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First global report of multiple herbicide resistance in *Eleusine indica* biotypes from Indonesia

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Abstract. *Budiawan A, Kurniaide D, Umiyati U, Widianto R. 2025. First global report of multiple herbicide resistance in* Eleusine indica *biotypes from Indonesia. Asian J Agric 9: 629-635. Eleusine indica* (goosegrass) is a major weed in maize cultivation and has evolved resistance to several herbicides worldwide. However, resistance to atrazine (a photosystem II inhibitor) and mesotrione (a 4-hydroxyphenylpyruvate dioxygenase inhibitor) has not previously been reported. This study aimed to verify farmer observations of reduced herbicide efficacy by screening 49 *E. indica* biotypes collected from six maize-producing provinces in Indonesia. A resistant biotype of the test sample was collected from maize fields following farmers' reports of lower efficacy of these herbicides. Greenhouse bioassays were conducted using atrazine (1,200 g a.i. ha^{-1}) and mesotrione (150 g a.i. ha^{-1}) at the 2-3 leaf stage. Resistance was classified based on plant survival three weeks after herbicide application. Screening results indicated the presence of 30 *E. indica* biotypes exhibiting resistance to atrazine, 13 of which demonstrated signs of developing resistance, and 6 were susceptible. For mesotrione, 4 biotypes were resistant, 2 were showing signs of resistance, and 43 were susceptible. A total of 4 biotypes originating from South Sulawesi exhibited resistance to atrazine and mesotrione in a multiple-resistant pattern. Notably, 4 biotypes from South Sulawesi exhibited multiple resistance to both herbicides. This study represents the first global report of *E. indica* multiple resistant to atrazine and mesotrione, particularly in Indonesia. The declining effectiveness of these key herbicides highlights the urgent need for Integrated Weed Management (IWM) strategies that combine chemical rotation, mixtures with complementary modes of action, and non-chemical practices to mitigate the further spread of resistance.

Keywords: Atrazine, *Eleusine indica*, maize weed, multiple herbicide resistance, mesotrione

INTRODUCTION

The emergence of herbicide resistance has evolved into a global agricultural crisis, representing a significant threat to the sustainability of crop production systems on a global scale. The evolution of weed resistance to herbicides is a process that occurs due to genetic mutations or changes in the plant's metabolic system. These changes enable weeds to withstand a type of herbicide or a group of herbicides with a similar mode of action (Gaines et al. 2020). Farmers have a higher propensity to employ herbicides as a control method compared to alternative techniques. However, the implementation of herbicide control frequently does not align with the application of suitable principles for utilization. The use of these chemicals over an extended period without implementing a rotation of active ingredients has been identified as a contributing factor to the emergence of resistant weed populations. The continuous application of the same active ingredient has been shown to effectively control sensitive weeds, while resistant weeds continue to develop (Ofosu et al. 2023).

This global issue is particularly concerning in maize production, where aggressive grass weeds such as *Eleusine indica* (L.) Gaertn. exert significant competitive pressure on crops (Jalaludin et al. 2010). In addition, the robust growth and prolific seed production have been identified as key contributors to its role as a limiting factor for agricultural productivity (Tampubolon et al. 2019a). The presence of the weed is a significant concern on production land, necessitating effective management and control measures.

In the context of maize cultivation, herbicides, such as atrazine (a photosystem II inhibitor) and mesotrione (a hydroxypropyl nitrosamine (HPPD) inhibitor), are frequently used to manage weeds, a practice that is particularly prevalent in Indonesia. These herbicides are among the primary active ingredients employed in the global management of weeds in maize crops. Should both begin to demonstrate a decline in efficacy against dominant weeds such as *E. indica*, the range of effective chemical options will undergo a substantial limitation. According to the International Herbicide-Resistant Weed Database, 37 cases of *E. indica* resistance to herbicides have been documented worldwide, with no recorded instances of resistance to atrazine and mesotrione (Heap 2025). In Indonesia, cases of *E. indica* resistance have been documented in relation to paraquat, glyphosate, and ammonium glufosinate used in oil palm cultivation (Tampubolon et al. 2019b; Ferdinans et al. 2023; Kurniadie et al. 2023). However, no instances of resistance to atrazine and mesotrione have been observed, particularly in the context of maize cultivation. A series of interviews with farmers revealed that the application of certain chemicals proved ineffective in controlling the proliferation of *E. indica*. Therefore, this study aims to map and confirm farmers' reports of *E. indica* resistance to atrazine and mesotrione herbicides.

