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Different Application Time of Atrazine and Mesotrione Mixture to Control Weeds on Grain Sorghum (*Sorghum bicolor* L. Moench)

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One obstacle in growing sorghum is weeds that may inhibit growth and yield. Herbicide is one of weed control measure that has been globally adopted in modern crop production. This study aimed to evaluate the efficacy and effectiveness of different application times of a formulation mixed of atrazine and mesotrione on grain sorghum. Seeds of sorghum var. B-100 were planted at the Agriculture Research Center of Bengkulu University, Indonesia. The application time of a herbicide were preemergence, early-postemergence, mid-postemergence, and late-postemergence. The trials were arranged in a completely randomized block design with 3 replications. Herbicide was applied at 1.0 x field rate (FR) in a 200 L ha^{-1} spray solution using a knapsach sprayer. The results showed that preemergence, early- and mid-postemergence application caused heavy injury on sorghum plants at 1 and 2 weeks after treatment (WAT), decreased to medium injury at 3 WAT, and recovered from the injury at 4 WAT. On the other hand, the late-postemergence application only caused medium injury at 1 and 2 WAT and recovered at 3 WAT. Preemergence, early- and mid-postemergence applications inhibited plant height, but late-postemergence application appeared to have no effect on plant growth. The early- and mid-postemergence application showed the highest efficacy which suppressed weed biomass 55.8 and 56.3 percent, respectively, while the late-postemergence was the most effective to increase the yield and biomass of biomass by 63.8 and 40.4 percent, respectively.

Keywords: atrazine, mesotrione, preemergence, postemergence, sorghum (*Sorghum bicolor* L. Moench)

Introduction

Sorghum (*Sorghum bicolor* L. Moench) is the fifth of the cereal crops after wheat, rice, barley, and corn. Many uses of grain sorghum are for food, animal feed, and for industry (Dahlberg *et al.*, 2011a). Seeds of grain sorghum have a good nutritional content, which are in 100 g contains carbohydrates protein, fat, calcium, phosphor, iron, vitamin B_1, and water of 73 g, 11 g, 3.3 g, 28 mg, 287 mg, 4.4 mg, 0.38 mg, and 11 g, respectively (Sirappa, 2003;

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Ramatoulaye *et a*l., 2016). Sorghum also contains juice in the stem that can be extracted and fermented into bioethanol (Prasad *at al.,* 2007; Almodares and Hadi, 2009; Rutto *et al.,* 2013). Leaf biomass of sorghum consists of 14-16 percent of the total fresh biomass can be used as forages for animal feed (Dahlberg *et al.,* 2011b).

Sorghum plants have a wide adaptability and tolerant to environment stress such as to drought and salinity (Saberi *et al.,* 2013). Because of the benefits and wide adaptability, grain sorghum is an appropriate crop to be cultivated in areas that have limitation for growing crops because of land's marginalities. One constraint of growing sorghum at marginal land is competition of weeds because weeds also known as adaptive plants under marginal conditions (Monaco *et al.,* 2013). Weeds compete with crops for nutrients, water, CO_2 and growing space, thus interfere plant growth and decrease the quality and quantity of yield. Loss of yields of agronomic and horticultural crops due to weed competition can be varied from 30 to 50% (Oerke, 2005; Simarmata *et al.,* 2015).

The harmful effects of weeds can be minimized by an appropriate control measure. One method of weed control considering relatively low cost and labor, and less time consuming is using herbicides (Monaco *et al.,* 2002). One of the recommended herbicide for cereal crops is mesotrione (James *et al.,* 2006). This herbicide was released to the market in 2001 (Mitchell *et al.,* 2001). Mesotrione is a phytotoxin herbicide extracted from buttlebrush plant. The mode of action inhibits the function of an enzyme *p-hydroxy-phenyl-pyruvate dehydrogenase* (HPPD). The symptoms on plants is bleaching due to inhibition of the carotenoid biosynthesis, thus photosynthesis was interfered and plant was died (Senseman and Armbrust, 2007).

