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Short Communication

Susceptibility of Brown-Winged Green Bug, *Plautia stali* Scott (Hemiptera: Pentatomidae) to Methyl Iodide Fumigation.

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Abstract: The susceptibilities of the egg, nymph and adult stages of the Brown-Winged Green Bug, *Plautia stali* Scott to Methyl Iodide (MI) were studied for the development of the MI fumigation condition. Following MI fumigation for 2 hours at 15℃, 100% mortalities of egg, 1st instar, 2nd−3rd instar and 5th instar nymph were obtained at dosages of 9.3, 6.2, 7.7 and 9.3 mg/l, respectively and survivors of the adult stage were observed at a dosage of 9.3 mg/l. Accordingly, the adult stage was considered less susceptible than other stages. Subsequently, MI fumigation for 2 hours using the adult stage of *P. stali* at 10 and 15℃ and 100% mortality was obtained at dosages of 15.4 and 10.8 mg/l, respectively. To develop the MI fumigation condition against a group of stink bugs infesting fruit and vegetable, additional susceptibility tests using other sting bug species are necessary.

Key Words: fumigation, methyl iodide, *Plautia stali*, susceptibility

Introduction

Methyl Bromide (MB) fumigation is applied to many kinds of imported plants and plant products when a quarantine pest is detected during import plant quarantine inspections. However, MB was listed as an ozone-depleting substance under the Montreal protocol in 1992 and the International Plant Protection Convention (IPPC) introduced a recommendation to replace MB or reduce its use as a phytosanitary measure in 2008 (IPPC, 2008). Under these circumstances, our laboratory has focused on Methyl Iodide (MI) as a potential replacement candidate and considered how best to expand the use of alternatives to MB. In terms of MI fumigation of fruit and vegetables, chemical injuries were examined for some commodities (Soma *et al*., 2007; Naito *et al*., 2011) and the effects of MI fumigation on the mortality of aphids, mealy bugs, spider mites and thrips have been reported (Naito *et al*., 2014 and 2015). However, other insect pests such as stink bugs are also found on fruit and vegetables during import quarantine inspections. Accordingly, we conducted basic mortality tests on the stink bug species, *Plautia stali* to develop the MI fumigation condition.

Materials and Methods

1. Test insect

Brown-Winged Green Bug, *Plautia stali* Scott: Eggs of *P. stali* were obtained from a fig tree (*Ficus carica*) in Yokohama city, Kanagawa prefecture, Japan in 2018. They were then placed into plastic containers (21.0×15.0×4.5cm) with a lid and an opening covered with wire mesh and kept under conditions of 25℃, 60 ± 10%RH and a 16L8D photoperiod in a climate chamber. After the eggs had hatched, husked peanuts and soybeans were provided and cotton soaked in water which was placed into a glass vial was given to the insects until adult stage. The water contained 500 mg of Sodium L - Ascorbate and 250 mg of L - Cysteine Hydrochloride Monohydrate per 1 *l*. After sufficient adults had been obtained, rearing for mortality tests commenced. Plastic containers dimensioned at 12.0 × 9.8 × 4.6 cm for eggs and 1st instar nymphs, 21.0×15.0×4.5 cm for 2−4th instar nymphs and 24.2 × 30.6 × 10.3 cm for 5th instar nymphs and adults were used to rear test insects under equivalent conditions in the chamber. Husked peanuts and soybeans on cleaning tissue paper (120 × 215mm, Kimwipe®) folded in half were fed to the developmental stages after the 2nd instar nymph and water as described above was given to the nymph and adult stages. To conduct mortality tests, the adults placed in containers were allowed to oviposit for 2−7 days. Eggs which were laid on the inner surface of the containers or cleaning tissue papers and nymphs having hatched were moved into the plastic containers

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and those were maintained under rearing conditions up to target stages. Periods after oviposition for MI fumigation of egg, 1st instar nymph, 2nd–3rd instar nymph, 5th instar nymph and adult stages were 1–4 days, 4–8 days, 8–16 days, 22–33 days and more than 40 days, respectively. For mortality tests, the test insects of each stage were placed into cylindrical plastic containers (15 cm in diameter × 9 cm high) which were covered with a nylon mesh lid and acclimated for 2–4 hours at 15℃ for the susceptibility test and one night at 10 and 15℃ for mortality verification test. The temperature conditions were configured to compare susceptibility between *P. stali* and other pest insects as reported in Naito *et al*. (2015). Cotton soaked in water as described above was given during both tests and a few peanuts and soybeans were fed for the test to verify mortality in the cylindrical containers.