Although there have been no scientific reports of *E. indica* resistance to atrazine and mesotrione either globally or in Southeast Asia (Busi et al. 2022; Heap 2025); field findings and farmer complaints indicate a decline in the effectiveness of these two herbicides in controlling this weed. This suggests the potential for initial resistance that has not yet been scientifically confirmed. Thus, this study is novel because it is the first to map suspected cases of *E. indica* resistance to the herbicides atrazine and mesotrione in maize fields in Indonesia. This study is designed to identify the locations of *E. indica* populations suspected of being resistant. The results of this study are expected to serve as an early warning for more sustainable herbicide management systems, while also contributing to global weed resistance databases such as the International Survey of Herbicide Resistant Weeds (weedscience.org).

MATERIALS AND METHODS

Plant materials

Eleusine indica biotypes resistant to atrazine herbicides were obtained from maize fields based on farmers' complaints that the weed was no longer effectively controlled using atrazine and mesotrione herbicides (6 provinces). The sampling covered in Indonesia, namely West Java (8 populations from Bandung, Sumedang, Cirebon, and Garut), Central Java (6 populations from Purbalingga, Kendal, Purworejo, and Klaten), East Java (9 populations from Jember, Mojokerto, Tuban, and Kediri), Lampung (6 populations from Pesawaran, North Lampung, South Lampung, and Central Lampung), South Sulawesi (17 populations from Bone, Soppeng, Wajo, Takalar, Jeneponto, and Gowa), and North Sumatera (3 populations from Langkat and Karo). The geographic location of the sampling sites was accurately determined using GPS (Table 1). The utilization of a geographically diverse collection of sampling sites facilitated a comprehensive evaluation of the potential distribution of herbicide resistance across Indonesia's primary maize production regions. Samples of *E. indica* susceptible biotypes were obtained from maize fields exposed to herbicides, but still susceptible to atrazine and mesotrione herbicides. Weed samples were collected as seeds, which were then subjected to a drying process under solar exposure for 14 days. This procedure was intended to enhance the maturation of the seeds. The seeds collected in the field were selected based on their physical characteristics, specifically the presence of brown pigmentation and the formation of seeds from the flowers. Furthermore, the selection of sample sites was informed by reports from farmers who expressed dissatisfaction with the efficacy of atrazine and mesotrione herbicides in managing *E. indica*.

Screening of *E. indica* resistance

The research was conducted in a greenhouse at Syngenta R&D Cikampek, Karawang, Indonesia, at an altitude of 27 meters above sea level from January to June 2025. This study used a completely randomized design with two replications. The factor was the biotype of *E. indica* obtained from six provinces, consisting of 49 samples. *Eleusine indica* seeds were sown in pots measuring 18 cm in diameter, each filled with soil medium that had been sterilized at 120°C for 6 hours, and a total of 30 seeds were planted in each pot. At the 7-day post-planting stage, a process of thinning and replanting was implemented to ensure the maintenance of a uniform population of 20 weed plants per pot. Herbicide treatments, including atrazine and mesotrione, were applied to the *E. indica* plants at the 2 to 3 leaf stage, at application rates of 1,200 and 150 g a.i. ha^{-1}, respectively, resulting in two experiments with the same experimental design. A semi-automatic backpack sprayer equipped with a flat-fan nozzle was used for the application of herbicide, operating at a pressure of 1 kg/cm^2 (15-20 psi) and applying a spray volume equivalent to 400 L ha^{-1}. The classification of resistance was determined based on the survival of the plants 3 weeks after the application of the herbicide.

