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Evaluation of Toxicity of Chlorinated, Organo Phosphorus and Pyrophosphate Oils Against *Tribolium castaneum*

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Abstract: The present research work dealt with the evaluation of toxicity of chlorinated, organophosphorylated and pyrophosphated oils, against *Tribolium castaneum*. Kerosene, castor, taramera (*Eruca sativa* seeds oil) and turpentine oils were chlorinated, phosphorylated and converted into pyrophosphate to evaluate their toxicity against red flour beetle, *Tribolium castaneum*. Chlorinated kerosene, castor, taramera and turpentine oils showed LC_{50}% values of 6.5, 6.0, 4.5 and 5.7%, respectively against *Tribolium castaneum* adults. While the phosphorylated kerosene, castor, taramera and turpentine oils showed LC_{50}% values of 4.5, 4.0, 4.5 and 3.7 %, respectively against *Tribolium castaneum* adults. Pyrophosphorylated kerosene, castor, taramera and turpentine oils showed LC_{50}% values of 2.3, 1.9, 1.9 and 2.0 %, respectively.

Key words: *Tribolium castaneum* · Stored grain pest · Chlorinated oils · Phosphorylates oils

INTRODUCTION

Pakistan is an agricultural country and wheat flour products are our basic food. The productivity of wheat, rice and other cereals is still very low in Pakistan, the cause is attributed to stored grain pests including *Tribolium castaneum* (Herbest). Recently the preparation of an inclusion complex of chlorpyrifos in cyclodextrin was explored to maintain its efficiency for long periods and to prevent overdosing [1]. The most important biological agent responsible for harvest losses are insects. In recent years, in order to reduce the toxicity of chlorpyrifos and phoxim, both of them have been used as components to form synergistic insecticidal compounds [2-4]. A large field of application for the organophosphorous is as floatation agents. Organic phosphorous compounds have been used in flame proofing textiles as well as plastics. Derivatives of organophosphorous compounds have been appreciated due to their greater toxic effects. These are used in different pesticides and several important insecticides world wide. The introduction of one or more chiral centers or asymmetric factor into the molecule usually changes the pesticidal activity [5] because enantiomers of the same chiral compound can degrade at significantly different rates [6-14] and have very different toxicological characteristics in the environment [15-19].

The present study dealt with the enhancement of toxicity of oils like kerosene, castor, taramera and turpentine by halogenations, phosphorization and finally their conversion to pyrophosphate derivatives as such. Because of the tested toxicology and small toxicological life span of these synthesized oil's, indicates the presence of chiral compounds in these formulations. The effectiveness of these synthetic compounds was determined by spreading uniformly on glass Petri plates containing *Tribolium castaneum* for successful control of this noxious stored grain pest.

As the oil samples were prepared by locally available materials so they are economical than the insecticides available in the market. The main object of this work was to prepare such compounds that should be environment friendly and toxic for insects and pests. These compounds have short active life and their by-products are supposed to be less toxic to the environment because they were derived from natural products, furthermore these compounds posses short active life i.e. decomposes very quickly.

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MATERIALS AND METHODS

Required Chemicals/Material: Chlorine gas (Commercially available), Phosphorous trichloride (Analytical grade) Ether (Analytical grade), Sodium metal (Commercially available), Kerosine Oil (Commercially available), Castor Oil (Commercially available), Taramera Oil (Commercially available), Turpentine Oil (Commercially available).

Chlorination of Oils: In order to enhance the toxicity of the selected locally available oils i.e. kerosene oil, castor oil, taramera (brasica) oil and turpentine oils were first halogenated, phosphorylated and finally converted into pyrophosphate compounds. The toxicity of these compounds was then tested against *Tribolium castaneum*. These oils were first chlorinated by passing chlorine gas through selected oils in a conical flask for about 15-20 minutes in order to chlorinate the unsaturated components naturally present in these oils. Portion of these chlorinated oils was kept for testing their toxicity and FTIR Spectroscopy, while the rest was used in phosphorylation. FTIR spectra of the chlorinated oils show peaks at 600-500/cm, which conforms the chlorination.

