Research Article

Phytochemical profile of *Atractylis gummifera* rhizome, acute toxicity to Wistar rat and rat poisoning effect in the field

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**Abstract:** This study aimed to valorize *Atractylis gummifera* rhizome (AGR) from a phytochemical study aspect of the aqueous and methanolic extract to determine the total phenolic, flavonoids, and tannins compounds and to evaluate the rat poison effect of the powder (AGRP), methanolic (MEAGR) and aqueous extracts against Wistar rats under laboratory and *Rattus norvegicus* under field conditions in Tiaret, Algeria. The methanolic and aqueous extracts gave a yield of 9.4% and 20% respectively from AGR. The total polyphenols [mg gallic acid Eq/g dry matter (DM)], flavonoids (mg quercetin Eq/g DM), and tannins (mg catechin Eq/g DM) quantities were 27.14 ± 0.01, 17.73 ± 0.04, 9.71 ± 0.01, and 20.60 ± 0.01, 4.46 ± 0.46, 11.12 ± 0.02 in the methanolic and aqueous extract.

The laboratory studies of toxicity showed that the three forms of plant rhizome have a poison effect against males and females of the Wistar rat with the highly toxic effect of AGRP. The results revealed a highly significant (< 0.01) increase in mortality with dose and time. Both LD₅₀ and LD₉₀ of the powder form were determined for males (471, 513 mg/kg/d) and females (471, 513 mg/kg/d) respectively. For the methanolic and aqueous extracts, the LD₅₀ values were 575 and 646 mg/kg/d for males and 676 and 708 mg/kg/d for females, respectively. While an LD₉₀ of 776 mg/kg/d was calculated for MEAGR in males, no LD₉₀ values were recorded for aqueous extract in either sex. Organs of dead rats were removed and examined for gross lesions. Histopathological examination confirmed the toxicity of the plant by pathological changes observed in the kidneys, liver, lungs, and small intestine, where hemorrhage and congestion were observed in the liver, kidneys, and lungs in all the subjects, also intestinal enteritis was present. In the light of the results obtained in the field test, the AGRP has proved its effectiveness in a significant reduction of rodent’s population in comparison with the chemical rodenticides. Powder of *A. gummifera* rhizome may be a good alternative to chemical rodenticides.

**Keywords:** *Atractylis gummifera*, phytochemical, histopathology, rat poison, field test

**Introduction**

Rodents are considered to be important crop pests by destroying crops or modifying the soil by their burrowing systems and galleries.

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(Singleton et al., 1999, Singleton et al., 2003). Most African rodents are herbivorous-granivorous and some of them can cause significant crop damage (Hubert, 1980, Granjon and Duplantier, 2009). They cause damage to stored food through direct damage, wastage, and contamination and so affect both grain quantity and quality. Post-harvest grain losses across all developing countries have been predictably estimated up
to 15%, and when combined with quality losses these could represent a total economic loss of 25 to 50% (Rickman, 2002). It involves not only the quality and quantity of grain damaged but, there may be additional effects which demand further studies. There are remarkably few studies quantifying postharvest losses caused by rodents. Although, post-harvest conservation is the only way to ensure the link between the harvest of the year and the permanent consumption; crops usually kept in inadequate conditions are attacked by molds, insects, and rodents (Kouahou et al., 1989).

In Algeria, cereal products, mainly wheat occupy a strategic place in the food system and the national economy (Djermoun, 2009). According to the Algerian Interprofessional Office for Cereals (O. A. I. C.), losses up to 35% were recorded in recent years (Aoues and Boutoumi, 2017). In a study of rodents from the Tiaret region in Algeria conducted by Adamou Djerbaoui et al. (2015), through a fieldwork sampling during 12 months in 4 localities, a total of 101 rodents belonging to 7 species: 26 Meriones shawii, 20 Psammomys obesus, 5 Gerbillus tarabuli, 22 Rattus rattus, 8 Rattus norvegicus, 12 Mus spretus, and 8 Mus musculus were captured.

Synthetic rat poisons are widely used for the control of rats in storage places. However, associated detrimental effects on the environment and health, and rat control failures have become a major concern and thus, impose alternative control methods. It has been reported that many plant materials have a raticidal effect. According to Abou-Hashem (2012), the extracts of Calendula aegyptiaca, Eucalyptus globules, and Rhus continus have raticidal effect with a high mortality rate. Moreover, the extract of Carica papaya L. is a good natural rodenticide that causes remarkable effects on target organs such as the liver, kidneys, and intestine (Pramestuti et al., 2019).

