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Original Research Article

Sensitivity of legumes and soil microorganisms to residue of herbicide mixture of atrazine and mesotrione

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Abstract

Mixing of herbicide compounds in one formulation is one way to prevent weed resistance, but it may increase herbicide persistence in the soil and cause eco-toxicity. The research was aimed to evaluate the sensitivity of legumes and the response of soil microorganisms to the residue of the herbicide mixture of atrazine and mesotrione. Herbicide mixture was applied on the soil surface in polybags at 0, 0.125, 0.25, 0.5, 1.0, and 2.0 x field rate (FR). Seeds of soybean, yard long bean, peanut, and mung bean were planted 2 weeks after herbicide application and grown in greenhouse for 4 weeks. Planting legumes was repeated in the same polybag at 8 and 14 weeks after herbicide application. The trials were arranged in a completely randomized design with three replications. Data observed were subjected to analysis of variance and means were further separated by LSD test ($P \leq 0.05$). Harmful effects of herbicide residues on legumes seedlings identified ascrop injury (CI), decreased greenness level of leaves (GLL), and decreased dry biomass weight (DBW) were observed at 2 and 8 weeks after application. After 14 weeks, CI and decreased GLL were evident only on soybean, but DBW of legume seedlings was remaining depressed. Similarly, a population of soil bacteria and fungi enumerated as colony forming unit (CFU) per gram of soil were inhibited up to 18 weeks. Efficacy of herbicide residues (EHR) counted as percent of reduction of DBW and CFU on legumes and microorganisms, respectively, were stronger by increasing the rates of herbicide applied andthe EHR persisted until 18 weeks after herbicide application.

Keywords: Atrazine, Ecotoxicity, Herbicide carryover, Legumes, Mesotrione

Introduction

Herbicides are being widely adopted over the world in order to increase crop production through prevention of weed competition with crops. One reason of herbicide use in crop cultivation is a shortage of agricultural labors that migrating from rural to urban areas as a result of industrialization (Hossain, 2015). Using herbicides for weed control and for tillage may improve not only crop yield, but also environmental conditions because herbicide reduces erosion, fuel consumption, greenhouse gas emissions, nutrient run-off, and conserves soil and water (Gianessi, 2013). Practically, weed control with herbicide is more effective because it can reach weeds that can not be controlled by other measures (Rashid et al., 2012). In Indonesia, herbicides were originally used on the large-scale plantation crops, but for the reasons above the use of herbicide were extended for agronomic and horticultural crops (Simarmata et al., 2017).

Global reports predicted that pesticide market continues to rise 6.56 percent AGR from the year of 2013 to 2018, and herbicide comprises about 40-45 % of pesticide markets (Stubler, 2016; McDougall, 2017). The increased market for herbicides should be followed by the discovery of new compounds. But in fact industry in weed control shifts to improving the nature of plants through genetic modification in order to become resistant to herbicides (Duke and Powles, 2008; Kraehmer and Drexler, 2009). Hence, the discovery of new herbicide compounds has slowed, but mesotrione was released in 2001 to prevent and remedy weed resistant problems (Mitchell et al., 2001; Kraehmer and Drexler, 2009).

Mesotrione (2- [4-(*methylsulfonyl*)-2-*nitrobenzoyl*] *cyclohexane*-1,3-*dione*) belongs to triketon family used for the control of broadleaf weeds in corn, sugarcane and sorghum (James et al., 2006). Mesotrione herbicide is inhibiting the synthesis of HPPD (4-hydroxyphenyl-pyruvate-dioxygenase) that plays a role in carotenoid biosynthesis. Mesotrione released to the market are mostly mixed formulation with other herbicides such as atrazine (James et al., 2006; Woodyard et al., 2009). Atrazine (2-*chloro*-4-*ethylamino*-6-*isopropylamino*-1,3,5-*triazine*) belong to the class of triazine inhibiting photosystem II (Senseman and Armbrust, 2007). Mixing of mesotrione and atrazine may increase herbicide efficacy, spectrum of weed control, and reduce the risk of weed resistance of atrazine (Senseman and Armbrust, 2007; Walsh et al., 2012). But, herbicide mixtures may also increase soil persistence that results in environmental toxicity or eco-toxicity (Robinson, 2008; Sondhia, 2014). Soil persistence of atrazine and mesotrione were more than 12 months with the half-life of 60 and 32 days, respectively (Senseman and Armbrust, 2007; Rahman et al., 2011). If the persistence of herbicides exceeds one growing season then the selection of crops for the following season is limited (Janaki et al., 2015). Growing legumes and vegetables in 10 to 18 months after atrazine or mesotrione application was not recommended (Curran and Lingenfelter, 2012). Residues of mesotrione after one year caused severe injury to some crops such as broccoli, carrot, cucumber, onion, and potato (Robinson, 2008). It was also showed that mixing atrazine with mesotrione accentuated the carryover injury from mesotrione on vegetable crops. In other study showed that sugar beet, lettuce, cucumber, green bean, pea, and soybean showed sensitivity to mesotrione carryover problem which was observed in crop injury, plant dry weight reduction, and yield loss (Riddle et al., 2013).

