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Efficiency of *Zingiber officinale* and *Myrtus communis* extracts against Red flour beetle, *Tribolium castaneum*

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ABSTRACT
Continues use of synthetic insecticides has resulted in many serious problems, such as the development of insect resistance, toxicity to non-target organisms and environmental pollution. Thus, there is a necessity for effective and safe alternatives which can control stored grain pests infestation. This study was conducted to evaluate the insecticidal activity of two plant extracts, ginger, *Zingiber officinale* and myrtle, *Myrtus communis* separately and in combination against adult of red flour beetle, *Tribolium castaneum*. The plant extracts prepared by using Soxhlet Extractor and Ethyl alcohol as solvent. Three concentrations (2.5%, 5%, and 7.5%) of each plant were prepared. We placed 0.5 mL of each concentration on filter paper in petri dish, in addition to the control treatment. Ten adult insects were put into each petri dish. The mortality rate was recorded at 24, 48 and 72 h after treatment. The results indicated that the mortality was varying from plant to plant as flowing: *M. communis* reached its maximum value of 3.33% at 7.5 g mL^{-1} after 72 h. In the case of *Z. officinale*, it was 4% at 7.5 g mL^{-1} after 72 h, while the mixture of *Z. officinale* and *M. communis* reached its maximum value (4%) at 7.5 g mL^{-1} after 48 h exposure time. Relationship between exposure time and concentrations on mortality of *T. castaneum* indicated that mortality was increased by elevating the concentration and exposure time. The results of the present study revealed that by considering the mean mortality as a main index, the combination of *Z. officinale* and *M. communis* was the most toxic, while *M. communis* the least toxic. Results suggested that the combination of the two extracts could become interesting alternative to conventional chemical insecticides against stored grain pests.

Keyword: *Tribolium castaneum*, plant extract, Baghdad, combination, *Z. officinale* and *M. communis*.
Article type: Research Article.

INTRODUCTION
Protecting stored food grains from insects is very serious problem throughout the world. The insect infestation affects the food grains in stores as well as during shipping and transportation (Upadhyay & Ahmad 2011). The insect pests damage the stored grain products, causing reduction in volume, weight loss, contamination by body parts and insect feces and overall loss of quality and quantity (Maedeh *et al.* 2012). Red flour beetle, *Tribolium castaneum*, is a serious insect pest attacking stored grains and foods throughout the world (Utono 2013). Both adults and larvae feed on broken grain and finely ground materials in mills, warehouses and all types of grain storages. This species is common storage pests of the genus *Tribolium* which cause the most damage, and one of the most economically important beetles (Khani & Basavand 2012). Currently, the chemical insecticides are the most popular and effective methods for the control of insects of stored products. However, their widespread use has caused some serious problems such as toxic residues on stored grains, the development of insect strains resistant to insecticides, and health hazards to human beings. Thus, there is an urgent need for safe suitable alternatives to these synthetic insecticides to control the insects of stored products (Ali & Mohammed 2013). Several plant extracts have been used as potential insect pest control agents due to their content of different

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substances and their effect as insecticides or repellents (Maedeh *et al.* 2012; Salhi *et al.* 2019; Naser AL-Isawi 2022; Hamdouch *et al.* 2022). The genus *Zingiber*, belonging to the family Zingiberaceae, includes about 50 genera and over 1200 species mostly grown in the tropical countries (Pintatum *et al.* 2020). It has been widely reported that rhizome of ginger is consumed worldwide for its medicinal value and flavor-enhancing agent (Yacout *et al.* 2007). *Zingiber officinale* (Zingiberaceae family) possesses a variety of components which have displayed insecticidal, anti-feedant, growth regulating and repellent activity against many insect pests (Abdulhay & Yonius, 2019). Previous studies investigated the insecticidal effects of *Z. officinale* against red flour beetle, *Tribolium castaneum*, Mediterranean flour moth, *Ephestia kuehniella* and Indianmeal moth, *Plodia interpunctella* (Maedeh *et al.* 2012), *Oryzaephilus surinamensis* and *Oryzaephilus mercator* (Amiri *et al.* 2016). *Myrtus communis* or common myrtle is often grown for its attractive foliage, flowers and berries (Qader *et al.* 2017). Since ancient times, myrtle has been used for a variety of purposes such as cosmetics, flavoring of food and drinks and extensive treatment of several diseases (Sumbul *et al.* 2011). It is reported to possess the potential of being insect growth regulatory and insecticidal against various insect species (Ghania *et al.* 2014). Several researches reported the insecticidal activities of *M. communis* against confused flour beetle, *Tribolium confusum*, cowpea weevil, *Callosobruchus maculatus* and khapra beetle, *Trogoderma granarium* (Khani & Basavand 2012; Tayoub *et al.* 2012). Therefore, this study was aimed to evaluate the effect of ginger, *Z. officinale* and myrtle, *M. communis*, separately and in combination, on the control of *T. castaneum*.

