



## RESEARCH ARTICLE

### PHOSPHOROTHIOATE DERIVATIVES OF HYDROXYBENZALDEHYDE AND THEIR TOXICITY AGAINST *RHYZOPERTHA DOMINICA* (F.) AND *TRIBOLIUM CASTANEUM* (H.)

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#### ABSTRACT

Organophosphates are commonly used for the control of insects in agriculture and public health in developing countries like India. Many of them have become less effective due to the development of resistance by the target insects. To combat this problem, novel phosphorothioate derivatives of hydroxybenzaldehyde were synthesized and screened for their toxicity to the stored product insect *Rhizopertha dominica* and *Tribolium castaneum* in comparison with the standard methyl parathion. The results indicate that the O, O-dimethyl phosphorothioates were more toxic than the diethyl derivatives and the toxicity was comparable with methyl parathion.

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## INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of major food crop including rice, sorghum, oat, pearl millet; malt barley which belongs to family Gramineae. They are well suitable for balanced diet and overall nutrition because they contain high rich proteins, vitamins and minerals etc. At the same time it is a potential breeding resources for the lesser grain borer, *Rhizopertha dominica* (F.), and the red flour beetle *Tribolium castaneum* (Herbst). The *Rhizopertha dominica* is a primary pest of stored wheat grain and *Tribolium castaneum* is a secondary pest of wheat flour in many part of the world. These insects are common and damaging pests of cereals, rice, and other substrates containing starch (Chittenden, 1991). These stored product pests are the most difficult ones to control with insecticide grain protectants including phosphine fumigation in developed and developing countries (Acda et al. 2000; Collins, 2006; Lorini and Galley, 1999; Zettler and Cuperus, 1990).

Many approved grain protectants are not effective against these insects due to the development of resistance in them, especially organophosphorus insecticides like chlorpyrifos methyl, fenitrothion, pirimiphos methyl, malathion, methoprene (Collins, 2006, Lorini and Galley, 1999; Guedes et al., 1996, 1997; Novarro et al., 1986; Zettler and Cuperus, 1990). However, organophosphorus (OP) insecticides are one of the most widely used classes of pesticides worldwide (Fulton and Key, 2001). Although they are acutely toxic to all animals, they are easily degradable in the environment, when compared with many other pesticides (Agrahari and Gopal, 2008). Currently, the main reason for developing new chemicals is to control or delay the resistance to existing pesticides, and to develop safer eco-friendly safer chemicals which are easily bio-degradable in the environment. Today, synthesis of phosphate esters, phosphorothioate is an important objective in organic synthesis. Since, they have found use in the preparation of biological active molecules (Varma et al., 1992; Smyth et al., 1992; Deloude et al., 1997; Babak and Fatemeh, 2006). These compounds have played a very important role in the molecular design and synthesis of modern effective pesticides (Burke et al., 1993, Smyth et al., 1994; Benayound et al., 1996). In agricultural science, novel derivatives of Strobilurins,

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Benzolphenylureas and other phosphate esters possess a wide range of bioactivities (Zhang *et al.*, 2013). In recent years a number of the salicylaldehyde derivatives have been introduced as potential antimicrobial agents (Martin *et al.*, 2012) chelating and extracting agents (Kurmaiah *et al.*, 1967). The derivatives of salicylaldehyde were identified as a characteristic aroma component of buckwheat (Janes and Kreft, 2008) and also an important component of Castoreum from *Castor canaadensis* and *Caster fiber* and also used as perfume. Moreover, salicylaldehyde isomers and their derivatives have been exploited as important component of the orchids like *Gastrodia elata* (Ha *et al.*, 2000). Despite their wide range of industrial, pharmacological activity and synthetic application, the synthesis of effective pesticides using salicylaldehyde and its isomers has received little attention. In view of the wide range of biological activities observed in salicylaldehyde and its isomers, it was aimed to study insecticidal activity of its phosphorothioate derivatives. In the present study we report the synthesis, characterization and insecticidal activity of phosphorothioates of the following compounds: 2-hydroxy, 3-hydroxy and 4-hydroxy benzaldehyde. Even though the preparation of dimethyl phosphorothioate of 3-hydroxy compound has been reported (Durand, G. *et al.*, 1994), this has not been assessed for its toxicity against stored-product insects. These six derivatives were screened for their toxicity towards stored product insects viz., *Rhizopertha dominica* and *Tribolium castaneum* by taking the organophosphate insecticide methyl parathion as standard test compound for comparison

