

# Effect of Seasonal Variation on the Persistence and Dissipation Behaviour of the Herbicide Mixture of Fomesafen + Quizalofop-Ethyl in Tropical Soybean Agroecosystem and Safety Risk Assessment

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## Research Article

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1 **Effect of seasonal variation on the persistence and dissipation behaviour of the herbicide mixture of**  
2 **fomesafen + quizalofop-ethyl in tropical soybean agroecosystem and safety risk assessment**

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10 **Abstract**

11 Weeds are the major limiting factor for optimum soybean production in India. The herbicide mixture of fomesafen  
12 and quizalofop-ethyl provides effective control of a broad spectrum of weeds, but its fate in the tropical soybean  
13 ecosystem is unknown and also the risks involved to the consumer and the environment are still unexplored. Hence,  
14 a supervised field trial was conducted following the post-emergence application of fomesafen 12% + quizalofop-  
15 ethyl 3% in two consecutive seasons. The dissipation of fomesafen followed biphasic double first order in parallel  
16 kinetics, whereas quizalofop-ethyl dissipation followed first order kinetics. A significant difference in the  
17 persistence of fomesafen was observed due to seasonal variation of meteorological parameters. However, the  
18 variation was significant only in plant, but non-significant in soil, in case of quizalofop-ethyl. The overall shorter  
19 persistence of both fomesafen and quizalofop-ethyl was recorded in warmer climatic conditions of Season I than  
20 Season II. The results thus indicated that care must be taken during application of this herbicide mixture in cold  
21 climatic regions, since both the herbicides may exhibit higher stability. The absence of end-point residues at harvest  
22 concluded that the formulation is safe for application in tropical agroclimate. The low chronic dietary toxicity and  
23 low soil ecological toxicity indicated that the herbicide mixture will offer no threat against consumer health and soil  
24 ecosystem. However, there was a concern about the toxicity against soil algal population which needs to be  
25 reconfirmed by further studies.

26 **Keywords** Fomesafen; Quizalofop-ethyl; LC-MS/MS; GC-MS/MS; Dissipation; Dietary risk; Soil ecological risk

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## 56 **Introduction**

57 Soybean (*Glycine max* L.) is considered as the most important seed legume, which occupies one of the largest  
58 monocultures in the world. Among all other field crops, it has the highest protein content (40-42%), which is a good  
59 substitute of animal protein also and the second highest oil content (18-22%) after groundnut. However, soybean  
60 production in India is severely affected by a number of monocotyledonous and dicotyledonous weeds, resulting in  
61 almost 50-76% yield reduction (Gharde et al. 2018). To compensate for such a huge yield loss, the herbicide mixture  
62 of fomesafen and quizalofop-ethyl has been introduced under the soybean cultivation in India. The mixture  
63 formulation (fomesafen 12%+quizalofop-ethyl 3%) controls a broad spectrum of weeds due to multiple modes of  
64 action. Fomesafen is a contact herbicide of the nitrodiphenyl ether chemical class. It controls broad leaf weeds by  
65 inhibiting the enzyme protoporphyrinogen oxidase (Alves et al. 2018). Quizalofop-ethyl is a systemic post-  
66 emergence herbicide of the aryloxy phenoxy propionate chemical family, which controls both annual and perennial  
67 grasses by inhibiting the enzyme acetyl coenzyme A carboxylase (Janaki et al. 2018).

68 Pesticide dissipation expresses a complex correlation with climate, since numerous interacting factors are  
69 involved. Meteorological parameters in totality determine the environmental concentration of pesticides by  
70 regulating their rate of degradation (Delcour et al. 2015). Besides, climate change is reported to alter the  
71 effectiveness and persistence of pesticides (Rhodes and McCarl 2020), often necessitating an increased pesticide  
72 application (Noyes et al. 2009). Therefore, conducting dissipation experiments under varying climatic conditions is  
73 necessary to predict the fate of pesticides accurately. This is extremely important especially for herbicides, since  
74 unlike other agrochemicals they are intended to persist longer in environment and change in climate thus tends to  
75 aggravate not only the risk of environmental contamination but also the detrimental effect induced by the herbicides  
76 on soil dwelling organisms. Again, the post-emergence herbicides may also contaminate the existing crop in field,  
77 making it unfit for domestic consumption as well as for export. Therefore, assessing the risk involved due to field  
78 application of herbicides is necessary to ensure the food and environmental safety. However, no information is  
79 available regarding the fate of the aforesaid herbicide mixture in soybean and its impact on dietary as well as soil  
80 ecological risk.

81 Therefore, the objectives of the current research are to assess the dissipation dynamics of the herbicide  
82 mixture (fomesafen 12%+quizalofop-ethyl 3%) in soybean and the impact of seasonal variation on its persistence.  
83 Besides, the risks involved towards soil ecological environment and consumer health are also evaluated.

## 84 **Material and methods**

### 85 **Chemicals and reagents**

86 The analytical standards of fomesafen and quizalofop-ethyl of >98% purity and their commercial formulation were  
87 supplied by Crystal Crop Protection Pvt. Ltd., India. Organic solvents were purchased from J.T.Baker, USA and the  
88 salts of ACS grade were procured from Merck Life Sciences Pvt. Ltd, Germany. The dispersive clean up sorbents  
89 viz. Primary Secondary Amine (PSA) and C<sub>18</sub> were purchased from Agilent Technologies, USA. Graphitized Carbon  
90 Black (GCB) was purchased from United Chemical Technology, Bellefonte, PA.

