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Bromine Uptake of Muskmelon and Cucumber Plants Following Soil Fumigation with Methyl Bromide

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Summary

Muskmelons and cucumbers were grown in soil fumigated with methyl bromide (MBr) to determine the effect of MBr on the growth, bromine (Br) content in the plant and the content of water soluble Br in the soil. The dry weight of the whole muskmelon plant was less at 600 g/m^3 MBr, while soluble solids of fruit were slightly higher at this dosage. Br content in muskmelon leaves at 0, 200, 400 and 600 g/m^3 MBr soil treatments was 203, 540, 3663 and 6783ppm, respectively. Br in the musk melon fruit was most concentrated in the pericarp, less concentrated in the outer flesh, and least concentrated in the inner flesh. On a dry matter basis Br content in the inner and outer flesh at 0, 200, 400 and 600 g/m^3 MBr treatments was as follows: the inner flesh was 47, 24, 109 and 228 ppm; the outer flesh was 60, 154, 137 and 323 ppm, respectively. Growth and yield of cucumbers grown in Iwata loam (IL) having 13.3% clay were not affected by dosage levels of MBr. Br content in the leaves, roots and fruit was significantly increased with increasing amounts of MBr. Growth and yield of cucumbers grown in Takamatsu light clay (TLiC) having 35.8% clay were not affected by dosage levels of MBr. On a dry matter basis Br content in leaves, roots and fruit was markedly higher in TLiC than in IL, and that in fruit at 0, 200, 400 and 800 g/m^3 MBr was 202 to 240, 590 to 900, 1080 to 1742, and 1750 to 2788 ppm, respectively. Uptake amount of Br by cucumber plants was significantly increased with time at all dosage levels of MBr, while water soluble Br in soil was inversely decreased.

Introduction

Methyl bromide (MBr) has been used by greenhouse growers to a great extent instead of steam sterilization. The reason for this is its easy application, its high toxicity to soil-borne diseases such as fusarium wilt of tomato, cucumber and muskmelon, bacterial wilt of tomato and eggplant, and *Phytophthora* and *Pythium*, and its rapid and extensive penetration into soil. However, this fumigant posseses certain disadvantages, principally its high human toxicity, necessitating considerable care in its use, and its breakdown in soil to inorganic bromide (Br) which is also toxic to some plants and can be taken up and accumulated in many crops. As to the intake of the Br ion, a joint FAO/WHO working pa ty recommended in 1969 a maximum acceptable daily intake of 1 mg Br ion per Kg body weight, with a tolerance level of 50 mg per Kg in raw cereals or whole meal flour (3). Aside from this recommendation the Food and Drug Administration (FDA) of the Department of Health, Education and Welfare, and the Environment Protection Agency in the U. S. A. established a tolerance level of Br ion in many crops in 1972 (14, 15, 16). In our country little is found about the Br residues in vegetable crops grown in soil fumigated with Br containing compounds. We have been concerned with the growth and uptake of inorganic Br in several vegetable crops grown in soil fumigated with MBr. This paper deals with the results of Br uptake of muskmelons and cucumbers.