## MATERIALS AND METHODS

### Instruments and reagents

2-Hydroxybenzaldehyde (salicylaldehyde), *O,O*-diethylchlorothiophosphate and *O,O*-dimethylchlorothiophosphate were purchased from Sigma Aldrich Chemical Co., Bangalore. Basic alumina was purchased from SD Fine Chemicals, Mumbai and silica gel pre-coated TLC plates of 0.2 mm thickness from Merck (Darmstadt, Germany). <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a 500 MHz Bruker Instrument with respect to TMS as reference.

### Chemical synthesis

General procedure for the preparation of phosphorothioates derivatives of hydroxybenzaldehydes Hydroxy benzaldehyde (4.88 g, 0.04 mM) was dissolved in acetone (100 mL) in a round-bottom flask. Finely powdered anhydrous potassium carbonate (10 g) was added to the solution. *O,O*-Dimethyl chlorothiophosphate (4.85 mL, 0.04 mM) /*O,O*-diethyl chlorothiophosphate (6.28 mL, 0.04mM) was added and the mixture was refluxed for 3 hours. The mixture was filtered and the organic solvent was evaporated off. TLC was done using a mixture of diethyl ether and petroleum ether (60-80°C) 15 + 85 v/v and the spots were visualized under UV light at 254 nm. The Rf value for the compound was recorded. The mixture was passed through silica gel, eluted with acetone and the solvent was evaporated off to obtain the product as an oily liquid.

The structure was characterized using NMR as given below. The reaction and structure of the phosphorothioates is given in Figure 1 and Table 1.

### Preparation of *O,O*-Dimethyl *O*-(2-formyl) phenyl phosphorothioate (1a)

Rf: 0.14, Yield: 99 % <sup>1</sup>H NMR (500 MHz) (Acetone-d<sub>6</sub>): δ 3.89 (d, 6H, *J*<sub>H-P</sub> = 13.35 Hz, P (S) (OCH<sub>3</sub>)<sub>2</sub>); 7.31-7.93 (m, 4H)Ar;10.38 (S,1H)-CHO ppm. <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 188.2 (-CHO); 187.9 C-2; 152.2 (d) C-1; 135.0 C-5; 128.5 C-3; 125.4 C-4'; 121.7 C-6; 55.2 (d) (-OCH<sub>3</sub>)<sub>2</sub> ppm.

### Preparation of *O,O*-Dimethyl *O*-(3-formyl) phenyl phosphorothioate (2a)

Rf: 0.05, Yield: 89 %  
<sup>1</sup>H NMR (500 MHz) (Acetone-d<sub>6</sub>): δ 3.88 (d, 6H, *J*<sub>H-P</sub> = 13.35 Hz, P (S)(OCH<sub>3</sub>)<sub>2</sub>); 7.44 -7.74 (m, 4H) Ar; 9.99 (S,1H)-CHO ppm. <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 190.7 (-CHO); 150.5 C-1; 137.5 C-3; 130.0 C-5; 126.7 C-4; 126.6 C-6; 120.9 C-2; 68.9 2 × -CH<sub>2</sub>-; 30.4 2 × -CH<sub>3</sub> ppm.

### Preparation of *O,O*-Dimethyl *O*-(4-formyl) phenyl phosphorothioate (3a)

Rf: 0.08, Yield: 95 % <sup>1</sup>H NMR (500 MHz): δ 9.97 (S, 1H -CHO); 7.85 (d, 2H, *J*=8.5 Hz, H3 & 5); 7.33 (d, 2H, *J*=8.5Hz, Ar H2 & 6); 3.88 d, *J*<sub>H-P</sub> = 14 Hz, 6H (-O-CH<sub>3</sub>)<sub>2</sub> ppm. <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 190.4 (-CHO); 154.9 (d) C-1; 133.2 C-4; 131.3 C-3 & C-5; 121.2 (d) C-2 & C-6; 55.0 (d) P(-OCH<sub>3</sub>)<sub>2</sub> ppm.

