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**RESEARCH ARTICLE**

# Differential response of grass weeds to ALS inhibiting broad-spectrum herbicide bispyribac-sodium

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**ABSTRACT**

An experiment on assessing the differential response of *Leptochloa chinensis* and *Echinochloa colona* to the varying concentrations of broad-spectrum herbicide bispyribac-sodium (BS) was carried out in the midland laterites of Kerala. Seedlings of both weeds at 4-5 leaf stage were treated with 50, 100 and 200% field recommended dose (FRD) of bispyribac-sodium, 10 SC, PI Industries) 0.0125, 0.025 and 0.05 kg/ha, respectively. Differential response by weeds was evaluated using amino acid content estimation and protein profiling by Sodium Dodecyl Sulphate - Poly Acrylamide Gel Electrophoresis. The amino acid content of *L. chinensis* was not influenced by the increasing concentration of BS, whereas it was found to decrease with an increasing concentration of BS in *E. colona*. The parameters examined to evaluate the varying response, *viz.* protein content, number of proteins expressed and molecular weight of proteins shown higher values in *L. chinensis* compared to *E. colona*, irrespective of the concentration. However, increase in concentration of BS reduced the amino acid levels in *E. colona*. The reduced effectiveness of BS in inhibiting the biochemical processes associated with amino acid and protein synthesis in *L. chinensis* might explain its poor performance. The study verified the contrasting expression of amino acids and proteins in *L. chinensis* and *E. colona*, despite being from the same family. The study validated the need to identify suitable herbicides for broad spectrum weed management in direct seeded rice, especially when *L. chinensis* dominated the grass weed flora.

**Keywords**: Direct-seeded rice, *Echinochloa colona*, Grass weeds, *Leptochloa chinensis*, Protein profiling

## INTRODUCTION

Bispyribac-sodium, chemically, sodium 2, 6-bis [(4, 6-dimethoxy-2-pyrimidinyl) oxy] benzoate, is a popular rice herbicide recommended against a wide range of weeds, including grasses, broadleaf weeds and sedges. It is absorbed through the roots and leaves and inhibits the enzyme acetolactate synthase (ALS) in susceptible weed plants. ALS, also referred to as acetohydroxyacid synthase, is the enzyme in the biosynthetic pathway leading to the production of branched-chain amino acids, *viz.* leucine, isoleucine, and valine. The absence of essential amino acids hinders protein synthesis and growth resulting in plant mortality (WSSA 2007). Bispyribac-sodium (BS), is prone to developing resistance to specific weed accessions rapidly (Tranel and Wright 2002). The fact has more relevance in the context that nearly 126 weed species have already developed resistance to ALS inhibitors (Tranel *et al.* 2012).

As rice fields contain complex weed flora, the knowledge on the response of weeds to applied herbicides has several practical implications, *viz.* developing appropriate weed management strategies, including the development of models capable of predicting the overall weed control level achievable with a specific herbicide (Khedr *et al.* 2018). *Leptochloa chinensis* (L.) Nees and *Echinochloa colona* (L.) Link are two major grass weeds infesting the rice fields of Kerala. *Leptochloa chinensis*, generally known as 'Chinese sprangletop' or 'Red sprangletop' or 'Asian sprangletop' has been reported as one of the most problematic grass weeds in direct-seeded rice fields (Chin 2001). *Echinochloa colona* (jungle rice or barnyard grass), a vigorous C4 annual species, is one of the world's most serious grass weeds in rice (Holm *et al.* 1991). It has been identified as a troublesome weed in 35 crops in more than 60 countries (Holm *et al.* 1991). Population density of two to six *L. chinensis*/$m^2$ can cause grain yield loss of 14 to 44% in rice (Bergeron 2017), and that for *E. colona* is 27 to 62% (Rao and Matsumoto 2017). Studies conducted by Sekhar (2021) revealed

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that season long weed competition in wet seeded rice (WSR) with *L. chinensis* as a major weed caused a yield reduction of 59.95%.

