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Acaricidal potential of some botanicals against the stored grain mites, *Rhizoglyphus tritici*

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Abstract
The present study was conducted to evaluate the susceptibility of *Rhizoglyphus tritici* against the comparative potential of ether extracts from *Azadirachta indica*, *Eucalyptus* sp., *Citrullus colocynthis*, *Allium sativum*, *Nicotiana tabacum*, *Curcuma longa*, *Nerium indicum*, *Syzygium aromaticum, Ocimum tenuiflorum* and *Cassia fistula*. The whole trial was executed under laboratory conditions with five concentrations from T1-T5 (0.5%, 1%, 2%, 4% and 8%), and four exposure periods (7, 14, 21 and 28 days). The percentage inhibition of mite population was both time-dependent and concentration-dependent. All the plant extracts exhibited significant acaricidal potential for adult mite as compared to control**.** The significant population inhibition percentage trend was observed of *C. longa* 91%, 95.54%, 94.97% and 97.46% and *S. aromaticum* 88.06%, 92.17%, 95.27% and 96.67% followed by *C. fistula* 92.03%, 93.27%, 93.22% and 92.07% while least population inhibition percentage was observed of *N. tabacum* 84.70%, 85..08%, 90.70% and 93.14% after 7, 14, 21 and 28 days. The median lethal concentrations were also calculated for all extracts, and it was observed that *S. aromaticum* was highly toxic to mites at lowest concentration 0.128, 0.028, 0.006 and 0.005 followed by *C. longa* 0.071, 0.036, 0.027 and 0.016 while least toxicity was observed in *C. colocynthis* 0.047, 0.030, 0.009 and 0.009 against *R. tritici*, and lethal concentration (LC_{50}) decreased with an increasing time of exposure of the *R. tritici* to the ether extract. It was concluded that acaricidal potential of plants is directly proportional to time exposure and during first and second week *C. fistula* and *S. aromaticum* followed *C. longa* but suddenly after two weeks acaricidal potential of *S. aromaticum* was boost up and it was concluded that *C. fistula*, *S. aromaticum* and *C. longa* proved to be more effective gainst stored grain mites as compared to the others plant extracts. Perhaps, this new study will provide the basis for further investigation in order to develop new and safer acaricides in field conditions.

Keywords: Acaricidal potential; *Rhizoglyphus tritici*; stored grains; mortality; plant extracts

Introduction
Wheat (*Triticum aestivum* L.) is a nutritious and economical source of food in Pakistan [1, 2], which contributes about 3.1% in total GDP. *T. aestivum* is cultivated on an area 9.045 million hectares having total production of 23 million tons annually [3]. It provides about 20% of the world food calories and food for nearly 40% of the world's population. The cereal grains grown globally on 23% cultivated area is of great importance in the manufacturing of bread, diet, and pharmaceutics but is also considered as an important product of international trade for the worldwide market [4].

Storage of wheat grains for longer period tends to deterioration. A wide range of factors is responsible for qualitative and quantitative losses of wheat while arthropod pests are considered to be the most destructive. Micro-arthropods have a wide range of adaptability being microscopic in body size and sometimes being abundantly present in wheat warehouses [5, 6]. Wheat grains tremendously affected by the attack of pests including pathogen and arthropods throughout the world, which cause much yield losses (25-50%) [7]. Contamination in warehouses owing the presence of microarthropods is never tolerable in developed countries [8]. Among all of the arthropods pests, stored grain mites are most destructive, which devastate the quantity and quality of wheat grains. Mite pests have been described throughout the world causing economic losses of processed food commodities [9-12] as well as in greenhouses and botanical gardens [13]. Likewise, *Rhizoglyphus tritici* (Acari: Acaridae) has been reported from wheat stored grain in Pakistan [14]. At the initial stages, the embryo of wheat grain ruined by an attack of mite, which leads toward low viability of grains [12], and at the end these grains lose acceptance from humans after the maximum existence of mite population. Moreover, mite

pests also cause secondary damage via contamination with their dead bodies and exuviae, as well as mycotoxin production after fungal growth in the stored chamber [15]. Thus, handlers of that infested grains face many health problems [16]. On the other hand, stored grain mites also act as carriers and vectors of different pathogenic diseases and become dangerous for ecosystem [17].

