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Dissipation kinetics of a new mixture formulation of bispyribac-sodium and metamifop in rice

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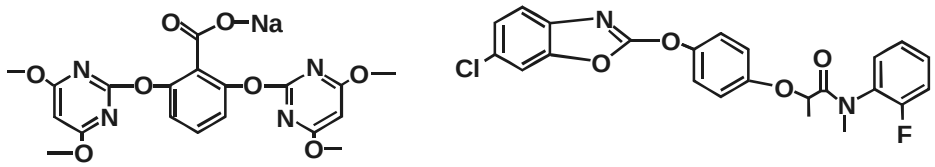
ABSTRACT

A supervised field trial on rice was conducted at the experimental farm, BCKV, West Bengal where the mixture formulation of bispyribac sodium 4% + metamifop 10% was applied at 14 days after transplanting of the rice seedlings at three doses viz. recommended dose i.e. @ 70 g a.i. ha^{-1} and double dose i.e. @ 140 g a.i. ha^{-1}and another is untreated control The residue was extracted by QuEChERS method and quantified by liquid chromatography electrospray ionization tandem mass spectrometry. The limit of quantification of both the compound was 0.02mg kg^{-1}in all the substrates (rice green plant, straw, grain, husk, and soil).The half-life ($T_{1/2}$) value of B-bispyribac sodium in soil ranged from 17.71 -23.16 days where as the $T_{1/2}$ value of metamifop in soil and rice green plant ranged from 12.54 - 15.84 and 1.91-2.71 days respectively. No residue of bispyribac sodium and metamifop was quantified in harvested rice straw, grain, husk and soil.

Keywords **:** Bispyribac sodium, half-life, metamifop, persistence, rice, soil

Rice is one of the most important food grain crops for its nutritional value throughout the world and India also. About 33 per cent of rice production is lost in India (Mukherjee, 2004) due to weed infestation while considering world scenario weeds cause about 10% yield (Oerke and Dehne, 2004) loss of rice. To reduce the production loss from the attack of weed, application of herbicide in agricultural field has become a very popular practice to the Indian farmers. Although the use of herbicide have a incontestable benefit in case of production but it's reaming residue contaminate the environmental compartment and different portion of plant, which is harmful to the environmental safety and human health (Nagami *et al*., 2004 ; Bhanti and Taneja 2007; Zhang *et al*., 2010; Ilboudo *et al*., 2014).

Bispyribac sodium(Sodium 2,6-bis[(4,6-dimethoxy-2-pyrimidinyl carboxybenzoate) (Fig. 1)is a postemergence systemic herbicide belonging to pyrimidinylcarboxy class and it works by interfering with production of acetolactate synthase (ALS) enzyme (Ding *et al*., 2009) necessary for the synthesis of branch chain amino acid *viz*. valine, leucine and isoleucine.The maximum residue limit (MRL) of bispyribac sodium in paddy under PFA is 0.05 ppm (Dureja *et al*., 2015)



(a) **(b)**

Fig. 1: Chemical structure of (a) bispyribac sodium and (b) metamifop

Metamifop (R)- 2- [4- (6- chloro- 1,3-benzoxazol -2 - yloxy) phenoxy]-2' – fluoro - N-methylpropionanilide is also a post-emergence herbicide (Fig. 1) belonging to Aryloxyphenoxy propionic acid group which is used to control a wide range of annual grass weeds in different cereal crops including rice. It inhibit the action of acetyl-CoA carboxylase (ACCase) (Kim *et al*., 2003; McCullough *et al*., 2016) which catalyzes the first committed step in de novo fatty acid biosynthesis (Sasaki *et al*., 1995; Focke *et al*., 2003). The inhibition of ACCase leads to the interruption of lipid biosynthesis and results in death of the plant. Recently a new mixture formulation of the herbicide of bispyribac sodium and metamifop is being introduced in India. Readymix formulation because of their multiple mode of action, synergistic action is gaining importance

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as an effective measure to control the wide array of weeds in a single application. As the mixture herbicide is newly introduced in the market, there is no systematic study available on its persistence dissipation behavior under Indian agro-climatic condition.

