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# STUDIES ON THE PERSISTENCE OF DICLOSULAM IN SOYBEAN CROP

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## ABSTRACT

*A three season field trial was conducted under West Bengal conditions during 2007–09 to evaluate the dissipation pattern of Diclosulam (84 WDG) in soybean at two application rates (26 and 52 g a.i./ha). The quantitative analysis of the herbicide residues was performed using HPLC with UV-VIS detector. The average recovery was found to be 90.67%, 88.67%, 88.33% and 89.33% for soybean cropped soil, soybean plant, soybean oil and de-oil cake, respectively. Following the first order kinetics, the herbicide dissipates in soybean cropped soil with half-life ($T_{1/2}$) values ranging between 5.28 - 6.02 days, 7.52 - 8.36 days and 6.27 - 6.84 days in three consecutive seasons, irrespective of the doses. Diclosulam residues were below detectable level (BDL) in plant samples irrespective of the treatment doses and the days in all seasons. No residues were detected in untreated control samples of field soil and plant during the entire study period. Furthermore, soybean oil and its de-oil cake were also analyzed and Diclosulam residues were found well below the detectable limit, irrespective of the season and treatments. So it may be concluded from the study that Diclosulam will not pose any residual toxicity problem in soybean crop.*

**Key words:** Diclosulam, dissipation, herbicide, persistence.

## INTRODUCTION

Diclosulam, a novel Triazolopyrimidine sulfonamide class of herbicide, is one of the new molecules which are highly effective for controlling broad-leaved weeds. It has been studied in field trials since 1990 and was first registered in 1997 in Argentina and Brazil. It was later registered in Bolivia (1998), Paraguay (1998) and the U.S. (2000). It is classified as a “not likely” human carcinogen. Diclosulam is not a developmental or reproductive toxicant. Based on the results of several subchronic, chronic and developmental reproductive toxicity studies, there was no evidence of neurotoxicity (source: http://www.epa.gov/opprd001/factsheets/appen_j.pdf).

Diclosulam inhibits acetolactate synthase (ALS), the enzyme responsible for biosynthesis of branch-chain amino acids and thereby cell division and growth of the weeds are quickly arrested. As an active

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ingredient, Diclosulam has activity both soil applied and post-emergence. Diclosulam can be soil applied in any tillage system since it does not require incorporation. The herbicide is a highly active, low dose compound. Its longevity in the soil makes Diclosulam ideal for control of broadleaf weeds in soybean and peanuts (Sheppard *et al*., 1997).

In soybean and peanut, Diclosulam is rapidly metabolized by facile conjugation with homoglutathione, which displaces the 7-fluoro substituent (Owen, 2000). The objective of the present work was to study the dissipation and the fate of Diclosulam residue in/on soybean in different seasons under West Bengal condition.

## MATERIALS AND METHODS

Field study was conducted at University Experimental Field, Mohanpur, BCKV for three consecutive seasons from August 2007 to November 2007 ($1^{st}$ season), August 2008 to November 2008 ($2^{nd}$ season) and August 2009 to November 2009 ($3^{rd}$ season) on soybean [variety- PK-472].

The climatic parameters of the soybean field in different seasons are presented in Table-1.

**Table-1. Climatic condition during the field study.**

| Parameters | Kharif,2007 | Kharif,2008 | Kharif,2009 |
|---|---|---|---|
| Average Maximum Temperature ($^0$C) | 31.85 | 32.38 | 32.18 |
| Average Minimum Temperature ($^0$C) | 23.98 | 23.38 | 23.35 |
| Average RH I (%) | 94.33 | 94.05 | 94.65 |
| Average RH II (%) | 70.85 | 68.53 | 69.45 |
| Average Rainfall (mm) | 236.85 | 130.28 | 169.45 |
| Other pesticides applied to trial plots | - | - | - |

### Application details and sampling details

The formulation Diclosulam 84 WDG was applied with a knapsack sprayer equipped with WFN 40 nozzle @ 26 g a.i.ha$^{-1}$ ($T_1$) and @ 52 g a.i. ha$^{-1}$ ($T_2$) in Randomized Block Designed (RBD) plots and untreated control ($T_3$) plots. Three replications were used for each treatment. Spraying of herbicide was done once one month after sowing of the soybean crop for three consecutive seasons. Soybean field soil and plant samples were collected at 0, 1, 3, 7, 15, 30 and 60 days after application of the herbicide for dissipation study in all the seasons.

