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Original Research

Response of lowland weeds and direct-seeded lowland rice (*Oryza sativa* L.) to varying herbicide and surfactant dose mixtures

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ABSTRACT

Herbicide efficacy could be enhanced by herbicide combinations and addition of surfactants. These substances improve absorption of herbicides by lowering the surface tension of spray droplets resulting in increased area of contact of the herbicide solution with the leaf surface. This experiment determined the tolerance of rice and efficacy of three post-emergence herbicides in varying combinations with surfactants against *Echinochloa crus-galli* (L.) Beauv., *Cyperus iria* L., and *Ludwigia octovalvis* (Jacq) Raven. The study was conducted at the Cambodian Agricultural Research and Development Institute (CARDI), in Cambodia from January to February 2019. Rice at 10 days after herbicide application (DAHA) showed some degree of susceptibility to Pyribenzoxim- and Quinclorac 50%+ Pyrazosulfuron-ethyl 7% + Fenoxaprop-P-ethyl 13% pre-mix-surfactant mixture but was fully recovered at 20 DAHA. Generally, rice was more tolerant at the 4-6 than at the 2-3 leaf stage. Phytotoxicity of the herbicides to *Echinochloa crus-galli* depended on the herbicide and surfactant concentration and the stage of growth of the weed at application time. *Cyperus iria* and *Ludwigia octovalvis* were relatively more sensitive to the pre-mix alone and where, in most instances, did not merit addition of surfactant.

Introduction

Significant shifts in weed species composition have occurred after the shift from transplanted to direct-seeded rice (Chauhan, 2012). In Cambodia, 19 weed species were recorded in varied agro-ecosystems and rice management practices. In some areas of Cambodia wherever direct seeding is practiced, grasses like *Echinochloa* spp. and *Leptochloa chinensis* (Linn.) have become more common, and are becoming hard to control in direct-seeded rice. The most common broadleaf

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species are *Melochia corchorifolia* and *Ludwigia octovalvis* while *Fimbristylis miliacea*, *Cyperus iria*, and *Cyperus difformis* are common sedges (Bush et al. 2016; Martin, 2017).

In direct-seeded rice, chemical weed control has become popular and will continue to be so because of labor insufficiency and rising wage rates. Herbicides are considered as alternative to other methods of weed removal with overwhelming importance (Matloob et al. 2015). Several studies have concluded that chemical weed control is possible because it is quick, easy, and economical. In Cambodia, chemical method is almost entirely dependent on post-emergence herbicides and heavily reliant on ACCase and ALS-inhibitor herbicides (Martin, 2017; Rodenburg and Johnson, 2009). The efficacies of these herbicides are increased by combining and adding surfactant in the spray solution. Surfactant, any substances in herbicide formulation or added to the spray tank, enhances herbicide uptake by keeping herbicide spray droplets moist, maintaining the herbicide in solution, and keeping it available for foliar uptake (Ramsey et al. 2005). Mehdizadeh et al. (2020) revealed that addition of adjuvants enhanced herbicide efficacy for weed control. Therefore, research is necessary to determine the efficacy of selected herbicide-surfactant mixtures against three weed species commonly associated with lowland rice in Cambodia.

Materials and Methods

This experiment was conducted in the greenhouse of the Cambodian Agricultural Research and Development Institute (CARDI) in Cambodia during dry season from January to February 2019. *Oryza sativa* CAR 15, short-term rice cultivar, and seeds of *Echinochloa crus-galli* (L.) Beauv., *Cyperus iria* L., and *Ludwigia octovalvis* (Jacq) Raven, which were collected from irrigated lowland sites, were dry-seeded in circular plastic pots.

The experiment was laid out in randomized complete block design (RCBD) with three replications. Seedlings were selected to have 2-3 leaf stage and 4-6 leaf stage of weeds and rice at the time of spraying. Herbicides: Pyribenzoxim at 30 and 22.5 g a.i ha^{-1}, Quinclorac 50%+Pyrazosulfuron -ethyl 7%+Fenoxaprop -P-ethyl 13% pre-mix at 280 and 210 g a.i ha^{-1} and Metsulfuron-methyl 10%+Chlorimuron-ethyl 10% pre-mix at 8 and 6 g a.i ha^{-1}. Surfactants: Alcoholpolyglycol at 0.03 and 0.02%, Alcohol ethoxylate at 0.1 and 0.05%, and Alquil poliglycol eter at 0.05 and 0.02% of the spray solution were admixed with the herbicide. The control treatments were the recommended dose of the herbicide sprayed at the 2-3 and 4-6 leaf stage of plants without surfactants.

