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Effect of Selected Pesticides on Survivality of *Exserohilum Longirostratum*, A Bioherbicide for Itchgrass

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ABSTRACT

Four commercial formulations of herbicides and four fungicides were evaluated for their toxic effects on germination of *Exserohilum longirostratum*. At the recommended rates, one of the herbicides recommended for grassy weed control was compatible with *E. longirostratum*. *E. longirostratum* conidia germination was not affected by Paraquat but was totally inhibited by Glufosinate, Glyphosate and Fenoxyaprop-p-ethyl. At lower concentrations, most herbicides were less toxic except Rumpas. The fungicide Benlate at recommended rate has no effect on *E. longirostratum*. Score and Topper reduced germination more than 50% at recommended rate and growth was distorted. Spore germination was totally inhibited at all concentrations with Dithane. Based on high germination and appresorium development, Sapol and Tween 20 is suitable as carrier for this fungus. Paraquat and Bentazone can be used with *E. longirostratum* either with tank mixing or as split applications but at concentration less than the recommended rate.

Keywords: *Exserohilum longirostratum,* herbicide, fungicide, spore, germination, fungi

ABSTRAK

Lima racun rumpai komersial dan empat racun kulat telah dinilai tahap toksiknya terhadap percambahan *Exserohilum longirostratum.* Pada kadar yang dicadangkan pengeluar, satu daripada racun rumpai adalah sesuai digabung dengan *Exserohilum longirostratum.* Percambahan spora *Exserohilum longirostratum* tidak terjejas oleh Paraquat terencat oleh Glufosinate, Glyphosate and Fenoxyaprop-p-ethyl. Pada kepekatan rendah, kebanyakan racun rumpai adalah kurang toksit kecuali Rumpas. Racun kulat Benlate pada kadar yang disyorkan tidak memberikan sebarang kesan terhadap *E. longirostratum.* Score dan Topper mengurangkan percambahan lebih dari 50% pada kadar yang disyorkan dan pertumbuhan pula terbantut. Percambahan spora pula sepenuhnya tidak berjaya pada semua kepakatan racun Dithane NT. Bersandarkan pada percambahan dan pembentukan 'appressorium', Sapol and Tween 20 adalah sesuai sebagai medium bagi kulat ini. Paraquat dan Bentazone boleh digunakan dengan *E. longirostratum* sama ada secara campuran tangki atau secara berasingan tetapi kepekatan haruslah lebih rendah dari yang dicadangkan.

Kata Kunci: *Exserohilum longirostratum,* racun rumpai, racun kulat, spora, percambahan, kulat, spora

INTRODUCTION

Green house studies have demonstrated that the effectiveness of *Exserohilum longirostratum* as a potential bioherbicide for the control of itch grass (*Rottboellia cochinchinensis*) (Ahmad *et al*., 2002). The itchgrass is a noxious and widely distributed annual grassy weed. It poses serious problems in annual and perennial crops in the tropical and subtropical regions all over the world (Alloub *et al*., 2009).

Integrated pest management requires the overall use of crop management systems of which pesticides and bioherbicides can be an integral part. The interactions between pesticides and bioherbicide must be known before a farmer can select the appropriate weed management strategy; presently this information is lacking. Pesticides may adversely affect spore survival and disease development.

Fungicide usage to control various plant diseases may directly affect efficacy of *E. longirostratum* or, alternatively, the few crops that are susceptible to *E. longirostratum* may be protected against this pathogen by using these fungicides which prevent spore germination or disease development. Previous work with *Colletotrichum spp*. has shown that under field conditions, some pesticides can be safely tank mixed or applied sequentially while other cannot. While spore germination provides an estimate of spore survival, it does not provide an accurate indication of disease potential (Kadir *et al*., 2003; Hailmi *et al*., 2011). Appressorial are involved with direct penetration of a host by most plant pathogenic fungi. The mechanism of host penetration by *Exserohilum spp.* has been studied extensively and is mainly affected by toxin production (Kadir *et al*., 2003; Ahmad *et al*., 2002).

Factors that influence appresorial formation include: temperature, nutrients, and hardiness of surface (Hailmi *et al.,* 2011). Tank mixing with pesticides may alter the chemical environment under which spore germination and appresoria formation occur.

The primary purpose of this investigation was to evaluate herbicides, fungicides, and selected adjuvants for their possible toxic effects on vegetative growth, and conidia germination of *E. longirostratum*. *E. longirostratum* is a potential biological agent for controlling itch grass (Kadir *et al*., 2003; Ahmad *et al*., 2002). In other report efficacy of *E. longirostratum* control crowfoot grass, *E. longirostratum* caused more than 80% mortality of *Dactyloctenium aegyptium* (Ninsuwan *et al*., 2011).The pesticides and adjuvants were selected because of their regular use in the most important crop production systems in Malaysia, where itchgrass cause severe yield loss. The objective of this study was to identify the best fungi-commercial herbicide and fungi-commercial fungicide combination in order to reduce the use of chemical herbicide and fungicide.

