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Analysis of mancozeb and carbofuran pesticides residues in the production area of shallot (*Allium cepa* L. *Var. ascalonicum*)

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Abstract

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The way to protect shallot from pests and diseases have encouraged the farmers to excessive use pesticides in shallot production area in Brebes Regency of Central Java, Indonesia. It can be observed through pesticide residues in soil and agricultural products. The purpose of this study was to determine the distribution of mancozeb and carbofuran pesticide residues in the shallot production area in Brebes Regency of Central Java, Indonesia. Research locations was choosed on purposive sampling, namely Kersana and Wanasari District. Pesticide residues analysis was carried out on seven soil samples and seven shallot products that consist of 3 samples from Kersana District and 4 samples from Wanasari District. Pesticide residue analysis used a gas chromatography method with an electron capture detector. Based on the analysis of soil samples, mancozeb residues were found in all samples from Wanasari District with concentrations of 0.037 to 1.132 mg/kg, and two examples from Kersana District with concentrations of 0.048 mg/kg and 0.083 mg/kg. Carbofuran residues was only found in one soil sample from Kersana District with a concentration of 0.017 mg/kg. In the shallot sample, mancozeb residues were identified in all samples from Wanasari District with concentrations rangeng from 1.274–2.195 mg/kg, and two samples of Kersana District with concentrations of 0.347 mg/kg and 0.497 mg/kg. The concentrations of mancozeb in all shallot samples from Wanasari District exceeded the maximum concentration of residual limit (RML) of mancozeb value (0.5 mg/kg). Carbofuran residues were not identified in all shallot samples from Kersana District, but were identified in two shallot samples from Wanasari District with concentrations (0.014 mg/kg and 0.018 mg/kg) that is still below the carbofuran RML (0.1 mg/kg).

Keywords: pesticide residue; mancozeb; carbofuran; shallot

Introduction

Shallot is one of the strategic agricultural commodities that support the national economy. National Development Planning Agency (2013) explained that shallotis ranked 7th out of eight main food commodities (seven other main food commodities are rice, corn, soybean, sugar, beef, chili and oil palm). In the Ministry of Agriculture's Strategic Plan for 2015-2019, shallots are listed as the third main commodity of horticulture after large chili and cayenne pepper. According to Santoso (2013), shallot is a type of horticultural vegetable that has high economic value, the economic value of shallots is higher than that of various other vegetables (Ngatindriatun et al., 2012).

Shallot (*Allium cepa* L. var. *ascalonicum*, *Allium ascalonicum*) (Wibowo, 2009) is one of the horticulture plants that is susceptible to plant pests and diseases. Some plant pests that can thwart harvest for instances *Thrips tobaci* Lind., *Laphygma exigua* HBN, *Spodoptera exigua*, *Agrotis interjectionis* and *Agrotis ifsilon* Hfn, *Acarina* sp., *Ditylenchus dipsaci* (Kuhn), *Acrolepia assectella* and *Pytobia cepae* (Wibowo, 2009; Hadisoeganda & Widjaja, 2008; Moekasan et al., 2012; Haryati & Nurawan, 2009). Onion thrips (*Thrips tabaci* Lindeman), are onion pests throughout the world and have been identified as number one pest in Canada (Allen, 2011).While some diseases that can cause shallot failure include purple spot disease caused by *Alternaria porri* fungus, dew flour disease caused by fungus *Peronospora destructor*, shoot out disease by *Phytophthora porri* fungus, wilt caused by *Fusarium oxysporium* fungus, root disease caused by *Bacterium solanacearium* and mosaic disease caused by viruses (Hadisoeganda & Widjaja, 2008; Moekasan et al., 2012; Wibowo, 2009). Pests and plant diseases that frustrate the harvest often make the shallot farmers disappointed, therefore the prevention and eradication of pests and plant diseases is the main task of farmers (Wibowo, 2009).

Pests attacks and plant diseases should be controlled through an Integrated Pest Management system. According to the Republic of Indonesia Government Regulation No. 6 of 1995, that crop protection is carried out through an Integrated Pest Management system (IPM). IPM is carried out through the prevention of the entry of plant disturbing organisms, control of pests and eradication of pests. Plant protection is carried out by using facilities and methods that do not interfere with health and or threaten human safety, cause disturbances and nature and or the environment. But in the field, the implementation of IPM in protecting crops has not run smoothly. Until now, the use of pesticides is still the main method for controlling pests. This is because pesticides are considered to be more effective, practical and faster than other ways to protect crops against crop failure (Mahmudah et al., 2012). Sembel (2012) argued that insecticides are still the most widely used chemicals to control pests, especially in developing countries.