Thus the objective of the present experiment was to find out the residue and dissipation behavior of the mixture formulation (bispyribac sodium + metamifop) 14%SE in the rice plant and soil in order to predict risk on their use.

MATERIALS AND METHODS

The reference standards of metamifop and bispyribac sodium (> 99% purity) and its formulation (14% SE) were provided by PI industries Limited. The HPLC grade solvents, *viz*., methanol, acetonitrile and ethyl acetate (J. T. Baker) were used for the experiment. Primary Seconday Amine (PSA: 40 µm particle size), C_{18} (40 µm particle size) (Varian) and Graphitized carbon black (GCB) (United Chemical Technology) were used for clean up purpose. All the reagents like acetic acid, magnesium sulphate, soduim chloride, sodium sulfate and ammonium acetate (Merck) were analytical grade.

A supervised field trial was conducted on rice (IET-4786) at the experimental research farm of BCKV, Nadia, West Bengal where mixture formulation of bispyribac sodium and metamifop14% SE was applied in rice field after 14 days of transplanting of the seedlings. The three different doses were recommended dose (T_1) *i.e.* 70 g a.i ha^{-1} + 100 ml ha^{-1} adjuvant (PIW-111), double the recommended doses (T_2) *i.e.* 140 g a.i ha^{-1} + 100 ml ha^{-1} adjuvant (PIW-111) and untreated control plot (T_3). The experiment was conducted in Randomized Block Design (RBD). The size of the experimental plot was 20square meter. Plots were separated from each other by buffer zone. The treatments were replicated thrice along with an untreated control. Rice green plant (0.1 kg) and soil (1kg) samples were collected at different time intervals on 0 (2 h after spraying), 1, 3, 7, 15, 30 and 45 days after the spraying of the herbicide. Rice straw (0.5 kg), grain (1.0 kg), soil (1 kg) samples were also collected from the treated and control plots at the time of harvest. All samples were packed in a box with ice pack after proper marking and it was transferred to the laboratory for analysis. Rice plant and straw samples were cut into small pieces and then grinded with mixer grinder (Bajaj GX7). Rice grain samples were grinded directly by mixer grinder. Soil samples were passed through 2 mm sieve and the samples were extracted and cleaned immediately after collection and stored at -20^{0}C for a minimum period before analysis.

The stock solution of bispyribac sodium (100 µg ml^{-1}) and metamifop (100 µg ml^{-1}) was prepared in methanol and acetonitrile respectively and stored in freeze at 4^{0}C. Intermediate standard solutions of the herbicides were prepared by appropriate dilution with the corresponding solvents.

Recovery studies were carried out in order to establish the reliability of the analytical method and to know the efficiency of extraction and clean up steps employed for the present study. The recovery experiment was conducted by fortifying bispyribac sodium and metamifop separately into different matrices of rice (*viz.*, plant, straw, grain, husk and soil) at 0.02 ppm, 0.05 ppm and 0.10 ppm level.

The representative samples of plant (5.0 g), straw (5.0 g), grain (5.0 g), husk (5.0 g) and Soil (10.0 g) were taken in a 50 ml polypropylene centrifuge tube (Tarson) separately and 10 ml Millipore water was added. The samples were then acidified with acetic acid topH 3.0. After 10 min, 10 ml ethyl acetate was added and shaken vigorously for 3 min. Then the samples were centrifuged for 5 min at 5,000 rpm and 4 ml of supernatant was collected to carry out the clean up procedure where PSA, C_{18} and GCB (for plant matrix only) were used. Representative 1.5 ml extracted plant matrix was taken in a 2 ml micro centrifuge tube where 25 mg PSA, 25 mgC_{18}, 25 mg GCB and 150 mg Magnesium sulphate was added as there was very little or no pigment, GCB was not used for cleanup of other matrices . The micro tubes were centrifuged at 8000 rpm for 8 minutes. One milliliter cleaned up extract was filtered through 0.2µm nylon membrane filter paper (0.2 µm ultipor N66 nylon 6, 6 membrane filter, Pall Corporation) and it was analyzed by LC-MS/MS.