MATERIAL AND METHODS

Fungal material

The single spore isolate of *E. longirostratum* used in this study was isolated from a naturally infected itchgrass. The infected itchgrass were taken to the laboratory and isolated using the protocol as outlined by Cai *et al.* (2009). For all experiments, fungal inoculum was produced on V-8 juice agar as best media for *Exserohilum* spp. (Hailmi *et al*., 2011).

Pesticides and surfactants

Five herbicides and four fungicides were tested at four concentrations (0.25, 0.5, 0.75, and 1 times the labeled rates (LR), as well as five surfactants on their effect on vegetative growth and spore germination. The experiment was conducted in CRD with 5 replicates for each treatment.

Germination study

Pesticides were prepared as a 10 x LR solution. Test suspensions were prepared then by serial dilution and consisted of a *E. longirostratum* conidia suspension in sterile water combined with appropriate concentration of pesticide, while the surfactant were simply mixed with a *E. longirostratum* conidia suspension The pesticides and the surfactant containing conidia suspensions were allowed to stand for 1 h before 0.5 ml of the test suspensions were spread onto water agar (1.4%). Control treatments include conidia suspension without pesticide or surfactants were included. The proportion of germinating conidia was determined by counting 50 conidia from each of the four replicates. Conidia were considered germinated when the germ tube was half the length of the conidia. The pesticide and surfactant experiments were performed twice.

Radial growth study

The pesticides were added at LR to molten V8 agar after sterilization, but before it was poured into petri plates and allowed to solidify. One 0.75-cm diameter agar disc from a 7 day-old culture of *E. longirostratum* was then placed in the centre of each plate. A control treatment without pesticide was included in each experiment. There are four replicated Petri plates per pesticide tested. Colony diameter was measured every day for seven days after inoculation (Ng *et al*., 2011). Conidia production was assessed 14 days after inoculation by flooding the plates with 10 ml of sterile water. The colony surface was carefully rubbed with rubber spatula and the conidia passed through a layer of cheesecloth. The number of conidia was determined with the aid of a hemacytometer.

Statistical analysis

All experiment was conducted in CRD design. All percentage data were transformed using the arcsine transformation before analysis. Data were analyzed after checking that they confirmed to the assumptions underlying an ANOVA, using the General Linear Models procedure of SAS. Treatments having significant differences were further subjected to the Least Significant Different Tests at *P< 0.005*.

RESULTS AND DISCUSSION

The herbicides tested influenced germination of *E. longirostratum* to various extend. Glyfosinate, Glyfosate, and Fenoxyaprop-p-ethyl were toxic at all concentrations (Figure 1). Germ tubes were stunted and shorter in Glufosinate, Glyphosate and Fenoxyprop-p-ethyl. In contrast, *E. longirostratum* produced normal germ tube and the fungus was compatible with Paraquat and Bentazone at concentration lower than 0.5X (Figure 1).

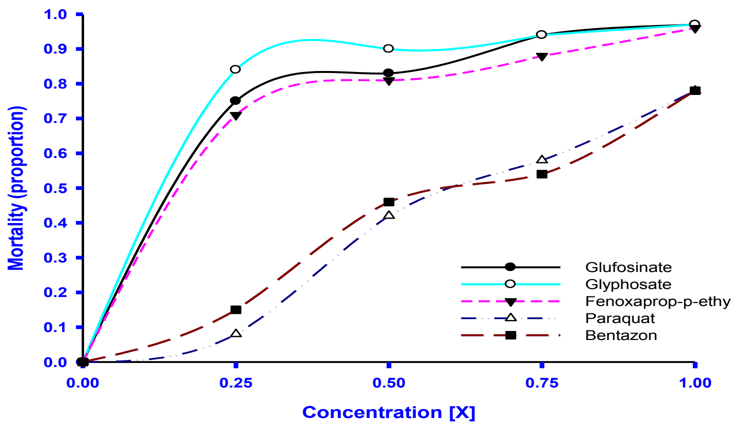

Figure 1 Influence of selected herbicides on conidia germination of *E. longirostratum*

Dithane NT was very toxic to *E. longirostratum* all concentration tested. *E. longirostratum* was able to germinate on Score and Topper but conidia germination were greatly reduced at higher concentration. Conidia germination was significantly higher on Benlate at all concentration (Figure 2). Thicker germ tubes were produced on Topper, Score, compared to Benlate and control.

