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Original article (Orijinal araştırma)

Comparative toxic potential of some plant extracts and spinetoram against *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae)

Bazı bitkisel özütlerin ve spinetoram ile kombinasyonlarının *Tribolium castaneum* (Herbst, 1997) (Coleoptera: Tenebrionidae)'a karşılaştırmalı olarak zehirlilik potansiyeli

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Abstract

The comparative toxicity of lemon grass, *Cymbopogon citratus* (Stapf) (Poales: Poaceae); neem, *Azadirachta indica* (A. Juss.) (Sapindales: Meliaceae); castor oil plant, *Ricinus communis* (L.) (Malpighiales: Euphorbiaceae); and yellow oleander, *Thevetia peruviana* (L.) Lippold (Gentianales: Apocynaceae) and spinetoram alone and in combinations were evaluated against *Tribolium castaneum* (Herbst, 1797) in the Entomology Laboratory of Punjab Bioenergy Institute, University of Agriculture, Faisalabad, Punjab, Pakistan from May to October, 2018. Different concentrations of plant extracts (5, 10 and 15%) and spinetoram (0.01, 0.02 and 0.03%) were applied in filter paper toxicity bioassay. The results of single applications of all treatments showed that spinetoram was more effective with the highest mortality of 79.8% by followed by 57.9% for *A. indica*, 49.5% for *C. citratus*, 40.1% for *T. peruviana* and 28.9% for *R. communis* extracts. In combinations, *A. indica*+spinetoram gave the highest mortality of 84.9% with a lower mortality of 25.2% observed for *R. communis*+spinetoram. LC_{50} values of spinetoram were comparatively low indicating that it is the more toxic than the plant extracts. Overall the results indicated that these plant extracts and spinetoram can be used as combined form in IPM for the efficient management of insect pests of stored grains.

Keywords: Concentrations, LC_{50}, management, mortality, synthetic insecticides, toxic

Öz

Limon otu (*Cymbopogon citratus* (Stapf) (Poales: Poaceae), neem (*Azadirachta indica*) (A. Juss.) (Sapindales: Meliaceae), hint otu (*Ricinus communis*) (L.) (Malpighiales: Euphorbiaceae) ve meksika zakkumu (*Thevetia peruviana*) (L.) Lippold (Gentianales: Apocynaceae) 'nun tek başına ve spinetoram ile kombinasyonlarının *Tribolium castaneum* (Herbst, 1797)'a karşı toksikolojik etkileri Mayıs-Ekim 2018 tarihlerinde Tarım Üniversitesi, Punjab Bioenergy Enstitüsü (Faisalabad, Punjab, Pakistan), Entomoloji laboratuvarında değerlendirilmiştir. Filtre kâğıdı yöntemi ile yapılan biyolojik testlerde farklı bitki konsantrasyonları (%5, 10 ve 15) ve spinetoramın (%0.01, 0.02 ve 0.03) farklı dozları uygulanmıştır. Tüm muameleler tek başına uygulandığında, spinetoram %79.8 oranla en yüksek öldürücü etkiye sahip olurken, bunu sırasıyla *A. indica* (%57.9), *C. citratus* (%49.5) ve *T. peruviana* (%40.1) izlemiştir. En düşük etki %28.9 oranla *R. communis* özütünde kaydedilmiştir. Özütler spinetoram ile birlikte uygulandığında, *A. indica* kombinasyonu %84.9'lik oranla en yüksek öldürücülüğe sahip olurken, nispeten düşük ölüm oranı (%25.2) spinetoramın *R. communis* ile kombinasyonunda gözlenmiştir. Spinetoramın LC_{50} değerleri denemede kullanılan bitki özütleriyle karşılaştırıldığında en yüksek değeri veren özüte göre düşük bulunmuştur. Sonuç olarak, bu çalışma bu bitki özütlerinin spinetoram ile kombinasyonun depolanmış tahıllardaki zararlı böcekler ile etkili mücadele için IPM'de kullanılabileceğini göstermiştir.