Pesticides have two opposite sides. On the one hand, pesticides can improve human welfare, but on the other hand pesticides are poisons that damage humans and the environment (Mahmudah et al., 2012). RI Government Regulation No. 6 of 1995 concerning plant protection and Minister of Agriculture Regulation No. 48 of 2006 concerning guidelines for good and Right food crop cultivation (Good Agriculture Practice), reminded that the use of pesticides is the last alternative and the negative impacts that arise must be minimized. The using of pesticides must meet six precise criteria, namely: the right type, the right quality, the right dose, on time, right on target, the right way and application tools. The use of pesticides that do not meet the criteria can cause adverse effects on human health and the environment.

The high demand for shallots makes opportunities and challenges at the same time especially for the development of domestic shallots production. According to Santoso (2013), the development of shallot agribusiness in the future must be directed to several objectives, including: meeting domestic consumption needs, meeting industrial raw material needs, replacing imported shallots and filling export market opportunities that are still wide open. However, the demand for fulfillment of high shallot products will encourage excessive use of pesticides, because shallots are one of the horticultural commodities that are susceptible to plant pests and diseases (Wibowo, 2009), so the use of pesticides cannot be avoided.

Brebes Regency, Central Java Province is the biggest production center for shallots in Indonesia and supplies around 30 % of the national shallots. In 2014, Central Java as the number one supplier contributed 42.09 % of the national shallot production concentrated in Brebes Regency. In 2014, Brebes Regency contributed around 73 % of shallot production in the province of Central Java (Central Java Statistics Center, 2015), or almost one third of national shallot production (Ministry of Agriculture, 2015).

The use of pesticides in the shallot production area of Brebes Regency, Central Java, is still the main choice for protecting shallots from pests and plant diseases. One type of pesticide that is widely used is the mancozeb compound, a dithiocarbamates compound which is commonly used as a fungicide. Whereas one type of insecticide used is carbofuran, a carbamate compound generally used to control soil caterpillars. Efforts to protect shallots from pests and diseases are driving farmers in excessive use of pesticides. This results in the leaving of pesticide residues in soil and agricultural products. The purpose of this research was to deter-

mine the distribution of mancozeb and carbofuran pesticide residues in the shallot production area in Brebes Regency of Central Java, Indonesia.

Materials and Methods

Location Selection

The area of the research was selected by purposive sampling, based on the area of shallot production (Statistics Center of Brebes Regency 2014), two districts were selected in Brebes Regency, Central Java Indonesia, namely Wanasari and Kersana Districts. Wanasari district was chosen by four villages (Kupu, Wanasari, Tanjungsari and Sidamulya) and Kersana district selected three villages (Kersana, Kemukten and Limbangan). Pesticide residue analysis site was carried out in the laboratory of Agrochemical Material Residues Agricultural Environment Research Institute, Bogor, Indonesia. The study was conducted from August to December 2017.

Sampling of Soil and Shallot Products

The samples analyzed included soil on the shallot growing area and shallot products taken from three villages in Kersana District and four villages in Wanasari District. Sampling soil and shallots referred to Akan (2013). Samples of soil were taken from the land where the shallots grow 6–12 hours after harvesting shallots. At each location 5 sampling points were determined. Soil samples were taken using a soil shovel with a depth of 0–20 cm. Samples were composited and taken about 1 kg, put into a clean plastic bag, labeled, transported to the laboratory. Samples of soil dried in an open space at room temperature until the moisture content was approximately 20 %. Samples of shallots were taken from the sample locations that have been harvested for ± 1000 grams, put in clean polyethylene bags, labeled, stored at 4°C until the sample extraction time.

Reagents and Standards

The analysis method of carbofuran (carbamate class) and mancozeb (group of dithiocarbamate) residues was carried out by Gas Chromatography (GC). The method referred to the AOAC method (2011) with modification that have been developed by this laboratory. All reagents used were analytical grade. Mancozeb (purity 81.4%) and carbofuran (purity of 99.5%) standard compounds were obtained from Chem Service Inc.

