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Weeding Efficacy of Melanized Formula with *Epicoccosorus nematosporus* on *Eleocharis kuroguwai* in the Field

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The study was conducted to determine the cultural conditions and the effect of inert fillers for melanization and sporulation abilities of sodium alginate pellets, and the weeding efficacy of the formula in the 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 produced more conidia with abundant mucilage than unmelanized pellets. Shaker culture of *Epicoccosorus nematosporus* with sodium alginate yielded a total of 55 mg per 100 pellets. Percentage of melanized pellets was highest, with 81.0% and 83.3% of melanization, when wheat bran and rice polish were amended and produced the conidia with 65.4 and 68.4 mg per 100 pellets, respectively. When 1 L of conidial suspension of 6.0×10^5 conidia per ml 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. The number of tuber formation of *Eleocharis kuroguwai* in the untreated control plots was 128.5 per plot, but those of the plots treated with conidial suspension and melanized pellets were 22.1 and 39.7, respectively, at the end of the season. Results of this study showed that melanization of mycelia-mixed sodium alginate are an important sporulation factor in *E. namatosporus* as a mycoherbicide.

Keywords **:** *Eleocharis kuroguwai, Epicoccosorus nematosporus,* formulation, melanization, weeding efficacy

Eleocharis kuroguwai is distributed widely and has been known to cause weed problem in rice production areas in Korea (Hong et al., 1995). Results of previous studies indicated that *Epicoccosorus nematosporus* has a potential as a mycoherbicide for controlling the rice field weed, *E. kuroguwai* (Hong et al., 1991, 1992). It is necessary to determine the suitable temperature and relative humidity to control the targeted weed before plant pathogen is applied (Hong et al., 2001, 2002a, 2002b, 2002c). The lack of field effectiveness of potential agents has been a common problem in different areas of biological control over the past 20 years. Biological control regulates pest populations by manipulating biological control agents, and control efficacy also depends on environment. To identify factors limiting control efficacy, a clear understanding of the ecological structures and the dynamics of plant pathosystems is needed. Constraints on disease development are those factors that retard or prevent the disease (Agrios, 1978).

Formulation and application methods are often of paramount importance in effective biological control. In addition, formulation may facilitate shipping and storage of the biological control agents. Several techniques have been employed for the delivery of biological control agents. In particular, reports on the incorporation of mycoherbicides into sodium alginate suggested that this method may have potential for use with biological weed control. Boyette and Walker (1986) obtained similarly high levels of control of velvetleaf (*Abutilon theophrasti*) and prickly sida (*Sida spinosa*) with a granular formulation of *Fusarium lateritium* applied at 1,120 kg/ha. The resulting granular preparation is lighter than liquids, and more uniform and less bulky than most organic matter preparations. The use of cheap agricultural products to increase sporulation and to provide higher populations suggests that lower application rates can be used to achieve higher levels of control efficacy. The reaction between aqueous solutions of sodium alginate and certain metal cat ions such as Ca^{++} to form gels has been used to formulate mycoherbicide (Allen et al., 1991). In some plant pathogenic fungi such as *Magnaporthe grisea* and *Colletotrichum lagenarium*, melanization of hypha was reported as an important factor to sporulation (Chida and Sisler, 1987a, 1987b; Kubo et al., 1984; Suzuki et al., 1982).

There have been few reports about the relationship between mycelial melanization and sporulation in *E. nematosporus.* In this study, the relationship between mycelial melanization of *E. namatosporus* and its sporulating

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ability and weeding efficacy was presented through the evaluation of disease severity of fingerprint stem blight pathogens of *E. nematosporus* with different degree of melanization. The purpose of this research was to determine: 1) the cultural conditions 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 pellets in the fields.

Materials and Methods

Formulation procedure. Isolate of YCSJ-112 of *E. nematosporus* from diseased water chestnut in Sangju in 1992 was used for this study. Sodium alginate pellets were prepared using a modified method by Walker and Connick (Walker and Connick, 1983) as shown in Fig. 1.