MATERIALS AND METHODS

Preparation of plant extracts

The leaves of myrtle plants were collected from gardens in University of Baghdad, Iraq. These fresh leaves were washed sufficiently with tap water several times and then placed under the shade for drying with constant stirring to prevent rotting, for 3 to 5 days. Afterward leaves were grinded to a fine powder with an electric blender. Dry rhizomes of ginger, *Zingiber officinale* were purchased from a local market in Baghdad, Iraq. The rhizomes were grinded into powder using an electric blender and these sieved to obtain fine powder. The powders of the two plants were placed into airtight containers separately to ensure that the active ingredients were not lost and then stored in a cool dry place until use.

Methanol extracts

Harborne method (1984) was used to obtain the organic extracts of both myrtle leaves and dry rhizomes of ginger. Using the Soxhlet extraction technique, 300 g of the powdered material from each plant was separately placed into a thimble. Then, solution of 1500 mL ethanol (80%) and distilled water was added to the solvent flask and operated for 8 h at 60 °C. The extracts were poured in clean glass dishes and placed in the electrical oven till dryness. The obtained residues of dried extract for myrtle (10 g) and ginger (8 g) were stored in the refrigerator at 4 °C until use. All concentrations of plant extract were prepared by using ethanol (99.9%) as a kind of solvent diluted with distilled water to reach the concentration of 80%. Ethanol (99.9%) was diluted by adding 80 mL alcohol measured by cylinder tool to 20 mL distilled water to reach the concentration of 80%. By this diluted ethanol, three concentrations of *Z. officinale, M. communis* and mixture of the two plants were prepared. The first concentration (2.5%) was prepared by adding 0.25 g of extract to 9.75 mL ethanol; second concentration (5%) by adding 0.50 g extract to 9.5 mL ethanol, while third concentration (7.5%) by adding 0.75 g extract to 9.25 mL ethanol. Each of these concentrations of different extract was kept in labeled tubes in the refrigerator until the insect treatment begins.

Insects rearing

T. castaneum adults were reared in plastic cans which contains fresh wheat in it, concealed with pieces of muslin fastened with rubber bands to avoid insect escape. Colonies were maintained for many generations in an incubator under conditions set at 25 ± 2°C and 65 ± 5 % relative humidity. The insects larvae were present in breeding medium. Therefore, these larvae were separated by hand and placed in another plastic container containing new wheat grains. They were preserved in the incubator under the same condition, inspected daily to follow their development and the emergence of adults.

Contact toxicity

The insecticidal activities of essential oils against adults of *T. castaneum* were determined by direct contact application. Plant extracts (the residue) was used, in which 0.5 mL solution of each plant extract was dropped on filter paper placed inside a petri dish (8 cm in diameter and 1 cm in height) with the help of a pipette. The filter paper was then air dried for a few minutes. Ten (10) adult insects were put into each petri dish, and the same number was also confined to filter papers treated with ethanol as an untreated check (control). Five replications were made for each treatment and control. The mortality of the insects was recorded at 24, 48 and 72 h after treatment. Experiments were conducted at room temperature (23 ºC). The mortality rate was assessed by means of direct observation, and when no leg or antennal movements were observed, the insects were considered dead (Ali & Mohammed 2013).

Statistical Analysis

The Statistical Analysis System (SAS 2012) program was used to detect the effect of different factors on the examined parameters. Least significant difference (LSD) and Duncan's Multiple range (1955) test (ANOVA) were used to significant compare between means in this study.

RESULTS AND DISCUSSION

The results of the mean mortality of the adult insects were given in Tables 1, 2 and 3. Then results were plotted as shown in Figs. 1, 2 and 3.

