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# Efficacy of Mixture of Pesticides on the Mortality and Energy Reserves of a Stored Grain Pest *Trogoderma granarium* Everts

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## ABSTRACT

*Trogoderma granarium* has become one of the most destructive insect pests of many amylaceous stored products. Its control has become difficult due to the emergence of resistance against almost all the known insecticides and fumigants. The development of resistance can be delayed by using binary combinations of insecticides. Keeping in view the importance of synergism as well as the concerns about environmental hazards and emergence of resistance against these insecticides, the current project was designed to figure out the lethal concentration ($LC_{50}$) of emamectin, abamectin and spinosad alone and in various conbinations such as abamectin:emamectin (1:1, 2:1, 3:1, 1:2 and 1:3) and abamectin:spinosad (1:1, 2:1, 3:1, 1:2 and 1:3) against two larval instars ($4^{th}$ and $6^{th}$) of MBDIN and Lahore populations of *T. granarium*. The $LC_{50}$ of abamectin, emamectin and spinosad for $4^{th}$ larval instars of Lahore population was 172, 185 and 196 ppm, respectively while $LC_{50}$ of these insecticides against $4^{th}$ larval instar of MBDIN population was 186, 192 and 198 ppm, respectively. Similarly, the $LC_{50}$ of abamectin, emamectin and spinosad for $6^{th}$ larval instars of Lahore population was 165, 180 and 174 ppm respectively, while $LC_{50}$ of these insecticides against $6^{th}$ larval instar of MBDIN population was 169, 184 and 179 ppm respectively. The abamectin was the most effective than emamectin and spinosad. Based on relative toxic unit, the 3:1 mixture of abamectin:emamectin showed higher toxicity among all the tested mixtures. The toxic effect of $LC_{20}$ of this 3:1 mixture on soluble and total proteins, total lipids, glucose, glycogen, free amino acid and trehalose contents was also recorded. The results indicated that contents of glycogen, trehalose, total lipids, free amino acids, soluble proteins and total proteins were significantly deceased in both larval instars except the free amino acids that increased in $4^{th}$ larval instar of both populations. The glucose contents increased in both larval instars of exposed groups of both populations with reference to unexposed group of the respective population. It is concluded that the mixtures of insecticides were more effective than the administration of insecticides alone to control *T. granarium*.

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**TR and FRS desingned and supervised the research. HM and SK performed the experiments. TR, FRS and MAS analyzed the data, TR wrote the article. FRS and MAS reviewed the field draft.**

**Key words**
***Trogoderma granarium*, Energy reserves, MBDIN, Abamectin, Emamectin, Spinosad**

## INTRODUCTION

Khapra beetle, *Trogoderma granarium* Everts is considered as one of the most economic insect pests of grains, cereals and other stored commodities particularly in subtropical and tropical areas of Africa and Asia (Burges, 2008). The feeding of larvae of *T. granarium* causes qualitative and quantitative damage to carbohydrate, protein and crude fat contents of stored products (Ahmedani *et al*., 2009). Contamination in grains with barbed hairs and skin of the larvae may cause a serious hazard to human health (Hosseininaveh *et al*., 2007; Ahmedani *et al*., 2009). Use of conventional and traditional chemical insecticides and fumigants was the main tool to control all types of stored grain insect pests but unplanned use of these insecticides have resulted in the development of resistance in *T. granarium* (Finkelman *et al*., 2006; Hafiz *et al*., 2017; Riaz *et al*., 2018; Shakoori *et al*., 2018).

The emergence of resistance can be delayed by adopting integrated pest management approaches and using the safer and selective biological insecticides. The reported data indicated that a single active ingredient fails to control various types of insect pest species that co-exist in a given storage commodity. Hence, the use of pesticide mixtures may provide a better control option for various insect species that are present in given storage structure. A pesticide mixture is actually a combined formulation of two or more pesticides into a single solution that can be applied simultaneously (O'Connor–Marer, 2000; Cloyd, 2011). In mixture, there is a possibility that these pesticides may develop synergism (Ware and Whitacre, 2004; Warnock

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and Cloyd, 2005; Cloyd *et al*., 2007) and these interactions developed when the toxicity of the combined pesticides is greater than the toxicity of pesticides applied alone to the target insect pest (Hewlett, 1968; O'Connor–Marer, 2000; Zhu, 2004). These mixtures may also be more effective against various developmental stages like eggs, larval instars, pupae and adult beetles of insect pests than individual application of a single insecticide (Blumel and Gross, 2001). Athanassiou *et al*. (2009) investigated that binary mixture of organophosphate (chlorpyrifos-methyl) and pyrethroid (deltamethrin) was most effective than spinosad or natural pyrethrum to control of psocids (Psocoptera) in stored grains. Similarly, Daglish (2008) found that the binary mixture of chlorpyriphos-methyl and spinosad was superior to the application of each insecticide alone to control various kinds of stored products insect species. In this context, the introduction of synergistic mixture of pesticides could be greatly beneficial, both economically as well as ecologically and combating resistance problem.

