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# **Research Article**

# Resistance of Spodoptera exigua Population from Nganjuk against Methomyl, Chlorfenapyr, and Emamectin Benzoate

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## ABSTRACT

Spodoptera exigua Hubner (Lepidoptera: Noctuidae) is the main pest of shallot causing significant economic losses. The continuous use of insecticides has resulted S. exigua to develop resistant against them. This study aims to determine the level of S. exigua resistance of Nganjuk and Bantul populations against methomyl, chlorfenapyr, and emamectin benzoate and temporal resistance of S. exigua of Nganjuk population to methomyl. Nganjuk population were sampled in June, September, and December. First instar larvae from the first generation were used tested using the leaf-dipping method. Temporal resistance test was carried out using the same concentrations. The resistance ratios of Nganjuk population to the insecticides methomyl, chlorfenapyr, and emamectin benzoate were 58.8; 8.8; and 2.5 fold respectively. The LC<sub>50</sub> values of S. exigua of Nganjuk population collected in June, September, and December were 1127.44; 50.62; and 366.76 mg [AI] liter<sup>-1</sup>, respectively. The results of this study indicated that the S. exigua of Nganjuk population was highly resistant to methomyl and its level has changed over time. Resistance management by rotating the type of insecticide is not sufficient but should also consider its change of resistance pattern over time.

Keywords: insecticide; resistance; Spodoptera exigua; temporal

# **INTRODUCTION**

Spodoptera exigua Hubner (Lepidoptera: Noctuidae) is a cosmopolitan and polyphagous insect that feeds on more than 90 plant species belonging to 18 plant families (Greenberg et al., 2001). In Brebes, S. exigua is the main pest on shallot across different shallot production centers around Indonesia (Triwidodo & Tanjung, 2020). S. exigua infestation occurs at both dry and rainy season. In Sulawesi, losses due to S. exigua has been estimated to reach 79.65% and in some occasions were able to cause crop failure when no management were done (Rauf, 1999). Larva feed on leaves and leave leaf epidermis that later cause leaves to break and disturb photosynthesis processes (Greenberg et al., 2001). Synthetic insecticides are still the main management option of farmers due to their effectiveness and fast results (Sisay et al., 2019). Registered insecti-

cides for S. exigua management under the Directorate of Fertilizers and Pesticides, Ministry of Agriculture has increased from 2011 to 2016 from 152 to 250 types (Ministry of Agriculture [Kementerian Pertanian], 2016). Several active ingredients are used in Brebes (Central Java), Nganjuk (East Java), and Bantul (Special Region of Yogyakarta) for S. exigua management, include chlorfenapyr, chlorpyrifos, methomyl, and emamectin benzoate (Aldini et al., 2020). Insecticide application on shallot in Nganjuk can reach 3-4 application per week (Fitriana et al., 2020) and 2-3 applications per week in Brebes (Moekasan & Basuki, 2007). Recent research have reported that farmers in Nganjuk and Brebes apply insecticide to manage S. exigua on calendar by applying at a 1–3 day interval (Aldini et al., 2020). Continuous use of the same synthetic insecticide over time can cause S. exigua to develop resistance (Insecticide Resistance Action Committee [IRAC], 2021).

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Spodoptera exigua have been reported to develop resis-tance against several insecticides across various countries. In China, S. exigua resistances against ema-mectin benzoate and abamectin have been reported and even cross resistances occurred between those two active ingredients (Che et al., 2015). S. exigua resistances against chlorantraniliprole (Lai et al., 2011) and tebufenozide in China (Jia et al., 2009). Resistant cases of endosulfan, chlorpyrifos, metho-myl, permethrin, and deltamethrin have been reported in Mexico (Garza-Urbina & Teran-Vargas, 1998). In Pakistan, S. exigua have been reported to be medium to high resistant against chlorpyrifos and pyrethroid between 2003 to 2007 and high resistance against deltamethrin between 2004 to 2007 (Ahmad & Arif, 2010). S. exigua resistance against insecticides have also been reported in Indonesia against chlorfenapyr, methomyl, and emamectin benzoate in Brebes, Nganjuk, and Bantul (Aldini et al., 2021). S. exigua resistance is an important challenge for shallot farmers because the increase of management cost. Overapplication and incorrect dose of insecticides application have been reported to reach 33.3% in Nganjuk and 43.3% in Brebes (Aldini et al., 2020).

