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Identification of Fly Larvas in White Rats (*Rattus norvegicus*) Carcass Treated with Various Dose of Organophospate

(IDENTIFIKASI LARVA LALAT PADA BANGKAI TIKUS PUTIH *(RATTUS NORVEGICUS)* DENGAN PERLAKUAN BERBAGAI DOSIS ORGANOFOSFAT)

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ABSTRACT

The growth of flies on the carcass could be an indicator in estimating Post Mortem Interval. The aim of this study was to determine the type of flies and their abundance on the carcass of white rats (*Rattus norvegicus*) given various doses of diazinon's organophosphate poison. This research was an experimental study using 12 female wistar white rats, aged 2-3 months, weighing 150-200 g. This study consisted of four treatments with three repetitions of each treatment. Variations in the dose of organophosphate given were treatment A (1.5 mL), B (2.5 mL), and C (3.5 mL). The parameters observed included the types and abundance of flies on the carcasses of white rats that were treated with organophosphate poisoning at different doses. Data analysis was carried out including the normality test, then continued with the homogeneity test and finally the analysis of variance test was carried out and the test results were not significantly different. The types of flies found on the carcass were *Sarcophaga haemorrhoidalis*, *Chrysomya albiceps*, *C. megacephala* and *C. bezziana*. The highest average abundance was *S. haemorrhoidalis* (34.3 larvae) and the lowest average abundance of fly larvae was *C. bezziana* (16.3 larvae). The conclusion of this study was that the variation in the dose of organophosphate does not affect the type, abundance and time of appearance of fly larvae on the carcass.

Keywords: *Chrysomya megacephala;* forensic entomology; organophosphate*;* *Sarcophaga haemorrhoidalis*

ABSTRAK

Pertumbuhan lalat pada bangkai dapat menjadi indikator dalam memperkirakan *Post Mortem Interval*. Penelitian ini bertujuan untuk mengetahui jenis dan kelimpahan larva lalat pada bangkai tikus putih (*Rattus norvegicus*) yang diberi variasi dosis racun organofosfat *merk* Diazinon. Penelitian ini bersifat eksperimental dengan menggunakan 12 ekor tikus putih galur wistar betina, umur 2-3 bulan, bobot 150-200 g. Penelitian ini terdiri dari empat perlakuan dengan tiga ulangan pada tiap perlakuan. Variasi dosis organofosfat yang diberikan yaitu perlakuan A (1,5 mL), B (2,5 mL), dan C (3,5 mL). Parameter yang diamati meliputi jenis dan kelimpahan lalat pada bangkai tikus putih yang diberi perlakuan diracun organofosfat dengan dosis berbeda. Analisis data dilakukan meliputi uji normalitas, kemudian dilanjutkan uji homogenitas dan terakhir dilakukan uji sidik ragam dan didaptkan hasil uji yang tidak beda nyata. Jenis lalat yang ditemukan pada bangkai yaitu *Sarcophaga haemorrhoidalis*, *Chrysomya albiceps*, *C. megacephala* dan *C. bezziana*. Kelimpahan rata-rata paling tinggi yaitu larva *S. haemorrhoidalis* (34,3 larva) dan kelimpahan rata-rata larva lalat paling sedikit yaitu *C. bezziana* (16,3 larva). Simpulan dari penelitian ini yaitu variasi pemberian dosis organofosfat berpengaruh atau tidak berpengaruh terhadap jenis, kelimpahan serta waktu kemunculan larva lalat pada bangkai.

Kata-kata kunci: *Chrysomya megacephala;* entomologi forensik, organofosfat; *Sarcophaga haemorrhoidalis*.

INTRODUCTION

Cases of unnatural death are often found in a rotting condition with fly larvae all over the body. Unnatural deaths can be caused by various factors, one of which is poisoning. Poisoning cases recorded by the *American Association of Poison Control Centers* (AAPCC) from 2012-2017 mention about 385 million cases of pesticide poisoning occur every year and cause about 11,000 deaths that occur globally (Boedeker *et al*., 2021). Cases of poisoning and deaths from pesticides are also common in Indonesia. Data from 2001-2007 stated that there were 4867 poisoning cases and 3789 of them died (Kurnia 2012). Among the pesticides that are most often associated with poisoning worldwide and have high toxicity are prganophosphates (Perwitasari *et al*., 2017).