Preparation of Soil Samples

Samples of soil that has been dried, finely ground, sieved with a 16 mesh sieve, weighed 25 grams, pour into a 250 mL Erlenmeyer glass, added 80 mL acetonitrile, shaken and allowed to stand for 24 hours then filtered. The next filtrate was clean up. Chromatography column was prepared (40 cm long, 2 cm diameter), cotton clogged column, filled with 50 mesh horizon to 5 cm high), moistened with hexane until submerged. The extracted filtrate was put in a column collected with round pumpkin.

The clean up filtrate was evaporated until a thick extract was obtained. Sodium disulphate was added to ± 2 grams, acetonitrile was added to 10 mL, then filtered. The filtrate was divided into two parts, one part (filtrate a) to test the mancozeb compound directly with the gas chromatography (GC) device, another part (filtrate b) derivatized for testing carbofuran compounds.

Against Filtrate b, derivatization was carried out. In the filtrate b in an erlenmeyer flask, 100 mL of distilled water were added, 2 mL of 0.5 N KOH and 1 mL of 1-fluoro-2,4-dinitrobenzene solution. Then closed and shaken for 30 minutes at high speed using a mechanical shaker. Then add 10 mL of 5% borax solution, shake it to mix, and heat it on steam for 30 min. The sample solution was cooled to room temperature by placing the flask in a container containing a little water for 10 min. The sample solution was put into a 250 mL separating funnel, added 10 mL hexane, then shaken, and allowed to stand. Then the water phase was removed (discarded), while the hexane phase was inserted into the sample bottle, for testing carbofuran with GC device.

Preparation of Shallot Sample

As many as 100 g of shallot samples are skinned, sliced, blended. Weighed with 25 g of fine sample, put into the grinded Erlenmeyer, added 75 mL acetonitrile, homogenized with Ultra Torrax device, filtered with filter paper, filtrate put into a separating funnel, added 50 mL hexane, shaken 5 minutes, allowed to stand, water phase issued. Against the hexane phase there is no need to clean up, but immediately evaporates until a thick extract was obtained. Into the extract was added acetonitrile to 10 mL, then filtered. The filtrate was divided into two parts, one part (filtrate a) to test the mancozeb compound directly with the GC device, another part (filtrate b) derivatized for testing carbofuran compounds.

Against Filtrate b, derivatization was carried out. In the b filtrate (in an erlenmeyer flask), 100 mL of distilled water were added, 2 mL of 0.5 N KOH and 1 mL of 1-fluoro-2,4-dinitrobenzene solution. Then closed and shaken for 30 min at high speed using a mechanical shaker. Then add 10 mL of 5 % borax solution, shake it to mix, and heat it to steam for 30 min, cooled to room temperature by placing the flask in a container containing a little water for 10 min. The sample solution was put into a 250 mL separating funnel, added 10 mL hexane, then shaken, and allowed to stand. Furthermore,

the water phase was released (discarded), while the hexane phase was inserted into the sample bottle, to be analyzed by GC device.

Analysis of Pesticide Residues with Gas Chromatography Device

The standard solution of mancozeb compounds is made using acetonitrile solvents. Made standard 100 ppm stock solution, then diluted to the concentration of the solution which gives the peak that can be read under standard conditions. The standard solution of carbofuran compounds was made using acetonitrile solvents at a concentration of 10 ppm, then derivatized according to the sample solution, then diluted to the concentration of the solution which gave the peak readable at standard conditions.

Tool Condition the variant type was 450 GC, the phase was N2-UHP, the flow rate was 28 mL/minute, VF 1701 capillary column was 30 m long, 0.25 mm in diameter and 0.25 µm thickness), and ECD detector. Injection temperature of 250°C, Detector temperature of 300°C.

A total of 2 µL of sample solution was injected into the GC device. Identification was done by comparing sample retention times with mancozeb and carbofuran standards. Determination of the level was done by calculating the area of the sample compared to the standard, with the following formula.

$$R = \frac{A_c}{A_s} \times K_s \times \frac{V_{ac}}{B_c}$$

Note :

R = Pesticide Residue (mg/kg)
A_c = Sample Area
A_s = Standard Area
K_s = Standard Concentration (ppm)
V_{ac} = End Volume of Sample
B_c = Weight Sample (g)

Results and Discussion

Identification of Mancozeb and Carbofuran Pesticide Residues in Soil and Shallot Products

Identification of carbamate compounds in the sample was done by comparing the retention time (Rt) of the sample with the standard. Under the standard conditions of this research, mancozeb compounds gave Rt 4.14 min while carbofuran compounds gave Rt 18.59 min. In different conditions, each compound will provide a different retention time. In the study conducted by Kaye et al. (2015), Rt Mancozeb was 6.72 min, in a study conducted by Andrade et al. (2011), the Rt for carbofuran was 11.62 min.

