

**FUMIGATION STANDARDS FOR LIQUID PHOSPHINE
(ECO2FUME -2% PHOSPHINE IN 98% CARBON DIOXIDE
W/W) FOR THE CONTROL OF QUARANTINE PESTS OF RICE,
PINEAPPLE AND BITTER GOURD**

I.K. WARSHAMANA¹, D.B. JAYALATHARACHCHI¹, P.R.A. WIJESINGHE¹,
G.T.S. PERERA¹, T.N.P. FERNANDO¹, W.R.U. WEERASINGHE¹,
S.P.S.K. SENADEERA¹, S.D.K. PRIYADARSHANI¹, R.A.P. RANAWEERA¹,
R.G.R. KUMARI¹, P. RANAWEERA², S.S. WELIGAMAGE²,
L. NUGALIYADDE³ AND R. COOKE⁴

¹ National Plant Quarantine Service, Department of Agriculture, Katunayake, Sri Lanka

² Horticultural Crops Research and Development Institute, Gannoruwa, Sri Lanka

³ Sri Lanka Organization of Agriculture Professionals, Department of Agriculture

⁴ Suren Cooke Agencies, Deans Road, Colombo, Sri Lanka

ABSTRACT

In response to Sri Lanka's commitment to develop alternatives to minimize the use of Methyl Bromide (MeBr) and to prevent risks involved in the use of metallic phosphides, the National Plant Quarantine Service (NPQS) initiated research programs to introduce safer and user friendly fumigation techniques for the control of pests of food products in accordance with the international standards. ECO2FUME, a cylinderized gas formulation of 2% Phosphine and 98% Carbon Dioxide by weight, is recognized as an alternate fumigant to MeBr in grain fumigation and its applications have being extended to quarantine purposes in fruits and vegetables globally. One of the added advantages of ECO2FUME is the ability to maintain the desired concentration during the fumigation when compared to fumigation with metal Phosphides. As an initial step, ECO2FUME, was tested against rice (*Oryza sativa* L) grains infested with different life stages of laboratory reared rice weevil (*Sitophilus oryzae*), bitter gourd (*Momordica charantia*) artificially infested with different life stages of melon fly (*Bactrocera cucurbitae*), and export quality Pineapple (*Ananas comosus*)(variety 'Mauritius') moderately infested with Mealy bugs (*Dysmicoccus brevipes*). Standard

protocols developed by the manufacturer were followed in testing the efficacy of the product against those pests. Among the different dose/exposure time regimes tested, 700 ppm /36 h for the control of rice weevil in rice, 300 ppm /7 h for mealy bugs in pineapple and 1,400 ppm /24 h for melon fly in bitter melon were identified as the best treatments to achieve the desired control without affecting the quality of the product.

Key words: ECO2FUME, Fumigation, Mealy bugs, Melon fly, Rice weevil

INTRODUCTION

The National Plant Quarantine Service (NPQS) is in the process of developing safer and user friendly fumigation techniques for the control of pests in foods, grains and other commodities for Quarantine and Pre-shipment (QPS) fumigation and Non-quarantine purposes. This procedure is being implemented in response to Sri Lanka's commitment to develop alternatives to minimize the use of MeBr and to prevent risks involved in the use of metallic Phosphides. At present, metal Phosphides are extensively used in Sri Lanka to control pests of stored grains. Metal Phosphides have several drawbacks like possibilities of explosions, inconvenience in handling, need for deactivation and disposal of unspent residue, resistance development in pests to phosphine and inability to top-up while fumigation to maintain the required concentration. (Bond *et al.*, 1984; Tom-Batchelor, 2004; Hills and Falls, 2012).

In addition, with the increasing global trades of perishable commodities, QPS fumigation is considered vital in terms of quality maintenance in fumigated commodities. Therefore, Liquid Phosphine (ECO2FUME: a cylinderized gas formulation of 2% phosphine and 98% carbon dioxide by weight), which is globally recognized as an alternate fumigant to Methyl Bromide (MB) in grain fumigation and to use in quarantine fumigation of fruits and vegetables, was selected for testing under local conditions (Zaebst *et al.*, 1988; Benhalima *et al.*, 2004; Tumaming *et al.*, 2012; Lee *et al.*, 2012; Juan, 2016). Moreover, it produces no waste by-products or dust residues, thus eliminating the hazardous deactivation and disposal issues, typically associated with traditional fumigants (Tumaming *et al.*, 2012). This experiment was conducted to determine the doses /time regime for ECO2FUME for post-harvest disinfestations of milled rice against Rice weevil (*Sitophilus oryzae*), Fruit flies (*Bactrocera* spp) in export quality bitter melon

and mealy bug (*Dysmicoccus brevipes*) in export quality pineapple. It is also expected that the developed protocols could be used to control pests in grains and for fumigation of bitter melon and pineapples, sent for quarantine inspection in order to ensure that these commodities exported from Sri Lanka are free from quarantine pests.

