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# EFFECT OF SMOKE ON *RHYZOPERTHA DOMINICA* AND *CALLOSOBRUCHUS MACULATUS* MORTALITY AND ITS SUSCEPTIBILITY TO PHOSPHINE

S.N. SINHA,[1]* S.P. SINGH[2] AND C. SRIVASTAVA[3]

[1]*Indian Agricultural Research Institute, Regional Station, Kamal 132001, India*
*[*e-mail:snsinha@nda.vsnl.net.in]*
[2]*Dep. of Bio Sciences, Inst. of Pesticide Formulation Technology, Gurgaon, 122001, India*
[3]*Div. of Entomology, Indian agricultural Research Institute, New Delhi 110012, India*

## ABSTRACT

Smoke generated by burning cow dung cake containing ~ 4% $CO_2$ gave 31.5, 32.0, 37.8, 57.8 and 80.0% mortality of adult *Callosobruchus maculatus* at 24, 48, 72, 96 and 120 h exposure, respectively. *Rhyzopertha dominica* appeared to be tolerant to smoke because only 32.5% of adults were killed after 120 h of exposure. Pre-treatment with smoke for 24 h increased susceptibility of *R. dominica* to $PH_3$. Only 40% of the *R. dominica* adults were killed with 1 g $m^{-3}$ $PH_3$ at 24 h exposure whereas complete mortality of the adults was achieved when insects were exposed to smoke for 24 h prior to $PH_3$ treatment. Mortality of *C maculatus* adults was found to be independent of smoke treatment using the same dose and exposure period of $PH_3$.

## INTRODUCTION

The lesser grain borer, *Rhyzopertha dominica* and the pulse beetle, *Callosobruchus maculatus* are two of the most important storage insects infesting a large number of cereal and pulse seeds. Even a single pair of pulse beetle per kg is sufficient to inflict injury to pulse seed above the minimum certification standard of 1.0% (NSP Annual Report 1999).

Regular use of DDVP, malathion and deltamethrin as space and residual insecticidal treatments of godowns (warehouses), and phosphine ($PH_3$) as a fumigant have resulted in the development of resistance in the Karnal strain of *R. dominica* collected from seed godowns (Saxena *et al.,* 1999; Srivastava *et al.,* 2000). Occurrence of resistance to $PH_3$ in different countries has been reported earlier (Champ and Dyte, 1976), while Taylor (1986), and Mills (1986), found a high incidence, and a high level of resistance, (10-fold), to $PH_3$ in *R. dominica*. Recently a field strain of *R. dominica* in the Indian sub-continent has been found to show an even higher degree of resistance to $PH_3$ (Champ, 1986; Alam *et al*., 1993; Rajendran *et al*., 2000). Varying degrees of resistance in field populations of *R. dominica*

collected from seed godowns have been detected (NSP report, 1999) together with a breakdown of well established control measures against this species.

Phosphine is the safest of all the fumigants for seeds (Strong and Lindgren, 1960; Zutshi, 1966; Ahmed, 1976; Krishnasamy and Seshu, 1990). Four repeated fumigations of wheat seed did not show any adverse effect on germination and vigour of wheat seed (Singh *et al*., 1999). However, growing resistance to $PH_3$ in various stored-product insect pests has become a major threat to the safe storage of seed.

Carbon dioxide ($CO_2$) has been used for killing insects in stored products (Jay and Pearman, 1973; Bailey and Banks, 1980; Jay, 1986; Kshrishnamurthy *et al*., 1986; White *et al*., 1990). It has also been used in combination with $PH_3$ to improve its efficacy against tolerant insects (Mueller, 1994; Kashi and Bond, 1975; Athie *et al*, 1998). Calderon and Navarro (1979; 1980) reported the synergistic effect of $CO_2$ to low oxygen ($O_2$) atmospheres on adult mortality of *R. dominica* and *Tribolium castaneum*. Jay (1971) described various methods of applying $CO_2$ for insect control in stored grain. Selection of the appropriate application technique is influenced by several factors including the cost of liquid $CO_2$, storage tank and vaporization equipment.

Smoke is the cheapest source of $CO_2$. Kitchen smoke has been in age-long use in Indian villages to protect corn seeds from insect pests from one season to another. Therefore, the present study was undertaken to determine the toxicity of smoke to *R. dominica* and *C. maculatus* and its effect on a tolerant field strain of *R. dominica* and a laboratory cultured strain *C. maculatus* to $PH_3$.