Herbicide residues in the soil also caused toxicity to soil biology. Long periods of herbicidal residues remaining in soil may eliminate sensitive strains of the microorganism so that population of microorganism decreased (Batisson et al., 2010; Sebiomo et al., 2011). Some reports of eco-toxicity of herbicide residues have been published, such as the residue of butachlor, 2,4-D, pretilachlor, and pyrazosulfuron-ethyl reduced the population of all soil bacteria. The effect was stronger with increasing concentration of herbicides applied (Latha and Gopal, 2010). Residues of metolachlor and mesotrione applied at 1 x of recommended field rate showed a toxic effect on microbial communities (Joly et al., 2012). Other research also reported that bacterial population decreased at 4 weeks after applied of atrazine and suppressed of some bacterial species (Stanley et al., 2013).

The research was assigned to investigate the sensitivity of four legumes included soybean, peanut, yard long bean, mung bean, and to evaluate the toxicity of soil microorganisms to soil residue of herbicide mixture of atrazine and mesotrione.

Materials and Methods

Bioassay of Legume Seedlings

The research was conducted from April to December 2015 in the greenhouse and Laboratory of Plant Protection, the University of Bengkulu, Indonesia. Four legumes included soybean (*Glycine max*), yard long bean (*Vigna unguiculata*), peanut (*Phaseolus vulgaris*), and mung bean (*Vigna radiata*) were bioassay to the residue of atrazine and mesotrione mixture. The planting media of ultisol soil was cleaned, air dried and sieved with a size of 2 mm mesh and then 5 kg of the soil was filled into a polybag. Total of 18 filled polybags, the combination of 6 herbicide levels with 3 replicates, were prepared for each legume commodity. Herbicides solutions were prepared in a sprayed volume of 200 L ha^{-1} at 0.125, 0.25, 0.5, 1.0, and 2.0 x FR (field rates) of formulated herbicide. A non-ionic surfactant (NIS) was added at a concentration of 1% v/v. Herbicide formulation of CALARIS$^{@}$ was ready mixed of atrazine and mesotrione in the ratio of 50 to 5 % w/v, respectively. Water without herbicide and surfactant was used as a control. The herbicide solutions were sprayed pre-emergence on the soil surface of polybags using

knapsack sprayer with a flat-fan nozzle at a pressure of 15 psi. Herbicides were incorporated into the soil by watering up to a field capacity. Herbicide trials were arranged in a completely randomized design (CRD) with three replications.

Three legume seeds in each polybag were planted in 3 cm holes two weeks after herbicide application. Crop maintenances were carried out for 4 weeks period included watering and controlling pests as needed. Data collected included crop injuries (CI), greenness level of leaves (GLL), dry biomass weight (DBW). CI was scored from 0 to 100 percent of injury as described in Table-1 (Stanley et al., 2014), GLL was measured using Chlorophyll Meter, and DBW were obtained from the entire crops in one poly bag and oven dried at 70 C for 72 hours. Efficacy of herbicide residue (EHR) was calculated as Eq. (1) modified from published research article of Widaryanto and Roviyanti (2017).

$$EHR = \frac{(DBWt - DBWc)}{DBWc} \times 100\ \% \quad .....(1)$$

where EHR is efficacy of herbicide residue, DBWt is dry biomass weight of treated seedling, DBWc is dry biomass weight of control or untreated seedling.

