Residues of several active insecticide ingredients in spinach (*Amaranthus tricolor* L.) and kale (*Ipomoea reptans* (L.) Poir.) in Bogor, West Java

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Abstract. Spinach and kale are popular vegetables in Indonesia. Spinach and kale production in Indonesia in 2021 will be 341,196 tonnes and 171,706 tonnes respectively. Farmers generally use insecticides to maintain the quality and quantity of vegetable production. The main problem with inappropriate use of insecticides is that there are residues in vegetable products. An interval between the last application and harvest time that is too short can cause high insecticide residues on agricultural products. The aim of this research is to analyze residue data for several active insecticide ingredients used on spinach and kale. The research carried out included field tests using pesticide formulations containing the active ingredients deltamethrin, profenofos, imidacloprid, chlorantraniliprole and carbofuran. Next, the residues of several active ingredients will be analyzed in the GIS Laboratory using GC. Data from residue analysis of several active ingredients will be compared with the maximum residue limits in National Standardization agency of Indonesia. The results obtained were residues of profenofos, imidacloprid and deltamethrin in spinach plants below MRL. Meanwhile, the residues of profenofos, imidacloprid and deltamethrin in kale were above the BMR. The profenofos residue in water spinach is 3.6546 mg/kg above the BMR 1 mg/kg based on BSN, the imidacloprid residue is 0.5195 mg/kg above the BMR 0.5 mg/kg based on the BSN and the deltamethrin residue is 0.9084 mg/kg above the BMR 0, 5 mg/kg based on BSN. Meanwhile, the active ingredients chlorantraniliprole and carbofuran were not detected. This is influenced by spraying frequency and climatic conditions. Apart from that, spraying intervals of 3 hsp and 7 hsp also affect residues on spinach and kale plants. The closer the spraying time to harvest time will affect the residue level in the plant. The physical and chemical properties that cause degradation of the active insecticide ingredient influence the reduction in residue.

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1 Introduction

Spinach (*Amaranth*) is a popular plant in some countries such as India because it is a source of several micronutrients such as carotene, vitamins A, B, C and D, iron, calcium and amino acids [1]. Kale (*Ipomoea*) is also a popular vegetable plant in Asian countries[2], widely traded and liked by consumers. Kale has an important meaning in fulfilling food nutrition because it contains a number of vitamins A, B, C and contains minerals, fiber and iron[3]. Nationally, kale is the most widely consumed vegetable commodity, namely 10.46 grams per capita per day or 73 grams per capita per week, followed by spinach at 9.26 grams per capita per day or 64 grams per capita per week. Meanwhile, the average consumption of kale and spinach by the people of West Java was 75 and 55 grams per capita per week respectively in the 2018-2021 period (data processed from BPS 2022). Kale and spinach remain the choice among several other types of vegetables such as cabbage, mustard greens, long beans, green beans, carrots, cassava leaves, eggplant, types of pumpkin and young jackfruit.

Spinach has the potential for nutritional diversity in improving diets and contributing to preventing obesity, eating disorders and micronutrient deficiencies or malnutrition which often occur in developing countries. Spinach is considered capable of becoming a source of income for small and medium scale farmers [4,5]. This is because the need for leafy vegetables such as spinach is likely to continue to increase and become daily merchandise in the market.

Spinach can be exploited for commercial purposes in the industrial world as a health supplement, pharmaceutical, food packaging and cosmetics. Natural hair dye derived from Amaranthus gangeticus or red spinach is used as a natural dye because red spinach contains anthocyanin compounds which come from flavonoid compounds [6,7]. The potential of *A. tricolor* spinach is believed to be useful in treating chronic inflammation associated with neurodegenerative disorders, neuroprotective or antidepressant, anti-inflammatory and has anti-microbial and antiviral effects [8].