## MATERIALS AND METHODS

### Rearing of test insects for bioassay

Adults of *R. dominica* were collected from the seed godowns at the Indian Agricultural Research Institute (IARI) Regional Station, Karnal. Cultures were maintained on *T. aestivum* wheat that was sterilized at 60ºC for 5 h and conditioned for a week in a desiccator maintained at 75% r.h. to increase the moisture content (m.c.) to 12-14%. Adults of *C. maculatus* were obtained from the culture maintained at the Division of Entomology IARI, New Delhi and were reared on green gram unexposed to insecticides. Both the test insects were maintained at 27±1ºC and 70% r.h.. These insects were used in the toxicological studies at IARI, New Delhi.

For field studies, *R. dominica* collected from seed godowns was reared at the IARI Regional Station, Karnal, on wheat under ambient conditions in the laboratory. The *C. maculatus* culture obtained from the Division of Entomology, IARI, New Delhi, was propagated and maintained at Karnal under laboratory conditions.

### Smoke generation and treatment

Burning cow dung cake was used to generate the smoke with the help of a simple device. A PVC pipe 10 cm in diameter and 100 cm long attached to a smoke

chamber of 1 $m^3$ volume were used for conducting the field-assays. A small hole bored in the PVC pipe was used to collect smoke samples for $CO_2$ measurement. In each set of treatments, 20 adult insects 8-10 d old for *R. dominica* and 1-2 d old for *C. maculatus* were placed in plastic vials (4 cm x 2.5 cm). Four replications were used for each treatment with an untreated control vial for each set. The differences in mortality between the treated vials and the untreated control were taken as real mortality. In all the vials 5 g of whole wheat or green gram were provided as food medium.

**Field and laboratory testing of phosphine**

Phosphine fumigations at doses of 0.5 g/$m^3$ and 1.0 g/$m^3$ were carried out in the 1 $m^3$ fumigation chamber. The experiments were conducted at ambient conditions with average air temperatures of 32-35ºC and an r.h. of 70% prevailing during the experimental period. The Karnal strain of *R. dominica* used in this study is known to have a 4.64-fold resistance to $PH_3$ (Saxena *et al.*, 1999) whereas the *C. maculates* obtained from, IARI, had not been previously tested for resistance. Fresh adults and adults previously exposed to smoke for 24 h were used to test their susceptibility to $PH_3$. The insects were smoked in a leak-proof PVC tube connected with the smoke chamber The smoke temperature was 30-32ºC. Smoked and fresh (non-smoked) insects were used for testing their susceptibilities to $PH_3$.

**Bioassay of phosphine**

The sensitivities to $PH_3$ of smoked and fresh field populations of adult *R. dominica* were evaluated at the Division of Entomology, IARI, New Delhi. $PH_3$ gas was generated by decomposition of aluminum phosphide tablets obtained from M/S Excell Industries Ltd. under the trade name of "Celphos". The air and fumigant mixture was prepared in a 10 -L flask fitted with a ground-glass stopper by placing a 3 g tablet, which on decomposition generates 1 g of $PH_3$ gas. The required doses of air-fumigant mixture were calculated and transferred to 250 mL fumigation flasks using the method described by Pradhan and Govindan (1953) with some minor modifications necessary to ensure accurate transfer of the air fumigant mixtures.

Thirty adult test-insect adults of both species at the same age groups as in the smoke-generation trials, were introduced into the fumigation flasks.. Three replicates were made for each concentration A paper strip with many folds was placed in each flask for insects to rest upon. The flasks were then closed with tapped ground-glass stoppers. The required doses of air-fumigant mixture, prepared as described above, were then introduced into the fumigation flasks through the taps. The insects were exposed for 24 h and then removed to petri dishes each containing a folded paper strip. Insects were allowed 48 h to recover before mortality was recorded. Percentage mortalities in the treatments were corrected for natural mortality in the control according to the formula of Abbott (1925). Then the data were subjected to Probit analysis (Finney, (1971) for calculating $LD_{50}$ levels.