Statistical analysis

The percentage of weed survival data was analyzed using Analysis of Variance (ANOVA) to test for differences between treatments. Furthermore, screening outcomes were categorized into 3 resistance levels, namely ≤2% survival, indicating susceptibility, 2 to 19% survival, suggesting developing resistance, and >20% survival, which was classified as resistant (Owen and Powles 2009).

RESULTS AND DISCUSSION

Atrazine refers to a triazine herbicide that functions by inhibiting photosystem II (PSII) through binding to the D1 protein, thereby disrupting electron transfer in photosynthesis and inducing weed death. The results indicated the presence of 30 biotypes of *E. indica* that exhibited resistance to atrazine (survival rate: >20%), 13 biotypes demonstrating signs of developing resistance (survival rate: 2-19%), and 6 susceptible biotypes (survival rate: <2%) (Figure 1, Table 2). Based on the results of the ANOVA test, the treatment with atrazine at a dose of 1200 g a.i. ha^{-1} showed significant differences between *E. indica* biotypes (Table 2). This distribution of *E. indica* resistant to atrazine was illustrated in Figure 2, which occurred in 11 locations in South Sulawesi, 6 locations in Lampung, 5 locations in Central Java, 5 locations in East Java, 1 location in West Java, and 2 locations in North Sumatra. The occurrence of atrazine resistance was extensively documented, with reports extending to Brazil in *Chenopodium album* L. and several other wild grass populations in the United States (Heap 2025). A number of studies have looked into *E. indica* and how it does not respond to atrazine and some other herbicides. For example, in a place called Guanxi in China, one study looked at 96 samples and found that 26 of them were not affected by atrazine, which is supposed to block PSII (Li et al. 2022).

Atrazine was first introduced as a herbicide in 1960 (Troyer 2001). Although there are no records of when it was first introduced to Indonesia, corn farmers in Indonesia have used it for more than 10 years. This could be one of the triggers for the emergence of resistant weeds. The continuous use of the same herbicide in monoculture corn cultivation systems throughout the year can accelerate the development of resistant weeds (Sharma et al. 2021; Bensch et al. 2023). That is related to the fact that most corn farmers in Indonesia's corn production centers use monoculture planting systems without crop rotation or appropriate herbicide rotation. This herbicide is the first choice of farmers because it controls a broad spectrum of weeds, is inexpensive, and can be used flexibly, both pre- and post-emergence (Blanco et al. 2013). This makes it difficult for farmers to rotate herbicides. A lack of knowledge about weed resistance causes farmers to continue using atrazine, which is more economically advantageous. By the late 1990s, as much as 69% of corn cultivation areas in the US predominantly used atrazine, and this figure remained at 57% in 2009 (Mitchell 2014).

Mesotrione, an HPPD (4-hydroxyphenylpyruvate dioxygenase) inhibitor, has been demonstrated to inhibit the biosynthesis of plastoquinone and carotenoids, resulting in leaf bleaching and plant death (Sherwani et al. 2015; Zimdahl 2018). Based on the results of the ANOVA test, the treatment with mesotrione at a dose of 150 g a.i. ha^{-1} showed significant differences between *E. indica* biotypes (Table 3). In this study, the results indicated the presence of 4 biotypes of *E. indica* that exhibited resistance to mesotrione, 2 biotypes demonstrating signs of developing resistance, and 43 susceptible biotypes (Figure 3, Table 2). Resistance to mesotrione was not as prevalent as to atrazine; its management remained crucial due to the potential for it to develop to a similar extent as, or even surpass, cases of resistance to atrazine. The distribution of *E. indica* resistant to mesotrione was displayed in Figure 4, which occurred in 4 subdistricts in South Sulawesi. Meanwhile, resistance to mesotrione was observed in biotypes originating from South Sulawesi.

