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Disinfestation of Pests on Cut Flowers with Gas Mixtures of Methyl Bromide, Phosphine and Carbon Dioxide

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Abstract : Each stage of eight species of insect and arthropod pests (*Tetranychus kanzawai, T. urticae, Thrips palmi, Trialeurodes vaporariorum, Myzus persicae, Aphis gossypii, Planococcus kraunhiae* and *Plutella xylostella*) on cut flowers of chrysanthemum and orchid was fumigated by mixture gas with methyl bromide (10 g/m³), phosphine (3 g/m³) and carbon dioxide (5%) for 3, 4 and 6 hours at 15 and 20 ℃. *T. kanzawai* egg was the most resistant stage to the mixture gas fumigation and the stage was killed completely at doses of 13 g/m³ of methyl bromide, 3 g/m³ of phosphine and 5% of carbon dioxide with 40% (v/v) loading at 15 ℃ for 4 hours and 20 ℃ for 3 hours. Thirteen percent of initial dose of methyl bromide was sorbed by cut flowers and packing materials, while no sorption was observed on phosphine, and carbon dioxide concentration increased due to respiration of cut flowers. No injury was observed on six cultivars of chrysanthemum and 4 cultivars of orchid fumigated at 15 ℃ for 4 hours. A slight injury was confirmed on both cut flowers when fumigated at 20 ℃ for 3 hours, followed by storage at 15 ℃ or above. Rating of the injury, however, could be acceptable in commercial trading. The use of three fumigants is available for not only avoiding flammability of phosphine, but also enhancing mortality for pests and reducing chemical injury on cut flowers with reduction of quantity of methyl bromide per cubic meter.
Key words : quarantine pests, *Tetranychus kanzawai, T. urticae, Thrips palmi, Trialeurodes vaporariorum, Myzus persicae, Aphis gossypii, Planococcus kraunhiae, Plutella xylostella*, quarantine treatment, mixture gas fumigation, methyl bromide, phosphine, carbon dioxide, sorption, chrysanthemum, orchid, phytotoxicity

Introduction

Various kinds of cut flowers (including cut leaves) have been imported from Netherlands, Thailand, U. S. A., Taiwan, Singapore, Australia, New Zealand, South Africa, etc. The amount of the import increased year by year and reached to 808 million stalks in 1994 made up orchid (22.5% of the total number of cut flower), fern (14.6%), Cleyera (12.7%), chrysanthemum (6.4%), Dianthus (4.2%), lily(3.3%), respectively (Yokohama Plant Protection Station, MAFF, 1995). In plant quarantine inspection at the port of entry of Japan, insect and arthropod pests of quarantine importance such as Tetranychidae, Thripidae, Pentatomidae, Aleyrodidae, Aphididae, Pseudococcidae and Yponomeutidae are often intercepted and they are mainly subjected to methyl bromide fumigation with quarantine schedule either 32.5 g/m³ for 2 hours at 20 ℃ or above or 48.0 g/m³ for 2 hours at 15 - 20 ℃ (MAFF, 1950). A valu-

able property of methyl bromide is the broad spectrum of various pests that it can control. However, the major disadvantage of methyl bromide for cut flowers is that the dose and exposure time required for a complete kill of pests sometimes cause a serious damage to certain species and cultivars of cut flowers, especially orchids and chrysanthemums growing under conditions of high temperatures and humidities. Moreover, methyl bromide was listed as an ozone depleting substance in the Fourth Meeting of Monteal Protocol in November 1992. It was also determined that the domestic production and consumption from 1995 was freezed at 1991 levels with the exception for the plant quarantine and preshipment treatment. It was further requested to make every effort to reduce emission as much as possible in plant quarantine field (TATEYA, 1993) and it is extremely urgent to find out alternatives to methyl bromide for the disinfestation.

Our objective was to study a disinfestation treatment by mixture gas fumigation with methyl bromide, phosphine and carbon dioxide against insect and arthropod pests on cut flowers with not only to avoid chemical injuries but also to reduce doses of methyl bromide.