Effect of plant extracts on *T. castaneum* after 24 h

Table 1. Toxic effects of three different plant extracts on mortality percentage of *T. castaneum* with different concentrations after 24 h of exposure.

Treatments	Mortality (%) (mean ±SE) Extract concentration(g/ml)				LSD
	Control	2.5	5	7.5	
Zingiber officinale	0.00 ± 0.00 $^{A c}$	1.00 ± 0.05 $^{A b}$	1.33 ± 0.07 $^{AB b}$	2.00 ± 0.07 $^{AB a}$	0.376 *
Myrtus communis	0.00 ± 0.00 $^{A c}$	0.66 ± 0.03 $^{B b}$	1.00 ± 0.05 $^{B b}$	1.66 ± 0.06 $^{B a}$	0.412 *
Zingiber officinale + Myrtus communis	0.00 ± 0.00 $^{A d}$	1.00 ± 0.05 $^{A c}$	1.66 ± 0.07 $^{A b}$	2.33 ± 0.12 $^{A a}$	0.398 *
LSD	0.00 NS	0.298 *	0.308 *	0.378 *	---

Note: Means with different big letters in the same column and small letters in the same row are significantly different. * ($P \leq 0.05$).

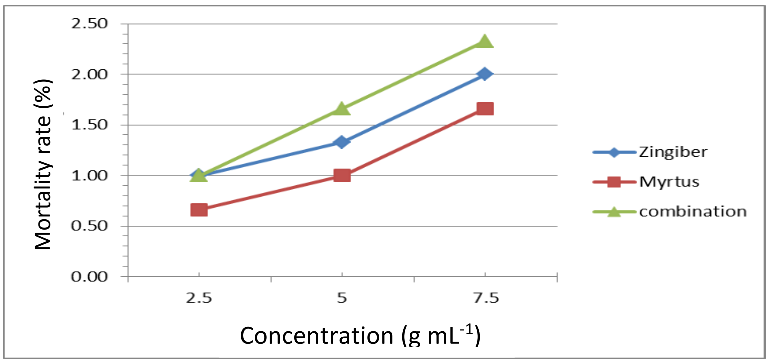

Fig. 1. Mortality rate in *Tribolium castaneum* with different plant extracts after 24 h of exposure.

The results of the mean mortality of *T. castaneum* adult treated with two plant extracts *Z. officinale* and *M. communis*, separately and in combination (T_{Z+M}) at concentrations of 2.5, 5 and 7.5 (g mL^{-1}) after 24 h, are represented in Fig 1. The combination extract of *Z officinale* and *M communis* exhibited the maximum mortality rate in *T. castaneum* (2.33%) at 7.5 g mL^{-1} throughout the 24 h followed by 1.66 % mortality at 5 g mL^{-1}, while the lowest (1.00 %) mortality at 2.5 g mL^{-1}. Thus, the results revealed that the mortality of adult insect with combination of the two plants vary in dose dependent manner. Furthermore, Z. *officinale* extract showed its highest mortality (2.00%) at 7.5 g mL^{-1}, while declined (1.00%) at 2.5 g mL^{-1}. In the case of *M. communis*, the highest mortality in adults (1.66%) was recorded at 7.5 g mL-1, while the lowest (0.66%) at 2.5 g mL^{-1}. In all cases, no mortality was observed in the control sample.

Effects of the plant extracts on *T. castaneum* after 48 hours

Table 2. Toxic effect of three different plant extracts on mortality percentage of *T. castaneum* with different plant extracts after 48 h of exposure.

Treatments	Mortality rate (mean ± SE) Extract concentration (g mL^{-1})				LSD
	Control	2.5	5	7.5	
Zingiber officinale (T_Z)	0.00 ± 0.00 A d	2.00 ± 0.08 AB c	2.66 ± 0.11 A b	3.66 ± 0.16 A a	0.417*
Myrtus communis (T_M)	0.00 ± 0.00 A c	1.66 ± 0.06 B b	2.00 ± 0.08 B b	2.66 ± 0.11 B a	0.409*
Zingiber officinale + Myrtus communis (T_{Z+} T_M)	0.00 ± 0.00 A d	2.33 ± 0.11 A c	3.00 ± 0.15 A b	4.00 ± 0.21 A a	0.514*
LSD	0.00 NS	0.369 *	0.407 *	0.394 *	---

Note: Means with different big letters in the same column and small letters in the same row are significantly different. * (P ≤ 0.05).