Soybean plant, soybean seed and cropped soil samples (for all seasons) were also collected at the time of harvest following standard sampling procedures. Soybean plant and seed (250 g) and field soil (1 kg) samples were collected from 5-7 places randomly in each treatment plot replication on each date of sampling. Samples from untreated control plots were collected in the same way. Soil samples were collected from a depth of 6″ with the help of soil auger.

**Residue Analysis**

Soil samples for the respective sampling dates were added to100 mL of a mixture of acetone: water (8:2), kept overnight and then shaken for 30 minutes using a mechanical shaker at 25°C. They were then filtered and the extract collected and the sample re-extracted using a further 100 mL mixture of acetone: water (8:2). The combined filtrate was concentrated by evaporating the acetone portion and then transferred to a 500 mL separatory funnel. Then 100 mL of distilled water was added to it. This mixture was partitioned thrice (100+50+50) with dichloromethane, and the dichloromethane fraction was collected through anhydrous $Na_2SO_4$. This combined fraction was concentrated to 1-2 mL in a Rotary Vacuum Evaporator at 40°C.

A chromatographic column was packed up with a mixture of 10 g Silica gel and Florisil (1: 1). Anhydrous sodium sulphate was placed at the bottom and top of the column using n-hexane. The residue was transferred into the column. Elution was done with 100 mL hexane followed by 100 mL of hexane: dichloromethane (8:2) mixture and then 100 mL methanol. Methanol fraction was evaporated to dryness in a rotary vacuum evaporator at 40°C and the volume was reconstituted in HPLC grade methanol for HPLC analysis.

Plant samples were homogenized with 100 mL mixture of acetone: water (8:2). They were then filtered and the extract collected, and re-extracted using 100 mL mixture of acetone: water (8:2). The combined filtrate was concentrated to evaporate the acetone portion and then transferred to a 500 mL separatory funnel. This mixture was partitioned thrice (100+50+50) with a hexane: ethyl acetate (9:1) mixture. The aqueous phase was then partitioned thrice (100+50+50) with dichloromethane. The same process as mentioned above was followed for the field soil.

The soybean seed samples (50 g) were ground in a grinder and subjected to Soxhlet extraction with 150 mL of hexane for 6 hrs. The oil portion dissolved in hexane was then partitioned thrice (100+50+50) with acetonitrile and the acetonitrile fraction collected over anhydrous $Na_2SO_4$ and the combined organic phase was evaporated in a rotary vacuum evaporator. The residue was collected in 1-2 mL of dichloromethane and then the same procedure followed for column chromatography as described above for the field soil. The deoil cake (10 g) obtained from the oil extraction step was analysed with the same procedure as for the field soil.

**Instrumental Parameters**

| | | | |
|---|---|---|---|
| i) | Column | : | Thermo $C_{18}$, 250 mm 4.6 mm |
| ii) | Mobile phase | : | Methanol: Water (1:1) |
| iii) | Flow rate | : | 0.5 mL/ min |
| iv) | Detector | : | UV-VIS detector |
| v) | Wavelength ($\lambda_{max}$) | : | 235 nm |
| vi) | Retention time | : | 5.36 ± 0.20 min |
| vii) | Injection volume | : | 20 μL |
| viii) | LOD (Limit of Detection) | : | 0.02 ppm |
| ix) | LOQ (Limit of Quantification) | : | 0.05 ppm |

**Linearity Check**

A calibration curve (Figure 1) was constructed by plotting seven concentrations (0.05–2.00 μg/g) of standard Diclosulam versus absorption. Also, to determine the interference of each substrate, a matrix match calibration standard for each substrate was prepared. In this study, the calibration curve was prepared by taking the areas corresponding to different concentrations of the matrix match calibration standard, against which final quantification was done.