### Preparation of *O,O*-Diethyl *O*-(2-formyl) phenyl phosphorothioate (1b)

Rf: 0.29, Yield: 68 % <sup>1</sup>H NMR (500 MHz) (CDCl<sub>3</sub>): δ 1.37 (dt, 6H, *J*=6 Hz, *J*<sub>H-P</sub> = 1Hz) 2 × -CH<sub>3</sub> 7.30-7.92 (m, 4H Ar); 10.39 (d,1H, *J*<sub>H-P</sub> = 0.5 Hz) -CHO ppm. <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 188.7 (-CHO); 152.8 (d) C-1; 135.2 C-5; 128.6 C-2; 128.3 C-3; 125.6 C-6; 122.2 C-4; 65.6 (d) 2 × -CH<sub>2</sub>-; 15.9 (d) 2 × -CH<sub>3</sub> (-OCH<sub>3</sub>)<sub>2</sub> ppm.

### Preparation of *O,O*-Diethyl *O*-(3-formyl) phenyl phosphorothioate (2b)

Rf: 0.21, Yield: 89 % <sup>1</sup>H NMR (500 MHz) (CDCl<sub>3</sub>): δ 1.38 (dt, 6H, *J*=7Hz, *J*<sub>H-P</sub> = 0.5 Hz), 2 × -CH<sub>3</sub>; 4.27 (Dq, 4H,) 2 × -CH<sub>2</sub>-; 7.74.46-7. (M, 4H) Ar; 10.00 (S,1H,-CHO) ppm. <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 190.8 (-CHO); 151.1 (d) C-1; 137.5 C-3; 129.9 C-5; 126.8 C-4; 126.5 C-6; 121.2 C-2; 65.0 (d) 2 × -CH<sub>2</sub>-; 15.6 (d) 2 × -CH<sub>3</sub> ppm.

### Preparation of *O,O*-Diethyl *O*-(4-formyl) phenyl phosphorothioate (3b)

Rf: 0.18, Yield: 97 % <sup>1</sup>H NMR (500 MHz) (CDCl<sub>3</sub>): δ 9.99 (S, 1H -CHO); 7.90 (d, 2H, *J*=8.5Hz, Ar 3H & 5H); 7.36 (d, 2H, *J*=7.5 Hz, Ar 2H & 6H); 4.27(M, 4h, 2 × -CH<sub>2</sub>-); 1.39 (t, 6H, *J*=7.0 Hz 2 × -CH<sub>3</sub>); ppm.

