Long-term application of diuron herbicides caused *Eleusine indica* weeds to become resistant to diuron

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Abstract. The presence of weeds during the vegetative growth of pineapple plants could reduce production by up to 88%, so weed control is necessary. One way of controlling weeds is by use of herbicides. However, continuous, long-term application of herbicides could result in environmental pollution and weed resistance to the herbicides. This research aimed to identify the resistance of *E. indica* weeds to herbicide diuron and to compare the growth characteristics of diuron-resistant and diuron-sensitive *E. indica*. The resistant weeds were taken from the pineapple plantations under continuous, long term application of diuron herbicides, while the sensitive weeds were taken from areas which have never been applied with diuron herbicides. This research was conducted at the University of Lampung, from February to October 2020. It consisted of two experiments, The experiment 1: the response of *E. indica* to diuron and The experiment 2: the growth characteristics of diuron-resistant and diuron-sensitive *E. indica*. In the experiment 1, experiments were arranged in a split plot design with five replicates and the treatments consisted of two factors. The first factor consisted of the diuron-resistant and the diuron sensitive *E. indica* and the second factor was diuron dosage (0, 1600, 3200 and 6400 g ha^{-1}). In the experiment 2, The resistant and sensitive *E. indica* were compared using t-test. The study revealed that the diuron-resistant *E. indica* needed a longer time to be 50% damaged than the sensitive one. The resistance index (RI) of *E. indica* to diuron was 2.80, indicating low level of resistance. The diuron-resistant and diuron-sensitive *E. indica* did not significantly showed different growth characteristics.

1. Introduction

Pineapple (*Ananas comosus* L.) fruit is one of the most important agricultural commodity of Indonesia, and Lampung Province is one of the pineapple production centers, with an annual production of 533,141 tons during 2011-2015 [1]. Weed infestation is among the factors that could lead to significant decrease in pineapple production. Eshetu et al. [2] reported that the presence of weeds in the vegetative growth stage of pineapple plants could reduce production by up to 88%. Therefore, weed control is necessary and the application of herbicides is considered the most effective. Hayata et al. [3] reported that chemical control using herbicides was more effective than other means.

The herbicides commonly used in pineapple plantations in Lampung are those containing diuron as an active ingredient. Diuron is a systemic and selective herbicide against broad-leaf weeds and grasses. This herbicide is able to inhibit weed growth by inhibiting electron transport in the photosystem II [4]. Diuron herbicide has been used in Lampung pineapple plantations since 1979 with the dosage twice the recommended dose and has been applied routinely [5].

The use of herbicides with the same mechanism for a long period of time could result in environmental pollution and weed resistance to the herbicides. Kumar et al. [6] reported that the use of

diuron in agriculture could result in physiological damage and disruption of seaweed (*Saccharina japonica*) growth. The continuous use of herbicides diuron (1250 and 2500 ppm) has been reported to cause an oxidative stress, increase cell metabolism, and increase cell death in the bladder of male rats [7].

Weed resistance is the ability of weeds to withstand the application of herbicides higher than the recommended dosage. The factors that can lead to the resistance include gene mutations, initial frequency of resistance alleles, inheritance, freshness of weeds, absence of herbicides, weed crossing systems, and gene flow [8].

Eleusine indica is one of the dominating weeds in pineapple plantations in Lampung. It was reported that *E. indica* from a pineapple plantation in Lampung showed moderate resistance to diuron, having a resistance index (RI) of 6.61 [9]. This is supported by Sari's research [10], showing that *E. indica* from pineapple plantations in Lampung has low resistance with RI of 4.92. It is important to know difference growth characteristics between the diuron-resistant and diuron- sensitive *E. indica* in order to get more understanding of the resistance mechanism. This current study aimed to identify the resistance of *E. indica* and to compare growth characteristics of the diuron-resistant and diuron-sensitive *E. indica* in pineapple plantations in Lampung, Indonesia.

