



Commercial Fumigant Fitness and Bio-Pesticides Potential against Resistant Strains of Quarantine Insect Pests

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ABSTRACT

Food spoilage of perishable and other grains are seriously proliferated during storage by stored grain insect pests under variable conditions. Resistance development in insect pests against fumigants is a serious threat to effective commercial use of fumigants in various quarantine treatments. Therefore, resistant strains of *Tribolium castaneum* (Herbst), *Rhizopertha dominica* (Fabricius) and *Sitophilus oryzae* (Linnaeus) were tested against phosphine and bio fumigants to check their fitness and potential following by F₁ generation. Phosphine fitness in term of mortality mean calculated at 300 ppm was minimum against weevil but highly effective against red flour beetle after 24hours while, at 400ppm showed same resistance level in all. At 500ppm, lesser grain borer (LGB) was more susceptible as compared to others as in rice weevil. *Nicotiana tabacum* (L.) and *Calotropis procera* (Aiton) oil both at 15% were most lethal to LGB, weevil and red flour beetle after 72hours following by effect of *Datura stramonium* (L.) and *Eucalyptus camaldulensis* (Dehnh) with respect to control. Similar response was noted at 10% concentrations of all plants but *E. Camaldulensis* (5%) was least effective against all insects after control. Resurgence response in F₁ generation in each experiment showed high multiplication rate at low concentrations and vice-versa also suppressed against *N. tabacum* and *C. procera*. Hence, developing PH₃ resistance can be managed with bio-pesticides up to extant and need more work to make it applicable in field.

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Authors' Contribution

AK and MIU conceived, designed and performed the experiments. MA and AA interpreted the data and wrote the paper. YI reviewed and edited the manuscript.

Key words

Phosphine, Biopesticides, *Tribolium castaneum*, *Rhizopertha dominica*, *Sitophilus oryzae*.

INTRODUCTION

Food commodities like food grains are stored all over the world as a major component of the food security till the next season harvest (Daglish *et al.*, 2015). Efficiency of the adopted prophylactic mitigations is highly flexible and directly proportional to the length of the storage (Benhalima *et al.*, 2004). In response to long time storage lead from mild to severe qualitative and quantitative losses due to proliferation by plenty of stored grain insect pests (Nenaah, 2014). Direct consumption not only effects the germination but also reduces the nutrients level as well as insect products like webbing, exuviae, body fragments, silk or other chemical secretions, dead and live insects may unfit food for human consumption (Nenaah, 2014; Rajendran and Sriranjini, 2008; Serin, 2016).

Among stored grain insect pests three insect pests as *T. castaneum*, red flour beetle (RFB), *R. dominica*, the lesser grain borer (LGB) and *S. oryzae*, the rice weevil (RW) are

documented as notorious polyphagous stored grain insect pests that has got resistance against commercial fumigants and is a serious threat for plenty of cereal crops including wheat (Ashouri and Shayesteh, 2009; Bughio and Wilkins, 2004; Daglish *et al.*, 2014; Lira *et al.*, 2015; Liu *et al.*, 2007; Nenaah, 2014). It is estimated that about 90% wheat grains or its products are being used for the human consumption where insect losses have been reported 9% in developed countries while more than 50% are recorded in the under developed (Brader *et al.*, 2002; Kwiatkowska *et al.*, 2014). Stored grain insect pests cause 20-30% losses in the tropical and sub-tropical region while 5-10% losses have been reported in the temperate zone (Nenaah, 2014; Rajendran and Sriranjini, 2008).

Wheat losses due to insect pests in Canada and United States of America were noted nearly about 20-26% while in Asia 6.5% losses were recorded in India (Pant *et al.*, 2014). Around the globe post-harvest losses due to pest infestation may accede from 10-40%. For 6-8 month storage duration of the small land holding farmers, the losses can be very high as much as 80% (Ogendo *et al.*, 2008; Pant *et al.*, 2014). Among other cereals, in a season all over the world maize production is infested by

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rice weevil cause losses 1-2% developed world and 14-50% in under develop countries (Suleiman *et al.*, 2015). In Pakistan, 2-6% food grains of the total grains produced are infested by store product pests during storage annually (Saleem *et al.*, 2014).