MATERIALS AND METHODS

Fumigation trials were conducted at the Treatment Technology Division of the NPQS, Katunayake, Sri Lanka from June to December 2017. The temperature and RH in the fumigation chamber ranged from 25 °C to 32 °C and 75% - 85%. The fumigation was done in 200 l capacity heavy duty plastic barrels.

Fumigation protocols for rice weevil (*Sitophilus oryzae*) in milled rice

Test insects Adult weevils of *S. oryzae*, collected from a warehouse in Gampaha, were used as mother stock of the pest. The adults were reared in 2 l plastic bottles containing milled rice. In order to obtain grains infested with eggs, larvae and pupae separately, adult weevils were introduced to three 2 l plastic bottles half filled with milled rice and kept for 5 days oviposition period as three batches namely; 42, 28 and 7 days prior to the date of fumigation.

Fumigation Procedure Rice samples (50 g) containing different life stages of the weevil (adults, pupae, larvae and eggs) were introduced separately into 50 ml plastic vials and the lids were covered with muslin cloths to prevent insects escaping from the vials. The plastic vials containing adults (50 adults/ vial), pupae, larvae, and eggs were then placed randomly inside 10 kg poly-sack bags containing milled rice as three 50 ml vials of each life stage separately (12 vials/10 kg rice bag). These poly sack bags were then kept in heavy duty plastic barrels at a rate of one per barrel.

As per the product protocols supplied by the manufacturer of ECO2FUME, 5 concentrations (140, 280, 490, 700 and 1,400 ppm)/ time regime (36 h) and 3 concentrations (2,100, 2,800 and 3,500 ppm) / time regime (24 h) were tested with two untreated controls (24 & 36 h). The adult containing vials in the treated and untreated chambers were opened two days after fumigation to count the number of dead and live adults. The vials containing pupae, larvae and eggs were kept in the laboratory under ambient conditions until adult emergence (in 42 days). The numbers of dead and live

adults in the vials kept in treated and un-treated barrels were recorded separately.

Confirmatory test for fumigation protocols for rice weevils in milled rice

Based on the results of the above experiment 700 ppm and 1,400 ppm ECO2FUME /m³ and 36 h exposure period were selected for pilot scale test against rice weevil in milled rice. Weevil infested discarded milled rice collected from ware houses (in Gampaha) were used for the fumigation. It was assumed that the discarded rice contained all life stages of weevils *i.e.* adults and rice grains infested with pupae, larvae and eggs. The infested rice was filled in 9 poly-sac bags with a capacity of 5 kg and the bags were sealed to prevent further contaminations. The poly-sac bags were placed inside three heavy duty 200 l plastic barrels at the rate of 3 bags/barrel. The treatments tested were (1) 700 ppm (50g ECO2FUME) phosphine for 36 h; (2) 1,400 ppm phosphine (100 g ECO2FUME) for 36 h; (3) untreated control. One-day after fumigation, 250 g of grain samples was withdrawn from each 5 kg poly-sac bag as 3 samples per bag and kept in 2 l plastic bottles separately in the laboratory. The live and dead adult weevils in the 2 l bottles were counted and discarded in biweekly intervals for 6 weeks. This procedure was adopted with the assumption that the live pupae, larvae and eggs in the fumigated and the control chambers would emerge within this period.

Proc Catmod was performed to check for normality and homogeneity and for data transformation for statistical analysis using Statistical Analysis Software (SAS) package version 8.2. The data on the dead and live weevils of different life stages were analyzed using chi-square <0.05 probability level and analysis of variance. The treatment means were compared by Duncan's Multiple Range Test at P<0.05 level.