Anahtar sözcükler: Konsantrasyonlar, LC_{50}, mücadele, öldürücü etki, sentetik insektisitler, zehirlilik

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Introduction

Insect pest infestations in stored commodities are increasingly important worldwide. Many different insect pests can infest the stored grain (Phillips & Throne, 2010). The red flour beetle, *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) is a cosmopolitan and the most widespread insect pest of wheat flour and cereals in Pakistan (Lee et al., 2002; Shafique et al., 2006; Nadeem et al., 2013) and worldwide (Ogendo et al., 2008). Quality of infested commodities is seriously impacted, for example., baking ability, nutritional value, decreased germination and increase proportion of damaged (Mondal, 1994). Both larvae and adults of *T. castaneum* feed on wheat flour and broken grain (Dars et al., 2001; Karunakaran et al., 2004), secrete quinines and other chemicals (Ho et al., 2008).

Synthetic chemicals (fumigants and other insecticides) have been the main means of control different insect pests in stored grains. With fumigants, such as methyl bromide and phosphine, have been mostly used in the management of different insect pests of stored grain. However, frequent and inappropriate use of these pesticides has resulted in adverse effects to human beings, and natural flora and fauna. Furthermore, some insect populations have developed resistance against these chemicals (Bell & Wilson, 1995; Benhalima et al., 2004). Many stored grain insect pests have also developed resistance against organophosphates (Hussain et al., 2005). Moreover, the residual effects synthetic insecticides in grains have been reported, leaving the food commodities unfit for human consumption (Benhalima et al., 2004; Phillips & Throne, 2010). Therefore, there is an imperative to develop and deploy bioinsecticides from plant materials and the bio-derived spinetoram, which are eco-friendly, safe to humans and can effectively manage the insect pests of stored commodities in storage facilities.

Plant extracts are potential substitute to synthetic insecticides (Copping & Menn, 2000; Mondal et al., 2006; Koul et al., 2008; Nenaah, 2011; Usha et al., 2011), having different effects, such as, fumigant action (Rajendran & Sriranjini, 2008), antifeedant action (Kamruzaman et al., 2005), toxicity (Islam et al., 2010), repellence (Dwivedi & Shekhawat, 2004; Susana et al., 2013; Hassan et al., 2017) and growth inhibition (Tatun et al., 2014). Being natural products and less persistent in nature, they are eco-friendly to surrounding flora and fauna (Isman, 2006; Sanna et al., 2004; Tapondjou et al., 2005; Saroukolai et al., 2010; Regnault-Roger et al., 2012). Many researchers have evaluated plant extracts for the management of different insect pests, for example, *Mentha royleana* Benth. (Lamiales: Lamiaceae) and *Artemisia absinthium* (L.) (Asterales: Asteraceae) extracts against *T. castaneum* (Zuhra et al., 2018), datura leaf extract against *Trogoderma granarium* (Everts, 1898) (Coleoptera: Dermestidae) (Ali et al., 2012), *Elletaria cardamomum* (L.) (Zingiberales: Zingiberaceae) against *Sitophilus zeamais* (Motschulsky), 1855 (Coleoptera: Curculionidae) and *T. castaneum* (Huang et al., 2000), *Abroma augusta* (L.) (Malvales: Malvaceae) and garlic extracts against *T. castaneum* (Mondal et al., 2006; Yang et al., 2010), *Azadirachta indica* (A. Juss.) (Sapindales: Meliaceae) extract against *T. granarium* (Odeyemi & Ashamo, 2005), *Datura alba* and some other plan extracts against *S. zeamais* and *Oryzaephilus surinamensis* (L., 1758) (Coleoptera: Silvanidae) (Rehman et al., 2018). Spinetoram is regarded as new member of spinosyn family and has been introduced commercially for the control of different insect pests (Sparks et al., 2008; Jones et al., 2010; Sial et al., 2011; Ali et al., 2017). Spinetoram is a product of secondary metabolites spinosyn J and L (Herbert, 2010). Another new chemistry insecticide, spinosad has also been used against *T. castaneum*. Spinetoram has been used by many researchers and proved comparatively more effective for the control of stored grain insect pests than spinosad (Sparks et al., 2008; Jones et al., 2010; Dripps et al., 2011; Yee & Alston, 2012; Saglam et al., 2016). To address the situation described above, experiments were conducted in the Entomology Laboratory of Punjab Bioenergy Institute, University of Agriculture, Faisalabad, Punjab, Pakistan from May to October, 2018 to evaluate toxic effects of some plant extracts and spinetoram on *T. castaneum*.