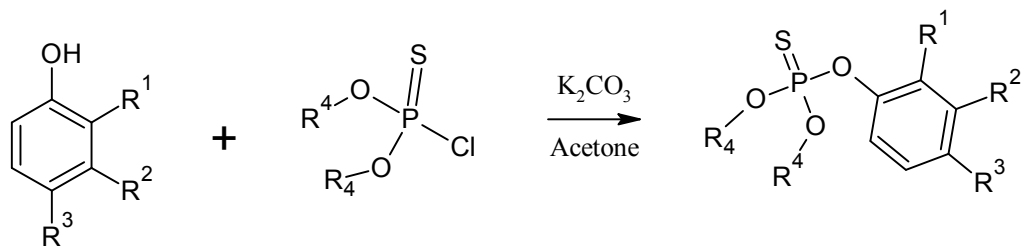


Figure 1. Preparation of phosphorothioates of hydroxy benzaldehydes

Table 1. Structure of the phosphorothioates of hydroxyl benzaldehydes

Dimethyl phosphorothioates of substituted benzaldehydes	
Compounds	Structural formula
1a O,O-Dimethyl O-(2-formyl)phenyl phosphorothioate	R <sup>1</sup> =CHO; R <sup>2</sup> =R <sup>3</sup> =H; R <sup>4</sup> =CH <sub>3</sub>
2a O,O-Dimethyl O-(3-formyl)phenyl phosphorothioate	R <sup>2</sup> =CHO; R <sup>1</sup> =R <sup>3</sup> =H; R <sup>4</sup> =CH <sub>3</sub>
3a O,O-Dimethyl O-(4-formyl)phenyl phosphorothioate	R <sup>3</sup> =CHO; R <sup>1</sup> =R <sup>2</sup> =H; R <sup>4</sup> =CH <sub>3</sub>
Diethyl phosphorothioates of substituted benzaldehydes	
1b O,O-Diethyl O-(2-formyl) phenyl phosphorothioate	R <sup>1</sup> =CHO; R <sup>2</sup> =R <sup>3</sup> =H; R <sup>4</sup> =C <sub>2</sub> H <sub>5</sub>
2b O,O-Diethyl O-(3-formyl) phenyl phosphorothioate	R <sup>2</sup> =CHO; R <sup>1</sup> =R <sup>3</sup> =H; R <sup>4</sup> = C <sub>2</sub> H <sub>5</sub>
3b O,O-Diethyl O-(4-formyl) phenyl phosphorothioate	R <sup>3</sup> =CHO; R <sup>1</sup> =R <sup>2</sup> =H; R <sup>4</sup> = C <sub>2</sub> H <sub>5</sub>

<sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 190.5 (-CHO); 155.1 C-1; 133.1 C-4; 131.1 C-3 & C-5; 121.2 C-2 & C-6; 65.0 (d) 2×-CH<sub>2</sub>-; 15.5 (d) 2 ×-CH<sub>3</sub> ppm.

#### Rearing of test insects

The lesser grain borer *Rhizopertha dominica* (F.) the red flour beetle *Tribolium castaneum* (Herbst) were reared for the present study. A small population of these insects was obtained from the entomology laboratory stock, Food Protectants and Infestation Control Department, CSIR-CFTRI, India. The rearing of above insect was maintained on whole wheat, flour wheat and cow pea seeds (food media) inside a growth chamber at 27 ± 2<sup>o</sup>C, L:D 12:12 and with 70 ± 5% RH (Rahman and Talukder, 2006). 50 pairs of 1-2 day old adults were placed in a glass jar (1.5-L) containing 250 gms of food media in glass containers covered by muslin cloth. Maximum of 7 days were allowed for mating and oviposition. Then the parent stocks were removed and food media containing eggs were incubated in a temperature/humidity controlled cabinet (27 ± 2<sup>o</sup> C and RH 70 ± 5%) in darkness to obtain same aged insects (Rahman and Talukder, 2006).

Thus subsequent progenies (6 days old) of the insects were used for all experiments. For dose mortality surface film assay was used through general concentrations of dimethyl and diethyl derivatives were selected as 100mg/ml as the stock doses. Then range of concentrations 0.004-0.050 mg/cm<sup>2</sup> was placed onto Whatman No. 1 filter circles (9 cm diameter) dried for a while and placed in petri-plates (bottom) of 6 cm diameter each of the peri-dishes (6 cm diameter) before releasing 30 insects (of 7days old *T. castaneum*, and *R. dominica*) in each petri-plates and covered with petri-plate tops as described in literature. There were three replicates for each concentration with equal number of untreated controls. Mortality of the insects was counted after 24 hours of exposure. At the end of exposure period, the insects were removed from the test chamber and were transferred to another set of glass tubes containing 10 g wheat. Insect mortality was assessed after 24 hours of termination of treatment.

Corrected mortality was calculated based on Abbott's formula. The probit analysis was done according to (Finney, 1947; Busvine, 1971) to find out the LD<sub>50</sub> and LD<sub>90</sub> values. The mean number and standard deviation of insects on the treated and untreated insects using Stats plus software. The Final corrected mortality estimated for the insects exposed is presented in Table-4.

## RESULTS AND DISCUSSION

Phosphorothioate derivatives (Fig 1) were synthesized by the reaction of the hydroxybenzaldehyde with the thiophosphoryl chloride, refluxing the mixture, checking the formation of the product by TLC and isolation of the compounds after work-up. The structures of the synthesized compounds were established by <sup>1</sup>H and <sup>13</sup>C NMR spectra. Toxicity of the products was tested against stored product insects. The phosphorothioate compounds were found to possess excellent insecticidal activity almost comparable to that of methyl parathion insecticide.