Cultural condition for melanization and sporulation of alginate pellets. The melanized alginate pellets were produced by placing 100 alginate pellets filled with the mycelia in a 250 ml Erlenmeyer flask with 100 ml distilled water, and cultured for 7 days on a rotary shaker at 28°C adjusted to 50, 100, 140, 180, and 220 rpm. Five flasks were used for each treatment with three replications. Conidial production on the melanized pellets was determined as follows: melanized pellets were uniformly spread on 9-cm-diameter Petri dishes, and placed for 48 hours in a growth chamber at 28°C, RH 60 ± 10% , and 4,500 lux of light intensity. Dry weight of the conidial matrix (mucilage) was measured after washing the sporulated pellets with 20 ml distilled water, and subsequent freeze drying at -40°C for 24 hours using a freeze drier (Model, FD-3-544, FTS Systems Inc. NY).

Effect of inert fillers on melanization and conidiation. Thirty-five grams each of four different media, PD broth, corn meal, wheat bran, and rice bran, in 1% sodium alginate solution (1 l) containing mycelial fragments of 10^5 cfu/ml were dropped into $CaCl_2$ gellant solution to form spherical pellets. One hundred pellets were placed in a 250 ml Erlenmeyer flask with 100 ml sterile distilled water and cultured on a rotary shaker at 150 rpm, 28°C for 7 days. Five flasks were used for each treatment with three replications. Conidial production and dry weight of conidial matrix were determined as described above.

Effect of weeding efficacy according to degree of melanization. Three different degree of melanization of sodium alginate pellets were produced by using the method described above. The degree of melanization was measured based on the color of the pellets: black, brown, and white. Each degree of the melanized pellets

