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# Incorporation of Bentazone with *Exserohilum rostratum* for Controlling *Cyperus iria*

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**Abstract: Problem statement:** Various methods are used to counter the population of *Cyperus iria* in rice fields and among the most common method are chemical approaches. Chemical control methods are preferred by rice farmers due to their efficacy, speed, ease of storage and affordable cost. The concern of chemical herbicides is on its effects on the environment. Less attention had been given to biological control methods. Limitations such as host specificity, formulations and storage requirements are some of the obstacles that have to be overcome before implementation. Although it is environmentally friendly, a total shift from chemical control methods to biological seems unreasonable. Therefore incorporation of biological control with reduced chemical herbicides is expected to produce a better and sustainable control of weeds. **Approach:** A study was conducted to determine the potential of using *Exserohilum rostratum* as a biocontrol agent (incorporated with chemical herbicide, bentazone) to control *Cyperus iria*. The biocontrol agent (*E. rostratum*) was confirmed to be pathogenic to *C. iria* by applying Koch's postulates. Preliminary laboratory experiments were carried out to ensure the interactions within the mixture was not inhibitory. **Results:** Bentazone was confirmed to be compatible with *E. rostratum* with an $ED_{50}$ value of 22064570.0 $\mu g_{ai}\ mL^{-1}$. In order to determine the efficacy of the incorporations, several mixtures were tested in a greenhouse environment. It was found that the mixtures of *E. rostratum* ($10^6$ spores $mL^{-1}$) with bentazone (0.4 $kg_{ai}\ ha^{-1}$) was significantly a better control of *Cyperus iria* compared to the usage of bentazone alone (0.4 $kg_{ai}\ ha^{-1}$) and spores alone ($10^6$ spores $mL^{-1}$). It was significantly more effective than the mixture of RBD palm oil (10%), Tween 80 (5%) and spores ($10^6$ spores $mL^{-1}$) which acted as the conventional bioherbicide approach. Synergistic effects were exhibited with 50% less herbicide concentrations from the recommended rates. The mixture exhibited 50% disease severity in 3.5 days compare to bentazone alone which requires 4.9 days and the conventional bioherbicide mixture was relatively less effective requiring 4.7 days. The mixture of spores alone required 7.6 days to achieve 50% disease severity. This suggests that the mixture can save 50% of the cost on bentazone while providing a faster control. **Conclusion:** The incorporations were confirmed to be successful in providing a better and environmentally friendlier method than the conventional methods of weed control.

**Key words:** *Exserohilum rostratum, Cyperus iria,* biological control, bentazone

## INTRODUCTION

Various methods are used to counter the population of *Cyperus iria* and among the most common methods are mechanical and chemical approaches. Mechanical control methods such as cutting, pulling, hoeing and ploughing are commonly used in fields. These methods are laborious if areas are large and the cost of production generally increases. It does not provide uniform and persistent control. The seeds are dispersed easily during mechanical operations causing the greater spread and colonization of weeds.

Chemical control methods are preferred by farmers due to their efficacy, speed, ease of storage and affordable cost. The concern of chemical herbicides is on its effects on the environment. Herbicides commonly used in fields with active ingredients such as bentazone have been reported to be highly soluble in water, risking contamination of groundwater (Kamrin, 1997).

Less attention has been given to biological control methods. Limitations such as host specificity, formulations and storage requirements are some of the obstacles that have to be overcome before implementation. Although it is environmentally friendly, a total shift from chemical control methods to biological seems unreasonable. Therefore incorporation of biological control with reduced chemical herbicides

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is expected to produce a better and sustainable control of weeds (Charudattan *et al*., 2002).

Thus, the objectives of the study were to:

- Determine the compatibility of *Exserohilum rostratum* with bentazone
- Evaluate the potential of incorporating bentazone with *Exserohilum rostratum* for controlling *Cyperus iria*
- Evaluate the effects of incorporating *Exserohilum rostratum* with reduced levels of bentazone on *Cyperus iria*

## MATERIALS AND METHODS

The pathogenicity of *Exserohilum rostratum* towards *Cyperus iria* was confirmed by applying Koch's postulates. Spore suspension ($10^6$ spores $mL^{-1}$) was sprayed onto *Cyperus iria* (2-6 leaf stage) grown from seeds. Infected leaves were surface-sterilized, then placed on a PDA or WA media and incubated for 5 days in room temperature (28±2°C). The conidia and conidiophores were scraped off from these leaves with a transfer needle and transferred onto glass slides for viewing under the microscope. The morphology and dimensions of the conidia and conidiophores were used for comparison with *Exserohilum rostratum* described in the literature. The test was repeated 3 times.

