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# DETERMINATION OF DIAZINON RESIDUE USED IN SHALLOT AGAINST ARMYWORM (*Spodoptera exigua*)

## *IDENTIFIKASI RESIDU PENGGUNAAN DIAZINON PADA BAWANG MERAH TERHADAP ULAT BAWANG* (Spodoptera exigua)

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## ABSTRACT

The research was carried out to identify the residue of diazinon in shallot crop which was grown in Yogyakarta Special Territory, Bantul Regency (Parangtritis, Samas and Sanden). Questionnaires were also distributed to farmers in the area to find out the background of diazinon application. Samples were taken from these locations and Bantul market, and were analyzed using Thin Layer and Gas Chromatography (TLC and GC) methods. TLC technique detected diazinon residues in all samples except Parangtritis sample from farmers plot with no diazinon spraying. GC results showed that all the samples diazinon residues were well below maximum residue limit (MRL). The least residue level (0.0029 ppm) was detected in one week after harvesting sample from Sanden. The highest residue level (0.0231 ppm) was detected in the harvesting time sample from Parangtritis.

Keywords: diazinon, MRL, residue, shallot, *Spodoptera exigua*

## *INTISARI*

*Penelitian ini bertujuan untuk mengidentifikasi residu diazinon pada bawang merah dari daerah Parangtritis, Samas dan Sanden, Kabupaten Bantul, DI. Yogyakarta. Kuesioner dibagikan kepada petani untuk mengetahui latar belakang penggunaan diazinon serta aspek lain yang berkaitan, seperti frekuensi dan dosis aplikasi. Sampel uji diambil dari ketiga lokasi tersebut ditambah sampel lain dari Pasar Bantul. Sampel dianalisis dengan metode thin layer chromatography (TLC) dan metode gas chromatography (GC). Deteksi residu diazinon dengan metode TLC menunjukkan adanya residu diazinon di semua lokasi, kecuali pada sampel yang berasal dari lahan petani di daerah Parangtritis tanpa menggunakan diazinon. Hasil analisis GC menunjukkan bahwa sampel uji mengandung residu diazinon dalam jumlah kecil, yaitu di bawah batas residu maksimum (MRL), sedang pada sampel hasil panen dari lahan petani di daerah Parangtritis tanpa menggunakan diazinon tidak terdeteksi. Residu terkecil (0,0029 ppm) terdeteksi pada sampel dari Sanden yang dianalisis satu minggu setalah panen. Residu terbesar (0.0231 ppm) terdeteksi pada sampel saat panen yang berasal dari Parangtritis.*

*Kata kunci: bawang merah, diazinon, MRL, residu,* Spodoptera exigua

## INTRODUCTION

People are encouraged to consume more vegetables and fruits, as these are good sources of vitamins and fiber and also healthy. But public opinion has it that most vegetables and fruits, especially those grown in Indonesia, bring about environmental and health problems, since their planting involved the use of chemicals, especially pesticides. Consequently, this has created a certain apprehension and fear in the public as to the presence of pesticide residues in their daily food.

On a wider-scale, insecticides, as one type of pesticides which are the most commonly used, are undoubtedly dangerous in the hands of untrained personnel, and it is reported that about 25 million peoples are poisoned annually by them (Rengam, 1992). Most of these people do not die, but sublethal effects can be serious and long-lasting. Exposure to these compounds has varying results: from skin irritations after pyrethroid insecticides use to complex systemic illness resulting from cholinesterase inhibition by organophosphates (O'Malley, 1997). People who apply the chemicals tend to suffer most, because of their inappropriate use of often unknown compounds, the use of the wrong and outdated technology, not using protective clothing, general inefficiency and wastage.

These problems are worst in developing countries, where advisory services and access to safer compounds and technologies are often unavailable. It is also suggested that pesticide companies in some developing countries exert unethical but tight hold on growers, where aggressive marketing and unscrupulous dealers are commonplace (Rengam, 1992). Irrespective of hazards to people, the insect pests themselves are becoming resistant to many insecticides, especially where large numbers of applications are required during one generation of particular crop, such as what happened in most vegetables plantings.

The problem identification of this research was to investigate the diazinon residue in shallot. Shallot is a daily consumed vegetable, and diazinon is moderately toxic to mammals with an acute oral LD50 of 250 mg/kg and 285 mg/kg in male and female rats, respectively and an acute dermal LD50 of 900 mg/kg and 455 mg/kg in male and female rats, respectively (Gaines, 1969). Diazinon become more toxic in the form of diaoxon, which is found in both plant and animal tissues (McEwen & Stevenson, 1989).