2. Fumigation

Fumigation was performed by the same method and devices described in Naito *et al*. (2014). The purity of the liquid MI was 99.5 % (FUJIFILM Wako Pure Chemical Corporation). The fumigation conditions were 2 hours at 10 and 15℃, respectively. The gas concentrations of MI and temperatures in the fumigation boxes were monitored with a gas chromatograph (GC-2014 with FID: Shimadzu) and a temperature recorder (Graphic logger CR-1016-A: Chino) during fumigation. Once the fumigation was completed, the air-fumigant mixture was exhausted with an aeration system for 1 hour and the tested insects and untreated controls were moved into the climate chamber. These tests were replicated three times.

3. Evaluation of mortality

The mortalities of the nymph and adult stages were determined by counting the number of living and dead insects 4–5 days after fumigation and that of the egg was evaluated by counting living nymph 5–7 days after fumigation. Regarding the susceptibility test, the mortalities were corrected by Abbott's formula (Abott, 1925) and arc-sine transformation was applied to the corrected mortalities before a *t* test for a dosage of 4.6mg/l and a Tukey-Kramer HSD test for dosages of 6.2, 7.7 and 9.3 mg/l by computer program, JMP (SAS Institute).

Results and Discussion

1. Susceptibility test at 15℃

Temperatures and gas concentrations during fumigation were within the range 14.3–15.5℃ and 4.5–4.8, 5.9–6.5, 7.2–8.3 and 8.9–9.8 mg/l at dosages of 4.6, 6.2, 7.7 and 9.3 mg/l, respectively. The results of the susceptibility test are shown in Table 1. Corrected mortalities among each stage at the same dosage were analyzed by a *t* test or Tukey-Kramer HSD test. Mortality at the dosage of 4.6 mg/l of the 1st instar nymph significantly exceeded that of the 2nd–3rd instar nymph ($p < 0.05$). At the dosage of 7.7mg/l, while the 1st and 2nd–3rd instar nymphs were killed completely, survivors at the egg, 5th instar nymph and adult stages were observed and the mortality of the adult stage was significantly less than other developmental stages ($p < 0.05$). At a dosage of 9.3 mg/l, survivors of the adult stage were observed and the eggs and 5th instar nymphs were killed completely. Accordingly, the adult stage was considered less susceptible than other stages.

Table 1 Corrected mortality of each stage of *Plautia stali* fumigated with MI for 2 hours at 15℃.

Dosage (mg/l)	egg		1st instar nymph		2nd - 3rd instar nymph		5th instar nymph		adult	
	n1)	mean (%) ± SD 2)	n1)	mean (%) ± SD 2)	n1)	mean (%) ± SD 2)	n1)	mean (%) ± SD2)	n1)	mean (%) ± SD 2)
0(control)	991	34.7 ± 1.8	1086	24.2 ± 7.3	725	50.9 ± 19.8	326	22.1 ± 6.5	244	26.2 ± 6.3
4.6	–	–	931	99.9 ± 0.1a	541	91.6 ± 6.2b	–	–	–	–
6.2	1134	91.5 ± 4.1b	930	100a	534	99.3 ± 0.9a	210	100a	246	84.5 ± 3.3b
7.7	1052	99.7 ± 0.2a	1083	100a	688	100a	235	99.4 ± 0.9a	241	96.5 ± 1.5b
9.3	1080	100a	–	–	–	–	219	100a	250	99.5 ± 0.8a

1) The number of insects tested were total of 3 replication.

2) Different letters following corrected mortalities were significantly different among developmenatal stages at each dosage ($p < 0.05$, *t* test at a dosage of 4.6 mg/l, Tukey-Kramer HSD test at dosages of 6.2, 7.7 and 9.3 mg/l).

Table 2 Mortality and CT value of the adult stage of *Plautia stali* fumigated with MI for 2 hours at 10 and 15℃.