Materials and Methods

Test Insects and Arthropods

Kanzawa spider mite, *Tetranychus kanzawai* KISHIDA and two - spotted spider mite, *T. urticae* KOCH were reared on kidney bean leaves at 26 ℃, 60% R.H. with a 12L : 12D photoperiod and egg, larva, pupa, first and second nymph, chrysalis and adult were prepared. *Thrips palmi* KARNY was reared on leaves of potted cucumber at 15 - 28 ℃ in the room and egg, larva and adult were prepared. Greenhouse whitefly, *Trialeurodes vaporariorum* (WESTWOOD) was reared on leaves of potted tomato at 15 - 28 ℃ in the room and egg, larva and adult were prepared. Green peach aphid, *Myzus persicae* (SULZER) and cotton aphid, *Aphis gossypii* GLOVER were reared on leaves of potted egg plant and cucumber at 15 - 28 ℃ in the room and larva and adult were prepared. Japanese mealybug, *Planococcus kraunhiae* (KUWANA) was reared on fresh pumpkin at 25 ℃, 70% R.H. with a 24D photoperiod and egg, which is the most resistant stage to methyl bromide fumigation (MISUMI et al., 1994) was prepared. Cabbage moth, *Plutella xylostella* (LINNAEUS) was reared on leaves of potted Chinese cabbage and egg, larva, pupa and adult were prepared.

These pests were placed for 2 days at fumigation temperatures of 15 ℃ and 20 ℃. The number of each stage per test was ca. 30 - 300 in the susceptibility test and ca.3,000 in the applied mortality test, respectively. The test was replicated 3 times.

Test Cut Flowers

Six cultivars of chrysanthemum (Shuhonochikara; white type, Shuhonohikari and Daikinko ; yellow type, Shuhonohana, Koshu, Hanashuho and Fukumusume; pink type) were obtained from a farmer at Shodoshima, Kagawa Prefecture and four cultivars of orchid, *Dendrobium phalaenopsis* (Panda and Sonia ; red type, swanwhite and KB white ; white type) were also obtained from a farmer at Tomishiro, Okinawa Prefecture.

Both chrysanthemum and orchid in three types of package, (a) chrysanthemums wrapped up by newspaper and then placed in carton (b) orchids packed in inner carton and then placed in master carton (c) orchids only placed in carton, for simulating to commercial package of import cut flowers were prepared for the test. These cartons were stored for 24 hours at fumigation temperatures of 15 ℃ and 20 ℃. Thirty - 50 stalks per test were used for phytotoxicity test. The test was replicated 2 - 3 times.

Fumigation

Six ℓ glass bins equipped with circulation and ventilation apparatus, and ports for gas application and sampling were used in the susceptibility test and a 0.96 ㎥ stainless steel fumigation chamber equipped with a 0.9 ㎥/min. of circulation and ventilation apparatus, special designed equipment for vaporising and mixing of methyl bromide and phosphine and ports for gas sampling, temperature probes and a manometer were used in large - scale mortality and phytotoxicity tests.

Each stage of the pests was placed in the 6 ℓ bin or 0.96 ㎥ chamber with ca. 40% loading of chrysanthemums and orchids. At first, 5% (v/v) of carbon dioxide was introduced from bottom injection site. Heavier specific gravity of carbon dioxide expelled extra air from upper exhaust port and then both ports were closed. Following carbon dioxide application, 10 g/㎥ of methyl bromide and 3 g/㎥ of phosphine were mixed in the special vaporizer and introduced into the fumigation bin and chamber. The fumigation was performed for 3 - 6 hours at 10 ℃ and 15 ℃. Gas chromatographs (FID for methyl bromide and TCD for phosphine and carbon dioxide) were used to monitor gas concentrations at time intervals of 0.5, 1, 2, 3, 4 and 6 hours during the fumigation. The circulation fan was kept on throughout the fumigation. After fumigation, the air - fumigant mixture was exhausted for an hour at 15 and 20 ℃ by the ventilation apparatus.

Assessment of Mortality and Phytotoxicity

After fumigation, test pests were stored under the rearing conditions as mentioned above. The eggs were observed under microscope after 7 days to count the number of eggs hatched and other stages were also examined whether they are survivor or not under microscope after 7 days. The number of treated insects for any given treatment was estimated by the number of survivor in untreated control.

Fumigated and unfumigated control cut flowers for assessment of phytotoxicity were put in water pot and then stored for 10 - 15 days at ambient temperature (3 - 22 ℃), 5 ℃ 10 ℃ and 15 ℃, respectively and examined by visible observation of symptoms on leaves, flowers and stems.