This research was first aimed to observe the insecticides usage pattern among shallot farmers in Bantul regency through questionnaire, and then to determine diazinon residue in shallot and give awareness on the dangers of residues in agricultural products when consumed by man.

## MATERIALS AND METHODS

**Time and Location of Research**. The research was conducted from February until June 2007, to study the resi-due of diazinon insecticide in shallot grown in Bantul Regency (Samas, Sanden and Parangtritis sites), from different farmer's plots. A sample from Bantul market was also included. Questionnaires were distributed among farmers in the above-mentioned sites to find the background of diazinon application and other aspects (e.g. frequency of application and concentrations) that could not be directly observed. In the laboratory of Pesticide Toxicology, Faculty of Agriculture, Gadjah Mada University, two methods were used to analyse diazinon residues (TLC and GC), which was taken after McMahon & Wagner (1999).

**Questionairre method**. Questionairre was administered in the three locations in Bantul (Samas, Sanden and Parangtritis), where farmers grew shallot. The objective was to know the types of insecticides which were used to control *S. exigua* on shallot, the percentage of diazinon application in each area, the frequency of diazinon application per week, and the variations of dosage. Fifteen respondents represent each area in Bantul (Samas, Sanden and Parangtritis) were interviewed.

**Sampling method**. Three samples were taken at harvesting time from three locations in Bantul (Samas, Parangtritis and Sanden), where farmers applied diazinon during rainy season. For the control, three samples were taken from same locations where farmers did not apply diazinon. One sample was bought from Bantul market randomly, 1 kg from four different origins/directions, in order to know whether any residues were detected. Samples from the field were taken diagonally in the amount of 1 kg/sample.

**Extraction method**. The three samples from area applying diazinon were taken and divided into six sub-samples. The three sub-samples were analyzed at harvesting time, and the other three were analyzed one week after harvesting. Sample from the market was analyzed directly, and weighed 100 g, then chopped and weighed 25 g from composite sample, which was then grinded and mixed. To the mixture 15 g $Na_2SO_4$ anhydrous, and 100 mL acetone were added, and were left for one night. Then the mixture was filtered using Buchner funnels and filter paper.

**Partition method**. Pour the above mixture into 1000 mL separatory funnel, add 250 mL of distilled water, and 50 mL petroleum ether (PE). Add 10 mL of NaCl. The mixture shacked until became homogenized, and then the separatory funnel was put in a stand ring.

The mixture was left in the separatory funnel until two layers appeared, the upper layer was organic in ether and the lower were acetone, water and dirt. After separation, upper layer was taken carefully and put in a flask. Extraction for the lower layer was done again by adding 50 mL PE and separated again. All organic phases in the PE were put in a rotary vacuum evaporator, where the temperature was adjusted to 40°C and the cycle to 150 rpm.

After the extract approximate dryness, each samples' extracts were put in volumetric flask and PE was added until 5 mL, and kept in refrigerator, ready for analysis with TLC. From each of these samples, 1 mL extract was taken and put in the microtube and heated in waterbath until the solvent evaporate and then 100 µL ethyl acetate was added. For injection in TLC analysis, only 10 µL was taken from each samples extract.

**Clean up method.** Before gas chromatography analysis, from each samples 1 mL extract was taken and cleaned up by using chromatographic column (pipette 10 mL) filled with glass wool (1 cm), then successively packed with $Na_2SO_4$ anhydrous (3 cm), florisil which had been heated 500° C for 7 hours (2 cm) and again with $Na_2SO_4$ (3 cm). Before pouring the extract, the column was cleaned with 10 ml PE extract (1 mL) which has been ready to be packed into column, and then the extract was cleaned with 25 mL eluent I (5% diethyl ether—DE—in PE with 2% ethanol). The rest of the extract was cleaned with eluent II (15% DE in PE) 25 mL. Afterward, eluents I and II were evaporated in rotary evaporator until dryness, and then PE was added until the volume reached 1 mL. The injection volume was 1.0 µL to Gas Chromatography.