After biomass harvested in 4 weeks, the polybag media were fallowed for 2 weeks and weeded before the subsequent planting. The 2nd and 3rd planting were carried out with the same crops as previously done, respectively at 8 and 14 weeks after herbicide application (Table 2). Planting methods, crop maintenances, and data collection were carried out similarly to the first planting.

Toxicity of soil microorganisms

Inoculation of fungi and bacteria from planting media were carried out in petridishes with the media of potato dextrose agar (PDA) and nutrients agar (NA), respectively. One liter of PDA stock media was prepared of 20 g of dextrose, 15 g of agar, and 200 g of potatoes. Potatoes were peeled, washed, and cut into cubes sized of 1 cm^2. The cut potatoes were cooked in a glass beaker containing up to 1 liter of distilled water. The stew solution was filtered into an erlenmeyer glass.

Table 1. Scores of crop injury due to herbicide residues (Standley *et al.*, 2014).

Crop Injury (%)	Effect on Crop
81-100	Complete killed
61-80	Very heavy damage and very heavy necrosis on leaf
41-60	Fairly heavy damage and fairly heavy necrosis on leaf
21-40	Medium injury and severe epinasty
1-20	Light injury and slight epinasty
0	No injury to crop

Dextrose was added to glass, heated until thickened and stored at room temperature. Media for bacteria inoculation was prepared by dilution of 20 g of NA and 15 g of agar in 1 liter of distilled water in a glass beaker, cooked and stirred homogeneously, and kept in the cabinet at room temperature. Inoculants were prepared from soybean media after the first, second, and third planting. One gram of soil sample was diluted homogenously in 9 ml distilled water to achieve a suspension of 1 x 10^{-1} g of soil. The dilutions were repeated to make a suspension of 1 x 10^{-5} gof soil. The stock media was preheated up to 50 C to allow the media to be melted. Streptomycin of 50 ppm was added to PDA media to prevent the growth of non-fungal microorganisms.

Inoculants suspension was pipette 1 ml into petridish, the media was poured up to two third of petridishes and shaken to homogenize the suspension and the media. The petridishes were wrapped and incubated in the storage cabinet at a room temperature. Similar protocols were done with NA media without antibiotics to enumerate bacterial population. The growth of fungi and bacteria were observed at 6 x 24 and 2 x 24 hours after inoculation, respectively.

Table 2.Time Schedule of 3 planting sequences of legume crops.

Week																		
-	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
*	FP-1		I				FP-2		II				FP-3		III			
						IM.1						IM.2						IM.3

***** Soil media preparation in polybags and herbicide application; FP = fallow period; I, II, and III =first, second, and third planting; IM = inoculation of microorganism.

The petridishes were placed upside down on top of the Colony Counter and each colony was marked and counted as colony forming unit (CFU) in 10^{-5} g of soil. Data were presented as CFU in one g of soil which was calculated by taking into account of the inoculants dilutions. EHR of fungi and bacteria were calculated as Eq. (1). Inoculation of fungi and bacteria, preparation of PDA and NA media, and culture methods of microorganisms were repeated similarly after the 2nd and 3rd planting or at 12 and 18 weeks after herbicide application, respectively.

Statistical Analysis

Data observed at each planting time, except for EHR, were subjected to one-way classification of analysis of variance (ANOVA). If the analysis showed a significant influence in F-test, means of the observed variables were separated by Fisher's LSD test ($P \leq 0.05$).

Results

Bioassay of Legume Seedlings

Seedlings of legumes were injured at 2 weeks after herbicide application (the first planting). CI was moderately heavy damage to completely killed (57 to 90 percent injury) which was characterized by necrosis on leaves and mortality of seedlings (Table 3). The effects of herbicides residue were also observed on significantly decreased GLL at all rates of herbicides. Due to increased CI and decreased GLL, the growth of legume seedlings became obstructed which indicated by depressed DBW. The EHR of legumes increased by increasing the herbicide rates which ranges from 27 to 83, 46 to 79, 36 to 86, and 38 to 72 percent from the low to the high rates of herbicides applied on soybean, yard long bean, peanut, and mung bean, respectively. On the second planting or 8 weeks after herbicide application, legumes seedlings were still injury, but the CI was slightly decreased and the GLL slightly increased compared to the first planting (Table 4). Even with the lowest of herbicide rates (0.125 x FR), the residues were still caused medium injury to soybean, yard long bean, peanut, and mung bean with CI of 40, 47, 47, and 43 percent, respectively. Fairly heavy damage of CI of legumes was observed at herbicide residues of 0.125 to 0.5 x FR, and a very heavy damage of CI observed at herbicide residues of 1.0 and 2.0 x FR. Soybean seedlings appeared more sensitive with a very heavily damaged injury from 0.25 to 2.0 x FR (CI = 67-73 percent). Level of the GLL remained low compared with control. DBW in all commodities were also suppressed by the residual of atrazine and mesotrione. In comparison with the first planting, there was a slight recovery on legume seedlings which was indicated by increased DBW at 0.125 x FR. The EHR of all legumes increased with increasing the rates of herbicides applied. The value of EHR of legumes seedlings ranged from 19 to 78 percent.