Thus, the infested grains need more care and treatment, which is mostly being done with chemicals. So, residues of chemicals are also hazardous for human health, along with resistance development in the target pest [18]. Researchers are diverting their attention towards natural pesticides which have a biological origin like plants and microorganism. Recently, many plant extracts have been tested for their toxic effects as an alternative of synthetic chemicals to control a different type of arthropods pests especially for stored food products [10, 19-21]. Hence, there is dire need to develop such type of alternative strategies which may keep our food grains safer for human consumption and also be environment-friendly.

In order to develop such kind of pest control strategy, plants and their derivatives are being used effectively against mites across the globe in different ways to test the effectiveness**.** Some of the recent studies have shown that the plant byproducts or extracts have shown significant importance, such as; *Azadirachta indica* [22]**,** *Satoreja hortensis* [23], *Datura stramonium, Citrullus colocynthis, Eucalyptus* sp., and *Melia azedarach* [19].

The objective of this study was to evaluate the comparative efficacy of ten plants in ether extract viz., *A. indica* (neem seed kernel), *Eucalyptus* sp. (sufeda leaves), *C. colocynthis* (kor tumma seeds), *A. sativum* (garlic bulbs), *N. tabacum* (tobacco leaves), *C. longa* (turmeric rhizomes), *N. indicum* (knair leaves), *S. aromaticum* (clove buds), *O. tenuiflorum* (tulsi seeds) and *C. fistula* (amaltas fruits) against stored grain mite (*Rhizoglyphus tritici*) of family Acaridae.

Materials and Methods

***Stored Grain Mites*:** The stored grain mites were reared in the Acarology Research Lab, University of Agriculture, Faisalabad, Pakistan, under the conditions of 27±2 °C temperature and 75±5% relative humidity (RH). Trials were conducted via completely randomized design (CRD). Mixed stages of mite (adults + nymphs) were used in treatments.

***Wheat*:** Wheat grains were used for culture of mites, without any chemical treatment. The moisture level of wheat grain was maintained up to 14 percent. The grains were cleaned and autoclaved prior to start the culture.

***Plant extracts*:** Ether plant extracts were prepared from following plants *A. indica*, *Eucalyptus* sp., *C. colocynthis*, *A. sativum*, *N. tabacum*, *C. longa*, *N. indicum*, *S. aromaticum, O. tenuiflorum* and *C. fistula* under lab conditions, using wheat as a host. All plants were ground into fine powder, and 50 g powder of each plant was processed to make homogenized mixture through Soxhlet Extractor apparatus in the ether for 4-6 hours. Then that mixture was dried through Rotary Evaporator, and moisture was maintained up to 100%. Serial dilutions were prepared with acetone at 8, 4, 2, 1 and 0.5 (%) concentration.

***Bioassay*:** Autoclaved wheat grains were cooled down in a tray, and 10% of wheat was kibbled separately. Whole wheat and kibbled wheat was mixed. This diet was spread in a tray and sprayed at the rate of 1ml.10g^{-1} of wheat with already prepared formulations of different concentrations i.e. 8, 4, 2, 1 and 0.5 percent. Treated 60g wheat was added to plastic jars. A batch of wheat was similarly treated with acetone only to act as a control. One hundred stored grain mites from the already developed culture were placed into each jar and the jars were re-closed with a fine mesh cloth lid. The jars were placed in a growth chamber with 27±2 °C temperature and 70±5% relative humidity. Six treatments (concentrations) 8, 4, 2, 1, 0.5 (%) and control were prepared and one jar for each data 3 times were prepared. After 7 days, 3 experimental units were selected for each treatment. Data were recorded after 7, 14, 21and 28 days and in this way, the efficacy of one plant extract after each one week up to one-month interval was checked.

Data collection: The data was recorded after 7, 14, 21 and 28 days of treatment application. For data collection, contents of the jar were processed through Berlese's funnel for at least 24 hours. The potential of each plant extract was determined on the basis of mite population inhibition. The percentage mite population inhibition was calculated by Püntener *et al*. [24], for each treatment as a proportion of the controls and expressed as: % age mortality of mite = No. of average mites in control - No. of alive mites/treatment x 100 No. of alive mites in control

Statistical analysis: Data regarding percentage mite population inhibition was subjected to statistical analysis. Analysis of variance (ANOVA) and means were compared by using Tukey's HSD test. While lethal concentration LC_{50} values were calculated by using Probit analysis with Minitab 16.2 version according to the description of Finney [25].