Due to the high incidence of death due to organophosphate pesticide poisoning, the determination of the Post Mortem Interval (PMI) in the identification of corpses is very important, especially in the case of unnatural death status (Primahatmaja and Sardjono, 2014). One of the identifications carried out by forensic experts in cases of unnatural death is post mortem interval (Vanin, 2018). Identification of the estimated time of death of the corpse is one of the regular procedures carried out by sending fly larvae on the corpse's body to the parasitology laboratory. This method is called forensic entomology, which is a method used to predict the length of time of death by utilizing the life cycle of flies that colonize carrion (Vanin, 2018). Utilization of the fly life cycle as a forensic indicator in estimating PMI because it is known that flies are the first insects to come to the carcass when it enters the decay process (Badenhorst and Villet, 2018).

Research on forensic entomology to estimate the time of death by utilizing the life cycle of flies that colonize carrion has been carried out by Jales *et al*. (2020) used white rats that were given the terbufos poison. The results of this study found second instar larvae from the Calliphoridae family, so it can be estimated that the age of the carcass is about two days. Research by Al-Qahetni *et al* (2020) by giving heroin treatment, found species of flies from the families Calliphoridae, Sarcophagidae and Muscidae, and It is known that the flies that colonized for the first time were from the Calliphoridae species, namely the discovery of the first instar which indicated the age of the carcass of about 0.5 days.

Based on the description, research on PMI using poisoned white rats has been carried out. However, PMI studies with organophosphate toxins have not been carried out a lot. So that this research is important as a reference in determining PMI in cases of death due to organophosphate poisoning, to reveal the type, abundance and time of appearance of fly larvae in white rat carcasses.

RESEARCH METHODS

Research Time and Place

This research was conducted in December 2021 – April 2022. Data collection for fly larvae was carried out in Kalisoka Hamlet, Pengasih District, Kulon Progo Regency, Province of Yogyakarta. The process of identifying fly larvae was carried out at the Ecology and Systematics Research Laboratory, Biology Study Program, Ahmad Dahlan University, Yogyakarta.

Tools and Materials

The tools used in this study include a cage measuring 31 x 22 x 13 cm as a place to keep rats during acclimatization, a 60 mL drinking bottle to feed the rats during acclimatization, Ddigital scales (ACIS®) to weigh the rats, the anemometer (Benetech®) to measure the speed of wind, to measure light intensity (AS803® lux meter), thermohygrometer (HTC-2®) to measure humidity and air temperature, pH indicator to measure carcass pH, 10 meter roll meter to measure land area, 1.5 m to measure distance between carcass, strimin wire measuring 20 x 20 cm to cover the carcass, *syringe* and sonde to poison the rats, *killing bottle* measuring 12 x 26 cm as a place for rat anesthesia. 120 x 20 mm petri dish for placing fly larvae during the identification process, 100 mL plastic bottle for collection of 3rd instar fly larvae from carcasses, 300 mL plastic cup for rearing 1st instar collected fly larvae, optilab for taking pictures of larvae flies, a stereo microscope to observe fly larvae, an 8 MP Samsung mobile phone camera for documentation during research and working board with it's paper to record research data.

The materials used in this study were 12 female white rats (*R. norvegicus*) aged 2-3 months weighing 150-200 g as experimental animals, Feed in form of pellets (AD2®) was used to feed rats during acclimatization, 10% ether and cotton to anesthetize mice. , 70% alcohol to preserve larvae, organophosphate pesticide (Diazinon®) as a poison in research treatment, masks and gloves as hand protection when *euthanizing* rats and taking larvae, and 8 x 20 mm label paper to label bottles.

Research Method

Preparation of Ethical Clearance. The Ethical Clearance (EC) file was sent to the Ahmad Dahlan University Research Ethics Committee. The EC was submitted with EC number 012110076

Animal Test Preparation. A total of 12 white rats were used with the characteristics of the wistar strain, female, 2-3 months old, healthy and weighing 150-200 g. White rats were acclimatized for one week, by being placed in a cage with a size of 31 x 22 x 13 cm. During the acclimatization process, the experimental animals were given feed in the form of AD 2 as much as 10% of the rat's body weight or about 10-15 g/ head/day. Feeding was carried out two times a day, in the morning and evening (Wolfenshon and Lloyd, 2013), and given water *ad libitum*. During acclimatization, the rat cage was cleaned every three days.