Atractylis gummifera (L.) (Asteraceae) is a thistle located in the Mediterranean regions; its ingestion continues to be a common cause of poisoning. The root powder of A. gummifera combined with henna is recommended as a hair softener and antidandruff. It is used to treat fever and colds by inhalation. The root is used in the treatment of vertigo, headache, and difficult deliveries by fumigation (Lahsissene et al., 2009).

This study aims to determine the phytochemical composition (polyphenols content, Flavonoids, and tannins) of the aqueous and organic extract of a local A. gummifera rhizome (Tiaret, Algeria), then to assess the rat poison effect of the powder and the methanolic and aqueous extracts of plant rhizome against Wistar rats in the laboratory and R. norvegicus under field conditions.

Materials and Methods

Plant material
The botanical identification of A. gummifera was confirmed at the Plant Protection Laboratory of the Faculty of Nature and Life Sciences, Ibn Khaldoun University, Tiaret, Algeria. A. gummifera rhizomes (AGR) were harvested during the period of December and January 2018 at the Cherb Erhil Mountains of Sidi Hosni (35° 28' 28'' North, 1° 31'' 3'' East), Tiaret, Algeria. The freshly harvested rhizomes were washed and shade dried in a ventilated place. Dried rhizomes were conserved in closed paper bags and reduced to a fine powder using an electric blender just before use.

Animal
One hundred and fifty-six healthy adult male and female Wistar rats aged between 10-12 weeks, and weighing 300 ± 4.5 g were used for each test. Animals were obtained from the Pasteur Institute in Algiers, Algeria. Experimental rats were individually kept in cages under animal house conditions (12 hours light/dark cycle, at 22 °C to 24 °C and relative humidity 60% and 70%), for three weeks acclimatization period. A standard pellets and clean drinking water were provided ad libitum. Animals were then
weighed and assigned to four groups of twelve rats each (six males and six females). Group 1 received bait supplemented with *A. gummifera* rhizome powder (AGRP), group 2 received bait supplemented with methanolic extract of *A. gummifera* rhizome (MEAGR), group 3 received bait supplemented with aqueous methanolic extract of *A. gummifera* rhizome (AEAGR), and control group (CTRL) received standard pellets. Each treatment group was divided into four subgroups of 12 rats receiving 200 mg/kg, 434 mg/kg, 600 mg/kg, and 800 mg/kg poisoned bait dose, and the control group included twelve rats. The study was organized in a completely randomized design (CRD) with three replicates.

**Preparation of plant extracts**

**Preparation of methanolic extract**

The methanolic extract was prepared according to the protocol proposed by Nostro et al. (2000), Navarro García et al. (2006), and Jaafreh et al. (2019) with some modification. 50 g of AGRP was mixed with 500 ml pure methanol in an Erlenmeyer flask, covered with aluminum foil, and shaken at room temperature for 24 hours. The extract was recovered after filtration of the mixture with filter paper. The solvent was removed from the filtrate by vacuum evaporation using a vacuum pump in a rotavapor at 40 °C. The crude extract was completely dried in a ventilated oven at 40 °C. The dried extract was stored at 4 °C in amber glass jars, tightly closed and stored in a refrigerator at 4 °C.

**Quantitative characterization of extracts: Determination of total polyphenols**

Total phenolic content was determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965). The total polyphenolic content was determined by spectrophotometry, following the protocol Applied by Li et al. (2008). 200 μl of the diluted extract was mixed with 1 ml of Folin-Ciocalteu reagent diluted 10 times in distilled water. After 4 min, 800 μl of 7.5% sodium carbonate solution was added and the final volume was adjusted to 3 ml with distilled water. After incubation for 2 hours at room temperature and in the dark, the absorbance was measured at 765 nm. Gallic acid was used as a positive control. Results were expressed in milligram equivalents of Gallic acid per gram of dry matter (mg AG/g DM).

**Determination of total flavonoids**

The determination of flavonoids was carried out according to the colorimetric method of Quettier-Deleu et al. (2000) described in Djeridane et al. (2006). One ml of plant extract (1 mg/ml) was mixed with 1ml of aluminum chloride methanolic solution (2% AlCl₃). After incubation for 10 min at room temperature, the absorbance measurement was performed at 488 nm. A blank was prepared by mixing 1 ml of extract solution with 1 ml of methanol for each extract. The concentration of Flavonoids contained in the various extracts was calculated by reference to a calibration curve, using Quercetin as the standard, and the concentration has been expressed in mg Quercetin equivalent/g dry matter.