Mesotrine herbicides are widely marketed in formulations mixed with other herbicide such as atrazine (Abendroth *et al*., 2006; Woodyard *et al*., 2009; Walsh *et al*., 2012). Atrazine includes in triazine class that can be applied pre- and postemergence. The mechanism of atrazine within plants is inhibiting photosystem II (Senseman and Armbrust, 2007). A formulation mixed of atrazine and mesotrione is recommended as a remedy to overcome the risk of weed resistance due to the intensive use of atrazine on cereal crops (Panacci and Covarelli, 2009; Woodyard *et al*., 2009; Walsh *et al.,* 2012). One of the mixed formulations of atrazine with mesotrione in Indonesia is Calaris@, which was mixed in a ratio of 500 to 50 g per liter, respectively (Hasanuddin, 2013).

The efficacy of herbicide on weed and the effectiveness of control measures on crops are influenced by many factors and one of the determining factors is time of application (Abit *et al*., 2010; Monaco *et al.,* 2013; Carles *et* *al*., 2017). Preemergence application has a high efficacy because herbicide inhibits weeds since germination, but on the other hand it can cause harmful effects to crops. Postemergence application directly ihibits the growing weeds but may cause injury to the growing crops (Monaco *et al*., 2013) Mesotrione was reported to cause injury on agronomic and vegetable crops, but the crops were recovery after 5 weeks of herbicide application (Abit *et al*., 2010; Riddle *et al*., 2013). The late-postemergence at 5 weeks after planting, crop injury may be prevented, but the efficacy of weed control may be decreased.

The objective of this study was to evaluate the efficacy and effectiveness of different application time of a formulation mixed of atrazine and mesotrione included preemergence, early-, mid-, and late-postemergence applications for weed control on grain sorghum.

Materials and Methods

The research was conducted at the Agriculture Research Center of Bengkulu University, Indonesia from October 2015 to February 2016. The experimental site was located at 03^{0} 45'08 "South and 102^{0} 16'59 East with the altitude at 12 meter above the sea level. Weed vegetation on the field was analyzed in 10 sampling plots using a wooden square sized 0.5 m x 0.5 m. The dominant weeds were determined based on the value of summed dominance ratio (SDR) calculated from the average of relative density, frequency and dominance of each weed species, such as Eq.1 (Widaryanto and Roviyanti, 2017).

$$SDR = \frac{Dr + Fr + D'r}{3} \qquad [1]$$

SDR = summed dominance ratio
Dr = Relatif density was calculated from absolute density of one species divided by total of absolute density of all species.
Fr = Relatif frequency was calculated from absolute frequency of one species divided by total of absolute density of all species.
D'r = Relatif dominance was calculated from absolute dominance of one species divided by total of absolute density of all species.

Field preparation was started by cutting the weeds and land was cultivated twice using a tractor. Experimental plots were formed with a size of 3 m x 2 m for each treatment. The research was conducted to evaluate the application time of a herbicide mixture of atrazine and mesotrione, included preemergence which applied one week before planting, early-postmergence applied 2 weeks after planting (WAP), mid-postemergence applied 3 WAP, late-postemergence applied 4 WAP, and unweeded as a control. The treatments were arranged in randomized completely block design (RCBD) with 3 replications as blocks. Herbicide was applied at 1.0 x recommended dose of field rate (FR) in 200 L ha^{-1} of spray volume, using a knapsack sprayer with flat-fan nozzle in a pressure at 15 psi.

