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Residue and Bio-Efficacy Evaluation of Controlled Release Formulations of Metribuzin Against Weeds in Wheat

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Abstract Controlled release formulations of metribuzin in polyvinyl chloride, (emulsion); carboxy methyl cellulose, CMC and carboxy methyl cellulose- kaolinite composite, CMC-KAO are reported. The MET-CMC-KAO-3 (T_9) formulation provided a superior control (76.1%) of weeds in field grown wheat in comparison to metribuzin 75 DF (57.14%) at the dose (350 g a.i. ha^{-1}) after 90 days of sowing. The treatment (T_9) reduced the dry weight of the weed flora after 30 days of sowing (4.0 g m^{-2}) and significantly superior over metribuzin 75 DF (6.0 g m^{-2}) and control (17.72 g m^{-2}). There were nil to negligible metribuzin residue in soil at harvest of wheat crop and were within prescribed limit of 10 mg L^{-1} in drinking water (EPA).

Keywords Controlled release metribuzin · Herbicidal activity · Residue

Metribuzin (4-amino-6-*tert*-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one), an asymmetrical triazine herbicide is used globally as a potent destroyer of *Phalaris minor* in wheat, *Cynodon dactylon* in potato, *Chenopodium album* in tomato and *Cyperus* spp. in sugarcane. It is weakly sorbed in soils and thus has potential for downward movement in water (Bedmar et al. 2004)· Several reports on the presence of metribuzin in ground waters suggest that its leaching to lower horizons of soil profiles is primarily responsible for the loss of its activity (Peek and Appleby 1991). Due to less availability of an effective dose for combating pests, repeat applications are necessitated which harm non-target organisms and cause ground water pollution. These limitations make it a good candidate for developing its controlled release formulations. Researchers all over the world have addressed to development of newer products and technologies that mitigate the undesirable effects of agrochemicals. Controlled pesticide release (CR) technology has emerged as an important application approach during the last three decades or so. It is owing to its ability to mitigate undesirable environmental effects encountered during applications by conventional methods for an effective pest control. Numerous examples in literature highlight such products to effectively combat pests (Mulqueen 1998; Kumar et al. 2003; Kumar et al. 2006; Choudhary et al. 2006a). Effect of different matrices on release of metribuzin from alginate band and sepiolite-gel CR formulations is reported (Maqueda et al. 2008). Earlier work carried out in this laboratory revealed that release of active ingredients was the slowest in polyvinyl chloride and the fastest in carboxy methyl cellulose (Choudhary et al. 2006b; Nisar et al. 2009). In view of the yield losses due to weeds and herbicide consumption, the controlled release (CR) formulations of metribuzin were developed by employing polyvinyl chloride, carboxy methyl cellulose and a composite of carboxy methyl cellulose- kaolinite. The purposes of this study were to evaluate the efficacy of a new formulation for weeds in wheat and the post-application residues of metribuzin with time.

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Materials and Methods

Commercial grade kaolinite (MCA Industries, New Delhi, India), carboxy methyl cellulose-Na (Merck India Ltd., Mumbai, India) and polyvinyl chloride-emulsion (Loba-Chemie Indo-Austranal Co., Mumbai, India) were used. Technical Grade, 94%, m/m and commercial granules 75 DF of metribuzin were obtained through the courtesy of Rallis India Ltd., Agrochemical Division, Ratnagiri, India. For routine work, laboratory grade and for HPLC analysis, analytical grade chemicals and solvents were employed.

Controlled Release Formulations were prepared as follows: Sodium carboxy methyl cellulose–kaolinite based formulations (MET-CMC-KAO-3) was prepared using a mixture (103.83 g) of sodium carboxy methyl cellulose, kaolinite and metribuzin (technical 3.83 g, 94%, m/m purity) was prepared in the ratio of 50:50: 3.83, m/m, to yield products with 3% a.i. It was mixed well in a mixer grinder. Water (250 mL) was added to obtain dough. To overcome stickiness, 51.0 mL of aluminium phosphate (0.5 M) was added as gelling agent. The resultant mass was dried at 40°C in an oven for 4–6 h and ground using a laboratory Wiley mill. It was sieved to obtain 30/60 mesh sized particles. For preparation of Sodium carboxy methyl cellulose (MET-CMC-3), a mixture of sodium carboxy methyl cellulose and metribuzin (technical 3.83 g, 94%, m/m purity) was prepared in the ratio of 100: 3.83 by weight to obtain 3% active ingredient and mixed well in a mixer grinder. The mixture was processed as per the method used for the preparation of MET-CMC-KAO-3. For preparing Polyvinyl chloride (MET-PVC-3), polymer (200 g) and metribuzin (technical 6.64 g, 94%, m/m purity) were dissolved in a compatible organic solvent and mixed thoroughly mixed with a spatula. Obtained slurry was dried in a Petri dish to yield a hard mass which was ground using a laboratory Wiley mill and sieved to obtain 30/60 mesh sized particles.