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Materials and Methods

1. *Muskmelons*

Some greenhouse growers sterilize soil beds with MBr using 2 or 3 times the amount recommended by the fumigant dealers. Therefore, 4 dosage levels of MBr were made as follows: 0 g/m^3 (not fumigated, 0 S for short), 200 g/m^3 (standard, 1 S), 400 g/m^3 (2 S) and 600 g/m^3 (3 S). The corresponding amount of MBr of each dosage was released from a canister into the soil under a vinylfilm cover continuously for 4 days. The soil used was Iwata loam and originally had a pH of 5.8 and 2.2 ppm of water soluble Br. After removing the cover, fumigated soils were well tilled to evaporate the residual fumigant. Sixteen liters of fumigated soil were fertilized with 7 g N from rape-seed cake, 10 g P_2O_5 from calcium superphosphate and rape-seed cake, 10 g K_2O from K_2SO_4 and rape-seed cake, 20 g CaO from $Ca(OH)_2$ and 8 liters of well decomposed rice straw, and put into a 40×40×20 cm box. Seedlings with 3 leaves of muskmelon cv. Spring No. 3 of Earl's Favourite grafted on Barnett Hill Favourite rootstock were planted in boxes filled with the fumigated soils, on May 7, 1974, and grown under uniform conditions in the greenhouse. The treatments were replicated 12 times. The plants were separated into leaves, stems, roots and fruit at harvest, and the fruit was further sectioned as shown in Fig. 1. The materials were dried at 70°C and finely milled. Mixed 1 g samples with 250 mg CaO in sufficient water to give a thin paste were ashed at 550°C overnight and the residues extracted with hot water. The whole extract was adjusted to pH 6.5~7.0 with HNO_3 or NaOH and filled up to 100 m*l*. An aliquot of this extract was colorimetrically determined by the fluorescein method (12). Soils for analysis taken at harvest were air dried and milled to pass through a 2 mm sieve. Water soluble Br was determined by the same method as used for plant materials after extracting 10 g of soil with 50 m*l* water and adjusting the soil extract to pH 6.0 with H_2SO_4 or NaOH.

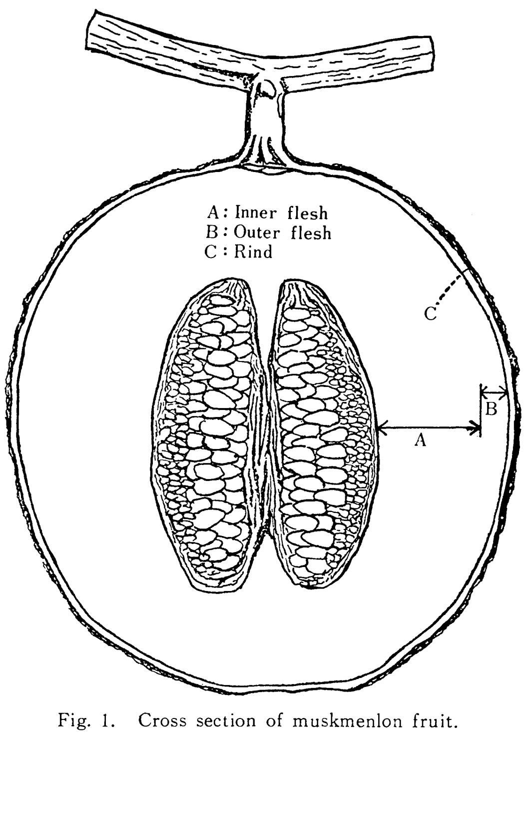

Fig. 1. Cross section of muskmenlon fruit.

2. *Cucumbers*

Expt. I

To determine the effects of soil fumigation with MBr on the growth and Br uptake of cucumbers, the treatment of 4 dosage levels of MBr, 0 g/m^3 (not fumigated, 0 S), 100 g/m^3 (0.5 S), 200 g/m^3 (standard, 1 S) and 400 g/m^3 (2 S), was made in 7 replications. The soil was the same as used in the muskmelon experiment, and was fumigated with MBr by the same method as in the muskmelon experiment. Sixteen liters of fumigated soil was fertilized with 3 g N from $(NH_4)_2SO_4$, 5 g P_2O_5 from calcium superphosphate, 3.5 g K_2O from K_2SO_4, 30 g CaO from $Ca(OH)_2$ and 8 liters of semi-decomposed rice straw, and put into a 40×40×20 cm box. One 3-leafed seedling of cucumber cv. Natsusairaku No. 3 was planted in each box filled with the fumigated soil on March 18, 1975, and grown in the greenhouse. The main stem was pinched at the 17 th node and lateral shoots from 6 th to 16 th node were pinched at the 1 st node. Fruits sized 70 to 100 g were picked April 14 through May 14. The fruits were sorted into early and late pickings as shown in Table 2. After all fruits were harvested, the leaves, stems and roots were separated, dried and milled. Br content of the plant and soil was determined by the same methods as in the muskmelon experiment.