**Data Analysis. a. Questionairre**. Descriptive analysis was applied to analyze the questionairre data from Bantul (Samas, Sanden and Parangtritis), to know the kinds of insecticides which were used to control *S. exigua* variations, and to know the frequency and concentrations of diazinon applied in the field.

**Data analysis. b. TLC**. Retention factor (Rf) is calculated using the following formula:

$$R_f \text{ value} = \frac{\text{distance from origin to component spot}}{\text{distance from origin to solvent front}}$$

**Data analysis. c. GC**. Confirming the sample chromatogram by comparing retention time (Rt) of samples with retention time of diazinon standard.

## RESULTS AND DISCUSSIONS

**Findings from the farmers' questionairres**. The questionairres were distributed to find out the background of diazinon insecticide usage in controlling *S. exigua* in Bantul. It targeted 45 respondents, where each area (Samas, Sanden and Parangtritis) had 15 respondents. Table 1 shows different kinds of insecticides to control *S. exigua* at three different areas in Bantul Regency. Diazinon as well as Thiodicarb insecticides were used in all of these areas.

Table 1. also shows that diazinon was very popular among farmers. Diazinon had a high percentage usage of more than 50% in the three locations compared to other insecticides. Parangtritis area had the highest percentage (73%), followed by Samas (60%) and Sanden areas (54%).

Most shallot farmers in the three locations applied diazinon twice per week. The highest percentage was the farmers from Parangtritis area (67%) who used diazinon twice per week, while 20% used it three times per week and only 13% used it once per week. In Samas area 60% farmers sprayed diazinon twice per week, 13% sprayed three times per week and 27% once per week. In Sanden area 53% applied diazinon twice per week, 40% applied it once time per week and only 7% applied three times per week (Table 2).

Table 3. shows that most farmers in Bantul Regency used 3 mL/L concentration. Parangtritis area had the highest percentage of this concentration (80%), followed by Samas area 67% and Sanden area 60%, although the recommended concentration on the label was 2 mL/L with spraying volume of 500 L/ha if the pest population density reaches economic threshold level (ETL). Unfortunately the farmers did not follow the instruction, they promptly sprayed if they find any numbers of insect pest.

**Thin layer chromatography (TLC) analysis.** With TLC as qualitative method to detect diazinon residues, it was confirmed that all samples of shallot contained the residue, except sample coming from plot in Parangtritis where diazinon was not used. Other sample sites where diazinon was not used still yield diazinon residues, which means that there were histories of diazinon usage in those areas.

**Gas chromatography analysis**. The correlation between diazinon concentrations with area width is quantitatively expressed in the formula y = 9450.71 x + 18540; where y = area width, x = concentration of diazinon residue (ppb) and $r^2 = 0.9516$ (Fig. 1). The values of diazinon residue in the samples were counted according to the area width of and the result divided by 5 (the weight of each samples was 25 g, further extracted into 5 mL).

Table 5. shows the highest residue count as 0.0231 ppm, detected in the sample analyzed at harvesting time from Parangtritis area. Amounts of 0.0091 ppm and 0.0097 ppm were detected in the samples from Samas and Sanden areas, respectively, where farmers did not use diazinon. Probably the diazinon residue was adrift in rainwater, especially as this study was done in rainy season. Residue was not detected in the sample from farmers' plot which did not use diazinon in Parangtritis area. Samples which were analyzed one week after harvesting showed that there were somewhat degradations in the samples from Parangtritis area from 0.0231 to 0.0149 ppm, in the samples from Samas area from 0.0144 to 0.0106 ppm and in the samples from Sanden area from 0.0105 to 0.0029 ppm. All samples were below Indonesia's Maximum Residue Limit (MRL) of diazinon on shallot (Anon., 1996) as shown by Table. 5.

Diazinon residues detected in samples at harvesting time from farmers plot in Parangtritis (0.0231 ppm), Samas (0.0144 ppm) and Sanden (0.0105 ppm) are all below maximum residue limits (0.5 ppm) although most farmers used high concentration 3ml/l and their average frequency of application is twice per week. Diazinon residue in shallot was very low because the amount of rainwater was very high in rainy season which then caused leaching, but the main factor might be degradation because diazinon has a rapid degradation rate (Copper, 1996). Diazinon residue detected in samples from farmer's plot which did not use diazinon (Samas and Sanden areas) might be due to drifts from other plots where diazinon was used, or the sites used to use diazinon or similar active ingredients in their farming practices. Quantitatively the amounts were far below the MRL.