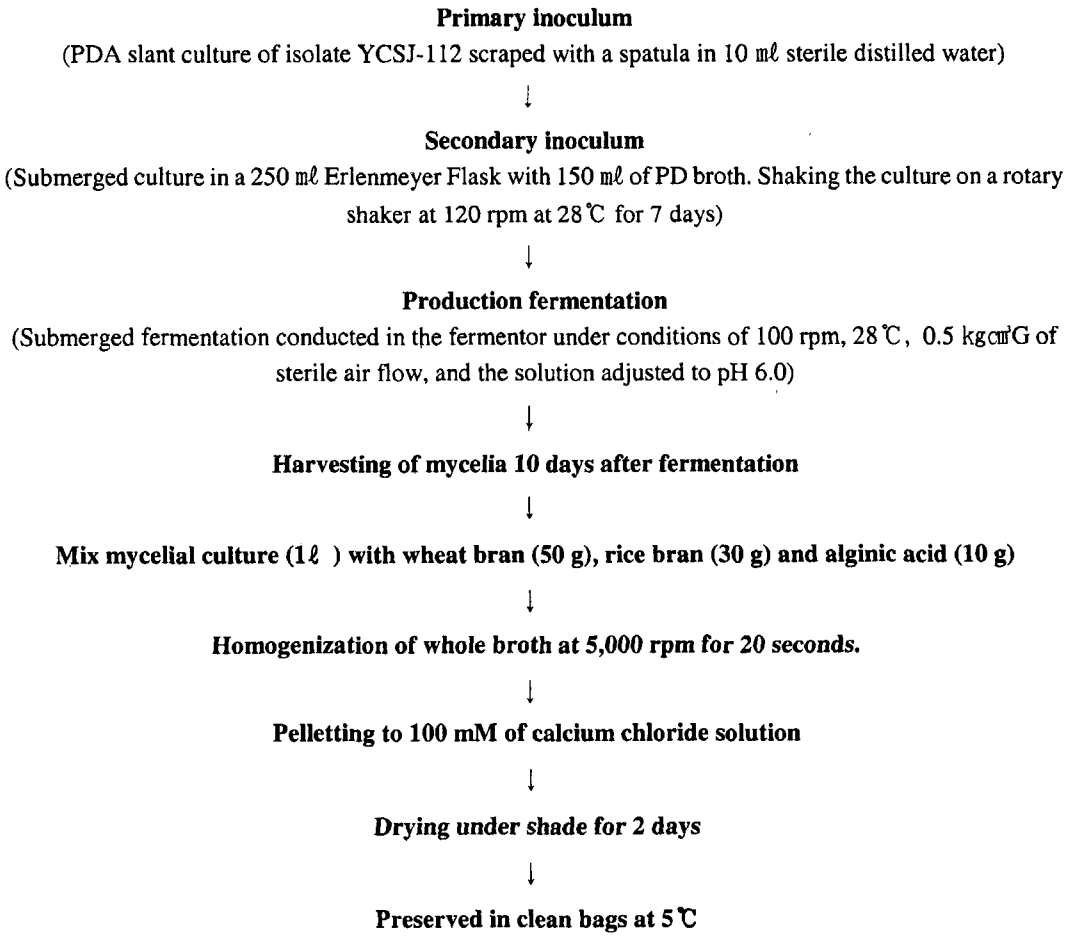

Fig. 1. Simplified process diagram for laboratory scale production of sodium alginate pellets.

was uniformly spread on a 40 × 50 cm glass tray. The tray was placed in a growth chamber at 28°C, RH 60 ± 10%, and 4,500 lux of the light intensity for 48 hours. The 30-day-old water chestnut plants grown in pots were inoculated with 50 g of sporulated alginate pellets per pot. The pots were arranged in a completely randomized design with four replications (= pots). Plant mortality was recorded 30 days after inoculation.

Weeding efficacy of *E. nematosporus* incorporated into the sodium alginate pellet in the field. The sporulated pellets were applied to 30-day-old *E. kuroguwai* plants in field plots at the rate of fresh weight/plot by hand on July 5, 1998. After application, the plots were conditioned as drained state for 5 days. Control plots were spray-inoculated with 1 l of conidial suspension of 6.0×10^5 conidia/ml of *E. nematosporus* in 0.5% dextrose solution. Plant mortality was examined by counting the number of diseased shoots in a plot of 50 cm × 50 cm, 30 days after inoculation.

Statistical analysis. Analysis of variance was done using the ANOVA procedure of Statistical Analysis System (SAS Software Co.). Data were analyzed statistically, and treatment means were separated by Duncan's new multiple range test for significance at $p = 0.05$.

Results and Discussion

Effect of shaking cultural condition on the melanization and conidiation. Percentage of melanized beads was increased at 80.6% with higher rpm up to 180 (Fig. 2). Conidia with abundant mucilage were produced after illuminating 4,500 lux of fluorescence light at 28°C for 48 hours. The average dry weight of conidia with mucilage was about 55 mg/100 pellets at 180-200 rpm. *E. nematosporus* isolate YCSJ-112 showed unusual variation of melanization in mycelia and sporulation among shaking conditions. Since the degree of melanization of *E. nematosporus* is different and the relationship between melanin production and sporulation has not been understood well, this study conducted experiments on the role of melanin for sporulation. Melanized mycelia of *E. nematosporus* produced abundant conidia. Melanization of the mycelia of *E. nematosporus* seemed to be important to produce conidia. It was observed that conidia were produced more from black mycelia than from brownish and whitish mycelia. When melanized, the conidiogenous cells were formed abundantly on the mycelial mats of the pellets. Several authors obtained abundant conidia from melanized mycelia of various fungi on basal media without added nutrients (Boyette and Walker, 1985; Walker, 1981; Walker and Connick, 1983; Weidemann, 1988).

