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Short Communication

Effects of Methyl Iodide Fumigation on Mortality of Carmine Spider Mite, *Tetranychus urticae* Koch, Kanzawa Spider Mite, *T. kanzawai* Kishida (Acari: Tetranychidae) and Green Peach Aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae).

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Abstract: Effects of methyl iodide (MI) on mortality were examined using *Tetranychus urticae, T. kanzawai* and *Myzus persicae*. As the result of 1 hour fumigation, egg stages of both species of spider mite were most susceptible with 100% mortality being obtained at a dose of 9.3 mg/l at 15°C. Mortality at the larva and nymph stage of *T. urticae* was however 98.4% under the same conditions. As the result of 2 hours fumigation, estimated LD_{50}' s at the larva and nymph stage, and nymph and adult stage of *T. urticae* were 1.05 and 2.51 mg/l, respectively. The nymph and adult stage of *T. urticae* was considered less susceptible than the larva and nymph stage. As a consequence of the mortality verification test, nymph and adult stages of *T. urticae* and *T. kanzawai* were killed completely at doses of 7.7 and 9.3 mg/l for 2 hours at 15°C, respectively. 100% mortality was achieved for nymph and adult stage of *M. persicae* at the dose of 3.1 mg/l for 2 hours at 15°C. The effects of MI on these insect pests were considered to be sufficient, since estimated numbers of each species treated were more than 1,000. To apply MI fumigation to many fruits and vegetables as an alternative to methyl bromide, further mortality tests are necessary using various insect pests.
Key Words: methyl iodide, fumigation, mortality, spider mite, aphid

Introduction

Methyl bromide (MB) as a fumigant is recognized as an important tool for the control of insect pests and diseases, especially quarantine pests and soil borne diseases. At the same time, it is also recognized as an ozone depleting substance under the Montreal protocol. Therefore, reduction in the use and emission of MB has been encouraged even in the quarantine and pre-shipment sector which is an exempted use under the protocol (UNEP, 2009).

Our laboratory has been tackling the development of alternatives to MB to reduce the use and emission of it in the Japanese quarantine sector. Methyl iodide (MI) is a pesticidal chemical and considered as one of the alternatives to MB. Effects of MI on mortality had been examined using stored product insects, forest insect pests and pine wood nematode associated with grains, logs and wooden materials (Naito *et al*., 2003; Goto *et al*., 2004; Soma *et al*., 2005; Soma *et al*., 2006; Soma *et al*., 2007a). Also damage to several fruits, vegetables, potted flowers and others by MI fumigation had been studied (Soma *et al*., 2007b; Naito *et al*., 2011; Shukuya *et al*., 2012). The effects of MI on mortality of insect pests which are living on fresh fruits and vegetables, however, have not been researched until now.

Meanwhile, MI as a fumigant is now facing unavailability overseas because of the withdrawal of

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pesticide registration for it in some countries other than Japan (UNEP, 2012). It has however been established that MI has high potential performance as a fumigant as a result of past research. Therefore, we have launched basic mortality tests with MI to examine its efficacy with respect to insect pests associated with fresh fruits and vegetables. We release a report of the effects to spider mite and aphid of MI fumigation at the beginning.

Materials and Methods

1. Test insects

Carmine spider mite, *Tetranychus urticae* Koch: Carmine spider mite which had been obtained from a laboratory strain at Kyoto university (Yamada *et al*., 2012) and then reared on kidney bean leaves in petri dishes (9 cm diameter) with soaking cotton in water at 25°C, 60-65% R.H., 16L8D photoperiod in a climate chamber since 2010 was used. Adult females were put on new leaves in petri dishes and allowed to oviposit for 2 days in the chamber, and then they were removed. Laid eggs on the leaves were maintained under rearing conditions up to target stages. 0-2 day-old egg, 2-4 day-old egg, larva and nymph stage (5-7 days after oviposition) and nymph and adult stage (8-10 days after oviposition) were provided for dose-response tests. For mortality verification tests, nymph and adult stages were prepared by putting a leaf infested with nymph and adult stages on a new leaf in a petri dish for 2 days to allow migration. Egg stages and other stages on the leaves in the petri dishes were acclimated to a fumigation temperature of 15°C in a fumigation room for 3-4 hours or one-night before fumigation.