Phosphorylation of Oils: 20.0 mL of chlorinated oil was taken in a quick fit flask fitted with reflux condenser. 10.0 mL of phosphorous trichloride was added into the flask, after 5 minutes 50.0 mL of ether was added into the flask and finally small pieces of sodium metal were added into the flask. The colour of the compound was changed after the completion of the reaction.

$$Pcl_3 + 3RX + \text{Ether} + 3Na\ (\text{Metal}) \rightarrow R3\text{–}P + 3NaCl$$

The phosphorylated oils were then separated by ether, which was then analysed by FTIR and preserved for insecticidal properties against *Tribolium castaneum*. FTIR spectra of the phosphorylated oils show peaks at 1100-1200/cm range, which conforms that the reaction has occurred.

Pyrophosphate Linkage: 10.0 mL Phosphorylated oils prepared in the above step were diluted in 50.0 mL of ether and reacted with 5.0 mL $POCl_3$ in a flask fitted with reflux condenser. The reaction was allowed to continue for overnight. The changes in the colour of the product indicate the formation of the product because of the following reaction.

$$R_3\text{—}P + POCl_3 + \text{Ether} \text{-------------------}\rightarrow R_4\text{—}P_2O_7$$

The reaction mixture was separated into two layers and ethereal layer was separated from the product layer by distillation. The pyrophosphate linked oils were then analyzed by FTIR spectroscopy and for insecticidal properties against *Tribolium castaneum*. The pyrophosphate peak on the FTIR spectrum appeared at 1600 to 1700/cm which confirms the reaction.

Biotoxicity Assay of Synthesized Oils Against *Tribolium castaneum*: The susceptible and resistance beetle strains were collected from Cell and Molecular Biology Laboratory, Department of Zoology, University of the Punjab, Lahore. Cultures were grown in sterilized jam jars at 30±1°C in laboratory at 65±5% relative humidity. The culture media used was wheat flour sterilized at 60°C for 70-80 minutes. Each jar was filled quarter with wheat flour and 100 beetle larvae were added. The jars were then covered with muslin cloth and tied with rubber band. Insects were left in culture medium for 2-3 days for egg laying and then were removed with the help of separating sieves and fine camel brush. Flour containing eggs was placed back in the same jar, in which the 6^{th} instars larvae emerged after 28±1 days. These larvae were then used for bio-toxicity assay.

Pre-sterilized Petri dishes were marked according to the concentration. Each concentration sample has three replicates. After adding the insects, 10 beetles (*Tribolium castaneum*) were added in the dishes and were covered with lids so that insects remained contact with the sample employed. Mortality rate was determined after 24 hours. The insect was considered as dead who did not response by the pressure exerted by the camel hair brush.

Observations were made and all the experiments were run in triplicates. At least three separate flasks were usually maintained for one treatment. Each time three readings were taken; their mean and standard deviation of the mean were calculated.

RESULTS AND DISCUSSION

Pest can be controlled through chemicals. Organophosphates and synthetic pyrethroids are still used for effective pest control. *Tribolium castaneum* has been reported to develop greatest resistance against different insecticides [20]. The increasing prevalence of resistance to insecticides suggests that other new insecticides may also be used for chemical control but they should possess many desirable properties including

Table 1: Effects of different chlorinated, organophosphorous and pyrophosphate oils on *Tribolium castaneum*