Results

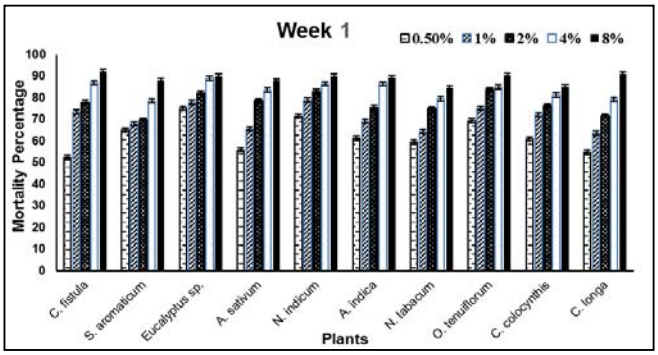

Fig 1: Potential of plants extract at different concentrations against *R. tritici* at the end of 1st week.

The acaricidal potential of ten plant extracts as expressed in fig.1. revealed that at the end of the 1st week the ether extract of *C. longa* (91.00%) had good acaricidal potential against stored grain mites followed by *C. fistula* (92.03%) at higher concentration (8%) while the ether extract *C. fistula* and *S. aromaticum* had 52.29% and 54.66% mortality percentage respectively at lower concentration (0.5%). Statistically, it was observed in the graph that the maximum population inhibition was found in higher concentration (8%) while the minimum population inhibition in (0.5%) that is statistically at par with 1%, 2% and 4%.

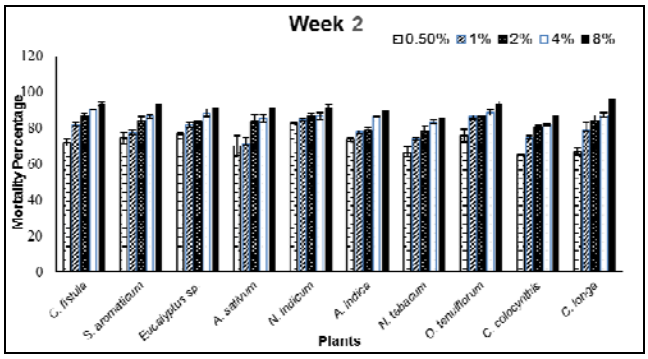

Fig 2: Potential of plants extract at different concentrations against *R. tritici* at the end of 2nd week.

The acaricidal potential of ten plant extracts as expressed in fig.2. revealed that at the end of the 2nd week the ether extract of *C. fistula* (93.27%) had significant acaricidal potential against stored grain mites followed by *C. longa* (95.54%) at higher concentration (8%) while the ether extract *C. fistula* and *C.longa* had 71.66% and 66.52% mortality percentage respectively at lower concentration (0.5%). Statistically, it was observed in the graph that the maximum population inhibition was found in higher concentration (8%) while the minimum population inhibition in (0.5%) that is statistically at par with 1%, 2% and 4%.

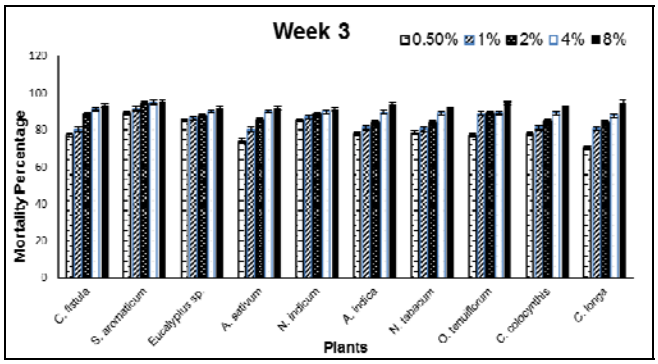

Fig 3: Potential of plants extract at different concentrations against *R. tritici* at the end of 3rd week.

The acaricidal potential of ten plant extracts as expressed in fig.3. revealed that at the end of the 2nd week the ether extract of *C. longa* (94.97%) had significant acaricidal potential against stored grain mites followed by *S. aromaticum* (95.27%) at higher concentration (8%) while the ether extract *C. fistula* and *S. aromaticum* had 70.62% and 89.18% mortality percentage respectively at lower concentration (0.5%). Statistically, it was observed in the graph that the maximum population inhibition was found in higher concentration (8%) while the minimum population inhibition in (0.5%) that is statistically at par with 1%, 2% and 4%.