Managing these insect pests with plenty of chemical insecticides employed like magnesium phosphide, aluminum phosphide, Methyl bromide, Deltamethrin, Pyrethroids, organophosphates and primiphos-methyl raised plentiful problems like food toxicity, deterioration, adulteration and contamination, insect resistance, environmental effects and lethal for the beneficial insect fauna (Chen and Chen, 2013; Eissa *et al.*, 2014; Fouad *et al.*, 2014; Nenaah, 2014).

Therefore, phosphine resistant strains of *T. castaneum*, *R. dominica* and *S. oryzae* are reported in different areas of the world which have adopted themselves to neutralize the phosphine effect like by changing respiration rate or chemistry of active compounds or going to inactive stage (Buglio and Wilkins, 2004; Sousa *et al.*, 2009; Tapondjou *et al.*, 2002). Two distinct levels for inheritance of phosphine resistance were diagnosed in red flour beetle where rph_1 and rph_2 genes were responsible for weak and strong resistance, respectively (Bengston *et al.*, 1999; Daghli *et al.*, 2015). Furthermore, homozygous beetles carried gene rph_1 showed weak resistance while, homozygous beetle insects possessed both genes showed strong resistance as genes were incompletely recessive and not sex linked (Bengston *et al.*, 1999). In eastern Australia a study conducted in 1980s sowed a little phosphine resistance development in field population of stored grain insect pest. Twenty two Brazilian populations of *Sitophilus* weevils were tested for phosphine resistance under FAO standard methods, 20 of which had inherited resistant (Pimentel *et al.*, 2009). In Morocco, 32% samples of stored grain insect pests population collected from various storage facilities were found having a significant number of phosphine resistant populations (Benhalima *et al.*, 2004).

Despite heavy dependence on phosphine and methyl bromide alternative strategies against massive use of these commercial fumigants need to adapt from *in-vitro* to *in-vivo* like use of plant extract, essential oils, leaf powders, seed oils, pathogenic fungi and other inorganic means like ozone, carbon dioxide and temperature (Betancur *et al.*, 2010; Rajendran and Sriranjini, 2008; Zandi-Sohani and Ramezani, 2015). Owing to several modes of action plant oils designated as potential fumigant of contact toxin, antifeedant, repellent and disruptors of cuticle as well as they are supposed good to suppress insect growth and fecundity significantly (Daghli and Pulvirenti, 1998; Zandi-Sohani and Ramezani, 2015). Plenty of biopesticides significantly influence the behavioral and physiological response

of stored product pests like lethal, growth regulatory, repulsive and reduce reproduction (Hanif *et al.*, 2015). Plant derived insecticides in the form of plant oils, extract, ash, leaf powders, seed oils and lectins has been purposed as possible part of integrated pest management (IPM) of stored grain insect pests (Demissi *et al.*, 2003; Lira *et al.*, 2015). However, profuse studies showed *N. tabacum*, *C. procera*, *D. stramonium*, *A. indica* and *E. camaldulensis* are the examples of promising phytochemical plants that possess potential to control the stored grain beetles (Gusmao *et al.*, 2013; Hanif *et al.*, 2015; Hasan *et al.*, 2014; Jemâa *et al.*, 2013; Sagheer *et al.*, 2013; Pant *et al.*, 2014; Saleem *et al.*, 2014). Medicinal plant belong to family Meliaceae, Solanaceae, Apiaceae, Laminaceae, Lauraceae and Myrtaceae actively own insecticidal or toxicological properties (cyanohydrins, monoterpenoids, thiocyanates, alkaloids and others) as fumigant (Nenaah, 2014; Rajendran and Sriranjini, 2008). *N. tabacum* at 10% concentration caused 6.69% insect mortality (Sagheer *et al.*, 2013). Similarly among other biopesticides highest mean mortality (14.36%) was recorded for *A. indica* (Hasan *et al.*, 2012). Studies for comparative efficacy of plant oils were found best against red flour beetle and other stored product insect pests (Gusmao *et al.*, 2013; Hanif *et al.*, 2015; Jemâa *et al.*, 2013).

