Studies on the effect of Carbon dioxide and Phosphine to Control Tribolium castaneum.H

R.Meenatchi[1], R.Brimapureeswaran[2], K.Alagusundaram[3], Alice R.P.Sujeetha[4] and M. Loganathan[5]
[1] Assist Professor, Institute of Crop Processing Technology, Thanjavur – 613 005
[2]Senior Research Fellow, Indian Institute of Crop Processing Technology, Thanjavur – 613 005
[3]Deputy Director General ICAR, New Delhi
[4]Associate Professor, Indian Institute of Crop Processing Technology, Thanjavur – 613 005,
[5]Associate Professor, Indian Institute of Crop Processing Technology, Thanjavur – 613 005

Abstract
Laboratory experiments were undertaken in a fumigation chamber to study the effect of carbon dioxide and phosphine combinations on different stages of Tribolium castaneum. Mortality of different stages of Tribolium castaneum (Herbst) was recorded after exposure for different times to the application rate of 98% CO_2 with phosphine at 3 g/m^3, 2 g/m^3, 1g/m^3 and 3g/m^3 phosphine alone as control. Mortality of all insects increased with increased exposure time. Tribolium castaneum old larva was more resistant than young larva. Tribolium egg and pupa was resistant than larva and adult stages.

Keywords: Carbon dioxide, *T. castaneum*, Fumigant toxicity, Phosphine, Synergistic effect.

I. INTRODUCTION

Red and confused flour beetles attack stored grain products such as flour, cereals, meal, crackers, beans, spices, pasta, cake mix, dried pet food, chocolate, nuts, seeds, and even dried museum specimens (Via 1999, Weston and Rattlingourd, 2000). The red flour beetle has developed resistance against a variety of insecticides in a number of Asian countries (CABI, 2007). Phosphine is the widely used fumigant against red flour beetle. Indiscriminate use of insecticides has led to the development of insecticide resistant strains of stored-product insects as well as insecticide residue problems in food grains (Bhatia, 1990). More recently, the worldwide phase out and ban of the fumigant insecticide methyl bromide, under the international agreement of the Montreal Protocol motivates the research into the search of alternative fumigants (Fields and White, 2002).

Carbon dioxide, a respiratory stimulant is a known adjuvant for fumigants including phosphine and methyl bromide. The advantages of using CO_2 in the mixture are to increase the toxicity of the fumigant, improve the distribution pattern and limit the levels of harmful residues in the treated commodity. Several studies on phosphine /CO_2 mixtures have been made in the past (Jones, 1938) and these were followed by investigations which showed that the addition of CO_2 to methyl bromide (MB) and phosphine (PH3) resulted in an increase in the susceptibility of some stored-product insects (Calderon and Leesch, 1983; Williams, 1985). The present study was undertaken at Indian Institute of Crop Processing Technology, Thanjavur during 2014-15. The study was conducted using 98% CO_2 with phosphine at 3 g/m^3, 2 g/m^3, 1 g/m^3 and phosphine alone @ 3 g/m^3. Mortality of various stages of T. *castaneum* at different time intervals were recorded.

II. METHODS AND MATERIALS

A. Culturing of Red flour beetle

Red flour beetle, T. castaneum was cultured and maintained in the Storage Entomology Lab, at IICPT, Thanjavur. Insects were cultured on whole maize and wheat flour at 30° C and 70% RH. Different stages of insects such as egg, pupa, young larva, old larva and adults were maintained separately to carry out mortality studies.

B. Lab model fumigation set up for toxicity studies

Phosphine fumigation lab model set up was designed and fabricated in the IICPT workshop. It consists of a circular outer acrylic cylindrical tube with the dimension of 37 x 27 cm (ht x dia) which is pasted in a flat acrylic tube at the bottom. Inside the fumigation chamber, four compartments were made by partitioning it with wire mesh and were fitted in a stand. Fumigation cups of 4 cm height and 6 cm diameter were made by cutting small acrylic tubes and are pasted with fine wire mesh at the bottom to carry insect mortality studies. At the top cover of the fumigation chamber, PVC end cap was used to make the set up air tight. Rubber septum is placed in the top cover for injecting phosphine. Provision for CO_2 gas inlet and outlet were also given for injecting and releasing CO_2 from the cylinder.