The herbicide selected for the evaluation was Basagran® 43.2% EC (Bentazone, Sodium salt). Bentazone was evaluated at concentrations of 0, 50, 100, 500 and 1000 $\mu g_{ai}\, mL^{-1}$. Bentazone was separately incorporated into PDA in disposable petri dishes. On solidification, each plate was seeded centrally with an 8 mm diameter PDA mycelia plug obtained from the margin of a 7 days old single spore culture. The dishes were then incubated at room temperature (28±2°C) for 7 days. The fungal growth was assessed by measuring colony diameter every 24 h at the right angles and recorded as the mean of two perpendicular radii minus the diameter of the inoculum plug. The colony growth rate was expressed as mm per 24 h by using simple linear regression (Gomez and Gomez, 1984). The $ED_{50}$ values of the above chemicals were determined by Probit Analysis. The experiment was repeated twice.

A volume of 15 ml PDA were poured into petri dishes and added Tween 80 (polyoxyethylene (20) sorbitan monoleate) at concentrations of 10, 5.0, 1.0, 0.5 or 0.1%. Each dish was inoculated with an 8 mm diameter PDA mycelia plug obtained from the margin of a 7-day old single spore culture. Dishes of each treatment were incubated for 7 days at room temperature (28±2°C) in the dark. The colony growth was assessed as described in the section above.

The effect of Tween 80 on conidial germination was evaluated by incorporating Tween 80 directly into 1.5% water agar at concentrations of 10.0, 5.0, 1.0, 0.5 or 0.1% and pH was adjusted to 5.9-6.2 using 1N HCl or NaOH to provide an environment suitable for fungal growth. Agar discs (8 mm diameter) were then plugged with a cork borer and placed onto supporting slides. Drops (10 µL) of each conidial suspension were placed on the discs and spread evenly on the surface using a spatula. The glass slides supporting the agar discs were placed in an incubator at 30°C in the dark for 24 h. After 24 h of incubation, staining with 12.5 µL of LCB was done to arrest germination of conidia. A conidium was considered to have germinated when the length of the germ-tube was greater than its width, or when a sessile appressorium was produced. Several randomly selected fields of view were examined using a compound microscope until a total of 200 conidia had been assessed.

The five types of mixtures formulated for field evaluation were:

- Mixture of fungus ($10^6$ spores $mL^{-1}$) and bentazone ($0.4\ kg_{ai}\ ha^{-1}$)
- Bentazone ($0.4\ kg_{ai}\ ha^{-1}$) alone
- Fungal spores ($10^6$ spores $mL^{-1}$) and RBD palm olein (10%) and Tween 80 (5%)
- Fungal spores ($10^6$ spores $mL^{-1}$) alone
- Control (distilled water)

The experiment was conducted on *Cyperus iria* (4 leaf stage) with 4 replications per treatment. Each replication consisted of 4 sub-samples. A 10 mL of each mixture was sprayed onto the weeds with a calibrated handheld sprayer. Assessments were done every 24 h for 7 days, for disease severity and analyzed by AUDPC to express the efficacy of the mixture. The data were also transformed into logit to enable the determination of disease progress rate and the number of days needed to reach 50% disease severity.

Disease severity was assessed on all plants within the replications by visual estimation of the percentage of diseased (necrotic) leaves. Modifications were done to the disease rating scale developed by (McGinnis *et al*., 1986) to suit the experiment (Table 1).

Area Under Disease Progress Curve (AUDPC) for each treatment was calculated from the disease severity ratings. Disease progress rate was determined by using regression of the transformed disease severity value on time. The number of days to reach 50% disease severity was determined from the linear regression of the disease progress curve in which data had been transformed by logit.

