Original Article: Plant bioassays to detect herbicides residues in intensive rice-based cropping patterns under Conservation Agriculture

Mohammad Hossain[a,*], Mahfuza Begum [b], Richard Bell [c], Abul Hashem [d], Moshiur Rahman [b], Sharif Ahmed [a]

[a]*Rice Breeding Innovations Platform, International Rice Research Institute, Bangladesh Country Office, Banani, Dhaka-1213*
[b]*Department of Agronomy, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh*
cAgricultural Sciences, Murdoch University, South St, WA 6150, Australia
[d]*Department of Agriculture and Food, 75 York Road, Northam 6401, Australia*
[b]*Department of Agronomy, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh*
[a]*Rice Breeding Innovations Platform, International Rice Research Institute, Bangladesh Country Office, Banani, Dhaka-1213*

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***Corresponding Author: Mohammad Hossai**
(mm.hossain@irri.org)

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ABSTRACT

Avoiding the negative effect of herbicide residues in soils on the growth of subsequent crops is especially challenging in intensive cropping sequences involving numerous crop species and short fallow periods between crops. For intensive rice cropping patterns in Bangladeshthe residual effect of six herbicides: glyphosate, pendimethalin, ethoxysulfuron-ethyl, carfentrazone-ethyl + isoproturon, isoproturon, and fenoxaprop-p-ethyl were investigated in the following crops of mungbean and mustard through plant bioassay approach. These herbicides in ten different combinations were applied to mustard, mungbean, summer rice, winter rice, and wheat in an on-farm Conservation Agriculture experiment, which was conducted for three consecutive years (2013–2016). No detrimental residual effects of any herbicides applied to the prior crops in the rotation were detected on the plant population, root, shoot growth, and chlorophyll content in the leaves of test crops. Moreover, plant biomass, seed production, and the germination rate of the harvested seeds of both crops were not hampered by the residues of tested herbicides. It is concluded that these six herbicides examined for controlling weeds in the prior rice, wheat, mustard, and mungbean crops under the minimum soil disturbance and crop residue retention could be considered safe for cultivating crops in the next season. Under the increased prevalence of herbicide use for controlling weeds in intensive rice-based cropping sequences in South Asia, the systematic assessment of herbicide residual risk to crops needs to be instituted as a routine practice in this region.
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Introduction

Owing to the declining availability of agricultural labour and degradation of the natural resources, Conservation Agriculture (CA) is gaining popularity in South Asia. Apart from saving the need for farm labour relative to the conventional practice, CA also improves soil health. However, the weeds proliferation is one of the significant obstacles to achieve an optimal yield of crops cultivated under the CA system (Hossain et al., 2021).

Farmers have recommended manual weeding at least six times to control weeds in CA fields which is very tedious, labour-intensive, and costly that consumes 25% of the total cost of production in this practice (Baudron et al., 2012). The acute shortage of labour at the peak period often leads to delay weeding causing drastic losses in yield. To resolve this problem, chemical weed control using herbicides is a prudent and economic choice in terms of effectiveness of labour and time. Typically, herbicides are sprayed on soil or on foliage to manage weeds for a specific period before breaking down. The repeated use of different herbicides in the same land season after season raises the chances of herbicide persistence in the soil (Wyk & Reinhardt, 2001). Their negative impact on the growth of subsequent crops is a severe concern. Herbicides should degrade into non-toxic by-products after the adequate weed control during the crucial time of the cropping season. Numerous studies have shown that herbicides do not persist in the soil due to their short half-life, and their persistence in the soil is further determined by soil physicochemical properties (Curran, 2016).

However, under some circumstances, herbicides remain active in the soil and have a residual impact on the performance of subsequent crops. Bangladesh, like developed nations, has seen an unanticipated rise in the area under weed management using an increasing diversity of herbicides in recent years (Ahmed et al., 2021). However, the fate of those herbicides and the risk to the subsequent crops in the rotation has not been systematically assessed. In triple cropping systems, the short follow period between the harvest of one crop and the sowing of the next exacerbates the risk of herbicide residue carry over to cause crop injury.