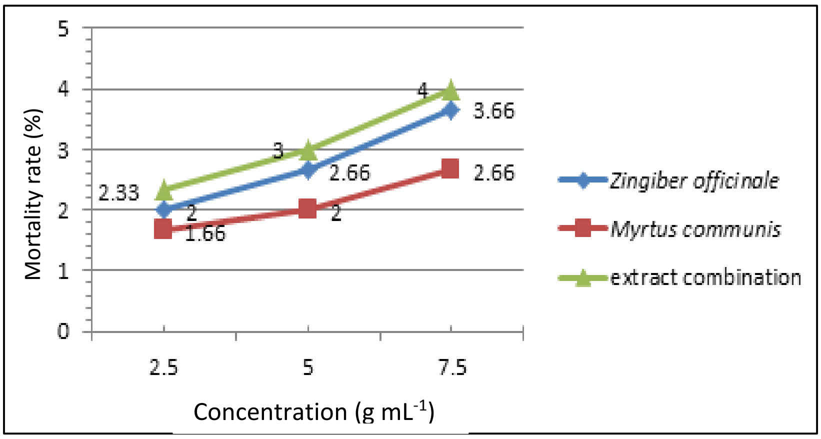

Fig. 2. Mortality rate (%) in *Tribolium castaneum* with different plant extracts after 48 h of exposure.

Fig. 2 shows the mortality rate of *T. castaneum* adult treated with two plant extracts, *Z. officinale* and *M. communis*, separately and in combination at 2.5, 5 and 7.5 g mL^{-1} after 48 h. Results reveal that mortality was increased after 48h from treatment. Maximum mortality was 4.00% recorded in the combination of the two plants extracts at 7.5 g mL^{-1} throughout the 48 h, while at the same concentration, the highest mortality rates (3.66% and 2.66%) were recorded in each separate *Z. officinale* and *M. communis* extracts respectively. On the other hand, minimum mortality (1.66%) was recorded in *M. communis* at 2.5 g mL^{-1} throughout the 48 h, while at the same concentration, lowest mortality rates (2.00% and 2.33%) were recorded in *Z. officinale* extract and the combination of two plants extracts respectively. Results revealed that mortality was increased by rising concentration. In control, no mortality was observed after 48 h exposure. Plant extracts caused mortality with the trend; combination of *Z. officinale* and *M. communis* > *Z. officinale* > *M. communis*.

Effect of plant extracts on *T. castaneum* after 72 h

Table 3. Toxic effect of different plant extracts on mortality percentage of *Tribolium castaneum* with different plant extracts after 72h of exposure.

Treatments	Mortality rate (mean ± SE) Extract concentration (g mL^{-1})				LSD
	Control	2.5	5	7.5	
Zingiber officinale (T_Z)	0.33 ± 0.01 A d	2.33 ± 0.10 AB c	3.00 ± 0.14 B b	4.00 ± 0.22 A a	0.437 *
Myrtus communis (T_M)	0.33 ± 0.01 A d	2.00 ± 0.08 B c	2.66 ± 0.11 B b	3.33 ± 0.15 B a	0.414 *
Zingiber officinale + Myrtus communis (T_{Z+} T_M)	0.33 ± 0.01 A c	2.66 ± 0.13 A b	3.66 ± 0.17 A a	3.66 ± 0.18 AB a	0.497 *
LSD	0.05 NS	0.372 *	0.416 *	0.405 *	---

Note: Means with different big letters in the same column and small letters in the same row are significantly different. * (P ≤ 0.05).