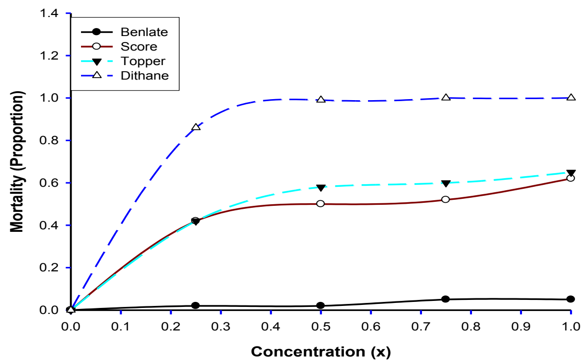

Figure 2 Influence of selected fungicides on conidia germination of *E. longirostratum*.

Germination of conidia under the influence of surfactants differed significantly within experiments. In general, germination of conidia was below 50% of the control at concentrations with Maxigreen, Polypol, and Tween 80. Germination was greater than 50 % with Tween 20 and Sapol at all concentrations tested (Figure 3).

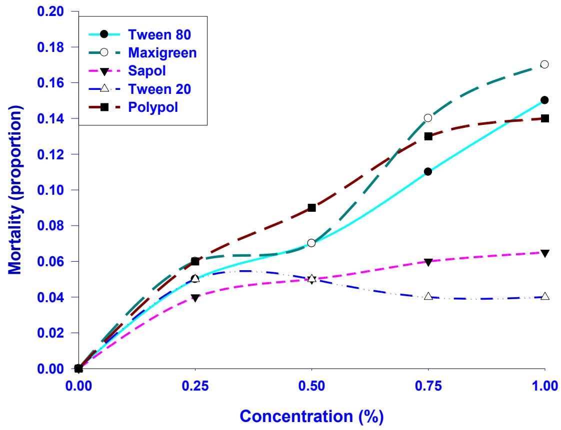

Figure 3 Influence of selected surfactants on conidia germination of *E. longirostratum*

Appressorium formation was higher with Sapol at concentration less than 0.25%, which is within the level used in most bioherbicides (Figure 4). Meanwhile tween 80 shows the lowest appressorium formation.

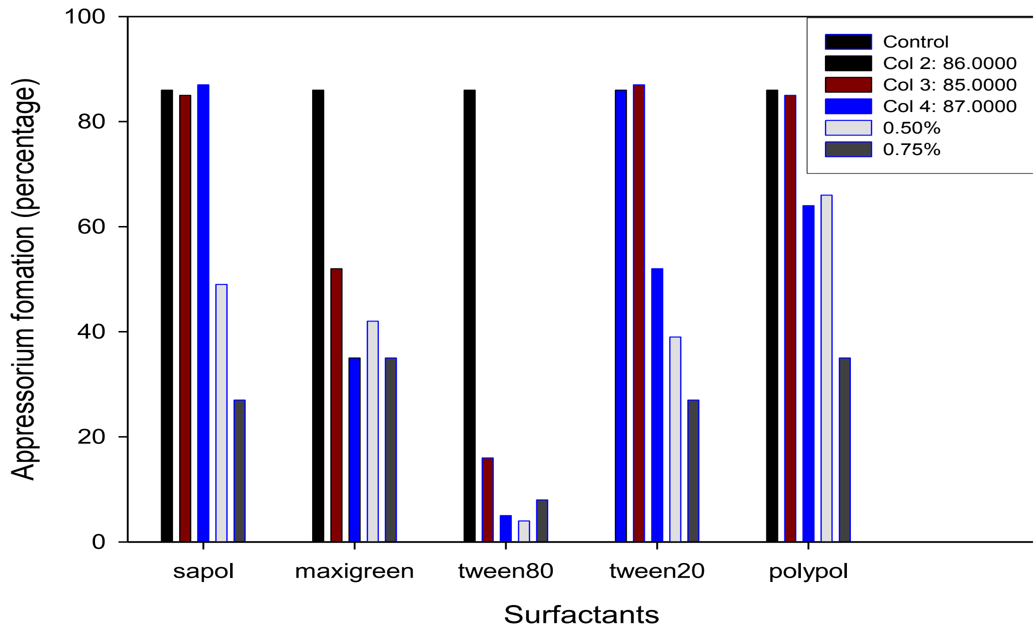

Figure 4 Influence of selected surfactants on appressorium development of *E. longirostratum*

The integration of bioherbicide with chemical pesticides is key to the development of an efficient weed control program. Any bioherbicide, even if used alone, will have to be resistant to, or tolerant of, herbicides and other pesticides used in crop production strategy (Greaves and MacQueen, 1990). Hence it is important to define compatibility of *E. longirostatum* with pesticides as mention in the result of this study.

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