Avermectin belong to family of natural insecticides that are derived from soil dwelling actinomycetes *Streptomyces avermitilis* (Yen and Lin, 2004; Kwon *et al*., 2010; Huter, 2011). Avermectin B1 is also known as abamectin and it is a mixture of avermectin $B_{1a}$ and avermectin $B_{1b}$ macro cyclic lactones discovered in 1967 (Bai and Ogbourne, 2016). Emamectin is a semi synthetic derivative 4'-deoxy-4'-epi-methyl-amino benzoate salt of abamectin (Jyot *et al*., 2014). Emamectin benzoate is currently being introduced as a newer broad spectrum insecticide due to its higher toxicity at lower doses (Yen and Lin, 2004). Avermectins are categorized as neurotoxins because their mode of action involves overstimulation of glutamate and/or gamma-amino butyric acid (GABA)-gated chloride channel (Duce *et al*., 1995; Bloomquist, 2003; Huter, 2011) so, they increase the permeability of membrane chloride ions which decreases the excitability of neurons (Yen and Lin, 2004). After exposure to avermectins, insect pest reduce feeding and irreversibly paralyzed which ultimately results into death (Grafton-Cardwell *et al*., 2005).

Spinosad, a mixture of spinosyns A and D in the ratio of (85:15) is derived as a fermentation product of soil actinomycete, *Saccharopolyspora spinosa* in 1982 (Mertz and Yao, 1990; Hertlein *et al*., 2010). It refers to a new class of insecticides that is registered for use in agriculture due to low mammalian and off-target toxicity profiles. The already reported material suggested that it may be useful in controlling phosphine resistant insect pests (Sarfraz *et al*., 2005). Spinosad has a unique mode of action as its acts in two ways. It primarily interfere with nicotinic acetylcholine receptor and then the gamma-amino butyric acid receptors (Salgado and Sparks, 2005; Scott, 2008). It causes mortality in insect pest by inducing hyper-excitation of the nervous system (Raghavendra and Velamuri, 2018). Moreover, spinosad may also be used in rotation with synthetic insecticides in order to manage the insecticide resistance management (Su, 2016).

Several studies across the globe have shown a significant synergistic potential of abamectin, spinosad and abamectin (Warnock and Cloyd, 2005; Kang *et al*., 2006; Willmott *et al*., 2013; El-Razik and Zayed, 2014; El-Sheikh, 2015) with other insecticides combinations against various insect pests. The available literature on the effectiveness of different grain protectants is lacking the efficacy assessment of abamectin formulation with emamectin and spinosad. Keeping in view the importance of synergism as well as the concerns about environmental risks and emergence of resistance against conventional insecticides, the current study was designed to (a) determine the toxicity of emamectin, abamectin and spinosad when administered alone (b) toxicity of various mixtures of abamectin and emamectin as well as various combinations of abamectin and spinosad (c) the toxic effect of the most effective mixture on energy reserves of 4$^{th}$ and 6$^{th}$ larval instars of *T. granarium*. These findings will assist in management of khapra beetle in grain storage commodities by avoiding the use of conventional insecticides and fumigants as well as their ineffective mixtures.

## MATERIALS AND METHODS

*Insect rearing*

Two populations of *T. granarium viz.,* Lahore population designated as LHR and Mandi Bahauddin population designated as MBDIN was used in this study. Both populations possessed different levels of susceptibility to abamectin, emamectin and spinosad. Their master cultures were obtained from Department of Zoology, University of the Punjab, Lahore and these populations were already used in the studies of Riaz *et al*. (2014, 2016, 2017 and 2018). The insects were reared in insectary of University of Central Punjab, Lahore at 35±2°C and 60±5% relative humidity on broken wheat by adopting the methodology of Riaz *et al*. (2014). The newly emerged 4$^{th}$ and 6$^{th}$ larval instars were used in this study.

*Insecticides used*

A technical grade of emamectin benzoate (1.9EC), abamectin (1.8EC) and spinosad (24SC) was purchased from the Agricultural Chemical Group of FMC Corporation Lahore, Pakistan.