Results and Discussion

Susceptibility to Mixture Gas Fumigation

Mortality data for each stage of eight species of the pests fumigated by mixture gas with three fumigants were shown in Table 1. The data showed that egg stage of the Kanzawa spider mite, two-spotted spider mite, *Thrips palmi*, Japanese mealybug and cabbage moth and pupal stage of greenhouse whitefly were not killed completely at 15 ℃ for 3 hours, while they were killed 100% at 15 ℃ for 4 hours. The data also showed that egg stages of both spider mites were less susceptible than other stages of the pests.

Results of the susceptibility between both spider mite eggs were shown in Table 2. They were fumigated with the same doses of three fumigants at 10 ℃ for 6 hours and the mortality of two - spotted spider mite egg was lower than that of Kanzawa spider mite egg in three replicate tests. It could, therefore, be said that two-spotted spider mite egg was the most resistant stage to mixture gas fumigation.

Applied Mortality Test

Table 3 shows the result of the large-scale applied mortality test conducted with two-spotted spider mite eggs, which is the most resistant stage.

Total numbers of 14,953 eggs at 15 ℃ for 4 hours and 13,429 eggs at 20 ℃ for 3 hours were killed completely by the mixture gas with 13 g/㎥ of methyl bromide, 3 g/㎥ of phosphine and 5% (v/v) of carbon dioxide with 40% loading of chrysanthemum and orchid.

Table 1. Mortality data for each stage of eight species of insect and arthropod pests on cut flowers of chrysanthemum and orchid fumigated by mixture gas with 10 g/m³ of of methyl bromide, 3 g/m³ of phosphine and 5% of carbon dioxide for 3 and 4 hours at 10 and 15 ℃ in a 6 ℓ fumigation bin.

Pests	Stages	Percent mortality (%)			
		3 hrs-10 ℃	4 hrs-10 ℃	3 hrs-15 ℃	4 hrs-15 ℃
Kanzawa spider mite	adult	100	-	-	-
	larva	96.9	100	-	-
	chrysalis	100	100	-	-
	egg	-	98.2	86.5	100
Two - spotted spider mite	adult	100	100	-	-
	larva	92.9	100	-	-
	numph	-	89.7	-	100
	egg	-	96.9	93.0	100
Thrips palmi	adult	100	100	-	-
	larva	100	100	-	-
	egg	-	94.2	95.9	100
Greenhouse whitefly	adult	100	-	-	-
	pupa	-	-	99.2	100
	larva	100	100	-	-
	egg	98.2	100	100	100
Greenhouse peach aphid	adult	93.4	100	-	-
	larva	92.5	100	-	-
Cotton aphid	adult	86.0	95.7	100	100
	larva	76.6	98.9	100	100
Japanese mealybug	egg	-	-	99.9	100
Cabbage moth	adult	100	-	-	-
	pupa	100	-	-	-
	larva	100	-	-	-
	egg	-	-	99.5	100

Table 2. Mortality data for Kanzawa spider mites and two-spotted spider mite eggs on chrysanthemum and orchid fumigated by mixture gas with 10 g/m³ of methyl bromide, 3 g/m³ of phosphine and 5% of carbon dioxide for 6 hours at 10 ℃ in a 6 ℓ fumigation bin.

Pests	Stag	Temp (℃)	Exposure (hour)	Percent mortality (%)		
				Rep1	Rep2	Rep3
Kanzawa spider mite	egg	20	6	100	98.0	100
Two-spotted spider mite	egg	20	6	97.5	98.9	98.0

The average of residual gas concentrations were 10.6 mg/ℓ of methyl bromide, 2.8 mg/ℓ of phosphine and 6.3% of carbon dioxide at 15 ℃ for 4 hours and 10.8 mg/ℓ of methyl bromide, 2.7 mg/ℓ of phosphine and 6.3% of carbon dioxide at 20 ℃ for 3 hours, respectively. Approximately 18% of methyl bromide and 8% of phosphine were absorbed to cut flowers and packing materials, while 1.3% of carbon dioxide increased during the fumigation due to respiration of cut flowers.