Bioassays were conducted to check phosphine fumigation prevalence and fitness as well as assessment of toxicological impact of indigenous biopesticides against resistant strains of *T. castaneum*, *R. dominica* and *S. oryzae* under laboratory conditions. Furthermore, to reflect their resurgence level F_1 progeny of surviving insects was also checked.

MATERIALS AND METHODS

Collection and rearing of test insects

Resistant population strains of *T. castaneum*, *R. dominica*, and *S. oryzae* were collected from grain market, Government godowns and reported flour mills located in Karachi (Longitude 67.02 East; Latitude 24.92 North). After collection, these insect pests population were kept in the separate jars covered with muslin cloths. Insect populations were regularly checked for their growth and were sieved and transfer to new uninfected sterilized wheat grains diet. At laboratory temperature $30\pm 2^\circ\text{C}$ and relative humidity at $65\pm 5\%$ was maintained for insect's maximum growth. Homogenous population of equal size and age was sieved out separate for each insect that later on was used for different bioassay studies (Hanif *et al.*, 2013, 2015; Pant *et al.*, 2014; Saleem *et al.*, 2014).

Plant collection

The fresh leaves of *Azadirachta indica* (L.), *Eucalyptus camaldulensis* (Dehnh) Myrtaceae, Myrtales), *Calotropis procera* (Aiton) (Apocynaceae, Gentianales), *Datura stramonium* (L.) (Solanaceae, Solanales) and *Nicotiana tabacum* (L.) (Solanaceae, Solanales) were collected from Food Quality and Safety Research Institute, Pakistan Agricultural Research Council, and Karachi University Campus Karachi (Longitude 67.02 East; Latitude 24.92 North) from 15 May to 15 June 2015 from their natural habitats and were identified by taxonomic specialists. Collected leaves were shadow dried under good ventilation and woody stems were separated. Dried samples were kept in separate plastic bags inside a refrigerator until the time for oil extraction (Hanif *et al.*, 2015; Saleem *et al.*, 2014; Ziaee *et al.*, 2014).

Essential oil extraction

Dried leaves were first ground into powder than essential oils were obtained using ethanol by steam distillation method. The ground powder of all collected plant material was run on Soxhlet's apparatus with ethanol as solvent in the flask. After that solvent was evaporated leaving the essential oil. The obtained essential oils were dried over sodium sulfate (Merck) and were kept in separate vials (volume 2 mL) with aluminum caps inside a refrigerator to be used later (Hanif *et al.*, 2013, 2015; Hasan *et al.*, 2014; Saleem *et al.*, 2014; Ziaee *et al.*, 2014).

Generation of phosphine gas

The phosphine gas was generated by FAO's method. The apparatus for generation of phosphine gas consisted of a 5 liter beaker, a collection tube (cylinder), an inverted funnel, Phostoxin® (aluminum phosphide) tablets and muslin cloth. The tube for collection of gas was sealed from one side with airtight rubber stopper and then was filled with 5% sulphuric acid (H_2SO_4) solution. Half of the beaker was also filled by 5% H_2SO_4 solution.

The gas collecting tube was placed carefully into the beaker over the inverted funnel in such a way that there is no loss of H_2SO_4 solution from the collection tube, while dipping into the beaker. Before generating phosphine gas all air in collection tube was removed within collection tube. Then phostoxin tablets (wrapped in aluminum foil goes down). When it was filled, 5 ml gas was suck out with the help of an air tight syringe and was injected into a sealed desiccator of known volume then 50 ml of gas was taken out from the desiccators and injected into phosphine meter for measuring gas concentration. With the help of phosphine meter required concentrations of 300 ppm, 400 ppm and 500 ppm of phosphine gas were obtained (Hanif *et al.*, 2013, 2015).