*Determination of $LC_{50}$*

Stock solutions (1000ppm) of emamectin, abamectin

and spinosad were prepared separately in acetone according to recommendation of WHO (2012). Ten different concentrations *i.e.,* 0-200ppm (at scale of 20ppm) of each insecticide were prepared and 1.0ml of each concentration was loaded on different filter paper and after through spreading, filter paper was air dried and placed in petri plate. For determination of $LC_{50}$, almost 15 individuals from each of 4th and 6th larval instars were introduced in these petri plates. Control plates were prepared by using acetone instead of insecticides. Plates were incubated at 35± 2°C and 60± RH. After 24 hrs, mortality was recorded in treatment and control plates and corrected mortality percentage was obtained by Abbott's formula (Abbott, 1925). The mortality was estimated using probit analysis statistical method of Finney (1971). Three sets of each abamectin:emamectin and abamectin:spinosad combinations were prepared in acetone. In set I, the concentration of both insecticides abamectin:emamectin was kept same. In set II, concentration of abamectin was increased and concentration of emamectin was decreased. In set III, concentration of abamectin was decreased and concentration of emamectin was increased. Same procedure was adopted for preparation of mixture of abamectin and spinosad (Table I). The aforementioned concentrations and procedure for administration of dose and evaluation of mortality was adopted for each set of the combinations of emamectin and abamectin as well as abamectin and spinosad. The possible antagonistic, additive or synergistic activity of these mixtures was calculated at $LC_{50}$ value by Relative Toxic Units (Otiltoloju, 2001).

$$\text{Relative Toxic Unit} = \frac{\text{Theoratical LC50 of mixture}}{\text{Observed LC50 of mixture}}$$

Theoretical $LC_{50}$ of mixture= ($LC_{50}$ of insecticide A alone×Percentage of A in Mixture)+($LC_{50}$ of insecticide B alone×Percentage of B in Mixture)

*Administration of $LC_{20}$*

The 4th and 6th larval instars of LHR and MBDIN was exposed to $LC_{20}$ of (3:1) mixture of abamectin:emamectin for 24 hrs to determine its toxic effects on energy reserves of *T. granarium*.

*Biochemical analysis*

Thirty individuals of each larval instar of these populations (treated and untreated groups) were homogenized in 1.5 ml of saline (0.89%) and centrifuged at 3,000g for 30 min at 4°C. The supernatants were used for the estimation of glucose contents by using *o*-toluidine procedure according to Hartel *et al*. (1969), trehalose contents according to Roe and Dailey (1966) and soluble proteins estimation was performed by Lowry *et al*. (1951). Similarly, total protein estimation was done by Lowry *et al*. (1951) but tissues were macerated in 0.5N NaOH and incubated at 70°C for 15 min followed by centrifugation at 3,000g for 30 min at 4°C. Tissue homogenate for free amino acid was prepared in ethanol (80%) and centrifuged at 461g for 10 minutes following by estimation according to Moore and Stein (1954) while glycogen was extracted in KOH (30%) and estimated by anthrone procedure of Consolazio and Lacono (1963). For estimation of total lipids concentration, homogenates was prepared by macerating larvae in hot ethanol followed by incubation at 65°C for overnight and centrifugation at 461g for 15 min. Zollner and Kirsch (1962) methodology was adopted for total lipids estimation.

**Table I. Concentrations of binary mixtures of emamectin, abamectin and spinosad for $LC_{50}$ determination against 4th and 6th larval instars of *T. granarium*.**

| Sets | Ratio | Pesticides active ingredient ratio in mixtures (ppm) | | | |
|---|---|---|---|---|---|
| | | Abamectin | Emamectin | Abamectin | Spinosad |
| Set I | 1:1 | 500 | 500 | 500 | 500 |
| Set II | 2:1 | 670 | 330 | 670 | 330 |
| | 3:1 | 750 | 250 | 750 | 250 |
| Set III | 1:2 | 330 | 670 | 330 | 670 |
| | 1:3 | 250 | 750 | 250 | 750 |

*Statistical analysis*

Statistical analysis was done in Minitab 16 and unpaired "t" test at 95% confident limit was used for effects of sub-lethal dose of mixture on metabolites of both larval instars of both populations. The data was considered non-significant at $p>0.05$ and significant at $p\leq0.05$.

## RESULTS

*Toxicity of insecticides*

The $LC_{50}$ of abamectin, emamectin and spinosad for 4th larval instars of Lahore population was 172, 185 and 196 ppm respectively while $LC_{50}$ of these insecticides against 4th instar larvae of MBDIN population was 186, 192 and 198ppm respectively. Similarly, the $LC_{50}$ of abamectin, emamectin and spinosad for 6th larval instars of Lahore population was 165, 180 and 174 ppm respectively while $LC_{50}$ of these insecticides against 6th instar larvae of MBDIN population was 169, 184 and 179ppm respectively. By comparing the $LC_{50}$ values of 4th larval instars of both populations, abamectin was found the most effective and spinosad was the least effective insecticide when administered alone. Similarly, the abamectin is the most effective insecticide for 6th larval instar of both populations but emamectin was the least effective for 6th

larval instar of both populations. Among larval instars, $LC_{50}$ of 4th instars of both populations was recorded at higher doses of insecticides than 6th instars. Among tested populations, both larval instars of MBDIN population were more tolerant than Lahore population (Table II and III).