C. Bioassay studies

Fifteen grams of the food material was taken in fumigation cups and 10 insects of various life stages were released and were secured with rubber band. After the treatment the insects were transferred with food material and are kept in environmental chamber at 25ºC and 60 per cent RH. The number of insects dead after fumigation at different time intervals were recorded and the percent mortality was calculated. Empty space test was conducted for different stages of insects to determine the effective combination of gases on the mortality of T. castaneum.

D. Statistical analysis

The mortality (%) was calculated for different combination of phosphine and CO_2 viz., 98 % CO_2 +3g/m^3 phosphine, 98% CO_2+2g/m^3 phosphine, 98% CO_2 + 1g/m^3 phosphine and 3g/m^3 phosphine (control) were used in the present study. Probit analysis using Polo plus software (V.2.0) with time as explanatory variable to derive the estimated hours for mortality (LT_{50} and LT_{99}) (LeOra , 1989).

III. RESULTS AND DISCUSSION

The LT_{50} and LT_{99} values for T. castaneum adults are presented in Table 1. The LT_{50} and LT_{99} values for eggs, old larvae, young larva, pupae, and adults are suitable to compare with different combinations of phosphine and CO_2 treatments with respect to time to produce maximal mortality (95% for confidential limit) for different life stages. The LT_{50} values for adults (Table 1) indicate that an atmosphere containing 98 % CO_2+ phosphine 3g/m^3 was most toxic to adults followed by 2g/m^3 and 1g/m^3 compared with control. As the concentration of phosphine increases time taken to achieve 100 per cent mortality decreases. 98 % CO_2 + 3 g/m^3 phosphine took 28.37 hours for LT_{99} compared to 98% CO_2 + 2 g/m^3 of the Phosphine which took at 38.80 hrs. Similar results were observed for young larva and pupa. LT_{50} and LT_{99} for Tribolium young and old larva which are presented in Table.2 &3.

LT_{50} and LT_{99} for young larva was less compared to old larva for 98 % CO_2 +3g/m^3 phosphine, 98% CO_2+2g/m^3 phosphine, 98% CO_2 + 1g/m^3 phosphine and 3g/m^3 phosphine alone (1.88,2.14,1.17 &12.87, 21.49 &55.04 hrs for young larva and 24.40, 33.94, 43.98 and 63.69,72.15, 80.70 hrs for old larva. Young larva was found to be susceptible than old larva (table 2 & 3). Pupa and eggs of T. castaneum was resistant than larva and adults. LT_{99} for pupa for the treatment 98 % CO_2 + 3g/m^3 phosphine was 27.88 hrs and eggs required longer exposure time at 80.01 hrs.

IV.CONCLUSION

From the toxicity studies, it is proved that addition of CO_2 to phosphine enhances the toxicity of phosphine by increasing its concentration than either phosphine or CO_2 alone. Application of Carbon dioxide to grains in combination with phosphine reduces the dose and time required for fumigation. In the present study, 98 % CO_2 + 3 g/m^3 phosphine and 98 % CO_2 + 2 g/m^3 phosphine could achieve 100% mortality to all stages of Tribolium castaneum in 5 days fumigation period instead of 7 days as a normal practice in warehouses. Synergistic effect was observed when CO_2 was added with phosphine than sole application of Phosphine alone. Addition of CO_2 reduces the time and dose required for phosphine fumigation.

