



## Response of *Isachne* to herbicides using bioassay techniques

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*Isachne miliacea* Roth. locally known as 'Chovverippullu, Naringa, Njammal, and Changanipullu' is one of the dominant weed in the low land rice ecosystems of Kerala. The weed belongs to the family Poaceae. It is seen both in *Kharif* (KAU 1988), and *Rabi* seasons (KAU 1990). Varghese (1996) has reported that *I. miliacea* alone can contribute to 61% reduction in the production of rice in Onattukara region. Presently, the weed is reported to be spreading to other rice growing regions of the state. Currently weed shift has become a common phenomenon in the rice ecosystems of Kerala. This can be attributed to the opportunistic germination, habit, fecundity and competitive ability of the weeds together with the natural resistance of some species to newer herbicides which are more specific in action.

The prostrate nature of *I. miliacea* and its ability to germinate from both seed and stem cuttings contribute to the fast spread of the weed in the rice ecosystems. The weed escapes attention in a mature rice field due to its prostrate nature, information on the sensitivity of the weed to new herbicides is also meagre. Hence a study was conducted to evaluate the sensitivity of *I. miliacea* to pre- and post-emergence herbicides using seed and whole plant bioassay techniques, respectively. According to Blacklow and Pheloung (1991), bioassay tests give practical information on the response of a species to specific herbicides. Zhang *et al.* (2012) used whole plant bioassay techniques with weed species for herbicide dose-response and resistance diagnosis.

The study was conducted during 2014-2015 in College of Horticulture, Vellanikkara, Thrissur to understand the response of *I. miliacea* to common herbicides. The sensitivity of *I. miliacea* to pre-emergence, post-emergence and non-traditional rice herbicides was tested using bioassay technique.

Seed bioassay technique was adopted to test the sensitivity of the weed to pre-emergence herbicides. Three sets of experiments were conducted in Petridishes of area 63.5 cm<sup>2</sup>, using Top of paper method (TP) and Between paper method (BP) as described by ISTA (1996). In the first set, herbicide soaked filter paper was placed at the bottom of Petri plates and seeds were placed on it (TP method). In the second set, the herbicide soaked filter paper was placed on top of the seeds and another water soaked filter paper was placed at the bottom (BP method I). In the third set, seeds were placed in between two filter paper soaked with herbicides (BP method II).

TP method gives the effect of the herbicide on the germinating radicle of the weed, BP method I gives the effect of the herbicides on the germinating plumule. In BP method II effect on both plumule and radicle is obtained.

To test the efficacy of pre- and post-emergence herbicides to *I. miliacea*, the weed was grown in mud pots of diameter 0.18 m. Four pots were arranged to test in an area of 0.16 sq.m and the water required for covering this area was calibrated using a hand sprayer. The quantity of herbicides required to cover 0.16 sq. m area was calculated (Table 1, 2 and 3). The weeds were allowed to spread in the pots after 15 days of spraying. The herbicides were sprayed when the weeds were in the vegetative phase using a hand sprayer. The herbicides were classified as susceptible, moderately resistant and resistant to *I. miliacea* based on phytotoxicity symptoms such as leaf scorching, leaf curling, tip burn, yellowing, dead plants and regrowth.

All the herbicides tested gave excellent control in BP method II where the seeds were placed between two herbicide soaked filter papers. The seed of *I. miliacea* showed zero germination for all the four pre-emergence herbicides tested by this method (Table 4). Pendimethalin was the only herbicide which gave better control in TP method as compared

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**Table 1. The recommended dose and quantity of pre-emergence herbicides used**

Herbicide	Recommended dose (kg/ha)	Quantity used in petriplate (ml)
Butachlor	1.25	0.008
Pretilachlor	0.50-0.75	0.004
Oxyfluorfen	0.15	0.0009
Pendimethalin	1- 1.50	0.008

**Table 2. The recommended dose and quantity of post-emergence herbicides used**

Herbicide	Recommended dose (g/ha)	Quantity used in 0.16 sq.m area (g)
Bispyribac sodium	25	0.0004
Pyrazosulfuron-ethyl	35	0.0005
Azimsulfuron	35	0.0005
Penoxsulam	25	0.0004
Fenoxaprop p-ethyl	60	0.0001

**Table 3. The recommended dose and quantity of non-traditional rice herbicides used**

Herbicide	Recommended dose (kg/ha)	Quantity used in 0.16 sq.m (ml)
Glyphosate	2	0.03
Diuron	3	0.04
Paraquat	3	0.04
Glufosinate ammonium	2	0.03

**Table 4. Seed bioassay using pre-emergence herbicides**

Herbicide	Seed germination percentage		
	Top paper TP I	Between paper BP I	Between paper BP II
Control	47.5 <sup>e</sup>	42.5 <sup>d</sup>	50.0 <sup>b</sup>
Pretilachlor	7.5 <sup>b</sup>	5.0 <sup>b</sup>	0.0 <sup>a</sup>
Oxyfluorfen	10.0 <sup>c</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>
Pendimethalin	22.5 <sup>d</sup>	32.5 <sup>c</sup>	0.0 <sup>a</sup>
Butachlor	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>