Phosphine fumigation bioassays for prevalence and fitness

Bioassays was followed the methods recommended by FAO method. A phosphine source was generated from an aluminum phosphide tablet and collected over acidified water. The source concentration was measured by gas chromatography using a gas density balance (Aerograph Model 90-P; Varian, Mount Waverley, Victoria, Australia). Adults of homogenous age was added to ventilate plastic soufflé cups which were then placed inside gas-tight desiccators and gas-tight syringes was used to inject the required amount of phosphine through a septum in the lid of each desiccators. Adults were fumigated at 300, 400 and 500 ppm and data was recorded after 24, 48 and 72 h until end-point mortality was assessed. Sterilized food was given to surviving adults in separate jar of each treatment to asses F_1 progeny after thirty days (Hanif *et al.*, 2013, 2015).

Screening bioassay for toxicological impact of indigenous bio pesticides against stored grains insect pests

Response of biopesticide oils in mortality bioassay was tested against test under laboratory conditions. For this purpose, different concentrations (5, 10 and 15%) of each botanical were applied on Whatman filter paper (Whatman No.1, qualitative filter paper has the pore size of 11 μm carrying 150mm diameter circles and 460mm x 570mm sheets) placed in having 20 sterilized cereal grains (wheat) for each treatment. Twenty insects for each insect strain were used for each treatment; the control was kept without any insecticide application. Each treatment was replicated three times; each contained about 60g of the wheat grains into each plastic vial (10mL). Whole experiment was performed in the laboratory keeping in an incubator at $30\pm 2^\circ C$ and $65\pm\% RH$. Data regarding mortality was noted at regular time intervals (24, 48 and 72 h). Surviving adults were shifted in separate vials (considering treatments) having sterilized food and data for F_1 progeny was recorded after 30 days for the completion of the experiment (Hanif *et al.*, 2013, 2015).

Data recording procedure for F_1 progeny development

Surviving adults along with food grains collected from mortality bioassay were shifted in separate vials (considering treatments) having additional sterilized food. Data for adult emergence as F_1 progeny was counted in number (adult beetles developed) after 30 days for the completion of the experiment (Hanif *et al.*, 2013, 2015; Mahmoud *et al.*, 2014).

Statistical analysis

Statistical analysis initially, the mortality data were transformed by arcSen in order to normalize the data.

Once normalized the data were analyzed by ProGLM to carry out an ANOVA; then the means were separated by Tukey test ($p=0.05$). For statistical analysis CRD Factorial was used for all collected data of percent mortality and F_1 progeny was subject to analysis of variance using Statistica-8 software (Hanif *et al.*, 2013, 2015).

RESULTS

Phosphine fitness against insects

Resistance inheritance level in three coleopteran pest strains was significantly ($P<0.05$) determined. Studies also conducted to evaluate the fitness of quarantine fumigant as well as to measure biocidal potential echelon of some plant oils for their management. Fumigant fitness was less affective at 300 ppm as rice weevil ($4.33\pm 0.47SE$) showed highest resistance compared to *T. castaneum* ($7.11\pm 0.42SE$) after 24 h but there was not abrupt change in mortality after 48 and 72 h. Population of *S. oryzae* was recorded most challenging among other coleopteran beetles at 500 ppm where insect mortality in *R. dominica* and *T. castaneum* was highest ($16.00\pm 0.29SE$), ($15.89\pm 0.56SE$) after all time intervals proved to possess weak resistance with respect to control treatment, respectively. Inheritance of phosphine resistance at 400ppm dose was same but averagely low in response showing that weevil has not been considerable affected as compare to red flour beetles and lesser grain borer as shown in Figure 1.