*Toxicity of binary combination of abamectin and emamectin*

The interaction and toxicity of binary combinations of abamectin and emamectin are presented in Tables (II and III). In set I (1:1) of abamectin and ememectin mixture, when concentration of both insecticides were same, synergistic relationship was observed among both insecticides in 4th and 6th larval instars of both populations.

In set II of these mixtures, when concentration of abamectin is increased and concentration of emamectin was decreased (2:1), a strong synergistic relation (RTU 2.57 and 2.29) was observed respectively in 4th larval instar of both populations. This synergistic relationship became stronger (RTU 3.27 and 2.67) by further increasing the concentration of abamectin and decreasing the concentration of emamectin (3:1) in 4th larval instars of both populations. Similarly, strong synergism (RTU 2.46 and 2.2) was noticed in mixture (2:1) against 6th larval instars of Lahore and MBDIN populations, respectively. These synergistic interactions become stronger (RTU 2.96 and 2.83) respectively in 3:1 mixture of set II against 6th larval instar of Lahore and MBDIN populations.

**Table II. Comparison of toxicity of abamectin, emamectin and spinosad alone and in mixtures against 4th larval instar of *T. granarium*.**

| Insecticides | Populations | Ratio | Theoretical $LC_{50}$ | Observed $LC_{50}$ | Relative toxic unit | Interaction*[1] |
|---|---|---|---|---|---|---|
| Abamectin | Lahore | 1:0 | - | 172 | - | - |
| | MBDIN | | - | 186 | - | - |
| Emamectin | Lahore | 1:0 | - | 185 | - | - |
| | MBDIN | | - | 192 | - | - |
| Spinosad | Lahore | 1:0 | - | 196 | - | - |
| | MBDIN | | - | 198 | - | - |
| Set I (Abamectin : Emamectin) | Lahore | 1:1 | 178.5 | 81 | 2.20 | Synergism |
| | MBDIN | | 189 | 105 | 1.8 | Synergism |
| Set II (Abamectin: Emamectin) | Lahore | 2:1 | 176.29 | 66 | 2.57 | Synergism |
| | MBDIN | | 187.98 | 82 | 2.29 | Synergism |
| | Lahore | 3:1 | 175.25 | 52 | 3.37 | Synergism |
| | MBDIN | | 187.5 | 73 | 2.67 | Synergism |
| Set III (Abamectin:Emamectin) | Lahore | 1:2 | 180.71 | 174 | 1 | Additive |
| | MBDIN | | 190.02 | 189 | 1 | Additive |
| | Lahore | 1:3 | 181.75 | 180 | 1 | Additive |
| | MBDIN | | 190.5 | 187 | 1 | Additive |
| Set I (Abamectin:Spinosad) | Lahore | 1:1 | 129 | 184 | 1 | Additive |
| | MBDIN | | 192 | 142 | 1.35 | Synergism |
| Set II (Abamectin:Spinosad) | Lahore | 2:1 | 115.05 | 171.91 | 1.18 | Synergism |
| | MBDINI | | 189.96 | 126 | 1.51 | Synergism |
| | Lahore | 3:1 | 108.25 | 178 | 1.35 | Synergism |
| | MBDINI | | 189 | 108 | 1.84 | Synergism |
| Set III (Abamectin:Spinosad) | Lahore | 1:2 | 143.95 | 188.08 | 1.31 | Synergism |
| | MBDINI | | 194.04 | 126 | 1.43 | Synergism |
| | Lahore | 1:3 | 150.75 | 190 | 1.94 | Synergism |
| | MBDINI | | 195 | 95 | 1 | Additive |

1* RTU =1 Additive action; RTU < 1 Antagonism or RTU >1 Synergism.

In set III of these mixtures, when concentration of abamectin was deccreased and concentration of emamectin was increased (1:2 and 1:3) an additive interactions were developed against 4th larval instars of both populations. In case of 6th larval instars, a weak synergism was noticed in (1:2 and 1:3) mixtures when applied to Lahore and MBDIN populations.