The recommendation for using herbicides is mainly crop-specific. Various herbicide reactions of different crop cultivars account for diversity in morphology, physiology, variation in germination, emergence, growth, and development (Li et al., 2020). Hence, it is essential to research the residual toxicity of herbicides on succeeding crops before recommending their use. As a result, this on-farm research used a bioassay method under the CA system to examine residual effects on mungbean and mustard of herbicides applied to three years continuous practice of mungbean-monsoon rice-wheat and mustard-winter rice-monsoon rice system in Bangladesh.

Materials and Methods

Location and environments of residual effect study

This on-farm experiment was conducted at the farmers' field at the Gouripur sub-district of Bangladesh (N: 24˚75', E: 90˚50', altitude: 18 m). The study was done from November to May in 2015–2016, after the completion of a three-year (2013–2016) on-farm CA experiment. Two CA experiments were conducted in different fields under mungbean-monsoon rice-wheat or mustard-winter rice-monsoon rice crop rotation.

The experimental land was flood-free medium-high terrain with a sandy clay loam soil texture (sand: silt: clay at 50: 23: 27%) having a pH of 7.2. November was the hottest month of the mustard season (November to mid-February, 2015-16), with the maximum and minimum temperatures of 29.9 and 18.1 °C, respectively. Temperatures began to fall in January, making this the coldest month on record. November was the driest month, with no rain. February found the most rainfall; amounting to approximately 20 mm. November had the most sunlight hours, while December had the fewest. During the mungbean growing season (mid-March to May 2016), the maximum and the lowest temperatures of 32.2 and 26.1 °C, respectively made April month as the warmest. May was the rainiest one having 484 mm rainfall, followed by March and April. March and May had the most and least sunlight hours, respectively.

Protocols of previous CA experiments

In the previous CA experiment, mungbean, mustard, wheat, monsoon rice, and winter rice were established after Conventional Tillage (CT) and Strip Tillage (ST). Weeds were controlled by six times hand weeding in CT (Control) and by herbicides in ST, where six herbicides (Table 1) were applied under different combinations (Table 2) for different crops.

Table 1. Six different herbicides used in previous CA experiments.

Herbicide name and chemical group	Dose (a.i. ha^{-1} per season)	Crops
Carfentrazone-ethyl+ Isoproturon (3:1 ratio) (Triazolinone/phenylurea)	21.5 kg	Wheat
Ethoxysulfuron-ethyl (Sulfonylurea)	618 g	Rice
Fenoxaprop-p-ethyl (Aryloxyphenoxy-propionate)	120 g	Mungbean
Glyphosate (Phosphonic acid)	1.0 kg	Rice, wheat, mungbean, mustard
Isoproturon (Phenylurea)	1.0 kg	Mustard
Pendimethalin (Dinitroaniline)	1.0 kg	Rice, wheat, mungbean, mustard

Table 2. Eleven different combinations of herbicides used in previous CA experiments

Herbicide combinations	*Crops*
No herbicide	*Rice, wheat, mungbean, mustard*
Glyphosate *Glyphosate followed by (fb) Pendimethalin*	*Rice, wheat, mungbean, mustard*
Glyphosate fb Ethoxysulfuron-ethyl *Glyphosate fb Pendimethalin fb Ethoxysulfuron-ethyl*	*Rice*
Glyphosate fb Carfentrazone-ethyl+Isoproturon *Glyphosate fb Pendimethalin fb Carfentrazone-ethyl+Isoproturon*	*Wheat*
Glyphosate fb Fenoxaprop-p-ethyl *Glyphosate fb Pendimethalin fb Fenoxaprop-p-ethyl*	*Mungbean*
Glyphosate fb Isoproturon *Glyphosate fb Pendimethalin fb Isoproturon*	*Mustard*

