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RESIDUAL EFFECT OF IMIDAZOLINONE HERBICIDE USED IN CLEARFIELD® RICE ON NON-CLEARFIELD RICE

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Abstract

A pot experiment was conducted in the glasshouse at Field 2, Universiti Putra Malaysia, Selangor to study the residual effect of imidazolinone herbicide used in Clearfield® rice which is resistant to imidazolinone on non-Clearfield rice. The experiment comprising of two factorials and was laid out in Randomized Completely Block Design (RCBD) with four replications. Treatments included six cultivated rice varieties, namely MR219, MR220, MR253, MR263, MR269 and MR220CL2 and six different time of combinations such as 3, 6, 9, 12, 15, 18 weeks of direct seeding after imidazolinone herbicide application and control (no imidazolinone herbicide application). Before direct seeding of rice, all pots were sprayed with imidazolinone according to the treatments (0.22 kgOnDuty™ WG Herbicide/ha). Imidazolinone herbicide application stunted rice emergence and growth for non-clearfield rice. Imidazolinone herbicide had significant effect on emergence and growth of non-Clearfield rice compared to control (untreated) except Clearfield® rice (MR220CL2) because it is already tolerant to imidazolinone herbicide. Applied herbicide significantly reduced the percentage of the germination, dry weight of shoot and root and number of tillers of non-Clearfield rice. Non-Clearfield rice showed chlorosis and necrotic symptom on leaves and finally died. Imidazolinone herbicide also had residual effect in soil even though 18 weeks after application. This is very harmful not only for environment but also for the biodiversity of all beneficial organisms.

Introduction

Rice is the third most important crop in Malaysia, grown mainly in eight granaries in Peninsular Malaysia covering an area of about 205,548 ha (Azmi and Baki 1995). Weed is a serious pest for rice causing annual yield loss by 15 - 21% worldwide (Hakim *et al.* 2011). The most serious problem found in paddy cultivation fields are caused by weed interference, especially weedy rice or red rice known as 'padiangin' in Malaysia.

In Malaysia for the effective management of weeds especially weedy rice (*Oryza sativa* L. complex) in direct-seeding is being done by using herbicide tolerant rice cultivars through the Clearfield® rice production system (Azmi *et al.* 2012). The Clearfield Production System (CPS) is the combination of using imidazolinone tolerant varieties MR220CL1 and MR220CL2 with imidazolinone herbicides, which can reduce weedy rice and other weeds, thus increase rice yield and reduce weed management cost for the farmers.

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There are two types of active ingredients containing in imidazolinone herbicides which are imazapic (52.5% w/w) and imazapyr (17.5% w/w). This chemical inhibits the acetohydroxy acid synthase in plant. Herbicide kills plant very slowly and the first symptom appears in the meristematic tissue followed by slow necrosis of the mature tissue. Imidazolinone herbicide can control a broad range of weeds and having a residual effect.

There are many benefits by using Clearfield® rice in order to overcome weedy rice but on the other hand, can cause abundant harmful effects to human, domestic animal, crop and also environment. Herbicides used in Clearfield® rice are highly toxic to human and animals even the field residues caused detrimental effect to other crops and also domestic animals. Before applying this herbicide in the field, the farmers should make sure that drift of herbicides do not enter to other cultivated rice and notify for those who did not use Clearfield® rice. However, the objectives of this research were to determine the effects of herbicide residue of imidazolinone on cultivated rice and to evaluate the response of cultivated rice on crop residue and to know how long imidazolinone herbicide is retained in soil.

Materials and Methods

A pot (24 cm × 23 cm) experiment was conducted in glasshouse, Field 2, Universiti Putra Malaysia, during the period of May to October, 2013. During the experimental period the temperature ranged between 28 and 34°C and the relative humidity was 70%. Five Malaysian local rice varieties, namely MR219, MR220, MR253, MR263 and MR269 and one Clearfield® rice variety MR220CL2 which represented as tolerance to imidazolinone herbicides. The factorial combination of treatments was laid out in a Randomized Complete Block Design (RCBD) with four replications. Experimental soil was collected from the rice field of Tanjung Karang, Selangor. Analyzed soil pH was 5.68. Well prepared 8 kg of soil was poured into each pot. The rice seeds were soaked into water for 24 hrs followed by incubation for 36 to 48 hrs to allow sprouting. After that 20 pre-germinated seeds were first direct seeded in each pot. The continuing direct seeding was followed for the week at 6, 9, 12, 15 and 18.