*Toxicity of binary combination of abamectin and spinosad*

The toxicity and interaction of binary combinations of abamectin and spinosad are presented in Tables (II and III). In set I (1:1) of abamectin and spinosad mixture, when the concentration of both insecticides in mixture was same, an additive interaction was developed in 4th larval instar of Lahore population and 6th larval instar of MBDIN population while a week synergism was noticed in 6th larval instar of Lahore population and 4th larval instar of MBDIN population.

In set II of these mixtures, when concentration of abamectin is increased and concentration of spinosad was decreased (2:1 and 3:1) a synergistic interaction was induced in both insecticides against both larval instars of Lahore and MBDIN populations.

In set III of these mixtures, when concentration of abamectin was decreased and concentration of spinosad was increased (1:2) synergistic interaction was noticed against 4th instar larvae of both populations and 6th larval instar of Lahore population while an additive relationship was observed in 6th larval instar of MBDIN population. By further decreasing the concentration of abamectin and increasing the concentration of spinosad (1:3), an additive interaction was seen in 6th larval instar of both populations

**Table III. Comparison of toxicity of abamectin, emamectin and spinosad alone and in mixtures against 6th larval instar of *T. granarium*.**

| Insecticides | Populations | Ratio | Theoretical $LC_{50}$ | Observed $LC_{50}$ | Relative toxic unit | Interaction* |
|---|---|---|---|---|---|---|
| Abamectin | Lahore | 1:0 | - | 165 | - | - |
| | MBDIN | | - | 169 | - | - |
| Emamectin | Lahore | 1:0 | - | 180 | - | - |
| | MBDIN | | - | 184 | - | - |
| Spinosad | Lahore | 1:0 | - | 174 | - | - |
| | MBDIN | | - | 179 | - | - |
| Set I (Abamectin:Emamectin) | Lahore | 1:1 | 172.5 | 88 | 1.96 | Synergism |
| | MBDIN | | 176.5 | 92 | 1.92 | Synergism |
| Set II (Abamectin:Emamectin) | Lahore | 2:1 | 169.95 | 69 | 2.46 | Synergism |
| | MBDIN | | 173.95 | 79 | 2.20 | Synergism |
| | Lahore | 3:1 | 168.75 | 68 | 2.96 | Synergism |
| | MBDIN | | 172.75 | 61 | 2.83 | Synergism |
| Set III (Abamectin:Emamectin) | Lahore | 1:2 | 175.05 | 107 | 1.64 | Synergism |
| | MBDIN | | 171.05 | 103 | 1.7 | Synergism |
| | Lahore | 1:3 | 176.25 | 120 | 1.47 | Synergism |
| | MBDIN | | 180.25 | 115 | 1.57 | Synergism |
| Set I (Abamectin:Spinosad) | Lahore | 1:1 | 169.5 | 148 | 1.15 | Synergism |
| | MBDIN | | 174 | 169 | 1 | Additive |
| Set II (Abamectin:Spinosad) | Lahore | 2:1 | 167.97 | 134 | 1.25 | Synergism |
| | MBDINI | | 172.3 | 151 | 1.14 | Synergism |
| | Lahore | 3:1 | 167.25 | 103 | 1.62 | Synergism |
| | MBDINI | | 171.5 | 132 | 1.29 | Synergism |
| Set III (Abamectin:Spinosad) | Lahore | 1:2 | 171.03 | 112 | 1.38 | Synergism |
| | MBDINI | | 175.7 | 144 | 1 | Additive |
| | Lahore | 1:3 | 171.75 | 169 | 1 | Additive |
| | MBDINI | | 176.5 | 98 | 1 | Additive |

and 4th larval instar of MBDIN population but 4th instar larvae of Lahore population presented a synergistic relationship.

By comparing the values of relative toxic unit, it was evident that (3:1) mixture of abamectin:emamectin was the most effective among all the other tested mixtures of insecticides for both larval instars of Lahore and MBDIN populations.

