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Detection of bispyribac sodium + metamifop 14% SE residue in soil by bioassay method

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ABSTRACT

Bioassay studies were carried out to assess the residual effect of herbicide mixture, bipyribac-sodium + metamifop 14% SE in soil with indicator plant. A screening trial with three test crops, *viz*. cucumber, sunflower and maize indicated that maize was the best indicator plant, because it recorded the highest regression co-efficient for the parameters tested such as fresh and dry weight of shoot, shoot length and root length. The shoot dry weight of maize was identified as the best parameter to detect the phytotoxic residue in soil. The bioassay conducted with maize as an indicator plant in the post experiment soil revealed that there was no significant difference among the treatments (bispyribac-sodium + metamifop at 60, 70, 80, 90 g/ha, bispyribac applied alone at 25 g/ha, hand weeding twice and weedy check) during both the seasons in germination percentage, shoot length, root length, fresh weight and dry weight of maize plant. Thus it can be inferred that the herbicide mixture, bispyribac-sodium + metamifop did not leave any phytotoxic residues in soil.

Key words: Bioassay, Bispyribac-sodium + metamifop, Indicator plant, Maize, Residue

The non-judicious use of herbicides is a source of concern, which has a growing interest in the environment, nature conservation and public health in general. Detection of herbicide residues is of great importance because of the risk of phytotoxicity on other species which are not direct object of the treatment, the risk involved in rotational crops due to the accumulation of phytotoxic residues in the field or herbicide drift during the application of the herbicide (Pestemer and Zwerger 1999). Plant bioassay is the viable alternative to the instrumental procedures for the determination of herbicide residue in soil. It is a simple, inexpensive, accurate and direct method of determining herbicide residues present in the soil at concentrations high enough to adversely affect crop growth yield and quality. Hernández-Sevillano *et al.* (1999) pointed out that it is a valuable tool that provides an overview of soil-plant-herbicide relationships. Instrumental methods such as gas chromatography or high performance liquid chromatography requires several solvent or solid phase extractions and clean up procedures before sample analysis and determine the total amount of active ingredient present in the soil (Szmigielski *et al.* 2012). The amount of residue extracted chemically may differ from the amount of residue biologically available to cause phytotoxic responses to bioassay species (Strachan *et al*. 2011). In contrast, bioavailable herbicide is determined by bioassay procedures (Szmigielski *et al.* 2009) because plant response varies with soil type and generally decreases in soils of high organic matter and clay contents and low soil pH. There are various procedures to undertake herbicide bioassays. Shoot and root bioassays with sensitive plants were suggested for herbicide bioassays (Vicari *et al.* 1994, Hermandez-Sevillano *et al*. 2001). For detecting the ALS herbicide residues, maize, sunflower and oriental mustard (Szmigielski *et al*. 2008) were used as indicator plants. Cotton (Grey *et al.* 2007) and sugar beet (Szmigielski *et al.* 2009) have been reported as the suitable indicator plants for detection of protox inhibiting herbicides in soil. With this back ground, the present bioassay study was planned to find out the residual effects in soil due to the application of bispyribac-sodium + metamifop, a combination product of broad spectrum herbicide, bispyribac-sodium (3.8%) and a grass effective herbicide metamifop (9.5%). Bispyribac sodium beongs to chemical group thiobenzoate inhibiting the biosynthesis of aminoacids and metamifop belongs to aryloxyphenoxypropionate inhibiting acetyl coenzyme-A carboxylase (ACCase) leading to growth retardation of weeds.

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MATERIALS AND METHODS

The bioassay experiments were conducted in Department of Agronomy, College of Agriculture Vellayani Thiruvananthapuram and field experiments were conducted in the farmers' field during *Kharif* 2014 and *Rabi* 2014-15 at Upaniyoor padashekaram, in Kalliyoor Panchayat, Nemom block, Thiruvananthapuram district, Kerala, India. Bioassay experiments comprised of two parts. The first part was the screening of indicator plants to identify the most sensitive indicator plant, among the three test crops *viz*. cucumber, sunflower and maize. The second part was the detection of phytotoxic residue of bispyribac-sodium + metamifop 14% SE in the post experiment soil using the most sensitive indicator plant identified.