# MATERIALS AND METHODS

# Spodoptera exigua Collecting

Spodoptera exigua larvae were collected from March to December 2022. Nganjuk populations were considered resistance while Bantul populations were susceptible (Aldini *et al.*, 2021). Sampling in March was done at Sanden, Bantul, Special Region of Yogyakarta. *S. exigua* larvae sampling from Nganjuk, East Java was done at June at Wilangan, September at Bagor, and Desember at Nganjuk. High *S. exigua* infestation ( $\pm$ 70%) was an indicator for sampling across Nganjuk. This was done to test temporal resistance of *S. exigua* at high infestation levels. Larvae sampling was done at one location at each district with a total of 250–350 larvae collected at each sampling point.

Spodoptera exigua population from Indonesia have

# Spodoptera exigua Mass Rearing

Mass rearing followed methods from Wibisono et al. (2007) with modification. Larvae were sampled from various locations and reared at Management Technology Laboratory, Sub Laboratory Pesticide Toxicology, Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta. Larvae were reared in  $20 \times 20 \times 10$  cm plastic containers. Holes were made on plastic containers and sealed with mesh clothes to ensure air circulations. Each containers contained 20–30 larvae with natural fed of  $2-3 \ge 20$ -days-old shallot leaf clumps replaced every 1–2 days. Five to six shallot tubers were planted in 40×40 cm polybags. Pupae were moved into imago containers of  $30 \times 30 \times 45$  cm. Imagoes were fed with 10% honey solution. Shallot plants in 12.5 cm tall and 9 cm diameter plastic containers were used for oviposition locations. One instar from F1 was used for bioassays.

been reported to be resistant against certain active ingredients. Populations from Nganjuk and Brebes have been reported to be resistant against methoxyfenozide (Wibisono et al., 2007). S. exigua were resistant against nine active ingredient, including methomyl (Moekasan & Basuki, 2007). Recent research have reported that S. exigua populations from Brebes, Nganjuk, and Bantul have resistant against chlorfenapyr, methomyl, and emamectin benzoate with high resistance against methomyl (Aldini et al., 2021). The aim of this study is to monitor resistance levels of S. exigua collected from Nganjuk and Bantul against methomyl, chlorfenapyr, and emamectin benzoate, and temporal resistance of S. exigua populations from Nganjuk against methomyl. Temporal resistance was only done for methomyl due to S. exigua showed the lowest resistance compared to emamectin benzoate and chlorfenapyr. This information will later be used to synthesize strategies for S. exigua resistance management.

## Insecticide

Insecticides used in this research were methomyl (Metindo; 80 active ingredient [AI] liter<sup>-1</sup>; PT Inti Everspring Indonesia) from group 1A based on its mode of action; chlorfenapyr (Arjuna; 200 gram active ingredient [AI] liter<sup>-1</sup>; PT Belirang Kalisari, Indonesia) belonging to group 13; and emamectin benzoate (Abenz; 22 gram active ingredient [AI] liter<sup>-1</sup>; PT Advansia Indotani, Indonesia) belonging to group 6 (Insecticide Resistance Action Committee [IRAC], 2021).

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### Bioassay

Preliminary testing were done on *S. exigua* populations from Nganjuk and Bantul using methomyl, chorfenapyr, and emamectin benzoate to determine testing concentrations for bioassay with mortality of 5-95% at 96 after insecticide exposure. Preliminary concentration for methomyl was between  $3.2 \times 10^{-1}$  to  $3.2 \times 10^{3}$  mg [AI] liter<sup>-1</sup>; chlorfenapyr between  $3 \times 10^{-1}$  to  $3 \times 10^{3}$  mg [AI] liter<sup>-1</sup>; and emamectin benzoate between  $2.2 \times 10^{-2}$  to  $2.2 \times 10^{2}$ 

resistance of RR = 10-40, high resistances of RR = 40-160, and very high resistance of RR > 160.