**Table IV. Concentration (µg/mg) of metabolite of 4th and 6th instar larvae of *T. granarium* after 24hrs exposure.**

| Parameters | Populations | Unexposed group | Exposed group |
|---|---|---|---|
| **4th instar larvae** | | | |
| Glucose (µg/mg) | Lahore | 70.24±0.020 | 73.16±0.022 |
| | MBDIN | 82.41±0.01 | 87.12±0.34 |
| Glycogen (µg/mg) | Lahore | 9.67±0.04 | 3.21±0.43 |
| | MBDIN | 10.42±0.16 | 4.39±0.23 |
| Trehalose (µg/mg) | Lahore | 7.19±0.027 | 5.62±0.15 |
| | MBDIN | 11.3±0.54 | 7.86±0.23 |
| Total proteins (µg/mg) | Lahore | 20.23±0.12 | 18.31±0.32 |
| | MBDIN | 23.81±0.38 | 21.21±0.34 |
| Soluble proteins (µg/mg) | Lahore | 16.91±0.42 | 15.21±0.43 |
| | MBDIN | 14.12±0.32 | 13.38±0.52 |
| Free amino acids (µg/mg) | Lahore | 3.21±0.18 | 4.65±0.27 |
| | MBDIN | 5.12±0.38 | 5.69±0.15 |
| Total lipids (µg/mg) | Lahore | 1.32 ±0.4 | 0.65±0.7 |
| | MBDIN | 2.74±0.10 | 1.98±0.05 |
| **6th instar larvae** | | | |
| Glucose (µg/mg) | Lahore | 66.23±0.020 | 71.45±0.022 |
| | MBDIN | 79.61±0.01 | 84.23±0.34 |
| Glycogen (µg/mg) | Lahore | 7.23±0.04 | 3.11±0.43 |
| | MBDIN | 11.79±0.16 | 8.42±0.23 |
| Trehalose (µg/mg) | Lahore | 9.16±0.027 | 7.27±0.15 |
| | MBDIN | 12.51±0.54 | 9.68±0.23 |
| Total proteins (µg/mg) | Lahore | 23.18±0.12 | 16.74±0.32 |
| | MBDIN | 24.77±0.38 | 21.94±0.34 |
| Soluble proteins (µg/mg) | Lahore | 18.63±0.42 | 14.21±0.43 |
| | MBDIN | 19.34±0.32 | 14.67±0.52 |
| Free amino acids (µg/mg) | Lahore | 4.62±0.18 | 3.99±0.27 |
| | MBDIN | 5.99±0.38 | 4.72±0.15 |
| Total lipids (µg/mg) | Lahore | 1.68±0.4 | 0.69±0.7 |
| | MBDIN | 2.94±0.10 | 2.03±0.05 |

*Biochemical analysis*

The effect of $LC_{20}$ of the most effective mixture 3:1 of abamectin:emamectin on concentration of various metabolites (Table IV) and percent change in concentration of exposed group with reference to unexposed group of the same population was shown in (Figs. 1 and 2). The contents of glycogen, trehalose, total proteins, soluble proteins and lipids in exposed groups of 4th larval instar of Lahore and MBDIN populations were decreased with respect to their unexposed group value. The glucose and free amino acids contents were raised significantly in 4th larval instars of both populations. Same trend of reduction in all aforementioned biochemical parameters were noticed in 6th larval instars of both populations except the glucose contents which showed a significant increase in 6th larval instars of both populations after treatment with insecticide mixture when compared with unexposed group values (Figs. 1 and 2).

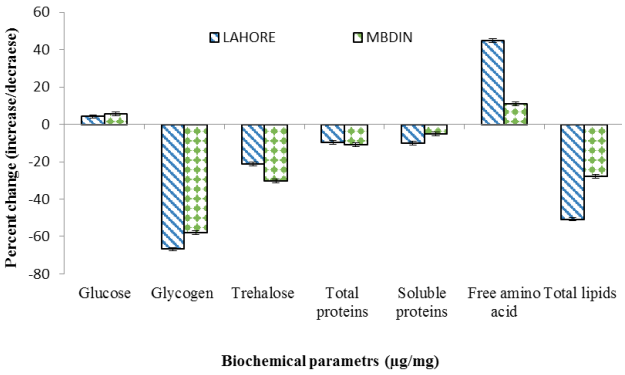


Fig. 1. Percent increase (+) or decrease (-) in biochemical parameters of 4th instar larvae of *T. granarium* after $LC_{20}$ exposure of 3:1 mixture of abamectin:emamectin with respect to unexposed group value.

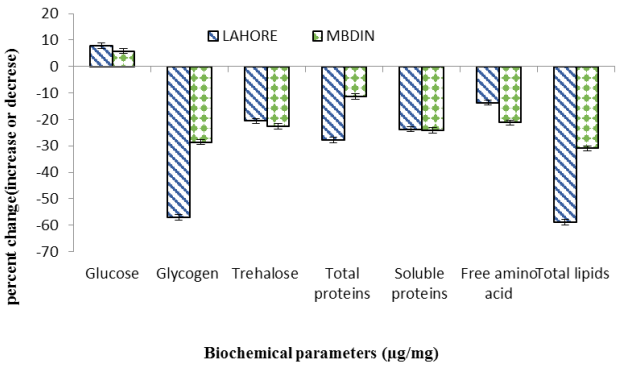


Fig. 2. Percent increase (+) or decrease (-) in biochemical parameters of 6th instar larvae of *T. granarium* after $LC_{20}$ (3:1 mixture of abamectin:emamectin) exposure with respect to unexposed group value.