**Determination of condensed tannins**

Condensed tannins dosing was achieved according to the vanillin method described by Julkunen-Tiitto, (1985). Fifty (50) μl of each extract were added to 1500 μl of 4% vanillin/methanol solution, vigorously mixed, then 750 μl of concentrated hydrochloric acid
(HCl) was added, allowed to react at room temperature for 20 min. The absorbance was measured at 550 nm against a blank. Different concentrations (0 to 1000 µg/ml) prepared from a stock solution of catechin were used to draw the calibration curve and catechin content was expressed in mg catechin equivalent of dry matter (mg Eq Cat/g DM).

Preparation of poisoned foods and animal treatment

The poisoned food (bait) was prepared by mixing 65% of ground wheat grain, 25% of ground peanuts, and 5% animal fat, all flavored with 5% sugar supplemented with AGRP, MEAGR, and AEAGR at different doses: 200 mg/kg, 434 mg/kg, 600 mg/kg, and 800 mg/kg. The bait was formulated in granules using Fackelmann 45311 Metal Meat Mincer.

An acute oral toxicity study was conducted according to OECD guidelines (Guidance, 2001). Standard food was removed twelve hours before treatment. Each rat received 15 grams/ day of supplemented bait the amount of feed consumed was calculated after the death of the animal. The animals were monitored for 36 hours every 12 hours and all signs of toxicity, coma, and the number of deaths was recorded. Since there is natural mortality in any treated population which is added to the mortality caused by toxic products, the percentage of mortality should be correctly by the formula of Abbott (Abbott, 1925).

Clinical signs

Treated animals were observed for clinical signs of toxicity and mortality.

Histopathology

Died rats were subjected to a full necropsy examination. Organs were then removed and examined for any gross lesion. Samples from the liver, lungs, kidney, and intestine were immediately fixed in 10% buffered formalin and sent to the Histopathology Laboratory at the Veterinary Institute of Tiaret University for further Histopathological examination. Tissue samples were routinely processed through an automatic tissue processor (Leica TP 1020). After that, the tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H & E) according to the technique described by Suvarna et al. (2018). Photomicrographs of selected lesions were taken using an optical microscope (Zeiss) equipped with a camera (Axio Cam EPc 5s).

Field test

Based on high mortality rates of Wistar rats obtained with the A. gummifera rhizome powder in the laboratory experiment, powder poisoned baits were evaluated as rat poison against Rattus norvegicus population under storage condition. The field test was carried out during the period from January to March 2020 at the dock of Sidi Hosni, district of Tiaret, Algeria before emptying the stocks of wheat harvested in summer according to the method described by Dubock (1982) using the consumption method to estimate the population density of rodent species before and after treatment.

A normal (untreated) food ball of 1000 grams was prepared and distributed in three storage places to calculate the pre-treatment consumption and estimate the number of the rodent population for three weeks. Then, AGRP poisoned bait balls were prepared, distributed according to the same procedure cited (1000 g per store) with the calculation of post-treatment consumption amount at the end of each week. The amount of bait was replaced weekly until consumption was stopped. The chemical rodenticide (RATICIDE 70®) was used as positive control under the same field conditions. One store was left without treatment to serve as a control and three test replicates were carried out for each store. The poisoned bait effect in reducing the numbers of rodent population was estimated using the following formula:

\[
\text{Population reduction} (\%) = \left( \frac{N_{RP_u} - N_{RP_p}}{N_{RP_u}} \right) \times 100
\]
Where \( N_{RP_{bt}} \) and \( N_{RP_{au}} \) are the number of rodent population before and after treatment, respectively.

**Statistical analysis**

The data obtained were analyzed using the SPSS v.20 software. Data were expressed as the Mean ± SE of the Mean (SEM) and were analyzed statistically using one-way Analysis of variance (ANOVA) followed by Dunnett’s post hoc test for multiple comparisons between the control and treated groups. Values of \( p \leq 0.05 \) were considered significant and highly significant when \( P < 0.01 \). We used independent samples t student test to compare the mortality between males and females. The measurement of the effect size (R) for two independent groups according to Cohen (1988), after a study has already been carried out in post hoc analyzes. The R is calculated as a function of the mortality caused by AGRP, EMAGR, and AEAGR. Cohen's 1988 effect size scale is: \( R = 0.8 \) (large), \( R = 0.5 \) (medium), \( R = 0.2 \) (small).