### 91 **Field experiment**

#### 92 Experimental design

93 Supervised field trials were conducted on soybean in two consecutive seasons at Jaguli Instructional Farm of Bidhan  
94 Chandra Krishi Viswavidyalaya (BCKV), Mohanpur, West Bengal, India (Latitude 22°93' N, longitude 88°59' E;  
95 altitude 9.75 m). The experimental region was constituted of new alluvial soil and the physico-chemical properties  
96 are represented in Table S1. Soybean seeds (variety: Pusa 16) were sown in 5m×4m plots at a spacing of 30 cm×10  
97 cm. The herbicide mixture (fomesafen 12%+quizalofop-ethyl 3%) was applied as post-emergence on 19<sup>th</sup> May 2017  
98 in Season I and 16<sup>th</sup> May 2018 in Season II at 225 g a.i. ha<sup>-1</sup> (T1 or recommended dose) and 450 g a.i. ha<sup>-1</sup> (T2 or  
99 double dose) doses. Separate plots were maintained as untreated control (T3) i.e. only water spray. The experiment  
100 was designed in randomized blocks, maintaining three replicates for each treatment including control. A total of 72.3  
101 mm and 41.4 mm precipitation was recorded in Season I and II respectively, of which no rainfall was recorded up to  
102 6 days after application of the herbicide mixture. The meteorological parameters were monitored in the regional  
103 meteorological station of BCKV (Fig. 1). The solar intensity and UV index were obtained from the Global Land  
104 Data Assimilation System and Tropospheric Emission Monitoring Internet Service, respectively (Fig. 1).

#### 105 Sample collection

106 Soybean plant and soil samples were collected at an interval of 0 (2 hours after application), 1, 2, 3, 4, 5, 7, 10, 13,  
107 15, 17 and 20 days after application of the herbicide mixture. Besides, soybean oil, deoiled cake and field soil  
108 samples were collected at harvest and analyzed separately for fomesafen and quizalofop-ethyl residues.

#### 109 **Analytical procedures of fomesafen and quizalofop-ethyl**

110 Extraction and clean up of soybean plant and soil

111 Representative 10 g homogenized plant and soil samples were taken separately in 50 mL polypropylene centrifuge  
112 tubes and 10 mL HPLC grade water was added. The extracting solvent was 10 mL acetonitrile for fomesafen,  
113 whereas quizalofop-ethyl residues were extracted with 10 mL acetonitrile + 1% acetic acid. A salt mixture of 1.5 g  
114 sodium chloride + 5 g magnesium sulphate was added, mixed thoroughly for two minutes and centrifuged at 5,000  
115 rpm for 5 minutes. The supernatant (2 mL) of both plant and soil samples were cleaned up separately by using 50  
116 mg PSA + 25 mg GCB + 150 mg magnesium sulphate and 25 mg PSA + 25 mg C<sub>18</sub> + 150 mg magnesium sulphate  
117 clean up combinations, respectively. The extract was filtered with a 0.2 μ membrane filter and analyzed in LC-  
118 MS/MS for fomesafen. However, for quizalofop-ethyl, 1.5 mL supernatant was evaporated to dryness after clean up  
119 and reconstituted with 1.5 mL ethyl acetate. The extract was filtered and analyzed in GC-MS/MS.

120 Extraction and clean up of soybean oil

121 A representative 100 g ground soybean seed was extracted with 450 mL hexane in soxhlet apparatus and the solvent  
122 was evaporated at 40 °C by using a rotary vacuum evaporator to collect the oil. Representative 2 g oil was dissolved  
123 in 20 mL hexane and partitioned thrice with 3x30 mL acetonitrile in a separatory funnel. The combined acetonitrile  
124 fraction was concentrated in rotary vacuum evaporator and reconstituted separately with acetonitrile (2 mL) for  
125 fomesafen and ethyl acetate (2 mL) for quizalofop-ethyl. Then the extracts were cleaned up by using 50 mg PSA +  
126 50 mg C<sub>18</sub> + 150 mg magnesium sulphate and filtered with 0.2 μ membrane filter before analysis.

127 Extraction and clean up of deoiled cake

128 The deoiled cake sample (2 g) was taken in a 50 mL centrifuge tube and 10 mL 5 % aqueous sodium chloride  
129 solution was added. The mixture was vortexed for one minute. Then 10 mL acetonitrile was added and vortexed  
130 again for 2 minutes, followed by roto-spin for 15 minutes at 50 rpm. The sample was centrifuged at 10,000 rpm for 5  
131 minutes. Then 6 mL supernatant was evaporated to dryness and reconstituted separately with acetonitrile (1.2 mL)  
132 for fomesafen and ethyl acetate (1.2 mL) for quizalofop-ethyl. Then the extracts were cleaned up by using 50 mg  
133 PSA + 25 mg C<sub>18</sub> + 150 mg magnesium sulphate and filtered with 0.2 μ membrane filter before analysis.