Progeny production

Insect resurgence in term of F_1 production/emergence was significantly ($P>0.05$) checked after 30 days to find the

response of tested strains to phosphine toxicity as shown in Table I. Progeny development counted in numbers was high with decrease in phosphine concentrations ($500 > 400 > 300$ ppm) against all insects with respect to control (0 ppm) treatment. Over all weevil multiplication rate was high ($32.22\pm 1.30SE$), ($21.67\pm 1.30SE$), ($11.00\pm 1.30SE$) at 300, 400 and 500 ppm with respect to control ($51.67\pm 2.25SE$) but squat in *T. castaneum* ($7.11\pm 1.05SE$), ($10.89\pm 1.05SE$), ($12.89\pm 1.05SE$) and *R. dominica* ($21.00\pm 0.74SE$), ($11.44\pm 0.74SE$), ($4.44\pm 0.74SE$), respectively.

Table I.- Concentration based response of commercial fumigant (Means \pm SE*) on rate of adult emergence (number) with comparisons to control against *R. dominica*, *T. castaneum* and *S. oryzae* after thirty days.

Test insects	Treatments	F_1 progeny (Means \pm SE*)
<i>S. oryzae</i>	500ppm	32.22 \pm 1.30B
	400ppm	21.67 \pm 1.30C
	300ppm	11.00 \pm 1.30D
	Control	51.67 \pm 2.25A
<i>R. dominica</i>	500ppm	21.00 \pm 0.74B
	400ppm	11.44 \pm 0.74C
	300ppm	4.44 \pm 0.74D
	Control	37.33 \pm 1.28A
<i>T. castaneum</i>	500ppm	12.89 \pm 1.05A
	400ppm	10.89 \pm 1.05B
	300ppm	7.11 \pm 1.05C
	Control	1.00 \pm 1.82D

*Means followed by same letters within treatments are not significantly different (Tukey test, $p=0.05$).

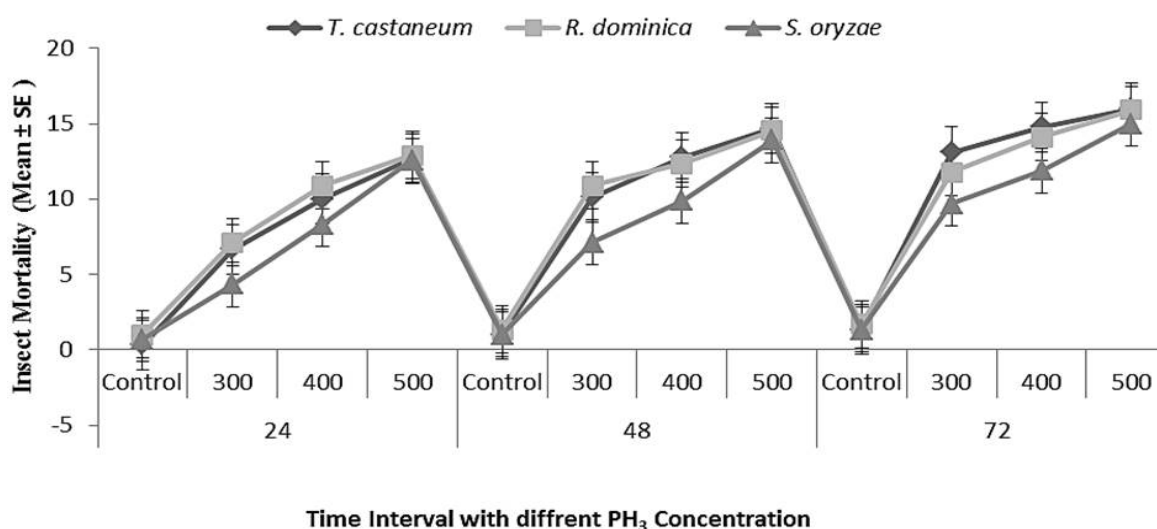


Fig. 1. End-point mortality of *R. dominica*, *T. castaneum* and *S. oryzae* against different phosphine (PH₃) concentration levels at room temperature after capricious exposure time periods.