Table 1. Mortality of *Tribolium castaneum* adults at different exposure times

Treatments	LT_{50} (hrs) (95% confidential limit)	LT_{99} (hrs) (95% confidential limit)
T1- 98 % CO_2 + 3 g/m^3 PH_3	3.81 (2.85 – 4.67)	**28.37** (18.80 – 59.95)
T2- 98 % CO_2 + 2 g/m^3 PH_3	4.79 (3.83 – 5.69)	38.80 (25.29 – 81.89)
T3- 98 % CO_2 + 1 g/m^3 PH_3	5.21 (4.12 – 6.23)	52.97 (33.18 – 99.68)
T4- 3 g/m^3 PH_3	4.94 (3.87 – 5.96)	50.71 (30.49 – 89.37)

Numbers in brackets give the 95% confidence range

Table 2. Mortality of *Tribolium castaneum* young larva at different exposure times

Treatments	LT_{50} (hrs) (95% confidential limit)	LT_{99} (hrs) (95% confidential limit)
T1- 98 % CO_2 + 3 g/m^3 PH_3	1.88 (1.23 – 2.41)	**12.87** (9.08 – 25.00)
T2- 98 % CO_2 + 2 g/m^3 PH_3	2.14 (1.35 – 2.76)	21.49 (13.63 – 53.74)
T3- 98 % CO_2 + 1 g/m^3 PH_3	1.17 (0.25 – 2.03)	55.04 (23.96 – 61..21)
T4- 3 g/m^3 PH_3	2.04 (1.16 – 2.77)	32.25 (19.05 – 54.15)

Numbers in brackets give the 95% confidence range

Table 3. Mortality of *Tribolium castaneum* old larva at different exposure times

Treatments	LT_{50} (hrs) (95% confidential limit)	LT_{99} (hrs) (95% confidential limit)
T1- 98 % CO_2 + 3 g/m^3 PH_3	4.29 (3.60 – 4.95)	**27.88** (19.81 – 48.23)
T2- 98 % CO_2 + 2 g/m^3 PH_3	5.87 (4.97 – 6.80)	45.84 (30.44 – 89.74)
T3- 98 % CO_2 + 1 g/m^3 PH_3	5.18 (4.18 – 6.17)	47.72 (29.74 – 110.14)
T4- g/m^3 PH_3	4.99 (4.21 – 5.79)	38.46 (25.56 – 76.39)

Numbers in brackets give the 95% confidence range

Table 4. Mortality of *Tribolium castaneum* pupa at different exposure times

Treatments	LT_{50} (hrs) (95% confidential limit)	LT_{99} (hrs) (95% confidential limit)
T1- 98 % CO_2 + 3 g/m^3 PH_3	24.40 (23.06 – 55.50)	**63.69** (53.81 – 79.29)
T2- 98 % CO_2 + 2 g/m^3 PH_3	33.94 (28.62 – 45.08)	72.15 (54.51 – 88.03)
T3- 98 % CO_2 + 1 g/m^3 PH_3	43.98 (34.58 –65.86)	80.70 (70.63 – 98.98)
T4- 3 g/m^3 PH_3	39.97 (33.03 – 54.81)	70.03 (59.73 – 87.05)

Numbers in brackets give the 95% confidence range

Table 5. Mortality of *Tribolium castaneum* egg at different exposure times

Treatments	LT_{50} (hrs) (95% confidential limit)	LT_{99} (hrs) (95% confidential limit)
T1- 98 % CO_2 + 3 g/m^3 PH_3	44..23 (33.76 – 55.58)	80.01 (73.81 – 106.29)
T2- 98 % CO_2 + 2 g/m^3 PH_3	60.68 (52.62 – 75.06)	95.15 (74.51 –118.60)
T3- 98 % CO_2 + 1 g/m^3 PH_3	71.97 (61.46 – 85.94)	102.70 (87.63 – 112.98)
T4- 3 g/m^3 PH_3	69.39 (58.03 – 84.88)	97.03 (82.73 –134.71)

Numbers in brackets give the 95% confidence range

(Aliniaz cent mortaee, 1971) reported that 100% CO_2 was more lethal to T. confusum and T. castaneum adults than 45–80% CO2. In addition, 92% CO2 atmosphere was more fatal to Lasioderma. serricorne larvae and adults than 35% CO_2 atmosphere. Therefore, susceptibility of insects to controlled atmospheres was dependent on the type of atmospheres, the insect species, and the developmental stage. Other studies have shown that the admixture of CO_2 could increase the toxicity of fumigants, mainly Methyl Bromide and phosphine (Monro et al., 1966; Dumas et al., 1969; Calderon and Leesch, 1983; Williams, 1985; Donahaye and Navarro, 1989).