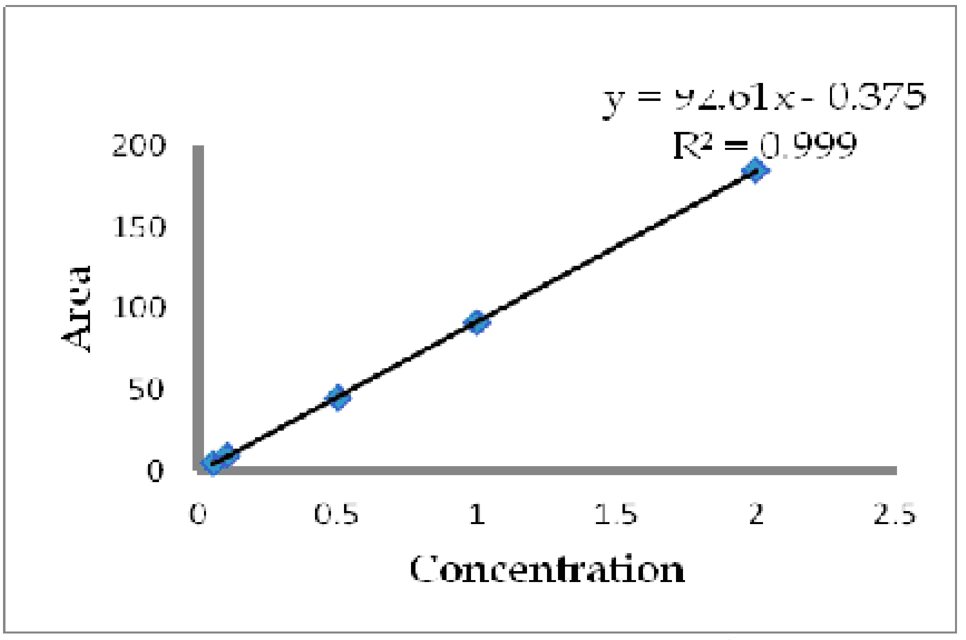


**Figure 1. Analytical standard calibration curve of diclosulam.**

## RESULTS AND DISCUSSION

Recovery studies were carried out to establish the reliability of the analytical method and to determine the efficiency of extraction and clean up steps employed for the present study. The average recovery was found to be 90.67%, 88.67%, 88.33% and 89.33% for soybean cropped soil, soybean plant, soybean oil and de-oil cake, respectively (Table-2). As the recovery percentage is more than 85% for all the substrates, the method can be adopted for residue and dissipation study of Diclosulam in different substrate of soybean.

The results of this field study of persistence of Diclosulam in soybean cropped soil have been summarized in Table-3 (season-I), Table-4 (season-II) and Table-5 (season-III). A straight line was found in all cases, when the log of residue was plotted against time, thereby establishing that first order reaction kinetics were involved in the dissipation process. The half-life values ($T_{1/2}$) in soybean cropped soil were in the range of 5.28-6.02 days, 7.52-8.36 days and 6.27-6.84 days in three consecutive seasons irrespective of dose used. More than 75% of the initial deposit was dissipated within 15 days irrespective of doses and seasons. Murdock and Witt (1999) reported that Diclosulam followed first order rate kinetics, the dissipation was relatively rapid, and half-life values ranged from 7-16 days in various seasons. Diclosulam residues were below detectable level (BDL) in plant samples irrespective of the treatment doses and days in all the seasons.

**Table-2. Recovery study of Diclosulam in different substrates.**

| Substrate | Amount fortified (ppm) | Amount recovered (ppm) | % Recovery | Average % recovery |
|---|---|---|---|---|
| | 0.05 | 0.046 | 92.00 | 90.67 |
| Field Soil | 0.10 | 0.089 | 89.00 | |
| | 1.00 | 0.910 | 91.00 | |
| | 0.05 | 0.043 | 86.00 | 88.67 |
| Plant | 0.10 | 0.091 | 91.00 | |
| | 1.00 | 0.880 | 89.00 | |
| | 0.05 | 0.043 | 86.00 | |
| Oil | 0.10 | 0.088 | 88.00 | 88.33 |
| | 1.00 | 0.910 | 91.00 | |
| | 0.05 | 0.045 | 90.00 | |
| Deoil Cake | 0.10 | 0.089 | 89.00 | 89.33 |
| | 1.00 | 0.890 | 89.00 | |