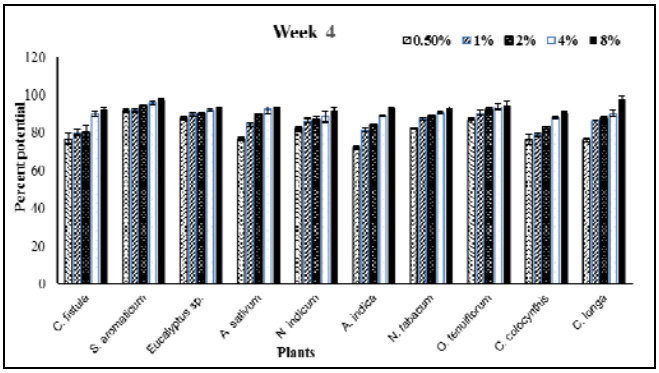

Fig 4: Potential of Plants extract at different concentrations against *R. tritici* at the end of 4th week

The acaricidal potential of ten plant extracts as expressed in fig.3. revealed that at the end of the 2nd week the ether extract of *C. longa* (97.46%) had good acaricidal potential against stored grain mites followed by *S. aromaticum* (96.67%) at higher concentration (8%) while the ether extract *C. longa* and *S. aromaticum* had 76.26% and 91.30% mortality percentage respectively at lower concentration (0.5%). Statistically, it was observed in the graph that the maximum population inhibition was found in higher concentration (8%) while the minimum population inhibition in (0.5%) that is statistically at par with 1%, 2% and 4%.

It was concluded that all plant parts in ether extract showed significant acaricidal potential at high concentration and after time exposure. During the first week plant used showed significant acaricidal potential which increased while in the last week acaricidal potential remained same as during the first week. The results from our study also concluded that all plants possess the ability and potential to act as a control agent against stored grain mites. They do not significantly decrease at the end of 4th week. Although the acaricidal potential of plant extracts decrease with the passage of time but in the case of our study, it was observed that acaricidal potential difference between a 1st week and the 4th week has no significant difference.

Table 1: The LC_{50} of plant extracts against *Rhizoglyphus tritici*

Time	LC_{50} (µl)	SE	F.L	p	χ^2
		S. aromaticum			
1st week	0.128	0.054	0.042-0.25	0.002	6.92
2nd week	0.028	0.019	0.004-0.08	0	1.15
3rd week	0.006	0.0002	0 -0.003	0	0.48
4th week	0.005	0.0001	0 -0.002	0	0.35
		Eucalyptus sp.			
1st week	0.016	0.014	0.001-0.06	0	1.20
2nd week	0.008	0.008	0.0002-0.04	0	0.47
3rd week	0.002	0.0001	0 -0.002	0	0.51
4th week	0.003	0.00001	0 -0.001	0	0.23
		C. fistula			
1st week	0.251	0.073	0.119-0.40	0.643	89.92
2nd week	0.042	0.022	0.011-0.09	0	1.55
3rd week	0.018	0.013	0.002-0.05	0	1.68
4th week	0.020	0.015	0.002-0.06	0	3.90
		A. sativum			
1st week	0.276	0.061	0.161-0.40	0.072	3.19
2nd week	0.064	0.031	0.018-0.14	0	3.88
3rd week	0.025	0.017	0.004-0.07	0	0.55
4th week	0.016	0.011	0.002-0.05	0	2.93
		N. indicum			
1st week	0.031	0.201	0.005-0.08	0	0.69
2nd week	0	0.0001	0 -0.004	0	1.41
3rd week	0	0.0001	0 -0.003	0	0.25
4th week	0	0.0004	0.00-0.005	0	0.58
		C. longa			
1st week	0.396	0.071	0.261-0.54	0.747	2.96
2nd week	0.115	0.036	0.053-0.19	0	4.18
3rd week	0.066	0.027	0.019-0.12	0	2.94
4th week	0.029	0.016	0.006-0.07	0	6.03
		N. tabacum			
1st week	0.202	0.064	0.089-0.34	0.104	1.13
2nd week	0.045	0.029	0.007-0.12	0	0.99
3rd week	0.005	0.006	0 -0.03	0	0.69
4th week	0.007	0.001	0 -0.01	0	0.59
		O. tenuiflorum			
1st week	0.055	0.028	0.013-0.12	0	1.69
2nd week	0.010	0.009	0.0006-0.04	0	3.86
3rd week	0.006	0.006	0.0003-0.03	0	8.98
4th week	0.001	0.0004	0 -0.004	0	0.54
		C. colocynthis			
1st week	0.112	0.047	0.036-0.22	0	2.08
2nd week	0.048	0.030	0.007-0.12	0	3.06
3rd week	0.009	0.009	0.0004-0.03	0	0.30
4th week	0.009	0.009	0.0004-0.04	0	0.49
		A. indica			
1st week	0.208	0.053	0.111-0.32	0	1.56
2nd week	0.020	0.016	0.002-0.07	0	1.74
3rd week	0.016	0.012	0.001-0.05	0	1.53
4th week	0.033	0.019	0.006-0.08	0	1.34