Preliminary field studies revealed that BS 0.025 kg/ha (100% field recommended dose) was effective against *Echinochloa colona* but not against *Leptochloa chinensis,* which is a major grass weed in wet seeded rice, thus preventing broad-spectrum weed control (Sekhar *et al*. 2020a). As BS is effective for other grass weeds, including *E. colona*, and not for *L. chinensis,* despite the fact that they both belong to the Poaceae family, the possibility of differential response is valid. In this context, the present study was aimed to assess the differential response of *Leptochloa. chinensis* and *Echinochloa colona* to the broad-spectrum herbicide BS at varying concentrations.

## MATERIALS AND METHODS

The study on assessing the differential response of *Leptochloa chinensis* and *Echinochloa colona* to BS at varying concentrations was carried out at College of Agriculture, Thiruvananthapuram, Kerala (midland laterites) during 2020. The mean temperature ranged between 28.6°C to 31°C and mean relative humidity ranged between 57.44% to 76.37%, with a direct normal irradiation of 72.93 - 318.75 W/m$^2$. No rainfall was received during the experimental period. Mature seeds of *L. chinensis* and *E. colona* were collected from lowland rice fields of the Integrated Farming System Research Station, Karamana, Thiruvananthapuram (8$^0$ N latitude and 76$^0$ E longitude). Panicles were collected from more than 50 arbitrarily selected plants. Shattered seeds were bulked up, cleaned, and stored at room temperature in airtight plastic containers for experimental purposes.

The experiment was conducted by sowing 20 seeds of each weed separately in pots (27 cm in diameter and 22 cm in height) filled with soil collected from rice fields (the soil was solarized to destroy the soil seed bank). The soil used for the experiment was sandy clay loam with medium texture and acidic in reaction with a pH of 5.3. The available N, P and K content was 175.6, 29 and 377.6 kg/ha, respectively. Seeds of both weeds were placed on the soil surface for germination and allowed to grow under open conditions. After one month, 10 seedlings of both weeds were retained in each pot. The seedlings at 4-5 leaf stage were treated with 50, 100 and 200% field recommended dose (FRD) of BS (Nominee Gold®, 10 SC, PI Industries), *i.e*., 0.0125, 0.025 and 0.05 kg/ha, respectively. The experimental design was a completely randomized block design (CRD) and three replicate pots were used per herbicide treatment and control. The specimens were collected three days herbicide application to study the early response, allowing a 48-hour period for the absorption and translocation of the herbicide, then stored on ice packs for analysis. ALS inhibitors mainly affects meristematic tissues, a key site for amino acid synthesis, and symptoms appear about 7–8 days after herbicide spray (Prakash *et al*. 2017). The differential response of *L. chinensis* and *E. colona* to varying concentrations of BS was evaluated using amino acid content estimation and protein profiling by Sodium Dodecyl Sulphate - Poly Acrylamide Gel Electrophoresis (SDS PAGE).

### Amino acid content

The amino acid content was estimated using standard procedures (Sadasivam and Manikam, 2007). Total free amino acids were estimated using ninhydrin reagent. Ninhydrin (triketohydrindene hydrate) reacts with an amino acid to form a purple colour complex (Riemann's purple) with maximum absorption at 570 nm. Ninhydrin oxidises the amino acid to aldehyde, releasing ammonia and carbon dioxide in the process, and then reduces to hydridatin. In the presence of ammonia, the hydridatin form condenses with Ninhydrin to form a purple complex. The tissue sample (1.0 mg) was homogenized in 1 mL of phosphate buffer (pH 7.0). It was then centrifuged at 10,000 rpm for 20 minutes. The pellet was resuspended in 1 mL of phosphate buffer (pH 7.0) after the supernatant had been removed. It was made up to a volume of 4 mL with distilled water and 1 mL of Ninhydrin reagent was added. The contents of the tubes were mixed by vortexing and were placed in a boiling water bath for 15 minutes. The test tubes were cooled in cold water and 1 mL of ethanol was added. After cooling, the absorbance was measured at 570 nm using a UV Visible Spectrophotometer. The concentration of amino acid was calculated using the standard curve for proline.