Sorghum seeds were planted in the hole of 3 cm depth, raw spaced of 75 cm, and the distance of 20 cm within a raw. Insecticide of *Carbofuron* granules was added into the planting hole before covered with soil. Plants were fertilized with nitrogen (N), phosphate (P_2O_5), and potassium chloride (KCl) fertilizers of 100 kg ha^{-1} for each, applied on an array of 5 cm apart from the crop rows. Nitrogen fertilizer was given 2 times, at the planting time and at 5 WAP with a half dose of each application, while phosphate and potassium chloride were given once at the planting time. Crops were watering daily in the first week, thinning at 1 WAP, pest-controlled as needed using *deltamethrin* 25 EC and *dithane* M-45. Weed control was done in accordance with the treatment. Harvesting was done at 100 days after planting or by considering the criteria of maturity seeds of grain sorghum.

Data collected included crop injury due to herbicide application, growth, yield, and biomass of sorghum, and weed biomass. Crop injury was observed visually at 1, 2, 3 and 4 weeks after herbicide application. The scores of crop injury were described in Table 1, which were ranged from 0.0 to 4.0, where a score of 0.0 indicated no injury and a score of 4.0 was severely injury and died (Simarmata *et al.,* 2015). Plant growth variables included plant height and number of leaves observed at 10 WAP. Sorghum yields were harvested from sample crops and measured as dried seeds with 10-12 percents of water content. Yield components included length of panicle and weight of 1,000 seeds obtained from dried seeds. Plant biomass was harvested from the whole plant sample, while the weed biomass was trimmed from 2 sampling plots using a wooden square sized of 0.5 m x 0.5m for each of experiment plot. Weeds biomass collected at the end of the experiment by cutting weeds above the soil surface and oven dried at 70 °C for 72 hours.

Data were subjected to one-way analysis of variance (ANOVA) and means of each parameter that was significantly influenced in F-test were further separated with Duncan's multiple range test (DMRT) at $P \leq 0.05$.

Table 1. Scores of crop injury (Simarmata *et al*., 2015)

Score	Level of injury	Description
0	No injury	0 - 5 % the shape or color of the young leaves is not normal
1	Light injury	6-10 % s the shape or color of the young leaves is not normal
2	Medium injury	11-20 % the shape or color of the young leaves is not normal
3	Heavy injury	21-50 % the shape or color of the young leaves is not normal
4	Very heavy injury	> 50 % the shape or color of the young leaves is not normal until it dries and the plant dies

Results and Discussion

Weed assessment

Weed assessment identified 10 weed species on the research site. The field was previously planted for maize and fallowed for three month before being used for this research. Based on the SDR values the ranks of dominant weed species were *Dichrocephala integrifolia, Eleuheranthera ruderalis, Imperata cylindrica, Cleome rutidosperma, Paspalum conjugatum, Borreria leavis, Eclipta prostrate, Mikania micrantha, Richardia brasiliensis,* and *Ageratum conyzoides* with SDR values of 15.97, 14.53, 13.80, 12.92, 8.81, 8.07, 7.04, 6.50, 6.41, and 5.99 percent, respectively (Table 2). Distribution of weeds composed of broadleaf weeds (B = 8 species) and grass weeds (G = 2 species).

Crop injury

The growth stage influenced sensitivity of crops to herbicide treatment. The height and the number of leaves of sorghum at early-postemergence (2 WAP) were 34-40 cm and 4-5 sheaths per plant, at mid-postemergence application (3 WAP) were 75-80 cm and 6-7 sheaths per plant, and at late-postemergence application were 90-95 cm and 8-9 sheaths per plant (Table 3). The older the growth stage of crops the more tolerant the crops or less injury due to a herbicide mixed of atrazine and mesotrione (Abit *et al.*, 2010).

