EFFECT OF PH_3 AND CO_2 MIXTURE AS A QUARANTINE FUMIGANT IN CUT FLOWERS

Min-Goo Park[1]*, Bo-Kyung Sung[1], Justin Tumambing[2]
[1]National Plant Quarantine Service (NPQS), Ministry for Food, Agriculture, Forestry and Fisheries, Anyang, Kyungki, Korea [*mgpark@korea.kr]
[2]Cytec Australia Holdings Pty Ltd, Baulkham Hills, Australia,

1. Introduction

Methyl bromide (MB) has been banned by Montreal protocol due to ozone depletion in developed countries since 2005 except for quarantine and pre-shipment(QPS) applications due to no appropriate alternatives yet available. However, according to European Union strategy for prohibition of QPS, all MB will be regulated and phased out so that there is greater need to develop MB alternatives. MB has also damaging effect to the quality of fresh products which need to be addressed as well. Especially, cut flowers in South Korea are exported mainly to Japan and other countries which were amounted up to 7,867 tonnes in 2009. Insect infestations in the flowers have caused importing countries to fumigate and downgraded or rejected consignments of flowers so that quarantine treatments without damages to flowers are an avoidable market requirement.

There are several fumigants for flowers over the world. Phosphine(PH_3) gas was identified as the most promising of different fumigants used (G.L.Weller, 1998). A non-flammable cylinder gas formulation of 2% PH_3 and 98% carbon dioxide(CO_2) was used as the fumigant treatment. Small scale experiment was conducted regarding mortality or sensitivity test of insects to PH_3 mixture and confirmation test including the phytotoxic evaluation to develop fumigation schedule for it.

2. Materials and methods

2.1. Materials

Test insect pests used were eggs and adults of *Tetranychus uriticae*, larvae and adults of *Aphis gossypii* and *Frankliniella occidentalis*. Test cut flowers were roses(Bordo and Grasia varieties), chrysanthemums (Baksun and Ford varieties) and lily(Orgast variety). The mixture gas is ECO_2FUME(2% PH_3 + 98% CO_2) manufactured by Cytec Industries. Gas-tight 12L glass desiccators were used and gas concentration during fumigation was monitored by Trace GC(Thermo Quest)-NPD. For confirmation test was conducted in 28m^3 container.

2.2.1. Method of sensitivity test to insects (15^oC)

The mixture gas was carried out at 15±1^oC in gas-tight 12L glass desiccators for 24 hours. The desiccators were sealed with glass stoppers containing a septum through which mixture gas was dosed at several levels (at least 5 doses) and gas sample were taken for analysis by gas chromatography. For mixture gas dosing, 50g/m^3 is equivalent to 1g PH_3/m^3 or 700 ppm/m^3. After 24 hours of fumigation, the desiccators were opened in the fume hood for aeration. Mortality of adult and nymph was assessed under a microscope after incubation for 1~3 days. Hatching rate of eggs was investigated after incubation for 5~7 days.

