muda area in 1994. Two fungi were isolated, an *Alternaria* sp. and *Colletotrichum gloeosporioides*. Both fungi were pathogenic, but further work with the *Alternaria* isolate was discontinued as it lost its virulence after subculturing.

The *C. gloeosporioides* isolate proved to be extremely virulent. Initial symptoms developed two days after inoculation. Infected leaves became desiccated and were easily dislodged. The fungus was also capable of causing stem lesions and when this occurred, death of the plant ensued.

Preliminary host-range testing indicated that the isolate was specific to *S. zeylanica*. The isolate could be cultured easily, both on solid, and in liquid, media. In liquid media (potato dextrose broth), approximately $10^8$ conidia/ml were produced after 3-4 days. The only drawback to its potential as a mycoherbicide would be the need for a long dew period for maximum disease development (Table 2). Our current target is to develop an invert emulsion compatible with the isolate to reduce dew dependence.

### *Mikania micrantha*

*Mikania micrantha* HBK (mile-a-minute) is a major weed of plantation crops in Malaysia. Yield depression

**Table 2.** Effect of dew period on infection of *Sphenoclea zeylanica* plants inoculated with *Colletotrichum gloeosporioides*.

| Dew period (h) | Infection (%) |
|---|---|
| 2 | 8.3 |
| 4 | 15.2 |
| 8 | 16.5 |
| 16 | 79.2 |

**Table 3.** Effect of single, weekly and fortnightly applications of *Cercospora mikaniicola* on disease severity (% infection) of *Mikania micrantha* after 4-8 weeks.

| | 4 weeks | 5 weeks | 6 weeks | 7 weeks | 8 weeks |
|---|---|---|---|---|---|
| Control | 0.9 | 2.8 | 6.0 | 9.4 | 8.5 |
| Single | 26.1 | 22.6 | 21.5 | 22.0 | 22.5 |
| Weekly | 33.9 | 35.1 | 43.5 | 50.7 | 50.9 |
| Fortnightly | 24.7 | 21.8 | 28.3 | 42.0 | 37.9 |

in both rubber (Watson *et al.* 1964) and oil palm (Turner and Gillbanks 1974) has been documented. *Mikania micrantha* can be controlled both manually and chemically. The most commonly used herbicide is 2,4-D amine but toxic effects on young rubber and oil palm can occur. Paraquat has been recommended as an alternative (Seth 1971) but has proven to be less effective (Mangoensoerarjo 1978). The adverse effects of herbicides and the high labour costs of manual weeding prompted a programme on biological control of *M. micrantha*. Accordingly the insect *Liothrips mikaniae* was imported into Malaysia. The project was not successful, however, because the insect failed to establish in the field (Liau *et al.* 1994). An investigation into the use of fungal pathogens was therefore initiated. Teoh *et al.* (1985) reported the isolation of *C. gloeosporioides*, but this species is also a major cause of secondary leaf-fall of rubber so it was not considered for investigation as a potential mycoherbicide. At our laboratory we have studied the possibility of using *Cercospora mikaniicola* as a mycoherbicide, but there are problems. It grows slowly and sporulates poorly on artificial media. Isolates differ markedly in this respect and tend to lose their ability to sporulate after repeated transfers. Disease development is also slow and to achieve 50% disease required weekly applications over a period of seven weeks (Table 3). We are currently assessing media that would support more rapid growth and increased sporulation. The effects of added nutrients to spore suspensions have also been assessed *in vitro*. Sucrose, glucose or yeast extract at concentrations of 0.1 to 5.0% did not have any effect on spore germination in water agar. On excised leaves, however, disease development was markedly increased, especially with yeast extract at 0.1%. Validation of these results via pot trials will be conducted when problems relating to growth and sporulation have been resolved.

## The future

Besides the above three weed species, studies on potential mycoherbicides for two other weed species from the family Gramineae are currently being pursued. In both cases the pathogens involved are from the genus *Colletotrichum*. Progress on the use of *C. caudatum* as a mycoherbicide for control of *Imperata cylindrica* is presented elsewhere (Caunter 1996 this Volume). The second grassy species being investigated is barnyard grass (*Echinochloa crus-galli*). Apart from serving as an alternate host of *Pyricularia oryzae*, no other pathogens have been recorded although *Curvularia* spp. are commonly isolated from diseased lesions. Recently we isolated *C. gloeosporioides* from *E. oryzicola*. This is a new record for Malaysia and spot inoculations indicate its pathogenicity on *E. crus-galli* also.

It is obvious that the use of fungi as biological control agents is still in its infancy in Malaysia. A number of reasons can be cited which are probably no different from those experienced in other countries. Insufficient funding is undoubtedly a major factor, but more important is the low number of researchers involved in this area of study. What is clearly needed to attract more research is a demonstration of the feasibility of using fungi as biological weed control agents. The choice of target weed is important to maximize success and therefore we intend to focus on rice-field weeds, namely, *S. zeylanica* and *Echinochloa* spp. as the humid environment of rice fields would alleviate to a large extent the moisture limitations characteristic of most mycoherbicides.

