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Bioassay for the detection of penoxsulam + cyhalofop butyl residue in soil

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ABSTRACT

Bioassay tests were carried out at Department of Agronomy, College of Agriculture Vellayani during 2014 and field experiments were carried out during kharif 2014 and rabi 2014-15 in farmers field at Kalliyoor Panchayat of Thiruvananthapuram district, Kerala. Response of three test crops, maize, cucumber and sunflower to penoxsulam + cyhalofop butyl revealed that maize was the most sensitive indicator plant and fresh shoot weight of maize was the most susceptible parameter to detect the phytotoxic residue of this herbicide in soil. Bioassay with maize plant in post experiment soil during both the seasons revealed that post emergence application of penoxsulam + cyhalofop even at a concentration of 135 g ha^{-1} did not cause any growth inhibition in fresh shoot and dry weight, shoot length and root length inferring that, the herbicide penoxsulam + cyhalofop butyl 6 % OD (oil dispersion) did not leave any phytotoxic residue in soil and is environmentally safe.

Keywords: Bioassay, indicator plant, maize, penoxsulam with cyhalofop butyl

Bioassays are used to measure the biological response of a living plant to herbicide and to quantify its concentration in a substrate (Rao, 2011). It is a tool that complements the analytical methods and provides information on herbicide residue and its phytotoxicity (Stork and Hannah, 1996). It can be able to detect the herbicide or herbicide residue present in the soil at concentrations high enough to affect the crop growth, yield and quality (Anon., 2001). It is a major tool for the quantitative and qualitative determination of herbicide residues (Ramani and Khanpara, 2010) and gives a general review of soil-plant-herbicide relationship. Bioassays have advantages in the study of herbicides, because it detects both the active substance and degradation products of the herbicide, it provides information based on the observation of response of plant to herbicide and is the simple, accurate, inexpensive and direct method for determining the herbicide residue in soil.

Biological test requires an indicator organism or species, which are sensitive to a specific herbicide or a class of herbicide. Selecting suitable plant species for bioassay is critical and the plant parameter measured in the bioassay should correlate well with herbicide concentration (Szmigielski *et al*., 2012). For detecting the ALS –herbicides residues, oriental mustard (Szmigielski *et al*., 2008), maize (Mersi and Foy, 1985) and sunflower (Hernandez-Sevillano *et al*., 2001) have been used as indicator plants. Cotton and sugar beet have been reported as the suitable indicator plants for the detection of protox inhibiting herbicides in soil (Grey *et al*., 2007; Szmigielski *et al*., 2009). Szmigielski *et al*. (2012) reported sugar beet as the best indicator plant for the detection of flucarbazone and sulfentrazone herbicides in soil. Cucumber was identified as the best indicator plant for the residue studies of pyrazosulfuron ethyl in soil (Yadav *et al*., 2013). Gowda *et al*. (2003) pointed out that seataria may be considered as the best indicator plant for detecting the residues of fluazifop-p-butyl. Cucumber and sorghum were used as indicator plants for the detection of residues and persistence of oxyfluorfen, oxadiargyl, quizalfop and fenoxaprop-p-ethyl (Ramani and Khanpara, 2010).

An ideal herbicide is one that brings about selective control of weeds for sufficiently long period to get a competitive advantage to the crop and at the same time, dissipates from the soil before the crop season without leaving any residue. Residual problem may arise when these herbicides persist in soil in its original or closely related phytotoxic form for a long time. Hence, it is necessary to check the ill effect of herbicides in the main crop as well as the succeeding crop. With this back ground, the present study was planned to find out the residual effects of post emergence application of penoxsulam + cyhalofop butyl 6 % OD, a combination product of broad spectrum, penoxsulam which belongs to the chemical group triazolopyrimidine sulfonamide inhibiting the biosynthesis of branched chain amino acids in susceptible plants, and cyhalofop butyl, a grass effective herbicide belonging to the chemical group aryloxyphenoxypropionate which inhibits the activity of acetyl coenzyme-A carboxylase (ACCase) leading to growth retardation of weeds using the most susceptible indicator plant.