A representative samples of rice plant (5.0 g), straw (5.0 g), grain (5.0 g), husk(5.0 g) and soil (10.0 g)was taken in a 50 ml polypropylene centrifuge tube (Tarson) separately and 20 ml acetonitrile : water (8:2) mixture was added to it. The tubes were kept undisturbed for 2 hrs and subjected to vortex for three minutes. After vortex, 5 g activated Sodium sulfate was added and the tubes were placed to rotospin for 30 minutes at 50 rpm. The tubes were then centrifuged for 10 minutes at 10,000 rpm. The supernatant (8 ml) was collected and concentrated in a nitrogen evaporator at 40^{0}C. The volume was reconstituted with 2 ml HPLC grade acetonitrile. The content was then transferred in 2 ml micro centrifuge tube and 25 mg PSA, 150 mg activated Magnesium sulfate & 25 mg C18 was added to clean up the substrates like Straw, husk, grain and soil. But in case of clean up of the matrix of green rice plant, 25 mg GCB was added extra, remaining other same to remove the coloured pigments from the matrix. The tubes were centrifuged again for 5 minutes at 5000 rpm. After centrifugation (1.0 ml) supernatant was collected and filtered through 0.2µm nylon membrane filter paper (0.2 µm ultipor N66 nylon 6,6 membrane filter, Pall Corporation) and finally analyzed by LC-MS/MS.

The residue of bispyribac sodium and metamifop was quantified by LC-MS/MS (Waters, Milford, MA, USA). Both herbicides were analyzed on a LC (Alliance 2695 separation module). Separation of analyte was performed by reversed phase Symmetry C_{18} (5µm; 2.1x 100 mm) column (Waters, USA). The mobile phase was composed of (A) 5 mM ammonium acetate + 0.1% acetic acid in water and (B) 5 mM ammonium acetate + 0.1% acetic acid in methanol. Gradient: 0.0 – 2.0 min – 95.0%A to 5 % A, 2.0-8.0 min 95.0% A at 10.0 min, it ends with 95% A for bispyribac sodium. In case of metamifop the linear gradient was 0.0 - 2.0 min – 95.0% A to 5 % A, 2.0-16.0 min95.0% A at 18.0 min, it ends with 95% A with a total flow rate of 0.3 ml min^{-1} and the injection volume was 20 µL. Positive mode of electrospray ionization (ESI) was used for the estimation of both the analytes. Optimized ms parameters were as follows capillary voltage: 0.50 kV; source temperature: 120 ^{0}C; desolvation temperature: 350^{0}C; desolvation gas (nitrogen) (L hr^{-1}): 650; collision gas (argon) (L hr^{-1}): 30. LC-MS/MS was operated by Mass Lynx software. Protonated parent ion of metamifop was m/z 441.08 and the product ions were m/z 288.23(used for quantification), m/z 123.07 and m/z 180.38. For bispyribac sodium, the ion transitions were 430.87 (protonated parention) >275.00 (used for quantification), 430.87> 412.84 and 430.87 >118.83.

RESULTS AND DISCUSSION

Two different calibration curves were obtained by plotting six calibration points *viz*.0.01, 0.02, 0.05, 0.10, 0.50 and 1.0 µg ml^{-1} of bispyribac sodium and metamifop respectively. The correlation coefficient (R^2) for both the calibration curve was 0.99. The standard chromatogram of the analytical standards of bispyribac sodium and metamifop at 1 µg ml^{-1} are shown in fig.-2

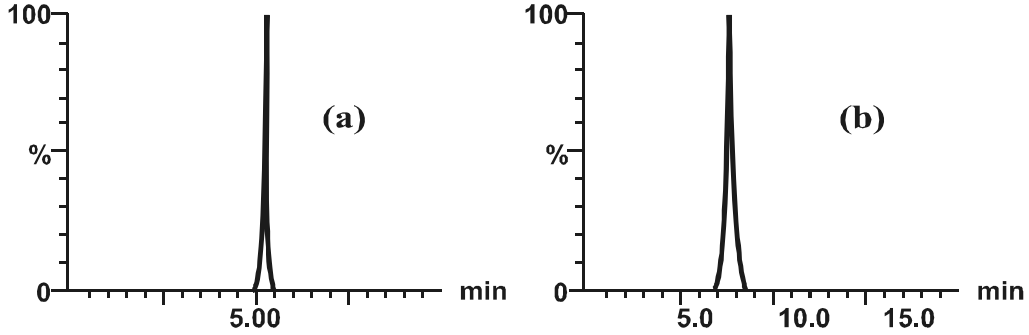


Fig. 2: a) analytical standard of bispyribac sodium, b) analytical standard of metamifop

The results of recovery study has presented in table 1. As the recovery was more than 85 % in all the cases, the method was adopted for residue study of bispyribac sodium and metamifop in rice. The limit of detection for both the compound was 0.01 µg ml^{-1}.