To Estimate metribuzin content in CR Formulations 50 mg samples of MET-CMC-3 and MET-CMC-KAO-3 each were taken in acetone (15.0 mL) and placed in a sonicated bath for 10 min. to disintegrate the granules. The containers were left at room temperature for 2 h, sonicated twice for 10 min each (2 times) and filtered. Acetone was removed from the filtrate in a rotavapor at 30°C under reduced pressure. The residue was taken in hexane and filtered quantitatively through a syringe filter (0.2 μm). The volume was made up to 15.0 mL and used for analysis. Fifty mg sample of the MET-PVC-3 formulation was refluxed in acetone for 6 h and filtered. Acetone was removed from the filtrate in a rotavapor at 30°C under reduced pressure. The residue was taken in hexane and filtered quantitatively through a syringe filter (0.2 μm). The volume was made up to 15.0 mL and used for analysis.

Encapsulation efficiency (EE)/Recovery of metribuzin in test formulation was calculated as follows:

$$\mathrm{EE/Recovery}\ (\%) = \frac{\text{Active ingredient present in test formulation}}{\text{Active ingredient incorporated in test formulation}} \times 100$$

Encapsulation efficiency/Recoveries of metribuzin from MET-CMC-3, MET-CMC-KAO-3 and MET-PVC-3 were 94.0, 96.80 and 90.90% respectively.

Field experiments were conducted to assess the bio-efficacy and residual behaviour of controlled release and commercial formulations of metribuzin against *Phalaris minor* Retz. and other weeds in wheat. Wheat variety PBW 343 was obtained from the Division of Agronomy of the Institute. It was raised in simple randomized block design during Rabi season (November–April), 2007–08 in plots of 2 m × 2 m in three replicates adopting the recommended agricultural practices. Four formulations of metribuzin *viz* MET-CMC-3, MET-CMC-KAO-3 and MET-PVC-3 granules (at three dosage level) and commercial 75 DF (at one dosage level) used in the study. MET-PVC-3@ 87.5 (T_1), 150 (T_2), 350 (T_3); MET-CMC-3@ 87.5 (T_4), 150 (T_5), 350 (T_6); MET-CMC-KAO-3@ 87.5 (T_7), 150 (T_8), 350 (T_9); g a.i. ha $^{-1}$ were broadcast and the commercial formulation, Metribuzin 75 DF@ 350 (T_{10}) g a.i. ha^{-1} sprayed at the time of sowing. The treatment (T_{11}) served as control.

Bioefficacy was evaluated based on the effect on weed population and their dry matter content. Weed population count and weed samples for dry matter production was taken to assess the effect of the treatments on weed growth. An area of 0.50 m^2 was randomly marked for recording population of different weed species. A metallic quadrate of 0.5 m × 0.5 m was placed at two places at 30 and 90 days after sowing (DAS) and at harvest. The weed number was expressed on square metre basis (m^{-2}). The data were transformed $\sqrt{(x + 0.5)}$ to minimize variation among the treatments before subjecting to statistical analysis. The dry matter contents of weeds collected from 0.5 m^2 area were recorded. These were initially sun dried followed by drying to constant weight an oven at 70°C. The total dry weight was recorded at 30 and 90 DAS and expressed as g m^{-2}.

The samples of soil were collected after crop harvest and were analyzed for metribuzin residues with GLC as per the procedure described below. After harvesting the wheat crop, soil sample was taken from three different locations of each plot. Around 500 g of soil was collected from tillage depth and thoroughly mixed and quartered. This was sieved and air dried for 48 h. One hundred g soil was taken into a 250 ml stoppered conical flask, 10 g each of ammonium sulphate and anhydrous sodium sulphate added and metribuzin extracted in acetone (150 mL × 3) employing an end-to-end shaker for 4 h. The sample was allowed to stand for 5 min and the supernatant decanted to another flask. The extracts was passed through a column of activated charcoal and anhydrous sodium sulphate to remove the impurities and filtered. Acetone was removed from the filtrate and residue dissolved in a known volume of hexane and metribuzin estimated by GLC. Untreated soil were spiked with known amount of metribuzin (1,000 ppm) and equilibrated for 1 h prior to extraction and subsequently subjected to the extraction procedure described above. It did not reveal any peak in the region of metribuzin. The extraction efficiency of metribuzin in soil based on the mean value of three replications was 98.2%, respectively. The limit of detection of metribuzin in soil was 0.010 $\mu g\ g^{-1}$.