Material and Methods

Collection and rearing of test insects

Tribolium castaneum adults were collected from flour mills and grain markets of Kasur and Faisalabad in Punjab, Pakistan. The insects were cultured in the Entomology Laboratory of Punjab Bioenergy Institute, University of Agriculture, Faisalabad, Punjab, Pakistan from May to October 2018 on sterilized wheat flour in small plastic jars at 30±2ºC and 65±5% RH. Fifty pairs of *T. castaneum* were released into jars containing 2 kg of wheat flour. The jars were covered with muslin, secured with a rubber band to prevent the insects escape. The insects were kept in the jars for abut 3 to 4 d to lay eggs. After oviposition, the insects were transferred to new jars of wheat flour using a camel hair brush to lay more eggs and then the flour combined with that in the original jar. Complete emergence of adult beetles was achieved after 30-35 d and these adults were used in toxicity bioassays.

Preparation of plants extracts

Leaves of four plant species (Table 1) were collected from different sites of the University of Agriculture Faisalabad.

Table 1. Description and altitude of collection of plant materials collected from May to October 2018

Common name	Scientific name	Description	Altitude (m)
Lemon grass	*Cymbopogon citratus* (Stapf) (Poales: Poaceae)	lofty perennial grass, silky heads, citronella fever grass	184
Neem	*Azadirachta indica* (A. Juss.) (Sapindales: Meliaceae)	pinnate leaves, purple-red when young, developing to a medium green color when mature, produces small, fragrant white flowers and olive-like fruits	168
Castor oil plant	*Ricinus communis* (L.) (Malpighiales: Euphorbiaceae)	perennial flowering plant, reproducing with a mixed pollination system which favors selfing by geitonogamy	184
Yellow oleander	*Thevetia peruviana* (L.) Lippold (Gentianales: Apocynaceae)	iornamental, medicinal shrub and is an evergreen shrub often planted close to stables and paddocks, highly toxic, a potent cardiotoxic plant	189

Plant materials were washed with distilled water, dried in the shade and ground into a powder using an electric mill. The plant powders were sieved using a suitable mesh sieve to get a fine powder. Plant materials were extracted with acetone as by mixing 50 g of plant powder with 100 ml of solvent as described by Valladares et al. (1997) and Ahmad et al. (2006). To minimize solvent evaporation during extraction process, conical flasks were plugged with cotton plugs covered aluminum foil. The flasks were then placed on rotary shaker at 220 rpm for 24 h. Filtration was performed with using the Whatman filter papers.

Bioassay of plant extracts and spinetoram against *Tribolium castaneum*

Completely randomized design (CRD) used for the bioassay. Dilutions (5, 10 and 15%) of the stock solution of each plant extract were prepared in acetone. Each dilution was applied on 40 g of wheat grain in jars using a glass pipette. Controls were treated with acetone only. The grain was shaken for 2 min to ensure uniform distribution of the applied treatments. After evaporation of the solvent for few minutes, twenty unsexed adults of *T. castaneum* were added to each jar, and the jar covered with muslin secured with a rubber to prevent insects escape. The jars kept in incubator under optimum conditions. Insect mortality was recorded at regular intervals from 24 to 72 h. Also, three dilutions (0.01, 0.02 and 0.03%) of a stock solution of spinetoram were prepared in acetone for a separate bioassay using the method given above. All experiments were performed with three replicates.

Percentage corrected mortality was calculated using Abbott's formula (Abbott, 1925) prior to statistical analysis as:

$$\% \text{ Corrected Mortality} = \frac{\text{Mo } (\%) - \text{Mc } (\%)}{100 - \text{Mc } (\%)} \text{ X } 100$$

where, Mo is the observed mortality and Mc is the mortality in control unit.

