Arab Gulf Journal of Scientific Research, 20 (3), pp 156-160 (2002)

ORIGINAL PAPER **(REF. 2125)**

K I Al-Mughrabi

Residue Studies of Methabenzthiazuron in Soil, Lentils and Hay

Abstract: Over two years, replicate plots of lentils (*Lens culinaris* L.) were treated before seeding with methabenzthiazuron at a rate of 0.5 kg a.i. ha^{-1}. In each year, representative soil, lentil and hay samples were randomly collected from plots of each treatment. Soil samples were tested for residues 24 hrs. after treatment and at harvest. Lentil and hay samples were tested at harvest. A cleanup step was conducted after extraction. Gas chromatograph equipped with a nitrogen/phosphorus detector was used to detect methabenzthiazuron. Overall average of residue levels in soil decreased significantly from 1.16 ± 0.15 mg kg^{-1}, 24 hrs. after treatment, to 0.12 ± 0.01 mg kg^{-1} at harvest. No significant difference in the maximum average residue was found in lentil and hay samples collected from various plots and tested at harvest (0.10 ± 0.01 and 0.19 ± 0.02 mg kg^{-1} in lentils and hay, respectively). Recovery tests were conducted with each group of samples tested in order to determine the efficiency of the analytical procedure.

Keywords: Methabenzthiazuron, lentil, hay, soil, residue analysis, chromatography.

دراسة متبقيات مبيد الأعشاب ميثابنزثيازورون Methabenzthiazuron في التربة وبذور وتبن العدس

خليل إسحاق المغربي

المستخلص: على مدار عامين من البحث، عوملت مقاطع مكررة بالمبيد ميثابنزثيازورون Methabenzthiazuron بمعدل 0.5 كغم مادة فعالة للهكتار قبل زراعة العدس. تم فحص عينات ممثلة من التربة بعد مرور 24 ساعة من الرش، وفحصت عينات ممثلة من التربة وتبن وبذور العدس عند الحصاد. وقد تم إستخلاص المبيد و أتبع ذلك بعملية تنظيف ، حيث إستخدم جهاز الفصل الكروماتوغرافي الغازي المزود بكاشف نيتروجين –فسفور (Nitrogen-Phosphorous Detector) للكشف عن المبيد. أظهرت النتائج أن مستويات المتبقيات للمبيد قد إنخفضت في التربة من 1.16 ± 0.15 جزء في المليون بعد 24 ساعة من الرش الى 0.12 ± 0.10 جزء من المليون عند الحصاد. ولم يكن هناك فرقاً معيارياً في الحد الأعلى لمعدل المبيد المتبقي في تبن و بذور العدس عند الحصاد (0.10 ± 0.01 و 0.19 ± 0.02 جزء من المليون على التوالي). هذا وقد تم إجراء إختبار إسترجاعي للمبيد للتأكد من مدى كفاءة طرق الكشف .

كلمات مدخلية: تربة، تبن، عدس، تحليل متبقيات، ميثابنزثيازورون.

Introduction

Methabenzthiazuron (1-benzothiazol-2-yl-1, 3-dimethyl urea) is a selective herbicide which is absorbed primarily through roots and, to a lesser extent, through the leaves. It controls annual grasses and broadleaf weeds pre- and post-emergence by inhibiting photosynthetic electron transport (PS II) (Ghafoor *et al.* 1987; Worthing, 1987 & 1991), which fits the shade adaptation reaction (Fedtke, 1974). The herbicide is used to control a broad spectrum of grasses and broad-leaved weeds in cereals, broad beans, garlic, peas and onions. It has also been used in orchards and vineyards in combination with other herbicides (Worthing, 1991). Full season competition from weeds reduces the yield of chickpea and lentil by 40-60%. The duration of its residual activity is about 3 months in soil. In plants, it is metabolised to water-soluble glycoside (Ahrens, 1994; Worthing, 1987 & 1991). Methabenzthiazuron is widely used around the world on a wide range of crops including lentils (*Lens culinaris* L.) and chickpeas (*Cicer arietinum* L.). No studies have so far been conducted on the fate of methabenzthiazuron in soil, lentils and hay under Jordanian conditions and hay after application for weed control under Jordanian conditions.