### Protein profiling

Protein samples were collected from leaves of all the treatments of both the weeds, three days after treatment application and analyzed by SDS PAGE as suggested by Sadasivam and Manikam (2007). A 10% resolving gel and a 5% stacking gel were prepared using 30% acrylamide stock solution, 10% SDS, 10% ammonium per sulphate solution, TEMED (N,N,N´N´-tetramethylethylene-1-diamine), and tris HCl (1.5M - pH 8.8 for resolving gel and 0.5M - pH

6.8 for stacking gel), along with distilled water. The sample buffer was composed of tris HCl (pH 6.8), bromophenol blue, β-mercaptoethanol, SDS, and glycerol. The electrophoresis buffer contained tris base, glycine, and SDS. The staining solution was prepared by mixing coomassie brilliant blue R 250, glacial acetic acid, methanol, and distilled water. Data generated were analyzed for completely randomised design using the online statistical analysis platform-KAU-GRAPES (General R-shiny based Analysis Platform Empowered by Statistics) (Gopinath *et al*., 2021).

## RESULTS AND DISCUSSION

Weeds in crop fields belonged to a wide range of types and categories, with some belonging to the same family while others were distinct. This made it impossible to predict the level of control that a specific herbicide would provide against a range of weeds. Information on the response of weeds to various herbicides is essential for developing effective management techniques as components of integrated weed management systems. The application of the herbicide BS resulted in a reduction of amino acid content in plants, as BS targets ALS, a key enzyme in the biosynthesis of branched-chain amino acids. Significant reduction in branched chain amino acids (BCAAs), *viz*. valine, leucine, and isoleucine levels in plants treated with ALS inhibitor herbicides, leading to a selective depletion of BCAAs as a proportion of the total free amino acid pool, coupled with a noticeable decline in protein synthesis was reported earlier by many researchers (Ray 1984, Anderson and Hibbard 1985).

There was no significant difference (p=0.05) between the relative amino acid contents at various concentrations of BS in *L. chinensis* (**Table 1**). The highest content of amino acid (0.3440 mg/g) in *L. chinensis*, was observed at 50% FRD of BS (0.0125 kg/ha). Considering the relative amino acid contents, it was inferred that the amino acid content at 50% FRD was statistically similar to that of 100 and 200% FRD, with an amino acid content of 0.3440, 0.2904 and 0.3234 mg/g, respectively (**Table 1**). This indicated that, regardless of the herbicide concentration, there was no observable non-inhibitory impact of BS on *L. chinensis*. Several authors have observed the inefficiency of BS in managing *L. chinensis* (Jacob 2014, Awan *et al*. 2015, Sekhar *et al*. 2020b). Compared to control (without herbicide spray), the amino acid content was higher in the *L. chinensis* plants treated with bispyribac-sodium. However, an increase in the concentration of BS had an effect on the amino acid content of *E. colona*. The amino acid content of *E. colona* decreased as the concentration of BS increased.

Critical appraisal of the data identified higher content of amino acid in *L. chinensis* compared to *E. colona*, irrespective of the concentration BS. The amino acid content in *E. colona* was 0.2437 mg/g with the 50% FRD (0.0125 kg/ha) of BS, whereas it was 0.3440 mg/g in *L. chinensis*. At 100% FRD, the amino acid content was 0.1520 and 0.2904 mg/g and at 200% FRD, it was 0.0627 and 0.3234 mg/g respectively, in *E. colona* and *L. chinensis*. The amino acid content in *E. colona* decreased by 32.38, 57.82 and 82.60%, respectively, at 50, 100 and 200% FRD of BS compared to control. However, in *L. chinensis*, amino acid content increased by 39.38, 28.20 and 35.52% at 50, 100 and 200% FRD (**Table 1**). The study implied that BS was ineffective in inhibiting amino acid synthesis in *L. chinensis*, as evident from the higher amino acid content compared to *E. colona*. Imidazolinone application ceases growth because of inhibited cell division and DNA synthesis and some of these changes relate in some way to the disruption of the synthesis of the branched-chain amino acids (Shaner 1991). A significant increase in protein content was specifically observed in *E. colona* control plants which could be attributed to inherent biological processes within the plant, such as natural growth and development cycles or regulatory mechanisms that modulate protein synthesis.