The use of high concentration of CO_2 appears to have a synergistic effect on these species as evidenced by significant decrements in LC_{50} and LC_{90} values for the adults and pupae. These results indicate that combination of CO_2 with other methods are more effective and CO_2 can act as an alternative to most commonly used commercial fumigants, methyl bromide and phosphine.

Acknowledgements

Authors would like to thank SERB, DST and MoFPI for funding the project and extends the sincere thanks to the Director, Indian institute of Crop Processing Technology, Thanjavur for encouragement and support for the successful completion of the project.

References

[1] Via S. Cannibalism facilitates the use of a novel environment in the flour beetle, Tribolium castaneum. Heredity, 1999, 82: 267-275.

[2] Weston PA, Rattlingourd PL. Progeny production by Tribolium castaneum (Coleoptera: Tenebrionidae) and Oryzaephilus surinamensis (Coleoptera: Silvanidae) on maize previously infested by Sitotroga cerealla(Lepidoptera: Gelechiidae) Journal of Economic Entomology, 2000, 93: 533-536.

[3] CABI. Tribolium castaneum (red flour beetle) datasheet. Crop Protection Compendium, 2007 Edition. CAB International Publishing. Wallingford, UK.

[4] Bhatia, S. K. Development of resistance to insecticides. In: Proceedings of Regional Workshop on Warehouse Management of Stored Food grains, Ministry of Food and Civil Supplies, New Delhi, Govt. of India, 1990, pp. 183-186.

[5] Fields, P. G. and White, N. D. G. Alternatives to methyl bromide treatments for stored-product and quarantine insects. Annu. Rev. Entomol. 2002, 47:331–59.

[6] Jones, R.M. Toxicity of fumigant CO_2 mixture to the red flour beetle. Journal of Economic Entomology, 1938. 31, 298-309.

[7] Calderon, M., Leesch, J.G. Effect of reduced pressure and CO2 on the toxicity of methyl bromide to two species of stored product insects. Journal of Economic Entomology, 1983,76, 1125-1128.

[8] Williams, P. Toxicity of methyl bromide in carbon dioxide enriched atmospheres to beetles attacking stored grain. General Applied Entomology, 1985, 17, 17-24.

[9] LeOra Software. POLO-PC: A Users' Guide to Probit or Logit Analysis. LeOra Software, 1987, Berkeley, CA, USA.

[10] Aliniazee MT. The effect of carbon dioxide gas alone or in combinations on the mortality of Tribolium castaneum (Herbst) and T. confusum du Val (Coleoptera, Tenebrionidae). J Stored Prod Res, 1971, 7, 243–52.

[11] Monro, H.A.U., Dumas, T., Buckland, C.TThe influence of vapour pressure of different fumigants on the mortality of two stored-product insects in vacuum fumigation. Journal of Stored Products Research,1966, 1, 207-222.

[12] Dumas, T., Buckland, C.T., Monro, H.A.UThe respiration of insects at reduced pressures. II. The uptake of oxygen by Tenebroides mauritanicus. Entomologica Experimentalis et Applicita 12, 389-402. EPA. 2001. Protection of stratospheric ozone: process for exempting quarantine and pre-shipment application of methyl bromide. United States Environmental Protection Agency, Federal Register, 1969, 66, 37752-37769.

[13] Donahaye, E., Navarro, S. Sensitivity of two dried fruit pests to methyl bromide alone, and in combination with carbon dioxide or under reduced pressure. Tropical Science, 1989, 29, 9-14.