Fumigation protocols for post-harvest disinfestations of pineapple against mealy bugs (*Dysmicoccus brevipes*)

A total of 90 pineapples (variety 'Mauritius'), moderately infested with Mealy bug (*D. brevipes*), was used for the experiment. The pineapples were placed inside 18 corrugated carton boxes (40 x 48 x 24 cm) at the rate of 5 fruits per box and placed them in 200 l heavy duty plastic barrels as one box per barrel. All the fruits were labeled according to the box number and fruit number and one randomly selected fruit per box was weighed. The number of mealy bugs (including colonies) in one randomly selected fruit

per box was counted. Five fumigation doses (100, 150, 200, 250, 300ppm)/ time regime (7 h) were tested with an untreated control in 3 replicates in a Randomize Complete Block Design (RCBD).

After fumigation two fruits from each box were kept at 26 °C (ambient) and the remaining 3 were stored in a cool incubator at 15 °C. One and 7 days after treatment, mealy bug populations (dead and live) were counted on randomly selected two fruits from a box – (one fruit kept under ambient condition and the other kept in the cool incubator). After counting mealy bugs, physicochemical properties of the same fruits were evaluated using the standard procedures (pH, brix value, hardness, outer and internal color, taste). After 14 days of treatment, mealy bug populations (dead and live) and physicochemical properties of the remaining fruit in the cool incubator were assessed following the same procedure.

Fumigation protocols for Mealy bug Based on the observations made from the above test, 300 ppm phosphine with 7 h exposure time was selected for confirmatory testing on mealy bug in pineapple. The testing procedure mentioned above was followed. The treatments were replicated 4 times with 5 fruits / treatment unit with an untreated control.

Proc Catmod was performed to check for normality and homogeneity and for data transformation for statistical analysis using Statistical Analysis Software (SAS) package version 8.2. The data on the dead and live mealy bugs and the fruit quality were analyzed using analysis of variance and the treatment means were compared by Duncan's Multiple Range Test and Dunnet Test (for brix Value) at $P < 0.05$ level.

Fumigation protocols for post-harvest disinfestations of melon flies (*Bactrocera cucurbitae*) in export quality bitter gourds

Preparation of insect life stages for fumigation *B. cucurbitae* colony maintained at the Horticultural Crops Research and Development Institute (HORDI) on cucurbits fruits were used to infest the bitter gourd fruits for testing. Around 130 un-ripened bitter gourds (grown for export market) were selected for the experiment. Seven, four and one day prior to fumigation, 32 un-ripened bitter gourds were separately exposed to adult

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melon flies for 1-day for egg laying. We expected that these bitter gourds would contain respectively, 5- 6-day-old mature larvae, 2-3 day-old young larvae and 1-day-old eggs by the day of fumigation. Same numbers of un-infested bitter gourds were used as the control.

Fumigation procedure As per the product protocols supplied by the manufacturer of ECO2FUME, two concentrations (75 and 100 g) and two time regimes (24 and 36 hours) were tested with two untreated controls in 2 replicates (Williams *et al.*, 2000). The infested and un-infested fruits were loaded separately into each barrel as follows: 4 fruits containing eggs; 4 with young larvae and 4 with mature larvae; and 4 un-infested fruits (in separated plastic bags). The barrels were loaded with ECO2FUME gas at estimated concentrations and kept for the required exposure period.

Fumigation protocols for melon fly Based on the observations made in the above test 1,400 ppm phosphine and 24 h exposure time was selected for confirmatory testing for the melon fly control. The above mentioned testing procedure was followed. The treatments were replicated 4 times with 4 fruits / treatment unit. Live and dead larvae were counted 3 days after fumigation in all fruits of each treatment. The quality of the bitter gourd fruits were assessed 2 days after fumigation.

Proc Catmod was performed to check for normality and homogeneity and for data transformation for statistical analysis using Statistical Analysis Software (SAS) package version 8.2. The data on the dead and live melon fly larval stages were analyzed using chi-square <0.05 probability level and analysis of variance as appropriate. The treatment means were compared by Duncan's Multiple Range Test at P<0.05 level.

RESULTS AND DISCUSSION

Fumigation protocols for rice weevil (*Sitophilus oryzae*) in milled rice

Adult mortality in treated vials was significantly higher than the untreated control. The estimated Chi square value determined to be higher than the critical value (P<0.0001), indicating the treatment effect is significant and the likely hood ratio is 0. We observed that all adult weevils in the vials, treated with 700 – 1,400 ppm phosphine for 36 h and 2,100 – 3,500 ppm phosphine for 24 h were dead on the 2nd day after fumigation (Table 1).

