Puccinia melampodii Diet. and Holow. as a Biological Control Agent of *Parthenium hysterophorus*

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ABSTRACT

***Parthenium hysterophorus* is an annual, extremely prolific weed of Asteraceae that causes severe economic losses, health problems and habitat destruction in many tropical and sub tropical countries. In Sri Lanka, it was first observed in Vavuniya district in 1999 and subsequently it has spread to many areas of the country.The fungus *Puccinia melampodii*, introduced from Australia, was evaluated for its efficacy as bio control agent against *P. hysterophorus* under local conditions to use as a component of integrated pest management programme. Life cycle studies revealed that *P. melampodii* completes its life cycle within 8-11 days at 25^0 C night and 28^0 C day temperature. Continuous fungal infection resulted complete destruction of Parthenium plants within 30 days. *In vitro* studies to determine the effect of temperature on teliospore germination of *P. melampodii* indicated that it occurs over a wide range of temperatures from 5 to30^0 C. The optimum temperature was 25^0 C and the fungus did not survive at 35^0 C after an inoculation period of 24 h and 48 h.Host specific studies of *P. melampodii* showed that, of 30 different plant taxa in 14 plant families tested, only *P. hysterophorus* were susceptible to the infection. Fungal infection at different growth stages of the Parthenium; rosette, preflowering and flowering stages, suppressed the plant growth, especially the leaf and flower production. Results revealed that the fungus *P. melampodii* can be effectively used as a potential bio-control agent against the Parthenium weed.**

Key words: Parthenium weed, *Puccinia melampodii*, teliospore germination, mycelium

INTRODUCTION

In Sri Lanka *Parthenium hysteophorus* was first identified in Vavuniya District in 1999 and subsequently it has spread through out the country. This herb belongs to the family asteraceae, which is a native of southern parts of North America, the West Indies and the central parts of South America. It has been introduced, over the past five decades, in to many countries including Australia, China, India, Israel, Madagascar, Mozambique, Nepal, South America and Vietnam (Towers *et al.*, 1977, Joel and Liston, 1986).

P. hysterophorus is known to suppress local vegetation by release of growth inhibitors through leaching, exudation of roots, decay of roots, decay of residues, etc. (Sukhada and Jayachandra, 1979, 1980a, 1980b). If not controlled, it can affect natural diversity and cause extinction of native flora.

As *P. hysterophorus* contains parthenin, a sesquiterpene lacone, poses a significant health risk to humans and toxic to livestock. If it is present as a pure stand, animals will feed on the weed. Toxic effects include dermatitis and skin lesions, emaciation, rupture of tissues, haemorrahages in internal organs and death (Ahmad *et al.*, 1988). Sheep are known to be more tolerant than cattle. Milk of cattle, buffalo and sheep as well as sheep meat can be tainted through feeding on the weed (Towers and Subba Rao, 1992, Tudor *et al*., 1982). *P. hysterophorus* causes allergic reactions in susceptible humans. Symptoms are allergic eczematous contact dermatitis through prolonged close contact with the weed and allergic rhinitis (hay fever) and allergic bronchitis through pollen (Mcfadyen, 1985, Towers and Subba Rao, 1992).

Manual and mechanical control of this weed is expensive and provides only short- term control requiring repeated

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applications. Application of weedicides can be effective if applied correctly but it is economically viable only for smaller intensive agriculture. Furthermore, the use of weedicides is not desirable in the vicinity of populated areas where *P. hysterophorus* is commonly found as a weed of disturbed land. On the other hand biological control of weeds is a cost effective, environmentally safe and ecologically viable method. Several potential effective bio-control agents, both insects and fungi have been identified and evaluated in Australia and India.

