SELECTED PLANT PATHOGENIC FUNGAL CULTURE FILTRATES (SECONDARY METABOLITES) AS POTENTIAL BIO-HERBICIDES

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Introduction

A weed is considered unwanted plant in a particular situation when it grows in a wrong place. Weeds remain problematic to agriculture, forestry, rural and urban landscaping, transport especially motorways and railway tracks, and fresh water ecosystem except their use in indigenous medicine as medicinal herbs. Weeds can be controlled by manually, mechanically, biologically and chemically. The discovery and introduction of the phenoxyacetic herbicides laid a novel era in the field of agricultural weed control. World consumption of herbicides is 44 % [1].

Herbicides are synthetic chemicals that cause many health issues to human, wild and farm animals. Moreover, application of such toxic chemicals not only may affect ecosystem functions such as productivity and food web interactions, and thus the services aquatic ecosystems provide [2], but also may cause unintentional injury to crop and other non-target vegetation and other organisms in an area by faulty application techniques. Consumers are not willing to by herbicides applied fruits and vegetables. Herbicide usage has resulted in plant evolution and adaptation by the selection of genetic traits conferring phenotypic resistance and allowing weedy plants to survive and reproduce in the presence of herbicides. Weeds have evolved resistance to 23 of the 26 known herbicide sites of action and to 163 different herbicides. Due to the hazardous nature of herbicides (Glyphosate, Atrazine, Metam and etc.), many herbicides have been banned in worldwide. The complexity of these situations has resulted in a need to develop a holistic sustainable eco-friendly weed management programme throughout the farming period. Ecofriendly secondary metabolites like ethylene-inducing peptide 1–like (NLP) proteins from microbes are now being targeted to produce novel herbicides and several successful findings have been reported in recent literatures.

Phytophthora infestans is the causal agent for late blight in potato and *P. infestans* producing NLPs and their mode of action have been identified [3]. *Botrytis cinerea* is also one of the Ascomycete fungi causing disease on crops called gray mold. *Botrytis cinerea* produces a range of cell-wall-degrading enzymes, toxins and other low-molecular-weight compounds such as oxalic acid [4]. *Fusarium solani* is implicated in plant disease as well as human disease. This species can decompose cellulose at an optimal pH of 6.5 and temperature of 30°C. Culture filtrates of *Fusarium solani* can be used to control weeds [5].

According to above information, this investigation was planned to study the potential of using the microbial toxins of *Phytophthora infestans*, *Fusarium solani* and *Botrytis cinerea* as an effective bio herbicide to control weeds.

Materials and Methods

Sample collection and Isolation of test fungi species

All the disease samples were collected (Research Field) and Isolation of *Phytophthora infestans*, *Fusarium Solani*, *Botrytis cinerea* were carried out at the Agriculture Research and Development Centre (National Potato Research Centre) Seetha Eliya, Nuwara Eliya, Sri Lanka located in 7°0'31"N 80°47'39"E.

Preparation of test fungi culture filtrates

Metabolites of *P. infestans* were prepared by inoculating a fungal agar plugs (1 cm^2) from the periphery of the 21 days old culture to pre-autoclaved Pea broth in 250 ml flasks under aseptic conditions. Conical flasks of PDB (Potato Dextrose Broth) were separately inoculated by one-week old *F. solani* culture and two weeks old *B. cinerea* cultures. Each flask was inoculated with the 1 cm^2 size agar plugs which were taken from the periphery of the fungal culture plates. The fungus *P. infestans* inoculated flasks were kept in the water bath shaker at 20 ℃ in 150 rpm for one month in the dark condition. *B. cinerea* and *F. solani* inoculated flasks were kept in Orbital shaker at the room temperature (18 ℃) in 150 rpm for twenty-one days (twelve ours light and twelve hours dark condition). The liquid cultures of filtered through the sterilized nitrocellulose membrane filters of 45 µm pore size by using vacuum condition.