Mesotrione was first introduced in 2001 and mixed with atrazine. The results of the study showed increased effectiveness in pre-emergence applications and an expanded spectrum of weed control (Armel et al. 2003). A mixture of the herbicides atrazine and mesotrione has been reported to control atrazine-resistant weeds (Walsh et al. 2012). Additionally, the increased price of these herbicide mixtures can be offset by their increased efficiency in controlling weeds. This efficiency can also prevent or slow the development of resistant weeds, making it easier for farmers to switch from single to mixed herbicides (Sumekar et al. 2023).

Table 1. Coordinates of *Eleusine indica* sampling location

Province	District	Coordinates
West Java	Bandung	7°02'24.7"S 107°54'23.2"E
West Java	Sumedang	7°00'07.1"S 108°06'01.5"E
West Java	Sumedang	6°53'49.8"S 107°49'48.6"E
West Java	Cirebon	6°45'34.6"S 108°24'36.2"E
West Java	Garut	7°10'39.1"S 107°56'22.0"E
West Java	Garut	7°10'44.0"S 107°59'30.6"E
West Java	Garut	7°09'39.2"S 107°57'57.1"E
West Java	Garut	7°13'52.8"S 107°49'46.0"E
Central Java	Purbalingga	7°22'19.2"S 109°23'06.7"E
Central Java	Kendal	6°55'38.4"S 110°08'12.6"E
Central Java	Kendal	6°55'52.2"S 110°08'19.7"E
Central Java	Purworejo	7°49'01.2"S 110°00'41.6"E
Central Java	Klaten	7°36'41.6"S 110°36'38.6"E
Central Java	Klaten	7°38'59.2"S 110°35'45.5"E
East Java	Jember	8°14'28.3"S 113°40'15.3"E
East Java	Mojokerto	7°33'22.1"S 112°27'57.5"E
East Java	Mojokerto	7°33'22.1"S 112°27'57.5"E
East Java	Jember	8°13'25.6"S 113°40'03.7"E
East Java	Tuban	6°50'04.6"S 111°59'39.2"E
East Java	Kediri	7°42'29.2"S 112°07'05.6"E
East Java	Kediri	7°46'03.3"S 112°12'16.7"E
East Java	Kediri	7°45'03.7"S 112°07'22.1"E
East Java	Kediri	7°42'24.1"S 112°06'48.2"E
Lampung	Pesawaran	5°10'22.4"S 105°10'44.7"E
Lampung	North Lampung	4°52'39.9"S 104°54'12.0"E
Lampung	South Lampung	5°19'25.5"S 105°10'32.0"E
Lampung	South Lampung	5°32'36.8"S 105°26'28.9"E
Lampung	Pesawaran	5°11'33.6"S 105°11'11.4"E
Lampung	Central Lampung	5°08'28.6"S 105°14'15.0"E
South Sulawesi	Bone	4°13'39.4"S 120°08'51.4"E
South Sulawesi	Bone	4°15'51.7"S 120°06'30.2"E
South Sulawesi	Bone	4°16'24.1"S 120°05'29.1"E
South Sulawesi	Soppeng	4°18'29.7"S 120°03'37.4"E
South Sulawesi	Soppeng	4°19'32.2"S 120°02'47.0"E
South Sulawesi	Soppeng	4°20'02.4"S 120°00'14.2"E
South Sulawesi	Soppeng	4°16'22.6"S 120°00'47.2"E
South Sulawesi	Wajo	4°13'16.6"S 120°01'13.4"E
South Sulawesi	Wajo	4°10'53.3"S 120°01'04.1"E
South Sulawesi	Wajo	4°11'53.0"S 120°06'05.0"E
South Sulawesi	Takalar	5°24'27.0"S 119°32'31.0"E
South Sulawesi	Takalar	5°22'51.8"S 119°27'04.9"E
South Sulawesi	Takalar	5°21'38.6"S 119°30'07.2"E
South Sulawesi	Takalar	5°24'47.9"S 119°27'17.6"E
South Sulawesi	Jeneponto	5°32'30.4"S 119°48'56.8"E
South Sulawesi	Gowa	5°30'27.0"S 119°46'54.3"E
South Sulawesi	Takalar	5°20'43.7"S 119°30'56.9"E
North Sumatera	Langkat	3°39'04.1"N 98°28'15.8"E
North Sumatera	Langkat	3°29'26.8"N 98°27'58.8"E
North Sumatera	Karo	3°15'02.0"N 98°22'15.0"E