Here, glyphosate was the pre-plant herbicide, pendimethalin was the pre-emergence, and the rest of all were post-emergence herbicides. Glyphosate was applied three days before tillage/planting. Pendimethalin was applied at 3 days after transplanting / seeding (DAT/S) in rice and wheat, but immediately after seeding of mustard and mungbean. Isoproturon was applied in mustard at 15 DAS. Ethoxysulfuron-ethyl, Carfentrazone-ethyl+Isoproturon and Fenoxaprop-p-ethyl were applied at 25 DAT/S in rice, wheat, and mungbean, respectively. A two-wheel tractor (2 WT) and a Versatile Multi-crop Planter (VMP) were used to prepare land for the CT and ST, respectively. The mechanical seeding of wheat, mustard, and mungbean was done simultaneously using VMP during the tillage operation

Test crops seeding

Immediately after the harvest of wheat and winter rice in fields under each cropping pattern, 30 kg mungbean seeds and 7 kg mustard seeds ha^{-1} basis were planted using VMP. Light irrigation was done after planting for optimal germination. No fertilizer was administered in the field. Sample seed germination was examined in the laboratory, and more than 80% germination was reported. Crops were maintained weed-free by manual weeding throughout the growth season.

Data transcription

A 2.0 m × 1.5 m quadrat was used to record the plant population m^{-2}. The quadrat was randomly placed at three locations within each plot. Plants were counted inside the quadrat, and an average of three quadrats was reported.

The chlorophyll content of the leaves was recorded using *SPAD 502 Plus Chlorophyll Meter*. The seedlings biomass was determined by drying them at 70 °C for 72 hours. The length of shoot and root were measured, and the following formula was used to determine the percent growth inhibition (Eliason et al., 2004). This data were collected at 25 DAS from randomly chosen 20 plants as follows:

$$\text{Growth Inhibition (\%)} = [1 - (\text{Lenth in Treatment}/\text{Lenght in Control})] \times 100$$

Visual assessment of phyto-toxicity (Rao, 1983) and crop vigor (Kingman & Moore, 1982) was done using the scale presented in Tables 3 and 4.

Table 3. Phyto-toxicity assessment scoring

Injury levels	Ratings
Normal growth without any injury	0
Trivial stunting/injury/discoloration	1
Some stand loss/stunting/discoloration	2
Injury more distinct but not insistent	3
Recoverable moderate injury	4
Persistent injury with uncertain recovery	5
Near severe injury irrecoverable	6
Severe injury stand loss	7
A few plants alive	8
Very few plants alive	9
Completely damage	10

Table 4. Crop vigor visual assessment

Crop growth	Ratings
Poor	1
Fair	2
Good	3
Excellent	4

The laboratory test was performed to determine the germination rates of harvested mungbean and mustard seeds. In this experiment, we tested 4 × 100 lots of seeds from each treatment plot. At 15 DAS, the number of normal seedlings was counted. The following formula was used to calculate the rate of seed germination.

$$Germination\ (\%) = \frac{Number\ of\ normal\ seedlings}{Number\ of\ seeds\ sown} \times 100$$

Data analysis

The data were analysed using analysis of variance, and treatment means were separated using the Duncans' Multiple Range Test at $p \leq 5\%$ significance level. All data were analysed using the STAR statistical software.

Results and Discussion

Data presented in Figure 1 revealed that there was no significant difference ($p > 5\%$) in the mean plant population of mungbean and mustard m^{-2} at 25 DAS. In the field, we recorded ≥85 plants m^{-2} of mungbean and mustard throughout the treatments.