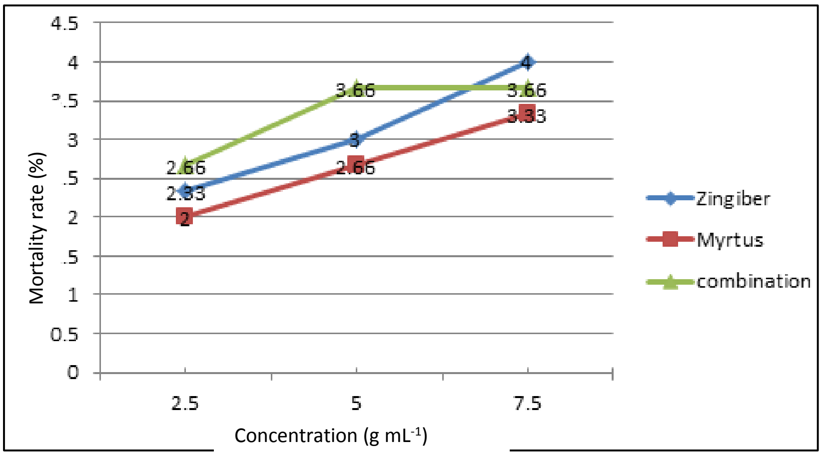

Fig. 3. Mortality rate (%) in *Tribolium castaneum* with different plant extracts after 72 h of exposure.

The results of the mean mortality in *T. castaneum* adult treated with the two plant extracts, *Z. officinale* and *M. communis*, separately and in combination at 2.5, 5 and 7.5 g mL^{-1} after 72 h, are represented in Fig. 3. The results showed that minimum mortality of *T. castaneum* adult (2.00%) was recorded in *M. communis* extract at 2.5 g mL^{-1} throughout the 72 h. Afterward, it increased to 2.66% and 3.33% at 5 and 7.5 g mL^{-1} respectively. According to these results, the toxic effect of *M. communis* extract on mortality rate of adult insects was gradually increased significantly by rising concentrations from 2.5 to 7.5 g mL^{-1}. A similar trend of increase in mortality rate was observed in the case of *Z. officinale* extract, while the mortality in control treatment was 0.33% in all concentrations throughout the 72 h. On the other hand, combination of the two plants extract recorded its highest mortality (3.66%) at 7.5 g mL^{-1}, whereas there was no difference between this rate and mortality at 5 g mL^{-1}. However, once comparing the mortality of the adult plants at 2.5 g mL^{-1}, the result revealed that the combination of the two plants extract caused higher mortality (2.66%) than each *Z. officinale* and *M. communis* extracts separately.

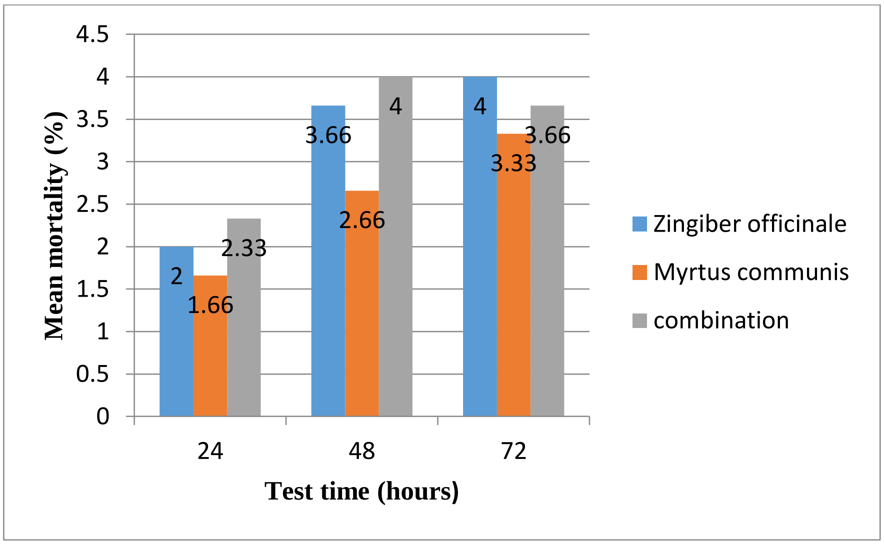

Fig. 4. Mortality of *Tribolium castaneum* exposed to the various plant extracts at different exposure intervals.