134 **Instrumental Condition**

135 LC-MS/MS

136 The analysis of fomesafen was performed with Waters 2695 separation module (Waters, USA) equipped with  
137 Symmetry C18 column (5  $\mu\text{m}$ ; 2.1 $\times$ 100 mm) and Micromass Quattro Micro API mass spectrometry. Masslynx V4.1  
138 software was used for data acquisition and quantification. The chromatographic separation was performed by using  
139 the binary mobile phase *viz.* mobile phase A (5 mM ammonium acetate + 0.1% acetic acid in water) and mobile  
140 phase B (5 mM ammonium acetate + 0.1% acetic acid in methanol) at a flow rate 0.3 mL minute<sup>-1</sup>. The gradient  
141 programming involved: 90% A + 10% B in 0-1 minute, 10% A + 90% B in 1-10 minute and 90% A + 10% B in 10-  
142 12 minute. Negative electrospray ionization with multiple reaction monitoring (MRM) scan type was used for  
143 analysis. The MRM transitions of fomesafen were: 436.98 > 195.01 (quantifier) with collision energy (CE) 41 eV;  
144 436.98 > 222 (qualifier) with CE 29 eV and 436.98 > 285.9 (qualifier) with CE 22 eV. The other parameters were:  
145 capillary voltage (1 kV), cone voltage (31 V), source temperature (120 °C), desolvation temperature (360 °C),  
146 desolvation gas (nitrogen) flow (650 L h<sup>-1</sup>) and collision gas (argon) flow (50 L h<sup>-1</sup>).

147 GC-MS/MS

148 The analysis of quizalofop-ethyl was performed by GC-MS/MS (Agilent Technologies, USA), equipped with 7890A  
149 GC, 7000 MS and Masshunter B.05.00 software. GC separation was performed in HP-5 MS capillary column (30  
150 m $\times$ 0.250 mm $\times$ 0.250  $\mu\text{m}$ ) and helium (purity  $\geq$  99.99%) was used as a carrier gas at a constant flow of 1 mL minute<sup>-1</sup>.  
151 Splitless injection mode was used to inject 1  $\mu\text{L}$  sample. A constant inlet temperature (285 °C) and auxiliary heater  
152 temperature (280 °C) were maintained. The oven temperature programme involved: Ramp 1 (150-220 °C, Rate: 50  
153 °C minute<sup>-1</sup>, Hold: 1 minute); Ramp 2 (220-250 °C, Rate: 10 °C minute<sup>-1</sup>, Hold: 2 minutes) and Ramp 3 (250-280 °C,  
154 Rate: 5 °C minute<sup>-1</sup>, Hold: 2 minutes). The mass spectrometry parameters involved electron impact (EI) ionization at  
155 -70 eV energy and source temperature of 230°C. The MRM transitions of quizalofop-ethyl were: 372.1 > 299.3  
156 (quantifier) and 299.2 > 91.2 (qualifier) with collision energy 10 and 25 eV, respectively.

157 **Recovery experiment**

158 The recovery experiment was carried out by fortifying all the substrates *viz.* soil, soybean plant, oil and deoiled cake  
159 at 0.01, 0.05 and 0.10 mg kg<sup>-1</sup> concentration levels separately for fomesafen and quizalofop-ethyl. An eight point  
160 calibration curve involving 0.003, 0.01, 0.02, 0.05, 0.10, 0.25, 0.50 and 1.0 mg kg<sup>-1</sup> concentration levels was used  
161 for quantification.

162 **Data analysis**

163 The degradation of pesticides is often reported to follow biphasic kinetics in the field (Gluhar et al. 2019).  
164 Therefore, the dissipation of fomesafen and quizalofop-ethyl were determined by comparing between two kinetic  
165 models as follows:

166 Single First Order (SFO):  $y = ae^{-bt}$  (1)

167 Double First Order in Parallel (DFOP):  $y = ae^{-bt} + ce^{-dt}$  (2)

168 Where, 'y' is the concentration (mg kg<sup>-1</sup>) at time 't' (days), 'a' is the initial concentration at time t=0 and 'b' is the  
169 dissipation constant (days<sup>-1</sup>) in SFO. In DFOP, 'a' and 'c' are the initial concentrations for two compartments and  
170 'b' and 'd' are the respective dissipation constants. The parameters of the kinetic models were estimated by using R  
171 Studio version 1.2.5042 (RStudio Team 2020) and associated package viz. mkin (Ranke 2020). The best fit model  
172 was selected based on  $\chi^2$  test as recommended by the FOCUS work group (FOCUS 2006).

173 **Safety evaluation**

174 Dietary risk assessment

175 The chronic dietary risk quotient (RQ<sub>d</sub>) was assessed as follows:

176 
$$RQ_d = \frac{\text{Estimated Daily Intake (EDI)}}{\text{Acceptable Daily Intake (ADI) X Average Body Weight}}$$
 (3)

177 The estimated daily intake (EDI) was calculated by multiplying the residue levels of fomesafen and quizalofop-ethyl  
178 in soybean plant with recommended consumption of soybean per day per person. The National Institute of Nutrition  
179 reported an intake of 30g and 75g person<sup>-1</sup> day<sup>-1</sup> of pulses for children (4-6 years) and adults (National Institute of  
180 Nutrition 2011). The average body weight of an Indian child and an adult are 18 kg and 60 kg (National Institute of  
181 Nutrition 2011). The acceptable daily intake (ADI) of fomesafen and quizalofop-ethyl is 0.003 and 0.0013 mg kg<sup>-1</sup>  
182 body weight day<sup>-1</sup> (PPDB 2021a, b), respectively. The RQ<sub>d</sub> > 1 signifies high risk for consumption and vice versa.