Screening of indicator plants: Screening of the best indicator plant was conducted in CRD with 8 treatments. The treatments comprised of seven different concentrations of bispyribac-sodium + metamifop, *viz*. 100 µL/L, 10 µL /L, 5 µL/L, 1 µL/L, 0.5 µL/L, 0.05 µL/L, 0.01 µL/L and 0 µL/L (control). Separate experiments were conducted for each test crop in three replications. Soil was collected from the herbicide free area, washed thoroughly with water and air dried. Then it was fortified with different concentrations of bispyribac-sodium + metamifop (as per the treatments) and mixed thoroughly and 300 g soil was taken in small plastic pots of 500 ml capacity separately. Ten seeds of each test species were dibbled in each pot at uniform depth of 2 cm. Germination count was taken at 4 DAS and then the plants were thinned to three per pot to avoid competition. At 14 DAS, the plants were uprooted from each pot without causing any damage to the roots. Shoot length and root length were recorded. The root system was removed using a sharp knife and the fresh shoot weight was recorded. Shoot dry weight was recorded after the plants were dried in hot air oven at 60 °C to constant weight. Data on shoot length, root length, shoot fresh and dry weight of indicator plants were statistically analyzed using ANOVA and regression equations were developed. The test crop which showed the highest R^2 value for all the tested parameters was selected as the best indicator plant and the parameter which showed the highest R^2 value was selected as the best parameter to detect the residual effects of herbicide mixture, bispyribac-sodium + metamifop. The response curve was also developed for the tested parameters of the best indicator plant.

Field experiments were laid out in randomized block design with seven treatments and three replications. The treatments were bispyribac- sodium + metamifop at 60, 70, 80 and 90 g/ha, bispyribac-sodium applied alone 25 g/ha, hand weeding twice and weedy check. The herbicides were applied at 15 DAS as per the treatment schedule using knapsack sprayer fitted with flat fan nozzle. The spray fluid was used at 500 L/ha for the study. The variety used was '*Kanchana'*, a short duration variety released from Regional Agricultural Research Station, Pattambi. The crop was fertilized with 70:35:35 kg/ha N, P and K, with one third N and K and half P applied on 15 DAS (days after sowing), one third N and K and half P on 35th day and remaining one third N and K on 55th day of sowing. All the Agronomic and plant protections were adopted as per package of practices recommendations of Kerala (KAU 2011).

Detection of phytotoxic residue: For the determination of bispyribac-sodium + metamifop residue in the soil, composite soil sample was collected from each treatment plot at a depth of 15 cm after the harvest of the crop. From this sample, 300 g soil was weighed and transferred into plastic containers of 500 ml capacity and 10 seeds of the most sensitive indicator plant, *i.e*. maize was dibbled in each pot at a uniform depth of 2 cm. Germination count was taken at 4 DAS and then the plants were thinned to three per pot to avoid competition. Observations on shoot and root length and shoot fresh and dry weight were recorded as in the screening trial described above.

The data generated were statistically analyzed using analysis of variance technique (ANOVA) and difference between the treatments means were compared at 5% probability level.

RESULTS AND DISCUSSION

Screening of indicator plants

The effect of different concentrations of herbicide mixture, on shoot length, root length, shoot fresh and dry weight of cucumber, sunflower and maize are presented (**Tables 1, 2** and **3**). The data on germination percentage of cucumber, sunflower and maize were not statistically analyzed, since no graded variation was observed among the treatments. In general, as the concentration of herbicide mixture increased, a decrease in the growth parameters were observed in the tested crops. Quadratic ($Y= a+ b X^2$) and logarithmic linear regression equation, $Y= a + b \ln (X)$ were fitted for shoot fresh weight, shoot dry weight, shoot length and root length for cucumber, sunflower and maize and among the two equations, logarithmic linear regression equation, $Y= a + b \ln (X)$ were best fitted and adopted for the study.

Table 1. Effect of different concentrations of bispyribac sodium + metamifop on the growth parameters of cucumber

Treatment (concentrations of bispyribac-sodium + metamifop)	Germination (%)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
100 µL/L	30.0	0.56	0.49	0.09	0.011
10 µL/L	40.0	0.80	0.79	0.11	0.012
5 µL/L	60.0	3.22	1.60	0.18	0.015
1 µL/L	66.0	4.36	1.48	0.21	0.018
0.5 µL/L	63.0	5.53	3.30	0.32	0.023
0.05 µL/L	74.1	7.82	4.50	0.60	0.042
0.01 µL/L	90.0	9.00	6.99	0.61	0.049
Control	90.0	9.54	7.03	0.76	0.057
LSD (p=0.05)	-	1.58	1.72	0.13	0.011

Table 2. Effect of different concentrations of bispyribac sodium + metamifop on the growth parameters of sunflower

Treatment (concentration of bispyribac-sodium + metamifop)	Germination (%)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
100 µL/L	76.7	2.16	0.65	0.28	0.036
10 µL/L	80.0	3.22	0.81	0.30	0.037
5 µL/L	80.0	5.42	0.87	0.38	0.039
1 µL/L	76.7	5.67	1.05	0.44	0.047
0.5 µL/L	80.0	7.73	1.53	0.49	0.048
0.05 µL/L	80.0	8.02	2.23	0.55	0.049
0.01 µL/L	86.7	14.08	4.08	0.80	0.056
Control	93.3	16.15	4.61	0.87	0.057
LSD (p=0.05)	-	1.950	0.520	0.156	0.0083