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## References


Anwar A.I., Soetikno S.S., Sivapragasam A., Tay B.L., Razali B. and Zubir A. (1994) Introduction and impact of *Neochetina bruchi* as a biocontrol agent of water hyacinth in Malaysia. In: *Proceedings of the Fourth International Conference on Plant Protection in the Tropics,* pp. 113-115. A. Rajan and Y.B. Ibrahim (eds). 28-31 March 1994, Kuala Lumpur, Malaysia.

Caunter I.G. (1982) Potential for biological control of water hyacinth with indigenous fungal pathogens. In: *Proceedings of the First International Conference on Plant Protection in the Tropics,* pp. 489-492. K.L. Heong, B.S. Lee, T.M. Lim, C.H. Teoh and Y. Ibrahim (eds). 1-4 March 1982, Kuala Lumpur, Malaysia.

Caunter I.G. (1994) Potential for mycoherbicides in Malaysia. In: *Appropriate Weed Control in Southeast Asia*, pp. 42-47. S.S. Sastroutomo and B.A. Auld (eds). CAB International, Kuala Lumpur.

Caunter I.G. and Mohamed S. (1990a) Effect of *Neochetina eichhorniae* on waterhyacinth and its interaction with *Myrothecium roridum*. In: *Proceedings of the Third International Conference on Plant Protection in the Tropics, Volume 6,* pp. 261-264. 20-23 March 1990, Genting Highlands, Malaysia.

Caunter I.G. and Mohamed S. (1990b) The effect of *Neochetina eichhorniae* and *Myrothecium roridum* alone and in combination for the initial control of waterhyacinth. *Journal of Plant Protection in the Tropics,* 7: 133-140.

Itoh K. (1991) *Life cycles of rice field weeds and their management in Malaysia.* Tropical Agricultural Research Center, Tsukuba, Japan.

Lee S.A. and Teoh C.H. (1994) Integrated weed management in Malaysia. In: *Appropriate Weed Control in Southeast Asia*, pp. 98-109. S.S. Sastroutomo and B.A. Auld (eds). CAB International, Kuala Lumpur.

Liau S.S., Tan C.L., Ooi P.A.C., Chung G.F., Lee S.A. and Tay B.L. (1994) Field releases of *Liothrips mikaniae* (Priesner) for the control of *Mikania micrantha HBK* - Experiences in Malaysia. In: *Proceedings of the Fourth International Conference on Plant Protection in the Tropics,* pp. 116-119. A. Rajan and Y.B. Ibrahim (eds). 28-31 March 1994, Kuala Lumpur, Malaysia.

Mangoensoerarjo S. (1978) Mile-a-minute (*Mikania micrantha* HBK) control in immature oil palm. In: *Proceedings of the Plant Protection Conference 1978*, pp. 381-387. Rubber Research Institute of Malaysia, Kuala Lumpur, Malaysia.

Mohamed A.Z., Lee B.S. and Lum K.Y. (1992) Developing a biological control initiative in Malaysia. In: *Proceedings of the Third International Conference on Plant Protection in the Tropics - Biological Control: Issues in the Tropics*, pp. 59-62. P.A.C. Ooi, G.S. Lim and P.S. Teng (eds). 20-23 March 1990, Genting Highlands, Malaysia.

Nag Raj T.R. and Ponnappa K.M. (1970) Blight of waterhyacinth caused by *Alternaria eichhorniae* sp. nov. *Transactions of the British Mycological Society*, 55: 123-130.

Ponnappa K.M. (1970) On the pathogenicity of *Myrothecium roridum-Eichhornia crassipes* isolate. *Hyacinth Control Journal*, 8: 18-20.

Puripanpinyo M. (1984) *Fungi pathogenic to waterhyacinth and their potential in biological control.* M.Sc. (Ag.) Thesis, Kasetsart University.

Rakvidhyasastra V., Iemwimangsa M. and Petchart V. (1978) Host range of fungi pathogenic to waterhyacinth (*Eichhornia crassipes* [Mart.] Solms.). *Kasetsart Journal,* 12: 114-118.

Seth A.K. (1971) Control of *Mikania cordata* (Burm. f.) B.L. Robinson in plantation crops using paraquat. *Weed Research,* 11: 77-83.

Singh K.G. (1973) *A Check-List of Host and Disease in Peninsular Malaysia.* Ministry of Agriculture and Fisheries, Malaysia.

Teoh C.H., Chung G.F., Liau S.S., Ibrahim G., Tan A.M., Lee S.A. and Mohammed M. (1985) Prospects for biological control of *Mikania micrantha* HBK in Malaysia. *The Planter,* 61: 515-530.

Turner P.D. and Gillbanks R.A. (1974) *Oil Palm Cultivation and Management.* Incorporated Society of Planters, Kuala Lumpur.

Watson G.A., Wong P.W. and Narayanan R. (1964) Effects of cover plants on soil nutrient status and on growth of *Hevea* IV. Leguminous creepers compared with grasses, *Mikania cordata* and mixed indigenous covers on four soil types. *Journal of the Rubber Research Institute of Malaysia,* 18: 128.