Morphological changes of weeds and rice after treatment were recorded and expressed through the modified visual rating system (Frans and Talbert, 1977) ranging from 0 to 100%. The observation concentrated on symptoms such as chlorosis, necrosis, stunting, wilting and curling of leaves, and other herbicide-related injury symptoms recorded at 5-day intervals starting from the fifth day after herbicide application (DAHA).

Weed Control Efficiency (WCE) was computed to analyze the effects of treatments on the weeds (Equation 1) while Percent Survival (PS) (Equation 2) and Percent Tolerance (PT) (Equation 3) were computed to analyze the effects of treatments on rice.

Equation 1: $WCE\ (\%) = \frac{Initial\ number\ of\ seedlings - Number\ of\ surviving\ seedlings}{Initial\ number\ of\ seedlings} x100$

Equation 2: $PS\ (\%) = 100 - \frac{Initial\ number\ of\ seedlings - Number\ of\ surviving\ seedlings}{Initial\ number\ of\ seedlings} x100$

Equation 3: $PT\ (\%) = 100 - \frac{Dry\ weight\ of\ control - Dry\ weight\ of\ treated}{Dry\ weight\ of\ control} x100$

The data were subjected to Analysis Of Variance (ANOVA) and Least Significant Difference (LSD) Test at 0.05 level of significance using Statistix 8.1 Analytical software.

Results and Discussion

Rice Injury

Rice showed some degree of injury to the combinations, initially, at 10 DAHA but fully recovered at 20 DAHA. At 2-3 leaf stage, Pyribenzoxim at 30 g a.i ha^{-1} admixed with surfactants (Alcohol ethoxylate, Alcoholpolyglycol and Alquil poliglycol eter) resulted in less than 80 % tolerance at 10 DAHA. ALS-inhibitor herbicide could suppress growth of rice by reducing biomass 23 to 37% in saturated soil (Bhagirath and David, 2011). Addition of non-ionic surfactants, improved the efficacy of ALS-inhibitor herbicides (Akbar and Hossein, 2016; Bunting et al. 2004).

The recommended dose (280 g a.i ha^{-1}) of Quinclorac 50%+ Pyrazosulfuron-ethyl 7%+ Fenoxaprop-P-ethyl 13% pre-mix with Alquil poliglycol eter caused 20 % reduction in tolerance at 2-3 leaf stage of rice. Quinclorac alone or combined with another herbicide, plus non-ionic surfactant resulted in more than 10% injury to rice at early stage of growth (Michael, 1995; Lo, 1988) while Pyrazosulfuron and Fenoxaprop did not injureed direct-seeded rice (Pal et al. 2012; Yadav et al. 2018; Abraham et al. 2012). Hence, response of rice to Quinclorac 50%+ Pyrazosulfuron-ethyl 7%+ Fenoxaprop-P-ethyl 13% pre-mix might have been due to Quinclorac, a growth regulator herbicide. Safety of Metsulfuron-methyl 10%+Chlorimuron -ethyl 10% pre-mix to direct-seeded rice corroborated with the results of Mahbub et al. (2017) even some injury symptoms were observed. These selective herbicides had some negative effects on rice because of metabolic degradation of active ingredient much more rapidly than weeds (Duke, 2014) and rice has already developed the resistance to different ALS-inhibitor herbicides (Rizzardi et al. 2002).

Pyribenzoxim

No injury symptom was observed on the *Echinochloa crusgalli* treated at 4-6 leaf stage while phytotoxic symptoms on grass treated at 2-3 leaf stage were observed as early as 5 DAHA. Degree of injury was dependent on dose of herbicide and surfactant with increasing dose showing higher level of injury. At 30 g a.i ha^{-1}, Pyribenzoxim resulted complete control of the weed at 20 DAHA when Alcohol ethoxylate at 0.1 % was added in the solution but the other combinations did not completely control the weed (Table 1). Surviving weeds were fully recovered at 15 to 20 DAHA. Bhagirath et al. (2013) also revealed that Pyribenzoxim alone could not provide complete control of barnyard grass. The addition of non-ionic surfactant to the spray solution enhanced almost 50% absorption of ALS-inhibitor than the herbicide alone (Patrick and Stephen, 2009). However, at the older leaf stage (4-6 leaf stage), the grass was already resistant to the herbicide even surfactant was added in the spray solution. Phytotoxic symptoms of *Cyperus iria* showed as early as 5 DAHA and complete control was observed at 10-15 DAHA with all herbicide-surfactant combinations including the herbicide alone applied at the 2-3 leaf stage. The control of rice flatsedge treated at 4-6 leaf stage was also observed as early as 5 DAHA, maximum with poor control at 10 DAHA and no control at 15-20 DAHA.