A Hewlett Packard 5890 II series Gas Chromatograph fitted with Ni^{63} electron capture detector was used. The oven, injector and detector temperatures were maintained at 160, 250, and 300°C respectively. Samples were resolved on a HP-1 [10 m × 0.50 mm (i.d.) × 2.53 µm film thickness] column using N_2 gas as mobile phase at 45 mL min^{-1}. Metribuzin content was estimated by using the formula:

$$\text{Residue of metribuzin } (\mu g\ g^{-1}) = As/Astd \times M/M1 \times V/V1 \times f$$

where As = Peak area of the sample, Astd = Peak area of the standard, M = Volume of standard injected (µL), M1 = Weight of the sample (g), V = Volume of the final extract (mL), V1 = Volume of the sample injection (µL), f = Recovery factor; 100% mean recovery.

Results and Discussion

The bioefficacy data against *Phalaris minor* Retz. and other weeds is reported in Table 1. The major weeds observed in the experimental field in order of their density at 30 and 90 days after sowing (DAS) were as follows; 30 DAS: *Chenopodium album* > *Phalaris minor* > *Coronopus didymus* > *Melilotus indica* > *Anagallis arvensis* > *Spergula arvensis;* 90 DAS: *Chenopodium album* > *Avena fatua* > *Phalaris minor* > *Melilotus indica* > *Coronopus didymus* > *Anagallis arvensis.*

Data in Table 1 reveal that total population of weeds per 0.25 m^2 varied significantly in different treatments. All the treatments resulted in a significant reduction of weed population as compared to untreated control. The observations were taken at 30 and 90 days after sowing. Most of the herbicidal treatments except T_1 and T_4 caused a significant reduction in weed population as compared to control. T_9 (MET-CMC-KAO-3 @ 350 g a.i. ha^{-1}) was the most effective treatment at both 30 and 90 DAS followed by T_3, T_5, T_6, T_8 and others. T_1 and T_4 at 30 DAS were at par with control. The remaining treatments were in between in performance. The weed population was invariably lower at 90 DAS as compared with the corresponding value at 30 DAS. All treatments employing 350 g a.i. ha^{-1} as well as commercial 75 DF were at par at 90 DAS and were significantly superior over control. T_3 was the most effective formulation. Amongst the 175 g a.i. ha^{-1} treatments, T_2 was the most effective though at par with T_5 and T_8 at 90 DAS. All the treatments employing 87.5 g a.i.

Table 1 Effect of controlled release and commercial 75 DF formulations of metribuzin on weed population and their dry weights

Treatment no.	Weed			
	Population (No. m^{-2})		Dry weight (g m^{-2})	
	30 DAS	90 DAS	30 DAS	90 DAS
T_1	5.16^{ab} (26.00)	3.46^{bc} (12.00)	3.75^{b} (14.0)	2.91^{abcd} (8.63)
T_2	4.99^{bc} (25.00)	2.64^{de} (7.0)	3.54^{b} (12.53)	2.07^{def} (4.37)
T_3	4.57^{bcd} (21.00)	2.23^{e} (5.00)	2.57^{de} (6.60)	1.78^{f} (3.20)
T_4	5.06^{abc} (25.67)	3.65^{b} (13.33)	3.63^{b} (13.17)	3.33^{ab} (11.13)
T_5	4.677^{bcd} (22.00)	2.88^{cd} (8.33)	2.89^{c} (8.40)	3.279^{abc} (10.8)
T_6	4.14^{bcd} (17.33)	2.75^{de} (7.67)	2.53^{de} (6.40)	2.43^{cdef} (5.93)
T_7	4.83^{bc} (23.33)	3.69^{b} (13.67)	3.49^{b} (12.20)	3.43^{ab} (11.77)
T_8	4.42^{bcd} (19.67)	3.46^{bc} (12.00)	2.79^{cd} (7.83)	2.68^{bcde} (7.17)
T_9	3.62^{d} (13.33)	2.44^{de} (6.00)	1.99^{f} (4.00)	1.91^{ef} (3.70)
T_{10}	4.02^{cd} (16.33)	2.99^{cd} (9.00)	2.45^{e} (6.00)	2.75^{bcde} (7.57)
T_{11}	6.0824^{a} (37.00)	4.58^{a} (21.00)	4.21^{a} (17.72)	3.654^{a} (13.37)