2.2.2 Method of confirmative test (8^oC)

Cut flowers usually exported to Japan was stored at 8°C, transported at 8°C for 1 day and sold at room temperature. So these conditions were selected for this trial. For fumigation, mixture gas at 100 and 200g/m^3 was used in 28m^3 refer-container consisting of 12 boxes(36(w)*100(L)*30(h)cm) of 3 cut flowers(5 varieties) in carton box used in export held for 24h at 8°C under normal atmospheric pressure. The test insects in petri dishes were inserted inside the selected seven boxes of cut flowers. Container was operated to assure mixing of the air and fumigant and keep the temperature setting uniformly within the container. Gas monitoring tubes were installed at 5 points (front down and up, middle up, rear down and up) in container to monitor the uniformity of the gas concentration inside the container. Concentration of gas mixture was determined by withdrawing a gas sample using a pump and injecting it with gas-tight syringe into a gas chromatography (HP6890). Mixture gas concentration was calculated from a standard curve. At the end of the treatment the concentration of mixture gas was remeasured to determine the concentration x time (CxT) dose. Following fumigation, the chambers were aerated using a fan for 2h, and then the commodity was moved to environmental rooms at 8±1°C for 1 day and moved to at 21±1°C for 6 days for storage before quality/phytotoxicity evaluation. The flowers were evaluated after the 1st, 4th and 7th day of storage for diameter of flower, chlorophyll in leaf, color of flower which developed after the fumigation. Diameter of 10 flowers per replication was measured at the widest site with venia-califers tester (Mitutoyo, Japan). Chlorophyll in 10 leaves for each replication was measured using chlorophyll-meter (Konica Minolta, Japan). Color of 10 flowers for each replication was measured using colorimeter (Techkon, Germany) and injury of flowers was subjectively scored as zero (none), one (slight, <5% affected), two (moderate, <25% affected), or three (severe, >25% affected). Mortality of adult and larva of the insects was assessed under a microscope after incubation for 1~3 days. Hatching rate of eggs was investigated after incubation for 5~7 days. Mortality test was conducted only at 100g/m^3.

3. Result and discussion

3.1. Sensitivity test to insects (15°C)

For the *T. urticae* species, the eggs were killed with gas mixture at 13.7mg/L or higher while the adults required lower dose of 7.5mg/L. This showed that the eggs were more tolerant than adults (Table 1). Mortality of gas mixture at 1~10mg/L for *A. gossypii* was 11.1~58.8% in adults and 0~9.3% in larvae, which indicates that larvae were more tolerant than adults (Table 2). Tolerance was greatest in larvae of *A. gossypii*. From this results, dose-response test was conducted more detail for *A. gossypii* so that LC_{99} value of mixture gas for *A. gossypii* was indicated 55mg/L(24hr, 15°C) and Ct product was 1,224 mg hr/L(table 3).

3.2. Confirmation test (8°C)

3.2.1 Mortality for the insects

Adults and larvae of *A. gossypii* and *F. occidentalis*, eggs and adults of *T. urticae* were killed with mixture gas at 100g/m^3 at 8°C (Table 4), which dosage was determined by based on the sensitivity test.

3.2.2. Damage for roses, chrysanthemums, lily(1day at 8°C followed by 6day at 20°C)

There was no damage in roses (Bordo, Grasia), chrysanthemums (Baksun, Ford), lily (Orgast) by the gas mixture at 100 and 200g/m^3 at 8°C, which was stored 1day at 8°C followed by 6 day at 20°C after fumigation. However, there was delaying flowering in

Bordo roses at 100 and 200g/m^3 and lily at 200g/m^3 which may not be concerned to damage, and there was no significant difference even if Bordo roses' color changed at 200g/m^3 (Table 5).

3.2.3. Mixture gas concentration during fumigation

Concentration of mixture gas at different points in container were 117.87~121.30 g/m^3 30 minutes after injection, which means that gas concentration equalized within 30 minutes in 28m^3 container. The final readings of mixture gas were 71.66, 167.59g/m^3 at 100 and 200g/m^3 and residue were 59.6%, 80.8%, respectively, which indicates that container was appropriate for fumigation whereas it is more leaky than stainless chamber. CT product was 2,197g hr/m^3 with mixture gas at 100g/m^3 (Table 6).

3.3. Summary and discussion

Matsuoka(2002) reported that egg of kanzawaii mite was more tolerant than larva or adult for PH_3, Yong biao(2008) showed that aphid was more tolerant than thrips for PH_3. On sensitivity test of our study, larvae of *A. gossypii* was most tolerant in all stage of *A. gossypii*, *T. urticae*, and *F. occidentalis* for PH_3. LC_{99} and LCT_{99} value of mixture gas for larvae of *A. gossypii* were 55mg/L and 1,224 mg hr/ml at 15°C. In confirmation test, all stages of *A. gossypii*, *T. uriticae*, and *F. occidentalis* were killed with mixture gas 100g/m^3 for 24hrs at 8°C, and the no damage was observed in roses, chrysanthemums and lily in 28m^3 container. This schedule has equivalence compared to PH_3 1.5g/m^3 for 24hrs at 15°C suggested by Soma etc(2002), because temperature is lower more or less but dosage is 33% higher. Also, this schedule is not much different from PH_3+CO_2 50g/m^3+Pestigas for 15hrs at 15°C defined by P. Williams(2000).