Bioassay of plant extract and spinetoram combinations

Combined effects were evaluated using the most effective concentrations of plant extracts and spinetoram against *T. castaneum* as determined in the bioassays dcribed above. Treated filter papers (with treatment combinations) were placed in separate Petri dishes along with broken grains and 20 adult beetles (10 pairs). Mortality was recorded at regular intervals from 24 to 72 h.

Statistical analysis

The recorded data was subjected to Abbott's formula and then analyzed by using STATISTICA and means of the treatments were compared by Tukey's HSD at α of 5%.

Results and Discussion

Figures 1, 2 and 3 shows the treatment and concentration effects, which were significant after 24, 48 and 72 h. of treatment application. The combined effect of time ($F_{2,24}$ = 206; $p < 0.05$) and concentration ($F_{2,24}$ = 20.8; $p < 0.05$) were also significant. The highest mortality of 79.9% was observed for 0.03% spinetoram and for the plant extracts, 57.9% for *A. indica* followed by 49.5% for *C. citratus* and 40.1% for *T. peruviana* after 72 h. Th lowest mortality 28.9% was for *R. communis* at 15% after 72 h. Exposure of 24 and 48 h was less effective with mortality at 24 h of 21.3% for spinetoram, 20.0% for *A. indica*, 18.3% for *C. citratus*, 11.2% for *R. communis*, 8.33% for *T. peruviana* and at 48 h of 45.0, 33.7, 29. 3, 17.7 and 15.3%, respectively.

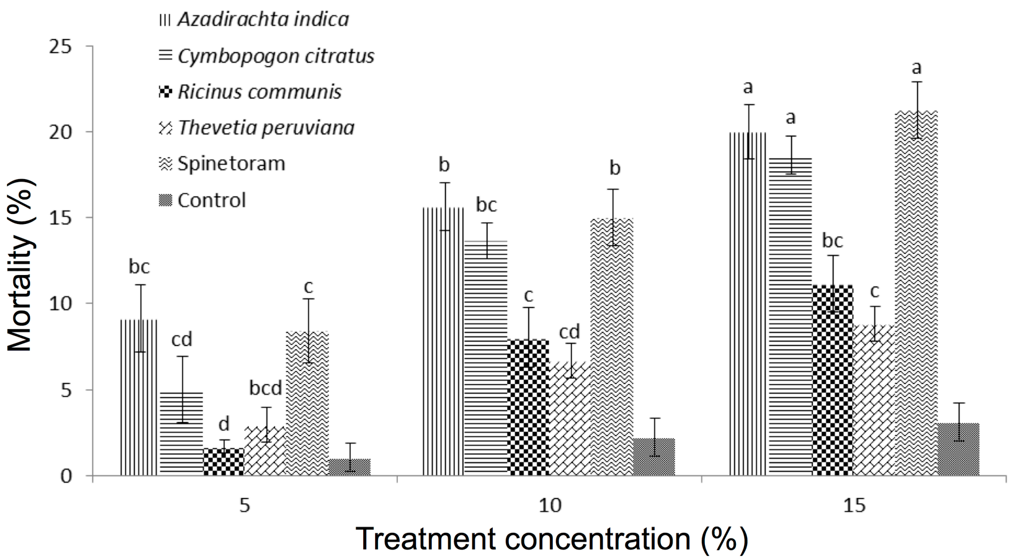

Figure 1. Efficacy of extracts of *Azadirachta indica*, *Cymbopogon citratus*, *Ricinus communis*, and *Thevetia peruviana,* and spinetoram against *Tribolium castaneum* adults after exposure of 24 h. Bars represent treatment means and error bars are 95% CI. The lowercase superscript letters above each bar represent post hoc pairwise comparisons between treatments. Treatment $F_{3,24}$ = 4.66, $p < 0.05$ and concentration: $F_{2,24}$ = 2.14, $p < 0.05$.