183 Soil ecological risk assessment

184 The soil ecological risk quotient (RQ<sub>s</sub>) of both fomesafen and quizalofop-ethyl was estimated for algae, earthworm  
185 and arthropods following the equation:

186 
$$RQ_s = \frac{\text{Effective Concentration of pesticide (EC)}}{\text{Predicted No Effect Concentration (PNEC)}}$$
 (4)

187 The predicted no effect concentration (PNEC) was obtained by dividing the toxicity value of respective target  
188 organism by corresponding assessment factor which was 50 for both the cases. The risk factor is considered high if  
189  $RQ_s > 1$ , moderate for 0.1-1 and low for  $RQ_s < 0.1$  (European Communities 2003).

## 190 **Results and discussion**

### 191 **Method validation**

192 The analytical methods of fomesafen and quizalofop-ethyl were validated following the SANTE guideline (SANTE  
193 2019). The calibration curves were linear with the coefficient of determination ( $R^2$ ) 0.99 (Figure S1-S2). An average  
194 recovery percentage  $>85\%$  along with repeatability ( $RSD_r$ ) and within-laboratory reproducibility ( $RSD_{wR}$ )  $<20\%$  for  
195 all the substrates proved that the methods were accurate and repeatable (Table S2). The matrix effect of both  
196 fomesafen and quizalofop-ethyl was within  $\pm 20\%$  (Table S2) and the specificity of the method was also found to be  
197 acceptable as the responses of both the compounds were  $<30\%$  of the matrix matched standard at the desired  
198 retention time in control samples. The limit of quantification (LOQ) of both the molecules was  $0.01 \text{ mg kg}^{-1}$ .

### 199 **Kinetics of dissipation of fomesafen and quizalofop-ethyl**

200 The dissipation of fomesafen in soil and soybean plant followed the biphasic DFOP kinetics (Fig. 2; Table 1). The  
201 biphasic degradation of fomesafen involved rapid initial photolysis from both soil and plant surfaces in the first  
202 phase. The compound then moved gradually into the soil column or plant matrix, which increased the chance of  
203 adsorption with soil colloids or plant cellular components and thereby reduced the rate of soil microbial or chemical  
204 degradation as well as the plant enzymatic metabolism. Hence, a slower second phase of fomesafen dissipation was  
205 observed. Similarly, Walker and Bond (1977) reported that herbicides dissipated more rapidly from the soil surface  
206 than an application incorporated into the soil column. Ugare et al. (2013) reported biphasic dissipation of several  
207 pesticides in plants due to adsorption with cellular components. Wu et al. (2014) reported biphasic dissipation of  
208 fomesafen in soil, where the rate of degradation decreased due to increased adsorption of the molecule with time.

209 Unlike fomesafen, the dissipation of quizalofop-ethyl followed SFO kinetics in both soil and soybean plant  
210 (Fig. 3; Table 1). A report from the European Food Safety Authority (EFSA 2008) described that the faster rate of  
211 deesterification is the reason why quizalofop-ethyl follows simple first order kinetics under field condition.  
212 Similarly, Mantzos et al. (2015) also reported SFO kinetics of quizalofop-ethyl decay in both soil and sunflower  
213 plant under field experimental condition.

#### 214 **Effect of seasonal variation on dissipation of fomesafen**

215 The average initial concentration of fomesafen in Season I and II ranged between 0.097-0.175 and 0.110-0.190 mg  
216 kg<sup>-1</sup> in soil (Fig. 2a) and 0.249-0.497 and 0.261-0.509 mg kg<sup>-1</sup> in soybean plant (Fig. 2b). A significant variation in  
217 the persistence of fomesafen between two consecutive seasons was observed in case of both soil and soybean plant  
218 (Table S3). The half-life of fomesafen was found to be lower in Season I (4.23-4.49 days in soil and 2.84-2.89 days  
219 in plant) than Season II (5.42-5.62 days in soil and 3.71-3.81 days in plant) (Table 1). A report from the Health  
220 Canada Pest Management Regulatory Agency (2018) described fomesafen half-life in soil may vary from less than 1  
221 to more than 4 months depending on the environmental conditions under field condition. The overall shorter  
222 persistence of fomesafen in Season I than Season II in our experiment was primarily due to faster rate of  
223 photodegradation of the molecule from both soil and plant surface, since the average intensity of UV radiation, total  
224 solar radiation and bright sunshine hour (BSSH) were observed to be much higher in Season I along with less cloud  
225 coverage (UV index: 12.34, Intensity of total solar radiation: 275.42 Watt m<sup>-2</sup>, BSSH: 10 hours, cloud coverage:  
226 39.06%) than Season II (UV index: 11.36, Intensity of total solar radiation: 209.24 Watt m<sup>-2</sup>, BSSH: 5.1 hours, cloud  
227 coverage: 78.13%) (Fig. 1). Photodegradation is reported to be the major decomposition pathway of fomesafen  
228 under field condition, which readily degrades the molecule even under relatively low sunlight conditions (Ahrens  
229 1994). Besides, both microbial degradation and chemical hydrolysis play an important role in fomesafen dissipation  
230 (Das et al. 2020; USEPA 2006) and the rate of degradation increases with an increase in temperature. Hence, higher  
231 soil temperature in Season I (34 °C) may also have contributed to some extent to the shorter persistence of  
232 fomesafen in soil by decreasing the adsorption of the molecule and augmenting the rate of dissipation as compared  
233 to Season II (31 °C) (Fig. 1). Similarly, Rauch et al. (2007) reported that fomesafen dissipation occurs more rapidly  
234 under warmer climatic conditions, while cold climatic conditions favour high concentrations.