Another popular vegetable plant that has good prospects and commercial content is water spinach (Ipomoea reptans (L.) Poir.). This vegetable plant has a nutritional value in 250 g of water spinach containing 9.9 g of carbohydrates, 0.17 mg of vitamin B1, 42.5 mg of vitamin C, 167.5 mg of calcium, 1 mg of zinc, 5.7 mg of Fe, 625.2 mg of potassium, 162.5 mg of sodium, flavonoids, alkaloids and steroids [9].

Efforts to increase kale and spinach production cannot be separated from pest and disease problems. Pests that attack spinach plantations include leaf caterpillars such as Spodoptera sp., Plusia sp., Hymenia recurvalis, grasshoppers, aphids (*Myzus persicae*), Thrips sp. and the mite Polyphagotarsonemus latus. Apart from that, spinach in the high and medium altitudes is also disturbed by leaf-cutting flies (Liriomyza sp.).

Pesticides are still quite important to use because they can prevent yield loss. If without pesticide application, the loss of agricultural production such as vegetables is 54% [10]. Globally, agricultural production will be reduced by 30-4% due to pest attacks if pesticides are not used [11]. FAO also states that 55% of human food potential in the world is lost due to pests, resulting in agricultural yield losses of 35% before harvest and 20% after harvest. Estimates of crop losses in developed countries are in the range of 10-30%, while in developing countries it is estimated that crop losses are in the range of 75% [12].

Although pesticides can play a role, their use is often excessive, especially in developing countries, and ultimately leaves serious health problems for consumers [13]. Improper use of pesticides such as applying harvest time intervals with the last pesticide application can cause residual effects on agricultural products such as vegetables and fruit [14]. Another problem is that the use of pesticides can cause residues in the human food chain, bioaccumulation and toxicity in mammals because their persistence in the environment is quite high [15].

Residues can arise both in plants and the environment due to high levels of spraying. In addition, spraying pesticides close to harvest time can also cause high levels of residue in agricultural products [16,17]. The aim of the research was to analyze the residues of several active insecticide ingredients applied to spinach and kale.

2 Methods

Insecticide testing activities on spinach and kale plants were carried out in April-July 2023 at the Experimental Garden of the Department of Plant Protection, Faculty of Agriculture. Bogor Agricultural University. The making of beds as experimental plots was made with a size of 2 m x 1 m with a distance between beds of 1 m. Each bed was fenced using plastic mulch to prevent the entry of other active insecticide ingredients into other beds. Furthermore, the land was given initial fertilization before planting and further fertilization when the kale was planted. After the initial fertilization, the kale was planted by sowing seeds in the furrows made above the bed while the spinach seeds were spread on the surface of the bed. The insecticides used were 5 (five) active ingredients including chlorantraniliprole (Ferterra), carbofuran (Furadan 3G), imidacloprid (Confidor 200 SL), deltamethrin (Decis 25 EC) and profenofos (Curacron 500 EC). Chlorantraniliprole (Ferterra) and carbofuran (Furadan 3G) were applied by sprinkling on the surface of the soil when planting spinach and kale. Chlorantraniliprole (Ferterra) and carbofuran (Furadan 3G) were applied only once during planting. The dose of Ferterra was 2 gr/bed and Furadan 1.5 gr/bed. Three other insecticides containing the active ingredients imidacloprid, deltamethrin and profenofos were applied by spraying. The concentrations of the three active ingredients used for spinach and kale were Confidor 200 SL 0.5 ml/L, Decis 25 EC 1 ml/L and Curacron 500 EC 2 ml/L, respectively. Spinach plants were sprayed twice for each spraying interval 3 and 7 days pre harvest (dph). The first and second spraying at 3 iph started at 21 days after planting (dap) and the second spraying at 28 dap after that the harvest was carried out at 31 dap, while the first and second spraying at 7 dph started at 16 dap and the second spraying at 23 dap after that the harvest was carried out at 30 dap. Meanwhile, the water spinach plants were sprayed 3 times for each spraying interval of 3 and 7 dph. The first, second and third spraying at 3 dph started at 33 dap, the second spraying at 40 dap and the third spraying at 47 dap after that the harvest was carried out at 50 dap, while the first, second and third spraying at 7 dph started at 31 dap, the second spraying at 37 dap, the third spraying at 44 dap after that the harvest was carried out at 50 dap. The harvest results were separated based on the type of active ingredient and spraying interval. Then a residue test was conducted at the Saraswanti Genetech Laboratory, Bogor using GC-MS. The results of the residue analysis in the form of residue levels obtained from the laboratory were compared with the maximum residue limit data obtained from the National Standardization Agency (7313: 2008; ICS 65.100.01).