Table 2. Analysis of variance results for atrazine in *Eleusine indica* biotypes

Variable	df	Sum of Squares	Mean Square	F value	F table 0.05	F table 0.01	Significance
Treatments	48	113.14	2.36	3.54	1.61	1.97	**
Error	49	32.65	0.67				
Total	97	145.79					

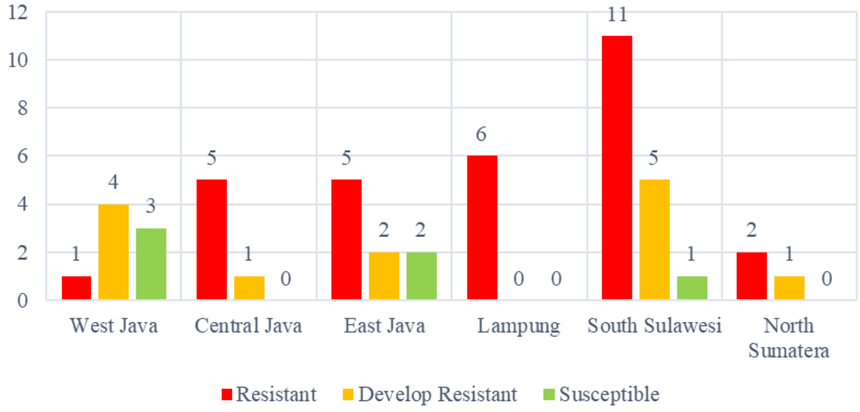

Figure 1. Distribution of atrazine resistance levels in *Eleusine indica* across major maize-producing provinces in Indonesia

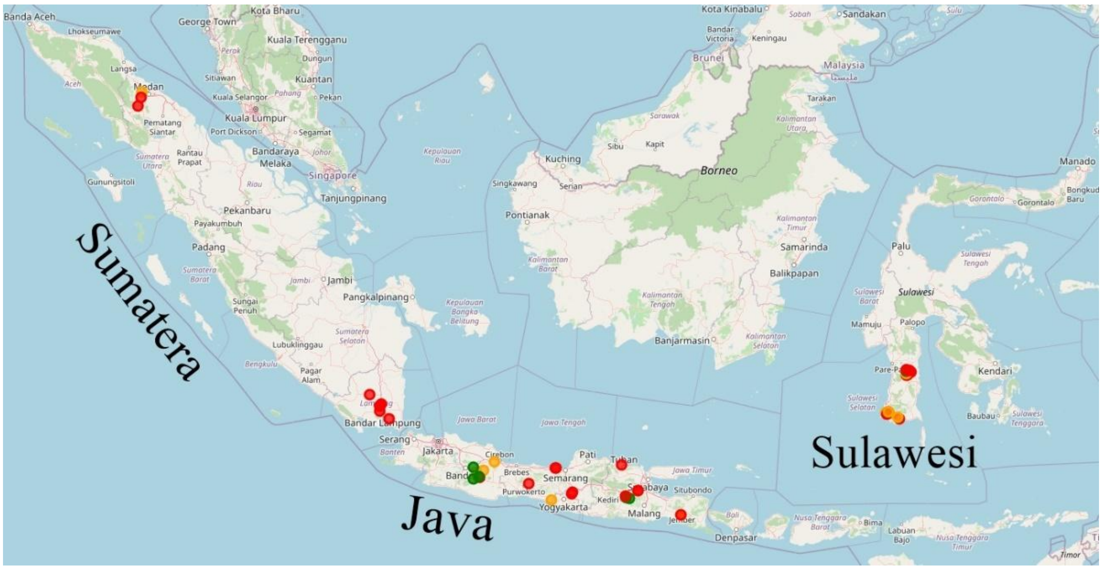

Figure 2. Geographical distribution of atrazine-resistant *Eleusine indica* populations

