Biological Weed Control with Plant Pathogenic Microorganisms.

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Contemporary biological control system includes the use of fungi to control weeds in agricultural ecosystems and forests. Fungal pathogens of weeds that are highly virulent and specific to target weeds, and able to be produced massively by artificial culture could be applied like chemical herbicides over the weeds.

The term mycoherbicide originated in the 1970's to differentiate this strategy from classical strategy of relying upon self-perpetuation introduced organisms for weed control. Interest in bioherbicides is hightened in particular by the increasing costs of chemical herbicides, lack of adequate chemical control for some weeds and the social concerns about the widespread use of pesticides. The approach of mycoherbicide is differed from the classical approach in which plant pathogens are released through natural spread. However, many pathogens have not been successfully used in practise as mycoherbicide to date despite the extensive researches and developments. Of the 80 weed control projects, 71 involved fungi, 6 involved viruses, and 3 each involved bacteria and nematodes. Charudattan added the list 153 mycoherbicide projects recently. One estimated that 30 weeds might be controlled with mycoherbicides by the year 2010, without taking into account the potential genetic engineering and other advances in biotechnology. Developed under such a definition, many mycoherbicides were considered which have shown their potentials in the laboratory or greenhouse, but most of them have been ineffective in the field. In addition, for some mycoherbicide candidates, control efficacy was not consistent from year to year or from field to field. These contradictions indicate lack of understanding of one or more important ecological factors or mechanisms contributing to the suppression of weeds by plant pathogens in the field. It is necessary to understand what are the suitable conditions of temperature and relative humidity to control the target weed with plant pathogen before applying in field. Nevertheless, under field conditions dew formation and its duration are difficult to predict. In the studies of biological control of weed with pathogens, there are some notable examples. Most weed species are hosts for many endemic pathogens, thus, a potential pathogen must be selected as a bio-control agent. Plant pathologist suggested that these pathogens must ; (1) be able to produce abundant and durable inoculum in artificial culture, (2) be genetically stable and specific for the target weed, and (3) be able to infect and kill the weed in environments of reasonable wide latitude. Several techniques have been employed for the delivery of bio-control agents. Formulation and application methods are often of paramount importance in effecting biological control. In addition, formulation may facilitate shipping and storage of the bio-control agent. Reports of the incorporation of mycoherbicides into sodium alginate suggested that this method may have potential for use with bio-control fungi. The resulting granular preparation is lighter than liquids, and more uniform and less bulky than most organic matter preparations. The reaction between aqueous solutions of sodium alginate and certain metal cations such as Ca^{++} to form gels has been used to formulate mycoherbicide.

Control efficacy of a mycoherbicide *Epicoccosorus nematosporus* on *Eleocharis kuroguwai* in field

E. kuroguwai is distributed widely and has caused weed problem in rice production areas in Korea (Hong et al., 1995). *E. kuroguwai* is a perennial sedge that propagates mainly by terminal tuber of rhizome. It is difficult to control the weeds because the tuber over winter in soil and sprouts irregularly. The fungus *E. nematosporus* was found to be involved as an epiphytotic on *E. kuroguwai* in Kyungpook, Sangju region (Hong et al. 1991). *Eleocharis kuroguwai* is distributed widely and has been caused weed problem in rice production area in Korea. This study was conducted to determine the efficacy of the *Epicoccosorus nematosporus* for control of *Eleocharis kuroguwai* and to evaluate the meteorological factors which affect the weeding efficacy in field condition. The best time to control the *E. kuroguwai* with *E. nematosporus* for biological control of field would be during July when the temperatures were 20.4-23.4 ℃ during the surface wetness duration of 12.6 -16.1 h and application time of 6:00 PM and 8:00 PM; weeding efficacy was 81-90%. On June 10 in Milyang area, where the field experiments was performed, mean temperature was 15.6 ℃ with 11.3 h of dew duration, and on Aug 20, the temperature was 21.3℃ with 15.4 h of dew duration. In these periods, the weeding efficacy was recorded at 61.8 and 60.8%, respectively. Time required for complete plant death was 25.8 and 25.6 days at the application times of June 10 and Aug 20. At the time of application of July 7, 18, and 27, mean temperature was 20.4-23.4 ℃ with 12.6-16.5 h of dew duration. The weeding efficacies of these periods were very high with 81.4-90.8 %. Three years of field observations showed that infection in field can occur at any time through the summer season, although total infection rates vary between months and between years. Disease progress was slowly from 24.4 % on 30 June to 49.2 % end of growing season. Results of this studies indicate that *Epicoccosorus nematosporus* has potential as a mycoherbicide for controlling the rice weed *Eleocharis kuroguwai* but has limitted by a meteorological factors .

Effect of the formula of *Epicoccosorus nematosporus* and its weeding efficacy on *Eleocharis kurowai* in field

Although *Epicoccosorus namatosporus* is selected as a potential mycoherbicide agent. But it should not be used effectively in field before resolving the safe bio- carrier of this fungus. So formulation and application methods are often of paramount importance in effecting biological weed control. The purpose of this research was to determine: 1) the cultural condition for melanization and sporulation of alginate pellets; 2) the effect of inert fillers on melanization and conidiation; and 3) the weeding efficacy of the sodium alginate pellet in field. Melanin production of *E. nematosporus* was affected by striking frequency. Percentage of melanized beads was increased to 80.6 % at higher rpm up to 180. The melanized pellets were produced conidia with abundant mucilage after illuminating 4,500 lux of fluorescence light at 28℃ for 48 h. When wheat bran and rice polish were amended and produced the conidia with 65.4 and 68.4 ㎎ per 100 pellets, respectively. When 1 ℓ of conidial suspension of 6.0×10^5 conidia per ㎖ was applied on 30-day-old plants in a plot, 74.5% of the plants were killed within 20 days, whereas its melanized sodium alginate pellets killed 57.8 % of the plants in the same period. Number of tuber formation of *Eleocharis kuroguwai* in the untreated

plots were 128.5, but those of the plots treated with conidial suspension and melanized pellets were 22.1 and 39.7 at the end of the season. Results of this study showed that melanization of mycelia mixed sodium alginate is an important sporulation factor in *E. namatosporus* as a mycoherbicide.