Expt. II

The purpose was to determine the effects of soil fumigation with MBr on the Br uptake by cucumber plants in relation to their growth and the Br content in soils during the course of the experiment. On Oct. 15, 1975 seedlings with 3 leaves of cucumbers cv. Natsusairaku No. 3 were planted in boxes filled with differentially fumigated soils, 0 g/m³ (0 S), 200 g/m³ (standard, 1 S), 400 g/m³ (2 S) and 800 g/m³ (4 S), and grown in the greenhouse. The soil used was Takamatsu light clay with an original pH 5.7 and 6.0 ppm water soluble Br. Cultivation and fertilization were similar to those in Expt. I with cucumbers. Plant samples of leaves, stems and roots were taken on Oct. 29, Nov. 14, Nov. 26 and Dec. 10. Fruits were picked when they weighed 70 to 100 g to determine the Br content. Soil samples were taken on Oct. 15, Oct. 29, Nov. 14, Nov. 26 and Dec. 10 to determine water soluble Br.

Results

1. Muskmelons

The effects of soil fumigation with MBr on the growth and Br in plant parts of muskmelons, and on the water soluble Br in soil are shown in Table 1. The early growth of plants tended to be restricted at 3 S. However no significant difference in the plant height was found among the treatments at the end of the experiment. The dry weight of a whole plant was less at 3S. The fresh weight of fruit was also less at 3S, while the soluble solids of fruit were slightly higher at 3 S. Br content in leaves and whole fruit was significantly increased with increasing amount of MBr. Br content in the inner flesh, outer flesh and pericarp tended to increase with increasing amount of MBr, and Br in fruit was generally most concentrated in the pericarp, less concentrated in the outer flesh, and least concentrated in the inner flesh. Water soluble Br was slightly higher at 3 S. No chlorosis or necrosis of the leaves caused by MBr was found even at 3 S.

2. Cucumbers

Expt. I

The effects of soil fumigation with MBr on the cucumber growth and water soluble Br in soil are given in Table 2. The number and weight of fruit per plant were not significantly different among the treatments. Growth was

Table 1. Effect of methyl bromide soil fumigation on growth and Br content of muskmelons and on soil Br content.

Treatment	Plant ht[W] at the end of expt. (cm)	Dry wt[X] of whole plant (g)	Fruit fresh wt (g)	Soluble solids of fruit (%)	Br in plant dry matter and in air dried soil (ppm)					
					Leaves	Whole fruit	Inner[Y] flesh	Outer[Y] flesh	Rind[Y]	Soil at the end of expt.
0 S	105[a]	248[a]	1271[a]	12.5[b]	203[c]	164[c]	47	60	89	7.2[b]
1 S	105[a]	259[a]	1303[a]	12.2[b]	540[c]	303[c]	24	154	467	8.6[ab]
2 S	105[a]	254[a]	1259[a]	12.2[b]	3663[b]	652[b]	109	137	1110	7.4[b]
3 S	101[a]	234[b]	1162[b]	13.5[a]	6783[a]	987[a]	223	323	1215	9.3[a]

W: Mean separation in columns by Duncan's multiple range test, 5% level.
X: Leaves + Stems + Roots + fruit.
Y: Not subjected to statistical analysis because composite materials were taken.

Table 2. Effect of methyl bromide soil fumigation on growth and Br content of cucumbers and on soil Br content.