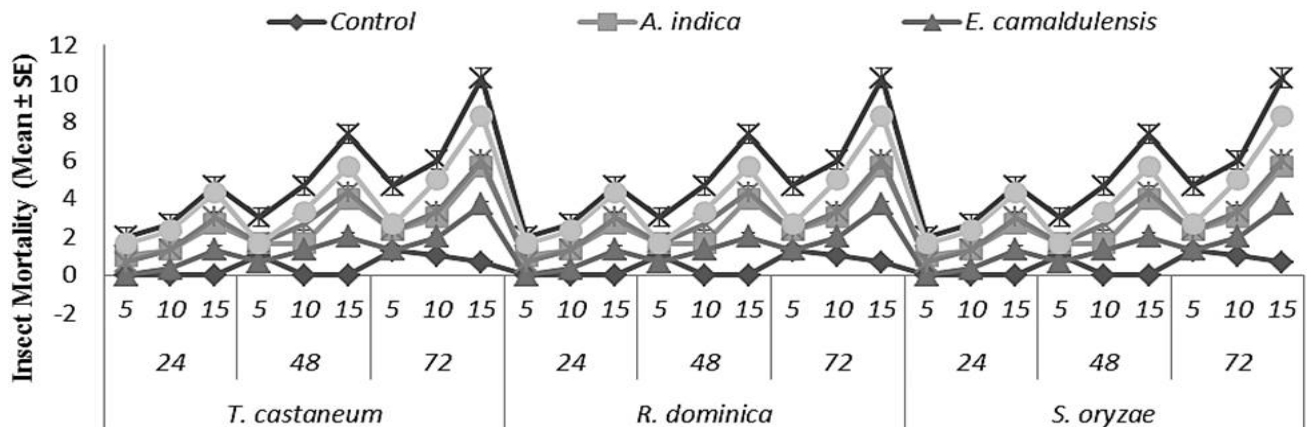


Fig. 2. End-point mortality of *T. castaneum*, *R. dominica* and *S. oryzae* exposed to time periods treated with biofumigants and their concentration levels under controlled conditions.

Table II.- Concentration based response (Means± SE*) of biofumigants on rate of adult emergence (number) with comparison to control against *R. dominica*, *T. castaneum* and *S. oryzae* after thirty days.

Test insects	Treatments	F ₁ progeny (Means± SE*)
<i>T. castaneum</i>	<i>A. indica</i>	35.67±1.37C
	<i>E. camaldulensis</i>	43.11±1.37B
	<i>N. tabacum</i>	18.89±1.37E
	<i>D. stramonium</i>	35.33±1.37C
	<i>C. procera</i>	24.78±1.37D
	Control	55.11±1.37A
<i>R. dominica</i>	<i>A. indica</i>	44.89±2.87AB
	<i>E. camaldulensis</i>	42.56±2.87BC
	<i>N. tabacum</i>	25.11±2.87D
	<i>D. stramonium</i>	35.00±2.87C
	<i>C. procera</i>	44.78±2.87AB
	Control	52.67±2.87A
<i>S. oryzae</i>	<i>A. indica</i>	39.78±2.35C
	<i>E. camaldulensis</i>	50.33±2.35B
	<i>N. tabacum</i>	20.89±2.35E
	<i>D. stramonium</i>	34.00±2.35CD
	<i>C. procera</i>	31.33±2.35D
	Control	63.78±2.35A

*Means followed by same letters within treatments are not significantly different (Tukey test, p=0.05).

Biocidal potential in biopesticides

Resistance inheritance in quarantine insect pests populations checked in the previous experiment was also encountered with some bio-pesticides (reported effective in early studies) were used. Significant (P<0.05) effect of bio-pesticides and their concentrations (5, 10 and 15%)

was found against all insect pests with exposure time. Plant derived oil of *N. tabacum* was highly lethal to a lesser grain borer (17.33±1.19SE) and rice weevil (15.33±0.90SE) after 72 h. Even 5% and 10% concentrations produced high toxicity in rice weevil; lessen grain borer and red flour beetle, respectively. Minute lethal effects were observed in oil of *E. camaldulensis* against all test insects especially red flour beetle proved high resistance at all concentrations and exposure time intervals, respectively. Average survival rate was originated against *A. indica* and *D. stramonium* but *C. procera* was the second most effective bio-pesticides against rice weevil LGB and RFB as shown in Figure 2. Overall results depicted that oils of *N. tabacum* and *C. procera* were potential insecticides at 15% rate against phosphine inherited resistant population of quarantine insect pests following by *A. indica*, *D. stramonium* and *E. camaldulensis*, respectively. Tested bio-insecticides have potential and efficient to cope the phosphine resistance inherited strains of these coleopteran beetles.