Table 1: Disease severity index

| Disease severity scale | Percentage of damage |
|---|---|
| 0 | 0 |
| 1 | 1-10 |
| 2 | 11-20 |
| 3 | 21-30 |
| 4 | 31-40 |
| 5 | 41-50 |
| 6 | 51-60 |
| 7 | 61-70 |
| 8 | 71-80 |
| 9 | 81-90 |
| 10 | 91-100 |

## RESULTS

The first aspect in the potential determination of a pathogen used as a bioherbicide in controlling a target weed is the degree of susceptibility of the weed to the pathogen. Pathogenicity test were carried out by applying Koch's postulates and *Exserohilum rostratum* were confirmed to be pathogenic to the target weed (*Cyperus iria*). The infected plant showed symptoms of straw colored brown margin with light brown lesions even after series of infection. The infected leaves were surface sterilized and isolations were done to see the presence of the fungus in the subsurface

When tested against *Exserohilum rostratum*, bentazone demonstrated the highest $ED_{50}$ value of 22064570.0 $\mu g_{ai}\, mL^{-1}$ (Table 2). Bentazone with a very high $ED_{50}$ value confirming the chemicals were not inhibitory to the colony growth.

The sticky nature of the spores and formation of clumps resulted in an uneven suspension of spores. In addition, the sedimentation of the spore subjected to gravitational force was also a factor contributing to the mentioned condition. Surfactants can be added to the solution containing spores to amend the condition (Chandramohan and Charudattan, 2000). The fungal colony growth was affected at all levels of Tween 80. The fungal colony growth demonstrated inhibitoriest effect of 51-56% inhibition at levels of 0.5 and 1.0% of Tween 80, respectively. Higher levels such as 5.0 and 10.0% were less inhibitory to the fungal colony growth with 29 and 28% inhibition, respectively. About 13% inhibition was recorded for levels of 0.1% were regarded as less inhibitory to the colony growth (Table 3). Based on the observations, the levels of Tween 80 also affected the discoloration on the fungus at all levels. However the spore germination of *Exserohilum rostratum* was not affected with 100% germination at all levels of Tween 80 (Table 3).

Table 2: Concentrations of selected ai for $ED_{50}$ determination on *Exserohilum rostratum*

| Herbicides | $ED_{50}$ ($\mu g_{ai}\, mL^{-1}$) | $R^2$ |
|---|---|---|
| Bentazone | 22064570 | 0.81 |
| Glufosinate | 18.3 | 0.93 |
| Glyphosate | 29.2 | 0.99 |
| Propanil | 8.2 | 0.80 |
| Trichlopyr | 81.8 | 0.86 |

Table 3: Effect of concentration of Tween 80 on fungal growth and spore germination

| | Concentration (v/v) | | | | |
|---|---|---|---|---|---|
| Percentage of inhibition | 0.1% | 0.5% | 1.0% | 5.0% | 10% |
| Colony growth (%) | 13 | 51 | 56 | 29 | 28 |
| Spore germination (%) | 0 | 0 | 0 | 0 | 0 |

The AUDPC analysis showed that the mixture of bentazone with spores was significantly higher AUDPC value at ($p<0.05$) compared to the other treatments (Table 4). Higher AUDPC value means that the treatment was more efficient in causing damage to the plant. The efficacy maybe explained by the reaction of the chemical compounds with the plant surface which facilitates penetration of fungal appressorium (Chandramohan and Charudattan, 2000).

The steepness of the slope represents the epidemic progress. The disease progress rate of the mixture of bentazone with spores was significantly higher than the other treatments. The disease progressed 0.92 units $day^{-1}$ whereby bentazone alone progressed 0.55 units $day^{-1}$. This represents swiftness of the mixture in controlling *Cyperus iria*. The mixtures of RBD oil, Tween 80 and spores representing conventional bioherbicide formulation had higher AUDPC value than bentazone alone and yet the disease progress rate of the mixture of RBD oil, Tween 80 and spores was less than bentazone alone. This was probably due to the mixture with spores being more damaging than bentazone alone. The infection rate was faster than the rate of damage caused by the herbicide.

By using linear regression of the transformed disease severity value on time, the days required for the mixture to display 50% damage severity was obtained (Table 4). It was estimated, the mixture of bentazone with spores needs 3.5 days ($R^2 = 0.95$) to achieve 50% severity whereas bentazone alone needed 4.9 ($R^2 = 0.92$) days and spores alone needed 7.6 days ($R^2 = 0.95$). Mixture of RBD oil, Tween 80 and spores were not significantly different from bentazone alone with 4.7 days ($R^2 = 0.95$). The damage was permanent and no regrowth was observable on the severely damaged plants even up to 4 weeks after spraying. The control was not affected and remained healthy throughout the experiment.