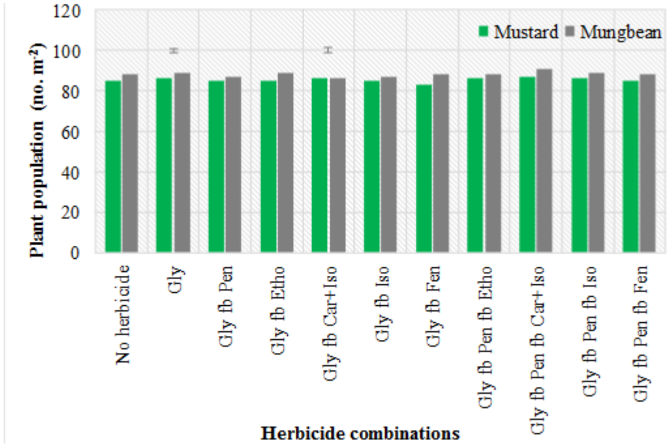

Figure 1. Residual effect of herbicides on the plant population of mustard and mungbean at p >5% **level.** Gly = Glyphosate, fb= followed by, Pen = Pendimethalin, Etho = Ethoxysulfuron-ethyl, Car = Carfentrazone-ethyl, Iso = Isoproturon, Fen = Fenoxaprop-p-ethyl.

The lengths of root (Table 5) and shoot (Table 6) of test crops were also unaffected (p >5%) by the residual toxicity of applied herbicides at 25 DAS. The negative values of percent growth inhibition (in parentheses) indicate that herbicide residues exerted no adverse effect on the root and shoot growth as well as the biomass of 25 days aged seedlings of both crops (Table 7), as compared with control.

Table 5. Residual effect of herbicides on the root growth of mungbean and mustard at 25 DAS

Herbicide combinations used in the previous CA experiments	Root length (cm)	
	Mungbean	Mustard
No herbicide	7.41	5.26
Gly*	7.3(-0.61)[1]	5.32(-1.06)
Gly fb Pen	7.39(-0.30)	5.68(-8.03)
Gly fb Etho	7.42(-0.15)	5.58(-6.13)
Gly fb Car+Iso	7.62(-2.85)	6.06(-15.22)
Gly fb Iso	7.42(-0.15)	5.91(-13.95)
Gly fb Fen	7.43(-0.16)	5.88(-11.83)
Gly fb Pen fb Etho	7.45(-0.45)	6.32(-20.29)
Gly fb Pen fb Car+Iso	7.43(-0.30)	5.93(-12.68)
Gly fb Pen fbIso	7.56(-2.10)	5.97(-13.53)
Gly fb Pen fb Fen	7.47(-0.90)	5.87(-11.84)
Standard deviation	0.09	0.30
Co-efficient of variance (%)	1.25	5.27
Standard error of mean difference	0.03	0.09

*Gly = Glyphosate, fb = followed by, Pen = Pendimethalin, Etho = Ethoxysulfuron-ethyl, Car = Carfentrazone-ethyl, Iso = Isoproturon, Fen = Fenoxaprop-p-ethyl; [1]percent growth inhibition in parentheses

Table 6. Residual effect of herbicides on the shoot growth of mungbean and mustard at 25 DAS

Herbicide combinations used in the previous CA experiments	Shoot length (cm)	
	Mungbean	Mustard
No herbicide	16.8	18.5
Gly*	22.0(-30.95)[1]	22.5(-21.62)
Gly fb Pen	22.7(-35.12)	20.6(-11.35)
Gly fb Etho	21.3(-26.78)	24.0(-29.72)
Gly fb Car+Iso	21.2(-26.19)	20.5(-10.81)
Gly fb Iso	20.6(-22.02)	22.5(-21.62)
Gly fb Fen	17.7(-5.35)	23.5(-27.02)
Gly fb Pen fb Etho	22.2(-32.14)	25.0(-35.13)
Gly fb Pen fb Car+Iso	18.6(-10.71)	21.0(-13.51)
Gly fb Pen fb Iso	20.3(-20.23)	23.3(-25.94)
Gly fb Pen fb Fen	16.9(-0.59)	20.5(-10.81)
Standard deviation	2.06	1.83
Co-efficient of variance (%)	10.3	8.36
Standard error of mean difference	2.98	2.41

*Gly = Glyphosate, fb = followed by, Pen = Pendimethalin, Etho = Ethoxysulfuron-ethyl, Car = Carfentrazone-ethyl, Iso = Isoproturon, Fen = Fenoxaprop-p-ethyl; [1]percent growth inhibition in parentheses.