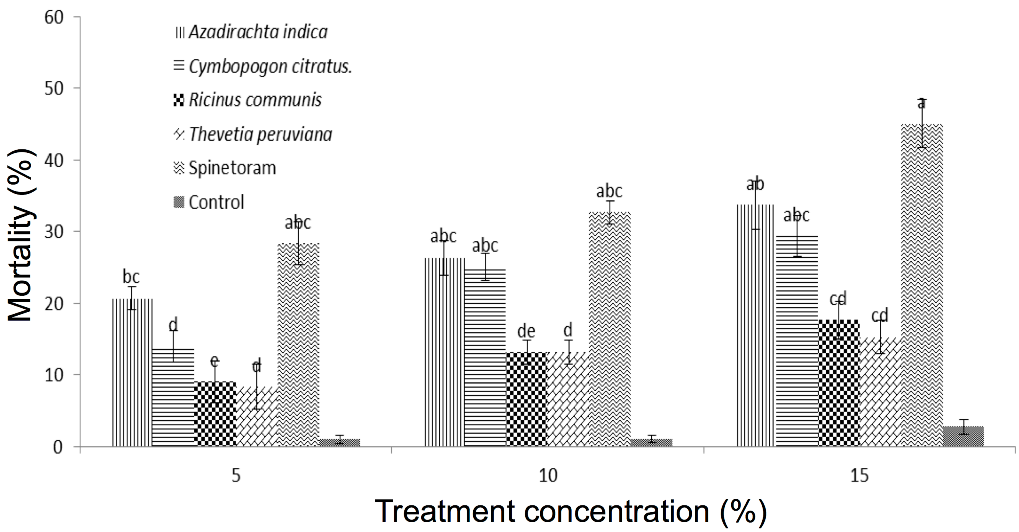

Figure 2. Efficacy of extracts of *Azadirachta indica*, *Cymbopogon citratus*, *Ricinus communis*, and *Thevetia peruviana,* and spinetoram against *Tribolium castaneum* adults after exposure of 48 h. Bars represent treatment means and error bars are 95% CI. The lowercase superscript letters above each bar represent post hoc pairwise comparisons between treatments. Treatment, $F_{3,24} = 12.3$, $p < 0.05$ and concentration $F_{2,24} = 11.5$, $p < 0.05$.

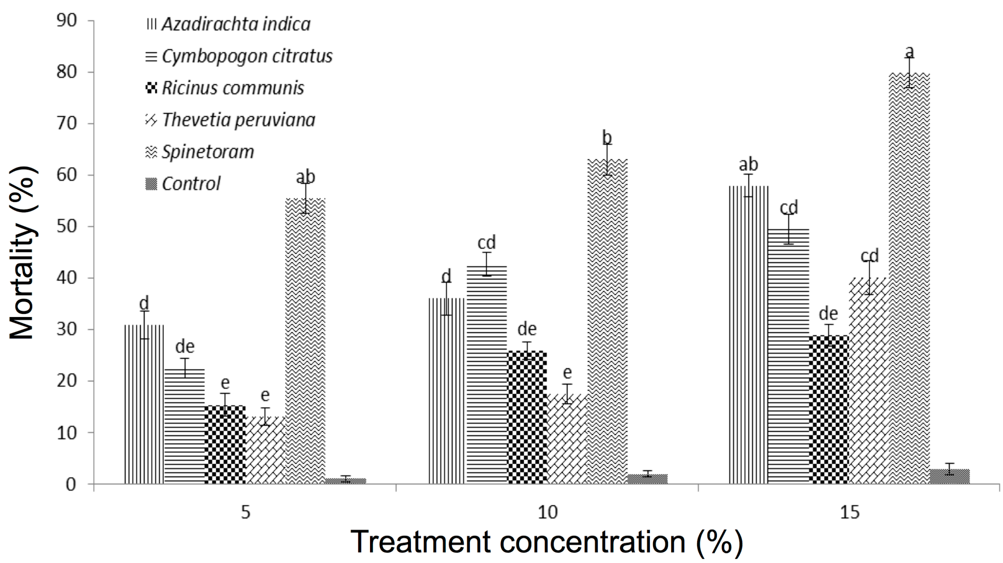

Figure 3. Efficacy of extracts of *Azadirachta indica*, *Cymbopogon citratus*, *Ricinus communis*, and *Thevetia peruviana,* and spinetoram against *Tribolium castaneum* adults after exposure of 72 h. Bars represent treatment means and error bars are 95% CI. The lowercase superscript letters above each bar represent post hoc pairwise comparisons between treatments. Treatment $F_{3,24} = 4.72$, $p < 0.05$ and concentration $F_{2,24} = 11.1$, $p < 0.05$.