2. Materials and Methods

2.1. Plant materials

Seeds were derived from *E. indica* weeds taken from pineapple plantations under continuous, long-term application of diuron herbicides and from those taken from areas which have never been applied with diuron herbicides. The former were reported to show some degree of resistance to diuron, while the latter were sensitive to diuron [10]. The seeds were sown on media composed of cocopeat and compost for 35 days until they germinated and grew to become weed seedlings. The weeds were transferred to plastic pots (20 cm in diameter) containing soils and composts, one weed per pot. The weeds were maintained until they produced physiologically-matured seeds.

2.2. Response of diuron-resistant and diuron sensitive E. indica to diuron application

This research was conducted in a greenhouse and The Weed Laboratory, Faculty of Agriculture, University of Lampung, Indonesia, from February to October 2020. The experiments were arranged in a split-plot design with five replications. The treatments were factorially arranged with 2 factors. The first factor consisted of the diuron-resistant and the diuron sensitive *E. indica* and the second factor was the diuron dosage (0, 1600, 3200 and 6400 g ha^{-1}). Herbicide application was conducted by spraying, starting from the lowest to the highest dose using a knapsack sprayer. Before the application, the spraying was calibrated.

Percent poisoning was determined by comparing the treated weeds with the untreated ones (control) at 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 days after treatment (DAT). The weeds were harvested at 20 DAT by cutting at the base of the stems and then dried in an oven at 80^{o}C for 48 hours. LT_{50}, the time required for a herbicide to cause 50% poisoning, was determined by probit analysis of the percent poisoning. ED_{50}, the dosage that is able to control the weeds by 50%, was calculated by converting weed dry weight to the proportion of weed damage [12]. Resistance index (RI) was determined by deviding ED50 of resistant weeds with that of sensitive weeds. The level of weed resistance was based on the criteria developed by Ahmad-Hamdani et al. [13] as the followings: RI <2 = sensitive; RI 2-6 = low resistance; RI> 6 – 12 = moderate resistance; and the RI> 12 = high resistance.

2.3. Growth characteristics of diuron-resistant and diuron-sensitive E. indica

The experiment was arranged in a completely randomized design with three replications, with one experimental unit consisting of nine individual weeds. In this experiment, the growth of diuron-resistant *E. indica* was compared with that of diuron-sensitive *E. indica*. Tiller number, branch number, panicle number, plant height, and seed weight were recorded. The different means were tested using T test.

3. Results and Discussion

3.1. Response of diuron-resistant and diuron sensitive E. indica to diuron application

Diuron herbicide application can cause poisoning of weeds with symptoms of yellowing and then the weeds are death. This research showed that the diuron-sensitive *E. indica* had higher symptoms of poisoning than the diuron-resistant *E. indica* as a response to diuron herbicide application. The sensitive weeds died completely after the application of the diuron herbicide at a dose of 3,200 and 6,400 g ha $^{-1}$. (Figure 1) Diuron could be applied by both preemergence and postemergence spraying. In response to the spraying, weed leaves turned yellow between the leaf veins and then turned brown from the base and outer leaf margins towards the middle [16]. Diuron will be translocated to all parts of the plant along with transpiration through the apoplast pathway, and not distributed to the phloem, and accumulation in the symplast tissue is very small [17].

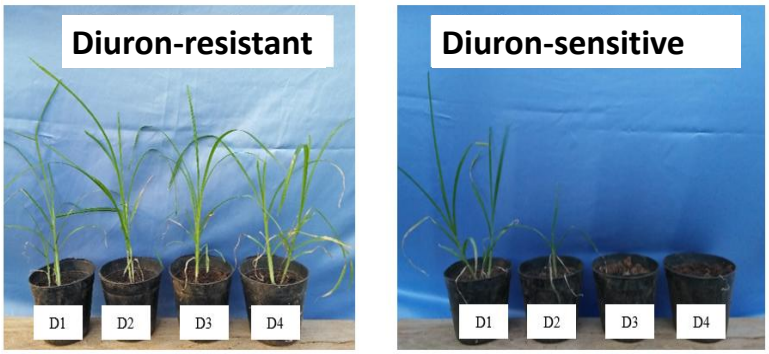

Figure 1. Response of diuron-resistant and diuron-sensitive *E. indica* to diuron application at 20 days after treatment (DAT). D1= without diuron. D2=1.600 g ha^{-1}. D3 =3.200 g ha^{-1}. D4=6.200 g ha^{-1}

LT_{50} is the time needed for the diuron herbicide to control *E. indica* weeds by 50%. The results of the study showed that the higher the dose used, the faster the herbicide is poisoned (Table 1). The sensitive weeds are poisoned more quickly than the resistant ones. For example, the diuron at 1,600 g ha^{-1} had the LT_{50} value of 87 days for the resistant weeds and 11 days for the sensitive ones (Table 1). Application of diuron at 6,400 g ha^{-1} resulted in LT_{50} of 58 days for the resistant weeds and 4 days for the sensitive ones.