Treatment and Carcass Placement. A total of 12 white rats were divided into four groups of treatment. Three groups were given organophosphate poison with different doses and one control group was treated with cervical dislocation. The dose of poison given for: Group 1 was 1.5 mL, Group 2 was 2.5 mL, Group 3 was 3.5 mL, and Group 4 underwent cervical dislocation The test animals were anesthetized by placing them in a *killing bottle* filled with cotton that had been moistened with 10% ether reagent. Test animals that had been anesthetized were then given organophosphate (diazinon®) as a poison. Meanwhile, the control animals were treated (sacrificed) with cervical dislocation. Oral administration of poison was carried out by holding the rat's neck and inserting the poison using a probe into the esophagus, then the poison was slowly released into the rat's digestive tract. Test animals for control treatment that had been anesthetized were dislocated their neck by holding the test animal by the tail and neck. Then the other hand pulls the tail hard, so that the neck bones bcome dislocated. The carcasses of white rats are placed on the ground with a distance of 2.5 m between the carcasses, then the carcasses will be fenced and covered with strimin wire so that they were not taken by wild animals. The placement of each treatment was arranged randomly using the method of Randomized Block Design.

Collection of Fly Larva. After being given different doses of organophosphate poison, the rats carcasses were observed for 10 days or until the carcasses were completely decomposed. The rat carcasses were checked every day, in the morning at 06.00-09.00 and in the afternoon at 15.00-18.00 to whether there were fly larvae on the carcass. Carcass checks are carried out in the morning and evening because the period of most fly activity is in the morning and evening (Byrd and Castner, 2010). The fly larvae were taken every day by taking the fly larvae using tweezers on each carcass. All of fly larvae that have been taken and are still in the 1st instar phase were reared until develop into the 3rd instar by means the 1st instar larvae being reared by being fed with rat meat. Before putting into plastic bootle, the fly larvae that have turned into 3rd instars were boiled in the warm water (60°C) for about five minuters. After boiled, the larva were put into plastic bottles filled with 70% alcohol to preserve the larvae and the bottles are labeled according to the treatment, date and time of larval collection.

Identification of Fly Larva. The fly larvae that had been collected from rat carcasses were then identified at the Ecology and Systematics Laboratory, Faculty of Applied Science and Technology, Ahmad Dahlan University. Examination of morphological characteristics was carried out by observing the posterior *spiracles* of the larvae using a stereo microscope. The observable characteristics were then matched with identification keys presented in *Identification of British Insects* (Smith, 1986) and fly larval identification keys according to Coban and Beyarslan (2013); Ghosh *et al*. (2017); and Matuszewski (2021). The number of larvae of each type of fly in each treatment was calculated to determine which fly species were abundant and not abundant.

Data Analysis. The data analysis that used in this research was descriptive and inferential analysis. Descriptive analysis was used to describe the fly species found in various doses of organophosphate poison given to the carcass of white rats, as well as to describe the species of fly larvae that first appeared and the time of their appearance on the carcass of white rats. Inferential analysis was used to calculate the abundance of each fly larvae species in various treatments found in white mouse carcasses. The first step in inferential analysis was to perform a normality test using the test of Kolmogorov-Smirnov One Sample. Furthermore, homogeneity test was carried out using the Levene test, and finally the One Way Analysis of variance to determine whether there was a significant difference between treatments and abundance.

RESULTS AND DISCUSSION

Fly Larva Species in White Rat Carcass

Based on the research that has been done, the types of flies were identified in the carcass of white rats consist of four types of flies that belong to two different families. The fly species are *Sarcophaga haemorrhoidalis* from the Sarcophagidae family; *Chrysomya albiceps, C. megacephala*, and *C. bezziana* of the Family Calliphoridae (Table 1).

