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Residue of paraquat in noni fruits

(Sisa baki paraquat di dalam buah mengkudu)

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Key words: paraquat, residue, *Morinda citrifolia* (noni), spectrophotometric method

Abstract

The residue of paraquat in noni (mengkudu) fruits was investigated using spectrophotometric method. Recovery study using paraquat fortified noni fruit produced satisfactory percentage recoveries of 78.5% and 81.7% for noni spiked with 0.25 μg/g and 0.50 μg/g respectively, demonstrating that the analytical method used was effective. In the field treatment, paraquat was applied at the rate of 0.941 kg/ha on three repeated applications and two controls were designated for comparative purposes. No paraquat residue was detected in the samples collected from both control and treated plots at all sampling periods except for samples from the treated plot at 60 days after last application (DALA). At 60 DALA, a paraquat concentration of 0.14 ppm was detected.

Introduction

Morinda citrifolia or noni or commonly known as mengkudu in Malaysia is a native plant of Queensland, Australia. Specific parts are used for their antihelmintic, antibilious, antibiotic, antiemetic and other medicinal purposes. In Malaysia, the fruit is taken as emmenagogue (Jaganath and Ng 2000) and popularly known as the most potential herb to cure diseases. Due to the resurging use of this herb in recent years for healthcare and therapeutic purposes, many suppliers and business activities related to it have been generated. This has created a good deal of competition in terms of quality of the noni products (Cheung et al. 2001). To generate more incomes for Malaysian farmers, noni is recommended to be planted as inter-crops especially in small holders' rubber estates. Even though this herbal product is comparatively safe to consume, the possibility of the presence of contaminants such as pesticides should not be neglected.

Paraquat or 1', 1'-dimethyl-4'4-bipyridilium ion is commonly commercialized in dichloride salt form. It is a non-selective, non-systemic, quick acting herbicide and desiccant, which is non residual because of its rapid inactivation by irreversible adsorption or contact with the soil (Calderbank and Slade 1975). Paraquat itself is a highly toxic substance. It caused giddiness, nausea, topical lesion in acute exposure whilst lung fibrosis and oedema were seen in chronic exposure cases (Bismuth and Hall 1995). In August 2002, the Pesticide Board of Malaysia had decided to ban the paraquat usage by disallowing the re-registration of the product. However, the existence of paraquat in the soil due to the previous and continuous application deserves attention considering its long half-life in soil. The reported half-life for

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paraquat in soil is about 23 weeks (Bismuth and Hall 1995).

Paraquat can be determined by various methods such as gas chromatography (Khan 1974), high performance liquid chromatography (Worobey 1987), spectrophotometry (Calderbank et al. 1961) and other apparatus. Despite being a conventional method as compared to others, colorimetric method by using spectrophotometer is much preferred due to its simpler analytical procedures and favourable results in recovery studies (Kuntom et al. 1999). The objective of this study was to investigate the residue of paraquat in noni fruits after several applications of paraquat as a post emergent and inter-crop weed control chemical.

Materials and methods

Study site

The study was carried out on a three hedge-row perimeter planting with 35 m of intercropping area located at Malaysia Rubber Board (LGM) Experimental Research Station in Tok Dor, Terengganu (N 04º 49', E 103º 12'). This system of rubber planting consisted of three rubber rows at 3 m apart on either side of the noni intercropping area. A total of 17 noni rows with planting distance of 5 m (between rows) x 4 m (within row) were used with the first and last noni rows 6.5 m from the inner rubber rows on either side in perimeter planting system. The soil type of the planting area was sandy clay. The rubber trees as an anchor crop were grown concurrently with noni the companion crop. There were 882 noni trees planted in this rubber-noni agroforestry system and they were used for various studies to evaluate the feasibility of cultivating medicinal/aromatic plants under rubber ecosystem.

Field treatment

A total of 119 noni plants were allocated for the herbicides treatment in a 35 m x 68 m area. The experimental design used was randomized complete block with three replications. Each replicate consisted of 10–14 noni trees. Three types of herbicide namely, paraquat, glyphosate and glufosinate were used to evaluate the efficacy of selected post-emergence herbicides. However for the purpose of this study, paraquat was the only herbicide investigated. Paraquat was sprayed on the soil three times (22.7.2002, 19.9.2002 and 4.2.2003) with the application rate of 0.941 kg/ha per application. One untreated plot within the experimental area was designated as Control 1 whereas an untreated plot nearby but outside the experimental area with the same acreage as control 1 was designated as Control 2. After the last application of paraquat, representative amount of mature noni fruits were randomly sampled for residual analysis. Samplings were made at 5, 9, 20, 30, 60, 90 and 120 days after last application (DALA).

Calibration curve

A standard calibration curve was prepared from a number of working standard solution containing 0.05–1.0 µg of paraquat. These solutions were added with 0.8 ml of 0.2% sodium dithionate and made up to 10 ml with saturated ammonium chloride (NH_4Cl). The absorbance was measured at 396 nm with a spectrophotometer Model GBC-UV/VIS 911A (GBC Scientific Equipment Pty Ltd, Australia). Saturated ammonium chloride was used as blank.