Screening the effect of microbial toxin on selected weeds

Pots of 5 Kg capacity were prepared by using black polythene. Each pot was filled with standard sterilized potting mixture. Matured seeds of most problematic weed species such as *Asclepias verticillata, Rorippa micrantha, Panicum repens*
and *Cyperus rotundus* were collected and dried in the normal room temperature for a week. Dried seeds were planted in pots as four seeds per pot and watered once in two days to maintain at field capacity. Only one species was planted in one pot. Each species was replicated four times for each treatment. Pots were kept under the natural environment. 400 mL of each treatment was prepared (Table 1) and sprayed equally to each replicates of every weed species. Hence, 25 ml of treatment was sprayed on each pot. Glyphosate (isopropylamine salt) 360 g/l SL was used for the treatment preparation. Glyphosate solution was made according to the standard {100-125 ml of Glyphosate should be dissolved in 10 L of water} (DOA, 2009). Treatments was applied in each pot using hand sprayer in an early morning of 6 a.m.

Table 1. Treatments for the pot experiment

Treatment Number	Treatment Materials
A-Treatment 1	Distilled water (Control)
B-Treatment 2	Glyphosate (Standard)
C-Treatment 3	*Fusarium solani* culture filtrate
D-Treatment 4	*Phytophthora infestans* culture filtrate
E-Treatment 5	*Botrytis cinerea* culture filtrate
F-Treatment 6	Mixture of *P. infestans* + *F. solani* + *B. cinerea* culture filtrate

Scoring of leaf yellowing

Before the treatment application, foliage of each plant was 100 % green color. After the treatment application, compared to the plants in the control, the yellow color portion of the foliage of each plant and the integrity of the yellow color of the foliage under each treatment were recorded as a percentage of yellowing from 0 % to 100 % using color chart (Fig. 1)

Figure 1. Visual scale scoring for weeds yellowing

Data collection and statistical analysis

Data of shoot and root growth and percentage of yellowing of part or whole plants by visual scoring were collected. Complete randomized design (CRD) was used to perform analysis of variance (ANOVA) in SAS software version 9.4. Duncan's least significant differences (LSD) test among the treatments were calculated to show the best treatment using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Secondary metabolites of different fungus on different weed species was significantly (P <0.05) varied compared to control. Application of mixture of secondary metabolites (Treatment 6) exhibited tremendous impact and highly significant on weed with the yellowing percentage of 80 % and 60 % on *R. micrantha* (Fig. 3) and *A. verticillata* respectively (Fig. 4). But individually, *F. solani* culture filtrate has given a considerable yellowing effect 40 % and 30 % on same species respectively than other weed species. None of the fungal secondary metabolites as mixture or individually was not significant on *C. rotundus* and the *P. repens* except synthetic chemical Glyphosate. Treatment 2 (Glyphosate) has reached its maximum 100% effect for the broad leaves (Species 1 and Species 2) within 7 days from the application and has taken 14 days to reach about 90% effect for the Sedge (Species 3) and the Grass (Species 4). From this experiment, it is concludable that the amount of application of synthetic nasty herbicide could be reduced if applied by mixing of secondary fungal metabolites as ratio wise fraction. Further experiment is needed to confirm the results.

The mode of action of different secondary metabolites varied. The sensitivity of plants to fungal phytotoxins in the culture filtrate can be assessed by morphological alterations and inhibition of weight increase [6]. The reduced of relative chlorophyll content (SPAD) can due to toxins translocate to the aerial parts. Agrios [7] stated that the toxins produced by pathogenic fungi inhibit enzymes that are directly or indirectly involved in photosynthesis. This inhibition causes chlorophyll degradation and/or the reduction of chlorophyll synthesis, resulting in the development of leaf chlorosis. Selected dicot weeds (Species 1 and Species 2) had a yellowing effect due to the *F. solani* toxin that cause for the chlorosis in leaves.