Flies generally have an affinity for bad smells. One source of the stench is carrion. Carcasses will become *bloating* during decomposition and produce ammonia gas (Meyer and Bryant, 2017). This ammonia gas is produced from the catabolism of inorganic bacterial subtraction around dead organisms and will attract flies to the carcass (Risriani *et al*., 2020). Flies have two receptors, namely *chemical detectors* and *visual detectors.* Both of these receptors function to detect odors, one of which is ammonia, so that it can help flies in detecting and find the location of the food (Cheng *et al.,* 2019). These two receptors are located on the antennae and when they detect a strong odor, either from poisoned treatment rats or neck dislocation treatment rays, the odor will become a sensory signal which can then guide the fly in finding the location of their feed.

Based on the description of the Table 1, the species *S. haemorrhoidalis, C. albiceps, C. megacepehala* and *C. bezziana* were found in all treatments and controls. The presence of four species of flies in the carcases of poisoned treatment rats was caused by the presence of a pungent odor in the form of ammonia gas from the decomposition process, as well as the smell of organophosphate poison residues in carrion meat which could attract the presence of flies to start colonization. Some of the larvae found in the carcass were also still in the stage of completing their life cycle even though the carcass was contaminated with organophosphate toxins. This is because the carcasses of rats that died due to *euthanasia* and then were poisoned, contain the poisonous substance (Eason *et. al.,* 2012). In general, the larvae of the Sarcophagidae and Calliphoridae families have higher levels of resistance to toxins than other fly families that play a role in forensic entomology (Badenhorst and Villet, 2018). Research conducted by Vanin (2018) states that the two families can withstand toxins in the form of liquid propoxur at a concentration of 10%. This resistance to toxins is due to the fact that both families have *neurosecretory* to neutralize the activity of chemical substances or toxins that enter the body.

The results of this study have similarities with the research conducted by Wardhani and Mulyanto (2019). The results report the presence of larvae of *S. haemorrhoidalis*, *C megacephala* and *C. bezziana* in carcasses whose deaths were caused by chemicals or poisons from *ciuciu oplosan* (alcoholic drink mixed with certain ingredients). However, there was one difference in the type of larvae found, namely the larvae of *C. albiceps* species. *Chrysomya albiceps* was not found in the research of Wardhani and Mulyanto (2019). The differences and similarities of the species found could occur due to several factors, one of which is the type of poison was used. This study used organophosphate toxins, while the research of Wardhani and Mulyanto (2019), used *ciu oplosan*. Due to the different toxic compounds was used, it is possible that these two poisons contain different active compounds so that they can affect the smell that attracts flies. The smell of chemicals in the form of morphine (heroine) and arsenic used in treated rats led to a reduction in the interest of flies to lay their eggs (Ghaffar *et al*., 2012; Mashaly *et al.,* 2020).

Table 1. Types of fly larvae found in white rat carcasses

No.	Family	Species	Treatment			
			Poison (mL)			Control
			1.5 ml	2.5 ml	3.5 ml	
1.	Sarcophagidae	*Sarcophaga haemorrhoidalis*	√	√	√	√
		Chrysomya albiceps	√	√	√	√
2.	Calliphoridae	*C. megacephala*	√	√	√	√
		C. bezziana	√	√	√	√

Based on the results of morphological identification and examination posterior *spiracles* of the fly larvae found, the types of flies obtained from this study were as follows:

1) ***Sarcophaga haemorrhoidalis.*** One of the third instar larvae identified in white rat carcasses was *S. haemorrhoidalis*. According to Showman (2016), the hallmark of *S. haemorrhoidalis* larvae is that the larvae have a much larger body size when compared with other larvae (10-22 mm), yellowish white color with a pointed head.peritreme. The posterior *spiracle* has three *slit* with the *spiracles* (the protective part of the *spiracles*) disconnected from the *dorso-ventral spiracle* (Laksmita *et al.,* 2015). Based on morphological identification and observation of posterior *spiracles* on larvae, it was identified as *S. haemorrhoidalis/* The larvae had a yellowish colorbodies with pointed heads. The end of the abdomen is a pair of posterior *spiracles* (Fig. 1a). Each spiracle has three slit *spilacular* and *peritreme* dark circular anterior *spiracle* with a pair of *papillae* (Fig. 1c).