Mancozeb compounds were identified in all soil samples from Wanasari District and two soil samples from Kersana District. While carbofuran compounds were only identified in one soil sample from Kersana District. In the shallot sample, mancozeb was identified in all shallot samples from Wanasari District and two samples from Kersana District. While carbofuran compounds are only found in two samples of shallots from Wanasari District.

Mancozeb Pesticide Residues and Carbofuran in Soil Samples

Mancozeb residues were found in all soil samples from Wanasari District and two soil samples from Kersana District (Table 1). Mancozeb concentration in soil samples from Wanasari District with a range from 0.037–1.132 mg/kg, with the highest level being an sample of Tkp (1.132 mg/kg). The highest mancozeb concentration in soil samples from Kersana District was Tlm (0.083 mg/kg), but this value was lower than the level of mancozeb soil sample in Wanasari District (Figure 1). Mancozeb residues were also found on vegetable farming land in North Sumatra, Poniman et al. (2017) reported that agricultural land in Karo Regency and Simalungan Regency as highland vegetable centers in North Sumatra Province were identified to contain mancozeb residues with a range of 0.0062–1.8433 mg/kg, this value was higher than the content of mancozeb residues in Wanasari District .

Table 1. Mancozeb and carbofuran residues in soil samples

District	Sampel Code	Concentration of Residues (mg/kg)	
		Mancozeb	Carbofuran
Kersana	Tkr	0.048	notdet
	Tlm	0.083	notdet
	Tkm	notdet	0.017
Wanasari	Tkp	1.132	notdet
	Ttn	0.142	notdet
	Twn	0.037	notdet
	Tsd	0.062	notdet

notdet: not detected, Limit of detection: 0.01 ppm

Table 1. was the data of the analysis results of mancozeb and carbofuran pesticide residues in 7 soil samples from two locations, namely Kersana and Wanasari Districts, three samples (sample code: Tkr, Tlm, Tkm) from Kersana District and four samples (Sample code: Tkp , Ttn, Twn, and Tsd) from Wanasari District. Pesticide residue analysis was carried out by gas chromatography.

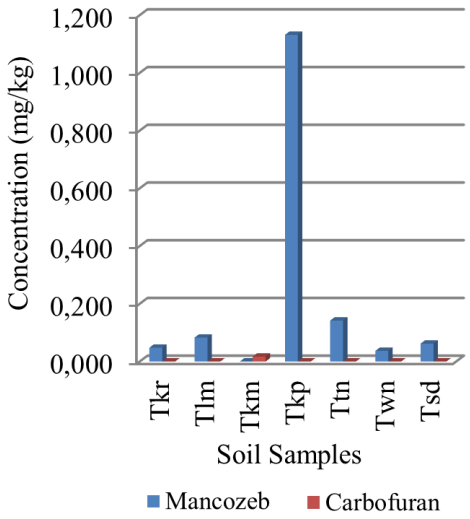

Fig. 1. Concentration of mancozeb and carbofuran residues in soil samples

Figure 1 was a description of the levels of mancozeb and carbofuran pesticide residues in 7 soil samples from two locations, three samples (sample code: Tkr, Tlm, Tkm) from Kersana District, and four samples (Sample code: Tkp, Ttn, Twn, and Tsd) from Wanasari District.

Carbofuran residues was only found in one soil sample from Kersana District, which was a low level of TKm which was 0.017 mg/kg. All soil samples from Wanasari District were not detected to contain carbofuran (Table 1). The use of carbofuran by farmers was not as intensive as mancozeb, so that the carbofuran residues in the land where the shallot grows was not as high as the mancozeb residue.

The high mancozeb residues compared to carbofuran residues (Figure 1) could be caused by several factors. The first could be caused by the factor of farmers who used mancozeb continuously, so that it accumulates in the soil. High levels in the soil are also influenced by the nature of mancozeb compounds, one of which is the solubility of compounds in water. Mancozeb compounds are fungicidal ditiocarbamate compounds whose solubility in water is very low at 6.2 mg/L (Rathore & Nollet, 2012). In addition, soil texture affects the absorption of pesticide compounds. The soil texture in this study was generally sufficiently clay, and clay will retain insecticidal residues in the soil, so that the concentration of pesticide residues in the soil was high.