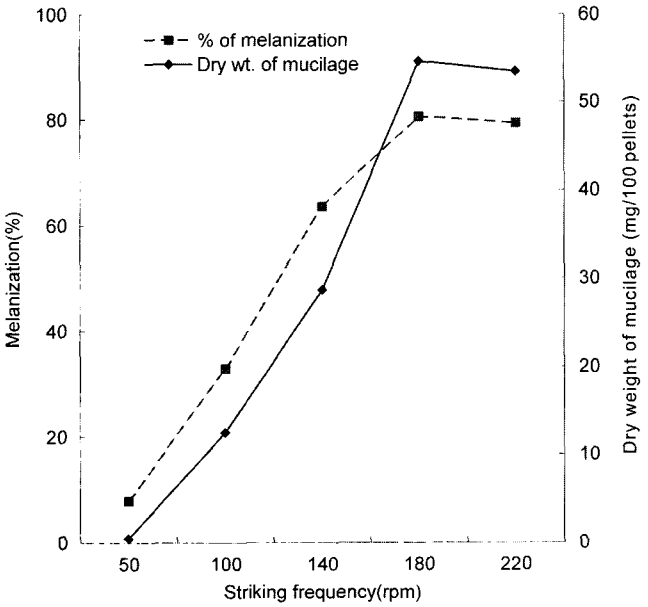

Fig. 2. Effect of the shaking culture condition on melanization and the conidiation ability of the sodium alginate pellets. Percent melanization was measured by percentage of the completely blackened beads. Dry weight of the mucilage was measured by washing sporulated pellets with 20 ml distilled water, freeze-drying at -40°C for 24 hours, and weighing.

Effect of inert filler on the melanization and conidiation. Percentage of melanized beads was high, 81.0% and 83.3%, respectively, when wheat bran and rice polish were used. On the other hand, percentage of melanization and conidial production was low in potato dextrose broth compare with the other media. Conidial production of pellets amended with wheat bran and rice polish were 65.4 and 68.4 mg per 100 pellets, respectively (Table 1). Fully-developed melanized beads produced abundant conidia, and killed over 82% of plants 30 days after their application.

Effect of melanization degree on weeding efficiency. Degree of melanization of the pellets was apparently related with sporulation of *E. nematosporus* and its weeding efficiency (Fig. 3). Completely melanized beads produced conidia, 72 mg more than brownish beads, and 12 mg more when wheat bran and rice polish were amended. Weeding efficiency of melanized pellets was 85% when treated with fully melanized and sporulated pellet, whereas, incomplete

Table 1. Effect of various inert fillers on melanization and conidiation ability of the sodium alginate pellet

Media (35 g/l)	Percent melanization	Dry wt. of mucilage (mg/100 pellets)[x]
PD broth	58.5 c	26.5[y] c
Corn meal	73.5 b	49.7 b
Wheat bran	81.0 a	65.4 a
Rice polish	83.3 a	68.3 a

[x] Dry weight of the mucilage was measured by washing sporulated pellet with 20 ml distilled water, freeze drying at -40°C for 24 hours, and weighing.

[y] Means were separated by Duncan's new multiple range test for significance at $p = 0.05$.

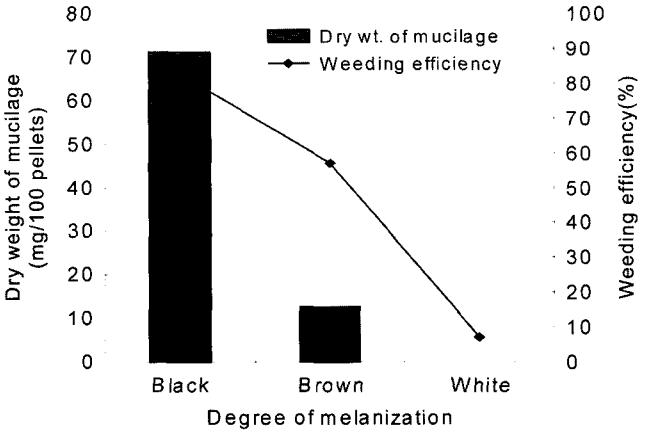

Fig. 3. Effect of the melanized sodium alginate pellets on conidiation and weeding efficiency in the greenhouse. Dry weight of the mucilage was measured by washing sporulated pellets with 20 ml distilled water, freeze-drying at -40°C for 24 hours, and weighing. Plant mortality was recorded 30 days after application.