## DISCUSSION

The study demonstrated that both larval instars of Lahore and MBDIN populations of *T. granarium* exhibited different levels of susceptibility against abamectin, emamectin and spinosad. Regarding the toxicity of insecticides, when administered alone, abamectin was found to be the most toxic from emamectin and spinosad. Our results are in consistent with the findings of Hussain *et al*. (2012) and Vojoudi *et al*. (2012) who reported that abamectin was more toxic to *Tribolium castaneum* than the other tested insecticides like spinosad, indoxacarb, buprofezin and azadirachtin etc. Abou-yousef *et al*. (2005) and Girgis *et al*. (2005) evaluated that abamectin was the most potent bio-compound in reducing the population of cotton leaf worm S*podoptera littoralis.* Willmott *et al*. (2013) reported the high efficacy of abamectin against western flower thrips. Similarly, Andric et al. (2011) also investigated that abamectin is more toxic to *T. castaneum* than spinosad. In contrast to these reports, Ahmed *et al*. (2005), Temerak (2007) and Megahed *et al*. (2013) reported that emamectin benzoate possessed more insecticidal activity than abamectin and spinosad against *Sitophilus littoralis*. Moreover, it was found that emamectin benzoate is toxic to a wide variety of insects like caterpillar pests (Lepidopterans); diamondback moth, *Plutella xylostella,* leaf miners, thrips and mites (Dunbar *et al*., 1998; Kodandaram *et al*., 2010; Shivalingaswamy *et al*., 2010). MacConnell *et al*. (1989) and Jansson and Dybas (1998) proposed that the abamectin residue on surface can easily be decomposed in sunlight which results in reduced toxicity to beneficial insects. Later on, the studies of Jansson and Dybas (1998) concluded that benzoate salt of emamectin protect it from decomposing in sunlight and thus providing more thermal stability and water solubility as compared with abamectin, so, emamectin is considered most effective than abamectin.

Several reports on efficacy of spinosad to control various types of insect pests indicated that it is very effective against house flies (Scott *et al*., 2000); eggplant flea beetle (Mcleod *et al*., 2002); German cockroach (Wei *et al*., 2001); *T. castaneum* and *Oryzaephilus surinamensis* (Fang *et al*., 2002; Hussain *et al*., 2005; Athanassiou and Kavallieratos, 2014); *Sitophilus oryzae* (Toews and Subramanyam, 2003) and rice moth (Huang and Subramanyam, 2004). The available literature documented the toxicity of abamectin, emamectin benzoate and spinosad against other insect pests but it is lacking the efficacy assessment of these insecticides against stored product insect pests especially *T. granarium.*

The current study demonstrated that each binary mixture (abamectin:emamectin and abamectin:spinosad) exhibited an additive or synergistic interactions when administered to both larval instars of tested populations. The information regarding the bio pesticidal mixtures that are safer to non-target material and highly toxic to test organisms is extremely valuable to control insect pests in storage commodities. Pesticide mixtures have the potential to suppress the insect pest population below the economic loss level. This suppression may be due to phenomenon of synergism or potentiation among pesticides that are combined together (Curtis, 1985; Comins, 1986; Ware and Whitacre, 2004; Warnock and Cloyd, 2005; Cloyd *et al*., 2007). The effects of binary mixtures of insecticides from same mode of action are usually easy to interpret, because the observed effects are often additive in nature (Lydy *et al*., 2004). For example, Bailey *et al*. (2000) observed that the binary mixture of chlorpyrifos and diazinon (both organophosphate) possessed additive interactions against cladoceran *Ceriodaphnia dubia*. Similarly, additive interactions were also observed in aquatic midge, *Chironomus tentans* when exposed to binary combinations of organophosphate insecticides like chlorpyrifos:azinphos methyl mixture and methidathion:diazinon mixtue (Lydy and Austin, 2004). In the same way, several reports have documented the synergistic interaction among classes of insecticides with almost similar type of mode of action. For example, Kulkrani and Hodgson (1980); Moreby *et al*. (2001) and Denton *et al*. (2003) noted that organophosphate and pyrethroid insecticides have synergistic interactions. They proposed that these synergistic interactions may develop due to similar type of mode of action. The organophosphates inhibit the activities of esterases so the insect ability to detoxify the pyrethroid will reduced resulting in increased toxicity of insecticides when combined. Similarly, when pyrethroids (Permethrin) and carbamates (carbamate propoxur) were combined, a synergistic interaction was observed against mosquito *Culex quinquefasciatus* (Corbel *et al*., 2003). These synergistic interactions were associated to the complementary mechanism of action of these insecticides, which may interfere with different components of the nerve impulse transmission.