Mancozeb Pesticide Residues and Carbofuran on Shallot Products

The maximum residual limit (MRL) of pesticides for shallots in Indonesia is found in SNI-7313 (2008) concerning the Maximum Limit of Pesticide Residues in Agricultural Products. In this reference, carbofuran MRL in shallots is listed as 0.1 mg/kg, while this reference does not include MRL for mancozeb compounds. The mancozeb (Ditiokarbamat) compound MRL in onion bulb according to the Codex Alimentariusis 0.5 mg/kg (2005).

In this research mancozeb residues were identified in all shallot samples from Wanasari District and two samples of Kersana District. Mancozeb content in the sample from Wanasari District ranged from 1.274-2.195 mg/kg (Table 2), all of these values exceeded the mancozeb MRL value (0.5 mg/kg) according to Codex Alimentarius (2005).Where as two samples from Kersana District contain mancozeb with levels of 0.347 mg/kg and 0.497 mg/kg respectively (Table 2), this value was still below the mancozeb MRL according to Codex Alimentarius (2005). Mancozeb identification in other vegetables, has been reported by several researchers. Kaye et al. (2015), reported that mancozeb was identified in tomato samples taken from agricultural land and markets in five districts in Uganda. It was found that the average mancozeb level on agricultural land was 1.03 ± 0.28 mg/kg, while the average level from the market 0.77 ± 49 mg/kg, this value exceeds mancozeb MRL (0.5 mg/kg).

Table 2. Mancozeb and carbofuran residues in shallots samples

District	Sampel Code	Concentration of Residues, mg/kg	
		Mancozeb	Carbofuran
Kersana	Bkr	0.347	notdet
	Blm	0.497	notdet
	Bkm	notdet	notdet
Wanasari	Bkp	2.120	0.018
	Btn	1.274	0.014
	Bwn	1.613	notdet
	Bsd	2.195	notdet

notdet: not detected, Limit of detection: 0.01 ppm

Table 2 was the data of the analysis results of mancozeb and carbofuran pesticide residues in 7 shallot samples from two locations, namely Kersana and Wanasari Districts, three samples (sample code: Bkr, Blm, Bkm) from Kersana District and four samples (Sample code: Bkp , Btn, Bwn, and Bsd) from Wanasari District. Pesticide residue analysis was carried out by gas chromatography.

In this research carbofuran residues were not identified in all shallot samples from Kersana District, but were identified in two shallot samples from Wanasari District (Table

2). Carbofuran content in two shallot samples from Wanasari District is 0.014 mg/kg and 0.018 mg/kg, this value is still below the carbofuran MRL (0.1 mg/kg) based on SNI-7313 (2008). Miskiyah & Munarso (2009) reported that samples from Brebes in Central Java were not detected to contain carbofuran residues, but samples from Cianjur West Java were detected to contain carbofuran with levels of 0.0011 ppm, this value was still below the specified MRL. Identification of carbofuran residues in other types of vegetables, reported by several previous researchers, Tuhumury et al. (2012), reported that carbofuran was identified in fresh spinach samples in Ambon City with concentration below the established MRL limit. Wispriyono (2013) reports that some vegetables from supermarkets and traditional markets in Depok are not identified as containing carbofuran residues.

In this research, mancozeb residues were more common in soil and shallot samples, compared to carbofuran residues, the content in the shallot sample (Figure 1) was much higher than the soil sample (Figure 2). The high mancozeb residue in the shallot sample was mainly due to intensive use of mancozeb by farmers during the planting period. Based on interviews with shallot farmers in Wanasari and Kersana Districts, it was known that during the planting period, 95.76 % of respondents were in Wanasari District and 96.36 % of farmers in Kersana District used pesticides containing mancozeb (Dhitane, Vondozeb, Detazeb and Delsene). While respondents who used carbofuran (furadan) pesticides in Wanasari District were only 4.24 % and 7.27 % in Kersana District.

Figure 2 was a description of the levels of mancozeb and carbofuran pesticides in 7 samples of shallots from two locations, three samples (sample code: Bkr, Blm and Bkm) from Kersana District, and four samples (Sample code: Bkp, Btn, Bwn, and Bsd) from Wanasari District.