* Average of three replicates

**Table-3. Dissipation of Diclosulam in soybean cropped soil in season-I.**

| DAA*days after application | Treatment | Residues in ppm | | | | Dissipation (%) |
|---|---|---|---|---|---|---|
| | | $R_1$ | $R_2$ | $R_3$ | Mean ± S.D | |
| 0 | $T_1$ | 0.86 | 0.81 | 0.77 | 0.81 ± 0.037 | - |
| 1 | | 0.74 | 0.68 | 0.72 | 0.71 ± 0.025 | 12.34 |
| 3 | | 0.61 | 0.54 | 0.57 | 0.57 ± 0.029 | 29.62 |
| 7 | | 0.27 | 0.23 | 0.26 | 0.25 ± 0.017 | 69.13 |
| 15 | | 0.13 | 0.11 | 0.12 | 0.12 ± 0.008 | 85.18 |
| 30 | | BDL | BDL | BDL | - | - |
| 0 | $T_2$ | 1.52 | 1.58 | 1.48 | 1.53 ± 0.041 | - |
| 1 | | 1.36 | 1.31 | 1.34 | 1.34 ± 0.021 | 12.41 |
| 3 | | 1.11 | 1.18 | 1.15 | 1.15 ± 0.029 | 24.83 |
| 7 | | 0.71 | 0.65 | 0.62 | 0.66 ± 0.037 | 56.86 |
| 15 | | 0.31 | 0.24 | 0.26 | 0.27 ± 0.029 | 82.35 |
| 30 | | BDL | BDL | BDL | - | - |
| $T_1$: Y = -0.057x+2.897<br>$T_{1/2}$ = 5.28 Days | | | $T_2$: Y = -0.050x+3.186<br>$T_{1/2}$ = 6.02 Days | | | |