Concentration (%)	%Mortality±SD Kerosene oil	%Mortality±SD Castor Oil	%Mortality±SD Taramera Oil	%Mortality±SD Turpentine oil
Chlorinated	Oil			
0	0.0±0.000	0.0±0.0	0.0±0.00	0.0±0.00
1	20.0±0.00	13.3±0.33	13.3±0.33	16.6±0.57
2	23.3±0.33	26.6±0.57	26.6±0.57	30.0±0.00
4	33.3±0.33	36.6±0.33	46.6±0.33	40.0±0.00
6	46.6±0.57	50.0±0.00	66.6±0.57	53.3±0.33
8	63.3±0.33	60.0±0.00	80.0±0.00	73.3±0.33
10	83.3±0.33	86.6±0.57	90.0±0.00	83.3±0.33
Organo	Phosphorylated	Oil		
0	0.0±0.000	0.0±0.0	0.0±0.00	0.0±0.00
0.5	20.0±0.00	13.3±0.33	13.3±0.33	16.6±0.57
1	23.3±0.33	30.0±0.00	26.6±0.57	33.3±0.33
2	33.3±0.33	43.3±0.33	36.6±0.57	46.6±0.57
4	46.6±0.57	50.0±0.00	46.6±0.57	56.6±0.57
6	63.3±0.33	66.6±0.57	66.6±0.57	73.3±0.33
8	83.3±0.33	76.6±0.57	93.3±0.33	90.0±0.00
Pyro	Phosphated	Oil		
0	0.0±0.000	0.0±0.0	0.0±0.00	0.0±0.00
0.5	13.3±0.33	23.3±0.33	26.6±0.57	26.6±0.57
1	26.6±0.57	43.3±0.33	36.6±0.57	36.6±0.57
2	46.6±0.57	53.3±0.33	53.3±0.33	50.0±0.00
4	76.6±0.57	70.0±0.00	73.3±0.33	63.0±0.00
6	100±0.00	73.3±0.33	93.3±0.33	75.0±0.00
8	100±0.00	93.3±0.33	100.0±0.00	93.3±0.33

high toxicity to insects, high biodegradability and effectiveness at low doses [21]. Synthetic pyrethroid (Fury) was most effective against 15 days old adults of *Tribolium castaneum*. The LC_{50} of Pakistani strain red flour beetle *Tribolium castaneum* was 405,648,560 and 121 ppm, respectively, whereas for insects susceptible FSS-II strain it was 856, 1088, 555 and 58 ppm, respectively [22].

Effect of Chlorinated Oils on Mortality Percentage of *Tribolium castaneum* Chlorinated kerosene, castor, taramera and turpentine oils showed LC_{50} values of 6.5, 6.0, 4.5 and 5.7 %, respectively against *Tribolium castaneum* adults (Table 1). 10% of chlorinated oils show mortality in the range of 83-90%. The phosphorylated kerosene, castor, taramera and turpentine oils showed LC_{50} values at 4.5, 4.0, 4.5 and 3.7 %, respectively against Tribolium castaneum adults. The effect of chlorinated oils (kerosene, Castor, Taramera and Turpentine) on the mortality percentage of *Tribolium castaneum* has been shown in Figs 1-4.

Effect of Organophosphorylated Oils on Mortality Percentage of *Tribolium castaneum*: At lower (0.5%) concentration of Organophosphorylated Oils, percentage mortality of beeatles was only 20.0±0.0, 13.3±0.33, 13.3±0.33 and 16.6±0.57 for kerosin, castor, taramera and turpentine oils, respectively. At higher (8.0%) concentration of organophosphorylated oils, percentage mortality of beatles was 83.3±0.33, 76.6±0.57, 93.3±0.33 and 90.0±0.00 for kerosin, castor, taramera and turpentine oils, respectively.

The effect of Organophosphorylated oils (kerosene, Castor, Taramera and Turpentine) on the mortality percentage of *Tribolium castaneum* has been shown in Fig. 5-8.

Effect of Pyrophosphated Oils on Mortality Percentage of *Tribolium castaneum*: 8% of the phosphorylated oils show over all 76 to 93.3% mortalities. Pyrophosphorylated kerosene, castoe, taramera and turpentine oils showed LC_{50} values at 2.3, 1.9, 1.9 and 2%, respectively. The best results were seen in case of 6% dose of Kerosene pyrophosphate for 100% mortality rates. The effect of Pyrophosphate oils (kerosene, Castor, Taramera and Turpentine) on the mortality percentage of *Tribolium castaneum* has been shown in Figs. 9-12.