This experiment was conducted in order to determine the insecticidal activity of the plant extracts used on *T. castaneum*. In all cases, considerable differences in insect mortality were found with different plant extracts. Results of the present study revealed that the natural plant extracts have the potential to control *T. castaneum* population in stored grains. Fig. 4 displays a comparison of the mortality of *T. castaneum* among the three different plant extracts used in this study at the highest (7.5 g mL^{-1}) concentration during the exposure times of 24, 84 and 72 h. The trend observed in this bar chart displays difference in the mortality rate as exposure times increased. Combination of *Z. officinale* and *M. communis* extracts was able to induce maximum mortality (4.00%) after 48 h compared to *Z. officinale* and *M. communis* extracts separately. Afterward, the mortality declined to 3.66% after 72 h of exposure time. The possible explanation for this decrease can be attributed to the death of most insects on the first and second days of treatment. This is in line with the finding of Al-Bayaty *et al.* (2013) who noticed a difference in the mortality rate of flour beetle larvae in the second and third days than in the first day. Furthermore, according to Fig. 4, *Z. officinale* extract showed toxicity to *T. castaneum* and reached its maximum mortality (4.00%) after 72 h. These finding are in agreement with previous studies reporting that *Z. officinale* exhibits the toxic, repellent and developmental inhibitory characteristics against *T. castaneum* (Chaubey 2011). Also, results of this study confirm the finding of Abdel-Rahman & Mahmoud (2018), who evaluated the toxic and the repellent effects of four essential plant oils including coconut oil, *Cocus nucifera* L., ginger oil, *Z. officinale* Rosc., Jojoba oil, *Simmondsia chinensis* L. and spearmint oil, *Mentha spicata* L. against the adult of red flour beetle. Their results showed that essential oil of *Z. officinale* was the highest effective oil against *T. castaneum*. Moreover, Ahmad *et al*. (2019) compared the efficacy of six common plant species namely *Z. officinale* (Ginger), *Azadirachta indica* (Neem), *Allium sativum* (Garlic), *Nicotiana tabacum* (Tobacco), *Eucalyptus globulus* (Eucalyptus) and *Cymbopogon citrates* (Lemongrass) against *T. castaneum* infesting stored rice, corn, wheat, and gram pulse. They found that *Z. officinale* (ginger) and *A. sativum* (garlic) were more effective against *T. castaneum* which resulting into 15 times higher adult mortality and 4 to 5 times reduction in grain weight losses when mixed with rice grains. Further, results of the present study are in line with the report given in the literature by Ali and Mohammed (2013), who reported that *Z. officinale* was the most toxic plant against *T. castaneum* insect as compared to other tested plant extracts. Several plant species have numerous chemical compounds that could be repellent or toxic for insect pests. It has been reported that *Z. officinale* contains a sesquiterpenes hydrocarbon; and the pungent odor appears to be responsible for its toxic effect on the insect pests (Atta *et al.* 2020). The insecticidal, repellent and fumigant properties of monoterpenes, the main component of essential oils, have been reported against stored products insects (Khani & Basavand 2012). The major constituents of monoterpenes such as 1,8-cineole, α-pinene, myrcene and ar-curcumene, were reported earlier as a toxic and repellent agents against some of insect pests (Maedeh *et al.* 2012). Therefore, the insecticidal activity of *Z. officinale* extract investigated in the present study may be attributed to these major components. On the other hand, *M. communis* extract exhibited insecticidal activity against *T. castaneum* for all intervals after treatment. However, least effective with highest mortality (3.33%) was found after 72 h. Although *M. communis* essential oil tested in our present study was less toxic than *Z. officinale* and the combination of two plant extracts but still showed potent toxicity against *T. castaneum*. These findings are in accordance with the earlier studies where essential oils extracted from *Laurus nobilis* L. (Lauraceae species) and *M. communis* L. (Myrtaceae species) has been proved to be an effective natural insecticidal against larvae and adults of *T. castaneum* (Senfi *et al.* 2014). In addition, results from our study are in agreement with those of Siddique *et al* (2013) who demonstrated that the essential oil of *M. communis* had repellency, fumigant and contact toxicities, against *T. castaneum* adult among five plant species belonging to family Myrtaceae used in their study. However, *M. communis* was most effective as repellent agent. Similarly, our findings are in accordance with study of KarabÖrkulü *et al*. (2011) who documented the fumigant toxicity of eight essential oils from aromatic plants including *M. communis*, and reported that this oil exhibited a moderate toxicity against Mediterranean flour moth, *Ephestia kuehniella*. Also, the findings of present study are agreement with those reported by Manzoor *et al.