Treatment	No. of[V] fruit/plant	Fruit fresh wt/plant (g)	Br in plant dry matter and in air dried soil (ppm)							
			Leaves	Roots	Early[W] picked fruit on main stem	Late[X] picked fruit on main stem	Early[Y] picked fruit on lateral shoots	Late[Z] picked fruit on lateral shoots	Soil just after fumigation	Soil at the end of expt.
0 S	18.4[a]	1450[a]	63[b]	41[c]	64[c]	67[c]	73[c]	85[c]	2.5[c]	5.4[a]
0.5 S	19.7[a]	1490[a]	78[b]	49[c]	79[bc]	97[b]	91[bc]	98[c]	3.9[bc]	5.1[a]
1 S	19.7[a]	1594[a]	110[b]	120[b]	91[b]	98[b]	106[b]	153[b]	5.5[b]	5.7[a]
2 S	21.1[a]	1569[a]	180[a]	241[a]	131[a]	189[a]	197[a]	246[a]	11.6[a]	5.5[a]

V: Mean separation in columns by Duncan's multiple range test, 5% level.
W: Apr. 14 to Apr. 24, X: Apr. 25 to May 6, Y: Apr. 24 to May 3, Z: May 4 to May 14.

not influenced by MBr levels although results are not shown. Br content in the fruit was significantly increased with increasing amounts of MBr. Br content tended to be slightly higher in the late picked and lateral shoot fruit than in the early picked and main stem fruit, respectively. The soil water soluble Br just after MBr fumigation was significantly increased with increasing amounts of MBr. However, the Br content at the end of the experiment was not significantly different among the treatments.

Expt. II

The effects of soil fumigation with MBr on

Table 3. Effect of methyl bromide soil fumigtion on growth and Br uptake of the cucumber plant.

Treatment	Plant ht[V] pinched at 17th node (cm)	Leaves dry wt/plant (g)	Stems dry wt/plant (g)	Fruit fresh wt/plant (g)	Amounts of Br uptake (mg/plant)[W]			
					Oct. 29	Nov. 14	Nov. 26	Dec. 10
0 S	162^{a}	41.8^{a}	19.9^{a}	683^{a}	4^{c}	15^{c}	21^{d}	60^{d}
1 S	152^{a}	42.5^{a}	20.0^{a}	760^{a}	24^{b}	36^{c}	99^{c}	159^{c}
2 S	153^{a}	41.5^{a}	20.7^{a}	729^{a}	51^{a}	62^{b}	202^{b}	244^{b}
4 S	158^{a}	46.5^{a}	21.5^{a}	747^{a}	62^{a}	178^{a}	355^{a}	523^{a}

V : Mean separation in columns by Duncan's multiple range test, 5% level.
W : Whole plant=Leaves + Stems + Roots + Fruit.

Table 4. Effect of methyl bromide soil fumigation on Br content in leaves and stems of cucumbers (ppm of dry matter).

Treatment	Leaves[V]				Stems			
	Oct. 29	Nov. 14	Nov. 26	Dec. 10	Oct. 29	Nov. 14	Nov. 26	Dec. 10
0 S	718^{c}	553^{c}	369^{d}	931^{c}	768^{c}	241^{d}	420^{d}	465^{d}
1 S	2533^{bc}	689^{bc}	831^{c}	2211^{b}	13280^{b}	3103^{c}	4890^{c}	2868^{c}
2 S	4468^{ab}	1268^{b}	1533^{b}	2146^{b}	20730^{a}	5515^{b}	9905^{b}	4915^{b}
4 S	5815^{a}	2971^{a}	2790^{a}	3638^{a}	20590^{a}	15825^{a}	17463^{a}	13288^{a}

V : Mean separation in columns by Duncan's multiple range test, 5% level.

Table 5. Effect of methyl bromide soil fumigation on Br content in roots and fruit of cucumbers (ppm of dry matter).