Table 7. Residual effect of herbicides on biomass (g) of 25 days aged 10 seedlings of mungbean and mustard

Herbicide combinations used in the previous CA experiments	Mungbean	Mustard
No herbicide	1.31	0.40
Gly*	1.32	0.47
Gly fb Pen	1.29	0.46
Gly fb Etho	1.36	0.43
Gly fb Car+Iso	1.42	0.41
Gly fb Iso	1.40	0.46
Gly fb Fen	1.39	0.47
Gly fb Pen fb Etho	1.43	0.46
Gly fb Pen fb Car+Iso	1.47	0.51
Gly fb Pen fb Iso	1.49	0.53
Gly fb Pen fb Fen	1.41	0.42
Standard deviation	0.11	0.081
Co-efficient of variance (%)	7.34	18.24
Standard error of mean difference	2.11	5.27

*Gly= Glyphosate, fb= followed by, Pen = Pendimethalin, Etho = Ethoxysulfuron-ethyl, Car = Carfentrazone-ethyl, Iso = Isoproturon, Fen = Fenoxaprop-p-ethyl.

Moreover, the chlorophyll content of mungbean and mustard leaves based on the mean SPAD meter indicates no significant (p >5%) difference between treatments due to the carryover impact of herbicides utilized in the earlier studies (Figure 2). Again, the mean visual score for the two fields experiment was 0.003 for phytotoxicity and 3.97 for crop vigor (data not given), which correspond to roughly 0 and 4, respectively. Visual examination of the toxicity symptoms on the morphology and robustness of mungbean and mustard plants revealed superior crop development without any toxic indications compared with the control treatment. Furthermore, the biomass of 25 day-old ten seedlings (Table 7), seed yield (Figure 3), and germination percentage (Table 8) of the harvested seeds of mungbean and mustard (mean values of two fields) did not differ statistically (p > 5%) across treatments. The results indicated that three years of continuous application of any herbicide, either alone or in combination with another, did not persist in the soil at sufficient levels to impair seedling growth, seed production, or seed germination of mungbean and mustard.

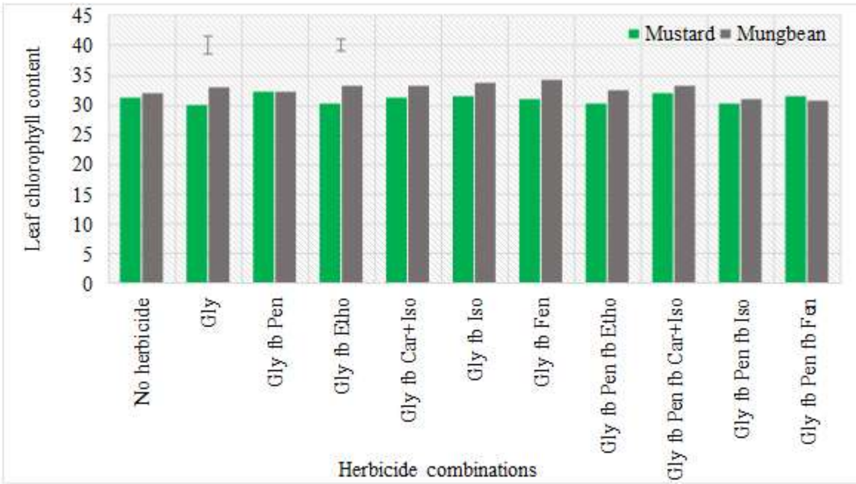

Figure 2. Effect of herbicide residues on the leaf chlorophyll content of mustard and mungbean at p >5% level. Gly = Glyphosate, fb = followed by, Pen = Pendimethalin, Etho = Ethoxysulfuron-ethyl, Car = Carfentrazone-ethyl, Iso = Isoproturon, Fen = Fenoxaprop-p-ethyl.