Isolation of host specific fungal isolate YK-201 to bulrush(*Scirpus hotarui* ohwi) and weeding effect of the plants caused by natural infection in paddy field

Brown stem blight of bulrush (*Scirpus hotarui* Ohwi), caused by isolate YK-201(*Alternaria* sp.), were observed at naturally occurred in rice paddy field, is first reported in korea. Typical symptom on stem having watersoaked brown blight were formed and which severely affected the seed malformation. Among the isolated fungi YK201 was the most virulence to bulrush plant in greenhouse test.

The fungus grew well at 25-28C and produced blackpigment on PDA at 15 days. It was the very interesting event that diseased plants were severly suppressed of seed forming caused by this host specific fungal organism. The infected shoots were ultimately killed within 20 to 25 days and severely inhibited seeding. Seeding inhibition rate was up to 85%. The fungus is non-pathogenic to 4 species of *Ghaminaceae* and 6 species of *Cyperaceae* excluding *Scirpus hotarui* . Further host range test is required to determine its potential as a bioherbicide for control of multiful weeds. Therefore, we conclude that the fungus may have a potential as a bilogical control agent of bulrush in rice paddy field

Pathogenicity and host range of a potential mycoherbicide isolate BWC 98-105(*Sclerotium* sp.), causing white root rot on white clover(*Trifoliorum repens*)

White root rot of white clover(*Trifoliorum repens*), caused by isolate BWC98-105, is first reported in Korea. Typical symptom on root having watersoaked brown rot were formed, resulting in complete blight of the top plant parts. The fungus grew well at 20-28 ℃ and produced sclerotia at 10 to 15 days after culture on PDA. Sclerotia were brown to dark brown in color and 1-2㎜ in length. When whiteclover plants were inoculated with mycelial suspension(10^5 cfu/㎖) of isolate YK 101, the plant shoots were killed within 4-6 days and the roots were completely blighted within 10-15 days in the field. The weeding efficacy of the fungus was maintained to next year, leading to a prominent reduction of the reshooting. The fungus was specifically parasite to whiteclover, but not to 5 lawn species under green house test. Therefore, we conclude that the fungus may have a potential as a biological control agent of whiteclover in lawn ground.

Weeding efficacy of phytotoxin extracted fungal isolate BWC98-105 (*Sclerotium* sp.) on the white clover (*Trifolium repens*)

Sclerotium sp.(isolate BWC98-105) causes stem blight, root rot in *Leghum* sp. and is presently being evaluated as potential mycoherbicide for the control of *Trifolium repens*. Bioassays had shown that the Sclerotinia produces phytotoxins biologically active against *T. repens*. Two biologically active compound, designated toxin I and toxin II were produced from culture filtrate of BWC98-105 isolate *Sclerotinia* sp. *in vitro*. The toxin I and toxin II were purified by means of liquid-liquid

extraction and C18 open column chromatography(300X30mm, i.d). To determine the purity, the purified toxin I and toxin II were analyzed by RP-HPLC. The analytical RP-HPLC column was a TOSOH ODS-120T(150×4.6㎜ i.d, Japan), the flow rate was set at 0.7mL/min. using linear gradient solvent system initiated with 15% methanol to 85% methanol in 50 min. with monitoring at 254nm. Under these RP-HPLC conditions, the toxin I and II eluted at 3.49 and 4.13 min. respectively. Toxin II shown to be most potent and host specific. But toxin I had a unique antibiotic activity to phytopathogenic bacteria like bacterial leaf blight on rice, played a less important role in producing disease on *Trifolium repens*. No toxin activity was detected in the water fraction after partitioning with several organic solvent. However, toxin activity was detected in the ethyl acetate and butanol fractions. In the leaf bioassay using toxin I , first appeared within 12 hr as a water soaked which subsequently developed into well-defined blighted leaves.

Evaluation of mycelium virulence of mycoherbicide agent, fungal isolate BWC-101 to *Aeschynomene indica* L.

A. summer annual that grows in the edges of rice paddies, ditches, and moist upland throught Korea. It produces by seed, grows from may to November and flowers in July to September. Brown stem blight of Indian joint-vetch (*Aeschynomene indica* L.) observed at naturally occurred in rice paddy field, is first reported in korea. The fungal isolate BWC-101 was successfully isolated from the diseased stems.

Symptoms first appeared on stems of *A. indica* in June and the lesions rapidly elongated, expanded around the stems, and blighted completely August. Typical symptoms on stem having watersoaked brown lesions were formed and which severely affected the seed malformation and the whole plants blighted.

The fungus BWC-101 was grew well at 25-28℃ , produced abundant aerial mycelial and dark brown sclerotinia on PDA at 15 days. The fungus was grown well in liquid culture media(PD broth) at 28℃ and fully grown within 4 days in 250 ml of flask. In host plant test, highly specific to *A. indica* but some Leguminosae were slightly infected by the pathogen. In pathogenicity test, mycelial mat was the most effective to control the plant of the several kinds of inoculums. Under paddies field condition, mycelial mat of the fungus at the size if 1㎠ gave around 90% of control effect. It was the very interesting event that diseased plants were severely blight of the whole body caused by this host specific fungal organism. Therefore, we conclude that the fungus may have a potential as a biological control agent of *A. indica* in rice paddy field.

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