The herbicide mixture of atrazine and mesotrione caused injury to cereal crops when applied at the early stage of plant (Abendroth *et al*., 2006; Abit *et al.*, 2010). In this study, sorghum plants were severely injured at 1 WAT with the preemergence, early-postemergence and mid-postemergence applications with the injury levels of 3.1, 3.9 and 3.5, respectively (Table 3). The injury dropped slightly at 2 WAT and then dropped very drastically at 3 WAT with the levels of 0.9, 0.9 and 0.3, respectively. Sorghum plants were recovery and become normal without injury symptoms at 4 WAT. On the other hand, with

Table 2. Weed assessment at the experiment sites calculated as SDR values (Summed Dominance Ratio) observed in 10 samples of wooden square sized of 0.5 m x 0.5 m

No.	Weed species (Group)[1]	Weed population[2]										Density (D)		Frequency (F)		Dominance (D')		SDR[3]
		01	02	03	04	05	06	07	08	09	10	Abso-lute	Relatif	Abso-lute	Relatif	Abso-lute	Relatif	
1	*Dichrocephala integrifolia* (B)	16	23	7	22	15	28	-	22	19	18	17	19,23	0,9	12,33	18,89	16,24	15,93
2	*Eclipta prostrate* (B)	9	-	5	8	6	3	7	-	2	6	4,6	5,20	0,8	10,96	5,75	4,94	7,04
3	*Eleuheranthera ruderalis* (B)	12	15	10	19	38	11	21	-	-	22	14,8	16,74	0,8	10,96	18,50	15,90	14,53
4	*Mikania micrantha* (B)	2	-	4	9	-	8	6	-	8	5	4,2	4,75	0,7	9,59	6,00	5,16	6,50
5	*Cleome rutidosperma*(B)	5	11	9	-	18	-	21	23	30	9	12,6	14,25	0,8	10,96	15,75	13,54	12,92
6	*Ageratum conyzoides* (B)	8	6	9	-	8	4	3	-	-	-	3,8	4,30	0,6	8,22	6,33	5,44	5,99
7	*Imperata cylindrica* (G)	-	22	18	24	12	-	16	20	7	19	13,8	15,61	0,8	10,96	17,25	14,83	13,80
8	*Paspalum conjugatum* (G)	11	12	10	14	-	-	17	7	-	-	7,1	8,03	0,6	8,22	11,83	10,17	8,81
9	*Borreria leavis* (B)	13	3	6	-	1	10	11	18	-	-	6,2	7,01	0,7	9,59	8,86	7,61	8,07
10	*Richardia brasiliensis* (B)	9	6	11	-	2	-	-	-	13	2	4,3	4,86	0,6	8,22	7,17	6,16	6,41
	Total	85	98	89	96	100	64	102	90	79	81	88,4	100	7,3	100	116,33	100	100

[1]B = Broadleaf, G = Grass; [2]Population in one wooden square sizes of (0.5 m x 0.5 m); [3]SDR = Summed Dominance Ratio calculated as Eq. 1.

the late-postemergence application, the plant only had light to moderate injury at 1 WAT with a score of 1.8. The level of injury decreased at 2 WAT with a score of 0.9 and the crops grew normal at 3 WAT.

Different harmful effect on sorghum plants due to different application time of a mixed herbicide of atrazine and mesotrione had been reported previously (Abit *et al*., 2010). The level of injury was closely related to the growth stage of crops, where herbicide application in the early stage was more sensitive (O'Sullivan *et al*., 2002). At the early stage, plant tissues were mostly very young and easily damaged by herbicides (Mitchell *et al*., 2001), thus crops were injured severely. With the late-postemergence application or 5 WAP, sorghum tissues developed stronger and more tolerant to herbicides. Sorghum plants were recovery from herbicidal injuries after 5 weeks of herbicide application (Abendroth *et al*., 2006; Abit *et al*., 2010).

Table 3. Crop injury of grain sorghum due to different application time of herbicide treatment

Time of herbicide application[1]	Growth stage		Score of crop injury[2]			
	Plant height (cm)	Number of leaves (sheaths plant^{-1})	1 WAT	2 WAT	3 WAT	4 WAT
Unweeded	-	-	0	0	0	0
Preemergence	0	0	3.1	2.4	0.9	0.2
Early-postemengence 2 WAP	35 - 40	4 - 5	3.9	3.1	0.9	0
Mid-postemergence 3 WAP	75 - 80	6 - 7	3.5	3.3	0.3	0
Late-postemergence 4 WAP	90 - 95	8 - 9	1.8	0.9	0	0