At 2-3 leaf stage, all herbicide-surfactant combinations injured *Ludwigia octovalvis* as early as 5 DAHA and resulted in complete control at 15 DAHA. Most of the combinations also had complete control of the weed treated at the 4-6 leaf stage except for 75 % of the dose with Alcoholpolyglycol and Alcohol ethoxylate. Pyribenzoxim inhibits branched chain amino acids synthesis or protein metabolism by eliciting lots of disputations (Zhou et al. 2007). Addition of Alcoholpolyglycol, Alcohol ethoxylate, or Alquil poliglycol eter benefited in improving the efficacy of ALS-inhibitor herbicides. At the recommended dose, Pyribenzoxim provided poor control on the grass. This might have been due to some of ALS enzyme genes were resistant to the herbicides (Olofsdotter et al. 2000). Mortimer and Hill (1999) indicated that *Cyperus iria* and *Ludwigia octovalvis* were more susceptible to ALS-inhibitor herbicides than grasses, thus, the resistance of these weeds to the herbicides seldom occurred (André et al. 2013).

Table 1. Weed Control Efficiency (%) of Pyribenzoxim admixed with surfactant sprayed at 2-3 or 4-6 leaf stage on weeds at 20 DAHA

Dose (g a.i ha^{-1})	Leaf Stage	Surfactant	Surfactant dose (%)	*E. crusgalli*	*C. iria*	*L. octovalvis*
30	2-3	Alcoholpolyglycol	0.03	71.1 c	100 a	100 a
			0.02	57.8 de	100 a	100 a
		Alcohol ethoxylate	0.1	100 a	100 a	100 a
			0.05	82.2 b	100 a	100 a
		Alquil poliglycol eter	0.05	57.8 de	100 a	100 a
			0.02	44.4 g	100 a	100 a
	4-6	Alcoholpolyglycol	0.03	0 n	37.4 hijk	100 a
			0.02	0 n	33.3 jkl	100 a
		Alcohol ethoxylate	0.1	0 n	55.6 cd	100 a
			0.05	0 n	51.1 cdef	100 a
		Alquil poliglycol eter	0.05	0 n	44.4 efghi	100 a
			0.02	0 n	35.6 ijkl	100 a
22.5	2-3	Alcoholpolyglycol	0.03	11.1 m	100 a	100 a
			0.02	11.1 m	100 a	100 a
		Alcohol ethoxylate	0.1	51.1 efg	100 a	100 a
			0.05	48.9 fg	100 a	100 a
		Alquil poliglycol eter	0.05	28.9 hi	100 a	100 a
			0.02	24.4 ijk	100 a	100 a
	4-6	Alcoholpolyglycol	0.03	0 n	20 mnop	15.6 d
			0.02	0 n	17.8 nop	15.6 d
		Alcohol ethoxylate	0.1	0 n	35.6 ijkl	44.4 b
			0.05	0 n	11.1 p	22.2 c
		Alquil poliglycol eter	0.05	0 n	17.8 nop	100 a
			0.02	0 n	17.8 nop	100 a
30	2-3	-	-	17.7 klm	100 a	100 a
	4-6	-	-	0 n	17.8 nop	100 a
		LSD (5%)		8	10.5	1.9
		CV (%)		21.1	9.4	1.3

Note: Means within a column with the same letter (s) are not significantly different at 5% level using Least Significant Difference (LSD) Test.

Quinclorac 50%+ Pyrazosulfuron-ethyl 7%+ Fenoxaprop-P-ethyl 13% pre-mix

Phytotoxicity of Quinclorac 50%+Pyrazosulfuron-ethyl 7%+Fenoxaprop-P-ethyl 13% pre-mix in combination with surfactant against *E. crusgalli* was observed as early as 5 DAHA progressing until 20 DAHA on the plants treated at 2-3 leaf stage while some plants recovered. Table 2 shows that the pre-mix alone at 280 g a.i ha^{-1} applied at 4-6 leaf stage did not control barnyard grass and only 35% control at the 2-3 leaf stage at 20 DAHA. Complete control of the grass was achieved when the surfactants were included in the spray solution. Droplet adhesion, spreading, and retention on leaf surfaces are increased by the addition of non-ionic surfactant (Basu et al. 2002). Poor control was obtained when dose of the pre-mix was reduced by 25%, even the surfactant was added. Phytotoxic symptoms on *Cyperus iria* were evident as early as 5 DAHA. The pre-mix alone at

280 g a.i ha^{-1} provided 100% control of *Cyperus iria* at 2-3 leaf stage but only 53% when applied at 4-6 leaf stage. Prefect control was also observed when admixed with any of the surfactants at the full or 75% of the herbicide dose applied at 2-3 leaf stage. Only Alquil poliglycol eter at 0.05% solution concentration provided 100% control when applied at 4-6 leaf stage and 76% at half its dose.