#### 235 **Effect of seasonal variation on dissipation of quizalofop-ethyl**

236 The average initial concentration of quizalofop-ethyl in soil ranged between 0.034-0.055 mg kg<sup>-1</sup> in Season I and  
237 0.035-0.053 mg kg<sup>-1</sup> in Season II (Fig. 3a). The half-life of quizalofop-ethyl in field soil was 1.63-1.70 days in  
238 Season I and 1.66-1.83 days in Season II (Table 1). The variation in the persistence of quizalofop-ethyl in soil  
239 between two consecutive seasons was found to be non-significant (Table S3). The reason underlying the fact is that  
240 the deesterification of quizalofop-ethyl to quizalofop acid, which is the major metabolic pathway of aryloxy  
241 phenoxy propionate (AOPP) herbicides, occurs rapidly in soil and depends primarily on soil solution pH. It has been

242 reported that pH is the key determining factor for degradation of quizalofop-ethyl in soil (Mantzos et al. 2017).  
243 Besides, Das et al. (2020) described that the rate of deesterification of quizalofop-ethyl reduces at lower pH. The pH  
244 of the soil in our experiment was acidic i.e. 6.4 (Table S1). Hence, quizalofop-ethyl showed an overall higher  
245 persistence in our experiment with half-life of 1.63-1.83 days in soil (Table 1) than the previously reported half-life  
246 of the molecule, which was 0.55-0.68 days (EFSA 2008; Mantzos et al. 2015).

247 The persistence of quizalofop-ethyl in soybean plant varied significantly between two consecutive seasons  
248 (Table S3). The estimated half-life was lower in Season I (1.76-1.77 days) than Season II (1.90-1.96 days) (Table 1).  
249 The dissipation of quizalofop-ethyl in soybean plant is represented in Fig. 3b. It has already been reported that the  
250 metabolism of AOPP herbicides in tolerant crop like soybean occur through deesterification by carboxy esterase  
251 enzyme and aryl hydroxylation by cytochrome P450 monooxygenase or oxidase enzymatic system (Koeppel et al.  
252 1990). The rate of such enzymatic degradation is highly dependent on temperature. A significant reduction of  
253 herbicidal activity was observed at higher temperature, which is due to increased metabolism of the compound  
254 (Coupland 1989). Refatti et al. (2019) also reported a 3 °C increment in temperature resulted in 30% reduction in  
255 cyhalofop-butyl activity due to constitutive upregulation of key genes responsible for detoxification of xenobiotics  
256 in higher temperature. In our experiment, higher atmospheric temperature in Season I (30.1 °C) (Fig. 1) thus induced  
257 an increased rate of metabolism of quizalofop-ethyl in soybean plant and thereby lower half-life than Season II (26.3  
258 °C).

#### 259 **End-point residue analysis at harvest**

260 The interval between the herbicide application and the crop harvest was 100 days. The end-point residues of both  
261 fomesafen and quizalofop-ethyl in soil and harvested soybean seed (oil and deoiled cake) were below the  
262 quantifiable limit, i.e. 0.01 mg kg<sup>-1</sup> (Figure S3-S4). Mandal et al. (2014) also reported that the residues of quizalofop  
263 ethyl in black gram were below the quantification limit (0.01 mg kg<sup>-1</sup>) at harvest. Besides, the residues in harvested  
264 crop products were also below the maximum residue limit (MRL) prescribed by the European Union i.e. 0.02 mg kg<sup>-1</sup>  
265 for fomesafen and 0.2 mg kg<sup>-1</sup> for quizalofop-ethyl. Hence, the application of the herbicide mixture in soybean crop  
266 was considered safe for consumer health.

#### 267 **Safety evaluation**

268 Dietary risk assessment

269 The dietary risk quotient (RQ<sub>d</sub>) of fomesafen ranged between 0.010-0.212 for adults and 0.014-0.283 for children  
270 (Table 2). The RQ<sub>d</sub> of quizalofop-ethyl ranged between 0.018-0.155 for adults and 0.025-0.207 for children (Table  
271 3). Since, the chronic RQ<sub>d</sub> of both fomesafen and quizalofop-ethyl on each sampling day were below 1 irrespective  
272 of the season and dose, the application of the herbicide mixture was therefore considered to impose no long-term  
273 risk of dietary toxicity. Pang and Hu (2020) also reported no dietary toxicity due to fomesafen application in  
274 soybean.