Statistical analysis done using Friedman test, The alphabets denote the effectiveness of the herbicide within the column

**Table 5. Whole plant bioassay using pre-emergence herbicides (DAS – Days After Spraying)**

Herbicide	Mode of action	2 DAS	4 DAS	8 DAS	15 DAS
Bispyribac-sodium	Inhibition of ALS	No discolouration	Leaf discolouration	Reddish colour at the tip of leaves, wilting started	Wilted, regrowth
Pyrazosulfuron-ethyl	Inhibition of ALS	No discolouration	No discolouration	Leaf discolouration	Not wilted
Azimsulfuron	Inhibition of ALS	No discolouration	Leaf discolouration	Reddish colour at the tip of leaves, wilting started	Wilted
Penoxsulam	Inhibition of ALS	No discolouration	Leaf scorching	Reddish colour at the tip of leaves, wilting started	Regrowth observed
Cyhalofop-butyl	Inhibition of ACCase	No discolouration	Leaf discolouration	Reddish colour at the tip of leaves, wilting started	Wilted
Fenoxaprop -p-ethyl	Inhibition of ACCase	No discolouration	Leaf discolouration	Reddish colour at the tip of leaves, wilting started	Wilted

[ALS - Aceto Lactate synthase, ACCase - Acetyl CoA Carboxylase]

to BP methods I and II. This may be because pendimethalin affects cell division of the root and is a root inhibitor and it is not translocated by xylem. Oxyfluorfen inhibited seed germination in both BP methods I and II, this may be because the chemical is more sensitive to the plumule than the radicle. The action of pretilachlor was similar to oxyfluorfen but less effective. Butachlor gave the best result in all the three methods indicating that both the germinating radicle and plumule were affected by the herbicide.

In the whole plant bioassay technique for post-emergence herbicides, best control was obtained for azimsulfuron followed by cyhalofop-butyl and fenoxaprop p- ethyl. Hence they were classified as susceptible herbicides for *I. miliacea* (Table 5).

Bispyribac-sodium and penoxsulam showed initial control but regrowth was seen after two weeks the weed showed moderate resistance to these herbicides. This may be due to faster metabolism of the weed or the insensitivity of the target enzyme to the ALS inhibitor as suggested Nady *et al.* (2012) in the case of *Echinochloa colona*

The weed was resistant to pyrazosulfuron. Similar effect of the herbicide has also been observed by Margo *et al.* (2010) on *Cyperus difformis*. This indicates variation in the target enzymes of the ALS inhibitors of pyrazosulfuron-ethyl.

All the four non-traditional rice herbicides tested, viz. glyphosate, diuron, paraquat, glufosinate ammonium gave excellent control of *I. miliacea* and no regrowth was observed after two weeks (Table 6). These herbicides can be recommended for control of the in paddy fields before land preparation.

The study clearly indicates that the pre-emergence herbicides butachlor and oxyfluorfen and the post-emergence herbicides azimsulfuron,

**Table 6. Whole plant bioassay using non-traditional herbicides**

Herbicide	Mode of action	2 DAS	4 DAS	8 DAS	15 DAS
Glyphosate	Inhibition of amino acids	Leaf discolouration	Leaf tip and shoot tip burned	Wilting started	Permanently wilted
Diuron	Inhibition of photosystem- I	Leaf discolouration	Leaf scorching	Wilting started	Permanently wilted
Paraquat	Inhibition of photosystem- I	Wilting Started	Permanently wilted	Permanently wilted	Permanently wilted
Glufosinate ammonium	Inhibition of glutamine synthetase	Leaf scorching	Leaf chlorosis	Leaf necrosis, wilting started	Permanently wilted

(DAS – Days After Spraying)

cyhalofop-butyl, and fenoxaprop-p-ethyl can be recommended in rice fields prone to the infestation of *I. miliacea*.

### SUMMARY

A study was conducted during 2014-2015 in College of Horticulture, Vellanikkara, Thrissur to understand response of the *Isachne miliacea* to common herbicides. Sensitivity of the weed to pre- and post-emergence herbicides were tested using seed and whole plant bioassay techniques, respectively. The pre-emergence herbicides butachlor and oxyfluorfen gave the best results. Among post-emergence herbicides, effective control was observed for azimsulfuron, cyhalofop-butyl and fenoxaprop-p-ethyl. The weed was found to be resistant to pyrazosulfuron. Regrowth was observed after two weeks for bispyribac sodium and penoxsulam. All the non-traditional rice herbicides tested, viz. glyphosate, diuron, paraquat, glufosinate ammonium gave excellent control of the weed.

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