[1]WAP = week after planting; [2]WAT = week after treatment

Growth and yield of grain sorghum

Different application time affected plant height significantly, but did not affect the number of leaves, length of panicle, and weight of 1,000 seeds (Table 4). The highest crop was observed with the late-postemergence application of 309.9 cm, followed by unweeded, preemergence, early-postemergence and mid-postemergence applications, respectively for 289.3, 285.1, 267.0 and 256.8 cm. The difference of plant height was due to the effect of crop injury which interfered the growth of crops (Abit *et al.*, 2010). Plant height appeared most depressed with the early- and mid-postemergence, whereas plant height was no longer affected with the late-postemergence application. The stronger response of sorghum with the late-postemergence was correlated to the growth stage that has reached 90-95 cm height, thus herbicide solution barely reached the maristematic region of the sorghum plant. The herbicide applied only about reached the bottom part of the stem. In these circumstances, the late-postemergence application might accelerate plant growth to be significantly higher than unweeded.

Different application time of herbicide mixture of atrazine and mesotrione also influenced sorghum yield significantly. The highest yield was found with the late-postemergence application of 108.3 g plant^{-1}, followed by the mid-postemergence, early-postemergence, preemergence, and unweeded of 98.2, 97.1, 92.3 and 66.1 g plant^{-1}, respectively (Table 4). Higher yield with the late-postemergence application were the impacts of uninterrupted both vegetative and generative part of the sorghum due to herbicide treatment, and on the other hand the emerged weeds were controlled. In contrast to untreated plots that exhibited high growth but weeds remained grew and compete with sorghum for nutrients, water and growing space (Monaco *et al*., 2013).

Table 4. Effect of different application time of herbicide on the sorghum growth, yield, and biomass, and on weed biomass

Herbicide application time	**Plant height (cm)**	**Number of leaves (sheaths plant^{-1})**	**Length of panicle (cm)**	**Weight of 1,000 seeds (g)**	**Yield (g plant^{-1})**	**Sorghum dried biomass (g plant^{-1})**	**Weed dried biomass (g m^{-2})**
Unweeded	289.3 b	11.5	19.5	39.3	66.1 c	298.5 d	227.7 a
Preemergence	285.1 b	12.2	19.9	41.6	92.3 b	394.8 bc	112.7 c
Early-postemergence (2 WAP)	267.0 c	12.3	18.7	43.3	97.1 b	387.1 c	100.6 c
Mid- postemergence (3 WAP)	256.8 c	10.8	18.2	38.3	98.2 b	406.8 b	99.6 c
Late-postemergence (4 WAP)	309.9 a	10.7	20.0	41.3	108.3 a	419.0 a	147.7 b
F-test from ANOVA	*	ns	ns	ns	*	*	*

Means followed by the same letters in one column are not different by DMRT at P≤ 0.05.
* or ns = significant or non significant effects of the application time from one-way analysis of variance (ANOVA) at P ≤ 0.05.

Biomass Production

Different application time of herbicide mixture of atrazine and mesotrione also affected sorghum and weed biomass. The depressed of weed biomass increased the production of sorghum biomass. The highest sorghum biomass production was observed in the late-postemergence application of herbicide mixture of 419.0 g plant^{-1}, followed by mid-postemergence, preemergence, early-postemergency and unweeded of 406.8, 394.8, 387.1 and 298.5 g plant^{-1}, respectively. While the highest weed biomass was observed in untreated plot of 227.7 g m^{-2}, followed by late postemergence, preemergence, early-postemergence, and mid-postemergence of 147.7, 112.7, 100.6 and 99.6 g m^{-2}, respectively (Table 4).