Treatment	Roots[V]				Fruit			
	Oct. 29	Nov. 14	Nov. 26	Dec. 10	up to[W] Nov. 14	Nov. 15[W] to Nov. 26	Nov. 27[W] to Dec. 10	Nov. 27[X] to Dec. 10
0 S	614^{d}	249^{d}	355^{c}	363^{c}	202^{d}	240^{d}	215^{c}	220^{c}
1 S	2239^{c}	829^{c}	1099^{c}	584^{c}	900^{c}	593^{c}	684^{c}	590^{c}
2 S	5144^{b}	1268^{b}	2728^{b}	1153^{b}	1742^{b}	1528^{b}	1100^{b}	1080^{b}
4 S	7906^{a}	4330^{a}	3900^{a}	2043^{a}	2788^{a}	2273^{a}	1750^{a}	1800^{a}

V : Mean separation in columns by Duncan's multiple range test, 5% level.
W : Fruit in main stem. X : Fruit in lateral shoots.

Table 6. Water soluble Br in soil fumigated with methyl bromide in relation to time after fumigation[W] (ppm of air dried soil).

Treatment	Oct. 15[V]	Oct. 29	Nov. 14	Nov. 26	Dec. 10
0 S	6.0^{c}	6.3^{c}	5.2^{c}	5.5^{c}	4.0^{b}
1 S	15.4^{b}	10.5^{b}	8.5^{b}	7.3^{bc}	4.6^{b}
2 S	21.5^{ab}	13.7^{ab}	10.4^{ab}	8.8^{ab}	6.5^{ab}
4 S	28.0^{a}	17.1^{a}	13.0^{a}	10.4^{a}	8.4^{a}

V : Mean separation in columns by Duncan's multiple range test, 5% level.
W : Soil was fumigated on Oct. 11.

of Br in the soil are presented in Table 3, 4, 5 and 6. Plant height, dry weight of leaves and stems, and fruit fresh weight were not influenced by MBr fumigation. Br uptake by a whole plant was significantly increased with increasing amounts of MBr. At the end of the experiment the amounts at 0, 1, 2 and 4S were 60, 159, 244 and 523 mg, respectively. The Br content in leaves, stems, roots and fruit was generally increased with increasing amounts of MBr. The content was higher in each plant part taken on Oct. 29, lower in the stems and roots taken on Dec. 10, and lower in the fruit taken between Nov. 27 and Dec. 10. Generally, Br was most concentrated in the stems, less concentrated in the leaves or roots, and least concentrated in the fruit. Water soluble Br was significantly increased in the soil with increasing amounts of MBr, and gradually decreased in each fumigated soil with time. Eventually the soil Br content between 0 and 4S on Dec. 10 resulted in the least difference-4.4 ppm.

Discussion

Numerous reports have been published on the effects of soil fumigation with MBr or other fumigants containing Br on the growth, yields and Br uptake in some crops. For instance, Kyrou (7) reported that MBr at dosages of 40 to 120 liters per 1000 m^2 resulted in increased yields of cucumbers by reducing the density of root-knot nematodes. Hayden et al. (4) also reported that preplant MBr fumigation at dosage of 2 lb. per 100 ft^2 gave a higher growth rate of young peach trees. However, Stelmach (13) found that MBr at dosage of 370 lb. per acre decreased the yields of beans and cabbage due to an excess uptake of inorganic Br resulting from the hydrosis of this fumigant. In general, favorable effects of MBr as a soil sterilant on the growth of crops can be attributed to the alleviation of injury from continuous cropping caused by soil-borne diseases and nematodes. In these experiments the growth of cucumbers was not influenced by MBr fumigation. One of the reasons appears to be attributable to the absence of soil-borne diseases and nematodes due to the use of new paddy soils. The growth of muskmelons was slightly inhibited by the 3S soil (600 g/m^3) treatment. However, the soluble solids of the fruit were slightly higher. As to the phytotoxic effects resulting from the use of MBr, Maw and Kempton (10) reported that the growth inhibition of crops may be caused by (i) the action on plants of the compound *per se*, (ii) the action of inorganic Br formed by the breakdown of MBr, or (iii) indirect action through effects of either MBr or inorganic Br on soil microflora, soil structure or composition. The growth of muskmelon did not appear to be associated with the above cause (ii), because chlorosis or necrosis caused by an excess of Br was not found in the leaves. However it was difficult to determine which of the cause (i) or (iii) was responsible for the growth inhibition. The reason that the soluble solids of the fruit were slightly higher at 3S soil seemed to be related to the fruit weight because small fruit has generally high soluble solids in greenhouse cultivation.