Means with the same letter are not significantly different

Values in parentheses are transformed values

ha^{-1} were at par 90 DAS. Except T_1 which was at par, the others inferior to commercial formulation. Thus at 90 DAS, all the treatments except T_4 and T_7 (87.5 g a.i. ha^{-1}) were at par with the commercial formulation. The effect in all treatments improved with increase in dose of a.i. Table 1 revealed that at 30 DAS, all the treatments significantly reduced the dry weight of the weed flora. T_9 was the most effective and significantly superior over all the other treatments. The commercial formulation (T_{10}) and the other 350 g a.i. ha^{-1} treatments (T_3 and T_6) were the next in order and were at par. The 87.5 g a.i. ha^{-1} (T_1, T_4, T_7) were the least effective and were at par, though superior over control. At 90 DAS, T_3 was the best treatment and at par with T_9. T_1, T_4, T_5, T_7 tended to be at par with control as well as the commercial formulation. The remaining formulations performed in between. The effect of such products in reducing dry weight was generally reflected. Overall, the bioefficacy against weeds brought out the CMC-Kaolinite composite to be the best matrix for preparing controlled release formulations of metribuzin. Whereas the commercial product was degraded/leached fast, being effective for the short period only, the CR products were more consistent and regulated the release of a.i. over longer periods. Similar results are reported with respect to the release of butachlor from polyvinyl chloride, thermocol and polystyrene (Kumar et al. 2003), diuron from granules based on lignin and alginate-bentonite matrix (Fernandez-Perez et al. 1999), alachlor from alginate-encapsulated (Johnson and Pepperman 1996), imidacloprid from a lignin matrix (Fernandez-Perez et al. 1998) carbofuran from carboxy methyl cellulose, carboxy methyl cellulose-clay (kaolinite, bentonite, fuller's earth) composites and polyvinyl chloride (Choudhary et al. 2006b) and acephate from carboxy methyl cellulose, carboxy methyl cellulose-kaolinite and polyvinyl chloride (Nisar et al. 2009). The addition of bentonite in alginate polymer and carboxy methyl cellulose decreased the release of carbofuran (Fernandez-Perez et al. 2000; Choudhary et al. 2006b). The results also highlight a need for choice of an appropriate matrix for achieving best results in a given situation. With more efforts, metribuzin products with better precision could be developed to achieve the desired release and bioefficacy.

The metribuzin residue in soil was carried out at harvest of wheat crop. The extracts of control samples revealed no interference in analysis by GLC under the test conditions. There were nil to negligible metribuzin residue in soil at harvest of wheat crop (T_{10}). The commercial formulation (T_{10}) treated plot revealed no detectable residue whereas the CR formulation revealed (0.017–0.065 µg g^{-1} soil). MET-PVC-3@350 g a.i. ha^{-1} (T_3) treated soil had recorded the highest residue i.e. at the tune of 0.065 µg g^{-1} whereas MET-CMC-3@350 g a.i. ha^{-1} (T_9) recorded the least (0.017 µg g^{-1}). In the other CR formulations the residue was recorded in the order- MET-PVC-3@175 g a.i. ha^{-1} (0.037µg g^{-1}) > MET-CMC-KAO-3@350 g a.i. ha^{-1} (0.033 µg g^{-1}) > MET-CMC-KAO-3@175 g a.i. ha^{-1}(0.018 µg g^{-1}) > MET-CMC-3@350 g a.i. ha^{-1} (0.017 µg g^{-1}).

Detection of trace amount of metribuzin in CR formulations further confirms the anticipated slow release. Higher values were observed in treatments @ 175–350 g a.i. ha^{-1} whereas the residue was non-detectable in 87.5 g a.i. ha^{-1} treatments. In case of Phorate and imidacloprid, similar observations have been reported by Kumar et al. (2003) and Ramprasad et al. (2007) respectively. The values can also be considered of no toxicological significance in view of the prescribed limit of 10 mg L^{-1} in drinking water (EPA). The formulations can thus be safely recommended for weed control.

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