275 Soil ecological risk assessment

276 The soil ecological risk quotients indicated that both fomesafen and quizalofop-ethyl are safe towards earthworm  
277 and soil arthropods (Table 2-3). However, a high risk for fomesafen and moderate to high risk for quizalofop-ethyl  
278 against green algae was observed (Table 2-3). Fomesafen has been reported to be toxic to green algae (Caquet et al.  
279 2005). A moderate toxicity against green algae *Pseudokirchneriella subcapitata* was also reported for quizalofop-  
280 ethyl (PPDB 2021b). Therefore, the risk associated with soil algal population due to application of this herbicide  
281 mixture can't be ignored and should be assessed extensively in different agro-climatic zones across the globe in  
282 future to establish a conclusive inference.

## 283 **Conclusion**

284 The degradation dynamics of the herbicide mixture i.e. fomesafen 12% + quizalofop-ethyl 3% was studied in the  
285 soybean ecosystem in two succeeding seasons at two treatment doses. A significant effect of seasonal variation on  
286 the dissipation of the herbicide mixture was observed. However, the variation in persistence was observed to be non-  
287 significant at two treatment doses irrespective of season. The overall low persistence of both the active ingredients  
288 indicated that the formulation is safe for application in the tropical agroclimatic environment and can be used as an  
289 alternative to the older molecules which in general leave considerable residues in harvested products. Both  
290 fomesafen and quizalofop-ethyl showed low chronic dietary toxicity to the children as well as adults. Besides, the  
291 absence of residues at harvest concluded that the harvested soybean products can be consumed safely if the  
292 formulation is applied at the recommended dose following good agricultural practices (GAP). However, the risk of  
293 acute dietary toxicity was not evidenced in this study. The assessment of soil ecological risk demonstrated that the  
294 herbicide mixture was safe towards earthworm and soil dwelling arthropods, whereas a high risk against soil algal  
295 population needs to be attended more carefully and sorted out as the future scope of research.

296 **Declarations**

297 **Ethics approval and consent to participate**

298 Not applicable.

299 **Consent for publication**

300 Not applicable.

301 **Availability of data and materials**

302 The datasets used and/or analyzed during the current study are available from the corresponding author on  
303 reasonable request.

304 **Competing interest**

305 The authors declare that they have no competing interests.

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307 No funding was received for conducting this study.

308 **Authors' contributions**

309 SD developed the experimental design and wrote the manuscript. DS developed and validated the analytical  
310 methods. AK conducted the field experiment, collected and prepared the samples. SM performed the statistical  
311 analysis and representation of the data generated. AK and AB contributed to the language editing and MK reviewed  
312 the manuscript. SR supervised the whole experiment and revised the manuscript for final submission. All authors  
313 read and approved the final manuscript.

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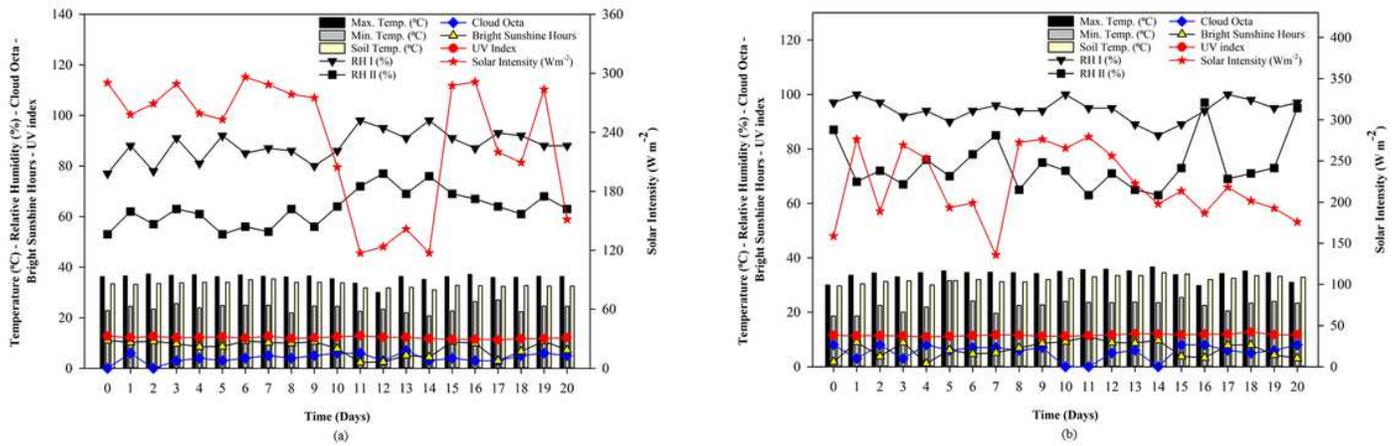
404 **Figure captions**

405 **Fig. 1** The temporal patterns of maximum, minimum and soil temperature; maximum and minimum relative  
406 humidity (RH I and RH II); cloud octa; bright sunshine hours; UV index and total solar intensity in Season I (a) and  
407 Season II (b)

408 **Fig. 2** Dissipation of fomesafen in soil (a) and soybean plant (b) applied at two treatment doses (T1 and T2) in  
409 Season I and Season II

410 **Fig. 3** Dissipation of quizalofop-ethyl in soil (a) and soybean plant (b) applied at two treatment doses (T1 and T2) in  
411 Season I and Season II