Table 3. Effect of different concentrations of bispyribac sodium + metamifop on the growth parameters of sunflower

Treatment (concentration of bispyribac-sodium + metamifop)	Germination, (%)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
100 µL/L	80.00	0.37	0.74	0.08	0.001
10 µL/L	86.67	3.94	1.50	0.13	0.026
5 µL/L	83.33	17.65	3.74	0.64	0.082
1 µL/L	86.67	29.48	7.57	1.08	0.133
0.5 µL/L	90.00	30.95	13.47	1.35	0.157
0.05 µL/L	90.00	32.80	18.45	1.40	0.181
0.01 µL/L	100.00	37.30	26.76	1.60	0.188
Control	100.00	37.69	27.93	1.64	0.192
LSD (p=0.05)	-	4.455	4.000	0.246	0.020

The different concentrations of bispyribac-sodium + metamifop significantly influenced the shoot fresh weight, shoot dry weight, root length and shoot length of cucumber. The percentage reduction in shoot fresh weight and dry weight, shoot length and root length of cucumber at 0.01 to 100 µl/L concentrations of bispyribac-sodium + metamifop ranged from 19.74 to 88.16, 14.04 to 80.70, 5.66 to 94.13 and 0.57 to 93.03%, respectively compared to control. Logarithmic linear regression equations developed for shoot fresh weight, shoot dry weight, shoot length and root length of cucumber were Y= 0.2714-0.06155 ln (X), Y= 0.0223 - 0.00414 ln (X), Y= 3.950 - 0.9871 ln (X) and Y= 2.385 - 0.6645 ln (X), respectively.

Similar to that of cucumber, the different concentrations of bispyribac-sodium + metamifop significantly influenced the shoot fresh weight, shoot dry weight, root length and shoot length of sunflower also. The percentage reduction in shoot fresh weight and and dry weight, shoot length and root length of sunflower at 0.01 µL/L to 100 µL/L concentrations of bispyribac-sodium + metamifop ranged from 8.05 to 67.82, 1.75 to 36.84, 12.82 to 86.63 and 11.50 to 85.90, respectively compared to control. Logarithmic linear regression equations developed for the shoot fresh weight, shoot dry weight, shoot length and root length of sunflower were Y= 0.4349 - 0.0513 ln (X), Y= 0.0434 -0.0022 ln (X), Y= 6.0154 -1.1373 ln (X) and Y= 1.4383 - 0.3132 ln (X), respectively.

The effect of different concentrations of bispyribac-sodium + metamifop on the growth parameters of maize was also statistically analyzed. The shoot fresh weight and dry shoot weight, root length and shoot length of maize were also significantly influenced by the different concentrations of bispyribac-sodium + metamifop. The percentage reduction in shoot fresh weight, shoot dry weight, shoot length and root length at 0.01 µL/L to 100 µL/L concentrations of bispyribac-sodium + metamifop ranged from 2.44 to 95.12, 2.08 to 99.48, 1.03 to 99.02 and 4.19 to 97.35, respectively compared to control. The logarithmic linear regression equations developed for shoot fresh weight, shoot dry weight, shoot length and root length of maize were Y= 0.7980 - 0.1890 ln (X), Y= 0.0977 -0.0230 ln (X), Y= 19.4270-4.4705 ln (X) and Y= 8.8401- 2.8056 ln (X), respectively.

Results revealed that, among the three indicator plants tested, *viz*. cucumber, sunflower and maize, maize plant was the most sensitive indicator plant to determine the residues of bispyribac-sodium + metamifop in soil, since it recorded the highest R^2 values (regression co-efficient values) for shoot dry weight, shoot fresh weight, root length and shoot length, the parameters tested (**Table 4, Figure 1a, 1b, 1c** and **1d**) and also the percentage reduction in the shoot fresh weight, shoot dry weight, shoot length and root length was more than in the case of cucumber and sunflower. Szmigielski *et al*. (2012) reported that, selecting a suitable plant species for bioassay is critical and parameter measured in the bioassay should correlate well with herbicide concentration.. Yadav *et al*. (2013) reported cucumber as the best indicator plant for the residue studies of pyrazosulfuron-ethyl in soil. The best parameter for the detection of residue in the soil was maize shoot dry weight (**Table 4, Figure 1d**), since it recorded the highest R^2 value (0.9548) compared to other tested parameters of maize. Vicari *et al*. (1994) and Stork and Hannah (1996) opined that plant height and dry or fresh weight of shoot has been found to be the sensitive parameters for the detection of sulfonyl urea herbicide residue in soil.