## **RESULTS AND DISCUSSION**

## Spodoptera exigua Resistance against Methomyl

Spodoptera exigua populations from Nganjuk had higher resistances against methomyl compared to populations from Bantul. Resistance ratio of Nganjuk was categorized as high resistances of RR =58.8 folds (Table 1) (Ahmad & Gull, 2017). The LC<sub>50</sub> of Nganjuk and Bantul populations against methomyl respectively were 1127.44 and 19.16 mg [AI] liter<sup>-1</sup>, while recommended concentrations was 320 mg [AI] liter<sup>-1</sup>. Methomyl was the earliest compound used to manage S. exigua compared to chlorfenapyr and emamectin benzoate. Methomyl was first registered in Indonesia in 1987. Methomyl was the most used active ingredients by shallot farmers (Aldini et al., 2020) and has been long used to manage S. exigua in Nganjuk with high application frequencies and dosages. The use of methomyl to manage S. exigua in Nganjuk reached 56.7% and 76.6% have been used above recommended doses (Aldini et al., 2020). Besides that, shallot was planted 3–4 times in a year in monoculture systems. Methomyl application with high frequencies and for a long time in monoculture systems can cause S. exigua resistances to occur faster. Meanwhile,  $LC_{50}$  of Bantul population was 16.67% of label recommendations. Resistance differences between Nganjuk and Bantul population may be due to different insecticide use behavior where Bantul farmers have been reported to use methomyl accordingly to label recommendation, less frequently, and rotate between insecticides with different mode of actions (Aldini et al., 2020).

mg [AI] liter<sup>-1</sup>. Highest concentrations used were 10-folds of recommended concentration from each brand. Water was used as solvents and untreated control for bioassays. Testings were done using leaf dipping method (Che *et al.*, 2015). Ten centimeter long shallots leaves were immersed in each treatment and concentration combination for  $\pm$  10 seconds and air dried for  $\pm$  30 minutes. Leaves were then placed in testing plastic containers with height of 15 cm and diameter of 9 cm. Ten newly emerged first instar larvae were placed in containers with five replications (Wibisono *et al.*, 2007; Yuliani *et al.*, 2020; Aldini *et al.*, 2021). Larva mortality was observed 96 hours after insecticide exposure. Temporal insecticide resistance of *S. exigua* populations from Nganjuk

against methomyl was done using the same method and concentrations as preliminary studies.

## Analysis

The LC<sub>50</sub> and LC<sub>95</sub> values were calculated using probit analysis (Finney, 1971) performed in Polo Plus (LeOra Software). The 95% confidence interval was use to determine LC<sub>50</sub> or LC<sub>95</sub> significance between groups and significant differences were determined when CI values did not overlap (Macron *et al.*, 1999). Resistance ratio (RR) were calculated by dividing LC<sub>50</sub> of Nganjuk population with LC<sub>50</sub> of Bantul population. Temporal resistance of *S. exigua* from Nganjuk population against methomyl was analyzed using similar procedures. Ahmad and Gull (2017) classified RR to six categories, including sus-

Methomyl is used widely across different countries and *S. exigua* resistances have been reported since the 19<sup>th</sup> century (Brewer & Trumble, 1994). In Mexico, *S. exigua* were resistant against methomyl with RR between 80–640 folds (Garza-Urbina & Teran-Vargas, 1998). *S. exigua* resistance against methomyl in China were reported at low levels of RR = 3.5–4.5 folds (Su & Sun, 2014). In Indonesia, recent reports of *S. exigua* resistances against methomyl in 2021 occurred in Brebes, Nganjuk, and Bantul with RR=16.5–226.1 folds (Aldini *et al.*, 2021).

ceptible RR = 1, very low resistances RR = 2–10, low resistance of RR = 11–20, moderate resistances RR = 21–50, high resistance of RR = 51–100, and very high resistance of RR > 100. Meanwhile, Lai *et al.* (2011) classified RR into six categories consisting of susceptible of RR d" 3, very low resistances of RR = 3–5, low resistance of RR = 5–10, moderate

from non	Notes: $LC_{50}$ and $LC_{95}$ value a = Resistance Ratio	Notes: $LC_{50}$
benzoa	Sanden	Bantul
Emam	Wilangan	Nganjuk
	Sanden	Bantul
Chlorf	Wilangan	Nganjuk
	Sanden	Bantul
Methot	Wilangan	Nganjuk
Insect	District	Regency

Table 1. Nganjuk and Bantul Spodop.

Active

Ingredien

Increase of S. exigua resistance against methomyl is correlated to increase of detoxification enzyme activity, such as cytochrome P450 monooxygenase or mixed-function oxidase (MFO) (Garza-Urbina & Teran-Vargas, 1998). The same enzyme also works for S. exigua against other insecticides. In Japan, S. exigua resistances against permethrin was due to increase P450 monooxygenase activity (Shimada et al., 2005). There are several enzyme involved in resistance mechanisms of S. exigua. S. exigua resistance against indoxacarb in China has been reported due to increase of glutathion S-transferase activity (Gao et al., 2014). Metabolic resistance dose can occur not only due to one enzyme but also several at a time and has been reported in China where S. exigua resistance to metaflumizone is caused by increase of esterase, glutathion S-transferase, and cytochrome P450 monooxgenase activity (Tian et al., 2014). This was caused due to the use of several insecticides simultaneously overtime.