Kanzawa spider mite, *T. kanzawai* Kishida: Cyclamen leaves infested with Kanzawa spider mite were collected from house plants at Musashino city in the metropolis of Tokyo in January 2013 and then inoculated to kidney bean leaves in petri dishes. The same methods which were used for carmine spider mite were applied to rearing and preparing for fumigation in the laboratory. After rearing for 2 months following collecting, the 0-4 day-old egg stage was used for dose-response test and nymph and adult stages were provided for the mortality verification test.

Green peach aphid, *Myzus persicae* (Sulzer): Green peach aphid which originated in a field strain at Makinohara city in Shizuoka prefecture was obtained from Japan Fumigation Technology Association in October 2012. Aphids were then inoculated to broad bean seedlings (ca. 15 cm high) and reared on the seedlings at 23°C, 16L8D photoperiod in an incubator. An infested leaf with aphids was put on a new seedling (10-15 cm high with 2-4 leaves) placed in a plastic cylindrical case (3 cm diameter, 5 cm high) with folded kitchen paper, and nymph and adult females were allowed to move to the new seedling and to reproduce their clones for 3 days in the incubator. These newly infested seedlings were put in cylindrical containers (11 cm diameter, 24 cm high) with two openings (7 x 6 cm) on the side and top face covered by mesh. These containers with infested seedlings were transferred to the fumigation room the day before fumigation and acclimatized overnight to a fumigation temperature of 15°C. Nymph and adult stages were used for mortality verification tests.

2. Fumigation

Fumigation was conducted using acrylic resin boxes of 29.5 liter volume (equipped with gas application and sampling ports, a gas circulation fan, gas exhaust port and temperature probe) in a temperature-controlled room at 15°C. Liquid MI (purity; 99% or more) was collected by a gas-tight micro syringe from a vial and injected into the properly depressurized box. To accelerate gasification of liquid MI, a crumpled piece of paper towel which was enfolded with a cruciform filter paper was set under the gas application port of the box. The box was returned to normal pressure after MI injection. Gas concentrations were measured by a gas chromatograph (GC-2014 with FID: Shimadzu) at the time intervals of 15, 30, 60 and 120 minutes after dosing. Temperatures in the boxes were also monitored with a temperature recorder (Graphic logger CR-1016-A: Chino) during fumigation. A gas circulation fan in the box was operated through fumigation. After fumigation was completed, air-fumigant mixture gas was exhausted for 1 hour using the ventilation system. Unloaded test insects and untreated control were returned to the rearing conditions of each species.

3. Evaluation of mortality

Mortality of the egg stage of spider mite was evaluated by hatching at 7 days after fumigation. Mortality of the larva, nymph and adult stages of spider mite was determined by counting the numbers of live and dead insects at 3 days after fumigation for the dose-response test. For the mortality verification test, fumigated and untreated control leaves were carefully removed from the petri dishes and placed on new leaves in petri dishes after fumigation, because fumigated leaves might be damaged by MI. 2 days after fumigation,

Table 1. Corrected mortality of egg stages of *Tetranychus urticae* and *T. kanzawai*, and larva and nymph stage of *T. urticae* fumigated with MI for 1 hour at 15℃.

Dose (mg/l)	*T. urticae*						*T. kanzawai*	
	Egg (0-2 day-old)		Egg (2-4 day-old)		Larva / Nymph		Egg (0-4 day-old)	
	n [1)]	mean% ± SD	n [1)]	mean% ± SD	n [1)]	mean% ± SD	n [1)]	mean% ± SD
0 (control)	122	1.5 ± 2.2	165	10.3 ± 11.6	44	4.5 ± 0.0	474	4.5 ± 4.1
0.8	120	2.6 ± 1.3	161	10.2 ± 5.2	60	34.4 ± 31.5	379	6.6 ± 4.0
1.6	120	11.9 ± 0.8	162	61.5 ± 1.5	62	49.8 ± 3.1	412	36.2 ± 1.8
3.1	134	81.8 ± 1.8	169	95.5 ± 3.9	66	58.0 ± 22.4	428	81.2 ± 6.8
4.6	125	98.4 ± 2.2	163	97.0 ± 1.5	66	64.2 ± 17.7	400	96.3 ± 0.2
9.3	129	100	170	100	55	98.4 ± 2.3	372	100

[1)] The number of insects tested were total of two replication.