No significant differences were observed among the number of adults emerged in vials (enclosed with pupae, larvae and eggs) treated with 700 – 1,400 ppm phosphine for 36 h and 2,100 – 3,500 ppm phosphine for 24 h. However it was observed a significant difference among the number of adults emerged from vials treated with 700 – 1.400 ppm phosphine for 36 h and 2,100 – 3,500 ppm phosphine for 24 h compared to the vials treated with 140- 490 ppm phosphine for 36 h. Considering the economic benefits, we selected 700 ppm (50 g ECO2FUME) / 36 h and 1,400 ppm (100 g ECO2FUME)/36 h for confirmatory testing (Table 2).

Table 1. Total number of dead and live adult weevils observed in 50 ml vials containing rice with adult weevils 2 day after fumigation with ECO2FUME (n= 12)

| Phosphine concentration (ppm)/ Exposure time (h) | Dead/ Live adult weevils (mean ±SE) observed in 200ml vials* | |
|-------------------------------------------------------------------------------|-----------------------------------------------------------------|------------------|
| | Dead | Live |
| 140 ppm /36 | 49±0.9 ^a | 1±0 ^a |
| 280 ppm /36 | 49± 1.0 ^a | 1±0 ^a |
| 490 ppm /36 | 49± 0.9 ^a | 1±0 ^a |
| 700 ppm /36 | 50 ^a | 0 ^b |
| 1,400 ppm /36 | 50 ^a | 0 ^b |
| 2,100 ppm / 24 | 50 ^a | 0 ^b |
| 2,800 ppm / 24 | 50 ^a | 0 ^b |
| 3,500 ppm / 24 | 50 ^a | 0 ^b |
| Control 36 | 0 ^b | 50 ^c |
| Control 24 | 0 ^b | 50 ^c |
| X ² (_{12; 0.001}) (estimated & critical pr 0.001) | 121.1 > 21.6 | 161.3 > 21.6 |

In a column means followed by same letters are not significant different at <5% probability level.

**Mean values were rounded to the nearest whole number*

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Table 2. Total number of live adult weevils observed in 2 L plastic bottles (containing rice with pupae, larvae and eggs) at 42 day after fumigation with ECO2FUME (n =12)

| Phosphine Concentration (ppm)/ Exposure time (h) | No. emerged adult weevils (mean ± SE)* | | |
|----------------------------------------------------------------|----------------------------------------|-------------------------------|-----------------------------|
| | Grain infested with pupae | Grain infested with larvae | Grain infested with eggs |
| 140 ppm /36 | 1±0 ^a | 1±0 ^a | 1±0 ^a |
| 280 ppm /36 | 1±0 ^a | 1±0 ^a | 1±0 ^a |
| 490 ppm /36 | 1±0 ^a | 1±0 ^a | 1±0 ^a |
| 700 ppm /36 | 0 ^b | 0 ^b | 0 ^b |
| 1,400 ppm /36 | 0 ^b | 0 ^b | 0 ^b |
| 2,100 ppm / 24 | 0 ^b | 0 ^b | 0 ^b |
| 2,800 ppm / 24 | 0 ^b | 0 ^b | 0 ^b |
| 3,500 ppm / 24 | 0 ^b | 0 ^b | 0 ^b |
| Control 36 | 7±1 ^c | 9±2 ^c | 6±1 ^c |
| Control 24 | 5±2 ^c | 6±1 ^c | 6±2 ^c |
| X ² _(12: 0.001) (estimated& critical) | 26.3> 13.4 | 32.4> 13.4 | 24.4 > 13.4 |

In a column means followed by same letter are not significant different at <5% probability level.

**Mean values were rounded to the nearest whole number*

In the confirmatory test, the adult mortality in treated vials was significantly higher than the untreated control and the estimated Chi square value was higher than the critical value at 0.001, indicating the treatment effect is significant and the likely hood ratio is 0. It was confirmed that both dose/ time regimes were equally effective in controlling all life stages of rice weevils (Table 3 and 4).