3 Result

3.1 Residues of the active insecticide ingredients use a spray application based on the interval and frequency of spraying on spinach plants

Table 1 shows that testing the active ingredient deltamethrin on spinach plants with a spraying interval of 3 days pre harvest (dph) left higher residues than profenofos and imidacloprid. Even though the residue level of the active ingredient deltamethrin is higher, it is still below the maximum residue limit (MRL) of 0.5 mg/kg based on the National Standardization Agency (NSA).

Profenofos residue with a spray interval of 3 dph had a residue level 6.07 times lower than the MRL while a spray interval of 7 dph had a residue level 35.71 times lower than the MRL. Imidacloprid residue with a spray interval of 3 dph had a residue level 1.87 times lower than the MRL while a spray interval of 7 dph had a residue level 5.30 times lower than the MRL. Deltamethrin residue with a spray interval of 3 hsp had a residue level 1.01 times lower than the MRL while a spray interval of 7 dph had a residue level 5.1 times lower than the MRL.

Table 1. Insecticide residues on spinach with intervals of 3 and 7 dph and MRL based on NSA.

Type of Insecticide Active Ingredient	Residue level at spray time interval (AOAC 2007.01)		MRL NSA (mg/kg)
	3 dph (mg/kg)	7 dph (mg/kg)	
Profenophos	0,1645	0,0280	1
Imidacloprid	0,2662	0,0943	0,5
Deltamethrin	0,4926	0,0974	0,5

Table 1 shows that the residue at a spraying time interval of 3 dph is higher than the residue level at a spraying time interval of 7 dph even though both residue levels are below the MRL based on BSN. This shows that the closer to harvest time with spray application, the potential for higher residues to occur. Another study showed that spraying the insecticide profenofos on cabbage increased residue levels because the spraying time was close to harvest time [18]. Apart from that, pesticide residues containing the active ingredients deltamethrin (decis 2.5 EC) and imidacloprid (curacron) were also found on vegetable plants sprayed by farmers with a spraying interval of 3 to 7 days before harvest [19].

The influence of the physical and chemical properties of the active ingredients of the pesticides used also affects the residue content in plants. Profenofos has non-systemic properties, is not absorbed by plant tissue but only sticks to the outside of the plant. Profenofos is an organophosphate pesticide that is easily degraded due to hydrolysis reactions, photolysis and the activity of microorganisms that affect profenofos residues in plants [20]. Therefore, the profenofos residue content in spinach plants with spraying of 3 and 7 hsp is the lowest below the MRL.

Profenofos cannot last long on spinach plants because it is degraded by weather factors, namely the effect of hydrolysis. The influence of weather during the last insecticide application (second application) on spinach influenced the low residue level in the plant. When insecticide application is carried out in the morning, moderate intensity rain occurs in the afternoon until evening. The hydrolysis process by rain after spraying on the same day affects the degradation of the active ingredients resulting in a reduction in residue on spinach plants.

Of the three active insecticide ingredients used, deltamethrin is the insecticide most widely used by Indonesian farmers to protect horticultural crops from pests. Even though residues of the active ingredient deltamethrin are toxic to mammals [21].

3.2 Residues of the active insecticide ingredients use a spray application based on the interval and frequency of spraying on kale plants

The treatment of the active ingredients profenofos, imidacloprid and deltamethrin on kale is the same as the treatment on spinach plants. It's just that the resulting residual level is different. Table 2 shows that the treatment of the active ingredients profenofos, imidacloprid and deltamethrin with a spray time of 3 dph had residual levels above the MRL.