Regarding the current results of mixtures, the toxicity of these binary combinations may be due to the unique mode of actions of these bio insecticides. Abamectin may act as an insecticide due to its contact and stomach action (Anonymous, 2003) while emamectin benzoate act on GABA gated chloride channels of the insect resulting in an increased chloride ion flux at the neuromuscular junction and causing rapid activation, inactivation and inhibition of neurotransmission (Jansson and Dybas, 1996). After its exposure, the insect stop feeding and irreversible paralysis and death of insect pest occur (MacConnell *et al*., 1989;

Jansson and Dybas, 1998). Spinosad possess a unique mechanism of action on nervous system of insect by acting on nicotinic acetylcholine receptors and also possessed an additional interference at H-Glutamate and GABA receptor sites causing a continuous activation of motor neurons and leading to reduced feeding, tremors of most muscles in the body followed by paralysis and death (Salgado, 1997; 1998; Semiz *et al*., 2006; Scott, 2008). Abamectin, emamectin and spinosad share some similarity in their mode of actions so, it was assumed that the both insecticides in binary mixture will interfere with these GABA gated chloride ion channels jointly and will induce paralysis and ultimately death of larvae. Their combined effect will result into additive interaction among both insecticides (Martin *et al*., 2003). In accordance to current findings, Ismail *et al*. (2007) observed synergistic interaction between spinosad and abamectin against two-spotted spider mites. Willmott *et al*. (2013) documented that spinosad and abamectin exhibited synergistic interaction against western flower thrips. Vojoudi et al. (2011) reported the synergistic interactions among spinosad and abamectin against *Helicoverpa armigera.* Although several pesticides are available in the market that are commonly used against whiteflies, thrips, spider, mites and aphids but very few documented reports are associated with efficacy of mixtures on stored grain insect pests. Moreover, no report is available about their efficacy against *T. granarium* so, more information is needed about impact of these pesticides mixtures against *T. granarium*.

The role of bio molecules including carbohydrates, protein and lipids are very crucial in performing various types of biochemical, physiological and behavioral responses in any organism (Yazdani *et al*., 2013). The decreased levels of glycogen, trehalose, soluble proteins, total lipids and total proteins in both larval instars of Lahore and MBDIN populations after exposure to the most effective mixture indicated that energy reserve are being utilized by the insect to cope the stress induced by mixture. The glycogen and trehalose is being converted to glucose (Shaurub and Aziz, 2015) which is noticeable due to increasing level of glucose and decreasing level of glycogen and trehalose in this study. The decreased level of lipids after treatment to mixture may be attributed to disturbance in lipid biosynthesis, metabolism and utilization as an energy source to survive during stress condition (Shaurub and Aziz, 2015). These findings are in consistent to Shakoori *et al*. (2016) and Shakoori *et al*. (2018a and b) after exposure to phopshine, esfenvalerate and λ-Cyhalothrin respectively working on *T. granarium.* Ali *et al*. (2011) also reported similar findings on working with *Rhyzopertha dominica* (F.) after treatment with Pyrethroid (Talsar). Similarly, the report of Hafiz *et al*. (2017) on *T. granarium* after treatment with deltamethrin supports the current findings.

The elevation in free amino acids contents and decrease in total and soluble protein contents are related to decreased activities of transaminases as proposed by Shakoori *et al*. (1994) after exposure of synthetic pyrethroid, Sumicidan Super to 6$^{th}$ larval instar of *T. castaneum*. Similarly, Shakoori *et al*. (2016) and Hafiz *et al*. (2017) reported an increase free amino acids concentration in larvae and adults of *T. granarium* after exposure to $LC_{20}$ of phosphine and deltamethrin, respectively. Ali *et al*. (2011) noticed significant increase in free amino acids concentration in adult beetles of *R. dominica* after exposure to melathion and Hussain *et al*. (2012) reported similar results in *T. castaneum* after exposure to abamectin. In order to provide energy, the normal metabolic processes were altered and proteins are degraded to amino acids which may enter the Citric acid cycle to continue the metabolic process (Bizhannia *et al*., 2005).

## CONCLUSION

The use of binary combinations of abamectin and emamectin was considered effective in controlling *T. granarium*. The metabolic abnormalities induced by $LC_{20}$ of mixture indicated that khapar beetle is highly sensitive to these binary mixtures of insecticides. By using synergistic approaches this notorious insect pest can be effectively controlled in godowns.

*Statement of conflict of interest*

The authors TR, FRS, HM, SK and MAS stated no conflicts of interest.

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