#### Insecticidal activity

All the Phosphorothioate derivatives were screened to choose the percentage mortality from the different doses on stored product insects was depicted in Table 1, 2 and 3. The percentage mortality was varied and dose dependent in both stored product pests. The highest percentage mortality 96.5%, 95.3%, 96.4% was exhibited by diethyl compounds such as 1b, 2b and 3b at different range of 0.047 mg/cm<sup>2</sup> to 0.050 mg/cm<sup>2</sup> on *Tribolium castaneum* (Table 1). The above results more or less similar on *Rhizopertha dominica* when exposed to diethyl compounds (Table 2). In case of dimethyl compounds highest mortality was noticed at the least dosage range from 0.23 to 0.044 mg/cm<sup>2</sup> from the Table 1 and 2. Hundred percent toxicity of standard methyl parathion was found at range of dose of 0.050 mg/cm<sup>2</sup> for *Tribolium castaneum* and 0.036 mg/cm<sup>2</sup> for *Rhizopertha dominica*. The results reported in Table 4 reveal the LC<sub>50</sub> and LC<sub>90</sub> values of the synthesized compounds and standard methyl parathion on stored product insects along with chi-square values and Feducial values.

Table 1. Toxicity range of Di-methyl and Di-ethyl derivatives on adults of *Tribolium castaneum*

1a		2a		3a	
Dosage (mg/cm <sup>2</sup> )	Percent mortality (mean±SD)	Dosage (mg/cm <sup>2</sup> )	Percent mortality (mean±SD)	Dosage (mg/cm <sup>2</sup> )	Percent mortality (mean±SD)
Di-methyl					
Control	1.1±1.50	Control	0.00	Control	0.00
0.009	3.3±1.70	0.009	4.44±1.33	0.009	2.2±0.57
0.013	7.7±1.52	0.011	10.5±2.15	0.011	3.3±2.17
0.017	15.5±2.73	0.013	24.4±7.33	0.013	5.4±5.33
0.021	37.7±3.51	0.015	31.1±3.33	0.019	14.4±3.32
0.023	44.4±1.52	0.020	42.2±1.52	0.022	28.8±1.22
0.025	47.7±2.51	0.024	54.4±2.53	0.025	38.9±3.53
0.027	57.7±4.88	0.027	61.1±1.15	0.029	50.0±1.17
0.029	70.1±2.51	0.031	68.8±0.05	0.031	68.8±1.05
0.031	84.4±2.94	0.035	76.6±2.64	0.033	83.3±2.05
0.033	87.7±1.52	0.039	87.7±1.63	0.035	87.6±2.53
0.035	94.4±1.15	0.047	98.8±0.57	0.044	96.8±1.57
Di-ethyl					
1b		2b		3b	
Dosage (mg/cm <sup>2</sup> )	Percent Mortality (mean±SD)	Dosage (mg/cm <sup>2</sup> )	Percent mortality (mean±SD)	Dosage (mg/cm <sup>2</sup> )	Percent mortality (mean±SD)
Control	0.00	Control	0.00	Control	0.00
0.009	5.5 ± 0.5	0.009	4.7 ± 1.4	0.009	3.3 ± 2.6
0.013	8.8 ± 1.6	0.013	7.8 ± 1.1	0.013	8.1 ± 2.9
0.017	13.3 ± 1.2	0.017	10.0 ± 2.0	0.017	11.7 ± 2.3
0.021	16.6 ± 1.6	0.021	13.3 ± 1.3	0.021	19.1 ± 2.2
0.023	21.1 ± 2.3	0.023	15.5 ± 1.0	0.023	27.7 ± 3.2
0.025	28.8 ± 1.4	0.025	21.1 ± 1.4	0.025	31.2 ± 1.4
0.027	30.0 ± 1.8	0.027	27.7 ± 1.5	0.027	35.1 ± 1.3
0.029	52.2 ± 2.0	0.029	32.2 ± 1.7	0.029	45.5 ± 2.4
0.035	61.1 ± 2.4	0.035	40.7 ± 1.6	0.035	57.7 ± 1.5
0.037	75.5 ± 2.6	0.037	51.2 ± 2.5	0.037	72.2 ± 2.4
0.040	85.5 ± 2.9	0.040	74.6 ± 2.8	0.042	88.8 ± 2.7
0.043	92.2 ± 1.8	0.043	86.4 ± 3.6	0.046	90.1 ± 2.1
0.047	96.5 ± 1.6	0.047	95.3 ± 2.4	0.050	96.4 ± 2.2