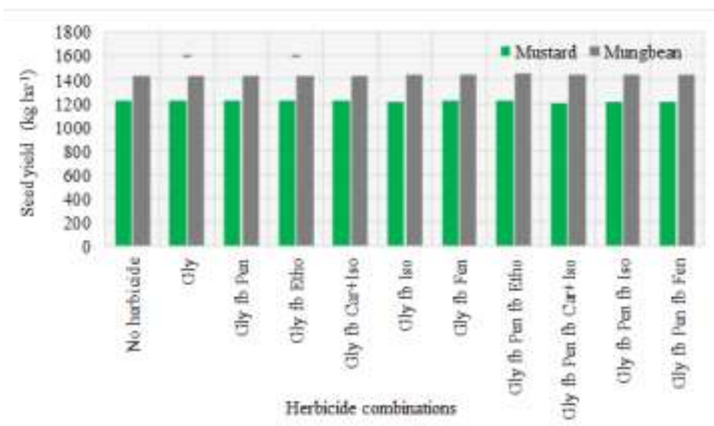

Figure 3. Residual effect of herbicides on the seed yield (kg ha^{-1}) of mungbean and mustard at p > 5% level. Gly = Glyphosate, fb = followed by, Pen = Pendimethalin, Etho = Ethoxysulfuron-ethyl, Car = Carfentrazone-ethyl, Iso = Isoproturon, Fen = Fenoxaprop-p-ethyl.

Table 8. Residual effect of herbicides on the percentage of seed germination rate (laboratory test) of mungbean and mustard

Herbicide combinations	Seed germination (%)	
No herbicide	89	87
Gly*	90	92
Gly fb Pen	89	90
Gly fb Etho	91	89
Gly fb Car+Iso	90	89
Gly fb Iso	89	88
Gly fb Fen	87	89
Gly fb Pen fb Etho	89	95
Gly fb Pen fb Car+Iso	91	86
Gly fb Pen fb Iso	93	88
Gly fb Pen fb Fen	90	94
Mean	90	90
Standard deviation	1.58	2.85
Co-efficient of variance (%)	1.76	3.18
Standard error of mean difference	0.53	0.96

*Gly = Glyphosate, fb= followed by, Pen = Pendimethalin, Etho = Ethoxysulfuron-ethyl, Car = Carfentrazone-ethyl, Iso = Isoproturon, Fen = Fenoxaprop-p-ethyl.

The results of this study occupying six herbicides (glyphosate, pendimethalin, ethoxysulfuron-ethyl, carfentrazone-ethyl + isoproturon, isoproturon, and fenoxaprop-p-ethyl) applied in 10 different combinations at their recommended rates to mungbean, mustard, rice, and wheat over 3 years had no noxious effect on the subsequent mustard and mungbean plant population (Figure 1), growth of shoot and root (Tables 5 and 6), leaf chlorophyll content (Figure 2), seedling biomass (Table 7), seed yield (Figure 3), and the germination rate of harvested seeds (Table 8). Moreover, no toxic symptoms were observed visually on the healthy plant growth across all the treatments. The prior study corroborates our conclusion by demonstrating that pendimethalin, pretilachlor, triasulfuron ethoxysulfuron, pyrazosulfuron ethyl, carfentrazone-ethyl, carfentrazone-ethyl+ isoproteuron, 2, 4-D amine herbicides applied in strip-tilled wheat did not show any adverse effect on germination, and seedling growth of succeeding mungbean (Shamim et al., 2020). Moreover, Zahan et al., 2016 observed no residual impact of these eight herbicides used in rice on the germination of following wheat, lentil, and sunflower in another field research under Conservation Agriculture. Chaudhari *et al. (2020)* also found no harmful residual effect of propaquizafop and imazethapyr on the plant population, height, and dry matter of wheat, chickpea, and mustard. According to the author, this might be due to the non-persistence of herbicides in the soil and crop is attributed to the degradation in the soil which is connected to the half-life (HL) of the herbicides studied. For example, after application in soil the HL (days) of glyphosate: 30−32 (Giesy et al., 2000), pendimethalin: 25−35 (Kočárek et al., 2016), ethoxusulfuron: 60 (Sondhia, 2019), isoproturon: 24 (Accinelli et al., 2005) and fenoxaprop-p-ethyl: 1.45–2.30 (Tandon, 2019), while the HL of carfentrazone-ethyl is 3.8-5.8 hours only (Han et al., 2007). On the other hand, mungbean and mustard need about 90, and 70 days to harvest, respectively and have escaped the residues to be toxic. In addition, residues of herbicide detected in the soil were below the European Union's Maximum Residue Limits (MRLs) for various herbicides (μg g^{-1}) including glyphosate: 1.3 (Helander et al., 2019), pendimethalin: 0.005 (Abdulra'uf et al., 2019), ethoxysulfuron: 0.001 (Sondhia, 2019), carfentrazone-ethyl: 0.003–0.005 (Han et al., 2007), isoproturon: 1.74 (Asma et al., 2002) and fenoxaprop-p-ethyl: <0.01 (Wu et al., 2015). Concerning herbicides half-life vs. crop growth duration and the trace detection levels of residue lower than that of MRLs showed these herbicides have a minimal probability of remaining in the soil until the next crop growing season.