Khalil I. Al-Mughrabi
New Brunswick Department of Agriculture, Fisheries and Aquaculture,
39 Barker Lane, Wicklow, New Brunswick E7L 3S4, Canada.
Tel:(506) 392-5199
Fax:(506) 392-5102
E-mail:khalil.Al-mughrabi@gnb.ca

Materials & Methods

Chemicals

The following chemicals were used: acetone and dichloromethane, all analytical grade (Merk, Darmstadt, Germany); sodium sulfate anhydrous

GR (May and Baker, U.K.); sodium chloride GR (Carlo Erba, Milan); glass wool prewashed with petroleum benzene in a soxhlet (Merk, Germany). Reference standard, analytical grade, methabenzthiazuron was purchased from the laboratories of Dr. S. Ehrenstorfer, Germany.

Gas Chromatograph Conditions

Gas liquid chromatographic (GLC) analyses of methabenzthiazuron in soil, lentils and hay were made on a Hewlett Packard model 5890 equipped with a 15 m long capillary column. Detection was done with a nitrogen/phosphorus detector. Injection was done at programmed initial oven temperature of 150°C, accelerating at a rate of 10°C min^{-1} to a final temperature of 220°C. The injection port, oven, and detector temperatures were 150, 220, and 220°C, respectively. Oven maximum temperature was 400 oC. Carrier gas (N_2) was set at a flow rate of 18.8 ml min-1. Recorder chart speed was set at 5 ml min^{-1}. Other operating conditions were: attenuation, 5; retention time, 1.3 min; peak width, 0.4 mm; threshold, 4; area rejection, 10,000 units using a Hewlett Packard 3392A automated integrator.

Herbicide Standard Solutions

A stock solution (1000 mg $litre^{-1}$) of methabenzthiazuron was prepared by dissolving the active ingredient (0.025 g) in acetone. Aliquots of this stock solution were used to prepare solutions containing 0.05, 0.1, 1.0, 5.0, 10.0, 15.0, and 20.0 mg $litre^{-1}$ of methabenzthiazuron in order to establish a calibration curve. The calibration curve gave a straight line over the range of 0.05-20.0 mg $litre^{-1}$ methabenzthiazuron.

Recovery Tests

In each year, a minimum of two recovery experiments (*i.e.* an untreated sample accurately fortified with a known amount of methabenzthiazuron) were run alongside each set of samples analysed each year in order to determine the efficiency of the analytical procedure.

Herbicide Application

In an experiment conducted over two successive years, pre-emergent application of methabenzthiazuron at 0.5 kg a.i. ha^{-1} (a rate commonly used by farmers in Jordan) was applied to lentils in order to determine herbicide residues. Methabenzthiazuron was applied to twelve plots, 3 x 6 m each. A non-treatment check plot was added. The treatments were arranged in a randomised complete block design. From each plot, soil samples, about 2 kg each, were taken from the top 10 cm soil, 24 hr after herbicide application and at harvest. Lentil plants (cv. Jordan^{-1}) were harvested 2 m from the front of the middle row to determine methabenzthiazuron residues in lentils and hay.

Extraction

A modified method based on that described by Zweig (1976) and Al-Mughrabi *et al.* (1999) was used for extracting methabenzthiazuron from soil, lentils and hay.

Soil samples

Prior to extraction, soil moisture was determined in all soil samples immediately after they were brought to the laboratory. From each sample collected, two sub samples (100 g each) were placed in a pre-weighed beaker and placed in an oven at 130°C overnight. The percentage of moisture content was calculated by multiplying the difference in weight by a factor of 100. Soil type, contents, EC and pH were also determined. Soil samples were then stored in a freezer until herbicide residues were extracted.