Resistant biotypes were confirmed in six provinces, although the level of occurrence varied from one place to another. This raised serious concerns, as herbicide resistance in weeds tends to be specific to each situation. The available data suggested that the actual spread of resistance might be wider than what was observed in those six provinces. A similar trend was seen with *E. indica*, which was first reported to be resistant to glyphosate in North Sumatra back in 2019 (Tampubolon et al. 2019b). By 2023, glyphosate resistance in *E. indica* had also been confirmed in wider areas: Lampung, Riau, East Java, West Java, Bangka Belitung Islands, and West Kalimantan (Ramadhanu et al. 2018; Ferdinans et al. 2023; Kurniadie et al. 2023).

The results demonstrated the presence of multiple resistance in 4 biotypes of *E. indica* resistant to atrazine and mesotrione originating from South Sulawesi. The combination of these 2 elements was a common practice in maize cultivation, particularly in Indonesia, where it was used to control *E. indica* synergistically. This study marks the first documentation of multiple resistance in this species on a global scale. The phenomenon of multiple herbicide-resistant weeds can be attributed to a combination of two or more resistance mechanisms working simultaneously, which often operate in a synergistic way. This phenomenon can be explained within the framework of Target Site Resistance (TSR) and Non-Target Site Resistance (NTSR) (Page et al. 2018; Murphy and Tranel 2019).

The presence of TSR in atrazine-resistant weeds, frequently associated with mutations in the chloroplast psbA gene, which codes for the D1 protein (QB-binding niche) in photosystem II. These mutations most often involve of Ser-264-Gly substitution, resulting in a decrease in the binding of atrazine to its specific location without altering the overall enzymatic target (Mikulka et al. 2024). These mutations confer high levels of resistance; however, they are often accompanied by a "fitness cost" in the form of reduced electron transfer rates and photosynthetic efficiency. NTSR plays a critical role in both herbicides, though it is most notably present in mesotrione. NTSR in atrazine and mesotrione resistance is commonly associated with increased detoxification and metabolism, such as conjugation by Glutathione-S-Transferase (GST) and/or oxidation by P450, which mimics the selectivity mechanism in maize (Evans et al. 2017; Zhou et al. 2025). This mechanism can act independently or in combination with TSR. In mesotrione, various populations of *A. tuberculatus* exhibit rapid ring hydroxylation (4-hydroxylation) mediated by P450, reduced translocation, and, in some cases, increased expression of detoxification genes. The result is broad resistance to multiple HPPD inhibitors despite no changes in the target (Kaundun et al. 2017). When a population carries NTSR for both herbicides (increased metabolism for atrazine and mesotrione), this facilitates multiple resistance even if TSR is present in only one of them.

Table 3. Analysis of variance results for mesotrione in *Eleusine indica* biotypes

Variable	df	Sum of Squares	Mean Square	F value	F table 0.05	F table 0.01	Significance
Treatments	48	30.81	0.64	18.63	1.61	1.97	**
Error	49	1.69	0.04				
Total	97	32.49					

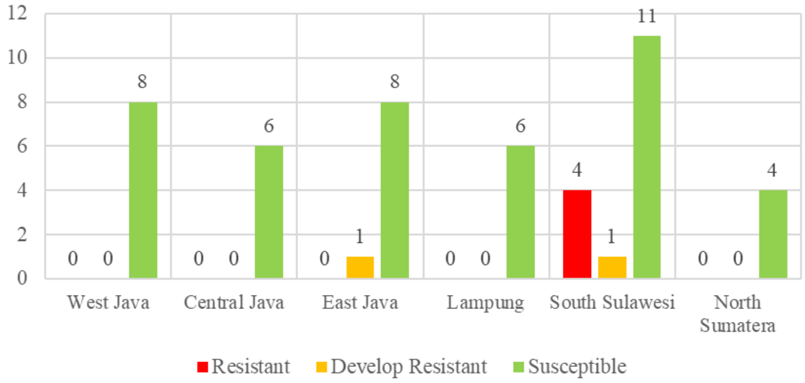

Figure 3. Distribution of mesotrione resistance levels in *Eleusine indica* across major maize-producing provinces in Indonesia