For the combined treatments, the effect of time and concentration were also significant (Figure 4). The results showed that the combined action was more effective with the highest mortality of 84.9% for *A. indica*+spinetoram followed by 61.1% for *C. citratus*+spinetoram and 52.7% for *T. peruviana*+spinetoram). The lowest mortality was 39.4% for *R. communis*+spinetoram.

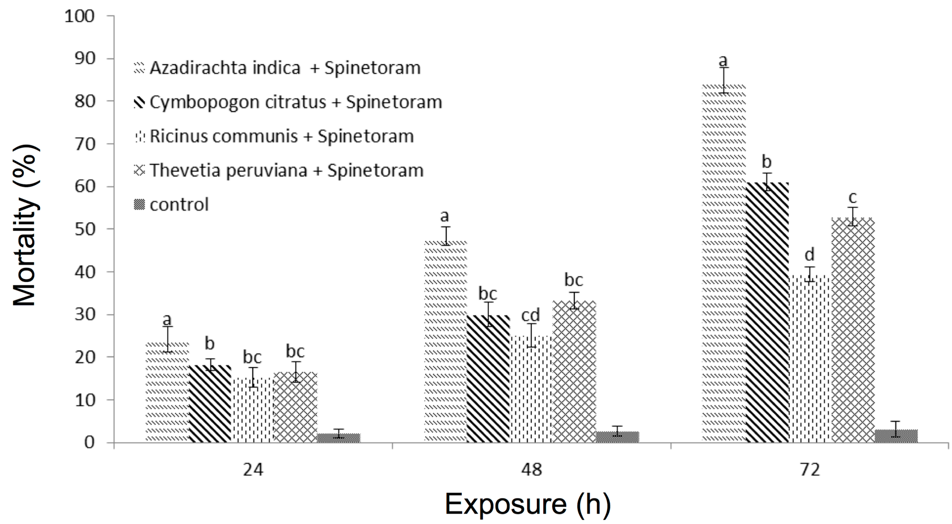

Figure 4. Combined action of optimal concentration of extracts of *Azadirachta indica*, *Cymbopogon citratus*, *Ricinus communis* and *Thevetia peruviana*, and spinetoram against *Tribolium castaneum* adults after different exposure times. Bars represent treatment means and error bars are 95% CI. The lowercase superscript letters above each bar represent post hoc pairwise comparisons between treatments. Time $F_{2,24}$ = 206, $p < 0.05$ and concentration $F_{2,24}$ = 20.8, $p < 0.05$.

LC_{50}

Probit analysis gave approximate LC_{50} values for plant extracts of 13.3% for *A. indica*, 14.7% *C. citratus*, 20.9% for *T. peruviana* and 39.7% for *R. communis* after 72 h. LC_{50} values after 48 and 24 h were higher, with the highest being 63.7% for for *A. indica* at 24 h (Table 2). The lower the value the greater the toxicity. The results indicated that extract of *A. indica* was more toxic than other extracts with extended exposure.

Table 2. LC_{50} of plant extracts and spinetoram against *Tribolium castaneum* after three exposure periods (24, 48 and 72 h)

Treatments	Exposure (h)	Slope	Z-value	P-value	R^2-value	LC_{50} (%)	Confidence limits (95%)	
Azadirachta indica	24	5.5	2.15	0.032	0.997	63.70	25.7	88.4
	48	6.5	2.03	0.042	0.998	44.00	19.9	76.3
	72	13.5	3.61	0.000	0.906	13.30	10.6	21.1
Cymbopogon citratus	24	6.5	2.74	0.006	0.982	41.40	23.0	82.4
	48	7.5	2.55	0.011	0.932	36.80	20.1	78.1
	72	13.5	3.78	0.000	0.906	14.70	11.7	24.0
Ricinus communis	24	4.5	2.40	0.016	0.964	48.30	24.5	84.6
	48	4.0	1.48	0.139	0.984	44.20	7.8	60.7
	72	6.5	2.38	0.015	0.911	39.70	20.4	79.8
Thevetia peruviana	24	3.0	1.50	0.133	0.988	70.10	4.1	82.7
	48	3.5	1.54	0.123	0.942	67.30	4.0	76.8
	72	18.5	4.05	0.000	0.985	20.90	16.1	39.9
Spinetoram	24	6.5	2.52	0.012	0.998	9.37	4.7	16.1
	48	8.5	2.38	0.017	0.914	4.42	2.8	8.2
	72	14.5	4.11	0.000	0.918	2.10	0.5	4.3