**Results**

**Extraction yield**

The yields of aqueous and methanolic extraction of AGR were 20% and 9.4%, respectively. It is well known that extraction yield depends on climatic factors, soil type, vegetative stage, plant part as well as the solvent used.

**Total phenolic content**

The quantitative analysis of phenolic content in both plant extracts was obtained from a calibration curve plotted using a range of gallic acid standard solution. Methanolic extraction of the AGR recorded a higher content of polyphenols with 27.14 ± 0.01 mg Eq AG/g DM extract compared to that obtained by aqueous extraction 20.60 ± 0.01 mg Eq AG/g DM extract. Our results revealed that polyphenols content was higher in MEAGR compared to the AEAGR extract (Table 1).

| Table 1 Phytochemical composition of *Atractylis gummifera* rhizome. |
|-------------------------|---------------------|---------------------|
| Phenolic compounds     | Methanolic extract  | Aqueous extract     |
| Total phenol (mg Eq AG/g DM) | 27.14 ± 0.01        | 20.60 ± 0.01        |
| Total flavonoids (mg Eq Qr/g DM) | 17.73 ± 0.04        | 4.46 ± 0.46         |
| Condensed tannins (mg Eq Cat/g DM) | 9.71 ± 0.01        | 11.12 ± 0.02        |

Data are presented as mean ± SD of three individual determinations. GAE = Gallic acid equivalents; Eq Cat = Catechin equivalents; Eq Qr = Quercetin equivalents; DM = Dry matter.

**Total flavonoids content**

Flavonoids content obtained by MEAGR and AEAGR extraction were 17.73 ± 0.04 mg Eq Qr/g DM and 4.46 ± 0.46 mg Eq Qr/g DM, respectively. These findings showed that flavonoids were the major component of polyphenols accounting for 65.25% of the methanolic extract compared to 21.65% in the aqueous extract (Table 1).

**Condensed tannins**

AEAGR and MEAGR contained very close amounts of condensed tannins; 11.12 ± 0.01 mg Eq Cat/g DM and 9.71 ± 0.02 mg Eq Cat/g DM, respectively. Condensed tannins represented 53.98% of polyphenols in the aqueous extract and 35.77% in the methanolic extract (Table 1).

**Assessment of acute toxicity**

All experimental rats showed clinical signs of acute toxicity regardless of the bait content. Animals showed dose-dependent digestive, respiratory and nervous disorders. Powder-based bait induced severe clinical signs and a high mortality rate compared to the two rhizome extracts, mainly in males than the females. Histopathological study of rat organs revealed severe tissue lesions compatible with acute toxicity.

**Clinical signs**

Rats in the control group did not manifest any clinical signs throughout the experimental period.
Acute toxicity of A. gummifera rhizome to Wistar rat

and no mortality was recorded in this group. Mobility disorders were observed a few hours after bait distribution in all rats (test groups), ranging from very low to immobility at the highest dose groups (800 mg/kg) within 36 hours. Digestive signs were observed in all dose groups within 0 to 12 hours characterized mainly by hypersalivation, vomiting, and diarrhea. Treated rats experienced tachycardia and hyperpnoea just before death. The temperature of treated rats deceased before death, where hypothermia was reported at an interval of 31 °C to 35 °C. It has been noted that the severity of clinical signs was dose dependent. These clinical signs lead the rat into a coma before death. Epistaxis was only observed in animals receiving powder-based bait.

Corrected mortality rate

No mortalities were observed in both sexes in the 200 mg/kg/d group treated with the powder (AGRP), methanolic (MEAGR), and aqueous extracts (AEAGR) of A. gummifera rhizome. While female rats appeared to be more resistant to the toxic effects of poisoned bait confirmed by the mortality rate of both sexes (Table 2). The t-test showed that the mortality rate in rats treated with A. gummifera (AGRP) significantly increased ($P < 0.05$) with a statistically higher mean in males; $33.33 \pm 9.12$ and $100 \pm 0.00$ than females; $6.66 \pm 3.33$ and $61.11 \pm 12.72$ at doses 434 mg/kg/d ($t = 2.72$) and 800 mg/kg/d ($t = 3.05$) respectively. While a significant difference ($P < 0.05$) was recorded at the dose 600 mg/kg/d ($t = 1.75^*$) in rats treated with MEAGR with a higher mean in males (30 ± 8.78) compared to females (11.11 ± 6.21).