Sorption by Fumigation Items

Residual gas concentrations after fumigation and the sorption ratios are shown in Table 4. Three types of the package of chrysanthemum and orchid were fumigated with 10 g/m³ of methyl bromide, 3 g/m³ of phosphine and 5% of carbon dioxide with 40% loading. Gas concentrations of three fumigants

were uniformed within only 6 minutes after appling doses. A 13% of initial dose of methyl bromide was sorbed by cut flowers and packing materials during the fumigation, while no sorption was observed on phosphine, and carbon dioxide concentration increased due to respiration of cut flowers.

Table 3. Mortality of two-spotted spider mite eggs on chrysanthemum and orchid fumigated by mixture gas with 13 g/m³ of methyl bromide, 3 g/m³ of phosphine and 5% of carbon dioxide for 3 and 4 hours at 15 and 20 ℃ with 40% loading in a 0.96 m³ fumigation chamber.

Pest stage	Temp (℃)	Exposure (h)	Rep	Number tested	Number dead	Percent mortality	Gas concentrations after fumigation		
							CH_3Br (mg/m³)	PH_3 (mg/m³)	CO_2 (%)
Kanzawa spider mite egg	15	4	Treat 1	5,772	5,772	100	9.5	2.6	6.3
			2	4,262	4,262	100	11.7	2.9	6.6
			3	4,919	4,919	100	10.5	3.0	6.1
			Total	14,953	14,953	100			
			Cont 1	1,443	36	2.5			
			2	1,066	40	3.8			
			3	1,259	31	2.5			
			Total	3,768	107	2.8			
	20	3	Treat 1	3,727	3,727	100	10.2	2.7	6.1
			2	5,240	5,240	100	11.9	2.8	7.0
			3	4,462	4,462	100	10.4	2.6	5.7
			Total	13,429	13,429	100			
			Cont 1	932	97	10.4			
			2	1,310	19	1.5			
			3	1,115	42	3.8			
			Total	3,357	158	4.7			

Table 4. Residual gas concentrations in a 0.96 m³ chamber containing cut flowers and packing materials. Fumigation at 10 g/m³ of methyl bromide, 3 g/m³ of phosphine and 5%(v/v) of carbon dioxide for 4 hours at 15 ℃ with 40% loading.

Fumigation items	Capacity of carton (ℓ)	Gas concentration			Absorption ratio		
		CH_3Br (mg/ℓ)	PH_3 (mg/ℓ)	CO_2 (%)	CH_3Br (%)	PH_3 (%)	CO_2 (%)
Empty chamber	-	9.9	3.0	5.0	1.0	0	0
Chrysanthemum in carton	78.4	8.7	3.4	5.5	13.0	0	0
Air space	-	8.6	3.4	5.5	-	-	-
Orchid in master carton	118.8	8.7	3.0	5.6	13.0	0	0
Orchid in carton	22.3	8.7	3.0	5.6	-	-	-
Air space	-	8.8	3.0	5.7	-	-	-

Phytotoxicity

Chrysanthemum

No injury was observed on three cultivars of 'Shuhonochikara' (white type), 'Shuhonohikari' (yellow type) and 'Fukumusume' (pink type) in three cultivars fumigated at 15 ℃ for 4 hours and then stored for 15 days under three types of storage. A slight injury, however, was observed on 'Fukumusume' (pink type) with symptoms, such as chlorosis of leave, partial withering of leave and gray colored spots on leave edge, while more severe symptoms, such as discoloration of tiny young petals and unopened flowers in addition to above mentioned symptoms were confirmed in several stalks of the test lot fumigated at 20 ℃ for 3 hours.

Orchids

No injury was observed in 'Panda' , 'Sonia' and 'Swanwhite' fumigated with both schedule at 15 ℃ for 4 hours and 15 ℃ for 3 hours and then stored for 10 days under three types of storage. A slight injury, however, with symptoms of chlorosis of buds, discoloration of tiny young petals, reduction of number of flowers was observed on all cultivars stored for 15 days after fumigation.

The rating of injuries in each cultivar were tended to relatively severe at conditions of high fumigation temperature and high storage temperature after fumigation. These data, however, showed clearly that phytotoxicity of cut flowers fumigated with mixture gas was drastically reduced comparing with that of fumigated with conventional schedules of methyl bromide alone(32.5 g/㎥ for 2 hours at 20 ℃ or above and 48.5 g/㎥ 2 hours at 15 - 20 ℃), and that the rating of injuries caused by mixture gas fumigation could be acceptable in commercial trading.