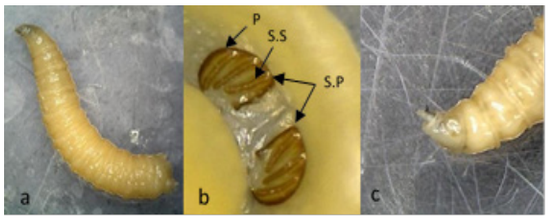

Figure 1. Larvae morphology *of Sarcophaga haemorrhoidalis*;
(a) Third instar larvae
(b) part *Posterior*
SP larvae (*posterior spiracle*);
P (*peritreme*);
SS (*spiracular slits*);
(c) *Anterior* larva.

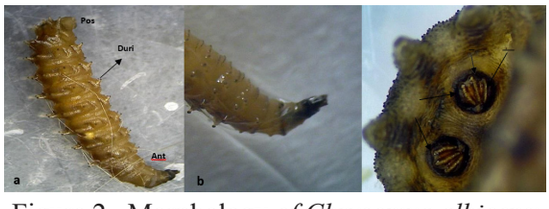

Figure 2. Morphology *of Chrysomya albiceps*; Pos (*posterior*), Ant (*anterior*)
(a) Morphology of third instar larvae
(b) *Anterior* larva
(c) *Posterior* larvae;
SP (*posterior spiracle*);
P (*peritreme*);
SS (*spiracular slits*).

2) *Chrysomya albiceps.* The next fly larvae were identified as *C. albiceps.* The larval types of *C. albiceps* were easy to spot and are commonly referred to as spiny maggots because they have many fleshy bumps along their bodies. The morphology of the third instar of this species is 10-15 mm in size, has 12 segments separated by spines arranged in rows on each segment. The head area is pointed and the anal segment is short. The larvae has an hairy body with visible *tubercles* and regularly located along the body segments. The spines of the first pectoral segment are partially slender and round. The *anterior* ends in a "Y" shape and the tip is tapered. The *posterior* of the larva has a pair of posterior *spiracles* protected by the anal *turbercle*. Each *spiracle* consists of three opening *spiracular slits*. The results of morphological identification and observation of posterior *spiracles* on the larvae showed that the larva's body looks segmented and brownish in color, there are spines that are located regularly along the body segments (Figure 2a).pair of *spiracles* is located in the caudal segment and is protected by the anal *turbercle* (Fig. 2c) and a pointed *anterior*. Each spiracle consists of three slit *spilacular slits.*

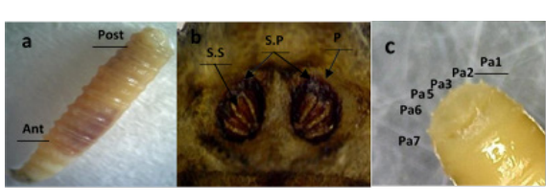

Figure 3. Larval morphology of *Chrysomya megacephala*; Pos (*posterior*), Ant (*anterior*)

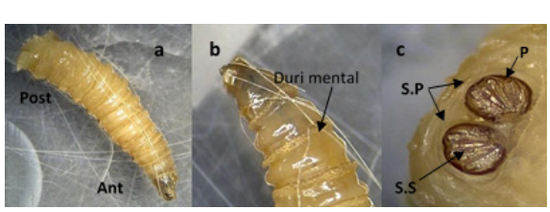

Figure 4. *Chrysomya bezziana*;
Pos (*posterior*), Ant (*anterior*) (a) *Anterior* larva; spines *Metal* (b)
The *posterior* the larva;
SP (*posterior spiracle*);
P (*peritreme*);
SS (*spiracular slits*) (c)

3) *Chrysomya megacephala.* The results of morphological identification and observation of the posterior *spiracles* of fly larvae showed the characteristics of the larvae of *C. megacephala*. *Chrysomya megacephala* are muscoid with *abdominal* without protrusions, approximately 8 mm long and brownish yellow in color. The anterior is pointed and posterior is blunt. The anterior *spiracle* has 12 marginal branches. Judging from the caudal segment, a pair of posterior *spiracles* that are close to each other and incomplete *peritreme* are clearly visible. Based on the identification results, the morphology of the *C. megacephala* was observed brownish yellow in color wich it's anterior pointed and her posterior blunt (Fig. 3a). A pair of anterior *spiracles* located adjacent to each other with incomplete *peritremes* (Fig. 3b), and *papillae* (Fig. 3c).