Progeny production

Although insect respond significantly (P<0.05) against different essential oils but F₁ adult emergence rate was little bit high in all insect strains especially in rice weevil in comparison to response observed against phosphine as shown in Table II. Resurgence response in term of offspring development counted in numbers was decline with raise in biofumigants concentrations (15>10>5%) in all insects. However, plant oil concentrations against LGB lessen affected the progeny with respect to red flour beetle. Similarly, among plant oils, *N. tabacum* and *C. procera* highly suppressed the progeny development of all insects following by *A. indica*, *D. stramonium* and *E. camaldulensis*, respectively.

DISCUSSION

The response to phosphine inherited resistance and important/essential constituents in the (isolated) biopesticides from *Nicotiana* sp., *Azadirachtin* sp., *Calotropis* sp., *Eucalyptus* sp. and *Datura* sp. were similar to previous studies performed against red flow beetle, lesser grain borer and rice weevil in different countries. However, results are significantly and/or slightly change with respect to insecticide used, concentrations, and exposure time tested in previous studies parameters. These changes might be due to an environmental variations (geographical, seasonal, climatic, agro ecological position), maturational status of the test animal or phonological state of pesticide source (plant) and soil variations where it was grown, time of year and climatic effects of bio-pesticides or plant part, genetic deference of strains and other chemo types (Nenaah, 2014).

In Australia mild phosphine resistant strains of *S. oryzae* are common but from some area high resistant weevil population has been documented (Daglish *et al.*, 2014). Weakly phosphine resistant phenotypes of *T. castaneum* were reported as 62.2% of the collected population of 115 samples. Alarming the resistance development and selection pressure in eastern Australia (Daglish *et al.*, 2015). Stored grains are frequently contaminated in result to exposure stored grain insect pests especially *T. castaneum*, *R. dominica* and *S. oryzae* throughout the storage period. Stored product insect pests are developing behavioral and genetically resistance with progress in the management practices. Two resistant non-sex linked genes (rph_1 and rph_2) were supposed to be responsible for resistance development (Bengston *et al.*, 1999; Price and Mills, 1988). Strange phosphine resistance (431 fold) in quarantine insect pests (*T. castaneum*) recorded after twenty hours exposure fumigant in sense of insect mortality but was low (12.3 fold) in week resistant strains (Bengston *et al.*, 1999).

Essential oils of *Azadirachta* sp. and *Eucalyptus* sp. tested against stored grain insect pests showed potential insecticidal action as well as antimicrobial or anti-oxidant action. United States Food and Drug Administration (FDA) have also categorized these biopesticides as GRAS can be used as food flavors and additives (Prakash *et al.*, 2015). Biological activates of biopesticides with peculiar chemical composition have a range of medical uses (Laribi *et al.*, 2015). Oil of *Alpinia purpurata* was highly effective against weevil as food deterrent or disrupted the nutritional status but biocidal action was slow when applied on the insect cuticle (Lira *et al.*, 2015). Among previous researches similar response of *Azadirachtin* (30.68%) and other biopesticides was noted at 20% concentration in