Inorganic Br which is present as a breakdown product of MBr can be taken up and distributed in the tissues of the plants (6, 9, 13). In our experiments Br content in the plant parts of muskmelons and cucumbers was significantly increased with increasing amounts of MBr. Br content in the muskmelon and cucumber leaves at the end of the experiments was high with 6783 ppm at 3S and 3638 ppm at 4S in Expt. II, respectively. However, there were no characteristic symptoms of phytotoxicity in these plants. This contrasted with the extreme sensitivity of carnations to Br (5) where the plants showed appreciable leaf damage and growth retardation although Br content in the leaves of plants grown at 4 S soil (800 g/m^3) was as low as 725 ppm.

Some experiments have been made to determine the distribution pattern of Br taken up in various tissues. For instance, Newton and Toth (11) found higher concentrations of Br in the stems (6320 ppm), compared with leaflets (2825 ppm) or roots (2100 ppm) in tomato plants grown in nutrient solution containing 20 ppm of inorganic Br. At the end of the experiment with cucumber plants grown at 4S soil in Expt. II, Br was most concentrated in the stems (13288 ppm), less concentrated in the leaves (3638 ppm) and roots (2043 ppm), and least concentrated in the fruit (1750 to 1800 ppm). This response is similar to the tomato results mentioned above. As to the detailed distribution pattern of Br in plants, with potatoes (1) less Br was found in the tubers than in the haulm, and in tubers Br was concentrated in the skin. Also, with lettuce (6) less than 10% of the total Br content of the plant was present in the center portion, representing 25% of the weight of the plant, and 60 to 85% of the total Br was contained in the 12 outermost leaves. In our experiment with muskmelons, Br was most concentrated in the pericarp (1215 ppm), less concentrated in the outer flesh (323 ppm), and least concentrated in the inner flesh (228 ppm) at 3 S soil with a similar pattern to the potatoes and lettuce mentioned above.

The composition of soil plays a role in the penetration of MBr. Chisholm and Koblitsky (2) reported that the degree of sorption of MBr by the soil decreased in the sequence peat>clay>sand. In our cucumber experiments Br content in the leaves, roots and fruit was markedly higher in Expt. II than in Expt. I. This was probably due to a potential difference in the retention of MBr between the two soils used in Expt. I and II. That is, Takamatsu light clay in Expt. II had 35.8% clay, while Iwata loam in Expt. I had only 13.3% clay. Thus the water soluble Br at 0, 0.5, 1 and 2S soils in Expt. I just after the fumigation was 2.5, 3.9, 5.5 and 11.6 ppm, respectively. In Expt. II that at 0, 1, 2 and 4S soil was 6.0, 15.4, 21.5 and 28.0 ppm, respectively.

The uptake extent of Br into plant tissues is closely related to the amount of inorganic Br in the soils (6). As shown in Table 3, 4 and 5, Br content in the cucumber plant parts and the uptake amount of Br by a whole plant reflected well the Br content of water soluble form in the soil. Br content in the late picked cucumber fruit was higher than that in the early picked one as shown in Table 2. Similar results were reported on young lemon trees by Martin et al. (8).