LC50 (µl), F.L= Fiducial limit (95%), *p*-value, S.E= Standard error, χ^2= Chi-square

The lethal concentration of ten plant extracts in Table (1) revealed that the LC_{50} value of *S. aromaticum* was 0.128 µl at the end of the 1st week while at the end of 4th week decreased up to 0.005 µl. The LC_{50} value of *Eucalyptus sp.* was 0.016 µl at the end of the 1st week while at the end of 4th week significantly decreased up to 0.003 µl. The LC_{50} value of *C. fistula* was 0.251 µl at the end of the 1st week while at the end of the 4th week was 0.020 µl. The LC_{50} value of *A. sativum* was 0.276 µl at the end of the 1st week while at the end of the 4th week was 0.016 µl. The LC_{50} value of *N. indicum* was 0.031 µl at the end of the 1st week while at the end of 4th week LC_{50} was significantly decreased up to zero. The LC_{50} value of *C. longa* was 0.396 µl at the end of the 1st week while at the end of 4th week LC_{50} was decreased up to 0.029 µl. The LC_{50} value of *N. tabacum* was 0.202 µl at the end of the 1st week while at the end of 4th week LC_{50} was decreased up to 0.0007µl. The LC_{50} value of *O. tenuiflorum* was 0.055 µl at the end of the 1st week while at the end of 4th week LC_{50} was decreased up to 0.001 µl. The LC_{50} value of *C. colocynthis* was 0.112 µl at the end of the 1st week while at the end of 4th week LC_{50} was decreased up to 0.009 µl. The LC_{50} value of *A. indica* was 0.208 µl at the end of the 1st week while at the end of 4th week LC_{50} was decreased up to 0.033 µl. Maximum LC_{50} was observed at the end of 1st week in the following plants i.e. *C. longa*, *A. sativum, C. fistula, A. indica* and *N. tabacum* while minimum LC_{50} was found in the case of *C. colocynthis, S. aromaticum, N. indicum* and *Eucalyptus* sp. The LC_{50} was significantly decreased in all plants; especially LC_{50} was quickly decreased in case of *C. longa*, *Eucalyptus*, *N. indicum*, at the end of the 4th week while others plants LC_{50} decreased slowly. It was concluded that LC_{50} decreased with an increasing time of exposure of the *R. tritici* to the plants part in ether extract.

Discussion

Potential of ether plant extracts was evaluated against *R. tritici* at different percent concentrations for one month. All extracts were toxic to *R. tritici*, and caused maximum inhibition during the observation period. Our results revealed that all plants extracts exhibited significant adult mite inhibition as compared to control**.** The significant population inhibition percentage trend was observed from *C. longa* followed by *C. fistula* while least population inhibition percentage was observed from *N. tabacum* during the whole observation period. Lethal concentrations were also calculated for all extracts, and it was observed that *S. aromaticum* was highly toxic to mites at the lowest concentration, followed by *C. longa* while least toxicity was observed in *C. colocynthis* against *R. tritici*, and their LC_{50} values decrease with the passage of time.

Our results supported by Numa *et al*. [26], who reported that the mortality of individuals was recorded at 24, 48 and 72 hours and concluded that *Cnidoscolus aconitifolius* leaf extract increased mortality in a concentration-dependent manner. The main metabolites identified included flavonoid- and sesquiterpene-type compounds, in addition to chromone- and xanthone-type compounds as minor constituents with potential acaricidal effects.

Likewise, it was observed that *Varroa destructor* was suppressed dramatically up to 100% by plant extracts within a short period of exposure. Razavi *et al*. [27] reported that extracts of *Zataria multiflora* and *Lepidium latifolium* were very effective to control *V. destructor* up to 86 and 100%, respectively, at 500 ppm after 12 days exposure. Thus, infestation rate was decreased up to 0% with *L. latifolium*,

while *Z. multiflora* shown only 13.74%. Both extracts showed the negligible effect on bees, and it can be concluded that these PDSS as biodegradable agents could be used for *V. destructor* control in honeybee colonies. Whereas, our results were confirmed by the studies of Gorji *et al*. [28], who reported that the garlic extract was effective and obtained a 96% success after two successive sprays in field experiment, and the administration of garlic extract was efficacious against *Dermanyssus gallinae* at the selected mite-infested locations.