beetle mortality while, minimum (20.24%) at 5% neem oil (Hasan *et al.*, 2014). Biopesticides (Spinosad) was lethal to *R. dominica* resulting 100% mortality at 0.5 mgkg⁻¹exposed 168 hours under controlled conditions (Eissa *et al.*, 2014). Fumigation with oil of *Achillea fragrantissima* at 60 uL L⁻¹ of air for 12 days exposure caused (91.3%) mortality in *S. oryzae* and *T. castaneum*. Lesser grain borer was highly susceptible (100%) at same rate of *A. beiber steinii* and *A. conyzoides*. Additionally, powder of mentioned plants when mixed with grains was more effective then oil application against these quarantine insect pests (Nenaah, 2014). Enriched plant extract of *A. indica* at 1000, 2000 and 4000EC caused successful beetle mortality also made it susceptible to the UV light exposure (Costa *et al.*, 2014). Significant toxicity of level of biopesticides used through topical applicant larval stage and on filter paper for adult red flour beetles recorded after 96 h exposure was 212 uL cm² and 796.8 uL cm² receptivity at 62.3 mg/mg dose (Nenaah, 2014).

Fumigant toxicity of *A. santolina* oil was dramatically increased and significant F₁ progeny of stored gain beetle was recorded (Nenaah, 2014). Lethal toxicity response of some biopesticides was result in delaying F₁ emergence and decrease in growth of the stored product weevil (Jumbo *et al.*, 2014). Percentage emergence of red flour beetle negatively correlated with the concentration of biopesticides. *N. tabacum* (10%) suppressed the F₁ generation (28%) at maximum level as compared to other botanical insecticides. As potential insecticide it affected the F₁ by minimizing pupation (40%) (Hasan *et al.*, 2012). Progeny production was censored in term of larvae, pupa and adult emergence of stored product beetle with increase in the concentration of *C. procerca* following by *Ammranthus hybridus*, *Salsola baryosma* and *Cumminum cimum* (Sagheer *et al.*, 2011). Basil oil of *Ocimum basilicum* fumigation produced strong lethal toxicity to *S. oryzae* at 3ml, 10% concentration inside a 1L plastic container. However, biopesticide used in packed rice caused very low mortality. Furthermore, F₁ progeny development was unaffected against basil oil fumigation (Follett *et al.*, 2014). Beetle progeny production collected from Faisalabad district of Pakistan was completely failed to develop as compared to Verhari strain against biopesticides extracted from four citrus species. *C. aurantium* derived insecticide (8%) killed 27.30% adult proved to be highly effective among others (Sagheer *et al.*, 2013).

Similarly, mortality rate was significant maximum (96.67%) when essential oils of two *Eugenia* sp. were applied on filter paper (Gonzalez *et al.*, 2014). Another example of biopesticides of Myrtales family (*Eucalyptus* sp.) was found to be litter bit effective against infestation

of *Sitophilus* sp. and *Tribolium* sp. When tested under laboratory conditions on homogenous population (Tapondjou *et al.*, 2005). Positive and improved synergistic insecticidal action of basil oil with other commercial fumigants, heat and irradiation application was suggested against stored grain insect pests (Follett *et al.*, 2013, 2014). Insecticidal activity of *D. stramonium*, *E. camaldulensis*, *Moringa deifera* and *Nigella sativa* oils were checked against three coleopteran beetles including *T. castaneum* and found effective. *D. stramonium* was highly toxic among all biopesticides treated against beetle and *T. castaneum* found second most susceptible (17.11%) insect after *Cryptolestes furrugines* (23.79%), respectively (Saleem *et al.*, 2014). Toxicological effects of *N. tabacum* at 10% concentration were high (6.69%) against *T. castaneum* mortality as well as its repellency (93.33%). While, *Pegnum hermala* and *Aussurea costa* were least effective so scientists nominated the *N. tabacum* and *Salsola baryosma* best for management of stored grain insect pest (Alvi, 2013). Fumigation toxicity of two *Eucalyptus* sp. against *C. maculates* was in wide rang (0.9-100%) in progeny production also for mortality bioassay (2.58-7.85). Furthermore, female oviposition was highly effected (6.3-100%) (Gusmao *et al.*, 2013). Insect mortality investigated in response to daily concentration variation proved that continuous phosphine treatment with respect to time and dose was less effected as compared diurnal interrupted treatments caused more mortality audit beetles of in the *S. oryzae* (Beckett, 2011).

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Statement of conflict of interest

The authors declare no conflict of interest.

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