In each of the two experiments, twelve samples, each consisting of three - 2 kg sub samples, were collected from the beginning, middle and end of each treatment plot. Each three sub samples were combined together, mixed thoroughly, and two samples of the combined sub samples were taken for herbicide extraction. Each soil sample (25 g) was mixed with distilled water (100 ml) and acetone (200 ml) in a 500 ml glass bottle with a screw cap, and then placed on a shaker at 100 rocking motions min-1. After approximately 20 hrs, NaCl (30 g) was added to the soil solution, and the mixture was then blended for two minutes in a Waring blender. Dichloromethane (150 ml) was added, and the mixture was then blended for 2-3 minutes. After the soil had settled down, the organic phase was separated, and a volume of 175 ml was transferred through a glass funnel containing anhydrous sodium sulfate (40 g) into a 250 ml round-bottom flask. The extract was evaporated using a rotary evaporator at 38-40°C to about dryness. Dichloromethane (3 ml) was added to dissolve the residues. The residues were concentrated to about 2 ml using a rotary evaporator and then transferred to a cleanup column.

Lentil and hay samples

Lentil or hay samples were collected from the beginning, middle and end of each treatment plot. Each three sub samples were combined together, mixed thoroughly, and two samples of the combined sub samples were taken for herbicide residue analysis. In each of the two experiments, twelve lentil or hay samples, 10 g each, were mixed with distilled water (100 ml) and acetone (200 ml) and ground for three minutes in Waring blenders. The blended mixture was then filtered through a Buchner funnel into a suction flask. One fifth of the filtrate was transferred to a separation funnel, and the solution was then extracted with dichloromethane (2 x 50 ml). The funnel was shaken for one minute (when emulsification occurred, it was destroyed by adding small portions of sodium chloride). After complete separation, the water was drawn into a beaker in order to be extracted for a second time. The combined dichloromethane phases were passed through a glass funnel filled with anhydrous sodium chloride into a 250 ml round-bottom flask. The funnel was washed with several portions of dichloromethane. The extract was concentrated to about 2 ml using a rotary evaporator at a bath temperature of 38-40°C. The concentrate was then transferred to a cleanup column.

Cleanup

Soil samples

Cleanup was processed in a glass column with a cork stopper. Glass wool was placed at the base, and then the column was half filled with dichloromethane. Florisil (10 g) deactivated with 8% w/w distilled water and anhydrous sodium sulfate (5 g) was added to the column. The solvent was then drained from the column. The residue of methabenzthiazuron was dissolved in dichloromethane (3 ml) and transferred for cleanup. The round-bottom flask was rinsed with dichloromethane (2 x 3 ml), and then the rinsed material was added to the column. The column was then eluted in dichloromethane (250 ml) in a clean round-bottom flask. The eluate was collected and then evaporated to about dryness. Acetone (3 ml) was added and the solution was then evaporated to about 1 ml. The residue was adjusted to 5 ml in acetone for gas chromatographic analysis. Samples, 2 µl each, were injected into the gas chromatograph 2-3 times. The standard solutions were injected before and after each sample.

Lentil and hay samples

Cleanup was processed in a glass column with a cork stopper. Glass wool was placed at the base, and an elution mixture (15 ml) made of dichloromethane and acetone (9:1) was added. Silica gel (4 g) containing 4% distilled water, and sodium sulfate (1 g) were transferred into the column. The extracted sample was then transferred into the column and eluted with the elution mixture (100 ml) into a clean round-bottom flask. The eluate was evaporated to about dryness. The residue was adjusted to 5 ml with acetone. Samples, 2 µl each, were injected in to the gas chromatograph 2-3 times. The standard solutions were injected before and after each sample.

Calculations

The amount of herbicide residue (mg kg^{-1}) detected in each experiment for each sample was calculated according to the following equation (Zweig, 1976; Al-Mughrabi *et al.*, 1992; Al-Mughrabi *et al.*, 1999): {[sample area or peak height/standard area or peak height] x [standard injection (ng)/sample weight (g)] x final volume (ml)/sample injection (µl)] x [100/100 - moisture (%)] x [100/percentage recovery]}.

Results & Discussion

Soil type was silt clay and consisted of clay (42%), silt (40%), sand (17%) and organic matter (0.7%), with electrical conductivity of 0.2 mv and pH value of 7.8.