Recovery study

Noni fruits taken from an untreated area were fortified with 0.05 µg/g, 0.1 µg/g, 0.25 µg/g and 0.5 µg/g of paraquat (98% purity) (Sigma Aldrich, Germany) in triplicates. The fortified samples were subjected to the process of extraction, clean-up and spectrophotometric determination procedure described below.

Sample extraction, clean-up and spectrophotometric determination of paraquat procedure

Noni fruits were chopped and a sample of 100 g was homogenized with 250 ml purified water for 2–3 min. Subsequently 25 ml of 97% concentrated H_2SO_4 (AR grade, Merck, Germany) was added to the homogenized samples to unbind the paraquat. Ten drops of octan-2-ol and glassbeads (anti-bumping and anti-foaming agent) were added to the mixture. The mixture was then refluxed for 5 h using electrothermal heating mantle (Electrothermal, England) and allowed to cool overnight. Subsequently 7 g of Duolite cation exchange resin (BDH, England) was mixed with 25 ml purified water and then packed into 9–10 mm glass column. The packed column was then washed with 25 ml of water and 20 ml of saturated NaCl (Merck, Germany).

The aqueous sample solution was percolated into the column at the flow rate 10 ml/min. The column was then washed with 25 ml of water, 100 ml of 2.5% NH_4Cl (Merck, Germany) and 25 ml of water sequentially. The paraquat residues that are trapped in the resin then were eluted with 75 ml of saturated NH_4Cl at a flow rate about 1 ml/min. The eluate was collected in volumetric flask and analysed for paraquat residue. About 9.2 ml of eluate was added to 0.8 ml of 0.2% sodium dithionate (Merck, Germany), a reducing agent. The solution was measured at the absorbance at the blue coloured radical ion obtained at 396 nm.

Results and discussion

Calibration curve

The calibration curve for the recovery of paraquat residues in noni fruits is shown in *Figure 1*. The equation derived from the curve is [P] = 12.99*absorbance – 0.11 with regression coefficient value of 0.98 (p <0.05) where [P] is the concentration of paraquat (μg/ml). The calibration curve is used for the quantification of paraquat residues in noni fruits.

Recovery studies of paraquat residue in noni fruits

Recovery studies of paraquat from the fortified noni fruits are shown in *Table 1*. Percentage recoveries of 78.6% and 81.7% were obtained for the fortification levels of 0.25 and 0.5 μg/g respectively. This indicated satisfactory reliability of the

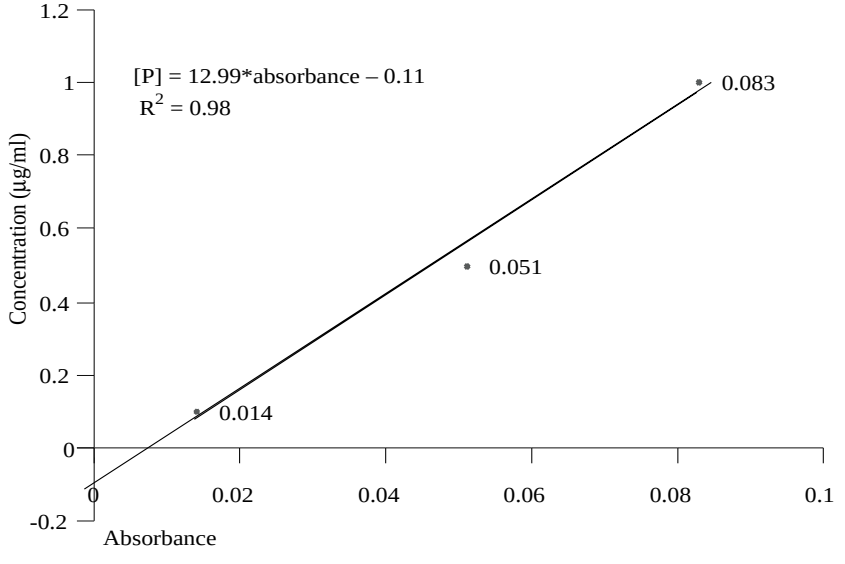

Figure 1. Calibration curve of paraquat

Table 1. Recovery of paraquat residue in noni

Amount added to spiked samples (μg/g)	Recovery (%)*	S.D.	R.S.D (%)
0.05	54.10	0.0314	14.10
0.1	62.00	0.0127	20.58
0.25	78.53	0.0349	6.29
0.5	81.66	0.1047	13.63

*Average of three replicates

method. However, recovery for the fortification level of 0.1 μg/g and 0.05 μg/g are lower (62.0% and 54.1%) than the fortification level mentioned above. It was observed that the percentage of recovery was higher with the increase in the fortification level. The minimum quantitative detectable level of paraquat concentration in noni fruits was 0.05 μg/g by using this method. The sensitivity of the spectrophotometer using this technique was 0.05 μg/g, which is similar to Kuntom et al. (1999), who reported the same level of sensitivity for paraquat in oil matrix by using the same technique.