Fumigation Procedure in Commercial Warehouse

Fumigation procedure for three fumigants is shown in Fig. 1. First, carbon dioxide is introduced from bottom injection site into warehouse through a vaporizer. Heavier specific gravity of carbon dioxide expells extra air from upper exhaust vent. Following introduction of carbon dioxide, humidified phosphine and vaporized methyl bromide are lead to a special designed gas mixing apparatus through mass flow controllers and introduced into warehouse. Phosphine is humidified prior to being lead to the mixing apparatus for avoiding flammability and air and air - phosphine mixture in injection duct must be expelled by vacuum pump and replaced with nitrogen gas before and after introduction of phosphine.

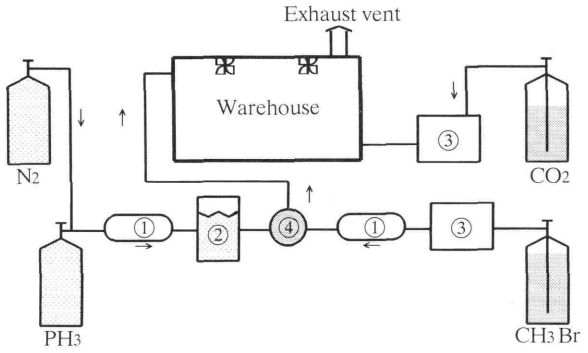

Fig. 1. Diagram of fumigation procedure in commercial warehouse by mixture gas with carbon dioxide, phosphine and methyl bromide.① mass flow controller ② fumidifier ③ vaporizer ④ gas mixing appatus

Phosphine damages to electrical and other machinery in fumigation facility. Meters, wiring, switches, fire alarm systems, and electrical systems should be taken to seal or cover before fumigation. For protection from a risk of inhaling excessive quantities of three fumigants, fumigators and other persons must be provided with positive pressure breathing apparatus with full face mask (self - contained breathing apparatus with portable air tank charged by compressor). Full face canister type respirators are not available for protection against accidental exposure to fumigants.

The use of carbon dioxide and methyl bromide - phosphine mixtures is available for not only avoiding flammability risk of phosphine but also enhancing of mortalities for the pests. That is, phosphine resulted in higher effect against egg and pupal stages and mixtures of methyl bromide with phosphine and carbon dioxide offer the possibility of reducing the quantities of methyl bromide per cubic meter (1/3 of dose of conventional fumigation schedule) and reduction of chemical damages on cut flowers caused by methyl bromide.

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和 文 摘 要

臭化メチル、リン化水素及び二酸化炭素混合ガスによる切り花害虫の消毒試験

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キク及びラン切り花に寄生するカンザワハダニ *Tetranychus kanzawai*, ナミハダニ *T. urticae*, ミナミキイロアザミウマ *Thrips palmi*, オンシツコナジラミ *Trialeurodes vaporariorum*, モモアカアブラムシ *Myzus persicae*, ワタアブラムシ *Aphis gossypii*, フジコナカイガラムシ *Planococcus kraunhiae*, コナガ *Plutella xylostella* の8種の各ステージを混合ガス（臭化メチル 10g/㎥, リン化水素 3g/㎥及び二酸化炭素 5%）で15及び20℃で3, 4及び6時間くん蒸し, 感受性及び完全殺虫基準を調査した。混合ガスくん蒸に対する耐性はナミハダニの卵が最も耐性で, このステージは, 臭化メチル 10g/㎥, リン化水素 3g/㎥及び二酸化炭素 5%, 15℃ 4時間及び 20℃ 3時間, 収容率 40%の基準で完全殺虫された。くん蒸中に臭化メチルは投薬量の 13%が切り花及び梱包材料に収着されたが, リン化水素はまったく収着されず, 二酸化炭素濃度は切り花の呼吸作用により増加した。

キク6品種及びラン4品種には 15℃, 4時間くん蒸では障害は認められなかった。20℃でくん蒸され, くん蒸後 15℃以上で保管された場合, わずかであるが障害が認められたが, 障害の程度は商業上許容される範囲であった。