Table 2. Toxicity range of di-methyl and di-ethyl derivatives on adults of *Rhyzopertha dominica*

Dosage (mg/cm <sup>2</sup> )	1a	2a	3a
Di-methyl	Percent Mortality (mean±SD)	Dosage (mg/cm <sup>2</sup> ) Percent mortality (mean±SD)	Dosage (mg/cm <sup>2</sup> ) Percent mortality (mean±SD)
Control	0.00	Control	0.00
0.001	4.4 ± 0.57	0.001	2.2 ± 1.3
0.003	7.7 ± 1.1	0.003	6.6 ± 1.8
0.005	14.4 ± 1.5	0.005	16.6 ± 1.2
0.009	21.1 ± 1.8	0.007	21.1 ± 1.4
0.011	33.3 ± 2.4	0.011	37.7 ± 2.4
0.013	46.6 ± 1.0	0.015	48.8 ± 1.6
0.015	61.1 ± 1.5	0.017	60.0 ± 2.1
0.017	75.5 ± 1.4	0.020	73.3 ± 1.7
0.020	81.1 ± 1.6	0.023	80.0 ± 2.4
0.021	83.3 ± 2.6	0.025	91.6 ± 1.5
0.023	97.7 ± 2.1	0.027	96.0 ± 1.8
di-ethyl			
1b		3b	
Dosage (mg/cm <sup>2</sup> )	Percent Mortality (mean±SD)	Dosage (mg/cm <sup>2</sup> )	Percent mortality (mean±SD)
Control	0.00	Control	0.00
0.003	4.6 ± 1.5	0.005	2.7 ± 2.6
0.005	8.8 ± 3.1	0.009	6.8 ± 2.1
0.009	12.3 ± 1.1	0.013	11.0 ± 2.0
0.011	15.5 ± 3.6	0.017	20.3 ± 1.3
0.013	25.1 ± 2.1	0.021	30.5 ± 1.0
0.015	34.4 ± 2.4	0.023	35.1 ± 1.2
0.020	40.0 ± 1.8	0.025	45.7 ± 1.5
0.025	45.5 ± 2.3	0.027	47.2 ± 1.7
0.027	57.1 ± 2.4	0.029	50.7 ± 2.9
0.030	65.5 ± 2.7	0.035	61.2 ± 2.4
0.035	72.5 ± 2.6	0.037	70.6 ± 2.8
0.038	80.2 ± 3.8	0.040	81.4 ± 3.9
0.040	95.5 ± 2.6	0.043	92.3 ± 2.4
0.042	97.6 ± 3.1	0.047	98.4 ± 3.8

**Table 3. Toxicity range of Methyl Parathion on adults of *Tribolium castaneum* and *Rhyzopertha dominica***

<i>Tribolium castaneum</i>		<i>Rhyzopertha dominica</i>	
Dosage (mg/cm <sup>2</sup> )	Percent mortality (mean±SD)	Dosage (mg/cm <sup>2</sup> )	Percent mortality mean±SD)
Control	0.00	Control	0.00
0.004	8.2 ± 1.4	0.002	3.5 ± 2.6
0.008	20.4 ± 3.5	0.004	7.4 ± 4.7
0.016	32.8 ± 1.1	0.008	14.5 ± 3.6
0.020	39.2 ± 3.6	0.012	26.0 ± 4.6
0.023	48.1 ± 5.7	0.016	37.6 ± 2.7
0.035	55.4 ± 2.5	0.020	49.8 ± 2.8
0.040	68.2 ± 5.8	0.024	66.1 ± 2.2
0.043	74.6 ± 3.2	0.028	80.7 ± 2.6
0.047	87.5 ± 2.9	0.032	93.0 ± 3.9
0.050	100.00	0.036	100.0

**Table 4. Toxicity level of Di methyl and Di ethyl derivatives to adults of *Rhyzopertha dominica* and *Tribolium castaneum***