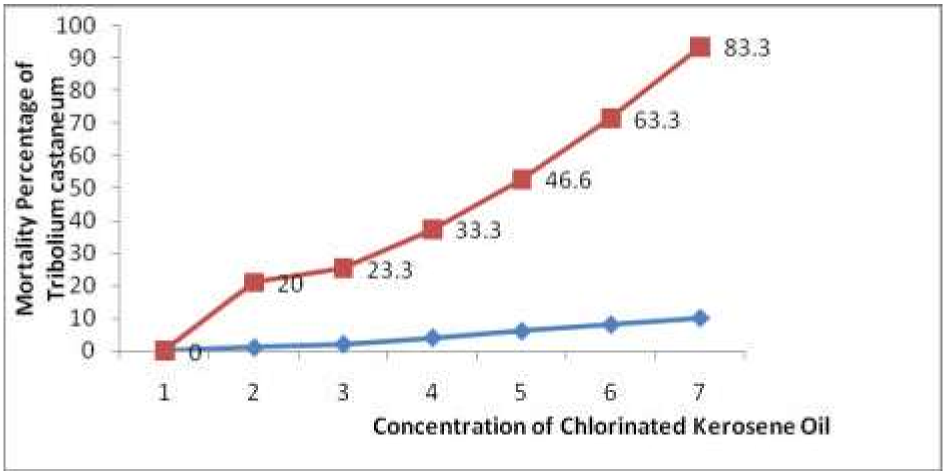

Fig. 1: Effect of Chlorinated kerosene oil on Mortality percentage of Tribolium castaneum

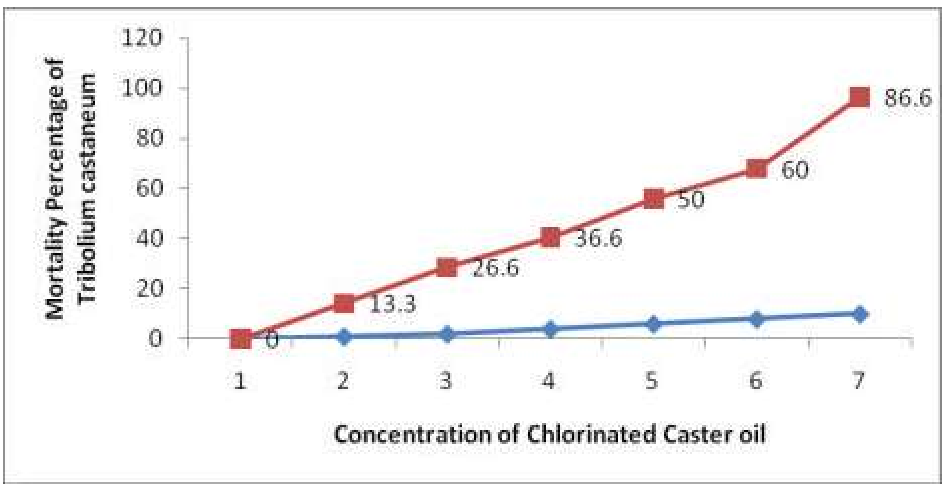

Fig. 2: Effect of Chlorinated Castor oil on Mortality percentage of Tribolium castaneum

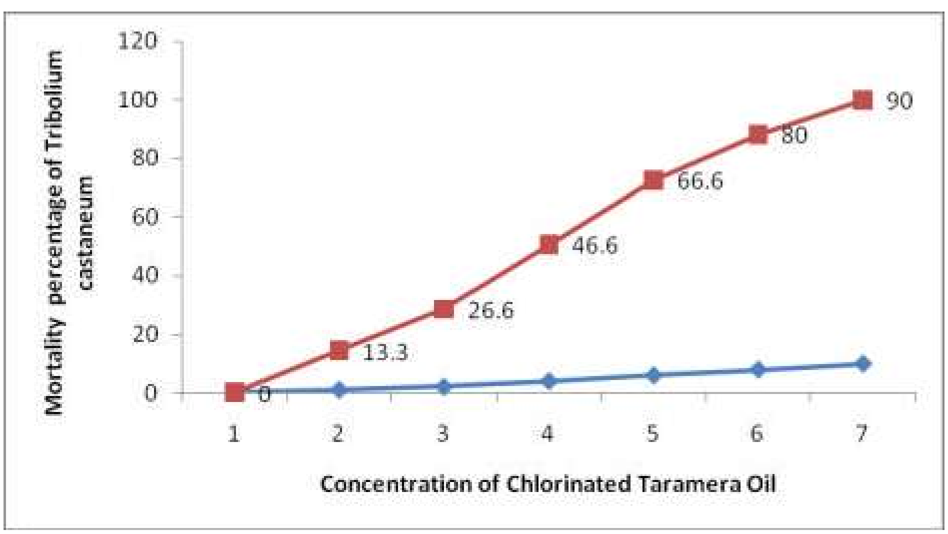

Fig. 3: Effect of Chlorinated Taramera oil on Mortality percentage of Tribolium castaneum