Differential expressions of proteins were observed in *L. chinensis* and *E. colona* with varying

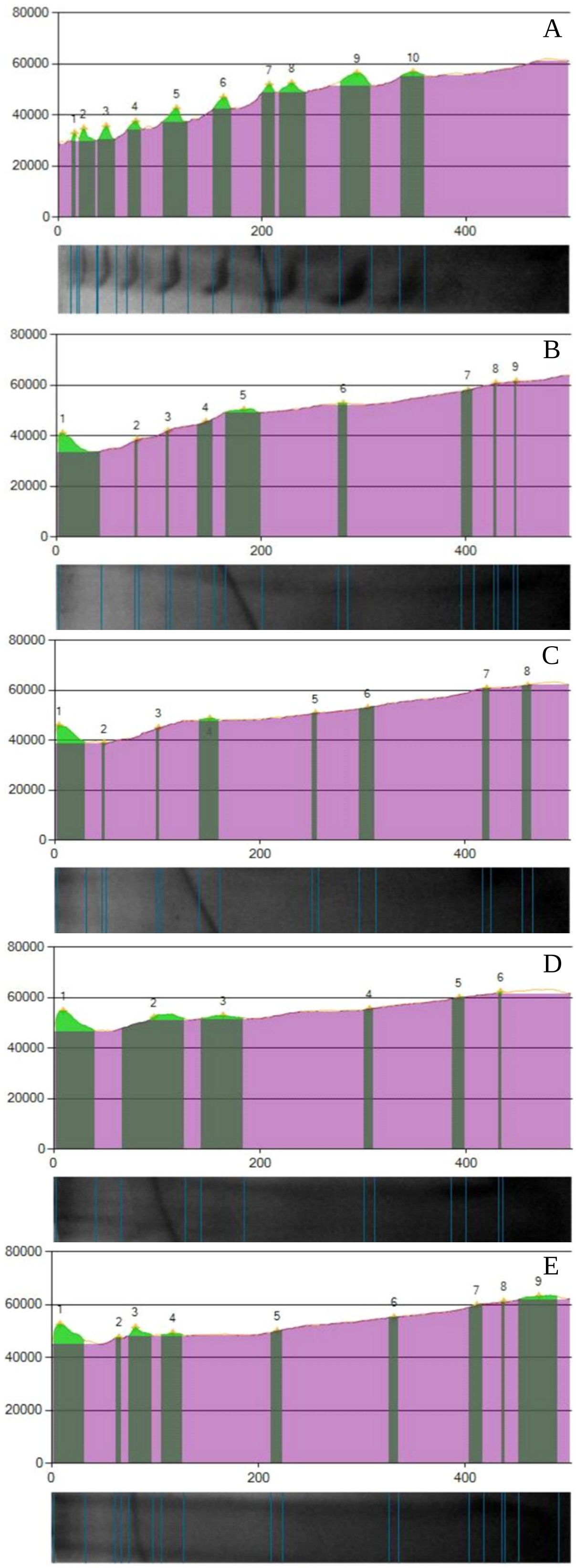
**Table 1. Differential response of *Leptochloa chinensis* and *Echinochloa colona* to bispyribac-sodium**