Accumulation of Br occurred in muskmelon and cucumber fruit to a substantially lesser extent than in leaves. Muskmelon fruit on the plants grown in soil fumigated with 600 g/m^3 MBr, which is a higher dosage but often being used by greenhouse growers, contained 228 ppm of dried tissue (23 ppm of fresh tissue) in the inner flesh and 323 ppm (32 ppm) in the outer flesh. Cucumber fruit on the plants grown in soil fumigated with 200 and 400 g/m^3 MBr in Expt. II contained 590 to 900 ppm of dried tissue (30 to 45 ppm of fresh tissue) and 1080 to 1742 ppm of dried tissue (54 to 87 ppm), respectively. Although there are no statutory limits on the Br content of foodstuffs in Japan except for cereal grains, maximum levels for Br in a range of foodstuffs have been proposed by the FAO Codex Committee on Pesticide Residues (FAO/WHO), including suggested limits of 20 to 30 ppm for various fresh fruits and 30 to 250 ppm for dried fruits. Also, in the U. S. A. tolerances for Br residues in plants grown in soil treated with various nematocides have been established by the FDA (14). These values are 75 ppm for melons and 50 ppm for cucumbers on a fresh weight basis. In a comparison of Br content in the muskmelon and cucumber fruit obtained by the authors with those by the FDA, Br in the muskmelon fruit was within the tolerance limit even in the soil fumigated with 600 g/m^3 MBr, while Br in the cucumber fruit was beyond the tolerance limit in the soil fumigated with 400 g/m^3 MBr. When we consume 200 g of cucumber fruit containing 50 ppm Br on a fresh weight basis, then Br intake by the human body will be 10 mg. This is substantially low compared with a maximum acceptable daily intake of 60 mg Br ion for 60 Kg body weight recommended by a joint party FAO/WHO. Thus Br content in the cucumber fruit of plants grown in soil fumigated with 400 g/m^3 MBr was beyond the tolerance limit established by the FDA. However, this level of Br appeared to be safe judging from the acceptable limit of daily intake of Br recommended by FAO/WHO.

Since MBr is being used on an increasing scale in Japan to fumigate soil beds for greenhouse crop production, it must be recognized that the harvest fruit or edible part will contain small quantities of Br. At the same time probably more attention needs to be given to lar components of the diet as Maw and Kempton (10) pointed out.

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臭化メチルの土壌くん蒸消毒がメロンとキュウリの臭素吸収に及ぼす影響

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摘　要

臭化メチル (MBr) の土壌くん蒸消毒が，メロンとキュウリの生育，植物体と土壌の臭素 (Br) 含量に及ぼす影響を明らかにするため，これら両野菜を栽培した．メロンの全乾物重は，MBr 600 g/m³ で少なくなったが，果実の可溶性固形物含量はやや高かった．メロンの葉の Br 含量は，MBr 0, 200, 400, 600 g/m³ で，それぞれ 203, 540, 3663, 6783 ppm であった．メロンの果実の Br 含量は，果皮が最も高く，果肉外壁部がこれに次ぎ，果肉内壁部が最も低かった．果肉の Br 含量を乾物重当たりでみると，MBr 0, 200, 400, 600 g/m³ の内壁部では，それぞれ 47, 24, 109, 228 ppm であり，外壁部では，それぞれ 60, 154, 137, 323 ppm であった．13.3% の粘土を含む磐田壌土で栽培したキュウリの生育，収量は，MBr の使用量によって影響されなかった．しかし，葉，根，果実の Br 含量は，MBr の使用量の増加とともに高まった．35.8% の粘土を含む高松軽埴土で栽培したキュウリの生育，収量は，MBr の使用量によって影響されなかった．乾物重当たりの葉，根，果実の Br 含量は，磐田壌土よりも高松軽埴土で著しく高く，MBr 0, 200, 400, 800 g/m³ における

高松軽埴土の果実のBr含量は，それぞれ 202-240，590-900，1080-1742，1750-2788 ppm であった．キュウリの Br 吸収量は，MBr のどの使用量区でも時間の経過とともに増加したが，土壌の Br 含量は逆に減少した．