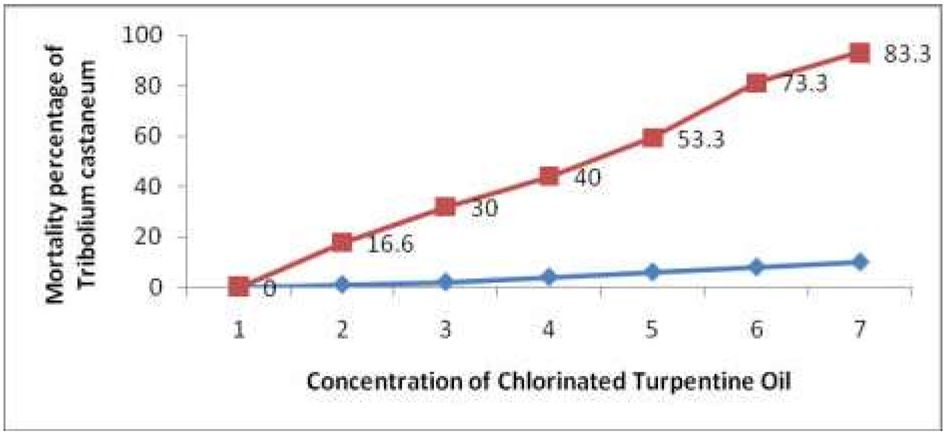

Fig. 4: Effect of Chlorinated Turpentine oil on Mortality percentage of Tribolium castaneum

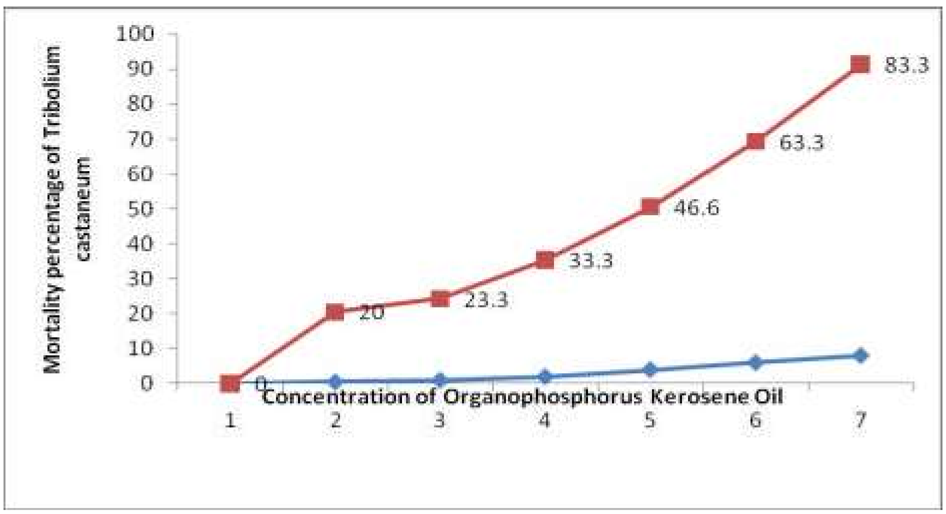

Fig. 5: Effect of Organophosphorylated kerosene oil on Mortality percentage of Tribolium castaneum