MATERIALS AND METHODS

The bioassay experiments were conducted in the crop museum, Department of Agronomy, College of Agriculture Vellayani, Thiruvananthapuram and field experiments were conducted in the farmers' field during

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kharif 2014 and *rabi* 2014-15 at Upaniyoor padashekaram, in Kalliyoor Panchayat, Nemom block, Thiruvananthapuram district, Kerala, India. Bioassay experiments comprised of two parts. First part of the experiment was to identify the most sensitive indicator plant, among the three test crops *viz*., cucumber, sunflower and maize. Second experiment part was to detect the residual effects of penoxsulam + cyhalofop butyl 6 % OD in the post experiment soil using the most susceptible indicator plant identified.

Screening of most sensitive indicator plant for the herbicide, penoxsulam + cyhalofop butyl 6 % OD was conducted in CRD with 8 treatments. The treatments comprised of seven different concentrations of penoxsulam + cyhalofop butyl *viz*., 100 µL L^{-1} (T_1), 10 µL L^{-1} (T_2), 5 µL L^{-1}(T_3), 1 µL L^{-1} (T_4), 0.5 µL L^{-1} (T_5), 0.05µL L^{-1} (T_6), 0.01µL L^{-1} (T_7) and 0 µL L^{-1} (control). Separate experiments were conducted for each test crop in three replications. Soil free of herbicide application was collected, washed thoroughly with water and air dried. Then it was fortified with different concentrations of penoxsulam + cyhalofop butyl (as per the treatments) and mixed thoroughly. 300 g soil was transferred to 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 was statistically analyzed using ANOVA and regression equations were developed. The test crop which showed the highest regression co-efficient (R^2) value for all the tested parameters was selected as the most sensitive indicator plant and the parameter which showed the highest R^2 value was selected as the most sensitive parameter to detect the residual effects of penoxsulam + cyhalofop butyl 6 % OD. The response curve was also developed for the tested parameters of the most sensitive indicator plant.

Field experiments were laid out in Randomized Block Design with seven treatments and three replications. The treatments comprised of penoxsulam + cyhalofop butyl at 120 (T_1), 125 (T_2), 130 (T_3) and 135 g ha^{-1} (T_4), penoxsulam applied alone 22.5 g ha^{-1} (T_5), hand weeding twice (T_6) and weedy check (T_7). The herbicides were applied on 15 DAS as per the treatment schedule using Knapsack sprayer fitted with flat fan nozzle. The spray fluid was used @ 500 L ha^{-1} 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^{-1} N, P and K, with one third N and K and half P applied on 15 DAS , 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 protection practices were adopted as per package of practices recommendations of Kerala Agricultural University (KAU, 2011).

For the determination of penoxsulam + cyhalofop butyl residue in soil composite soil sample was collected from each treatment plot at a depth of 15 cm after the harvest of the crop. From this composite 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 analysed using analysis of variance technique (ANOVA) and difference between the treatments means were compared at 5 per cent probability level.

RESULTS AND DISCUSSION

Identification of indicator plant for penoxsulam + cyhalofop butyl

The effect of different concentrations of penoxsulam + cyhalofop butyl, on shoot length, root length, shoot fresh and dry weight of cucumber, sunflower and maize are presented in table 1. 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 the herbicide increased a decrease in the growth parameters were observed in the tested crops. Quadratic (Y= a+ b X2) 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) was best fitted and adopted for the study.