The highest residue level in kale plants among the three active ingredients used is profenofos, namely 3.6546 mg/kg. This is influenced by spraying frequency and climatic conditions. Meanwhile, there is little influence on the physical and chemical properties of the

active ingredient profenofos, such as the nature of being easily degraded in nature. Profenofos in kale plants is not degraded quickly due to the influence of climate and the influence of spraying frequency. The climatic influence in question is the weather/climate conditions when spraying or after spraying there is no rain so that the profenofos degradation process is reduced. The level of pesticide degradation in each country is influenced by climate so there are differences in the pesticide degradation process. Climatic elements that influence pesticide degradation in nature include average temperature, average amount of rainfall, average amount of sunlight emitted and the presence of microbes. The degradation process with water will help reduce residues on plants [22].

The frequency of spraying profenofos on kale is done 3 times during the growing season. One of the factors causing high levels of residue in agricultural products is the application of pesticides more than once in one planting period. Spraying kale plants 3 times resulted in an increase in residual level. Moreover, it is supported by weather conditions so that the hydrolysis process does not reduce the high levels of profenofos residues [26].

Table 2. Insecticide residues on kale with intervals of 3 phi and 7 phi and MRL based on NSA

Type of Insecticide Active Ingredient	Residue level at spray time interval (AOAC 2007.01)		MRL NSA (mg/kg)
	3 dph (mg/kg)	7 dph (mg/kg)	
Profenophos	3,6546	0,0903	1
Imidacloprid	0,5195	0,0846	0,5
Deltamethrin	0,9084	0,3000	0,5

During testing of the active pesticide ingredients on kale plants, the weather/climate experienced light to moderate intensity rain. Rain occurred only once, namely during the second spraying. The second spraying was carried out in the morning and in the afternoon it rained with light to moderate intensity rainfall. Meanwhile, during the third spraying there was no rain until harvest time.

The time interval for spraying near harvest time also affects the residue level of the plant. The factors causing high levels of residue in agricultural products are not only the application of pesticides more than once in one planting period, but also the spraying of pesticides close to harvest time [23]. Table 2 shows that the residue level of kale with a spray interval of 3 dph is higher than a spray interval of 7 dph. The level of pesticide residue on plants is determined by the type of pesticide, dose and frequency of application, and application time. The closer the application of pesticides is to harvest time, the more pesticides remain on agricultural produce and have not completely disappeared from the plants. If spraying is carried out far from harvest time, the residue level may be lower [24]. The time interval for spraying insecticides on mustard plants before harvest tends to reduce insecticide residue levels and plant quality characteristics [25].

Apart from profenofos, imidacloprid and deltamethrin residues also exceeded the MRL at the spraying time of 3 dph. The residual level of profenofos is 3.6546 mg/kg higher than MRL 1 mg/kg, the residual level of imidacloprid is 0.5195 mg/kg higher than BMR 0.5 mg/kg and the residual level of deltamethrin is 0.9084 higher than BMR 0, 5 mg/kg based on BSN. This means that the residual level of the active ingredient profenofos is 3.6 times the BMR, imidacloprid is 1 times the MRL and deltamethrin is 1.8 times the MRL, so it will definitely have an impact on human health (table 2).

Pesticide residues in vegetables and fruit are a problem of considerable concern. Moreover, multi-residue pesticide contamination in different commodities [26,27]. Pesticides should be used within safe limits as given by different regulatory bodies to avoid adverse effects of pesticides on humans [28].

3.3 Residues of the active insecticide ingredients of the insecticide carbofuran and chlorantraniliprole

The results of GC testing at the GIS Laboratory showed that the residues of carbofuran (furadan 3G) and chlorantraniliprole (ferterra) in kale and spinach were still below the Limit of Detection (LOD) and were therefore not detected (table 3). The residual levels of chlorantraniliprole and carbofuran were not detected because the active ingredient residues were not readable by the instrument. This contains two possibilities, namely that no residue of the active ingredients carbofuran and chlorantraniliprole were found in spinach and kale or the possibility that there was residue but it was below the LOD of the testing equipment.