Figure 4. Geographical distribution of mesotrione-resistant *Eleusine indica* populations

The documented trend of weeds exhibiting multiple resistance to PSII and HPPD inhibitor herbicides has been observed in numerous studies. A case study of *Amaranthus palmeri* S.Watson in Kansas demonstrated multiple resistance to 2,4-D, ALS, EPSPS, PS II, and HPPD inhibitors (Shyam et al. 2021). Research on *A. palmeri* indicates the presence of a non-target site resistance mechanism. The administration of cytochrome P450 and Glutathione S-Transferase (GST) enzyme inhibitors resulted in a lower biomass compared to the absence of enzyme inhibitor administration. This finding suggests a predominant role for metabolic resistance, which is facilitated by cytochrome P450 and GST enzyme activity. In China, some weed types that were already resistant to glyphosate and glufosinate were also found to be resistant to atrazine (Chen et al. 2024). Then, in Iowa, USA, a test using different doses of herbicide showed that the weeds had resistance not just to PSII but also HPPD inhibitor (Hamberg et al. 2024). That research added more to what was already known and also helped in identifying and grouping *E. indica* in Indonesia that didn't respond well to atrazine and mesotrione. Consequently, this result emphasizes the necessity of implementing integrated weed management strategies to prevent the spread of resistance and maintain the effectiveness of weed control in maize farming systems.

Cases of *E. indica* resistant to both atrazine and mesotrione highlight the urgent need for Integrated Weed Management (IWM) to slow the spread and evolution of multiple resistance. In such biotypes, target-site mutations and enhanced metabolic detoxification act together, rendering mode-of-action herbicide programs ineffective over time. IWM offers a coordinated framework that reduces selection pressure by rotating herbicides with different modes of action, applying mixtures with complementary activity, and integrating non-chemical tactics such as crop rotation, competitive cultivar selection, narrow row spacing, manual removal, and the use of mulches or cover crops (Barbieri et al. 2022; Pavlović et al. 2022). These combined strategies not only suppress weed-resistant populations but also interrupt their life cycle through repeated, diverse disturbances, making it harder for resistant individuals to dominate. The effectiveness of IWM in managing weed resistant depends on routine field monitoring, the documentation of herbicide usage history, herbicide usage history, and flexibility in adjusting tactics according to weed conditions, climate, and cropping systems at each location (Casimero et al. 2022).

The findings of this study demonstrate a novel observation: the occurrence of multiple resistance exhibited by *E. indica* to both atrazine and mesotrione. The prevalence of atrazine resistance is most frequently observed in South Sulawesi. In comparison, mesotrione resistance is exclusively detected in this province, indicating South Sulawesi as the region with the highest incidence of resistance compared to other provinces. However, *E. indica*, which is resistant to both atrazine and mesotrione, was found at four sites in South Sulawesi and establishing this region as the location with the most serious incidence of resistance. To keep maize cultivation working for the long term, it became important to try better ways of handling weed resistance. One such approach is through the implementation of integrated weed management strategies. Some of those ways included rotating herbicides using HPPD-based mestinon, ACCase inhibitor, and doing other things like rotating crops or using machines instead of just spraying chemicals all the time. In Indonesia, studies showed that resistance was happening over and over again, mostly because farmers kept using the same herbicides, like atrazine and mesotrione, too often. Changing up the herbicides and trying different farming methods like mixing herbicides with different modes of action or using mechanical or natural control was seen as a way to slow down the resistance. The local mapping data from this study gave a useful starting point for figuring out better and more flexible weed control plans in maize fields.

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