Shoot fresh weight, shoot dry weight, root length and shoot length of cucumber were significantly influenced by different concentrations of penoxsulam + cyhalofop butyl (Table 1).The percentage reduction in shoot fresh weight and dry weight, shoot length and root length of cucumber at 0.01 to 100 µL L-1 concentrations of penoxsulam + cyhalofop butyl ranged from 26.39 to 90.28, 20.0 to 94.29, 15.37 to 94.30 and 35.57 to 93.13

per cent respectively compared to control. Logarithmic linear regression equation developed for shoot fresh weight, shoot dry weight, shoot length and root length of cucumber were Y= 0.2582 - 0.0554 ln (X), Y= 0.0135 – 0.0031 ln (X), Y= 6.0011 – 1.4327 ln (X) and Y= 2.3952 – 0.5766 ln (X) respectively. Similarly, the different concentrations of penoxsulam + cyhalofop butyl significantly influenced the shoot fresh weight, shoot dry weight, root length and shoot length of sunflower also (Table 1). The percentage reduction in shoot fresh weight, shoot dry weight, shoot length and root length of sunflower at 0.01 µL L^{-1} to 100 µL L^{-1}concentrations of penoxsulam + cyhalofop butyl ranged from 15.21 to 83.07, 9.09 to 56.82, 2.72 to 91.24 and 12.53 to 98.16 respectively, compared to control. Logarithmic linear regression equation developed for the shoot fresh weight, shoot dry weight, shoot length and root length of sunflower were Y= 0.4537 -0.0711 ln (X), Y= 0.2809 - 0.0021 ln (X), Y= 6.1079 – 1.7063 ln (X) and Y= 1.5453 - 0.5972 ln (X) respectively.

The effect of different concentrations of penoxsulam + cyhalofop butyl 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 penoxsulam + cyhalofop butyl. The percentage reduction in shoot fresh weight, shoot dry weight, shoot length and root length at 0.01 µL L^{-1} to 100 µL L^{-1} concentrations of penoxsulam + cyhalofop butyl ranged from 1.06 to 94.36, 16.11 to 90.60, 13.05 to 95.17 and 17.23 to 96.16 respectively compared to control. The logarithmic linear regression equation developed for shoot fresh weight, shoot dry weight, shoot length and root length were Y= 1.0621 – 0.2030 ln (X), Y= 0.0726 – 0.0126 ln (X), Y= 26.0430 – 5.0312 ln (X) and Y= 10.2452- 2.5908 ln (X).

Results revealed that, among the three indicator plants tested *viz*., cucumber, maize and sunflower, maize plant was the most sensitive indicator plant to determine the residues of penoxsulam + cyhalofop butyl 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 2) 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 (Fig 1a, 1b, 1c and 1d).The best plant parameter selected for bioassay has to be very sensitive and correlate well with herbicide concentration. Gowda *et al*. (2003) opined that in soil bioassay, fresh weight of setaria seedlings showed wide range of response and high R^2 value (0.93) compared to other parameters (shoot length and dry weight) and was selected as the most sensitive parameter for detecting the fluazifop-p-butyl residue in soil. Several research reports revealed that plant height or plant dry or fresh weight were the sensitive parameters for the detection of sulfonyl urea herbicide residue in soil (Blacklow and Pheloung, 1991; Gunther *et al*., 1993; Vicari *et al*., 1994; Stork and Hannah, 1996).