* DAA Days after application **BDL below detectable limit

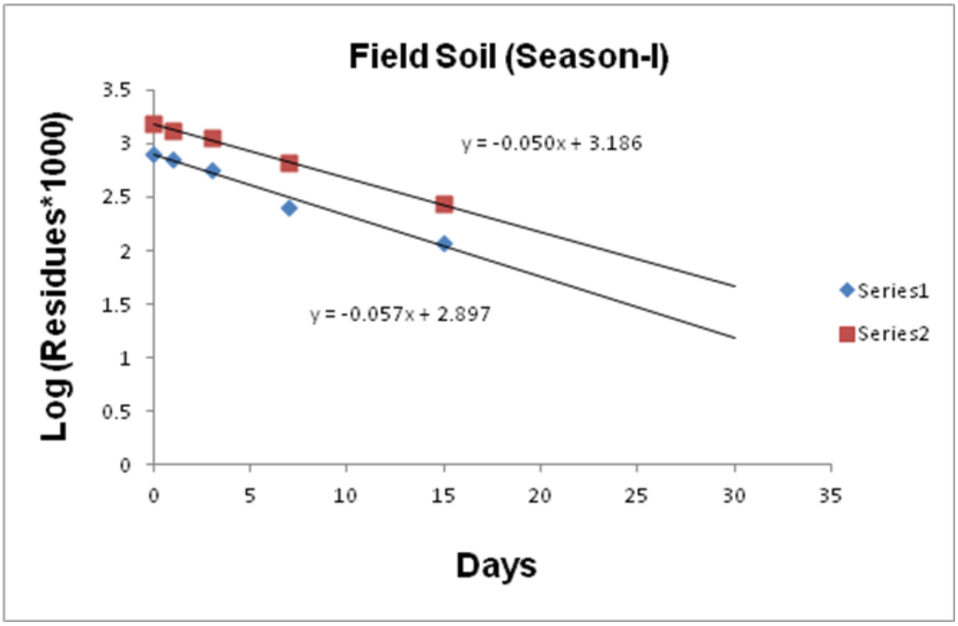


**Figure 2. Linear plot of dissipation of Diclosulam in field soil in season-I.**

**Table-4. Dissipation of Diclosulam in soybean cropped soil in season-II.**

| DAA* | Treatment | Residues in ppm | | | | Dissipation (%) |
|---|---|---|---|---|---|---|
| | | $R_1$ | $R_2$ | $R_3$ | Mean ± S.D | |
| 0 | | 0.93 | 0.95 | 0.89 | 0.92 ± 0.025 | - |
| 1 | | 0.75 | 0.70 | 0.73 | 0.73 ± 0.021 | 20.29 |
| 3 | | 0.67 | 0.59 | 0.62 | 0.63 ± 0.033 | 31.88 |
| 7 | $T_1$ | 0.47 | 0.43 | 0.45 | 0.45 ± 0.016 | 51.45 |
| 15 | | 0.18 | 0.25 | 0.20 | 0.21 ± 0.029 | 76.81 |
| 30 | | BDL | BDL | BDL | - | - |
| 0 | | 1.63 | 1.60 | 1.54 | 1.59 ± 0.037 | - |
| 1 | | 1.45 | 1.39 | 1.38 | 1.41 ± 0.031 | 11.11 |
| 3 | | 1.33 | 1.28 | 1.32 | 1.31 ± 0.022 | 17.82 |
| 7 | | 0.81 | 0.72 | 0.75 | 0.76 ± 0.037 | 52.20 |
| 15 | $T_2$ | 0.48 | 0.44 | 0.46 | 0.46 ± 0.016 | 71.28 |
| 30 | | BDL | BDL | BDL | - | - |
| $T_1$: Y = -0.040x+2.931 $T_{1/2}$ = 7.52 Days | | | | $T_2$: Y = -0.036x+3.192 $T_{1/2}$ = 8.36 Days | | |