Table 3. Total number of live and dead weevils in the samples (250 g) drawn from the treated and untreated poly-sac bags 2 days after fumigation with ECO2FUME (n=9)

| Concentration phosphine ppm Exposure time (h) | Dead /Live adult weevils (mean ± SE)* | |
|-----------------------------------------------------------------|---------------------------------------|------------------|
| | Dead | Live |
| 700 ppm /36 h | 15± 2.4 ^a | 0 ^a |
| 1,400 ppm/36h | 17 ± 3.1 ^a | 0 ^a |
| Control 36h | 1± 0 ^b | 7±1 ^b |
| X ² _(12: 0.001) (estimated & critical) | | 32.3 > 11.3 |

In a column means followed by same letter are not significant different at <5% probability level.

**Mean values were rounded to the nearest whole number*

Table 4. Total number of dead and live adults (mean \pm SE)observed in 250 g samples drawn from poly-sack bags in treated and untreated chambers 14, 28 and 42 days after fumigation with ECO2FUME (n= 9)

| Concentration phosphine ppm/ Exposure time (h) | 14d after fumigation | 28d after fumigation | 42d after fumigation |
|---------------------------------------------------------|------------------------------|---------------------------|----------------------------------|
| | Dead/Live* | Dead/Live* | Dead/Live* |
| 700 ppm /36h | 23 \pm 4.1 /0 ^a | 0/0 ^a | 0/0 ^a |
| 1,400ppm /36h | 31 \pm 2.5 /0 ^a | 0/0 ^a | 0/0 ^a |
| Control 36h | 0/21 \pm 2 ^b | 0/14 \pm 1 ^b | 1 \pm 1/7 \pm 1 ^b |
| X ² (12: 0.001) (estimated & critical) | 25.6 > 12.1 | 34.5 > 12.1 | 24.3 >12.1 |

*In a column means followed by same letter are not significant different at <5% probability level.
* Mean values were rounded to the nearest whole number*

Fumigation protocols for post-harvest disinfestations of pineapple against mealy bugs (*Dysmicoccus brevipes*)

The pineapples were found to contain an average of 22.2 \pm 5 mealy bug colonies/fruit before fumigation. Mealy bug populations (live) on pineapples treated with 300 ppm phosphine (21.3 g/Phosphine/ m3) and 7 h exposure were found to be significantly lower (p<0.0001) than those on other treatments and untreated control (Table 5). Hence, this treatment was selected for confirmatory tests.

Table 5. Mean number of dead and live mealy bugs on pineapples treated with different dosages and time regimes and stored at 15 °C and ambient temperature (26 °C) , 1, 7 and 14 days after fumigation with ECO2FUME

| Phosphine ppm | 1 day after | | | | 7 days after | | | | 14 days after | |
|---------------|--------------------------|------------------------|---------------------------|--------------------------|--------------------------|----------------------------|--------------------------|---------------------------|---------------------------|------|
| | 15 °C | | Amb. T | | 15 °C | | Amb. T | | 15 °C | |
| | Dead | Live | Dead | Live | Dead | Live | Dead | Live | Dead | Live |
| 100 | 13 \pm 5 ^a | 0 ^a | 48 \pm 35 ^a | 0 ^a | 16 \pm 2 ^{ab} | 3.3 \pm 0.1 ^b | 29 \pm 10 ^a | 0 ^a | 1 \pm 0 ^a | 0 |
| 150 | 23 \pm 9 ^a | 0 ^a | 13 \pm 4 ^b | 0 ^a | 27 \pm 17 ^a | 0.6 \pm 0.6 ^b | 20 \pm 10 ^a | 0 ^a | | - |
| 200 | 33 \pm 26 ^a | 1 \pm 0 ^a | 27 \pm 20 ^{ab} | 0 ^a | 38 \pm 17 ^a | 1 \pm 0 ^b | 40 \pm 8 ^a | 0.6 \pm .3 ^b | 1 \pm 0 ^b | 0 |
| 250 | 18 \pm 6 ^a | 0 ^a | 6 \pm 2.5 ^b | 0 ^a | 23 \pm 9 ^a | 0.6 \pm 0.3 ^b | 17 \pm 10 ^b | 0 ^a | | - |
| 300 | 18 \pm 7 ^a | 0 ^a | 4 \pm 1 ^b | 0 ^a | 31 \pm 28 ^a | 00 | 14 \pm 9 ^b | 0 ^a | 0 ^a | /0 |
| Cont | 7 \pm 3 ^b | 6 \pm 3 ^b | 8 \pm 5 ^b | 31 \pm 18 ^b | 8 \pm 4 ^b | 11 \pm 4 ^c | 7 \pm 2 ^b | 24 \pm 10 ^c | .6 \pm 0.6 ^b | 0 |
| CV % | 23.2 | 17.8 | 18.5 | 13.3 | 17.6 | 15.2 | 17.3 | 18.1 | 12.3 | |