Determination of herbicide residue

Perusal of data on germination percentage, shoot length, shoot fresh and dry weight and root length of maize grown in soil collected after the harvest of both kharif and *rabi* crop were statistically analysed to find out the residual effect of penoxsulam + cyhalofop butyl in soil and are presented in table 3. Though shoot fresh weight of maize was selected as the best parameter to detect the herbicide residue, other growth parameters were also assessed. Results of the study 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 could be inferred that the herbicide, penoxsulam + cyhalofop butyl 6 % OD applied at 120, 125, 130 and 135 g ha^{-1} did not leave any phytotoxic residue in soil and are environmentally safe. It is an ideal herbicide provides selective weed control for sufficiently long period of time and dissipates before the cropping season without leaving any phytotoxic residue. The pre-plant soil application of trifluralin at the recommended dose (0.5 to 1 kg ha^{-1}) had no problem of residue in soil after the harvest of jute crop as evidenced from bioassay studies with *Avena fatua* (Sarkar *et al*. 2005). Bioassay with baby corn, cucumber and soya bean indicated that the residues from pre- emergence herbicides *viz*., acetochlor, alachlor, clomazone, isoxafutole, metribuzin, oxadiazon, pendimethalin + oxadiazon and metribuzin + pendimethalin did not have any phytotoxic effect or growth retardation in the tested plants (Pornprom *et al*., 2010). Poddar*et al*. (2014) reported that application of oxyfluorfen at different concentrations (150 to 300 g ha^{-1}) for the control of weeds in DSR did not hamper the population of succeeding crops of lentil, linseed and coriander after the rice in two years of study, indicating that oxyfluorfen did not leave any phytotoxic residue in soil.

From the study, it can be concluded that, maize was the best indicator plant among the three test crops, and shoot fresh weight of maize was the most sensitive parameter to detect the phytotoxic residue of penoxsulam + cyhalofop butyl in soil. Bioassay study with maize during kharif 2014 and *rabi* 2014-15 revealed that post emergence application of penoxsulam + cyhalofop butyl @ 120, 125, 130 and 135 g ha-1did not cause any growth inhibition in the growth parameters of maize, viz.,

Table 1: Effect of different concentrations of penoxsulam + cyhalofop butyl on the growth parameters of tested indicator plants

Treatments	Growth parameters of indicator plants														
	Cucumber					Sunflower					Maize				
	Germination (%)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Germination (%)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Germination (%)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
T_1	33.33	0.83	0.62	0.07	0.002	96.67	1.74	0.15	0.207	0.019	86.67	2.50	1.04	0.106	0.014
T_2	33.33	1.18	1.10	0.10	0.004	100.00	2.19	0.36	0.327	0.026	100.00	10.91	2.70	0.543	0.047
T_3	50.00	4.68	1.44	0.17	0.012	100.00	3.10	0.53	0.373	0.027	90.00	27.23	5.81	1.060	0.064
T_4	53.33	7.20	1.73	0.24	0.014	93.33	3.99	0.77	0.398	0.027	100.00	34.17	13.10	1.270	0.077
T_5	56.67	9.66	3.24	0.43	0.022	93.33	8.09	1.50	0.520	0.029	100.00	38.47	14.67	1.613	0.113
T_6	56.67	11.41	4.96	0.46	0.024	100.00	10.61	2.59	0.577	0.036	93.33	42.61	21.51	1.730	0.114
T_7	56.67	12.33	5.80	0.53	0.028	100.00	19.33	7.12	1.037	0.040	93.33	44.98	22.44	1.860	0.125
T_8 (control)	46.67	14.57	9.03	0.72	0.035	100.00	19.87	8.14	1.22	0.044	100.00	51.73	27.11	1.880	0.149
SEm (±)	**#**	**0.655**	**0.438**	**0.040**	**0.002**	**#**	**0.303**	**0.445**	**0.0767**	**0.0042**	**#**	**2.261**	**1.675**	**0.168**	**0.008**
LSD (0.05)		**2.775**	**1.857**	**0.119**	**0.0063**		**0.909**	**1.336**	**0.2300**	**0.0127**		**6.777**	**5.023**	**0.5027**	**0.0247**

 # - mean value

Table 2: R^2 values of different parameters of tested indicator plants, Y= a + b ln (X) to identify the most sensitive indicator plant for the herbicide, penoxsulam + cyhalofop butyl 6% OD

Parameters	R^2 values		
	Cucumber	Sunflower	Maize
Shoot fresh weight	0.9042	0.7007	0.9854
Shoot dry weight	0.9625	0.8787	0.9725
Shoot length	0.9424	0.7301	0.9644
Root length	0.8243	0.5917	0.8993