L. octovalvis response to the mixtures was increased from 5 to 20 DAHA. Variations in degree of response and progression depended on the kind and concentration of the surfactant. This broad leaf weed was very sensitive to the pre-mix alone or in combination with any of the surfactants at 2-3 or 4-6 leaf stage at 100% and 75% of the herbicide dose. Thus, addition of the surfactants provided not advantage on herbicide efficacy. Quinclorac 50%+ Pyrazosulfuron-ethyl 7% + Fenoxaprop-P-ethyl 13% pre-mix was composed by three herbicide modes of action, growth regulator, ALS-inhibitor and ACCase inhibitor, respectively. In general, sedges and broadleaf weeds naturally tolerate to ACCase inhibitor herbicides because of a less sensitive ACCase enzyme content. Quinclorac normally offers good efficiency to barnyard grass. However, Quinclorac 50%+ Pyrazosulfuron -ethyl 7% + Fenoxaprop-P-ethyl 13% pre-mix alone did not control barnyard grass because biotypes of this grass might present resistance to Quinclorac and Pyrazosulfuron. Application of ACCase inhibitor herbicides in rice fields has promoted efficient control to the grass (Mariot et al. 2010). *C. iria* and *L. octovalvis* naturally tolerate to Quinclorac and Fenoxaprop (Lo, 1988; Kukorelli et al. 2013), thus, sedge and broad leaf weeds were controlled by Pyrazosulfuron. Non-ionic surfactant plays key role in improving efficacies of several ACCase and ALS-inhibitor herbicides (Devilliers et al. 2001; Sanyal et al. 2006; Bunting et al. 2004).

Metsulfuron-methyl10%+ Chlorimuron -ethyl 10% pre-mix

Early response of *E. crusgalli* to Metsulfuron-methyl 10% + Chlorimuron-ethyl 10% pre-mix was seen at 5 DAHA with recovery from the symptoms at 10 DAHA. The mixtures did not control the grass at 4-6 leaf stage while only 11-33% control was observed on grass treated at the earlier stage (Table 3). Metsulfuron-methyl was known as killer of broad leaf weeds and sedges, thus it was not effective against barnyard grass (Larry et al. 1990; Gopinath and Kundu, 2008). Chlorimuron, grass killer, was frequently tolerated by ALS genes of barnyard grass in paddy field (Juliano et al. 2010).

Similar response of rice flatsedge at 2-3 leaf stage to the herbicide-surfactant combinations was observed. Phytotoxicity was evident as early as 5 DAHA and complete control at 10 DAHA, even the herbicide alone. However, the combinations did not result complete control on the weed treated at 4-6 leaf stage. Chlorimuron-ethyl provided good control of broad leaf weeds and sedges (David, 1996). *Ludwigia octovalvis* treated either at 2-3 or 4-6 leaf stage, complete control was observed with all combinations at both doses of the herbicide. Phytotoxic symptoms occurred as early as 5 DAHA and complete control of the weed sprayed at 2-3 or 4-6 leaf stage was observed at 15 or 20 DAHA. No advantage is obtained by the addition of surfactant at 8 g a.i ha^{-1} of the herbicide.

Table 2. Weed Control Efficiency (%) of Quinclorac 50%+ Pyrazosulfuron-ethyl 7% + Fenoxaprop-P-ethyl 13% pre-mix admixed with surfactant sprayed at 2-3 or 4-6 leaf stage on weeds at 20 DAHA.