*In a column means followed by same letter are not significant different at <5% probability level,
Amb. T = Ambient temperature, * Mean values were rounded to the nearest whole number*

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In the confirmatory test (live) mealy bug populations on pineapples treated with 300 ppm phosphine (21.3 g/Phosphine/ m³) and 7 h exposure were found to be significantly lower (zero) than those in untreated control (Table 6). Hence, this dose / time regime was selected as the best for post-harvest disinfestations of pineapple against mealy bugs (*D. brevipes*)

Table 6. Mean number of dead and live mealy bugs on pineapples treated with 300 ppm phosphine for 7 h exposure and stored at 15 °C and ambient temperature, 1, 7 and 14 days after fumigation with ECO2FUME

| Phosphine ppm | 1 day after* | | | | 7 days after* | | | | 14 days after* | |
|---------------|-------------------|-------------------|-------------------|--------------------|------------------|-------------------|------------------|--------------------|----------------------|-------------------|
| | 15 °C | | Amb. T | | 15 °C | | Amb. T | | 15 °C | |
| | Dead | Live | Dead | Live | Dead | Live | Dead | Live | Dead | Live |
| 300 | 56±9 ^a | 0 ^a | 47±8 ^a | 0 ^a | 0 ^a | 0 ^a | 0 ^a | 0 ^a | 0 ^a | 0 ^a |
| Contr | 3±2 ^b | 48±3 ^b | 9±2 ^b | 31±18 ^b | 3±1 ^b | 53±4 ^b | 4±1 ^b | 64±10 ^b | 0.6±0.6 ^b | 87±5 ^b |
| CV% | 11.2 | 10.8 | 11.5 | 23.3 | 17.6 | 19.6 | 13.5 | 13.7 | 10.4 | |

*In a column means followed by same letter are not significant different at <5% probability level, Amb. T = Ambient temperature, * Mean values were rounded to the nearest whole number*

In addition, the organo-leptic properties of the fruits treated with 300 ppm Phosphine for 7 h and stored in a cool cabinet under 15 °C and under ambient temperature were found to be equally superior as those in the untreated control (Table 7 a,b,c,d). No significant differences among the Brix values, pH value, stiffness value and taste / appearance was observed among the concentrations.

Table 7a. Pineapple fruit quality (Brix Value) 1, 7 and 14 days after fumigation with ECO2FUME and stored under 15 °C and under ambient temperature

| Dose phosphine PPP | Brix value* | | | | |
|-----------------------|-------------|----------|--------------|----------|---------------|
| | 1day after | | 7 days after | | 14 days after |
| | 15 °C | Amb. T | 15 °C | Amb. T | 15 °C |
| 100 | 9.4±0.7 | 10.2±1.3 | 12.0±1.0 | 10.8±0.9 | 10.4 |
| 150 | 9.8±0.4 | 9.7±0.3 | 11.2±0.3 | 8.0±0.2 | - |
| 200 | 10.1±0.5 | 9.2±0.4 | 11.4±0.3 | 11.0 | 10.1±2.3 |
| 250 | 9.5±0.5 | 9.3±0.7 | 10.8±0.8 | 11.4 | - |
| 300 | 9.8±0.7 | 9.0±0.5 | 10±1.4 | 12.8±0.2 | 5.2 |
| Control | 8.8±0.8 | 9.3± | 11.4±0.3 | 11.4±0.2 | 7.8 |
| | Ns | Ns | Ns | Ns | Ns |
| CV % | 22.2 | 25.8 | 12.3 | 14.1 | 11.0 |

* Mean values were rounded to the nearest whole number, Amb. T = Ambien temperature