Table 2. Corrected mortality of larva, nymph and adult stages of *Tetranychus urticae* fumigated with MI for 2 hours at 15℃.

Dose (mg/l)	*T. urticae*			
	Larva / Nymph		Nymph / Adult	
	n [1)]	mean% ± SD	n [2)]	mean% ± SD
0 (control)	108	13.0 ± 7.3	174	29.9 ± 5.8
0.8	113	41.9 ± 4.3	171	29.3 ± 8.4
1.6	108	54.9 ± 9.7	166	35.8 ± 9.1
3.1	106	77.4 ± 5.3	163	77.0 ± 7.2
4.6	100	99.2 ± 1.1	158	96.7 ± 5.7
6.2	93	100	157	100

[1)] The number of insects tested were total of two replication.

[2)] The number of insects tested were total of three replication.

the numbers of live and dead insects were counted. All stages were checked under stereo microscope.

Mortality of aphid was also confirmed by counting the numbers of live and dead insects at 2-3 days after fumigation using stereo microscope.

Mortalities were corrected by Abbott's formula (Abbott, 1925) and results of dose-response tests for spider mites were analyzed by probit analysis using computer program, Polo Plus ver.1.0 (LeOra Software, Petaluma, CA, USA).

Results and Discussion

1. Dose-response test

Corrected mortalities of egg stage, and larva and nymph stage of spider mites for 1 hour fumigation at 15°C are shown in Table 1. Mortalities of 2-4 day-old eggs of *T. urticae* were higher than those of 0-2 day-old eggs at the doses of 0.8-3.1 mg/l, and mortalities of egg stages of *T. urticae* were approximately the same figure at the dose of 4.6 mg/l. Mortalities of 0-4 day-old eggs of *T. kanzawai* indicated a similar trend with egg stages of *T. urticae* considering differences of age in days. 100% mortalities were obtained with egg stages of both species at the dose of 9.3 mg/l. Mortalities of larva and nymph stage of *T. urticae* were about 34-98% at the doses of 0.8-9.3 mg/l. Since 100% mortality could not be attained with larva and nymph stage of *T. urticae* at the dose of 9.3 mg/l, larva and nymph stage was considered less susceptible than egg stages. In addition, mortalities of the larva and nymph stage of *T. urticae* fluctuated between replications. It was considered that this variation was caused by the short treatment time of 1 hour. The duration of fumigation was therefore changed to 2 hours thereafter.

Results of larva and nymph stage, and nymph and adult stage of *T. urticae* for 2 hours fumigation at 15°C are shown in Table 2. Relatively stable mortalities were obtained compared with those of 1 hour fumigation in all plots. Mortalities of nymph and adult stage were lower than those of larva and nymph stage at the doses of 0.8 and 1.6 mg/l, although approximately the same figures were shown at the doses of 3.1 and 4.6 mg/l. 100% mortalities were obtained from both stages of *T. urticae*

Table 3. Estimated LD_{50} and LD_{95} of *Tetranychus urticae* and *T. kanzawai* fumigated with MI for 1 or 2 hours at 15°C.

LD (mg/l)	*T. urticae*				*T. kanzawai*
	Egg (0-2 day-old) [1]	Egg (2-4 day-old) [1]	Larva / Nymph [2]	Nymph / Adult [2]	Egg (0-4 day-old) [1]
LD_{50} (95%CL)	2.33 (2.16-2.49)	1.47 (1.11-1.80)	1.05 (0.64-1.42)	2.51 (1.50-2.99)	2.00 (1.58-2.33)
LD_{95} (95%CL)	3.94 (3.36-4.38)	3.42 (2.73-4.93)	4.94 (3.52-9.08)	4.46 (3.91-6.02)	3.90 (3.24-5.53)

[1] Estimated LD's were based on the results of 1 hour fumigation.
[2] Estimated LD's were based on the results of 2 hours fumigation.

at the dose of 6.2 mg/l for 2 hours at 15°C.