Table 1. LT_{50} (lethal time) values of *E. indica* due to diuron application. LT_{50}= the time needed to cause 50% mortality

Dose (g ha^{-1})	Origin of Weeds	Regression	LT_{50} (days) Resistant	Sensitive
1600	Resistant	y = 1,6323x + 1,8324	87	
	Sensitive	y = 3,0283x + 1,8026		11
3200	Resistant	y = 1,6433x + 1,9239	74	
	Sensitive	y = 6,0101x + 1,0491		5
6400	Resistant	y = 1,72x + 1,9605	58	
	Sensitive	y = 5,7249x + 1,3321		4

Note : y = probit value of weed poisoning percentage, x = log of day of observation, and LT = anti log of x value.

Weed resistance is formed because of the ability of weeds to withstand the pressure of selecting one type of herbicide that is used repeatedly for a long time [18]. ED_{50} values of the resistant and the sensitive *E. indica* were 6.80 and 2.43 g ha^{-1}, respectively, so the value of the RI (resistance index) was

2.80 (Table 2). The weeds having RI of 2.8 is considered to heve low resistance [13]. There are five possible mechanisms in the occurrence of resistance, namely mutations in the herbicide site of action, deactivation of active ingredients to become non-phytotoxic, reduced translocation of herbicides, immobilized herbicides into vacuoles or cell walls, and dilution of herbicides [19]. To inhibit the resistance of weeds to herbicides, integrated control is needed by using several methods. Alcantara et al. [20] reported that the use of herbicides with different modes of action could delay the section of herbicides resistance.

Table 2. ED_{50} (Effective doses at 50% mortality) and RI (resistance index) values of *E. indica* due to diuron application.

Weeds	**ED_{50}**	**Resistant Index (RI)**	**Levels of Weeds Resistance Based on Ahamd-Hamdani *et. Al.* (2012)**
Resistant	6,80	2,80	Low Resistant
Sensitive	2,43		

Note : RI = ED_{50} of resistant weeds divided by that of sensitive weeds.

3.2. Growth characteristics of diuron-resistant and diuron-sensitive E. indica

The t-test showed that for all of the variables, there was no significant difference in growth characteristics between the diuron-resitant and diuron-sensitive *E. indica* (Table 3). This results suggests that mutations in resistant genes of *E. indica* had no significant effect on the growth characteristics. This is in line with research of Delye et al. [14] who reported that mutations in Asn_{2041} which cause resistance to the herbicide ACCase inhibitor did not have a significant effect on the germination of the weed *Alopecurus myosuroides.* Lestari et al. [15] also reported the insignificant effects of mutation on growth variables in green bean plants, such as plant height, number of productive branches, and number of pods.

Table 3. The growth characteristics of *Eleusine indica* weeds that are resistant and sensitive to the herbicide Diuron.

	Number of Tillers		**Number of Branches**		**Total Panicles**		**Plant Height (cm)**		**Seed Weight (gram)**	
	R	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**
$\bar{X}$	4.65	5.59	2.95	3.23	4.42	3.31	11.31	8.95	1.07	0.93
S	2.75	2.02	1.91	2.25	2.73	1.78	3.04	4.37	0.50	0.40
Df	52									
T cal.	- 0.28		-0.09		0.34		0,44		0.23	
T table	2.01									

Note : R = Resistant and S = Sensitive

4. Conclusion

E. indica resistant to diuron herbicide has no significant effect on the growth characteristics. *E. indica* that resistant to diuron needed a longer time to be 50% damaged than the sensitive weeds, Resistance index (RI) value for *E. indica* that treated with diuron was 2,80 and thus it is classified as low resistance level.

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