When the weed was first detected in Sri Lanka in 1999 in Vavuniya District, the Department of Agriculture has been vigilant and launched an awareness programme immediately in 2000. This resulted the detection of the weed in varying population sizes from several areas in the Districts of Vavuniya, Trincomalee, Kurunegala, Kandy, Rathnapura, Badulla, Ampara and Thissamaharamaya. Localized small patches have also been reported from Matale, Bandarawela, Moneragala, Jaffna and Puttlam districts.

Since it was a newly identified problem in Sri Lanka, the Department of Agriculture launched an island wide weed management programme using cultural and chemical methods to eradicate the weed. Biological control of the weed is only cost effective, environmentally safe and ecologically viable method as practiced in other countries. Therefore, the main objective of this study is to evaluate the efficacy of bio control agents in controlling *P. hysterophorus* under local conditions and to use them as a component in the integrated pest management programme in future.

MATERIALS AND METHODS

1. Maintenance of *P. hysterophorus* and cultures of biological control agents

1.1 Maintenance of *Parthenium hysterophorus* plants

A separate cage (block) was arranged in a plant house of National Plant Quarantine Service (NPQS), Katunayake to establish the weed culture. Seeds of *Parthenium* were sown in the cage in order to obtain sufficient number of plants continuously and to maintain the cultures of biological control agent, *Puccinia melampodii* for all investigations in this study.

1.2 Culturing and Maintenance of *Puccinia melampodii*

Air dried spores (teliospores) of *P. melampodii* introduced from Australia were used to establish the fungus culture on *P. hysteroporus* plants which were grown in a separate cage covered with a minute nylon mesh at average 24^0 C night and 28^0 C day temperatures. Healthy *Parthenium hysteroporus* plants were inoculated placing infected leaf pieces bearing telia of the fungus over healthy leaves. The fungus was multiplied on *Parthenium* plants through continuous re-inoculation on to new young plants. A sufficient innoculum source was obtained which was air dried and stored under refrigerated conditions to use as the innoculum for laboratory and plant house investigations.

2. Studies with *Puccinia melampodii*

2.1 Pathogenicity of *P. melampodii* on *P. hysteroporus* plants

Four week old *Parthenium* plants were inoculated with 10^4/ml fungal suspension. Observations were made for a period of one month to determine pathogenic effects of fungus on weed plants.

3. Effect of temperature on teliospore germination

The plants inoculated with *P. melampodii* were used to obtain the teliospores for this study. Teliospores were removed from infected leaves of the plants under a microscope. Thirty five plates were inoculated with teliospores at the rate of 100 per plate. There were 7 treatments

based on the temperature at which the plates were maintained in the growth room. They were 5, 10, 15,20,25,30 and 35^{0}C. The treatments were replicated 5 times. Germination of teliospores was observed after 24h and 48h of inoculation and germination ratio was evaluated (as described by Parker,1989).

4. Host specific studies with *Puccinia melampodii*

Pathogenic effect of *Puccinia melampodii* on *Parthenium* with 30 different plant taxa from 14 families available in agricultural eco systems was tested. In this study one or several leaves (depending on the size) of each plant were placed in a Petri dish containing water agar medium with Chloromphinicol as an antibiotic to prevent the medium from contamination by other pathogens. Spore suspension of the fungus was prepared using water and PWS Tween 20 as a surfactant. Leaves in plates were inoculated with a few drops of spore suspension at the rate of 10^4 teliospores/ml of water and covered with the lid of the petri dish. Underside of the petri dish was covered with a moistened filter paper and placed under sterilized laboratory conditions in a growth chamber [20^0 C in the darkness and 25^0 C in the light (3 fluorescent bulbs were lit)] where humidity was 90%. The samples were examined weekly for 3 weeks for the development of the fungus on leaves of test plants.