Residues of paraquat in noni fruits

Residue was not detected for both untreated and treated plots at all sampling periods except for the sample from the treated plot of 60 DALA. Similar results were also obtained in field trials of paraquat in soft fruits such as apples, strawberries, blackcurrants and many other crops (Summers et al. 1980). In this study, paraquat was applied at the concentration of 0.22–1.77 kg per acre during the growing seasons and no residues were detected in any of the samples examined. This phenomenon could be due to the fact that paraquat was not applied directly to the fruits, but was sprayed to the soil for controlling weeds. In another trial where paraquat was applied at higher dosage (equivalent to 5 kg per acre) around the base of the barks of apple trees, no residue was also detected in the harvested fruits (Calderbank and Yuen 1965).

At 60 DALA, the paraquat residue of 0.14 ppm were detected in noni fruits. In view of the fact that the crops were not directly sprayed with paraquat, assumption could be made that the residues found in the fruits were due to the root uptake of the plants from the soil. Being a cationic herbicide, paraquat is unlikely to leach into soil water because of its strong adsorption capacity to soil particles. However paraquat could leach into soil water depending on certain factors such as paraquat concentrations in soil and the soil characteristics itself. At high concentrations in soil, paraquat is loosely bound compared to low concentrations where it is tightly bound to soil particles (Tucker et al. 1967) and this loosely bound paraquat can be washed away by water (Rai et al. 1997). This loosely bound paraquat might be desorbed by leaching since the experimental plot received frequent rainfalls. The leachates could contaminate the soil water and made it available for the roots during the nutrient uptake (Riley et al. 1976). There could be a possibility that 60 DALA is the critical time for the paraquat to be absorbed by root and translocated to the fruits.

Paraquat being a cationic herbicide binds strongly with soil particles especially soils with high clay content. On the contrary paraquat has a weaker binding affinity with sandy soils compared to clayey soils (Calderbank and Slade 1975). A study done by Cheah et al. (1997) demonstrated that desorption of paraquat did happen in sandy loam soil due to the lower content of clay in the sandy loam compared to muck soil. In view of the fact that the soil type of the experimental plot was sandy clay, which contained about 55% sand, desorption of paraquat could have probably occurred in the soil. This could have provided chances for root uptake of residues by plant.

It would be more meaningful if this study had taken into consideration the paraquat residue in soil and the soil characteristics such as clay content, type of clay and the organic matter content in order

Table 2. MRLs of certain crops that are listed by Codex Alimentarius for paraquat

Crop	MRL (ppm)
Maize	0.1
Olives	1.0
Passion fruit	0.2
Potato	0.2
Rice	10
Rice, polished	0.5
Sorghum	0.5
Soya bean (dry)	0.1
Sunflower seed oil, crude	0.05
Sunflower seed oil, edible	0.05
Vegetables (except otherwise listed)	0.05

to explain the binding affinity of paraquat with soil. Besides the factors mentioned above, the physical changes of the soil such as soil cracking or earthworm activity can contribute to the mobility of paraquat to deeper layer of the soil and made it available in soil water (Fryer et al. 1975). At 90 and 120 DALA, paraquat residue in noni fruits had probably degraded to the level lower than the limit of quantification (0.05 μg/g).

The degradation of paraquat in plants did not occur metabolically but most of the paraquat on the plant surface was decomposed when the plants were exposed to sunlight (Slade 1966). Therefore the assumption can be made that photodegradation had decreased the concentration of paraquat residue in fruits well below the quantification levels. The level of paraquat found in this study is not known to cause health hazard because there is no MRL for paraquat in noni fruits in Codex Alimentarius *(Table 2).* It is important to give consideration in setting the MRLs for this herbal plant to protect the consumer's health in view of the fact that it is widely used all over the world.

Conclusion

The presence of paraquat residue in noni was not evidenced throughout all the sampling intervals except for samples at 60 DALA where the concentration of paraquat residue was 0.14 ppm. The method used by Kuntom et al. (1999) for the determination of paraquat in palm oil is proven applicable in noni fruits as reflected by the satisfactory results of the recovery study.

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Abstrak

Kehadiran sisa baki paraquat, sejenis racun rumpai dikaji di dalam buah mengkudu. Kaedah spektrofotometrik yang diubah suai telah digunakan dalam kajian ini untuk mengesan sisa baki paraquat. Kajian perolehan semula sisa baki paraquat yang telah dijalankan menunjukkan kadar perolehan semula daripada 78.6% hingga 81.7% untuk paraquat pada kepekatan 0.25 μg/g dan 0.5 μg/g. Bagi tujuan kajian ladang, paraquat yang digunakan untuk mengawal rumpai disembur pada kadar 0.941 kg/ha sebanyak tiga kali pada waktu yang berbeza dan dua kawalan digunakan untuk tujuan perbandingan. Sampel dianalisis pada kadar masa 5, 9, 20, 30, 60, 90 dan 120 hari selepas semburan terakhir (DALA). Tiada sisa baki paraquat dikesan pada semua kadar masa bagi kawalan dan sampel dari kawasan yang disembur kecuali bagi sampel 60 hari dari kawasan yang disembur yang dikesan mempunyai sisa baki paraquat pada kadar 0.14 ppm.

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