Table 3. Granular insecticide residues on spinach and kale

Type of active ingredient	Residual level		LOD	MRL NSA 7313: 2008 (ICS 65.100.01)
	Spinach	Kale		
chlorantraniliprole	Not detected	Not detected	0,001	0,5
carbofuran	Not detected	Not detected	0,00087	0,5

Carbofuran is an insecticide from the carbamate group which is widely used in the field [29]. Carbofuran is a carbamate insecticide that usually has low toxicity to mammals when compared to organophosphates but is very effective in controlling insects.

Carbofuran is an active insecticide ingredient which is generally sprinkled on the surface of the soil. Carbofuran of the furadan type is granular and works by entering plant tissue through absorption from the soil by the plant. Carbofuran that is applied to the soil will then be carried to all plant tissues including leaves and fruit through plant roots [24].

Carbofuran can enter from the outside of plants through the epidermis of stems, bark and roots. Carbofuran is lipophilic so it can enter more quickly through the lipid components of the cuticle [30]

Furadan is an insecticide with the active ingredient carbofuran which is applied at the same time as planting. Furadan is sown on the same day after the kale and spinach seeds are planted or sown. Carbofuran residues that were not detected in spinach and kale were probably caused by the long harvest time and leaching by rainwater while in the field. The degradation period for carbamates in the environment is around 2 weeks so it is necessary to adjust the spraying time. So the frequency of spraying the carbamate group is once every two weeks [24]. This possibility affects the residual levels of chlorantraniliprole and carbofuran in spinach and kale which were not detected because they were only applied once at the beginning of planting. Carbofuran will tend to accumulate if several sprays are applied and will decrease more slowly during the spraying time [31]. Apart from the influence of the frequency of application of carbofuran and chlorantraniliprole. Another possibility that causes pesticide residues to not be detected is that the tool cannot detect the residue because it is below the LOD level.

The residual results of the active ingredient carbofuran in spinach and kale were not detected. Apart from being influenced by the degradation period in the environment which was only 2 weeks, it may also have been influenced by the distance between the application of carbofuran and chlorantranilipro to harvest time. The application interval between carbofuran and chlorantraniliprole on spinach plants is 30 days and on kale plants is 40 days.

3.4 Residues in spinach and kale

Spinach and water spinach cultivation was carried out simultaneously using the same treatment. Spinach and kale seeds were spread on the beds, each bed was separated using plastic mulch. The use of plastic mulch was so that when spraying, insecticide drift would not be carried by the wind to other beds and/or prevent the flow of spray water from moving from one bed to another (Figure 1). The pesticide dosage and spraying interval on kale were the same as those on spinach. However, the results of the residue content in the two plants were different. When compared, the average spinach residue content was lower than the kale residue content. This is likely influenced by the frequency of spraying. kale plants were sprayed 3 times, while spinach plants were sprayed 2 timesIn addition, the possibility of kale having a higher residue level is influenced by plant characteristics. Although spinach and kale are both hyperaccumulator plants. The characteristic of hyperaccumulator plants is that they accumulate certain metal elements in high concentrations in their shoots [32].

Fig. 1. Condition of kale and spinach plants fenced using plastic mulch

4 Conclusion

The residue content of imidacloprid, deltamethrin and profenofos insecticides in spinach plants sprayed 3 or 7 days before harvest (dap) was still below the maximum residue limit (MRL) based on NSA. Meanwhile, the residue content of imidacloprid, deltamethrin and profenofos in kale plants was higher than the MRL when sprayed 3 dap, while the residue content of deltamethrin, imidacloprid and profenofos pesticides in kale plants at 7 dap showed a low residue content of the MRL. Carbofuran and chlorantraniliprole insecticides applied to spinach and kale plants showed residue content below the MRL based on NSA..

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