After 14 weeks of herbicide application (the third planting), CI was observed only on soybean seedlings, which scores ranges from 27 to 51 percent which described a fair damage of seedlings (Table 4). The GLL appeared to be recovery, but at the highest rate of herbicide applied (2.0 x FR), the GLL of soybean, yard long bean, and peanuts seedlings were still lower compare to GLL of control. The residue effects of atrazine and mesotrione at all rates of application were still evident on depressed DBW. The EHR was higher compare with EHR at 2 and 8 weeks after herbicide applied. Based on the EHR, the most sensitive legume to the residue of atrazine and mesotrione was soybean, followed by peanut, mung bean and yard long bean with the EHR values of 21-38, 14-31, 11-27, and 15-19 percent, respectively.

Table 3. Crop injury (CI), greenness level of leaf (GLL), dry biomass weight (DBW), and efficacy of herbicide residue (EHR) of legumes at 2 weeks after application of atrazine and mesotrione mixtures (the 1st planting).

Legume Commodity	Herbicide Rates (x Field Rate)	CI (%)	GLL	DBW (g/polybag)	EHR (%)
Soybean	0.000	0 b	30.67 a	0.84 a	0
(*Glycine max*)	0.125	83 a	22.60 b	0.61 b	27
	0.250	87 a	15.96 c	0.50 b	40
	0.500	87 a	12.90 c	0.27 c	67
	1.000	90 a	9.20 c	0.19 c	77
	2.000	90 a	10.46 c	0.14 c	83
Yard long bean	0.000	0 c	44.96 a	1.81 a	0
(*Vignaunguiculata*)	0.125	63 b	28.56 b	0.97 b	46
	0.250	67 b	26.23 b	0.77 c	57
	0.500	72 b	16.73 c	0.65 c	64
	1.000	84 a	10.73 d	0.39 d	78
	2.000	90 a	8.63 d	0.38 d	79
Peanut	0.000	0 d	34.70 a	1.53 a	0
(*Phaseolus vulgaris*)	0.125	63 c	25.90 b	0.98 b	36
	0.250	67 c	27.06 b	0.89 b	42
	0.500	77 b	25.56 b	0.47 c	69
	1.000	78 b	24.63 b	0.48 c	69
	2.000	90 a	11.33 c	0.22 d	86
Mung bean	0.000	0 d	30.73 a	0.74 a	0
(*Vignaradiata*)	0.125	57 c	24.63 b	0.44 b	38
	0.250	73 b	24.50 b	0.25 c	65
	0.500	85 ab	12.13 c	0.23 c	68
	1.000	87 a	10.90 c	0.23 c	68
	2.000	90 a	8.33 c	0.20	72

Means followed by the same letters in the same column of one commodity were not significantly different by LSD (P≤0.05).

Table 4. Crop injury (CI), greenness level of leaf (GLL), dry biomass weight (DBW) and efficacy of herbicide residue (EHR) of legumes at 14 weeks after application of atrazine and mesotrione mixtures (the 3rd planting).