Management of *T. castaneum* has been mainly achieved with fumigants such as methyl bromide and phosphine, and some dusts chemicals such as permethrin (Price & Mills, 1988). However, frequent and inappropriate use of such insecticides has resulted in problems such as environmental pollution, residues in food and hazardous effects on non-target organisms (Benhalima et al., 2004). These problems have motivated researchers to search for alternative management options such as the use of plant extracts and bio-derived insecticides such as spinetoram.

The research reported here, was were to evaluate the toxic effects of four plant extracts and spinetoram against *T. castaneum*. Concentrations as well as exposure period effects were found to be significant in bioassays. After 72 h at the highest concentration, the highest toxicity was 79.8% for spinetoram and the lowest 28.9% was for *R. communis*. These findings are close to Khoshnoud et al. (2008) who used plant extracts against two stored grain insect pests and recorded increased mortality at highest concentration and exposure period similar to the present study. Likewise, the findings are consistent with Mamun et al. (2009) and Ahmed et al. (2018), who recorded increased mortality at increased plant extract concentrations confirming the toxicity. The highest mortality (79.8%) was close to Mostafa et al. (2012) who used *Cucumus sativus* and recorded 80% mortality of *T. castaneum.* In present study, the extract of *A. indica* gave mortality up to 57% at highest concentration after exposure of 72 h is similar to Padín et al. (2013) who examined the toxicity of some plant extracts against *T. castaneum* and recorded mortality up to 57% with extract of *Matricaria chamomilla* L. LC_{50} values in the present study are close to Mamun et al. (2009) who evaluated neem oil and some other plant oil against the same target insect. Slight difference may be due to different concentrations of plant extracts. The LC_{50} are similar to the findings of Reddy et al. (1999) who recorded similar but somewhat different values due to difference in insect species in both studies. Findings of the present study are close to Huang et al. (2000) who recorded values up to 51 and 52%, close to the 48.3% for *R. communis*. Slight difference may be due to difference in plant extracts and concentrations, used. The toxicity (70%) in the bioassays with spinetoram was also close to Vassilakos et al. (2014) who recorded 72.4% against *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrichidae). A slight difference may be due to different species. Findings of the present study are close to Hameed et al. (2012) who evaluated the insecticidal activity of a bio-base insecticide spinosad and two extracts, neem (*A. indica*) and *Nerium oleander* L., against *T. castaneum*. Mortality values of *T. castaneum* were up to 50%. Slight differences may be due to difference in new chemistry insecticide than in the present study (spinetoram). Mortality finding of the present study were close Vassilakos et al. (2012) who found mortality similar to the present study with application of spinetoram against the adults of *Sitophilus oryzae* (L., 1763) (Coleoptera: Curculionidae), *R dominica*, *Prostephanus truncatus* (Horn, 1878) (Coleoptera: Bostrichidae), *Tribolium confusum* Jacquelin du Val, 1863 (Coleoptera: Tenebrionidae), *Sitophilus granarius* (L., 1758) Coleoptera: Curculionidae) and *O. surinamensis.* The mortality trend was similar to the present study, increased mortality was found at increased concentrations.

It is concluded that spinetoram and the tested plant extracts can provide effective control of *T. castaneum*. However, the efficacy of spinetoram was enhanced when combined with plant extracts, especially in case of *A. indica.* Therefore, it is suggested that the combine application of plant extracts and spinetoram at higher concentration along with longer exposure periods can be an effective alternative to synthetic insecticides for eco-friendly management of stored commodity insect pests.

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