Powder

Results showed that the highly significant increase ($p < 0.01$) in mortality rate was dose and time-dependent in both sexes in comparison with the control group. The average mortality of 100 ± 00 was obtained in 800 mg/kg/day dose groups within 12 and 36 hours for males and females. At 600 mg/kg/day dose groups, the mortality average was 100 ± 00 in males compared to 50 ± 00 in females 36 hours post-treatment. The average mortality of 50 ± 9.62 and 20 ± 00 was recorded in the 434 mg/kg/day dose groups for males and females, respectively (Tables 3 and 4).

Table 2 The mortality rate according to the t test for independent samples in males and females Wistar rats treated with AGRP, MEAGR, and AEAGR during 36 hours.

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Doses (mg/kg/d)</th>
<th>Corrected mortality ± SE (%)</th>
<th>t - value</th>
<th>df</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGRP</td>
<td>0</td>
<td>0.08 ± 0.08</td>
<td>8.730</td>
<td>19</td>
<td>0.393</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.00 ± 0.00</td>
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<tr>
<td></td>
<td>434</td>
<td>33.33 ± 9.21</td>
<td>2.722*</td>
<td>10.05</td>
<td>0.021</td>
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<td>600</td>
<td>66.66 ± 16.66</td>
<td>1.769</td>
<td>12.13</td>
<td>0.102</td>
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<td></td>
<td>800</td>
<td>100.00 ± 0.00</td>
<td>3.055*</td>
<td>8</td>
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</tr>
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<td>MEAGR</td>
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<td></td>
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<tr>
<td></td>
<td>200</td>
<td>0.00 ± 0.00</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>434</td>
<td>16.66 ± 6.21</td>
<td>1.512</td>
<td>16</td>
<td>0.150</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>30.00 ± 8.78</td>
<td>1.756</td>
<td>16</td>
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<tr>
<td></td>
<td>800</td>
<td>61.11 ± 7.34</td>
<td>0.812</td>
<td>16</td>
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<tr>
<td>AEAGR</td>
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<td>0.08 ± 0.08</td>
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<td></td>
<td>200</td>
<td>0.00 ± 0.00</td>
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<td></td>
<td>434</td>
<td>5.55 ± 3.92</td>
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<tr>
<td></td>
<td>600</td>
<td>7.40 ± 4.89</td>
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</tr>
<tr>
<td></td>
<td>800</td>
<td>38.88 ± 10.75</td>
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</tbody>
</table>

* Corrected mortality is calculated by Abbott’s formula. * indicate significant at $p < 0.05$. AGRP: Atractylis gummifera rhizome powder, MEAGR: Methanolic extract of A. gummifera rhizome, AEAGR: Aqueous extract of A. gummifera rhizome.
Methanolic extract

Our results showed that the methanolic extract bait affected the mortality rate; it confirmed its toxicity highly significantly (p < 0.01) depending on the dose and duration of treatment compared to the control group in the intervals of 0 to 12 h and 24 to 36 h. Average mortality rates obtained within 36 hours using bait supplemented with MEAGR were, 33.33 ± 9.62 and 16.67 ± 9.62 in the 434 mg/kg/d dose group, 50 ± 9.62 and 33.33 ± 9.62 in the 600 mg/kg/d dose group, and 83.33 ± 9.62 and 66.33 ± 9.62 in the 800 mg/kg/d dose group for males and females, respectively (Tables 3 and 4).

Aqueous extract

Baits supplemented with the AEAGR showed average mortality of 66.67% ± 00 and 50 ± 9.62 in rats for the high dose level group 800 mg/kg/d, 33.33 ± 9.62 and 16.66 ± 9.62 in the 600 mg/kg/d dose group, and 83.33 ± 9.62 and 50 ± 9.62 in the 800 mg/kg/d dose group, within 36 hours for males and females, respectively. Although, an average of 16.66 ± 9.62 was recorded in both sexes at the dose of 434 mg/kg/d (Tables 3 and 4). There was a highly significant (p < 0.01) increase in the mortality rate caused by AEAGR, depending on dose and duration of treatment at the interval of 12-24 and 24-36 h, respectively compared to the control group in males and only at the interval of 24-36 h in females.