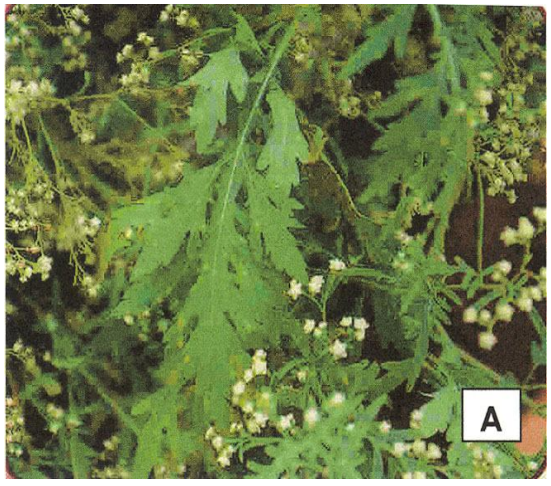
5. Effect of *P. melampodii* infection on different growth stages of *Parthenium*

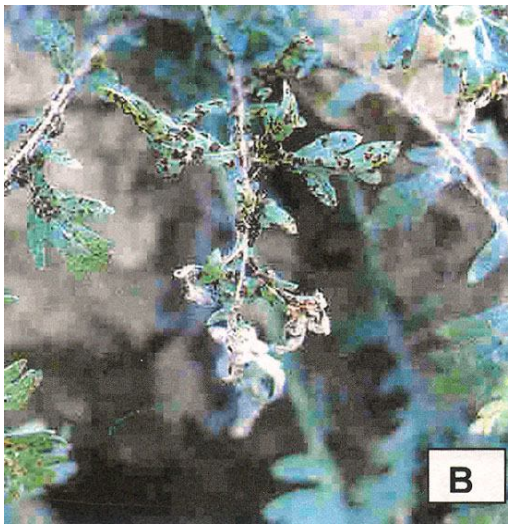
Seeds of *Parthenium* were germinated in 30 pots of 2 litres each containing sterilized soil (a mixture of top soil and compost) at three different intervals to obtain three different growth stages of plants (4 weeks old with average of 6cm height without flowers; 6 weeks old with average of 10 cm height without flowers and 8 weeks old with average of 25 cm height with 1 or 2 flowers). Each plant was inoculated by spraying a suspension containing 10^4 /ml of fungal spores and a surfactant on to the foliage at 27 $\pm 2^0$ C in a plant house. There were three treatments (3 growth stages) with five replicates and five plants from each growth stage were maintained under same conditions without inoculation as control treatments. Inoculated and control plants were maintained for 50 days and records were taken on plant height, number of leaves and number of flowers. Dry weight of each plant was recorded separately after drying in an oven at 55-65^0 C for 3 hours. Data were subjected to the analysis of variance and means were compared using Least Significant Difference Test at $p \geq 0.05$.

RESULTS AND DISCUSSION

Pathogenicity of *P. melampodii* on *P. hysteroporus* plants

Leaves of inoculated plants showed symptoms of chronic leaf spots 3-5 days after inoculation. Telia infection was observed on the lower leaf surface and it was spreading outward from the centre of spots causing yellowing and twisting of leaves 8-9 days after the inoculation (Plate1). Continuous infection of the fungus completely destroyed the leaves with black spots due to necrosis and die back in 30 days after inoculation. The fungus completed its life cycle within 8-10 days at 28^0 C day and 25^0 C night temperatures.





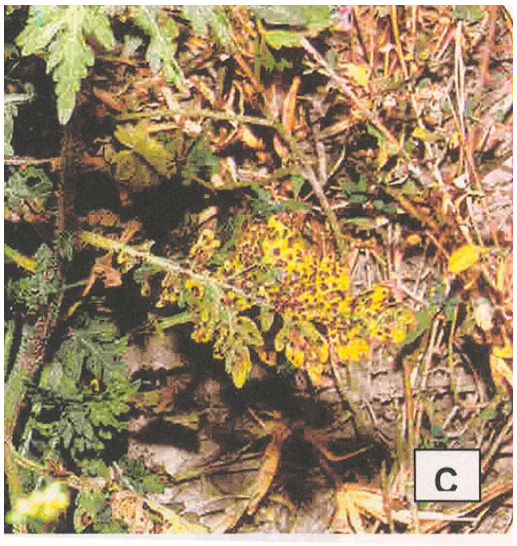


Plate 1. Leaves of *Parthenium* plants showing mild infection (A) after 3-5 days, yellowing and twisted leaves (B) after 8-9 days and yellow necrotic leaves (C) after 15 days of inoculation of *Puccinia melampodii*