## RESULTS AND DISCUSSION

### Sensitivity to smoke

It is evident from Table 1 that there was a significant effect of smoke on the mortality of *C. maculatus*, reaching 80% mortality after 120 h exposure. However, *R. dominica* appeared to be more tolerant to smoke when compared to *C. maculatus,* with only 32.5% of adults killed after 120 h exposure. The $CO_2$ measured in the smoke ranged between 3.6 to 4.2%. Other gasses such as methane, and $O_2$ were not measured. The results indicate that smoke may significantly reduce populations of *C. maculatus* in traditional storage. Storey (1978) reported that adults of *C. maculatus* when exposed to 1% $0_2$, 9-10% $CO_2$ with the rest nitrogen ($N_2$) required only two days exposure to reach 100% kill.

TABLE 1
Effect of smoke on the mortality of *Callosobruchus maculatus* and *Rhyzopertha dominica* adults

| Insects | Per cent mortality* (hours after treatment) | | | | |
|---|---|---|---|---|---|
| | 24 | 48 | 72 | 96 | 120 |
| *C. maculatus* | 33.75±2.72 | 37.5±4.04 | 45±6.50 | 72.5±9.74 | 80.0 |
| *R. dominica* | 3.75±1.25 | 6.25±1.25 | 13.75±3.75 | 27.5±2.50 | 32.5±1.50 |

* Mean of three experiments each with four replicates containing 20 insects.

### Sensitization of phosphine toxicity by smoke

Smoke treatments for 24 h appeared to increase the susceptibility of *R. dominica* to $PH_3$, reducing considerably the exposure time required to control the insects. The findings given in Table 2, show that 1 g $PH_3/m^3$ gave 40 to 52% kill of non-smoked *R. dominica* adults within 24 h exposure while total kill was obtained at the same dose when the insects were exposed to smoke for 24 h prior to $PH_3$ treatment. However, in the case of *C. maculatus* adults, exposed to $PH_3$, mortality under the experimental conditions was found to be independent of previous smoke treatment since complete kill of both non-smoked and smoked *C. maculatus* adults was recorded within 24 h of exposure to $PH_3$ at both dosages, showing that *C. maculatus* adults are highly susceptible to $PH_3$.

The toxicity of smoke on field and susceptible strains of *R. dominica* adults was found to be 14.5% and 11.5%, respectively after 24 h exposure (Table 3). Therefore, the remaining live insects were bio-assayed with $PH_3$. Susceptibility of $PH_3$ smoked live insects and non-smoked insects to $PH_3$ (Table 4) showed that the $LD_{50}$ of $PH_3$ for *R. dominica* Karnal strain with and without previous smoke treatments were 0.264 and 0.413 mg/L, respectively, thus clearly showing that after the smoke treatment, susceptibility of the insects to $PH_3$ was increased. Similar findings were obtained with the susceptible strain of *R. dominica*. Due to the high mortality of smoked

*C. maculatus*, sufficient live beetles were not available for bioassay with $PH_3$. However without the smoke treatment, the Karnal and susceptible strains of *C. maculatus* showed $LD_{50}$ of 0.199 and 0.156 mg/L, respectively. It may be noted that Athie *et al.*, (1998) also reported that mixtures of $PH_3$ plus 10 and 20% $CO_2$ reduced the resistance levels of *R. dominica* and *S. oryzae*.

TABLE 2
Effect of smoke on the susceptibility of *Rhyzopertha dominica* and *Callosobruchus maculatus* adults to phosphine

| Insect species | Dose of $PH_3$ ($g/m^3$) for 24 h exposure | Duration of smoke treatment (in h) | Mean adult mortality (%) 1999* | 2000** |
|---|---|---|---|---|
| R. dominica | 0.5 | 0 | 0 | - |
| (field population) | | 24 | 7.5±4.33 | - |
| | 1.0 | 0 | 40±2.00 | 52± |
| | | 24 | 100 | 100 |
| *C. maculatus* | 0.5 | 0 | 100 | - |
| | | 24 | 100 | - |
| | 1.0 | 0 | 100 | 100 |
| | | 24 | 100 | 100 |

Mean of *8 and **12 replicates with 20 insects in each lot.

These results indicate that smoke, which is a cheap, eco-friendly and household product holds good promise in helping to control insect infestations, in addition to reducing the tolerance levels of field populations of *R. dominica* to $PH_3$. However, the method of using smoke in practice needs to be standardized. Unlike fumigants, the movement of smoke in the intergranular spaces of seeds and grain may be difficult. Other constituents of smoke may also be important and its insecticidal properties need to be more fully assessed.

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