Statistical analyses were made with the Statistical Analysis System (SAS) (SAS Institute Inc., 1983). A least significant difference test was applied in order to separate experimental means for samples collected in each experiment and for the average values of the two experiments (Chew, 1976; Petersen, 1977).

The average residue values in mg kg^{-1} of methabenzthiazuron in soil, lentils and hay are shown in Table 1. The average residue decreased significantly from 1.16 ± 0.15 mg kg^{-1}, 24 hr after treatment, to 0.12 ± 0.01 mg kg^{-1} at harvest. Recovery tests conducted with soil samples tested 24 hr after treatment and at harvest averaged 75.5% and 68.8%, respectively (Table 2). Low residues of methabenzthiazuron averaging 0.1 ± 0.01 and 0.19 ± 0.02 mg kg^{-1} in lentils and hay, respectively, were detected at harvest (Table 1). This may be due to metabolism of methabenzthiazuron to water-soluble glucosides in plants (Worthing, 1987 & 1991; Weed

et al., 1995). Recovery tests conducted with lentil and hay samples analysed at harvest averaged 79.2% and 83.3%, respectively (Table 2). No detectable residues were found in the untreated checks (Table 1). Our results are not in variance from those reported by other scientists around the world. Hassink *et al.* (1994) and Schmidt (1977) did not detect methabenzthiazuron in the harvested cereal and oilseed crops. They concluded that accumulation in the soil was most unlikely because under adequate rainfall, degradation was very good. However, Pestemer and Malcomes (1981) reported that in field trials with winter cereals where methabenzthiazuron was applied, about 50% of the applied dose was degraded from the top 5 cm of soil in 120 days when applied in spring. Using the leaching and degradation of C_{14}-labeled methabenzthiazuron, Kubiak *et al.* (1988) identified 40% of the applied herbicide in the top 0-10 cm soil 127 days after application. The average time for residual activity of methabenzthiazuron in soil is approximately 3 months (Worthing, 1987 & 1991). Our findings suggest that under our experimental conditions, low levels of methabenzthiazuron remain in soil, lentils and hay if applied at a rate of 0.5 kg a.i. ha^{-1}. Therefore, we recommend that methabenzthiazuron be applied *at a* lower rate in order to reduce its residue level in the harvested lentils and hay, and to prevent its accumulation in soil. In a similar experiment conducted under similar experimental conditions by Al-Mughrabi *et al.* (1999), the researchers studied the fate of the herbicides fluazifop-butyl and metribuzin in soil. Although the two herbicides were applied at 0.5 kg a.i. ha^{-1}, no residues were detectable at harvest. This indicates that their accumulation in soil is less likely to happen compared to methabenzthiazuron.

Table 1. Average residue values of methabenzthiazuron in soil, lentils and hay.

Sample	Average Residue (mg kg^{-1})[1] ± S.E.[2]		
	Year 1	**Year 2**	**Grand**
Soil - 24 hrs.	$1.28 \pm 0.17^{a*}$	1.04 ± 0.09^{a}	1.16 ± 0.15^{a}
Soil - harvest	0.10 ± 0.01^{b}	0.14 ± 0.02^{b}	0.12 ± 0.01^{b}
Lentils	0.09 ± 0.01^{bc}	0.11 ± 0.01^{bc}	0.10 ± 0.01^{bc}
Hay	0.14 ± 0.01^{bcd}	0.22 ± 0.02^{bcd}	0.19 ± 0.02^{bcd}
Untreated check	< 0.01 **	< 0.01	< 0.01

[1] Average value of 24 samples (twelve samples, each replicated twice).

[2] Standard error.

* Mean values followed by the same letter within each column are not significantly different (P = 0.05) from each other according to the LSD test.

** The lowest detectable level of methabenzthiazuron.

Table 2. Recovery values (%) of methabenzthiazuron in hay, lentils and soil.