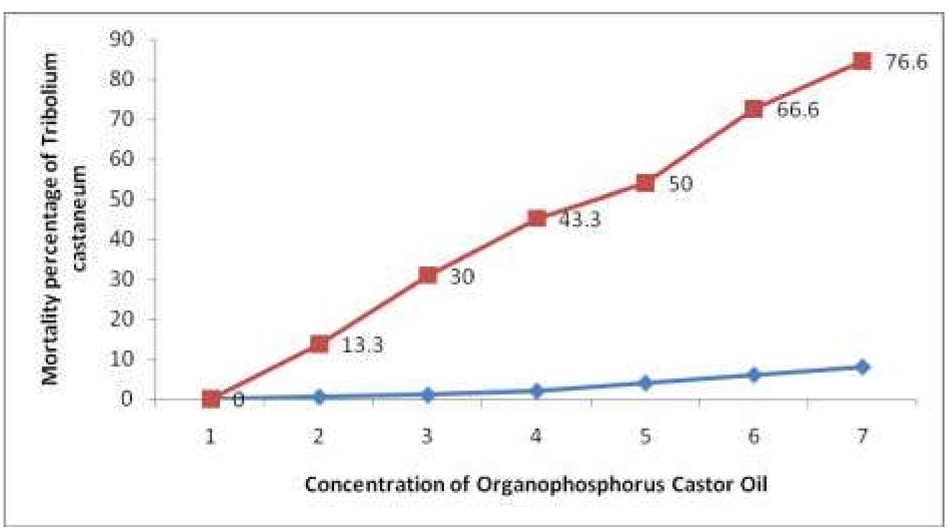

Fig. 6: Effect of Organophosphorylated Castor oil on Mortality percentage of Tribolium castaneum

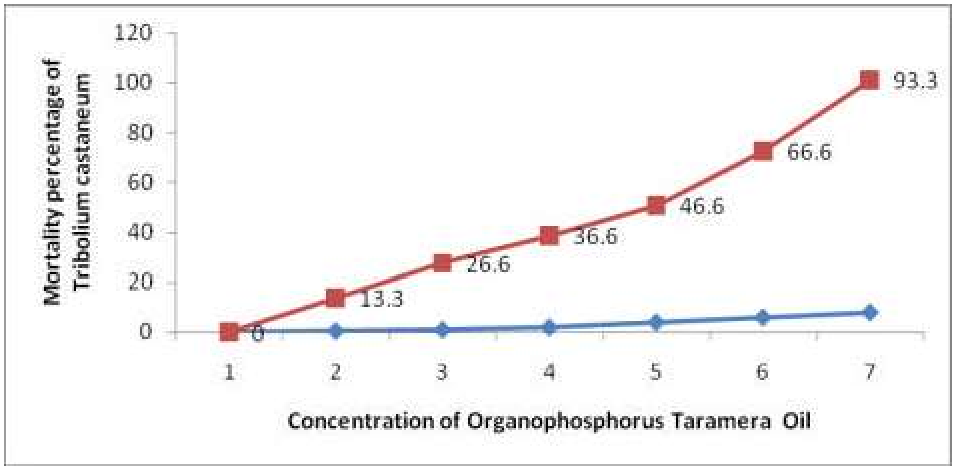

Fig. 7: Effect of Organophosphorylated Taramera oil on Mortality percentage of Tribolium castaneum

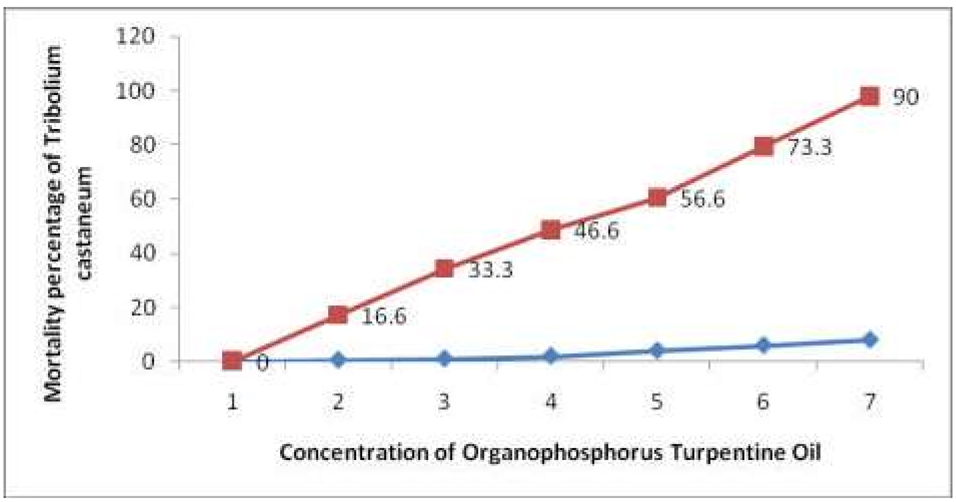

Fig. 8: Effect of Organophosphorylated Turpentine oil on Mortality percentage of Tribolium castaneum