Sorghum biomass is an important component in cultivation of sorghum as it can be used to feed ruminants and can also be extracted to obtain sugar (Almodares and Hadi, 2009; Dahlberg *et al*., 2011b). An increase in sorghum biomass was due to depressed competition with weeds. The heaviest biomass observed with the application of late-postemergence because less harmful effect of herbicide. Likewise, mid-, early-postemergence, and preemergence application produced higher biomass compare with unweeded because emerged weeds in unweeded plot remained to compete with sorghum plants (Bollman *et al*., 2006).

The increase of biomass and yield were the impact of depressed of weeds observed at the end of the study (Bollman *et al*., 2006). The weed biomass of unweeded plot reached 227.7, followed by the late postemergence, preemergence, early post emergence, and mid-postemergence of 147.7, 112.7, 100.6 and 99.6 g m^{-2}, respectively. It appeared that the lowest weed biomass was found in the mid-postemergence and this was not significantly different from premergence and early-posemergence application. The difference of weed biomass showed the efficacy of different application time of herbicide to control weeds.

Efficacy and Effectiveness

Efficacy is the ability of herbicides alone or mixtures to control weeds that can be measured in decreased weed biomass, while the effectiveness of weed control to increase the growth, yields, and the production of crop biomass. Time of application determined the efficacy of herbicide mixed of atrazine and mesotrione on sorghum plants (Carles *et al*., 2017). Table 5 showed that the highest efficacy was observed in the mid-postemergence of 56.3, followed by early-postemergence, preemergence and late postemergence of 55.8, 50.5 and 35.1 percent, respectively. On the other hand, the effectiveness of herbicide with different application time measured by increase of sorghum yield and biomass. The highest effectiveness on yield observed with the late-postemergence applications of 63.6 percent, followed with the mid-postemergence, the early-postergence, and preemergence of 48.6, 46.9 and 39.6, respectively. While the most effective of application time of herbicide mixture on sorghum biomass production observed in the late postemergence of 40.4 percent, followed by mid-postemergence, preemergence, and early-postemergence of 36.3, 32.3 and 29.7 percent, respectively.

The increased efficacy and effectiveness on sorghum biomass and yield was strongly correlated with life stage of grain sorghum when herbicide applied (Abit *et al*., 2010). The efficacy with different application time ranged from 35.1 to 56.3 percent, while the effectiveness ranged from 39.6-63.6 and 29.7-40.4 respectively to yields and sorghum biomass. Despite the lowest efficacy of the late-postemergence, but it provided the highest effectiveness on yield and biomass (Panacci and Covarelli, 2009). This can be explained with the application of late-postemergence with a plant height of 90-95 cm and 8-9 leaves plant^{-1}, thus plant did not experience to herbicidal toxicity and certainly allowed to the optimum of vegetative and generative phases of sorghum.

Table 5. Herbicide efficacy and effectiveness counted as suppressed weed biomass and as increased sorghum yield and biomass, respectively

Herbicide application time	Herbicide efficacy[1] (%)	Herbicide effectiveness[1]	
		sorghum yield (%)	sorghum biomass (%)
Preemergence	50.5	39.6	32.3
Early-postemergence (2 WAP)	55.8	46.9	29.7
Mid-postemergence (3 WAP)	56.3	48.6	36.3
Late-postemergence (4 WAP)	35.1	63.8	40.4

[1]Reduction of weed biomass; [2]Increased of sorghum yield and biomass counted from untreated plot.

Conclusion

A formulation mixed of atrazine and mesotrione herbicides caused a heavy injury on grain sorghum at 2 weeks after treatment (WAT). The injury level decreased slightly at 3 WAT and no injury was observed at 4 WAT. The late-postemergence application caused only a medium injury at 2 WAT and no injury was observed at 3 WAT. The mixed formulation of atrazine and mesotrione inhibited plant height, but late-postemergence applications appeared to have no effect on plant growth parameters. The early and mid-postemergence application showed the highest efficacy which suppressed weed biomass by 55.8 and 56.3 percent, respectively. The most effective application time was the late-postemergence which increased the yield and sorghum biomass by 63.8 and 40.4 percent, respectively.

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