Imidazolinones were mixed with surfactant and recommended rate of herbicide was sprayed in the pots by knapsack sprayer (Table 1). The calibration was done before imidazolinone application. Pots were divided into 6 groups where each group consisted of 48 pots and represented the time of direct seeding of pre-germinated rice seeds. Among 48 pots, 24 were treated with imidazolinone herbicide and 24 were untreated. Pre-germinated seeds were planted at 3, 6, 9, 12, 15, and 18 weeks after imidazolinone treatments.

Table 1. Recommended rate for On Duty™ WG herbicide and surfactant.

Item	Recommended rate
OnDuty herbicide (Imidazolinone) Active ingredients : Imazapic(52.5%) & Imazapyr (17.5%) w/w	220 g/ha/200 l water
Surfactant	30 ml Tenagam in 20 liter water

The irrigation was applied manually using tap water from hose pipe; one time in every two days intervals from the beginning of germination. After two weeks of establishment, water was applied daily for 2 - 3 cm from the base on soil surface. NPK green fertilizer (15 : 15 : 15) at the rate of 160 kg/ha was applied during final soil preparation. Fertilizer was top dressed at 15 days after direct seeding. Standard agronomic practices were adopted and crop protection measures were carried out as and when necessary.

Data on germination percentage was recorded after 4 weeks. Average tiller numbers were counted and recorded during harvesting time. Shoot and root samples were then oven dried to a constant weight at 70^{o}C for 72 hrs and their weights were recorded. The data were analyzed with ANOVA procedure and the mean comparison was carried out using Tukey's Multiple Range test from Statistical Analysis System software (SAS, version 9.3).

Results and Discussion

The analysis results for percentage of seed germination showed significant ($p \leq 0.05$) differences between herbicide treated rice seedling with non-treated (control) rice seedlings in all six different time of direct seeding (Table 2). Germination percentage of all rice varieties except MR220CL2 (herbicide resistant Clearfield® rice) were significantly affected by the residual effect of imidazolinone in all different times of direct seeding. Imidazolinone herbicide treated rice seed germination ranged between 0.0 and 5.0%.The highest value (5.0%) was in MR253 and MR269 at 18 and 15 WAT, respectively and the lowest (0.0%) in all vareities at 3 and 6 WAT except Clearfield® rice, while the germination percentage of untreated rice seedling (control) and MR220CL2 (Clearfield® rice) ranged from 92.5 to100% (Table 2).

Table 2. Effect of imidazolinone residue on germination percentage of different rice varieties seedlings at 3, 6, 9, 12, 15 and 18 weeks after treatment application.

Variety	Different time of direct seeding (Week after herbicide treatment)					
	3WAT	6WAT	9WAT	12WAT	15WAT	18WAT
No herbicide						
MR219	93.75a	97.5a	100a	96.25a	100a	98.75a
MR220	98.75a	98.75a	98.75a	98.75a	97.5a	98.75a
MR253	90a	96.25a	96.25a	98.75a	97.5a	96.25a
MR263	100a	96.25a	96.25a	100a	100a	100a
MR269	97.5a	97.5a	96.25a	96.25a	97.5a	100a
MR220CL2	100a	100a	96.25a	97.5a	100a	100a
With herbicide						
MR219	0b	0b	2.5b	2.5b	3.75b	2.5b
MR220	0b	0b	1.25b	1.25b	3.75b	2.5b
MR253	0b	0b	1.25b	2.5b	1.25b	5b
MR263	0b	0b	1.25b	1.25b	1.25b	3.75b
MR269	0b	0b	2.5b	2.5b	5b	3.75b
MR220CL2	92.5a	96.25a	96.25a	96.25a	96.25a	96.25a

Means within column with the same letters are not significantly different at $p < 0.05$ in Tukey's Studentized Range (HSD) Test.

Germination percentage of treated rice seedling except MR220CL2 was seriously reduced and delayed by the imidazolinone residues in the soil. So, non-Clearfield rice varieties are found very sensitive to imidazolinone herbicide. The active ingredients (imazapyr and imazapic) in this herbicide negetatively impacted to germinate the rice seed. Present findings were found consistent with the findings of Pannaci *et al*. (2006). They reported that one of the familiar vegetable spinach showed very sensitive to imazamox soil residues.