Table 3: Residual effect of penoxsulam + cyhalofop butyl on the growth parameters of maize (*kharif* 2014 and *rabi* 2014-15)

Treatments	*Kharif* 2014					*Rabi* 2014-15				
	Germination (%)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	Germination (%)	Shoot length (cm)	Root Length (cm)	Fresh weight (g)	Dry weight (g)
Penoxsulam + cyhalofop butyl @ 120 g ha^{-1}	73.33 (59.71)	28.00	24.20	2.14	0.244	66.67 (55.08)	27.03	24.73	2.36	0.284
Penoxsulam + cyhalofop butyl @ 125 g ha^{-1}	70.00 (57.00)	25.07	25.00	2.17	0.270	80.00 (63.93)	26.77	25.00	2.11	0.270
Penoxsulam + cyhalofop butyl @ 130 g ha^{-1}	80.00 (63.44)	30.37	31.77	1.98	0.264	66.67 (55.78)	26.90	23.93	1.94	0.282
Penoxsulam + cyhalofop butyl @ 135 g ha^{-1}	73.33 (59.71)	27.50	29.23	2.10	0.306	70.00 (57.29)	27.07	24.93	2.37	0.281
Penoxsulam @ 22.5 g ha^{-1}	73.33 (59.21)	29.17	26.33	2.27	0.266	66.67 (54.78)	26.13	25.47	2.67	0.246
Hand weeding twice at 20 and 40 DAS	66.67 (55.08)	28.37	24.93	2.09	0.263	63.33 (52.78)	25.53	25.30	2.42	0.293
Weedy check	60.00 (50.85)	28.07	26.00	2.06	0.257	70.00 (56.99)	26.03	24.17	2.14	0.263
SEm (±)	**4.600**	**2.149**	**2.430**	**0.086**	**0.017**	**4.951**	**0.426**	**0.462**	**0.243**	**0.027**
LSD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

Figures in the parentheses are angular transformed values.

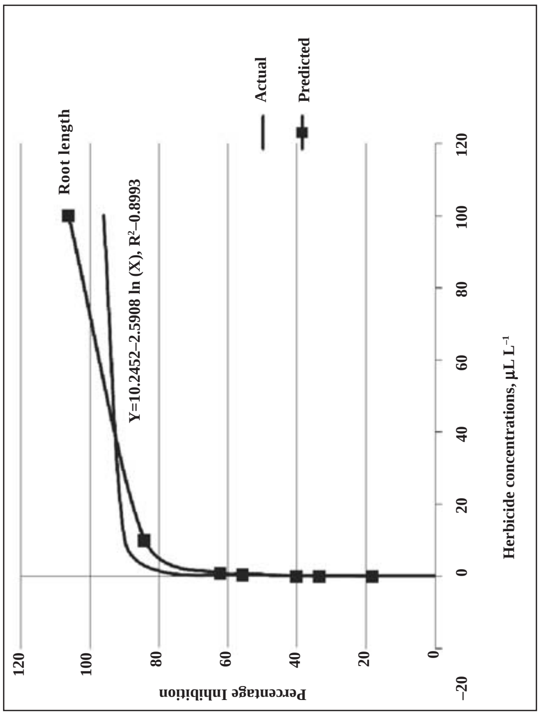

Fig.1a: Percentage growth inhibition in the root length of maize, as influenced bydifferent concentrations of penoxsulam + cyhalofop butyl