We determined that concentrations and repellency have direct relation irrespective of the plant extracts. Similar findings were reported by Bashir *et al*. [19], who observed the efficacy of crude aqueous extracts from *Eucalyptus* sp*., A. indica, D. stramonium, M. azedarach* and *C. colocynthis* in laboratory at five concentrations 100, 50, 25, 12.5 and 6.25% at 7, 14, 21, and 28 days' time periods against *R. tritici*. Concentration and exposure period dependent LC_{50} levels and efficacy of plants extract increased with increasing concentrations and time exposure for all extracts i.e. *A. indica, M. azedarach, D. stramonium* and *C. colocynthis* extracts were significantly and equally effective.

These results are supported by Hanifah *et al*. [29], who evaluated the different concentrations of leaf extract of *Cymbopogon citratus* (lemongrass) and ethanolic extract of *A. indica* against two species of house dust mite. They stated that lemongrass caused maximum mortality than the ethanolic extract of *A. indica*. Maximum mortality and concentration are directly related. Lemongrass caused 91% mortality.

Current findings are also in conformity with the results of Singh *et al*. [30], who studied the repellent property of three plants leaf extracts i.e. *A. indica,* A. Juss, *Eucalyptus globules* L. and *Ocimum basilicum* L., against aphids and mealybugs. The concentrations of 1, 2, 4, 8 and 10% were used and data were taken at 12 and 24 h after the release of aphids and mealy bugs. The highest repellency was recorded by *A. indica* leaf extract, which was 99.0 and 97.0% after 24 h for aphids and mealy bugs, respectively. While minimum repulsion was seen by *O. basilicum* leaf extract against aphids and mealy bugs, that was 91.0% and 88.0%, respectively.

Results from the present study on the toxicity of different plant leaf extract against mites were highly related to results from prior studies and conclusions of various researchers who reported insecticidal and potential of *M. azedarach* extracts against arthropod pests [31-33], who reported that effectiveness of the plant extracts against mites was dependent on concentration and exposure interval and has direct relation

Percent efficacy was ranged from 72.2 to 92.5, 73.5 to 88.9, 78.1 to 93.8, and 54.7 to 91.2 when mites were exposed to different concentrations of *Eucalyptus* sp. extract for periods of 7, 14, 21, and 28 days, respectively. It was also reported the acaricidal activities of leaf extract of *Eucalyptus* sp., which caused 55.6% and 62.6% mortality of *T. urticae* exposed to leaf extract by adhesive tape and residual film methods, respectively. Moreover, *Alfaroa mexicana, Calotropis procera, Solanum Xanthocarpum* and *Eucosmophora echinulata* extracts proved to be a regulator in the control of plant parasitic nematodes and soil-inhibiting fungi [34].

Our results are supported by the findings of Shyma *et al*. [35], who testified that the crude extracts of *Alluvium sativum* and *Carica papaya* seeds have good acaricidal properties and could be a potential component of alternative *Rhipicephalus micro plus* tick control strategy. They stated that inhibition of oviposition at the highest concentration of *Azadirachta indica*, *Calotropis procera*, *Datura stramonium*, *Alluvium sativum*, and *Carica papaya* extract-treated ticks were 20.73, 71.34, 77.17, 85.83, and 100.00%, respectively. As well, *A. altissima* extract showed stronger toxicity against *Sarcoptes scabiei* than against *Psoroptes cuniculi*, indicating that the acaricidal activity of *Ailanthus altissima* bark extract is both time-dependent and concentration-dependent [36]. Whereas *Solanum incanum* and *Strychnos spinosa* were also lethal to ticks as described by James *et al*. [37].

On the other hand, it can be observed from Table (1) that the LC_{50} decreased with an increasing time of exposure of *R. tritici* to the ether extract, and these results were verified by the findings of Numa, Carlos Augusto, Wendel José Teles, Wei-Bing [26, 38-40], who reported that increasing the exposure time, lead towards decline in the LC_{50} of plant extracts. However, the lethal concentration LC_{50} between a 1st week and 4th week indicated that there were no significant differences.

Previous studies have revealed that extracts of different plant parts can kill a variety of plant and animal pests and pathogens. Thus, the results of our study confirmed that plant extracts are also toxic to stored grain mites. However, further studies are needed to robustly assess the most effective use of this natural resource against stored grain mites as well as against other insect pests.

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