Sample	Average Recovery (%)[1]		
	Year 1	**Year 2**	**Grand**
Soil - 24 hr	81.0 (79.0 - 81.2) [2]	70.0 (68.1 - 75.0)	75.5 (68.1 - 81.2)
Soil - harvest	65.6 (65.0 - 71.0)	72.0 (68.0 - 77.0)	68.8 (65.0 - 77.0)
Lentils	77.0 (73.0 - 82.0)	81.4 (77.0 -85.0)	79.2 (73.0 - 85.0)
Hay	84.0 (82.0 - 86.0)	82.6 (81.0 - 84.0)	83.3 (81.0 - 86.0)
Untreated check	78.0 (75.0 - 81.0)	81.0 (79.0 - 83.0)	79.5 (75.0 - 83.0)

[1] Average of a minimum of two recovery experiments.

[2] Range of recovery values (%).

Acknowledgements

I would like to thank Dr. B. Abu-Irmaileh for his help in conducting the fieldwork, and Dr. I. Nazer for supervising the lab work, Faculty of Agriculture, University of Jordan, Amman, Jordan.

References

Ahrens, W.H. (Ed.) (1994) *Herbicide Book*, 7th edn. Weed Society of America, Campaign Illinois, USA.

Al-Mughrabi, K.I., Abu-Irmaileh, B.E. & Nazer, I.K. (1999) Residues of the herbicidesmetribuzin and fluazifop-butyl in soil, lentil seeds and straw. *Arab J. Pl. Prot.* **17** (2), 66-70.

Al-Mughrabi, K.I., Nazer, I.K. and **Al-Shuraiqi, Y.T.** (1992) Effect of pH of water from the King Abdallah Canal in Jordan on the stability of cypermethrin. *Crop Prot.* **11**, 341-344.

Chew, V. (1976) Comparing treatment means: A compendium. *Hort Sci* **11**, 348-357.

Fedtke, C. (1974) Influence of methabenzthiazuron on ATP-level and protein synthesis in wheat.Pestic. Biochem. *Physiol.* **4** (4), 386-392.

Ghafoor, A., Yaqub, M., Sadiq, M. and **Jamil, F.F.** (1987) Effect of different herbicides on chlorophyll contents and total carotenoides in wheat. *Pakis. J. Agr. Res.* **8** (2), 239-240.

Hassink, J., Klein, A., Kordel, W. and **Klein, W.** (1994) Behaviour of herbicides in non-cultivated soils. *Chemosphere* **28** (2), 285-295.

Kubiak, R., Fuhr, F., Mittelstaedt, W., Hansper, M., and **Steffens, W.** (1988) Transferability of lysimeter results of actual field situation. *Weed Sci* **36** (4), 514-518

Pestemer, W., and **Auspurg, B.** (1986) A predictive model for the assessment of metribuzin and methabenzthiazuron residues in soil and their effects on succeeding crops. *Weed Res.* **24** (4), 275-286.

Pestemer, W., and **Malcomes, H.P.** (1981) Effects of plant protection systems applied to winter cereals on biological activity in soils. II. Degradation of chlortoluron and methabenzthiazuron. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz, Sonderheft* **9**, 313-320.

Petersen, R.G. (1977) Use and misuse of multiple comparison procedures. *Agron. J.* **69**, 205 403.

SAS Institute Inc. (1983) *SAS User's Guide: Statistics.* Cary, NC, USA.

Schmidt, R.R. (1977) Effect of many years' use of tribunil on rotational crops, weed flora and degradation behaviour in three different soils. *Pflanzenschutz Nachrichten Bayer* **30** (3), 304-324.

Weed, D.A.J., Kanwar, R.S., Stoltenberg, D.E. and **Pfeiffer, R.L.** (1995) Dissipation and distribution of herbicides in the soil profile. *J. Environ. Qual.* **24** (1), 68-79.

Worthing, C.R. (Ed.) (1987) *Pesticide Manual.* 8th ed. British Crop Protection Council, Croydon, UK pp 1081.

Worthing, C.R. (1991) *Pesticide Manual.* 9th ed. British Crop Protection Council, Croydon, pp1140

Zweig, G. (Ed.) (1976) *Analytical Methods for Pesticides and Plant Growth regulators, Vol. VIII.* Academic Press, NY., pp 453-471

(Received 07/10/2001, in revised form 02/08/2002)