Dosage	10℃				15℃			
	Number of insects[1)]		Mortality (%)	CT value (mg・h/l) [2)]	Number of insects[1)]		Mortality (%)	CT value (mg・h/l) [2)]
	Treated	Dead			Treated	Dead		
10.8	–	–	–	–	943	943	100	17.7
Control	–	–	–	–	334	63	18.9	–
12.4	336	334	99.4	20.8	898	898	100	20.4
Control	347	45	13.0	–	296	51	17.2	–
13.9	550	549	99.8	23.7	–	–	–	–
Control	453	57	12.6	–	–	–	–	–
15.4	813	813	100	25.7	–	–	–	–
Control	486	43	8.8	–	–	–	–	–
17.0	558	558	100	27.7	–	–	–	–
Control	363	28	7.7	–	–	–	–	–

[1)] The number of insects tested were total of 3 replication.

[2)] CT value = $(7.5C_{15} + 22.5C_{30} + 45C_{60} + 30C_{120}) / 60$ (Cn: gas concentration after n minutes)

2. Mortality verification test at 10 and 15℃

Temperatures during fumigation at 10 and 15℃ were within the range 9.7−10.7℃ and 14.6−15.4℃, respectively. The gas concentrations were 10.0−10.8, 11.5−12.4, 13.0−14.0, 13.9−16.2 and 15.6−17.3 mg/l at dosages of 10.8, 12.4, 13.9, 15.4 and 17.0 mg/l, respectively. The results of the mortality verification test, which used an adult stage with MI fumigation for 2 hours at 10 and 15℃ are shown in Table 2. The minimum dosages and CT values for which 100% mortality was obtained at 10℃ were 15.4 mg/l and 25.7 mg・h/l and at 15℃, 10.8 mg/l and 17.7mg・h/l, respectively.

3. Application of MI fumigation against stink bugs

Regarding insect pests which infest fruit and vegetables, Naito *et al*. (2015) evaluated treatment schedules with MI fumigation by categorizing insect pest groups and commodity groups. Regarding the former in terms of treatment schedules, one group was for aphids, spider mites and thrips, while another was for mealybugs and the estimated dosages for the group of mealybugs far exceeded those of another group. Naito *et al*. (2015) also reported that the estimated dosages and CT values which obtained 100% mortality with MI fumigation for 2 hours for *Tetranychus kanzawai* and *T. urticae* at 10℃ were 13.9 mg/l and 24.3 mg・h/l and the equivalent for *T. kanzawai, T. urticae, Frankliniella intonsa* and *Thrips tabaci* at 15℃ were 9.3 mg/l and 16.3 mg・h/l. Those were close to the values for which 100% mortality was obtained for the adult stage of *P. stali* because the dosages and average CT values were 15.4 mg/l and 25.7 mg・h/l at 10℃ and 10.8 mg/l and 17.7 mg・h/l at 15℃, respectively. When treatment schedules for a group of stink bugs are considered, a susceptibility test using other stink bug species should be conducted. As a result, if *P. stali* is the most tolerant species among stink bugs, it may be appropriate to apply stink bugs to the same treatment schedules for the group of aphids, spider mites and thrips because the susceptibility of *P. stali* to MI seems to be close to these insect pests.

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和 文 摘 要

ヨウ化メチルくん蒸に対するチャバネアオカメムシの感受性（英文）

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青果物に寄生する害虫のヨウ化メチルくん蒸の殺虫条件を検討するため、チャバネアオカメムシを対象に卵、幼虫及び成虫のヨウ化メチルに対する感受性を比較した。各発育ステージについて、15℃、2時間のヨウ化メチルくん蒸を行った結果、100% 殺虫されたヨウ化メチル単位薬量（以下、「薬量」という。）は、卵が 9.3mg/l、1 齢幼虫が 6.2mg/l、2 - 3 齢幼虫が 7.7mg/l、5 齢幼虫が 9.3mg/l であり、成虫は 9.3mg/l で生存虫が確認されたため、成虫の感受性が最も低いと考えられた。次に本種の成虫を対象に 100% 殺虫される薬量を確認するため、10℃及び 15℃、2 時間くん蒸により殺虫試験を行った結果、10℃では薬量 15.4mg/l、15℃では薬量 10.8mg/l で 100% 殺虫されたことを確認した。今後、カメムシ類に適用可能な消毒処理基準を設定するため、他のカメムシ類についても同様の試験を行い、チャバネアオカメムシと感受性を比較する必要がある。

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