These results are in agreement with results obtained by Parker *et al.* (1994). Green house experiment to evaluate the host specificity of *P. melampodii* was conducted using predominantly two *P. melampodii* strains collected from *P. hysteroporus* in Mexico. These strains were proven to be pathogenic and virulent towards the Australian biotype of *P. hysteroporus.* All inoculated plants first develop macroscopic symptoms in the form of leaf chlorosis after 7-9 days, followed by telia formation after another 2-3 days.

Effect of temperature on teliospore germination

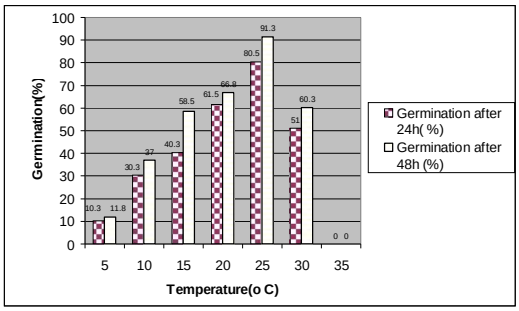


Figure 1- Effect of temperature on the germination on the teliospores of *Puccinia melampodii* at 24 h and 48 h after inoculation

Significantly higher percentage teliospore germination of 91.3 and 80.5% were observed at 25^0 C after 24 and 48 hours of inoculation respectively (Figure 1). Positive relationship was observed between temperature and percentage teliospore germination from 5-25^0 C and optimum temperature for the germination of teliospores was 25^0C at both incubation period 24h and 48h after inoculation. At 35^0C, no teliospore germination was observed.

The results indicate that with respect to teliospore germination, *P. melampodii* is well suited to climates with higher temperatures similar to the conditions of weed infested areas in Sri Lanka.

Host specific studies with *Puccinia melampodii*

Table 1. Susceptibility of different plants to *Puccinia melampodii* infection

Plant type	Family	Infectivity
Alocasia indica (Roxb.) Schott-	Araceae	-
Amaranthus viridis L	Amaranthaceae	-
Ananas comosus(L) Merr.	Bromiliaceae	-
Basella alba L.	Basellaceae	-

Bidens chinensis Willd.	Compositae	-
Cassia auriculata L.	Leguminosae	-
Carica papaya L.	Caricaceae	-
Cichorium intybus L.	Compositae	-
Calocynhtis citrullus	Cucurbitaceae	-
Cyperus rotundus L.	Cyperaceae	
Chrysanthemum spp.	Compositae	-
Dimorphocalyx glabellus Thw.	Euphorbiaceae	-
Dregea volubillis(L.f.) Hook.F.	Asclepiadaceae	-
Emilia sonchifolia (L) DC	Compositae	-
Glycine max (L) Merr.	Leguminosae	-
Gossypium herbaceum L.	Malvaceae	-
Hellianthus annus L	Compositae	-
Ipomoea aquatica Forsk.	Convulaceae	-
Ipomoea batatus(L.) Lam.	Convulaceae	+
Lasia spinosa (L.)	Araceae	-
Oryza sativa L.	Graminae	-
Parthenium hysterophorus L.	Asteraceae	-
Pisum sativum L.	Leguminosae	-
Sorghum nitidum (Vahl) Pers.	Graminae	-
Spillanthes paniculata Wall. Ex. Dc	Compositae	-
Tagetes erecta L.	Compositae	-
Tagetes patula L.	Compositae	-
Xanthium straumarium L.	Compositae	-
Zea mays L.	Graminae	-
Zinnia sp.	Compositae	±*