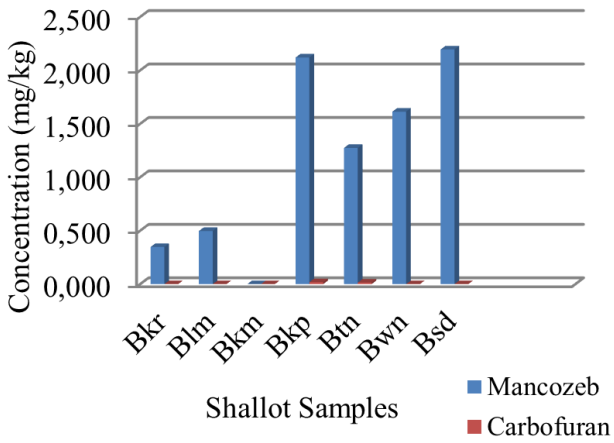

Fig. 2. Concentration of mancozeb and carbofuran residues in shallot samples

Mancozeb is a dithiocarbamate compound that functions as a fungicide (Rathore &d Nollet, 2012), usually used to treat diseases caused by fungi, including purple spot disease (caused by *Alternaria porri* fungus), dew flour disease (caused by *Perenospora destructor* fungus), shoot out disease (caused by fungus *Phytophthora porri*) (Wibowo, 2009). Fungicides with active ingredients are mancozeb in the form of flour, very low water solubility of 6.2 ppm (Rathore & Nollet, 2012), therefore before being used it is usually suspended first with water, so the continuous use of mancozeb without regulation can cause a buildup of residue on the land the place of growing shallots and is very possible to accumulate residues on the shallot bulbs. Sulaeman et al. (2016), suggested that land contaminated with pesticides greatly contributed to the content of pesticide residues in agricultural products.

High carbamate residues in agricultural products can threaten human health and the environment. The effects of carbamate pesticide residues on health problems are lethargy (malaise), muscle weakness, dizziness, sweating, headache, excessive saliva, nausea, vomiting, stomach ache, diarrhea, nervous system depression, blurred vision and weight loss (Rathore & Nollet, 2012). Doull et al. (1986), argued that organophosphate and carbamate pesticides have anticholinesterase activity by inhibiting the action of acetylcholinesterase, so that there is no hydrolysis of acetylcholine, resulting in accumulation of acetylcholine, which causes dizziness, nausea, weakness, chest pain and others. Ahouangninou et al. (2012) have tested the potential risk of a number of pesticides impact on health by calculating the health risk index (HRI) and its impact on the environment by calculating the environmental risk index (ERI), the results showed that mancozeb provided the second highest risk (HRI 3499) after chlorpyrifos (HRI 3538), but mancozeb has a low environmental risk (ERI 30); Carbofuran has a low health impact (HRI 925), but it provides the highest environmental risk (ERI 576), WHO classifies carbofuran as a 1b category (very dangerous) (Ahouangninou et al., 2012). Mancozeb is known to cause cancer, distrupt of the reproductive and endocrine system (Poniman et al., 2017). The discovery of soil and shallot samples containing mancozeb with levels exceeding the Mancozeb MRL according to Codex Alimentarius(2005), shows that efforts to achieve self-sufficiency in food for shallot commodities, have the potential to threaten the health of the environment and the community, if no remedial efforts are made on the shallot production process, especially in using of pesticides.

Conclusions

Mancozeb residues were found in all soil samples from Wanasari District with concentration of 0.037-1.132 mg/kg, and two soil samples from Kersana District with levels of 0.048 mg/kg and 0.083 mg/kg. Carbofuran residue was found in one soil sample from Kersana District with a concentration of 0.017 mg/kg, all soil samples from Wanasari District were not detected to contain carbofuran. At the shallot sample, mancozeb compounds were identified in all samples from Wanasari District with concentration ranging from 1.274-2.195 mg/kg, and two samples of Kersana District with levels of 0.347 mg/kg and 0.497 mg/kg. Mancozeb levels in all shallot samples from Wanasari District exceeded the mancozeb MRL value (0.5 mg/kg) according to Codex Alimentarius (2005),Carbofuran residues were not identified in all shallot samples from Kersana District, but were identified in two shallot samples from Wanasari District with concentration of 0.014 mg/kg and 0.018 mg/kg, this value is still below the carbofuran MRL (0.1 mg/kg) according to SNI-7313 (2008).

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