Diazinon residues decreases in samples from diazinon-treated farmers' plots of all sites were also proofs of diazinon's rapid degradation rate. The existence of diazinon residue in Bantul Market shallot samples, which was very low, show that degradation of diazinon still continue until after the produce is in the market. This advantage does not mean that the use of diazinon should be encouraged. On the contrary, this situation must be maintained with careful planning and use of diazinon in Bantul regency.

Table 1. Types of insecticides which were used to control *Spodoptera exigua* on shallot from 15 February-15 April 2007

| Area (district) | Insecticides | % farmers use diazinon |
|---|---|---|
| 1. Sanden | Diazinon-Thiodicarb-Deltamethrin-Carbaryl | 54 |
| 2. Samas | Diazinon-Thiodicarb-Deltamethrin | 60 |
| 3. Parangtritis | Diazinon-Thiodicarb-Carbaryl | 73 |

Table. 2. Weekly diazinon application frequency in Bantul Regency

| Frequency per week | Sanden area (%) | Samas area (%) | Parangtritis area (%) |
|---|---|---|---|
| Once times | 40 | 27 | 13 |
| Twice times | 53 | 60 | 67 |
| Three times | 7 | 13 | 20 |

n=15 farmers

Table 3. Variation usage of diazinon concentrations by farmers in Bantul Regency

| Concentration (mL/L) | Sanden (%) | Samas (%) | Parangtritis (%) |
|---|---|---|---|
| 1 | 0 | 0 | 0 |
| 2 | 40 | 33 | 20 |
| 3 | 60 | 67 | 80 |
| 4 | 0 | 0 | 0 |

n = 15 farmers

Table 4. Concentration of diazinon standard solution and area width

| No | Concentration (ppb) | Area width |
|---|---|---|
| 1 | 200 | 203400 |
| 2 | 100 | 111451 |
| 3 | 50 | 936701 |
| 4 | 25 | 34492 |
| 5 | 12.5 | 16149 |

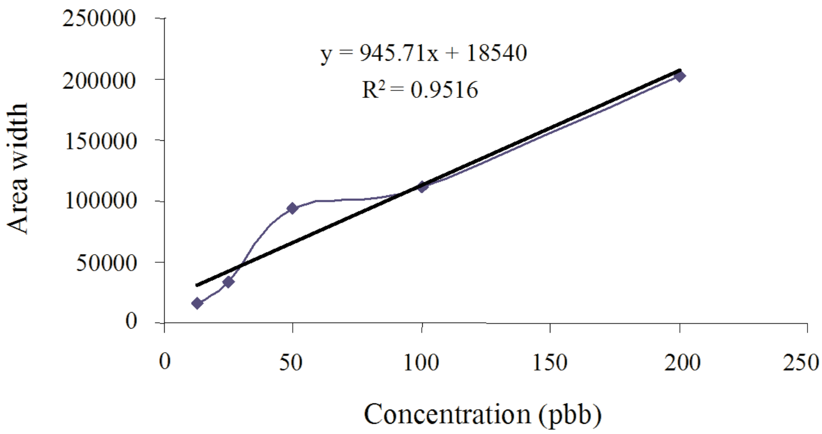


Fig.1. Calculating the residue amount by correlating area width with concentration (in ppb)

Table 5. Diazinon residue in sample and MRL in Indonesia

| Location | Residue (ppm) | MRL (ppm) |
|---|---|---|
| 1. Sample from Samas area not using diazinon | 0.0091 | 0.5 |
| 2. Sample from Samas area at harvesting time. | 0.0144 | 0.5 |
| 3. Sample from Samas area one week after harvesting. | 0.0106 | 0.5 |
| 4. Sample from Sanden area not using diazinon | 0.0097 | 0.5 |
| 5. Sample from Sanden area at harvesting time. | 0.0105 | 0.5 |
| 6. Sample from Sanden area one week after harvesting. | 0.0029 | 0.5 |
| 7. Sample from Parangtritis area not using diazinon | NDL | 0.5 |
| 8. Sample from Parangtritis area at harvesting time. | 0.0231 | 0.5 |
| 9. Sample from Parangtritis area one week after harvesting. | 0.0149 | 0.5 |
| 10. Sample from Bantul Market. | 0.005 | 0.5 |

MRL-Maximum Residue Limit NDL-No Detectable Level

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