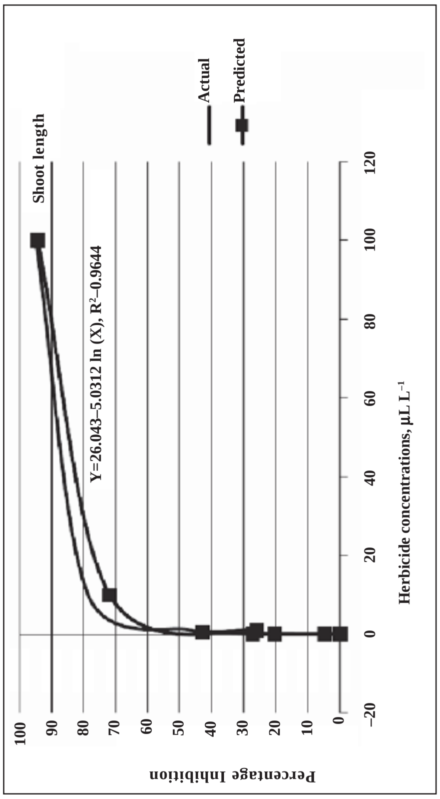

Fig.1b: Percentage growth inhibition in the shoot length of maize, as influenced by different concentrations of penoxsulam + cyhalofop butyl

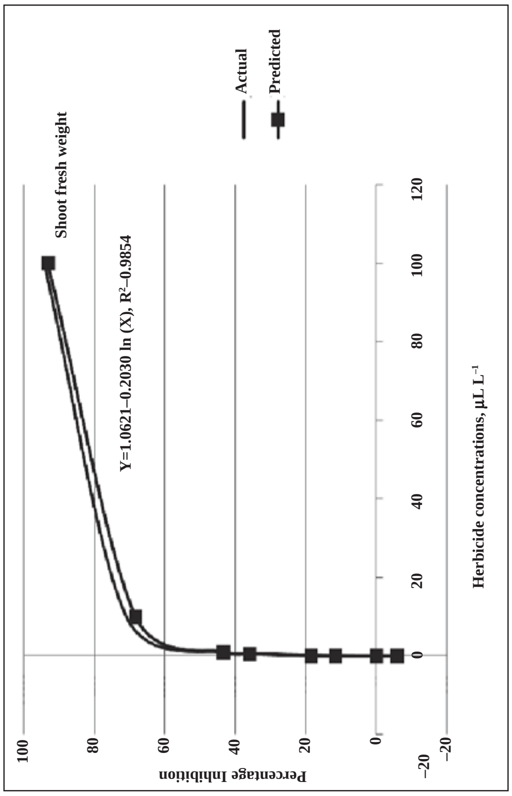

Fig.1c: Percentage growth inhibition in the shoot fresh weight of maize, as influenced by different concentrations of penoxsulam + cyhalofop butyl

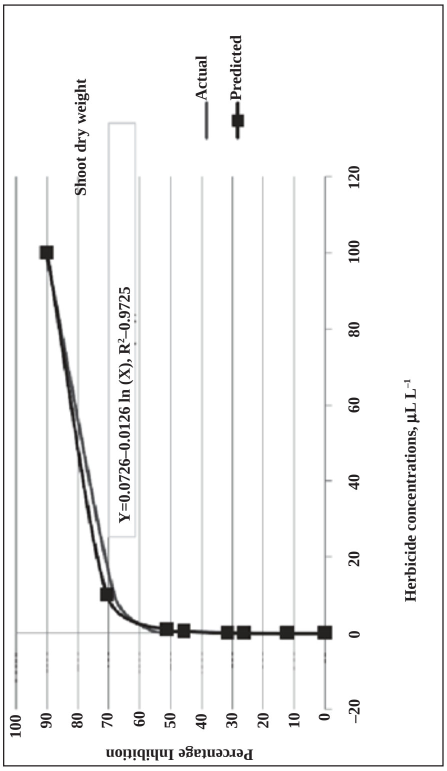

Fig.1d: Percentage growth inhibition in the shoot dry weight of maize, as influenced by different concentrations of penoxsulam + cyhalofopbutyl

germination percentage, shoot fresh weight, shoot dry weight, shoot length and root length inferring that the herbicide is environmentally safe without leaving any phytotoxic residue in soil..

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