Table 4. R^2 values of different parameters of tested indicator plants, Y= a + b ln (X)

Parameter	Cucumber	Sunflower	Maize
Shoot fresh weight	0.7861	0.8245	0.9379
Shoot dry weight	0.7501	0.8772	0.9548
Shoot length	0.9325	0.8454	0.9310
Root length	0.8039	0.6670	0.8408

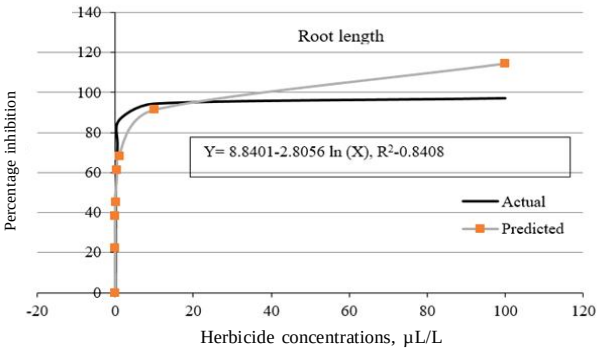

Figure 1a. Percentage growth inhibition in the root length of maize, as influenced by different concentrations of bispyribac-sodium + metamifop

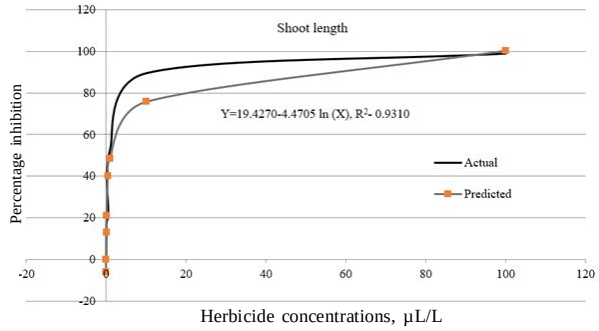

Figure 1b. Percentage growth inhibition in the shoot length of maize, as influenced by different concentrations of bispyribac-sodium + metamifop

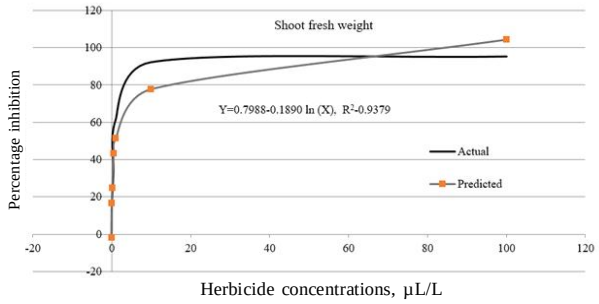

Figure 1c. Percentage growth inhibition in the shoot fresh weight of maize, as influenced by different concentrations of bispyribac-sodium + metamifop

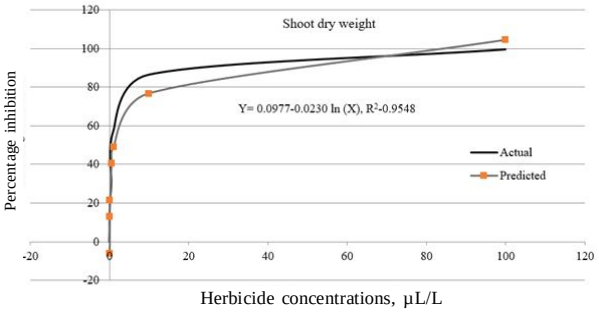

Figure 1d. Percentage growth inhibition in the shoot dry weight of maize, as influenced by different concentrations of bispyribac-sodium + metamifop

Herbicide residue in post experiment soil

Results revealed that there was no significant difference among the treatments during both the seasons in the parameters studied, *viz.* germination percentage, shoot length, root length, fresh weight and dry weight of maize plant. Thus, it can be assumed that the herbicide mixture applied at 60, 70, 80 and 90 g/ha did not leave any residue in soil. Ramani and Khanpara (2010) reported that the post-emergence herbicides *viz*. oxadiargyl 90 g/ha, quizalofop-ethyl 40 g/ha and fenoxaprop-P-ethyl 75 g/ha when applied at 60 DAS showed no reduction in germination percentage, plant height and dry weight of indicator plants, sorghum and cucumber indicating no residual phytotoxic effect.

It was concluded that maize was the best indicator plant among the three test crops to detect the phytotoxic residue of bispyribac-sodium + metamifop in soil and shoot dry weight of maize was adjudged as the most sensitive parameter to detect the phytotoxic residue of bispyribac-sodium + metamifop in soil. Results of the bioassay study with maize plant as the indicator plant during *Kharif* 2014 and *Rabi* 2014-15 indicated that post-emergence application of bispyribac-sodium + metamifop at 60, 70, 80 and 90 g /ha did not leave any phytotoxic residue in the soil to cause any growth inhibition in the growth parameters of maize, germination percentage, shoot fresh weight, shoot dry weight, shoot length and root length.

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