The dry weight of shoots for all rice varieties except MR220CL2 (with herbicide) were aslo seriously affected by the residual effect of imidazolinone in all different time of direct seeding (Table 3). The dry weight of shoot for herbicide treated rice seedling ranged between 0.02 and 0.11 g, while the dry weight of shoot for untreated rice seedling (control) and MR220CL2 (with herbicide) ranged between 0.99 and 4.48 g (Table 3).

Table 3. Effect of imidazolinone residue on dry weight of shoot for different rice varieties seedlings at 3, 6, 9, 12, 15 and 18 weeks after treatments.

Variety	Different time of direct seeding (Week after herbicide treatment)					
	3WAT	6WAT	9WAT	12WAT	15WAT	18WAT
No herbicide	gm					
MR219	1.32bc	1.59a	1.85a	3.78a	1.5c	3.94a
MR220	1.95a	1.35ab	1.85a	2.64b	1.67c	2.85a
MR253	1.24bc	1.32ab	2.04a	4.48a	1.6c	3.74a
MR263	1.42b	1.35ab	2.06a	4.23a	2.08bc	4.09a
MR269	1.16bc	1.19b	2.26a	3.96a	2.09bc	4.00a
MR220CL2	0.99c	1.36ab	1.76a	3.78a	2.76ab	3.41a
With herbicide						
MR219	0.04d	0.04c	0.04b	0.05c	0.06d	0.06b
MR220	0.02d	0.04c	0.04b	0.05c	0.06d	0.05b
MR253	0.02d	0.04c	0.04b	0.05c	0.08d	0.05b
MR263	0.04d	0.05c	0.05b	0.05c	0.11d	0.07b
MR269	0.04d	0.05c	0.07b	0.06c	0.08d	0.04b
MR220CL2	1.33bc	1.44ab	1.95a	3.77a	3.21a	3.38a

Means within column with the same letters are not significantly different at $p < 0.05$ in Tukey's Studentized Range (HSD) Test.

The shoot dry weight of treated rice seedlings contained the lowest amount as compared to untreated rice seedlings and MR220CL2. The imidazolinone herbicides translocated in both the xylem and phloem to the site of action at the growing points but the residue of these herbicides stunted the growth of treated rice plant and finally reduced the dry weight of rice shoot (Fig. 1). The observed residual effect of imidazolinone herbicide to dry matter production of treated rice seedlings indicates the disturbance of photosynthetic ability which has also been reported by Heiser (2007).

Highly significant reduction in root dry weight was also observed for herbicide treated rice seedling ranging between 0.02 and 0.04 g, while the dry weight of root for untreated rice seedling (control) and MR220CL2 (with herbicide) ranged between 0.21 and 3.54 g (Table 4).

In soil roots are in direct contact with imidazolinone herbicide. Thus, root growth of treated rice seedling in this experiment was severely inhibited by imidazolinone residue. This herbicide is responsible for the blocking of the channel of important enzyme which substrates the access of active site. It may be probable that residue of imidazolinone affected final cell size as well as rate of cell production and thereby resulting in reduced root dry weight. Root pruning and dark color was observed due to residual effect of imidazolinone. The symptom is in conformity with the report by Ferrell *et al.* (2004).

The number of tillers of all rice varieties except MR220CL2 (with herbicide) were seriously affected by residual effect of imidazolinone in all different time of direct seeding (Table 5). The number of tillers for untreated rice seedling (control) and MR220CL2 (with herbicide) ranged between 1.0-2.0 but for herbicide treated rice seedling did not able to produce any tiller. The rice

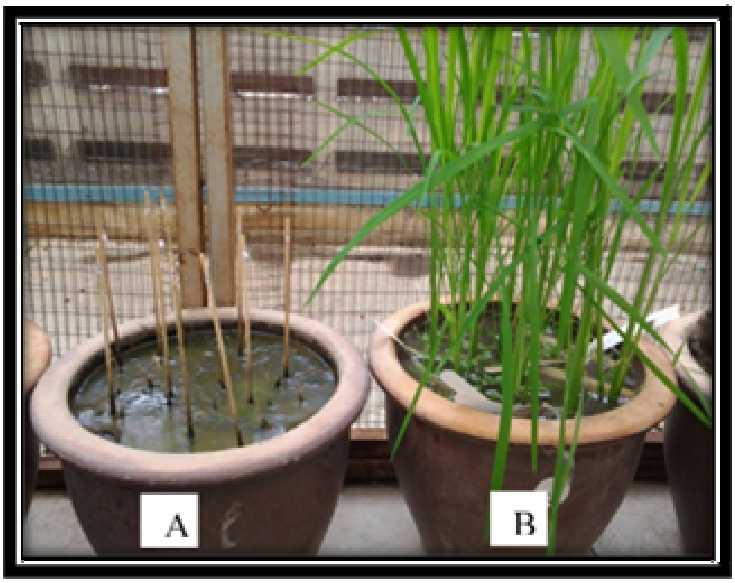

Fig. 1. Stunted symptom of treated pot (A) compared to control (B).