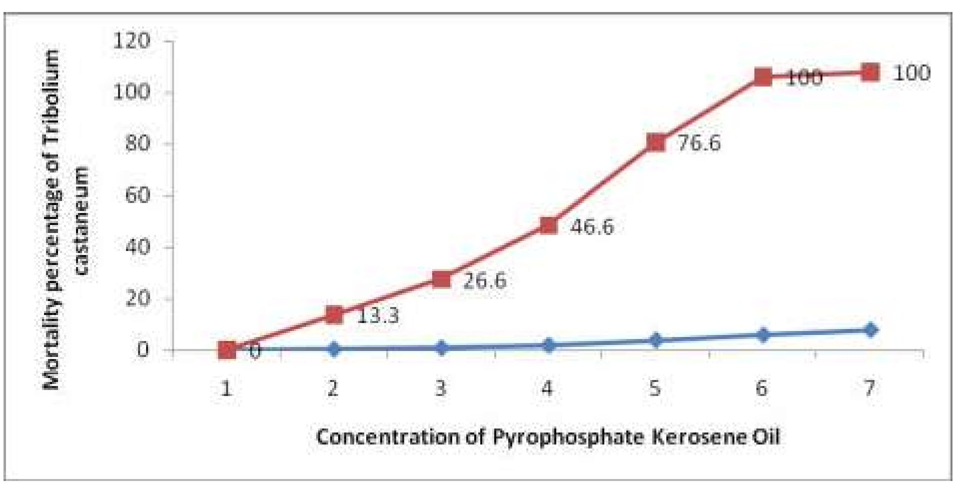

Fig. 9: Effect of Pyrophosphated kerosene oil on Mortality percentage of Tribolium castaneum

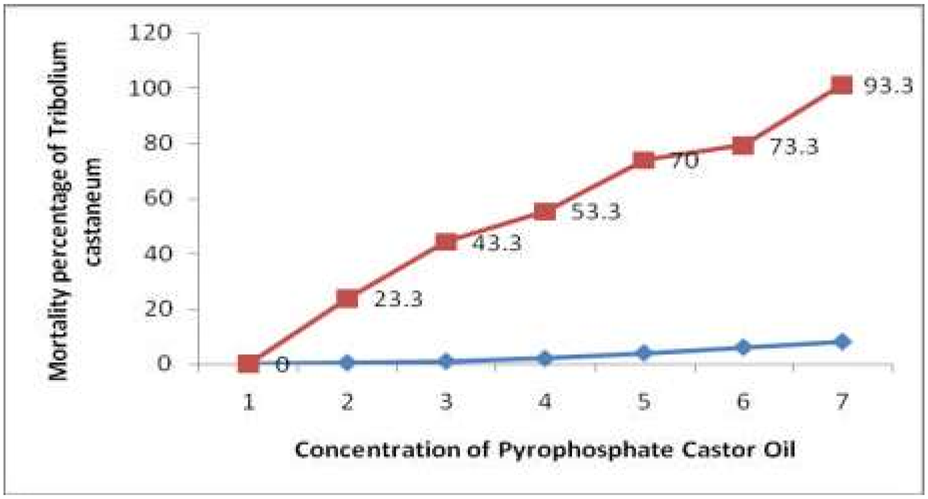

Fig. 10: Effect of Pyrophosphated Castor on Mortality percentage of Tribolium castaneum