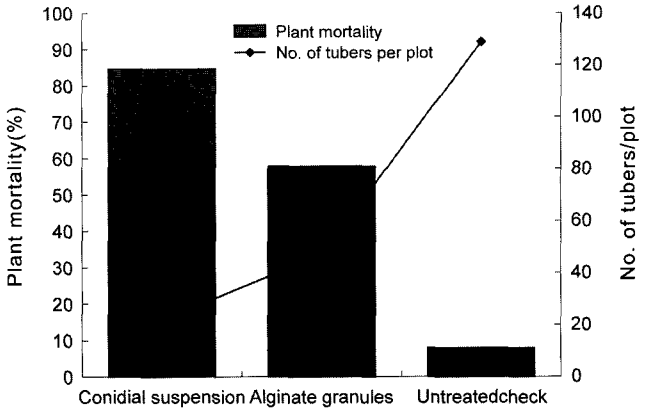

Fig. 4. Comparison of weeding efficacy by sodium alginate pellet application and conidial suspension of *E. nematosporus* in the fields. Tubers were collected from three spots sized 50 × 50 × 30 cm in each plot with four replications. The plant mortality was evaluated 30 days after application.

melanized pellets had less than 60%.

Many fungi has problems because the spore drying technology is not well developed yet (Fravel et al., 1985; Lewis and Papavizas, 1985; Papavizas and Lewis, 1979; Walker and Connick, 1983). In this study, the pellets were generally used within 50-60 days after their production. During the period, the viability of the pellets was only 40-50% when stored at 4°C for 6 months. Despite poor preservation ability at room temperature, sodium alginate granules have several advantages including light weight and uniform particle size that could be applied with existing equipment. In addition, use of cheap agricultural product in the formulation to enhance conidial production could contribute to less application rates to achieve acceptable levels of control over those of non-amended formulations. The production of melanin was shown to be affected by many factors such as temperature, light intensity, and nutrients in sodium alginate pellets. Hyakumachi et al. (1987) speculated that melanin might be correlated with fungal survival, and that lack of melanin results in loss of pathogenicity. In *E. nematosporus*, however, there have been no reports about the effect of melanin on its weeding efficacy to *E. kuroguwai,* except for the relationship between melanin and survival in soil.

Comparison of the weeding efficiency between conidial suspension and melanized alginate pellets. When 1 l of conidial suspension of 6.0×10^5 conidia/ml 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 (Fig. 4). The number of tuber formation of *E. kuroguwai* in the untreated control plots was 128.5 per plot, but those of the plots treated with conidial suspension and melanized pellets were 22.1 and 39.7, respectively, at the end of the season. One of the difficulties encountered in killing weeds is that the weeds have great regeneration potentials as shown in *E. kuroguwai* (Hong et al., 1991, 1992) and bindweed (*Convolvulus arvensis*) (Kim and Kwon, 1985; Ormeno-Nunez et al., 1988; Swan, 1983). To obtain effective control, several factors have to be considered including the development of shoots, reshoots, and underground tuber. Ideally, the shoots must be killed before regeneration occurs. In the fields, *E. kuroguwai* plants form underground tubers initially from early August to late September (Hong et al., 1996, 1997). Control strategy against *E. kuroguwai* has to be established not only to suppress the aerial part of seedlings but also to inhibit underground tubers which will serve as inoculum the following year. Under field conditions, a long period of inoculum is required to obtain satisfactory control. Therefore, long periods of survival in the field are crucial for effective weed control by melanized *E. nematosporus*.

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