Table 4: Disease progress development of mixtures towards *Cyperus iria*

| Treatment | AUDPC | Disease progress rate (units day$^{-1}$) | Days to reach 50% severity | $R^2$ |
|---|---|---|---|---|
| Bentazone + spore | 344a | 0.92a | 3.5a | 0.95 |
| Bentazone alone | 228b | 0.55b | 4.9b | 0.92 |
| Oil + Tween 80 + spore | 271c | 0.35c | 4.7b | 0.95 |
| Spore alone | 169d | 0.34c | 7.6c | 0.95 |
| Control | 0e | N/A | N/A | N/A |

Means within column denoted by different alphabets are significantly different at $p<0.05$ according to DNMRT. N/A = Not available

## DISCUSSION

*Exserohilum rostratum* was reisolated even after series of infections. The morphology of the conidia and conidiophores were similar before and after the inoculation as well as in the literature (Zhang *et al*., 2003; Boger and Sandmann, 1989) confirming the pathogenicity.

Therefore, incorporation of biocontrol with chemical control will lessen the dependency on the chemical herbicide. Theoretically, the incorporation of *Exserohilum rostratum* with lower levels of herbicide should be able to improve or provide same levels of control. The results confirm that bentazone was compatible with *Exserohilum rostratum* reflecting its potential to be incorporated with *Exserohilum rostratum* for weed control.

This herbicide formulation was targeted to be used for controlling *Cyperus iria* in rice fields. The safety of the mixture towards rice is a concern. It was reported that bentazone were inactivated by rice and do not possess any threat (Chandramohan and Charudattan, 2000). A host range test was conducted for *Exserohilum rostratum* as biological control agent by. The rice was rated resistant to the fungus according to the study. This suggests that the mixture should be ineffective against rice representing its appropriateness to be used in rice fields.

The potentials of incorporation of bentazone with *Exserohilum rostratum* for controlling *Cyperus iria* were confirmed to be prospective. The fungus *Exserohilum rostratum* ($10^6$ spores mL$^{-1}$) was pathogenic to *Cyperus iria* according to Koch's postulates. The damage was enhanced by the presence of bentazone which was confirmed to be compatible with the fungus ($ED_{50}$ = 22064570.0 $\mu g_{ai}$ mL$^{-1}$). Bentazone was not detrimental to the fungus and synergistic interactions were exhibited in the field experiment. The chemical herbicide (bentazone) facilitated the fungus to penetrate the surface of *Cyperus iria* and speeded the infectivity. This condition hastened the effectiveness of the control. The mixture exhibited 50% disease severity in 3.5 days which was faster than the other mixtures.

There were reports on the control of seven grasses with a mixture of three fungal pathogens, *Drechslera gigantea*, *E.longirostratum* and *Exserohilum rostratum* (Chandramohan and Charudattan, 2000) which broadens the spectrum of weed control. One of the pathogens, *Exserohilum rostratum* was used in this study. The prospect of mixing bentazone into other fungal mixtures to speed up the control and even broaden the target range can be considered as long as it is confirmed to be compatible.

## CONCLUSION

According to the AUDPC analysis, Bentazone alone (0.4 $kg_{ai}$ ha$^{-1}$) achieved an AUDPC value of 288 and spores alone ($10^6$ spores mL$^{-1}$) was recorded at 169. The efficacy of the mixture of bentazone with spores was 19% more effective than bentazone alone and 104% more effective than spores alone with an AUDPC value of 344. The mixture provided significantly better control than the conventional methods. The dosage used was 0.4 $kg_{ai}$ ha$^{-1}$ which was 50% less than the recommended dosage (0.8 $kg_{ai}$ ha$^{-1}$ for young weeds). This also suggests that this mixture may save 50% on the cost of chemical herbicides leading to higher profitability.

Bioherbicides generally are target specific. The potential of mixing bentazone into other fungal mixture to speed up the control and even broaden the range can be considered. Although the results seem promising, further work is needed to develop this incorporation for mass production, shelf-life extensions, storage requirements, safety and market acceptance. These aspects of work have to be answered if the technique was to be implemented.

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