Estimated LD_{50} and LD_{95} for each stage of *T. urticae* and *T. kanzawai* for 1 or 2 hours fumigation at 15°C are shown in Table 3. LD values of larva and nymph stage of *T. urticae* for 1 hour fumigation were not analyzed because of fluctuation in mortalities. LD_{50}' s for 0-2 and 2-4 day-old egg of *T. urticae* were 2.33 and 1.47 mg/l, respectively. LD_{95}' s for them were 3.94 and 3.42 mg/l, respectively. Susceptibility of 0-2 day-old egg of *T. urticae* was significantly lower than 2-4 day-old egg at LD_{50}, because overlapping wasn't observed in the 95% confidence limit of both stages. There was however no significant difference between them at LD_{95}. LD_{50} and LD_{95} of 0-4 day-old egg of *T. kanzawai* were 2.00 and 3.90 mg/l, respectively. LD values between egg stages of *T. urticae* and *T. kanzawai* could not be compared directly because of difference of age in days of each egg stage. It was however considered that susceptibility of egg stages of both species were at a comparable level, because their 95% confidence limits overlapped at LD_{50} and LD_{95}, respectively. LD_{50}' s and LD_{95}' s of larva and nymph stage, and nymph and adult stage of *T. urticae* were calculated at 1.05, 4.94 mg/l and 2.51, 4.46 mg/l, respectively. Susceptibility of nymph and adult stage was significantly lower than larva and nymph stage at LD_{50}, while there was no significant difference at LD_{95}. It was considered that the nymph and adult stage of *T. urticae* was more tolerant than the larva and nymph stage of it.

As a result of the dose-response test, it was clear that egg stages of *T. urticae* and *T. kanzawai* were the most susceptible of all stages. Goto *et al*., (2004) has reported that egg stages of four species of stored product insects were highly affected by MI and the results of Naito *et al*., (2003) has also suggested that egg stages of forest insect pests might be more susceptible than other stages of them. MI might be more highly effective in mortality to egg stage than other stages of various insect pests. Also, Aung *et al*., (2001) reported that 100% of mortalities were obtained for 2nd instar larva of California red scale, *Aonidiella aurantii*, on lemon and egg stage of Codling moth, *Cydia pomonella*, on nectarine with MI at 40 and 25 mg/l at 21°C for 2 hours, respectively. Accordingly, it is highly possible that two species of spider mite are killed completely at a relatively lower dosage of MI for 2 hours at 15°C compared with California red scale and Codling moth.

2. Mortality verification test

Results of mortality verification tests for 2 hours fumigation at 15°C are shown in Table 4. Effects on mortality of nymph and adult stages of *T. urticae* and *T. kanzawai* were evaluated, and nymph and adult stages of *M. perscicae* were also provided for this test.

It was revealed that the nymph and adult stage of

Table 4. Corrected mortality of nymph and adult stages of *Tetranychus urticae, T. kanzawai* and *Myzus persicae* fumigated with MI for 2 hours at 15°C.

Dose (mg/l)	*T. urticae* [1]				*T. kanzawai* [1]				*M. persicae* [1]	
	Nymph / Adult male		Adult female		Nymph / Adult male		Adult female		Nymph / Adult	
	n [2]	mean% ± SD	n [2]	mean% ± SD	n [2]	mean% ± SD	n [2]	mean% ± SD	n [2]	mean% ± SD
0 (control)	1,039	13.7 ± 3.4	279	9.5 ± 4.1	2,991	27.9 ± 12.3	565	26.1 ± 12.0	2,738	22.1 ± 17.2
1.6	-	-	-	-	675	16.9 ± 18.7	229	27.4 ± 3.4	1,225	98.7 ± 1.3
3.1	-	-	-	-	1,032	80.9 ± 17.9	194	83.3 ± 16.7	1,118	100
6.2	1,158	99.8 ± 0.4	278	100	1,058	99.2 ± 1.3	229	100	-	-
7.7	1,387	100	293	100	1,036	99.9 ± 0.2	267	100	-	-
9.3	-	-	-	-	1,708	100	229	100	-	-

[1] Fumigation tests of *T. urticae, T. kanzawai* and *M. persicae* were carried out four, three and five replication, respectively.
[2] The number of insects tested were total of estimated figures which were based on survival rate of untreated control of each replication.

M. perscicae was most susceptible within them. 100% mortality was obtained at the dose of 3.1 mg/l and the estimated number of test insects was over 1,100. 100% mortalities for the nymph and adult male stage, and the adult female stage of *T. urticae* were achieved at the doses of 7.7 and 6.2 mg/l, respectively. The adult female stage of *T. kanzawai* was killed completely at the dose of 6.2 mg/l, although the dosage of 9.3 mg/l was required for obtaining 100% mortality for the nymph and adult male stage. Adult female stages of both species of spider mite were considered more susceptible than nymph and adult male stages of them, and the nymph and adult male stage of *T. kanzawai* was also considered more tolerant of it than *T. urticae*. The estimated number of nymph and adult male stage of *T. urticae* was more than 1,300 at the dose of 7.7 mg/l and the same number of *T. kanzawai* was over 1,700 at the dose of 9.3 mg/l.