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Spodoptera exigua Resistance against Chlorfenapyr Spodoptera exigua from Nganjuk were more resistant to chlorfenapyr than ones from Bantul based on their LC<sub>50</sub> of 22.33 and 2.54 mg [AI] liter<sup>-1</sup> while lable recommended concentration of this compound was 300 mg [AI] liter<sup>-1</sup>. The RR from Nganjuk population was 8.8 fold and categorized as low (Table 1) (Lai et al., 2011). This slight increase should be an indicator to precautious chlorfenapyr use to manage S. exigua to not cause resistances. Chlorfenapyr was registered in Indonesia in 1999 and has been used to manage S. exigua in shallot since. The LC<sub>95</sub> of Nganjuk and Bantul populations were 335 and 262 mg [AI] liter<sup>-1</sup> respectively, was categorized as low resistance and to be effective against S. exigua causing farmers to choose this com by 93.3% of farmers in Nganjuk while only 34.8% of farmers in Bantul (Aldini et al., 2020). However, application frequency and duration of use will also effect S. exigua resistance against chlorfenapyr. Chlorfenapyr effectiveness is caused by several factors, such as farmers' rotation among active ingredients, how insecticides are applied, doses used, and application frequency (Glass et al., 1986). As much as 63.3% farmers in Nganjuk and 50% farmers in Bantul rotate between insecticides (Aldini et al., 2020).

enapy ectin myl ate ticide calculated signit can 350 250by 350 350 350 Ins 50 D dividing  $\mathbf{C}$ 0.390.730.65 0.82 .39 Slope  $C_{50}$  $(\pm 0.1557)$  $(\pm 0.0616)$  $(\pm 0.0704)$  $(\pm 0.1169)$  $(\pm 0.1094)$  $(\pm SE)$ (0.0730)and llowed  $C_{95}$ fro LC<sub>50</sub> (CI milar 22.33 2.54 0.20 lganjuk  $\mathbb{N}$ 19.16 0.08 letter 95%; (660.ndod **t**S ba  $\bigcirc$ 

tera exigua population resistances against methomyl, chlo

namectin l	benzoate	
	T OZOZO ~ LAIPL I -1	RF
L 95 (	/	LC <sub>50</sub>
79.86 (	19.52 – 1115.79) a	58.8
6.59	(0.58 H 588.66) a	
0.34	(0.14 H 1.91) a	8.8
0.26	(0.09 H 2.23) a	
0.04	(0.01 H 0.13) a	2.5
0.11	(0.02 — 5.37) a	
pping 95%	6 confidence intervals	
	namectin   LC <sub>95</sub> (C 79.86 ( 0.34 0.11 0.11 nd LC <sub>05</sub> B	CI 95% co Bantu

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Chlorfenapyr's wide adoption can reduce its effectiveness against S. exigua when application do not follow recommendations. High S. exigua resistance against chlorfenapyr with RR=69.3 fold was reported in Wilangan, Nganjuk, while population from other locations were reported with low to moderate (Aldini et al., 2021). The high application frequency and long duration of an active ingredient can cause S. exigua resistance against those active ingredients, including chlorfenapyr.

The  $LC_{50}$  value of Nganjuk and Bantul population were 0.20 and 0.08 mg [AI] liter<sup>-1</sup>) respectively while label recommendation were 22 mg [AI] liter<sup>-1</sup>. The  $LC_{50}$  were 110 and 275 fold lower compared to the recommended concentrations with Nganjuk RR=2.5 fold and categorized as very low resistance (Table 1) (Ahmad & Gull, 2017). That value implied that emamectin benzoate was still effective and can be used as an alternative rotation option.