4) Chrysomya bezziana. The identification of the next fly larvae found was *C. bezziana*. The morphology of the third instar fly larvae of *C. bezziana* is muscoid with a characteristic tapering to the anterior (Fig. 4a). The body segments are clearly visible and at the posterior, a pair of posterior *spiracles* clearly visible. The anterior *spiracle* of the third instar larvae are *palmate* with *papillae* and equipped with *mental* between the *prothorax* and *mesothorax* that curves inward (Fig. 4b). Pair of posterior *spiracles* are close together, *spiracular slits* are straight with the *dorsal* outer spiracles slightly *divergent*. The wall *peritreme* are thick, dark and not fused *ventro-medially* (Fig. 4c).

Abundance of Fly Larva in White Rat Carcass

Based on the results of the collection of fly larvae in white rat carcasses, there were differences in the abundance of fly larvae found in the poisoned and control treatments rats. The average abundance of fly larvae found in the control treatment was dominated by *C. albiceps* (346.3 larvae), while in the poisoned treatment the dominant larvae were *S. haemorrhoidalis* (60.3 larvae). The smallest average abundance of fly larvae found was a species of *C. bezziana* (16.3 and 2.3 larvae) (Figure 5).

The average abundance of fly larvae was found in the control treatment compared to the poisoned treatment, which could be due to differences in the treatment given. The treatment was poisoned, and the test animals were *euthanized* and then organophosphate poison was inserted to her gastrointestinal, while in the control, the test animals were *euthanized* by cervical dislocation. Giving toxins or compounds to test animals can cause carrion meat to contain toxins or compounds given (Nurokhman *et al*., 2018), so that fly larvae will not develop optimally. This will have an impact on the abundance of fly larvae on the carcass. Another study also stated that organophosphate compounds content in carcasses will affect the development of larvae to pupae. The total *anticholinesterase* compound *terbufos* contained in the carcass can cause muscle paralysis, resulting in longer fly development or even death (Yan-Wei *et al*. 2010). The larvae

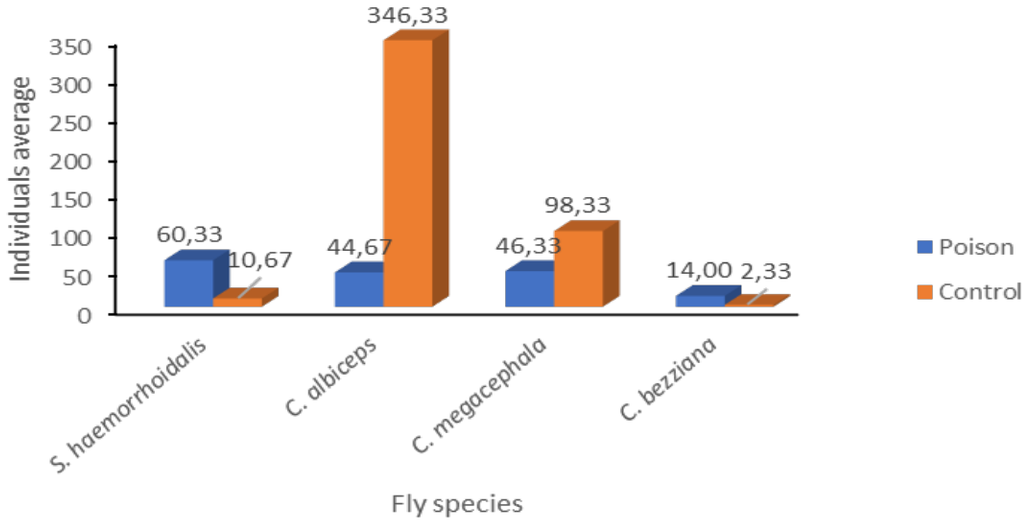

Figure 5. Average abundance of fly larvas in the white rats carcass

that were collected in this study showed that the larvae were able to survive. This is because the two families of fly found have an ability to neutralize the effects of the poison substances contained in the carcass.