As a result of the mortality verification test, the estimated numbers which were killed completely were calculated to be more than 1,000 of each species. According to Couey & Chew (1986), efficacy of MI fumigation for 2 hours at 15°C is evaluated at 99.7% or more with a 95% confidence limit for these insect pests.

3. Applicability of MI fumigation

Soma *et al.*, (1997) reported that egg and adult stages of two-spotted spider mite, *T. urticae*, were killed completely with phosphine at the dose of 2 mg/l for 24 hours at 15°C and Matsuoka *et al.*, (2002) also reported that all stages of Kanzawa spider mite were controlled completely with phosphine at the dose of 1.5 mg/l for 24 hours at 15°C. Also, according to Yamada *et al.*, (2012), it is necessary to apply an ethyl formate and carbon dioxide mixture (EF+CO_2) at the dose of 350 mg/l for 3 hours at 15°C to obtain 100% mortality for carmine spider mite and Kanzawa spider mite. Our research has identified that MI has more advantageous characteristics which are applicable with short-term fumigation than phosphine and very low dosage compared with EF+CO_2.

In addition, according to Soma *et al.*, (2007b) and Naito *et al.*, (2011), damage to several fruits and vegetables by MI fumigation has been examined and it has been confirmed that there was no damage on strawberry, pumpkin, cauliflower and Brussels sprouts at the dose of 48.5 mg/l for 3 hours at 15°C. Slight damage was observed on orange, okra and broccoli at the doses of 30 or 48.5 mg/l for 2 or 3 hours at 15°C. Especially the latter commodities may be able to avoid those unfavorable cases, since it is considered possible to reduce the dosage of MI to around one-half or less than those reports by limiting the range of applicable pests such as aphid and spider mite only.

And these undamaged or slightly damaged commodities with MI fumigation are strongly associated with species of mealy bug, scale insect, thrips and whitefly in addition to spider mite and aphid in Japanese import quarantine. For example, the percentage of these pest groups detected at import inspection in 2011 were about 80% on orange and strawberry, about 60% on okra, and about 30% on Brussels sprouts, cauliflower and squash or pumpkin based on the number of times all pests were detected on each commodity (PPS, 2011). To further explore the applicability of MI fumigation in the Japanese quarantine sector, it is necessary to confirm its efficacy with respect to these insect pests.

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和 文 摘 要

ヨウ化メチルくん蒸によるナミハダニ赤色型、カンザワハダニ及びモモアカアブラムシの殺虫効果（英文）

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ナミハダニ赤色型*Tetranychus urticae*、カンザワハダニ*T. kanzawai*及びモモアカアブラムシ*Myzus persicae*を供試してヨウ化メチルくん蒸の殺虫効果を調査した。感受性試験の結果、ハダニ類の卵は感受性が高く、ヨウ化メチル9.3mg/l、1時間、15℃で100%の殺虫率となった。ナミハダニ赤色型の幼虫及び若虫では、同じ条件で98.4%の殺虫率であった。2時間くん蒸の結果、ナミハダニ赤色型の幼虫及び若虫、並びに若虫及び成虫のLD_{50}は、1.05及び2.51mg/lとなり、若虫及び成虫は、幼虫及び若虫よりもヨウ化メチルに対する感受性が低いものと考えられた。ナミハダニ赤色型及びカンザワハダニの若虫及び成虫を供試した殺虫確認試験の結果、それぞれ7.7mg/l及び9.3mg/l、2時間、15℃で100%の殺虫率となった。また、モモアカアブラムシの若虫及び成虫は、3.1mg/l、2時間、15℃で100%殺虫された。それぞれの種で1,000頭以上の100%の殺虫が確認されたことから、これらの害虫に対するヨウ化メチルの殺虫効果は十分と考えられた。臭化メチル代替剤として青果物のヨウ化メチルくん蒸を検討するため、今後、さらに種々の供試虫を用い殺虫試験を行う必要がある。

[1)]現在、横浜植物防疫所川崎出張所