*DAA Days after application **BDL below detectable limit

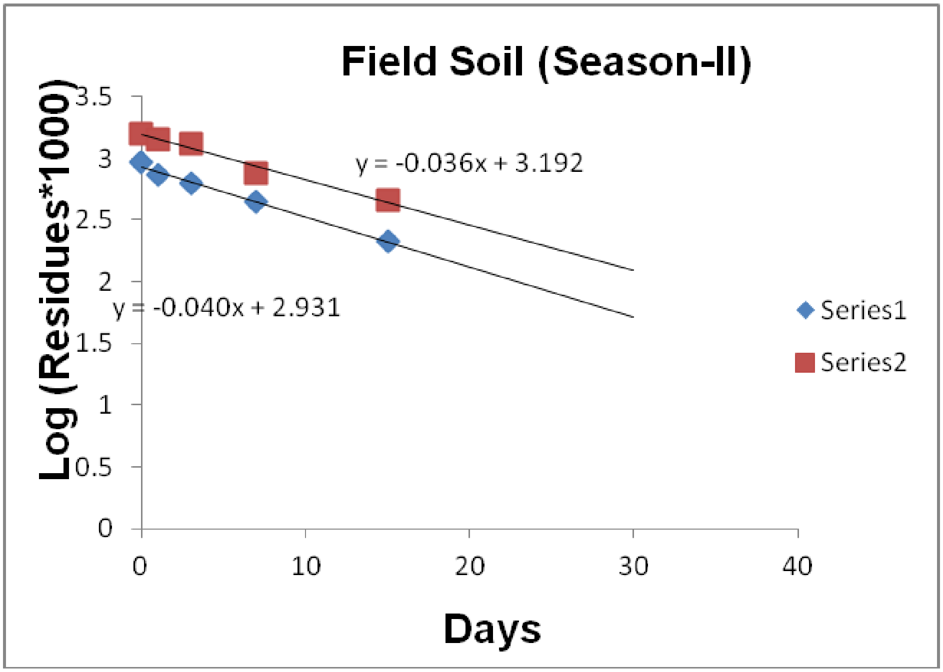


**Figure 3. Linear plot of dissipation of Diclosulam in field soil in season-II.**

**Table-5. Dissipation of Diclosulam in soybean cropped soil in season-III.**

| DAA* | Treatment | Residues in ppm | | | | Dissipation (%) |
|---|---|---|---|---|---|---|
| | | $R_1$ | $R_2$ | $R_3$ | Mean ± S.D | |
| 0 | $T_1$ | 0.84 | 0.89 | 0.86 | 0.86 ± 0.021 | - |
| 1 | | 0.78 | 0.73 | 0.75 | 0.75 ± 0.021 | 12.79 |
| 3 | | 0.65 | 0.60 | 0.61 | 0.62 ± 0.022 | 27.91 |
| 7 | | 0.41 | 0.33 | 0.35 | 0.36 ± 0.034 | 58.14 |
| 15 | | 0.18 | 0.15 | 0.16 | 0.16 ± 0.012 | 81.40 |
| 30 | | BDL | BDL | BDL | - | - |
| 0 | $T_2$ | 1.65 | 1.59 | 1.63 | 1.62 ± 0.025 | - |
| 1 | | 1.37 | 1.44 | 1.41 | 1.41 ± 0.029 | 12.76 |
| 3 | | 1.20 | 1.15 | 1.18 | 1.18 ± 0.021 | 26.95 |
| 7 | | 0.62 | 0.56 | 0.60 | 0.59 ± 0.025 | 63.79 |
| 15 | | 0.34 | 0.36 | 0.37 | 0.36 ± 0.012 | 77.57 |
| 30 | | BDL | BDL | BDL | - | - |
| $T_1$: Y = -0.048x+2.927<br>$T_{1/2}$ = 6.27 Days | | | $T_2$: Y = -0.044x+3.184<br>$T_{1/2}$ = 6.84 Days | | | |

*DAA Days after application **BDL below detectable limit

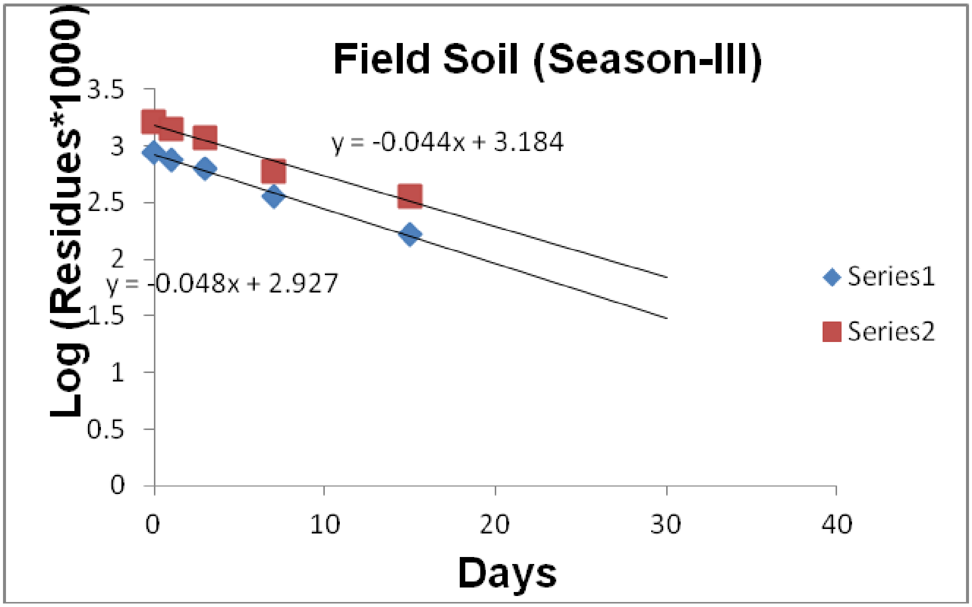


**Figure 4. Linear plot of dissipation of diclosulam in field soil in season-III.**

Diclosulam residues were found well below the detectable limit irrespective of seasons and treatments in all the substrates at harvest time. This may be due to rapid metabolism of

Diclosulam in the soybean plant leaving non-toxic metabolites in the plant system as described by Kramer and Schirmer (2007). The MRL value of Diclosulam in soybean in India has not yet been established. On the basis of above facts it may be concluded that Diclosulam in this formulation does not pose any residue toxicity problem in soybean at harvest.

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