The effect size

During three time-intervals, efficiency comparison according to the Cohen’s effect size scale after 36h of treatment revealed a value of R = 0.99, 0.95, 0.94 in males and R = 0.99, 0.92, 0.87 in females for AGRP, EMAGR, and AEAGR respectively (Tables 3 and 4). Hence, AGRP seems to be the most effective (R = 0.99) plant-based extract which could be compared with the chemical rat poison in the cereal storage field test.

Table 3 Dose- and time- dependent variations of corrected mortality in males Wistar rats treated with AGRP, MEAGR, and AEAGR during 36 hours.

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Dose (mg/kg/d)</th>
<th>Corrected mortality ± SE (%)</th>
<th>0-12 h</th>
<th>12-24 h</th>
<th>24-36 h</th>
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<td>AGRP</td>
<td>0</td>
<td>0.00 ± 0.00*</td>
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<tr>
<td></td>
<td>200</td>
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<tr>
<td></td>
<td>434</td>
<td>0.00 ± 0.00*</td>
<td>50.00 ± 9.62b</td>
<td>50.00 ± 9.62b</td>
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<tr>
<td></td>
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<td>0.00 ± 0.00*</td>
<td>100.00 ± 0.00b</td>
<td>100.00 ± 0.00b</td>
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<tr>
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<td>800</td>
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<td>100.00 ± 0.00b</td>
<td>100.00 ± 0.00b</td>
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<td>MEAGR</td>
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<td>0.25 ± 0.25a</td>
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<td></td>
<td>200</td>
<td>0.00 ± 0.00*</td>
<td>0.00 ± 0.00*</td>
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<tr>
<td></td>
<td>434</td>
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<td>0</td>
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<td>0.25 ± 0.25a</td>
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<td>66.66 ± 9.62b</td>
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</tr>
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</table>

Means followed by the same letters in each columns are not significantly different from the control group (Dunnett’s test, P < 0.05). AGRP: Atractylis gummifera rhizome powder, MEAGR: Methanolic extract of A. gummifera rhizome, AEAGR: Aqueous extract of A. gummifera rhizome.
Acute toxicity of A. gummifera rhizome to Wistar rat

Table 4 Dose- and time-dependent variations of corrected mortality in females Wistar rats treated with AGRP, MEAGR, and AEAGR during 36 hours.

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Dose (mg/kg/d)</th>
<th>Corrected mortality ± SE (%)</th>
<th>0-12 h</th>
<th>12-24 h</th>
<th>24-36 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.00 ± 0.00a</td>
<td>0.00 ± 0.00a</td>
<td>0.25 ± 0.25a</td>
</tr>
<tr>
<td>AGRP</td>
<td>0</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>200</td>
<td></td>
<td>0.00 ± 0.00a</td>
<td>0.00 ± 0.00a</td>
<td>0.00 ± 0.00a</td>
</tr>
<tr>
<td></td>
<td>434</td>
<td></td>
<td>0.00 ± 0.00a</td>
<td>0.00 ± 0.00a</td>
<td>20.00 ± 0.00b</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td></td>
<td>0.00 ± 0.00a</td>
<td>50.00 ± 9.62b</td>
<td>50.00 ± 0.00b</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td></td>
<td>16.66 ± 9.62b</td>
<td>66.66 ± 9.62b</td>
<td>100.00 ± 0.00b</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td></td>
<td>3.35*</td>
<td>32.61**</td>
<td>82060.68**</td>
</tr>
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<td></td>
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<td>&lt; 0.001</td>
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<tr>
<td></td>
<td>R</td>
<td></td>
<td>0.74</td>
<td>0.96</td>
<td>0.99</td>
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<td>MEAGR</td>
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<tr>
<td></td>
<td>200</td>
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<td>0.00 ± 0.00a</td>
<td>0.00 ± 0.00a</td>
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</tr>
<tr>
<td></td>
<td>434</td>
<td></td>
<td>0.00 ± 0.00a</td>
<td>0.00 ± 0.00a</td>
<td>16.66 ± 9.62a</td>
</tr>
<tr>
<td></td>
<td>600</td>
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<td>0.00 ± 0.00a</td>
<td>33.33 ± 9.62b</td>
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<tr>
<td></td>
<td>800</td>
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<td>44.44 ± 5.55b</td>
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<td>&lt; 0.001</td>
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<tr>
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<td>R</td>
<td></td>
<td>0.98</td>
<td>0.95</td>
<td>0.92</td>
</tr>
<tr>
<td>AEAGR</td>
<td>0</td>
<td></td>
<td>0.00 ± 0.00a</td>
<td>0.25 ± 0.25a</td>
<td>0.00 ± 0.00a</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td></td>
<td>0.00 ± 0.00a</td>
<td>0.00 ± 0.00a</td>
<td>0.00 ± 0.00a</td>
</tr>
<tr>
<td></td>
<td>434</td>
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<td>0.00 ± 0.00a</td>
<td>0.00 ± 0.00a</td>
<td>16.66 ± 9.62a</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td></td>
<td>0.00 ± 0.00a</td>
<td>0.00 ± 0.00a</td>
<td>16.66 ± 9.62a</td>
</tr>
<tr>
<td></td>
<td>800</td>
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<td>50.00 ± 9.62b</td>
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<td>F value</td>
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<td>8.68**</td>
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<tr>
<td></td>
<td>P value</td>
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<td>&lt; 0.402</td>
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<td>0.53</td>
<td>0.87</td>
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</table>