Compounds	Insects													
	<i>R. dominica</i>							<i>T. castaneum</i>						
	1a	2a	3a	1b	2b	3b	Std	1a	2a	3a	1b	2b	3b	Std
<i>LD</i> <sub>50</sub> (mg/cm <sup>2</sup> )	0.013	0.015	0.019	0.023	0.028	0.031	0.017	0.025	0.023	0.027	0.030	0.033	0.030	0.024
<i>LD</i> <sub>90</sub> (mg/cm <sup>2</sup> )	0.023	0.026	0.034	0.041	0.044	0.048	0.028	0.035	0.040	0.038	0.043	0.047	0.045	0.047
Fiducial limits	0.013, 0.014	0.010, 0.015	0.007, 0.024	0.022, 0.025	0.027, 0.029	0.029, 0.032	0.016, 0.018	0.024, 0.025	0.022, 0.028	0.026, 0.028	0.029, 0.030	0.032, 0.034	0.029, 0.031	0.022, 0.016
Intercept ± SE	3.10 ± 0.08	3.30 ± 0.03	3.31 ± 0.01	3.22 ± 0.033	2.76 ± 0.02	2.70 ± 0.01	2.96 ± 0.06	1.82 ± 0.01	3.16 ± 0.01	1.74 ± 0.03	2.21 ± 0.02	2.08 ± 0.04	2.43 ± 0.02	3.61 ± 0.04
$\chi^2$	99.5	49.5	82.1	41.8	31.3	21.9	65.8	29.9	3.3	37.3	77.8	25.5	37.3	13.6

Our result indicates that dimethyl phosphorothioate derivatives showed good insecticidal property for both *Rhyzopertha dominica* and *Tribolium castaneum* compared to diethyl phosphorothioates. Particularly compounds 1a and 2a were very toxic to *Rhyzopertha dominica* than *Tribolium castaneum* (Table 1, 2 and 4). The Table 3 and 4 reflects that modest effect of methyl parathion on both the insects compared to our synthesized compounds. Further data reported in Table 4 revealed that out of 6 compounds tested, compounds 1a: (*LC*<sub>50</sub> 0.013 mg/cm<sup>2</sup>), 2a: (*LC*<sub>50</sub> 0.015 mg/cm<sup>2</sup>) were effective than recommended insecticide methyl parathion (*LC*<sub>50</sub> 0.017 mg/cm<sup>2</sup>) but not so much difference between 2a and 3a (*LC*<sub>50</sub> 0.019 mg/cm<sup>2</sup>) against *Rhyzopertha dominica*. The compounds 2b, 3b on *Tribolium castaneum* was less effective compare to 1b and other compounds. The compound 3a showed middle range toxicity in the contact bioassay on insect pests. Compound 1a produced high levels of toxic on *Rhyzopertha dominica* and *Tribolium castaneum*. This dual toxicity action on both insects makes compound 1a as a potential control agent for economical important pests. The order of insecticidal activity was 3b < 2b < 1b < methyl parathion < 3a < 2a < 1a for both *Rhyzopertha dominica* and *Tribolium castaneum* with slight changes in order of methyl parathion. Although, the insecticidal order is similar but varied in LC values in both insects. However, the results procured from the bioassay on two product insects indicate that these phosphorothioate derivatives can be used to design new compounds endowed with insecticidal activity. Further from our studies concludes 1a and 1b is the active and best compound for control of stored product insects especially on *Rhyzopertha dominica* and *Tribolium castaneum*.

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#### REFERENCES

- Acda, M.A., Bengston, M., and Daglish J. 2000. Response to phosphine of susceptible and resistance strains of *Rhyzopertha dominica* (Fabricius) coleopteran: Bostrichidae from the Philippines. *Asian Life Sciences*, 9: 102-113.
- Agrahari, S., Gopal, K. 2008. Inhibition of Na<sup>+</sup>-K<sup>+</sup>-ATPase in different tissues of freshwater fish *Channa punctatus* (Bloch) exposed to monocrotophos. *Pest. Biochem. Physiology*, 92: 57–60.
- Benayound, F., Hemmond, G.B. 1996. *Chemical. Communication*, 1447. doi 10: 1039/cc3960001447.
- Burke, T.R., Smyth, M.S., Namizu, M., Otaka, A., Roller, P.P. 1993.. Doi 10.1021/j000058 a009. *Journal of organic chemistry*. 58: 1336
- Burke, T.R., Smyth, M.S., Otaka, A. and Roller, B.P. 1993. 34-4125 doi: 10: 10616150040-4039 (00)60508-7. *Tetrahydron letter*.
- Chittenden, F.H. 1911. The lesser grain borer and the larger grain borer. *Bulletin of United State Bureau of Entomology*, 96: 29-47.
- Collins P.T., Daglish G. J., Benstron, M., Lambkin, T.M. and Pavic, H. 2002. Genetics of resistance to Phosphine in *Rhyzopertha dominica* (Coleoptera: Bostrichidae). *Journal of Economic Entomology*, 95: 862-869.
- Collins, P.J., Daglish, G.J, Pavic, H. and Kopittke, R.A. 2005. Response of mixed – age cultures of phosphine-resistance and susceptible strains of lesser grain borer, *Rhyzopertha dominica*, to phosphine at a range of concentration and exposure periods. *Journal of Stored Products Research*, 41: 373-385.