Table 1. Toxicity of mixture gas to eggs and adults of *Tetranychus uriticae* for 24hrs at 15°C

Stage	Dosage (mg/ℓ)	No.[1]	Mortality (%)[2]	Stage	Dosage (mg/ℓ)	No.[1]	Mortality (%)[2]
Egg	2.0	81	2.1	Adult	5.0	60	98.2
	5.8	93	94.8		7.5	60	100
	9.8	78	98.9		10.0	60	100
	13.7	96	100		12.5	60	100
	17.6	76	100		15.0	60	100
	23.5	69	100		20.0	60	100

[1] Sum of three replicates, [2] Corrective mortality: (mortality for the treated-mortality for the control)/(100-mortality for the control)*100

Table 2. Toxicity of mixture gas to larvae and adults of *Aphis gossypii* for 24hrs at 15°C

Dosage (mg/ℓ)	Adults		Larvae	
	no.[1]	Mortality(%)[2]	no.[1]	Mortality(%)[2]
1	28	11.1	122	0
2	27	30.4	139	0.3
4	26	26.7	138	0.2
6	26	42.3	171	2.4
8	22	38.2	189	2.0
10	23	58.8	155	9.3

[1] Sum of three replicates, [2] Corrective mortality: (mortality for the treated-mortality for the control)/(100-mortality for the control)*100

Table 3. Toxicity of mixture gas to larvae of *Aphis gossypii* for 24hrs at 15℃by Probit analysis

no.	LC_{50} (95%CI)	LC_{99} (95%CI)	Slope	DF	Chi Square(x^2)	Temp.
1,646	23.5 (18.1~42.5)	54.9 (33.4~180.6)	6.30	14	120.33	15°C

※ The residue of gas after 24hr was about 86%

Table 4. Result of mortality for the insect

Dosage (g/m^3)	*Aphis gossypii*		*Tetranychus uriticae*						*Frankliniella occidentalis*			
	no[1]	Mortality(%)	no.			Mortality(%)			no.		Mortality(%)	
			adult	larvae	eggs	adult	larvae	eggs[2]	adult	larvae	Adult	larvae
Control	250	0	520	611	477	0	0	2.5	130	175	0	0
100	799	100	1,027	1,396	679	100	100	100	336	382	100	100

[1] Mixed with stages(larvae and adults), [2] 100-((number of the hatched/number of egg)×100)

Table 5. Mean(±SD) effects of exposures to mixture gas at $100g/m^3$ and $200g/m^3$ at 8°C on cut flowers. Cut flower evaluation 7th day after treatments (1 day at 8±1°C followed by 6 day at 20±1°C).