Emamectin benzoate was first registered in Indo-

Chlorfenapyr has been adopted in many countries to manage S. exigua. From 2009 to 2012, S. exigua resistance against chlorfenapyr was low RR=0.4-7 in seven provinces of China (Che et al., 2013) while another study using another population in China had RR value less than 2 from 2011 to 2012 (Zhang et al., 2014). In the Province of Jiangsu no resistance of S. exigua against chlorfenapyr was found based on RR=0.9–2.2 kali (Huang et al., 2021) while populations from Shanghai also showed similar results with LC<sub>50</sub> between 3.49–10.1 mg [AI] liter<sup>-1</sup> and RR=1–3 fold (Che et al., 2015). Another study from Japan showed no S. exigua resistance against chlorfenapyr with RR=0.95 fold (Shimada et al., 2005). A study from Pakistan showed RR=2.2-32 between 1998–2007 (Ahmad et al., 2018) indicating

nesia in 2001. Emamectin benzoate use in Nganjuk was lower than methomyl and chlorfenapyr to manage S. exigua with 26.3% farmers using it compared to 56.7% and 93.3% for methomyl and chlorfenapyr respectively (Aldini et al., 2020). Tests showed that RR of emamectin benzoate was lower compared to methomyl and chlorfenapyr due to its lower use to manage S. exigua or only used as a rotational active ingredient. Research showed that rotation was not based solely on rotational programs, but by recommendation from pesticide sellers (23.3%) or other farmers (41.1%) (Aldini et al., 2020). Rotation should be done between different active ingredients to reduce chances of S. exigua populations to develop resistance against methomyl, emamectin benzoate, and chlorfenapyr at the same time.

increases of resistance that should be monitored.

Increase of detoxification enzymes have been correlated with S. exigua resistances against chlorfenapyr. In Australia, Helicoverpa armigera Hubner resistant against chlorfenapyr was due to increase of esterase activity (Gunning et al., 2005). The usage of the same insecticide on Lepidopteran indicate increase of the same enzyme activity. Increase of esterase caused S. exigua to develop resistance against metaflumizone (Su & Sun, 2014; Tian et al., 2014). Other studies have shown that multiple enzymes are involved in chlorfenapyr resistances, such cytochrome P450 monooxgenase in Tetranychus urticae Koch from Belgia (Van Leeuwen et al., 2005). S. exigua resitance against chlorfenapyr is also correlated to

Emamectin benzoate has been used across several countries to manage S. exigua and resistance have also been reported. In Shandong, China, S. exigua resistance against emamectin benzoate was categorized as very low to moderate (Zhang et al., 2014) while resistance from populations from Jiangsu are categorized as low (Huang et al., 2021). Meanwhile, S. exigua resistance against emamectin benzoate from seven provinces di China ranged from very low to very high based on RR=4-348 fold between 2009 to 2012 (Che et al., 2013). In Nanjing, S. exigua have been reported to be resistant to emamectin benzoate with RR=1,110 fold and even showed cross resistance to abamectin (202 folds) (Che et al., 2015). Different S. exigua resistance against ema-

esterase, glutathion S-transferase, cytochrome P450 monooxygenease (Tian et al., 2014).

# Spodoptera exigua Resistance against Emamectin Benzoate

Emamectin benzoate was still effective against S. exigua populations from Nganjuk and Bantul.

mectin benzoate was shown from eight locations across China with RR=1.7-104.1 fold (Wang *et al.*, 2018). Management of emamectin resistant S. exigua populations requires planning. In Indonesia, recent study reported that S. exigua from Nganjuk was resistant against emamectin benzoate with RR= 10.3-46.7 fold and RR=7.2-51.7 fold from Brebes

populations, while low resistance of RR=1-6.7 fold was shown from Bantul populations (Aldini *et al.*, 2021).

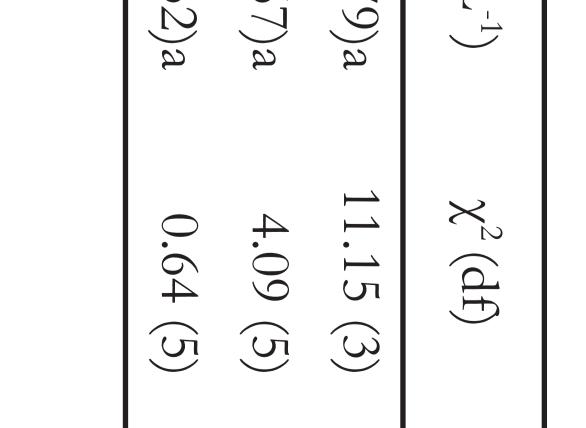
Several studies has shown biochemical resistant of *S. exigua* against emamectin. Increased mortality after treatment of PBO and DEF syngergist with abamectin application indicated increase of esterase and cytochrome P450 monooxgenase as the mechanism of *S. exigua* resistance against abamectin which has similar mode of action as emamectin benzoate (Ahmad *et al.*, 2018). Cross resistance in insects is commonly reported for different active ingredients with similar mode of action. In China, cross resistance between emamectin benzoate and abamectin against *S. exigua* with high RR of 202 fold indicating that emamectin benzoate resistance is cause by similar detoxification enzymes of abamectin (Che *et al.*, 2015).