+ = Fungal infection

- = No microscopic symptoms observed on the development of the fungus

±*= Six days after inoculation signs of bacidiospores or mycelium growth not observed.

of 30 plant taxa tested only *P. hysteroporus* and *Zinnia* sp. were observed as susceptible hosts for the rust infestation (Table 1). Seven days after inoculation, leaves of *P. hysteroporus* became yellowish with young teliospores. Microscopic observations revealed that the fungal growth of leaves of Zinnia spp. was negligible and no signs of bascidiospores or mycelium growth observed six days after inoculation.

According to this study, *P. melampodii* is a microcyclic rust species that does not require an alternate host but completes its reduced life cycle on one host, *P. hysteroporus*.

The degree or extent of infection on non-target species in a field situation may also be of crucial importance. For example, the strain of *P. xanthi* which attacks *Xanthium strumarium* is also known to attack some cultivars of sunflower in the field in Australia (Alcorn and Kochman, 1976). However, there are no records of any commercial damage to sunflower crop.

Effect of *P. melampodii* infection on different growth stages of *Parthenium*

Table 2. Effect of *Puccinia melampodii* inoculated at three different growth stages of *Parthenium* plants after 50 days of inoculation.

Growth parameters	4 week old plants		6 week old plants		8 week old plants	
	Inoculated	Control	Inoculated	Control	Inoculated	Control
Mean plant height(cm)	12.8 b	30.3 b	23.8 b	48.5 a	47.8 b	91.5 a
Mean No. of leaves	12.5 b	22.3 b	15.0 b	25.8 a	20.5 b	30.0 a
Mean number of flowers	0.0 b	2.0 a	0.0 b	4.0 a	1.3 b	19.6 a
Mean dry weight of plant(g)	1.2b	5.5 a	2.9 b	6.1 a	3.5 b	7.5 a

Means with the same letter are not significantly different at p= 0.05

Table 3. Percentage reduction in plant growth of *Parthenium* plants of three different growth stages after 50 days after inoculation with *Puccinia melampodii.*

Plant growth parameters	4 week old plants	6 week old plants	8 week old plants
Plant height (cm) (%)	57.7	50.9	47.8
Mean No. of leaves (%)	43.9	41.9	31.7
Mean No. of flowers (%)	100	100	93.4
Mean Dry Wt. of plant (%)	78.2	52.5	53.3

Compared to the control plants, plants inoculated at 4 and 6 week of age did not produce any flower after 50 days of inoculation. The plant height has reduced as a result of the fungal infection. Number of leaves and flowers and dry weight of inoculated plants were significantly lower than the control plants (Table 2). Parker *et al.,* (1994) have reported similar results in the fields in Mexico where *P. melampodii* has caused the disease incidence and severity on *P. hysteroporus* of up to 50%. Green house experiments showed that repeated inoculation with the rust had a severe impact on *P. hysteroporus*, causing reduction in the number of leaves (ca 30%), in plant height(ca50%) and in root and stem dry weight (70-80%) and, furthermore, it prevented flowering.

This study indicates that infection of *P. melampodii* at any stage of *Parthenium* plant can cause considerable reduction in plant growth especially in the production of leaves and flowers. 50 days after inoculation, reduction in leaf production was 43.9% at 4 weeks, 41.9% at 6 weeks and 31.7% at 8 weeks old plants and reduction in flower production was 100% in plants inoculated at 4 and 6 weeks stage and 93.4% at flowering stage (Table 3). Since flowering and seed production was severely affected by *P. melampodii* spread of the weed would be controlled effectively.

CONCLUSION

Results of all above investigations show that the fungus *P. melampodii* can be used effectively to control growth and spread of *Parthenium* in the country. Mass scale production of this bio control agent can be done in the plant protection centre of the Department of Agriculture for the field

application in future weed management programmes.

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