| Concentration | Amino acid content (mg/g) | | Protein content (mg/g) | | No. of proteins expressed | | Molecular weight of total proteins (kDa) | |
|---|---|---|---|---|---|---|---|---|
| | *L. chinensis* | *E. colona* | *L. chinensis* | *E. colona* | *L. chinensis* | *E. colona* | *L. chinensis* | *E. colona* |
| Bispyribac-sodium 0.0125 kg/ha (50% *FRD) | 0.3440 | 0.2437 | 0.5401 | 0.2061 | 7 | 7 | 639.86 | 377.3 |
| Bispyribac-sodium 0.025 kg/ha (100% FRD) | 0.2904 | 0.1520 | 0.4762 | 0.1420 | 6 | 4 | 460.76 | 248.82 |
| Bispyribac-sodium 0.05 kg/ha (200% FRD) | 0.3234 | 0.0627 | 0.4827 | 0.1009 | 8 | 3 | 629.06 | 107.84 |
| Control (no herbicide) | 0.2085 | 0.3604 | 0.5021 | 0.4599 | 8 | 6 | 610.86 | 622.27 |
| LSD (p=0.05) | 0.037 | 0.018 | - | 0.059 | - | - | - | - |

concentrations of BS from lower to higher concentrations (**Table 1**). However, a statistically significant reduction was not observed in the protein content, the number of proteins expressed and the molecular weight of proteins in *L. chinensis* with the application of BS from a lower to a higher concentration.

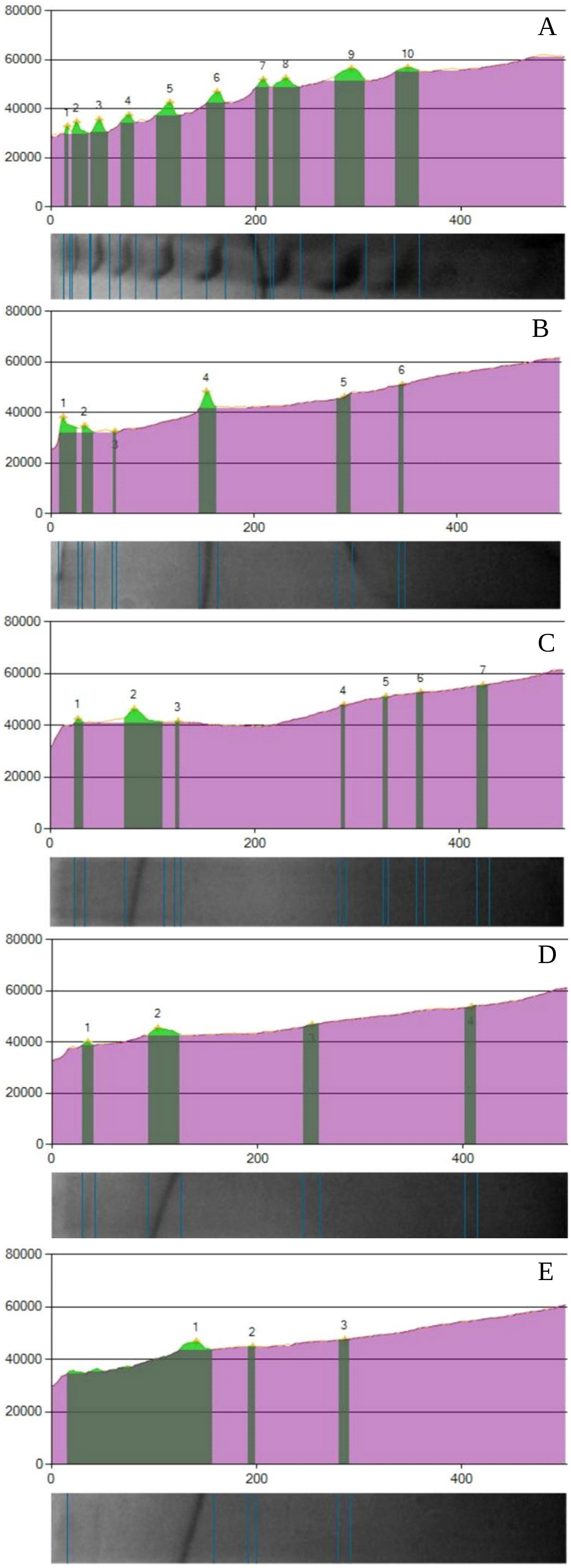
In *E. colona*, the protein content and molecular weight of total proteins were found to decrease with BS application compared to control. The number of proteins expressed, the molecular weight of proteins and protein content diminished with increasing concentration of BS. The negative correlation of total protein content, number of proteins expressed and the molecular weight of proteins with the concentration of BS could be due to the fact that this herbicide is known to induce oxidative stress. The application of herbicides, even if selective, can cause biochemical and physiological changes, resulting in oxidative stress (Langaro *et al*. 2016). Drop in protein levels as a biochemical and physiological consequence with the primary action of ALS inhibitors has been observed by Sidari *et al*. (1998). Rhodes *et al*. (1987) proposed that an increase in protein turnover caused the drop in protein content which could be due to accelerated degradation of existing proteins. The lowest protein content of 0.1009 mg/g was registered at 200% FRD in *E. colona*. This was statistically comparable with 100% FRD with a protein content of 0.1420 mg/g. The highest protein content (0.4599 mg/g) and molecular weight of total proteins (622.27 kDa) were registered in control without any herbicide application. Among various concentrations, the application of BS at 50% FRD recorded the highest protein content (0.2061 mg/g) and molecular weight of total proteins (377.3 k Da) in *E. colona*. In general, a higher molecular weight of total proteins was observed in *L. chinensis* compared to *E. colona* in herbicide-treated plants (**Figure 1** and **2**).