Table 7b. Pineapple fruit quality (pH value) 1, 7 and 14 days after fumigation with ECO2FUME and stored under 15 °C and under ambient temperature

| Dose phosphine PPP | pH value* | | | | |
|--------------------------|------------|----------|--------------|----------|---------------|
| | 1day after | | 7 days after | | 14 days after |
| | 15 °C | Amb. T | 15 °C | Amb. T | 15 °C |
| 100 | 3.7±0.03 | 3.7±0.03 | 3.6±0.01 | 3.8±0.03 | 3.9 |
| 150 | 3.6±0.01 | 3.7±0.01 | 3.6±0.06 | 3.8±0.02 | - |
| 200 | 3.6±0.03 | 3.7±0.04 | 3.6±0.03 | 3.8 | 3.8±0.04 |
| 250 | 3.6±0.01 | 3.7±0.03 | 3.6±0.04 | 3.8 | - |
| 300 | 3.6±0.03 | 3.7±0.02 | 3.7±0.07 | 3.8±0.01 | 3.62 |
| Control | 3.7±0.02 | 3.7±0.04 | 3.7±0.02 | 3.9±0.02 | 4.0 |
| | Ns | Ns | Ns | Ns | Ns |
| CV % | 11.6 | 10.6 | 12.3 | 14.1 | 11.0 |

* Mean values were rounded to the nearest whole number, Amb. T = Ambien temperature

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Table 7c. Pineapple fruit quality (stiffness) 1, 7 and 14 days after fumigation with ECO2FUME and stored under and under ambient temperature

| Dose phosphine PPP | Stiffness* | | | | |
|-----------------------|-------------|-----------|--------------|-----------|---------------|
| | 1 day after | | 7 days after | | 14 days after |
| | 15 °C | Ambient | 15 °C | Ambient | 15 °C |
| 100 | 4.95±0.15 | 4.4±0.56 | 4.61±0.21 | 3.53±0.07 | 2.6 |
| 150 | 4.91±0.08 | 5.0 | 3.65±0.18 | 3.85±0.13 | - |
| 200 | 4.98±0.01 | 4.96±0.01 | 4.0±0.24 | 3.75 | 2.55±0.25 |
| 250 | 5.0 | 5.0 | 4.18±0.17 | 3.55 | - |
| 300 | 5.0 | 4.96±0.03 | 4.01±0.049 | 4.05±0.1 | 2.85 |
| Control | 4.59±0.24 | 4.85±0.15 | 4.25±0.10 | 4.1 | 2.3 |
| | Ns | Ns | Ns | Ns | Ns |
| CV % | 12.9 | 10.1 | 12.3 | 14.1 | 11.0 |

* Mean values were rounded to the nearest whole number, Amb. T = Ambient temperature

Table 7d. Pineapple fruit quality (taste / appearance) 1, 7 and 14 days after fumigation with ECO2FUME and stored under 15 °C and under ambient temperature

| Dose phosphine PPP | Fruit quality | | | | |
|-----------------------|---------------|--------|--------------|--------|---------------|
| | 1 day after | | 7 days after | | 14 days after |
| | 15 °C | Amb. T | 15 °C | Amb. T | 15 °C |
| 100 | Good | Good | Good | Rotten | Good |
| 150 | Good | Good | Good | Rotten | Good |
| 200 | Good | Good | Good | Rotten | Good |
| 250 | Good | Good | Good | Good | Good |
| Control | Good | Good | Good | Rotten | Good |

Amb. T = Ambient temperature

Based on the above observations, 300ppm phosphine and 7h exposure and storage under 15 °C can be recommended for the quarantine treatment of pineapple for mealy bug control without any quality deterioration.

Fumigation protocols for post-harvest disinfestations of melon flies (*Bactrocera cucurbitae*) in export quality bitter gourds

No larvae were observed 3 days after fumigation in the un-infested bitter gourd samples in treated and untreated containers, indicating that the field collected

samples were free from melon fly damage (Table 8). The estimated Chi square value for mortality of eggs, young larvae and mature larvae were greater than the critical values indicating the treatment effects were significant at P 0.0001 with a likely hood ratio is 0. We observed a few live larvae in bitter gourds treated with 1,000 ppm and 36 h exposure. Eggs, young and old larvae in cucurbits treated with 1,400 ppm for 24 h found dead at the time of inspection, indicating that this concentration is effective in controlling all life stages of melon fly.