In contrast to the poisoned treatment, in the control treatment the test animals were *euthanized* with cervical dislocation. *Euthanasia* with cervical dislocation will cause the carcass to decompose naturally so that fly larvae can develop optimally because there is no influence from chemical substances. Overall, larvae of the species of *S. haemorrhoidalis, C. albiceps, C. megacephala, C. bezziana* were present at all dangerous doses. However, according to Figure 5, it is known that the abundance of fly larvae of each species in each rat poisoned treatment decreased. According to Yan-Wei *et al*. (2010), the effect of *terbufos* (organophosphate) can cause muscle paralysis, resulting in longer fly development or even death. So this causes the abundance of larvae to decrease at high doses of organophosphates. Wang *et al*. (2019), which used variations in *methamphetamine* in rabbits as test animals , also found that the higher dose of *methamphetamine* cause higher mortality of the fly larvae, and thus treatment affecting the abundance of the larvae.

The most abundant number of fly larvae was found in treatment at a dose of 1.5 mL diazinon, was dominated by *S. haemorrhoidalis* with an average abundance (34.3 larvae). The abundance of fly larvae at a dose of 1.5 mL was because this dose was the lowest among other treatments. This lowest dose has a low content of organophosphate compounds in the carcass, so that the toxic effects of organophosphates do not have much impact on the development of fly larvae. Treatment at doses of 2.5 and 3.5 mL, cause the number of larvae abundance decreased. The abundance of fly larvae at a dose of 2.5 mL was also dominated by *S. haemorrhoidalis* with an average abundance (18 larvae). Furthermore, at a dose of 3.5 mlL the average abundance of *S. haemorrhoidalis* was eight larvae.

The factors that influence the poisoned treatment was dominated by *S. haemorrhoidalis*, could be caused of the organophosphate poison content in the carcass meat did not affect the development of *S. haemorrhoidalis*. It is known that flies from the Sarcophagidae Family are attracted to carcasses in almost all conditions or locations of carcasses, such as wet or dry environments, exposed to or protected by sunlight, including carcasses exposed to drugs/*toxins* (Nurokhman *et al*., 2018). Research conducted by Jales *et al.* (2021), stated that the growth of fly larvae from Families Calliphoridae and Sarcophagidae on carrion meat given terbufos caused greater mortality of individual larvae for Family Calliphoridae. This indicates that Sarcophagidae are more resistant to similar poisons. This is different from the research conducted by Wardhani and Mulyanto (2019), which used a *ciu oplosan* a traditional beverage (alcoholic beverages mixed with other ingredients) found that the type of fly larvae was dominated by the Calliphoridae family. This explains that the content of the type of poison used can affect the abundance of fly larvae on the carcass. The results of this study are in the same line as those research conducted by Jales *et al.* (2021) using terbufos poison and research conducted by Putra and Astuti (2021) using liquid mosquito repellent, which is also a type of pesticide poison. The results of those studies found that larvae of *S. haemorrhoidalis* were found more abundant than other species.

The smallest number of larvae found in this study was *C. bezziana*. The few number of this species larvae could be due to the organophosphates content in the carcass causing the development of these larvas were disrupted so that only a few larvae were found. The results of this study are different from the research conducted by Wardhani and Mulyanto (2019) which found that the larvae of *C. bezziana* were quite abundant. This difference could be due to organophosphate poison and *ciu oplosan* contain different compounds. The residue of the organophosphates left on the treated carcasses also causes the carcass aroma mixed with the organophosphate poison for a while. This can reduce the interest of *C. bezziana* fly to lay eggs on carcasses.. Overall, as stated by Wang *et al*. (2019), the same drugs may have different effects on the development of different types of flies. The difference in the dose of poison given to each treatment was then statiscally analyzed to test whether or not there was a significant difference between treatments on the number of larvae or the abundance of larvae found in the carcass.