A total of seven proteins were expressed in both *L. chinensis* and *E. colona* at 50% FRD of BS. As the concentration increased from 50 to 200% FRD, the total number of proteins expressed in *E. colona* was found to decrease from seven to three, whereas it decreased to six at 100% FRD and then increased to eight at 200% FRD in the case of *L. chinensis.* Among the varying concentrations of BS, 100% FRD resulted in lower protein content, molecular weight of total proteins and the number of proteins expressed in *L. chinensis* compared to its higher and lower concentrations. There was also a reduction in protein content, low molecular weight of total proteins and




A – Marker; B – Control; C – bispyribac-sodium 0.0125 kg/ha (50% FRD); D – bispyribac-sodium 0.025 kg/ha (100% FRD); and E – bispyribac-sodium 0.05 kg/ha (200% FRD)

**Figure 1. Protein profiling by SDS PAGE (*Leptochloa chinensis*)**




A – Marker; B – Control; C – bispyribac-sodium 0.0125 kg/ha (50% FRD); D – bispyribac-sodium 0.025 kg/ha (100% FRD); and E – bispyribac-sodium 0.05 kg/ha (200% FRD) (*FRD – Field recommended dose)

**Figure 2. Protein profiling by SDS PAGE (*Echinochloa colona*)**

less number of proteins expressed in *L. chinensis* at 100% FRD of BS compared to control, which was not observed at 50 and 200% FRD. This confirmed that increasing the concentration of BS did not have much effect on *L. chinensis*.

The parameters analyzed to assess the differential response of grass weeds to BS registered a higher value in *L. chinensis* than in *E. colona* regardless of the concentration. The differential response of the grass weeds, *viz. Echinochloa crus-galli* and *E. colona* to BS was earlier reported (Riar *et al*. 2012, Kaloumenos *et al*. 2013, Khedr *et al*. 2018). The poor performance of BS in *L. chinensis* could be attributed to its lower efficiency in inhibiting the biochemical processes related to amino acid and protein synthesis. The study confirmed the differential expression of amino acids and proteins in *L. chinensis* and *E. colona*, even though they belong to the same family. In a flora dominated by *L. chinensis*, the use of BS may not be suitable for broad spectrum weed management, emphasizing the importance of assessing the weed species present and identifying appropriate herbicides when implementing chemical control methods.

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