Following QuEChERS method (Lehotay *et al*., 2010) with some modification we got good recovery for metamifop but in case of bispyribac sodium, the recovery less than 30%.We tried buffer QuEChERS for bispyribac sodium but sill the result was below the

Table1: Recovery study of metamifop and bispyribac sodium (*Average of five replicates)

Samples	Spiked levels (mg kg^{-1})	Recovery* (%)	
		Bispyribac sodium	Metamifop
Rice plant	0.020	92	90
	0.050	89	86
	0.500	88	88
Straw	0.020	90	90
	0.050	86	83
	0.500	86	90
Husk	0.020	88	88
	0.050	90	86
	0.500	86	84
Grain	0.020	90	96
	0.050	89	98
	0.500	88	94
Soil	0.020	90	98
	0.050	90	93
	0.500	88	90

acceptable range. So we changed the extracting solvent acetonitrile by ethyl acetate but yet the result was not satisfactory. Then we acidified the water first and extracted with ethyl acetate using anhydrous sodium chloride. This modification gave the successful outcome with good recoveries. Thus the acidification process has great importance in liquid liquid extraction of bispyribac sodium from water.

Dissipation and residue of metamifop and bispyribac sodium under field condition

Dissipation of both herbicides followed the first-order kinetics ($C_t = C_0 e^{-kt}$) where C_t denotes the concentration of the pesticide residue at the time of t , C_0 denotes the initial deposit after application and k is the degradation constant under field condition and its environmental persistence was characterized by the half life ($t_{1/2} = \ln 2/k$).

The initial deposit and subsequent residue of metamifop and bispyribac sodium in soil at 0, 1, 3, 7, 15and 30 days after spraying are presented in fig.- 3.

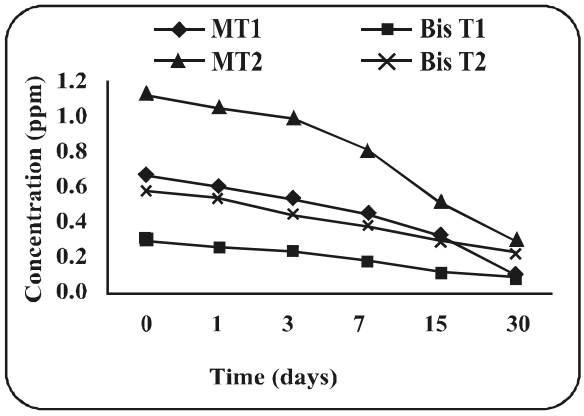
The mean initial deposit of metamifop on soil was 0.67 mg kg^{-1} for single dose and it was dissipated to 0.60, 0.53, 0.45, 0.32 and 0.11 mg kg^{-1} at 1,3,7,15 and 30 days respectively. For double dose the initial residue was 1.12 mg kg^{-1} and it was gradually dissipated to 1.05, 0.99, 0.81,0.51 and 0.30 mg kg^{-1} at 1,3,7,15 and 30 days respectively. The half-life values of metamifop in soil were 12.54 and 15.84 days for single and double dose respectively.