# Figures



**Figure 1**

The temporal patterns of maximum, minimum and soil temperature; maximum and minimum relative humidity (RH I and RH II); cloud octa; bright sunshine hours; UV index and total solar intensity in Season I (a) and Season II (b)

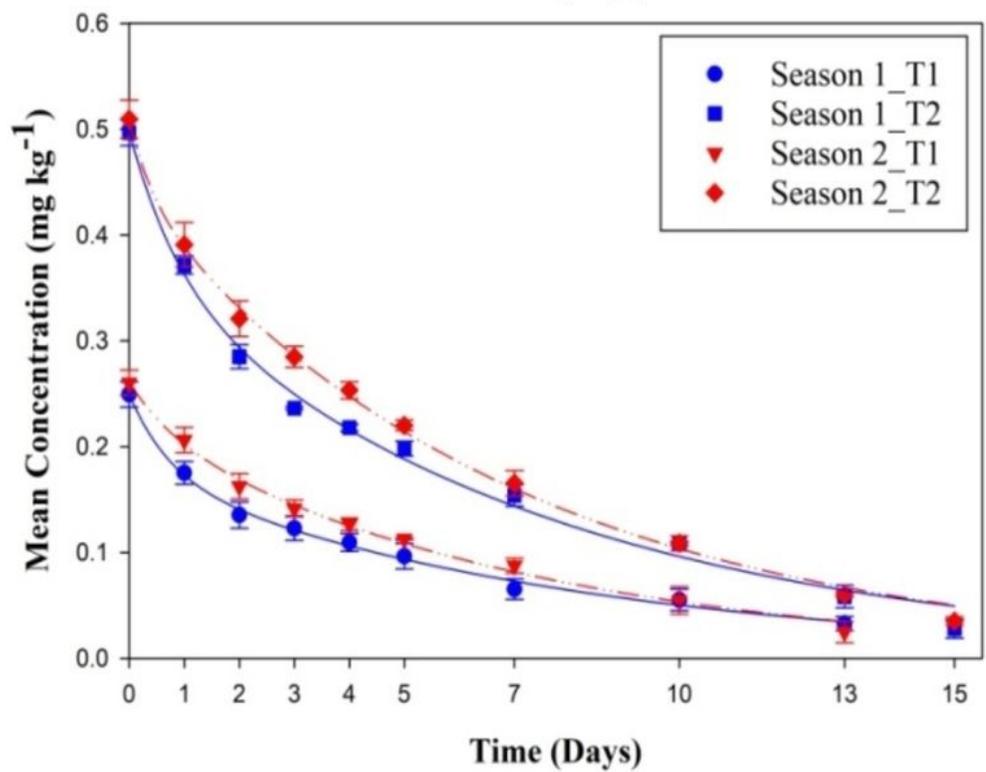
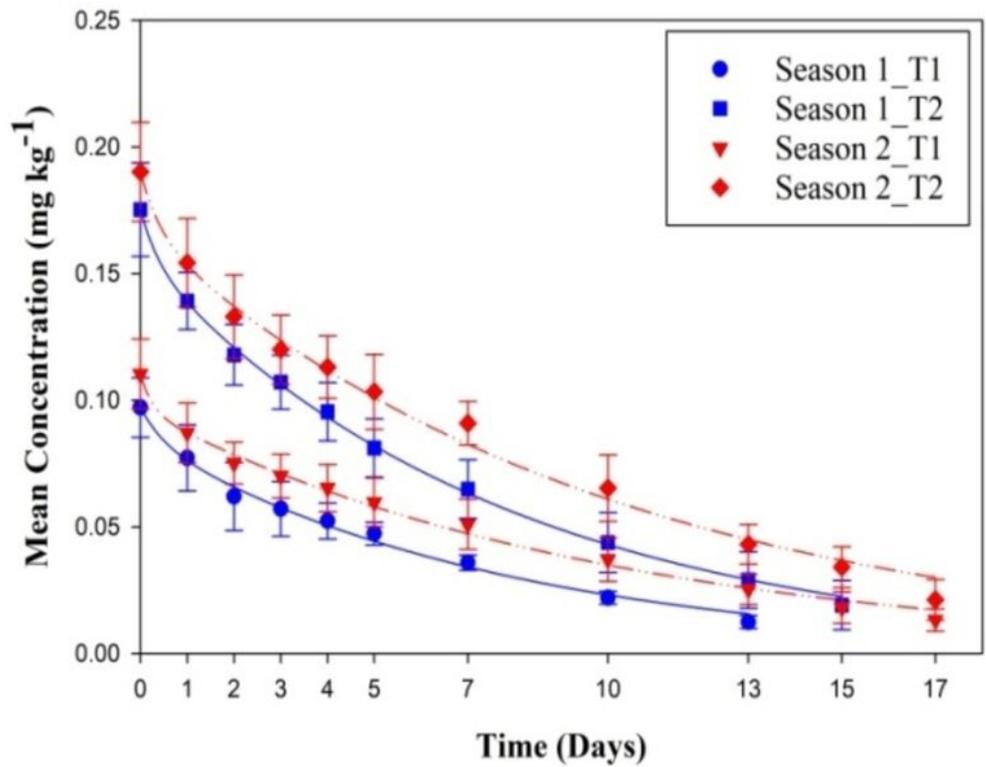