	$LC_{50}$ (CI 95%; mg [AI <sup>c</sup> ] L <sup>-1</sup> ) $LC_{95}$ (CI 95%;	LC <sub>50</sub> (CI 95%	Slope (±SE)	Ŋ	Time <sup>a</sup>	District	Regency
	(660.16 – 2608.00)a 79.86	1127.44 (660	0.39 (±0.0616)	250	June	Wilangan <sup>b</sup>	Nganjuk
89.22)b	(26.95 H 89.22)b 6.37	50.62	0.78 (土0.0904)	350	September	Bagor	Nganjuk
		366.78 (197.84 H 709.36)a	0.78 (土0.1250)	350	December	Kota	Nganjuk
			Slope (±SE) 0.39 (±0.0616)	n 250	June	District Wilangan <sup>b</sup>	Regency Nganjuk

# Spodoptera exigua Temporal Resistance against Methomyl

Temporal resistance of Nganjuk S. exigua population against the same concentrations used in the response bioassay showed different responses against methomyl. The  $LC_{50}$  of samples taken at June, September, and December were 1127.44 (Table 1); 50.62; and 366.76 mg [AI] liter<sup>-1</sup> respectively (Table 2). In general, farmers in Nganjuk plant shallot at similar time and use insecticdie at similar rates and using the same compounds. Insecticide application in Nganjuk used high application frequency with intervals of 1–3 times (Aldini et al., 2020; Fitriana et al., 2020). Methomyl applications in June are done in high intensity due to high S. exigua adaptation to methomyl. Methomyl application caused S. exigua lower population of susceptible ones causing populations to consist of resistant individuals which later increase pest management cost for farmers. As much as 76.6% farmers in Nganjuk use insecticide doses that exceeded label recommendations (Aldini et al., 2020). Harvest happen in September. Some shallot farmers rotate to emamectin benzoate

which may increase susceptibility of *S. exigua* populations against methomyl. This study did not show multiple resistance between methomyl and emamectin benzoate. Insecticide and fungicide applications in December were done in balance frequency



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due to high pest and pathogen infestation. Methomyl is still used to manage S. exigua because its long history of use by farmers. Less farmers in Nganjuk (36.7%) rotate between active ingredients (Aldini et al., 2020). Rotation was done due to peer recommendation (41.1%), but not to solely rotate (Aldini et al., 2020). Increased methomyl application have caused S. exigua resistances. Different response of S. exigua against methomyl overtime is caused by farmers methomyl use behavior. Insecticide application methods, doses, frequency, persistence, chemical characteristics, and economic threshold effect insect resistances (Glass et al., 1986). One factor that determines farmers' pesticide use behavior in Nganjuk is climate. More insecticides are applied more during dry season while fungicides are used more in rainy seasons. Rainfall negatively affect insect population (Chen et al., 2019). S. exigua population was shown to be 78 times higher in the dry season compared to rainy season (Rauf, 1999) causing farmers to focus on managing pathogens during the rainy season. The reduce of selection pressure on S. exigua using insecticide may reduce its resistance. Sampling were done on June, September, and December that were the dry season, transition between dry and rainy season, and the rainy season. Average rainfall in the District of Tanjunganom, Regency of Nganjuk from 2015–2021 in June, September, and December were 28.57; 58.71; and 257.14 mm (Badan Pusat Statistik [BPS], 2023).

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Monitoring of *S. exigua* resistance against insecticides is important to determine insecticide effectiveness over time and to inform effective insecticide rotations over time. This step can hopefully contribute to insecticide resistance management.

# CONCLUSION

*S. exigua* populations from Nganjuk were resistance against methomyl, chlorfenapyr, and emamectin benzoate with RR of 58.8; 8.8; and 2.5 folds respectively. *S. exigua* resistance against methomyl in Nganjuk varied from each season (June, Septem-ber, and December 2022) with  $LC_{50}$  of 1127.44; 50.62; and 366.76 mg [AI] liter<sup>-1</sup> respectively.

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