Table 8. Dead / live larvae in treated and untreated bitter gourds fruits at 1 and 3 days after fumigation with ECO2FUME (4 fruits / sample)

| Treatment Phosphine ppm / Exposure time (h) | Dead/ live larvae \pm SE * | | | | | | | |
|---------------------------------------------------------------------|------------------------------|------|--------------------------|---------------------------|--------------------------------------------|--------------------------|----------------------------------------------|--------------------------|
| | Un-infested | | Infested with eggs | | Infested with 3 day old young larvae | | Infested with 6 day old mature larvae) | |
| | Dead | Live | Dead | Live | Dead | Live | Dead | Live |
| 1,000 ppm; 36h | 0 | 0 | 65 \pm 18 ^a | 2.0 \pm 11 ^b | 27 \pm 9 ^a | 0 ^a | 38 \pm 11 ^a | 0 ^a |
| 1,400 ppm; 24h | 0 | 0 | 47 \pm 11 ^a | 0 ^a | 68 \pm 6 ^a | 0 ^a | 59 \pm 9 ^a | /0 ^a |
| untreated control 36h | 0 | 0 | 4 \pm 1 ^b | 82 \pm 4 ^c | 0 ^b | 43 \pm 3 ^b | 0 ^b | 76 \pm 56 ^b |
| untreated Control 24h | 0 | 0 | 1 \pm 1 ^b | 28 \pm 7 ^c | 0 ^b | 69 \pm 21 ^b | 0 ^b | 95 \pm 8 ^b |
| X ² (_{10: 0.001}) estimated & critical) | | | 144.7> | 112.7> | 144.7> | 222.4> | 144.7> | 131.4> |
| | | | 23.2 | 14.1 | 23.2 | 23.2 | 23.2 | 23.2 |

* Mean values were rounded to the nearest whole number

It was observed that the un-infested fruits taken from the field (grown for export market) were free from melon fly damage (Table 9). The estimated Chi square value for mortality of eggs, young larvae and mature larvae were greater than the critical values, indicating the treatment effects were significant at P= 0.0001 with a likely hood ratio is 0. The treated fruits, having eggs, young and old larvae showed 100% mortality indicating that the treatment schedule was effective in eliminating all life stages of the melon fly. The appearance (color /hardness) of the infested and un-infested fruits subjected to ECO2FUME fumigation found to be better than those in the untreated control. This could be partly due to the influence of CO₂ (reducing the respiration) and the indirect effect of the melon fly damage (Table 10).

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Table 9. Dead and total larval counts (mean ±SD) of the bitter gourds in treated and untreated fruits 1 and 3 days after fumigation in confirmatory testing (mean values were rounded to the nearest whole number)

| Treatment Phosphine ppm / Exposure time (h) | No. dead / live larvae* | | | |
|---------------------------------------------------------|-------------------------|-----------------------|-----------------------------|---------------------------|
| | Un- infested fruits | Infested with eggs | Infested to young larvae | Infested to old larvae |
| 1,400 ppm/ 24h exposure | 0 | 0/0 | 20±1.2/0 | 9±1.1/0 |
| Untreated control | 0 | 0/48±5.6 | 6±0.9/50±3.9 | 1±0/40±4.0 |
| X ² (10: 0.001) (estimated & critical) | | 101.7> | 98.2 > | 86.4> |
| | | 11.3 | 11.3 | 11.3 |

* Mean values were rounded to the nearest whole number

Table 10. Fruit quality of the bitter gourds in treated and untreated fruits 2 days after fumigation with ECO2FUME

| Treatment Phosphine ppm / Exposure time (h) | Un-infested fruits | Infested with eggs | Infested to young larvae | Infested to mature larvae |
|------------------------------------------------|-----------------------|-----------------------|-----------------------------|------------------------------|
| 1,400 ppm/24h exposure | fresh/ green | fresh/ green | slightly fresh | Part rotten |
| Untreated control | yellowing | part Rotten | Rotten | Rotten |

CONCLUSION

The following dose / time regimes of ECO2FUME can be recommended for the fumigation of rice for the control of Rice Weevil (*Sitophilus oryzae*), pineapple for the control of Mealy bugs (*Dysmicoccus brevipes*) and bitter gourd for the control of Melon Fly (*Bractocera cucurbitae*); 700 ppm (50 g ECO2FUME/m³) /36 h, 300 ppm (21.2g ECO2FUME/m³) /7 h 1,400 ppm (100 g ECO2FUME/m³) /24 h, respectively.

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