Table 4. Effect of imidazolinone residue on dry weight of root for different rice varieties seedlings at 3, 6, 9, 12, 15 and 18 weeks after treatments.

Variety	Different time of direct seeding (Week after herbicide treatment)					
	3WAT	6WAT	9WAT	12WAT	15WAT	18WAT
No herbicide			gm			
MR219	0.66a	0.34a	0.22a	0.81a	0.17bc	3.54a
MR220	0.66a	0.28ab	0.22a	0.54b	0.22b	1.88ab
MR253	0.60ab	0.24ab	0.21a	0.72ab	0.2b	3.34a
MR263	0.65a	0.28ab	0.20a	0.67ab	0.23b	2.37a
MR269	0.58ab	0.23ab	0.21a	0.55b	0.21b	2.54a
MR220CL2	0.61ab	0.22ab	0.17a	0.73ab	0.28b	2.17ab
With herbicide						
MR219	0.04c	0.03c	0.02b	0.02c	0.03cd	0.03b
MR220	0.06c	0.02c	0.02b	0.03c	0.02d	0.04b
MR253	0.03c	0.03c	0.03b	0.02c	0.04cd	0.04b
MR263	0.03c	0.03c	0.03b	0.02c	0.05cd	0.04b
MR269	0.03c	0.03c	0.04b	0.03c	0.04cd	0.02b
MR220CL2	0.41b	0.21b	0.22a	0.31b	0.43a	1.5ab

Means within column with the same letters are not significantly different at $p < 0.05$ in Tukey's Studentized Range (HSD) Test.

plant normally started to produce tillers at 20 days after sowing. In this experiment, the growth of all the imidazolinone treated rice seedlings were stunted and did not produced any tiller and gradually died at four weeks after sowing. The rice seedlings remained in their vegetative stage for two weeks and no more growth and development were occurred and finally died due to the effect of imidazolinone residue. These results are in accordance with the findings of Braverman *et al.* (1985) who observed that; metolachlor used in rice stunted the growth of rice and the rice remained in its vegetative stage longer that delayed maturity and affected tillering stage.

Table 5. Effect of imidazolinone residue on number of tillers for different rice varieties seedlings at 3, 6, 9, 12, 15 and 18 weeks after treatments.

Variety	Different time of direct seeding (Week after herbicide treatment)					
	3WAT	6WAT	9WAT	12WAT	15WAT	18WAT
No herbicide						
MR219	1.25a	1.25a	1.25a	1.00a	1.25a	1.5a
MR220	1.25a	1.50a	1.25a	1.00a	1.25a	1.25a
MR253	1.25a	1.50a	2.00a	1.00a	1.75a	1.5a
MR263	1.25a	1.25a	2.00a	1.25a	1.00a	1.25a
MR269	1.25a	1.50a	1.25a	1.5a	1.25a	1.00a
MR220CL2	1.25a	1.50a	1.25a	1.25a	1.5a	1.00a
With herbicide						
MR219	0b	0b	0b	0b	0b	0b
MR220	0b	0b	0b	0b	0b	0b
MR253	0b	0b	0b	0b	0b	0b
MR263	0b	0b	0b	0b	0b	0b
MR269	0b	0b	0b	0b	0b	0b
MR220CL2	1.25a	1.25a	1.25a	1.5a	1.75a	1.5a

*Means within column with the same letters are not significantly different at $p < 0.05$ in Tukey's Studentized Range (HSD) Test.

Different time of direct seeded have been evaluated to measure the residual effect of imidazolinone that was used in Clearfield® rice and non-Clearfield rice. Imidazolinone herbicide had significant effect on emergence and growth of non-Clearfield rice compared to untreated (control) except Clearfield® rice (MR220CL2) because it was tolerant to imidazolinone herbicide. The dry weight of shoot and root, and number of tillers were significantly reduced and gradually appeared chlorosis and necrotic symptoms and finally the rice plant died by the effect of imidazolinone. Based on the overall results, it is concluded that the herbicide imidazolinone has residual effect in soil until 18 weeks after application.

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