* (2011) who reported that the effect of ethanolic extracts of habulas (*M. communis*), lemongrass (*Cymbopogon citratus*), mint (*Mentha longifolia*), datura (*Datura stramonium*) and Bakain (*Melia azedarach*) had repellent and lethal effects against red flour beetle (*T. castaneum*), pulse beetle (*Callosobruchus chinensis*) and saw-toothed grain beetle (*Oryzaephilus surinamensis*). They demonstrated that *M. communis* extract showed maximum repellency (64.05%) against *T. castaneum*. In the case of lethal effects, maximum mortality of *O. surinamensis* was recorded for *M. communis* (50.7%). In view of the abovementioned, regarding to *M. communis* extract, the difference with the present study may be due to several reasons such as laboratory and environmental conditions, pest species, dose of the treatment, commodity differences and methodology of research. The toxic effects of the myrtle could be attributed to the major constituents such as 1.8-cineole, linalyl acetate and linalool (Ayvaz *et al.* 2010). 1,8-cineole isolated from *Artemisia annua* was effective both as fumigant and a contact poison to adult insects and larvae of *T. castaneum* (Tripathi *et al.* 2001). Rozman *et al*. (2007) evaluated the fumigant activity of nine compounds of the essential oil of aromatic plants against adults of *T. castaneum*, *Rhyzopertha dominica* and *Sitophilus oryzae*. They found that 1.8- cineole, linalool and linalyl acetate have high toxicity against *S. oryzae* and *Rhyzopertha dominica* when applied for 24 h at the lowest dose (0.1 mL/720 mL volume). However, *T. castaneum* was highly resistant to the essential oils tested and only 1,8-cineole achieved 92.5% mortality when applied at the highest concentration (100 mL/720 mL volume) after 7 days of exposure. By comparing the mean mortality values in Figs. 1, 2 and 3, it is well obvious that insecticidal activity of plant extracts varied with extract concentration and exposure time. The toxicity of plant extracts were increased significantly by elevating the extracts concentration and exposure time from 24, 48 and 72 h, which is in consistent with other authors' reports (Ogendo *et al.* 2008; Maedeh *et al.* 2012; Atta *et al.* 2020). Based on our knowledge, no study has been previously reported on the insecticidal activity of plant extracts (*Z. officinale* and *M. communis*), separately and in combination against *T. castaneum* adult at the same time. However, the insecticidal efficiency of *Z. officinale* or *M. communis* extracts has been reported against insects at other studies. The toxicity of these plant extracts against *T. castaneum* could depend on several factors among which are the chemical composition of the plant extract and insect susceptibility. From our observations, *T. castaneum* adults were resistant to contact toxicity of the different plant extracts. The difference of sensitivity may be attributed mainly to the phenotypic resistance (modifications in the target site), metabolic resistance (ability to detoxify insecticides) or behavioral modification. Behavioral changes that minimize contact between insect and insecticide may cause a severe impact on the insecticide application efficacy, especially if resistance is selected by physiological features (Martins 2012). In our study, we found that *T. castaneum* had a tendency to be less active (behavioral resistance mechanism) and had a limited movement. These finding are in agreement with those demonstrated by Khani & Basavand (2013). They reported that, in the fumigant toxicity test, *T. castaneum* was less active and exhibited a limited movement that may reduce breathing and *M. communis* oil penetration via the insect spiracle. Taking all of the above into account, the insecticidal effect of the plant extracts at the end of the test has the following order: combination of *Z.r officinale* and *M. communis* > *Z. officinale* > *M. communis*. Moreover, the toxic effects of these extracts depend on the concentration and time exposure. The insecticidal activity of the combined extracts (*Z. officinale* and *M. communis*) can be attributed to major components as well as potential synergistic action of their different constituents.

CONCLUSION

The findings of the present study indicate that all tested plant extracts were effective to some degree in reducing the number of adult *Tribolium castaneum*. Differences in insect mortality were observed depending on extracts concentrations and exposure time. Treatments with higher dose (7.5 g mL^{-1}) had comparatively higher and significant total mortality of adult *T. castaneum* than those treated with smaller dosage (2.5 g mL^{-1}). When the lethal effect was compared, combination of *Z. officinale* and *M. communis* was the most toxic plant, while *M. communis* the least toxic. Results suggested that plant extracts may have potential as a control agent against this major stored product insect, as these substances are not only an inexpensive, but also have non-toxic effects on non-target organisms and less environmental influence in terms of insecticidal hazard.

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