Cut flower	Dose	Diameter of flower (cm)	Chlorophyll	Color				Damage[1]
				L	a	b	Hue Value	
Rose[2] (Grasia)	Control	17.9(±3.8)	43.2(±5.0)	50.5(±8.4)	40.9(±12.4)	0.15(±5.7)	65.1(±11)	0
	$100g/m^3$	16.8(±2.1)	45.3(±5.9)	49.3(±4.0)	44.8(±4.5)	-1.5(±2.0)	66.8(±3.4)	0
Rose[3] (Bordo)	Control	66.6(±8.4)	55.8(±3.1)	35.2(±6.4)	42.9(±10.5)	14.8(±9.9)	59.0(±5.6)	0.1(±0.3)
	$100g/m^3$	46.2(±4.9)	59.5(±3.8)	34.4(±3.9)	50.8(±1.9)	16.4(±5.0)	63.7(±3.4)	0
	$200g/m^3$	49.8(±16.8)	57.0(±2.9)	38.5(±5.2)	36.2(±17.4)	21.5(±10.6)	60.3(±4.7)	0.4(±0.5)
Chrysanthemum (Ford)	Control	17.0(±3.3)	49.7(±6.6)	80.9(±8.8)	-2.9(±0.9)	6.98(±1.9)	81.3(±9.2)	0
	$100g/m^3$	17.3(±2.8)	51.1(±5.1)	79.2(±7.8)	-3.1(±0.6)	6.89(±1.0)	79.6(±7.8)	0
	$200g/m^3$	17.5(±3.6)	48.7(±2.3)	81.9(±7.9)	0.08(±3.6)	7.96(±2.0)	82.4(±7.5)	0
Chrysanthemum (Baksun)	Control	83.6(±9.6)	61.1(±5.9)	87.3(±1.9)	-2.5(±0.3)	6.05(±1.3)	87.5(±1.9)	0
	$100g/m^3$	92.7(±8.8)	56.3(±6.0)	85.5(±5.4)	-2.6(±0.6)	5.31(±1.0)	85.7(±5.4)	0
	$200g/m^3$	94.4(±7.4)	64.1(±4.2)	87(±2.9)	-2.7(±0.4)	4.96(±0.7)	87.1(±2.9)	0
Lily[3] (Orgast)	Control	74.8(±44.8)	51.8(±5.8)	76.5(±7.1)	-12(±3.3)	25.7(±7.8)	82.7(±4.1)	0
	$100g/m^3$	60.4(±37.7)	49.3(±4.4)	78.7(±3.1)	-9.6(±4.2)	21.8(±10.4)	82.9(±2.5)	0
	$200g/m^3$	39.7(±19.1)	53.4(±4.3)	72(±5.8)	-12(±2.7)	27.6(±6.0)	78.2(±3.9)	0

[1] Damage score: zero(none), one(slight), two(moderate), three(severe) (Evaluation was conducted to observe 10 flowers each treatment). [2] No evaluation on $200g/m^3$ due to no samples. [3] On the result of SAS for diameter, color, effect of flowers, diameter has a significant difference between the control and treated, but the color and effect no significant difference between the control and treated.

Table 6. Mixture gas concentration during fumigation

Hour	$100g/m^3$(container)						$200g/m^3$(chamber)		
	1(rear upper right)	2(middle upper middle)	3(rear down right)	4(front upper left)	5(front down left)	Mean	1	2	Mean
0.5hr	121.30	117.87	120.96	120.71	120.82	120.33	192.02	222.09	207.06
24hr	71.32	73.04	70.83	71.72	71.40	71.66	150.32	184.86	167.59
Residue(%)*	58.8	62.0	58.6	59.4	59.1	59.58	78.3	83.2	80.75

* Residue(%) : Concentration at 24hr/Concentration at 0.5hr after injection

Reference

1. Soma, Y., I. Matsuoka, H. Naito, Y. Tsuchiya, T. Misumi, and F. Kawakami. 2002. Disinfestation of export Japanese pears "Nijisseiki" by phosphine fumigation. 2. Large scale fumigation of Japanese pears by phosphine from generator. Res. Bull. Pl. Prot. Japan 38: 9-12.
2. P.Williams. 2000. Improved fumigation for Export Wildflowers. RIRDC
3. Matsuoka, I. 2002. Disinfestation of export Japanese pears "nijisseiki" by phosphine fumigation. 1. Tolerance of Japanese pears "Nijisseiki" and mortality of kanzawa spider mite. Res. Bull. Pl. Prot. Japan 38: 5-8
4. G.L.Weller. 1998. Cut flower disinfestation: Assessment of replacement fumigants for methyl bromide. Postharvest Biology and Technology 14: 325-333
5. Yong-Biao Liu. 2007. Fumigation with phosphine for postharvest insect control on lettuce and broccoli. Proceeding of the Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions. pp. 128