Dose (g a.i ha^{-1})	Leaf Stage	Surfactant	Surfactant dose (%)	*E. crusgalli*	*C. iria*	*L. octovalvis*
280	2-3	Alcoholpolyglycol	0.03	100 a	100 a	100 a
			0.02	100 a	100 a	100 a
		Alcohol ethoxylate	0.1	100 a	100 a	100 a
			0.05	100 a	100 a	100 a
		Alquil poliglycol eter	0.05	100 a	100 a	100 a
			0.02	73.3 c	100 a	100 a
	4-6	Alcoholpolyglycol	0.03	0 n	51.1 cdef	100 a
			0.02	0 n	42.2 fghij	100 a
		Alcohol ethoxylate	0.1	0 n	55.6 cd	100 a
			0.05	0 n	48.9 cdefg	100 a
		Alquil poliglycol eter	0.05	0 n	100 a	100 a
			0.02	0 n	75.6 b	100 a
210	2-3	Alcoholpolyglycol	0.03	65.6 cd	100 a	100 a
			0.02	46.7 fg	100 a	100 a
		Alcohol ethoxylate	0.1	62.2 d	100 a	100 a
			0.05	53.3 ef	100 a	100 a
		Alquil poliglycol eter	0.05	33.3 h	100 a	100 a
			0.02	26.7 hij	100 a	100 a
	4-6	Alcoholpolyglycol	0.03	0 n	15.6 op	100 a
			0.02	0 n	11.1 p	100 a
		Alcohol ethoxylate	0.1	0 n	28.9 klm	100 a
			0.05	0 n	22.2 mno	100 a
		Alquil poliglycol eter	0.05	0 n	57.8 c	100 a
			0.02	0 n	55.5 cd	100 a
280	2-3	-	-	34.6 h	100 a	100 a
	4-6	-	-	0 n	53.3 cde	100 a
LSD (5%)				8	10.5	1.9
CV (%)				21.1	9.4	1.3

Note: Means within a column with the same letter (s) are not significantly different at 5% level using Least Significant Difference (LSD) Test.

Table 3. Weed Control Efficiency (%) of Metsulfuron-methyl 10% + Chlorimuron-ethyl 10% pre-mix admixed with surfactant sprayed at the 2-3 or 4-6 leaf stage on weeds at 20 DAHA

Dose (g a.i ha^{-1})	Leaf Stage	Surfactant	Surfactant dose (%)	*E. crusgalli*	*C. iria*	*L. octovalvis*
8	2-3	Alcoholpolyglycol	0.03	33.3 h	100 a	100 a
			0.02	28.9 hi	100 a	100 a
		Alcohol ethoxylate	0.1	33.3 h	100 a	100 a
			0.05	24.4 ijk	100 a	100 a
		Alquil poliglycol eter	0.05	20 jkl	100 a	100 a
			0.02	20 jkl	100 a	100 a
	4-6	Alcoholpolyglycol	0.03	0 n	53.3 cde	100 a
			0.02	0 n	46.8 defg	100 a
		Alcohol ethoxylate	0.1	0 n	42.2 fghij	100 a
			0.05	0 n	33.3 jkl	100 a
		Alquil poliglycol eter	0.05	0 n	42.2 fghij	100 a
			0.02	0 n	37.8 hijk	100 a
6	2-3	Alcoholpolyglycol	0.03	20 jkl	100 a	100 a
			0.02	15.6 lm	100 a	100 a
		Alcohol ethoxylate	0.1	17.8 klm	100 a	100 a
			0.05	13.3 lm	100 a	100 a
		Alquil poliglycol eter	0.05	13.3 lm	100 a	100 a
			0.02	11.1 m	100 a	100 a
	4-6	Alcoholpolyglycol	0.03	0 n	35.6 ijkl	100 a
			0.02	0 n	28.9 klm	100 a
		Alcohol ethoxylate	0.1	0 n	26.7 lmn	100 a
			0.05	0 n	28.9 klm	100 a
		Alquil poliglycol eter	0.05	0 n	40 ghij	100 a
			0.02	0 n	37.8 hijk	100 a
8	2-3	-	-	0 n	100 a	100 a
	4-6	-	-	0 n	40 ghij	100 a
LSD (5%)				8	10.5	1.9
CV (%)				21.1	9.4	1.3

Note: Means within a column with the same letter (s) are not significantly different at 5% level using Least Significant Difference (LSD) Test.

Conclusion

In response to the herbicide-surfactant combinations, rice showed degree of phytotoxicity at the earlier stage of growth but tolerance at the latter growth stage. In some cases, dose of herbicide significantly differed the response of rice, while surfactant dose mostly resulted consistently. In general, addition of surfactant to the herbicide solution had more suppression on rice growth than the herbicide alone applied at 2-3 leaf stage. Type and concentration of the surfactant and growth stage of the weed can affect herbicide performance especially against *E. crusgalli*. The grass at 4-6 leaf stage tolerates to the combinations while degree of control is observed at 2-3 leaf stage of application. Reducing concentrations of herbicide and surfactant decreased control efficiency on barnyard grass. Application of the recommended dose of the herbicides alone at 2-3 leaf stage is enough to control *C. iria* and *L. octovalvis*, thus addition of surfactants provides no advantage on herbicide efficacy but will maintain the efficacy of the herbicide at lower dose. *L. octovalvis* at latter growth stage was also controlled by the herbicide alone.

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Conflicts of Interest

No conflicts of interest have been declared.

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