- Deloude L., and Laszio, P.J. 1996. doi: 10.1021/j09660633pp *Organic Chemistry* 61: 6360.
- Fulton, M.H. and Key, P.B. 2001. Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator or organophosphorus insecticides exposure and effects. *Environmental. Toxicology and Chemistry*.20: 37-45.
- Guedes, R.N.C., Dover, B.A. and Kamabhampati, S. 1996. Resistance to Chloropyrifos-Methyl Primiphos-Methyl and Malathion in Brazilian and US population of *Rhizopertha dominica* (coleopteran: Bostrichidae). *Journal of Economic Entomology*, 89: 27-32.
- Guedies, R.N.C., Kambhampathi, S. and Dover, B.A. 1997. Organophosphate resistance and its biochemical mechanism in Brazilian and US population of the lesser grain borer, *Rhizopertha dominica*. *Resistant Pest Management News letter*, 9: 24-25.
- Janes, D., and Keft, S. 2008. Salicyladehyde is a characteristics aroma component of buck wheat groats. *Food Chemistry*, 109(2): 293-298.
- Kurmaiah, N., Suryanarayana, D. and Pandu Ranga Rao, V. 1967. Studies on the use of Salicyladehyde as a cheating and extracting agent. *Fresenius Zeitschrift fur analytische chemie*, 230(3): 199-204.
- Lorini, L., Collins, P.T., Daghish, G.S., Nayak, M.K., and Pavic, H. 2007. Detection and characterization of strong resistance to Phosphine in Brazilian *Rhizopertha dominica* (F.) Coleoptera: Bostrichidae). *Pest Management Science*, 59: 1191-1196.
- Martin, K., Jarmila V., Nabila, G R. and Jirina, S. 2012. Antimicrobial activity of Salicylanilide Benzenesulfonates. *Molecules*, 17: 492-503.
- Navarro, S., Carmi, Y., Kashanchi, Y. and Shasya, E. 1986. Malthion resistance of stored product insects in Isreal. *Pytoparasitica*, 14: 273-280.
- Rahman, A. and Talukder, F.A. 2006. Bio-efficacy of some plant derivatives that protect grain against the pulse beetle *Callosobruchus maculates*. *Journal of Insect Science*, 6(3), 1-10.
- Smyth M.S., Ford, H. Jr. and Burke. T.R.1992. 33. 4137-4140 doi 10: *Jr.Tetrahydron Letter* 1016/30040-40 39(00)74672-7.
- Smyth, M.S., Burke, T.R. 1994, Doi 10.1039/cc 9960001447. *Tetrahydron letter* 35: 551.
- Varma R.S., and Meshram, H.M. 1997. doi 10. 1016150040-40. 39. *Tetrahedron let.* (97), 10143-5. 38:7973.
- Zhang, T., Xie, R., Zhang, T., Mei, X., Yang, J and Jun, N. 2013. Design, synthesis and bioactivities of novel oxime ether derivatives. *Journal of Pesticide Science*. 38(2): 88-90.
- Zettler, L.J., and Cuperus, G.R. 1990. Pesticide Resistance in *Tribolium castaneum*. (Coleoptera: tenebrionidae) and *Rhizopertha dominica* (Coleopteran: Bostrichidae) in wheat, *Journal of Economic Entomology*, 23: 1677-1681.

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