During growth of *P. infestans,* several hydrolytic enzymes which are interfering with various plant physiological functions including cell wall-degrading activity. Therefore, mixture of the culture filtrates may have broad spectrum (interfering more than one physiological function at a time) of phytotoxic compounds from *Phytophthora infestans*, *Fusarium solani* and *Botrytis cinerea*. So that the plant leaves may get higher chlorosis than the other culture filtrates when applied individually

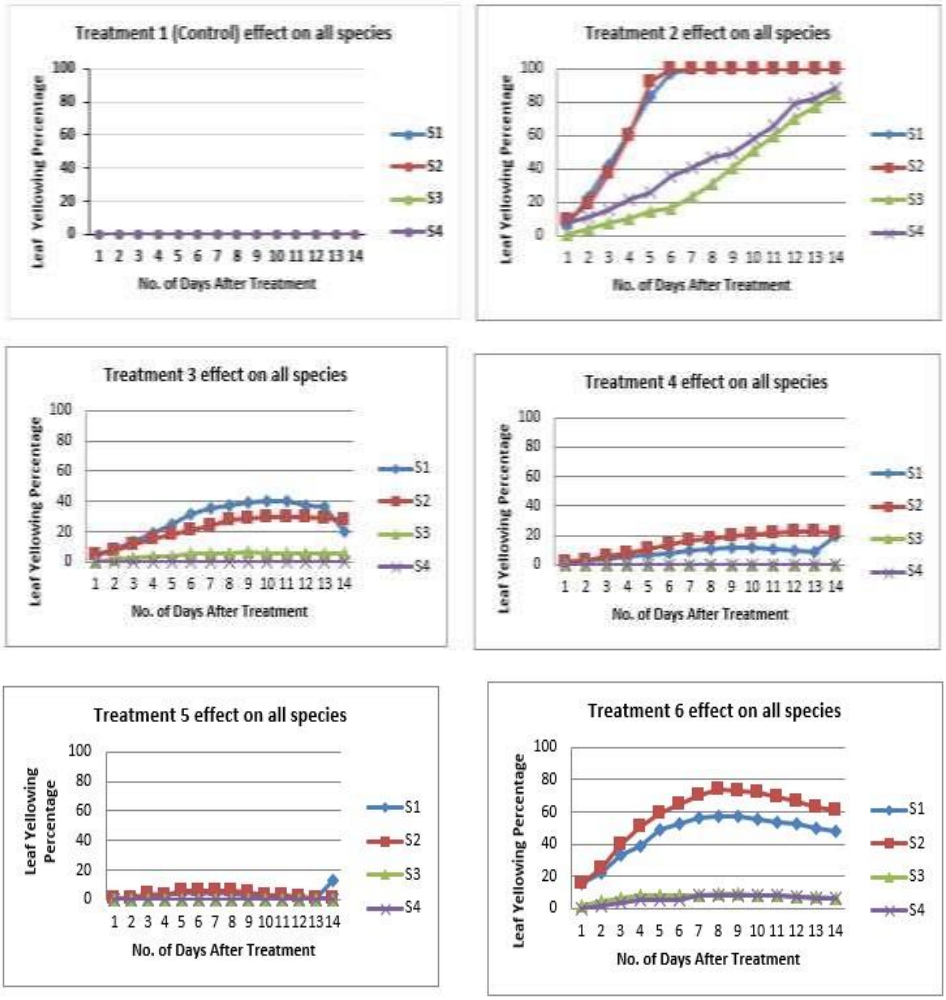

Figure 2. Yellowing effect of fungal culture filtrates on the weeds

Figure 3: *Rorippa micrantha* response to the fungal secondary metabolites (A) control (T1), B-*Fusarium solani* (T3), C- *Phytophthora infestaans* (T4), D- *Botrytis cinerea* and E- Mixture of culture filtrates (T6) after 7 days

Figure 4: *Asclepias verticillata* response to the fungal secondary metabolites (A) control (T1), B-*Fusarium solani* (T3), C- *Phytophthora infestaans* (T4), D- *Botrytis cinerea* and E- Mixture of culture filtrates (T6) after 7 days

Conclusion

In the application of mixture of the cultural filtrates gave the highest yellowing response on all the selected weed species. From this experiment, it is concludable that the amount of application of synthetic nasty herbicide could be reduced if applied by mixing of secondary fungal metabolites and synthetic chemical as ratio wise fraction. Further experiment is needed to confirm the results.

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