Figure 2

Dissipation of fomesafen in soil (a) and soybean plant (b) applied at two treatment doses (T1 and T2) in Season I and Season II

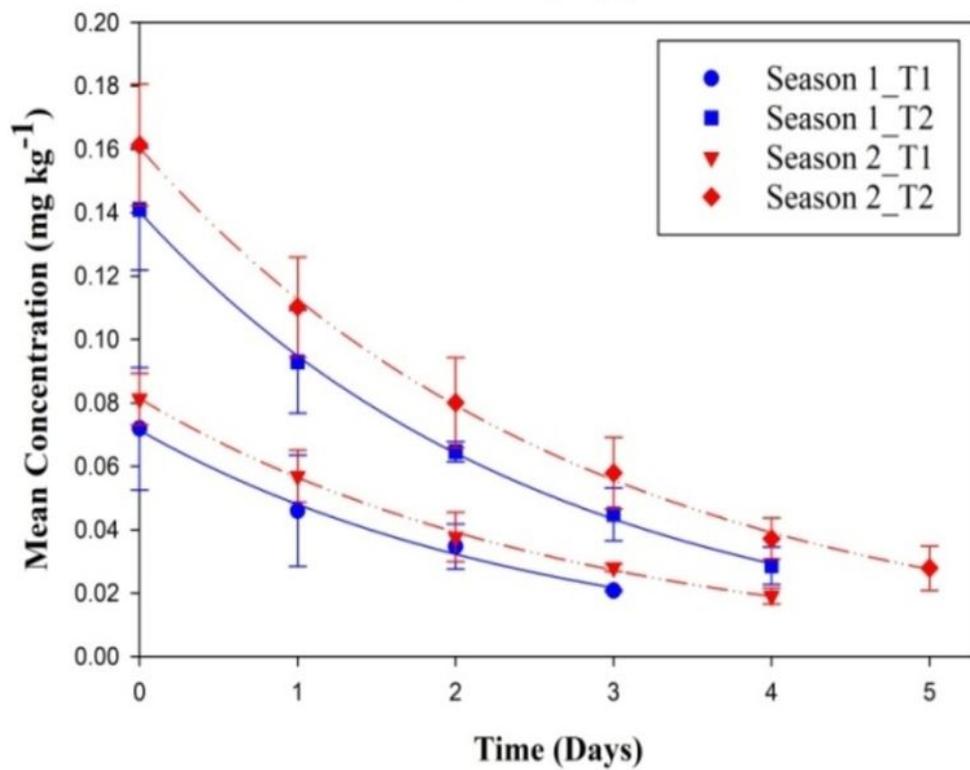
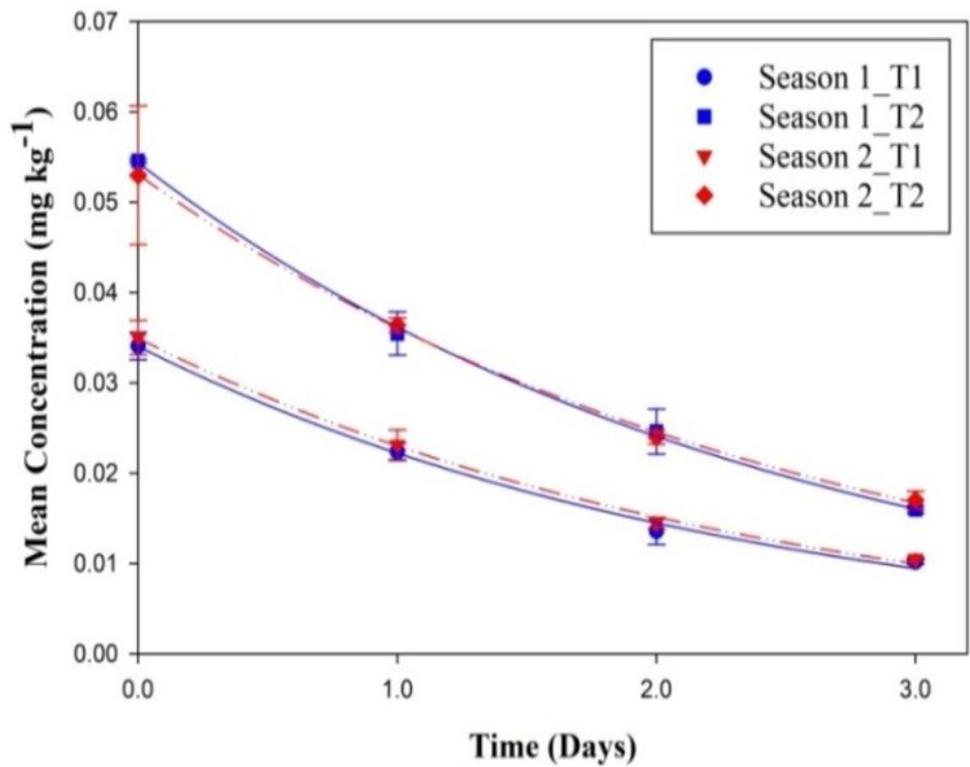


Figure 3

Dissipation of quizalofop-ethyl in soil (a) and soybean plant (b) applied at two treatment doses (T1 and T2) in Season I and Season II

## Supplementary Files

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