Based on the results of the Anova test it can be seen that the value of Sig. 0.633 > 0.05. This means that there is no effect between treatments and the average abundance of fly larvae. This is likely because of all treatments of carcasses were placed in the same location and abiotic conditions. The dose of

organophosphate given in his study is not toxic on larval development, so that there are still fly larvae that can develop well in the carcass. This is also in line with the opinion of Badenhorst and Villet (2018), which state that flies from the Calliphoridae and Sarcophagidae families more resistance to poisons than other fly families that play a role in forensic entomology. However, the resistance of these two larvae to poison varies depending on the type of poison was used. Research conducted by Vanin (2018), states that the two families can withstand toxins in the form of liquid propoxur at a concentration of 10%.

The average temperature measured was 26.4°C and the normal temperature range for the development of *S. haemorrhoidalis* was 25°C (Showman and Connelly, (2011). The smallest number of the larvae of *C. bezziana* found in this study could be cause of the environmental temperature conditions did not match with the optimal temperature required for *C. bezziana*. This causes the larvae of *C. bezziana* can not complete itslife cycle optimally. The optimum temperature for the development of *C. bezziana* is 30°C.

The average air humidity measured in this study was 66.2%. The measured air humidity was suitable for the larval development of the Calliphoridae and Sarcophagidae families, which ranged from 40-80%. According to Mashaly *et al*. (2018), *S. haemorrhoidalis* larvae can grow well at 55-73% humidity. While the *C. bezziana* found in this study were few, their presence was still supported by the appropriate air humidity of 61-75% (Szpila *et al*., 2014).

The average light intensity measured in this study was 4182.2 lux. The abundance of *S. haemorrhoidalis* in this study could also be influenced by the appropriate light intensity for the development of these larvae, which ranged from 3216.1 to 5296.6 lux (Mashaly *et al.*, 2018). While the larvae of *C. bezziana* were found to be few in this study due to the nature of these larvae tend to avoid exposure to sunlight because the optimal light intensity required is 1382.1-3142.1 lux (Szpila *et al*., 2015).

The measured of wind speed at the location of the carcass was 0.97-1.57 m/s. The range area of flies ranges between 1000-2000 meters and is strongly influenced by wind speed (Sucipto, 2011). Flies have sensitivity to strong winds, so flies tend to be less active in looking for food when wind speeds are high (Sucipto, 2011). The presence of wind can help spread the smell of carrion, and can be a factor attracting the arrival of Calliphoridae and Sarcophagidae families.

Based on the measurement results of abiotic factors, it can be seen that although there is a suitability of abiotic factors for the development of fly larvae, not all larvae can develop well. The presence of other factors, such as competition and food availability, tended to have a greater influence on the abundance of larvae in carcasses. This is in accordance with the statement from Bambaradeniya (2019), which states that the suitability of abiotic factors is not the main benchmark in seeing the abundance of larvae in carcasses. However, it is the presence of competitors and the availability of food that will most likely affect the abundance of fly larvae on the carcass (Bambaradeniya, 2019).

Table . Abiotic factors that measured in research

Day observation	pH of Carcass	Air temperature (°C)	Air humidity (%)	Wind velocity (m/s)	Light intensity (lux)
1.	7	26	62	1.27	3572
2.	7	26	62	1.57	6868
3.	8	27	70	1.53	1180
4.	8	27	67	1.07	7838
5.	7	26	70	0.97	1453
$\sum$	37	132	331	6,41	20.911
$\bar{x}$	7,4	26,4	66,2	1,282	4,1822

CONCLUSION

Based on the research that has been done, the conclusions that can be drawn are: a) There are four types of fly larvae found in the carcass of white rats (*R. norvegicus*) with various doses of organophosphate treatment, namely *S. haemorrhoidalis*, *C. albiceps*, *C. megacephala*, and *C. bezziana*; and b) The most abundant fly larvae was *S. haemorrhoidalis* with an average abundance of (34.3 larvae) and the smallest abundance of fly larvae was *C. bezziana* (16.3 larvae).

SUGGESTION

Suggestions that can be given for further research are: a) Future research can be developed by conducting research in different seasons such as dry season to determine whether or not the type and abundance of fly larvae obtained with the results of this study; b) Future research can use a recording device, such as video recorder, to make it easier to determine the time of first arrival of fly larvae in determining PMI; and c) This research can be developed by conducting a toxicology of the content of organophosphate toxins in the larvae found.

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