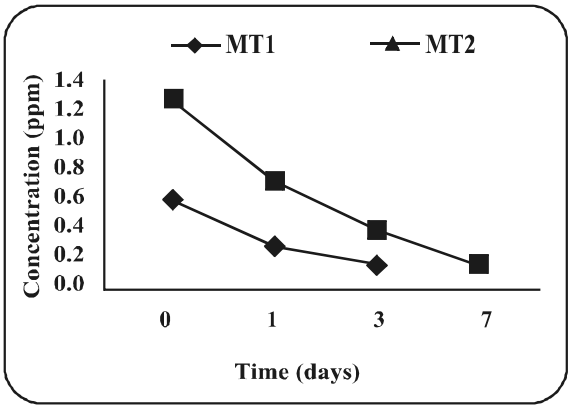
The residue of bispyribac sodium was decreased following the first order kinetics from 0.30-0.09 mg $kg^{-1}$for single dose and 0.58-0.22 for double dose. The half-life values of bispyribac sodium in soil were 17.71 and 23.16 days for single and double dose respectively.

The initial residue of metamifop on soil is greater than that of bispyribac sodium. The rate of dissipation of metamifop in soil is also greater than bispyribac sodium and probably for which both residues goes below detectable limit at the same day (45day) after application of the mixture formulation.

Dissipation of herbicides in soil is subjected by different factor such as soil type (Tao and Yang 2011), pH, organic matter content (Long *et al.*, 2014), type of interaction between soil and active ingredient (adsorption, absorption etc.) and environmental condition etc.

Initial deposit of metamifop in rice plant was 0.58 mg kg^{-1}. It was dissipated to 0.29 and 0.18 at 1 and 3 days for single dose. But for double dose, the initial residue was 1.20 mg kg^{-1} which was dissipated to 0.70, 0.40 and 0.18 at 1, 3 and 7 days. The dissipation kinetics of metamifop in rice plant is presented in fig.- 4. The half life values were 1.91 and 2.71 days for single and double dose. In case of bispyribac sodium no residue was found in rice plant irrespective of any doses.





T1 (70g a.i. ha^{-1}); T2 (140g a.i. ha^{-1})

Fig. 3: Dissipation curve of metamifop (M) and bispyribac sodium (Bis) in field soil at two different doses

Fig. 4: Dissipation curve of metamifop (M) in rice plant at two different doses

Residue of bispyribac sodium persisted in soil upto 30 days but no residue was detected in plant probably due to the strong interaction of pesticide with soil and absorption of this compound through plant tissue is low. In addition different factors such as metabolism (Hall *et al*., 2001), temperature (Walker, 1976), type of mixture (Fogg *et al*., 2003), crop type & climatic condition (Arnold and Briggs, 1990) play a significant role in degradation of herbicide.

Harvest residue of metamifop and bispyribac sodium

To confirm about the presence of herbicide residue in harvested straw, grain, husk and soil, we analyze the samples of four different locations (BCKV, Nadia; TNAU, Coimbatore; Agriculture Research Station, Garikpadu; IGKV, Raipur) of India at the time of harvest but no residues of metamifop and bispyribac sodium was detected in any substrate of rice. The residue study of bispyribac sodium and metamifop in rice was done separately by Wu and Mei, 2011 and De-yang *et al*., 2011 and they also reported no residue of either bispyribac sodium or metamifop in any of the substrate of the harvested rice which qualifies our findings.

A simple modified QuEChERS method was developed and applied for the extraction and cleaned up of bispyribac sodium and metamifop in rice. The results of the residue experiment clearly shows that the residue of bispyribac sodium and metamifop goes below detectable limit (0.02 ppm) at the time of harvesting the rice. So it can be conclude that application of this mixture herbicide to rice cultivation will not pose any residual toxicity to any of the substrate of rice and rice grain is safe for human consumption.

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REFERENCES

Arnold, D. J. and Briggs, G. G. 1990. Fate of pesticides in soils: predictive and practical aspects. *In. Environmental Fate of Pesticides*. John Willey and Sons Ltd., pp. 101-02.

Bhanti, M. and Taneja, A. 2007. Contamination of vegetables of different season with organophosphorous pesticides and related health risk assessment in northern India. *Chemosphere*, **69**: 63-68.

DeYang, K., Shi, L. L, Dan, Z. J. and Wu, W. Z. 2011. Residue and degradation of metamifop in paddy field. *J. Econ. Rur. Env*., **27**: 104-07.

Ding, G., Li, X., Zhang, F., Chen, J., Huang, L. and Qiao, X. 2009. Mechanism-based quantitative structure-activity relationships on toxicity of selected herbicides to *Chlorella vulgaris* and *Raphidocelis subcapitata*. *Bull. Env. Contam. Toxicol*., **83**: 520-24.