Legume Commodity	Herbicide Rates (x Field Rate)	CI (%)	GLL	DBW (g/polybag)	EHR (%)
Soybean	0.000	0 d	43.86 a	1.22 a	0
(*Glycine max*)	0.125	27 c	39.93 a	0.96 b	21
	0.250	30 c	32.40 b	0.89 b	27
	0.500	43 b	32.56 b	0.83 bc	32
	1.000	41 b	28.46 b	0.77 c	37
	2.000	51 a	21.90 c	0.76 c	38
Yard long bean	0.000	0	40.50 a	1.99 a	0
(*Vignaunguiculata*)	0.125	0	44.13 a	1.69 b	15
	0.250	0	42.56 a	1.64 b	18
	0.500	0	39.66 a	1.69 b	15
	1.000	0	33.96 b	1.65 b	17
	2.000	0	33.76 b	1.60 b	19
Peanut	0.000	0	51.13 a	1.73 a	0
(*Phaseolus vulgaris*)	0.125	0	47.00 a	1.48 b	14
	0.250	0	45.00 a	1.44 b	17
	0.500	0	47.63 a	1.28 c	26
	1.000	0	46.06 a	1.14 c	34
	2.000	0	38.93 b	1.19 c	31
Mung bean	0.000	0	37.83 a	0.87 a	0
(*Vignaradiata*)	0.125	0	36.20 a	0.77 b	11
	0.250	0	29.50 a	0.72 bc	17
	0.500	0	35.53 a	0.69 bc	20
	1.000	0	34.96 a	0.70 bc	19
	2.000	0	31.26 a	0.63 C	27

Means followed by the same letters in the same column of one commodity are not significantly different by LSD (P≤0.05)

After 18 weeks or after completing of the third planting, the effect of herbicide residues was still evident slightly by depressed the population of the CFU of fungal and bacteria (Table 5). The residues of herbicides mixture of atrazine and mesotrione still inhibited the growth of microbial colonies compared with controls. The population of fungi and bacteria were (79.2-31.0) and (91.6-42.2) x 10^5 CFU per gram of soil with the herbicide rates from 0.125 -2.0 x FR. These populations increased at 6 and 12 weeks after herbicide application. There was a significant recovery in fungal growth indicated by decreased EHR to become 19, 22 and 38 percent at the residue of herbicide applied at 0.125, 0.25 and 0.5 x FR. However, the EHR remained high at the residue of 1.0 and 2.0 x FR which were 68 and 68 percent, respectively. The EHR on bacteria were also decreased to become 14, 41, and 45 percent at the residue of herbicide applied at 0.125, 0.25, and 0.5 x FR, respectively. At the higher rates of herbicide applied of 1.0 and 2.0 x FR, the values of EHR remaining high with 56 and 60 percent reduction compared to control, respective.

Table 5. Enumeration of fungal and bacterial population as colony forming units (CFU) and efficacy of herbicide residue (EHR) influenced by residue of atrazine and mesotrione mixtures at 6, 12, and 18 weeks after applied (after the 1st, 2nd, and 3rd planting, respectively).

Micro-organism	Herbicide Rates (x FR)	After 6 weeks		After 12 weeks		After 18 weeks	
		CFU ($x10^5$ g^{-1} soil)	EHR (%)	CFU ($x10^5$ g^{-1} soil)	EHR (%)	CFU ($x10^5$ g^{-1} soil)	EHR (%)
Fungi	0.000	74.4 a	0	91.4 a	0	98.4 a	0
	0.125	16.0 b	78	77.0 b	16	79.2 b	19
	0.250	12.8 bc	83	63.6 c	30	76.4 b	22
	0.500	13.8 bc	81	55.2 c	40	61.0 b	38
	1.000	11.8 c	84	28.6 d	69	31.2 c	68
	2.000	8.4 c	89	22.2 d	76	31.0 c	68
Bacteria	0.000	87.2 a	0	99.6 a	0	106.4 a	0
	0.125	14.2 b	84	39.2 b	61	91.6 a	14
	0.250	10.6 bc	88	30.4 bc	69	63.2 b	41
	0.500	11.0 b	87	27.8 c	72	59.0 b	45
	1.000	7.4 bc	92	18.8 c	81	46.4 c	56
	2.000	5.0 c	94	17.6 c	82	42.2c	60

Means followed by the same letters in the same column of fungi and bacteria are not significantly different by LSD (P≤0.05)

Discussion

Results of this research showed that soybean, yard long bean, peanut, and mung bean were sensitive to the residue of the herbicide mixture of atrazine and mesotrione. The effects of the herbicide residues on legume seedlings were very harmful at 2 and 8 weeks after application observed as increased level of CI and decreased GLL and DBW. The harmful effects decreased slightly at 14 weeks after herbicide application indicated that the active amount of herbicide mixed with atrazine and mesotrione reduced in the soil. The higher the herbicide rates applied increased the level of CI and decreased the GLL and DBW. The values of EHR on legumes counted from the reduction of DBW remained high until 14 weeks after herbicide application.