Sondhia (2019) reported that herbicides applied to soybean had no impact on the dry matter accumulation of succeeding wheat, barley, spinach, pea, raya, canola, and sugarbeet due to herbicides used in prior crops being completely degraded. Moreover, no significant change in the growth and development of succeeding maize and barley (Mehdizadeh et al., 2016) and bottle gourd (Singh & Kulshrestha, 2007) was observed by the residues of sulfosulfuron herbicide. Similarly, another research claimed that residues of pyrazosulfuron-ethyl herbicide applied in rice had no detrimental effect on the germination and growth of the following wheat crop (Zahan et al., 2020) might be due to non-persistence in soil. Herbicides persistency in soil might be short-lived due to the cultural activities of various crops, such as flooding for irrigation and microbial degradation, as the primary mechanisms by which herbicides are dissipated from the soil (Lim et al., 2015). Thus, one might argue that many herbicides used for weed control are safe in terms of residual toxicity in soil. The explanation for this may be because the herbicides used have completely degraded in the soil or that their presence is at a measurable level that does not negatively impact the germination of subsequent crops.

In contrast, according to Sondhia (2005), sorghum and cucumber plants were very sensitive to metribuzin and could detect residues even at 0.010 and 0.046 mg kg^{-1}in the post-harvest soil of potato crops. Again, pendimethalin was found to be sufficiently persistent in the soil to negatively affect cabbage (Arora & Gopal, 2004). Mehdizadeh *et al.* (2019) noticed that metribuzin residues in soil had a significant impact on the root and shoot biomass of oilseed rape. In another study, Mehdizadeh *et al.* (2016) sulfosulfuron and tribenuron methyl residues in soil decreased sugar beet dry biomass. According to Kandel *et al.* (2018), application of sulfentrazone + cloransulam-methyl + S-metolachlor and flumioxazin + chlorimuron ethyl + S-metolachlor herbicides posed phytotoxicity at different stages of soybean growth. There is a very scares study to show any residual toxicity on the succeeding crops under the CA practices. Such results imply conducting future long-term studies on the residual effect of herbicides using all the available herbicides to be applied in all types of crops across Bangladesh under CA.

Conclusions

On a sandy loam soil, ten combinations of six herbicides: glyphosate, pendimethalin, ethoxysulfuron-ethyl, carfentrazone-ethyl + isoproturon, isoproturon, and fenoxaprop-p-ethyl along with one control applied to over three years to rice, wheat, mustard, and mungbean had no toxic effect on the plant population, leaf chlorophyll content, seed yield, and germination of harvested seeds of succeeding mustard and mungbean relative to the control treatment. Thus, the study concluded that herbicides used in preceding crops left insufficient residues to affect mustard and mungbean crops. The long-term field trials across the country are essential to validate this result. Under the increased prevalence of herbicide use for weed control in intensive rice-based cropping sequences in South Asia, systematic assessment of herbicide residual risk to crops needs to be instituted as routine practice in this region. Furthermore, there is need to study the herbicide residue effects on the soil microbiology.

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Conflict of interest

No conflicts of interest have been declared.

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