Dureja, P., Balasingh, S. and Parmer, B. S. 2015. Pesticide maximum residue limit (MRL): background, Indian scenario. *Pest Res. J*., **27** : 4-22

Focke, M., Gieringer, E., Schwan, S., Ja¨nsch, L., Binder, S. and Braun, H. P. 2003. Fatty acid biosynthesis in mitochondria of grasses: malonyl-coenzyme A is generated by mitochondrial-localized acetyl-coenzyme A carboxylase. *Pl. Physiol*., **133**: 875-84.

Fogg, P., Alistair, B. A., Boxall, A. B. A. and Walker, A. 2003. Degradation of pesticides in biobeds: The effect of concentration and pesticide mixtures. *J. Agril. Food Chem*., **51**: 5344-49.

Hall, J.C., Hogland, R. E., Zablotowicz, R. M. and Van Eerd, L. L. 2003. Pesticide metabolism in plants and microorganisms. *Weed Sci*.,

51: 472-95.

Ilboudo, S., Fouche, E., Rizzati, V., Toe, A. M., Gamet-Payrastre, L. and Guissou, P. I. 2014. *In vitro* impact of five pesticides alone or in combination on human intestinal cell line Caco-2. *Toxicol. Reports,* **1** : 474-89

Kim,T.J., Chang, H.S., Kim, J.S., Hwang, I.T., Hong, K.S., Kim, D.W., Cho, K.Y., Myung, E.J. and Chung, B. J. 2003. Metamifop: Mechanism of

herbicidal activity and selectivity in rice and barnyard grass. *The BCPC Int. Cong.: Crop Sci. Tech.*, Vol 1 and 2. *Proc. Int. Cong.* held at the SECC, 10-12 November, 2003 in Glasgow, Scotland, UK, pp. 833-38.

Lehotay, S. J., Son, K. A., Kwon, H., Koesukwiwat, U., Fu, W., Mastovska, K. Hoh, E. and Leepipatpiboon, N. 2010. Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables. *J. Chromatograph*., **1217:** 2548-60.

Long, Y.H., Li, R.Y. and Wu, X. M. 2014. Degradation of S-metolachlor in soil as affected by environmental factors. *J. Soil Sci. Pl. Nutri.*,**14** : 189-98 .

McCullough, P. E., Yu, J., Czarnota, M. A. and Raymer, P. L. 2016. Physiological basis for metamifop selectivity on bermuda grass (*Cynodon dactylon*) and Goose grass (*Eleusine indica*) in cool season turf grasses. *Weed Sci*., **64** : 12-24.

Mukherjee , D. 2004. Weed management in rice. *Agr. Today*.,**11**: 26–27.

Nagami, H., Asanuma, S., Yajima, N., Usuda, M., Hirosawa, M. and Shimizu, S. 2004. Dymron Herbicide Residues in Paddy Soil and Channel Water. *Bull. Env. Contam.Toxicol*.,**73** : 326–30.

Oerke, E. C. and Dehne, H. W. 2004. Safeguarding production - losses in major crops and the role of crop protection. *Crop Protec*., **23**: 275-85.

Sasaki, Y., Konishi, T. and Nagano, Y. 1995. The compartmentation of acetylcoenzyme-A carboxylase in plants. *Pl. Physiol.*, **108**: 445-49.

Tao, L. and Yang, H. 2011. Fluroxypyr biodegradation in soils by multiple factors. *Env. Monit Assess*, **175** : 227-38.

Walker, A. 1976. Simulation of herbicide persistence in soil: Simazine and prometryne. *Pest. Sci*., **7:** 41–9.

Wu, S. and Mei, J. 2011. Analysis of the herbicide bispyribac-sodium in rice by solid phase extraction and high performance liquid chromatography. *Bull. Env. Contam. Toxicol*., **86**:314-18 .

Zhang, C. Z., Zhang, Z. Y., Liu, X. J., Jiang, W.and Wu, Y. D. 2010. Dissipation and environmental fate of herbicide H-9201 in carrot plantings under field conditions. *Food Chem*., **119**: 874-79.