In the herbicide manual book, it was described that persistence of atrazine and mesotrione in the soil were more than 12 months, but the amount of active compound decreased over time (Senseman and Armbrust, 2007). A half life of atrazine and mesotrione remained in active form in the soil were 60 and 32 days, respectively (Barchanska et al., 2017). Decreasing the amount of herbicide residues after a half life allowed some tolerant legumes to grow with lower herbicidal effects. After 14 weeks of herbicide application, it was assumed that half of atrazine and mesotrione applied still remained in active form, therefore herbicidal injuries were still evident on sensitive crops such as soybean (Abdul-ghany et al., 2013).

A formulation mixed of atrazine and mesotrione has been marketed and recommended to control grass and broad leaf weeds on cereal crops such as corn, sorghum, and sugarcane (Woodyard et al., 2009). One

goal of mixing atrazine and mesotrione is to prevent the risk of atrazine resistant weeds due to intensive uses of atrazine for a long time (Walsh et al., 2012; James et al., 2006). But mixing herbicides compound may cause harmful effects from herbicide carryover and limit the crop election in the subsequent planting season (Curran and Lingenfelter, 2012). Some crops have been reported injured and yield reduced by the residual effects of atrazine and mesotrione including broccoli, carrot, cucumber, onions, and legumes (Robinson, 2008; Riddle et al., 2013). The previous study reported that a cocktail herbicide of atrazine and mesotrione inhibited growth and formation of nodules on navy bean (Abdul-ghany et al., 2013).

Long persistence of atrazine and mesotrione residue in the soil also caused ecotoxicity to soil microorganism. Two categories of soil microorganisms in responding to herbicides residues are sensitive and tolerant strains. The sensitive strains will be eliminated due to herbicide treatments whereas the tolerant strains were unaffected (Batisson et al., 2010). The growth of fungi and bacteria measured as colony forming units (CFU) were inhibited by herbicide residue at 6, 12, and 18 weeks after application. The higher herbicidal rates applied appeared to suppress the larger number of fungal and bacterial populations. As described in the published research article that some tolerant strains remaining to grow which indicated by increased population of bacteria and fungi over time after herbicide application (Stanley et al., 2013). Some tolerant strains of bacteria that have the capacity to degrade the active form of herbicidal compounds within the soil have been identified in previous reports such as *Bacillussp.* and *Pseudomonas sp.* These strains can be used in bioremediation to reduce the persistence of the herbicide in soil and to minimize its impacts on the natural ecosystem (Sebiomo et al., 2011; Stanley et al., 2013).

The growth of fungal and bacterial populations appeared to be partial recovery after 12 and 18 weeks of herbicides application (after the second and third planting). The lower the dose of herbicide applied showed the higher number of fungi and bacteria populations. The declining active ingredient of herbicides in soil media after 12 and 18 weeks provided the opportunity for some tolerant strains of microorganisms to grow progressively (Stanley et al., 2013). However, the negative influence of herbicidal residues was still evident to inhibit the growth of microbial colonies compared with controls.

When herbicide compound have been decomposed and have no toxic properties, hence it can be a source of organic matter for both sensitive and tolerant soil microorganisms and can increase the growth of fungi and bacteria populations progressively (Stanley et al., 2013). Inhibited population of bacteria and fungi from the carryover or residues of herbicide mixture of atrazine and mesotrione provided valuable information in order to conserve natural ecosystem. Persistence of pesticide carryover within ecosystems for a long period of time could be overcome by some ways such as rotating the atrazine and mesotrione application or by cultural practices such as using other weed control measures.

The residue of a herbicide mixture of atrazine and mesotrione in soil caused toxicity either to legumes or soil microorganisms observed from 2 to 18 weeks after application. The most sensitive legume was soybean, followed by mung bean, yard long bean, and peanut which was indicated by CI, decreased GLL and DBW until 14 weeks of herbicide application. The EHR after 14 weeks of herbicide application on soybean, peanut, mung bean, and yard long bean were 37, 17, 34, and 19 percent, respectively. Herbicide residue of atrazine and mesotrione inhibited the bacterial and fungal population indicated by decreased of CFU per gram of soil. The EHR on fungi and bacteria after 18 weeks of herbicide application were 68 and 56 percent, respectively.

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