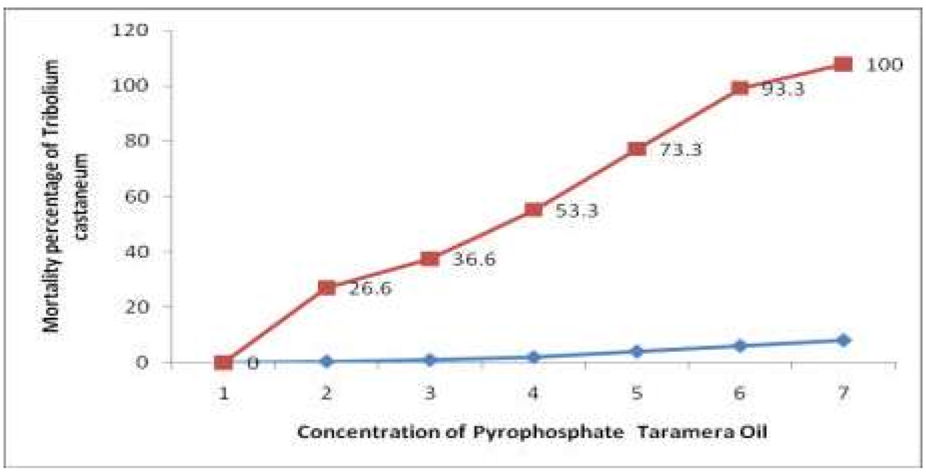

Fig. 11: Effect of Pyrophosphated Taramera oil on Mortality percentage of Tribolium castaneum

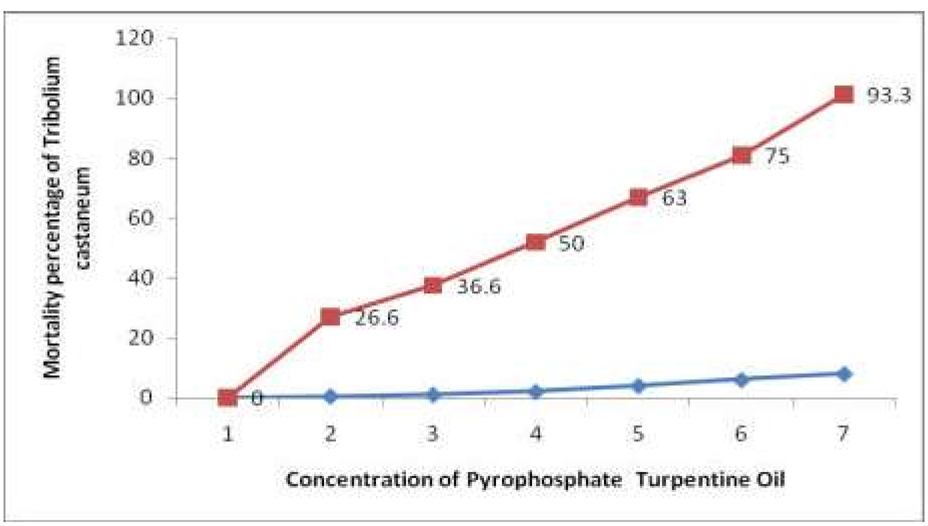

Fig. 12: Effect of Pyrophosphated Turpentine oil on Mortality percentage of Tribolium castaneum

It is clear from the Table 1 that treating the oils with chlorine, phosphorous trichloride and phosphorous oxychloride enhances the toxicity of the oils. The most appropriate results were obtained when 2% pyrophosphate castor oil gave around 50% mortality rate.

Further work is needed to elucidate the structure of the active components in these synthesized samples and to study their activity, mode of action, resistance mechanism, residual properties and safety in use as well as their action against other insects and pests.

CONCLUSION

Chlorination, phosphoralization and their pyrophosphate estrification of oils enhances the toxicity of Kerosine, Taramera and castor and terpine oils. Further work is needed to elucidate the structure of the active components in these synthesized samples and to study their activity, mode of action, resistance mechanism, residual properties and safety in use as well as their action against other insects and pests. These insecticides were developed from local raw materials for the first time and may be used as insecticides and pesticides, once their toxicity is fully established. Our objectives were achieved successfully. Project will be useful to save the foiegn exchange and develop the well trained and skilled manpower in the country.

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