Means followed by the same letters in each columns are not significantly different from the control group (Dunnett’s test, P < 0.05). AGRP: Atractylis gummifera rhizome powder, MEAGR: Methanolic extract of A. gummifera rhizome, AEAGR: Aqueous extract of A. gummifera rhizome.

LD₅₀ and LD₉₀ determination
The LD₅₀ and LD₉₀ values obtained from the log-dose and probit regression curve of corrected mortality percentage (CM %) according to Tainter and Miller (1944) are shown in (Table 5). The A. gummifera powder (AGRP) recorded an LD₅₀ and LD₉₀ of 471 (406.79-427.21), 513 (507.32-518.68) mg/kg/d and 500 (498.42-501.22), 613 (606.5-619.5) mg/kg/d in males and females respectively. For the methanolic (MEAGR) and the aqueous (AEAGR) extracts, the LD₅₀ values of males and females respectively were 575 (571.19-578.21) and 646 (642.22-649.78) mg/kg/d and 676 (668.19-683.81) and 708 (703.39-712.61) mg/kg/d. An LD₉₀ was calculated for MEAGR in males; however, no LD₉₀ values were determined for AEAGR in either sex.
Table 5 LD50 and LD90 values for AGRP, MEAGR, and AEAGR in Rattus norvegicus after 36 h of treatment.

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>LD value (mg/kg/d)</th>
<th>LD values (95% confidence intervals) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Males</td>
</tr>
<tr>
<td>AGRP</td>
<td>LD50</td>
<td>417 (406.79 - 427.21)</td>
</tr>
<tr>
<td></td>
<td>LD90</td>
<td>513 (507.32 - 518.68)</td>
</tr>
<tr>
<td>MEAGR</td>
<td>LD50</td>
<td>575 (571.79 - 578.21)</td>
</tr>
<tr>
<td></td>
<td>LD90</td>
<td>776 (768.63 - 783.37)</td>
</tr>
<tr>
<td>AEAGR</td>
<td>LD50</td>
<td>676 (668.19 - 683.81)</td>
</tr>
<tr>
<td></td>
<td>LD90</td>
<td>ND</td>
</tr>
</tbody>
</table>


Field test
The aim of the field test of AGRP in cereal storage stores was to find a simple, easy to use a natural alternative to chemical rat poisons to protect stored food. The field trial showed that the bait poisoned by AGRP induced a 60% reduction in Rat population at three storage sites based on the consumption method compared to a 75% reduction for the chemical rodenticides (Table 6). A highly significant reduction ($p < 0.01$) in the consumption of baits treated with AGRP was noted very close to that recorded with the chemical rodenticides, which confirms the effectiveness of our tested plant product.

Table 6 Field evaluation of AGRP baits against Rattus norvegicus in the durum storage (dock) stores.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Consumption (g/store)</th>
<th>Population reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control store</td>
<td>1000 ± 0.00a</td>
<td>0</td>
</tr>
<tr>
<td>AGRP</td>
<td>1000</td>
<td>400 ± 104.08b</td>
</tr>
<tr>
<td>RATICIDE 70%</td>
<td>1000</td>
<td>250 ± 11.54b</td>
</tr>
</tbody>
</table>

F value 161.17**

Means followed by the same letters in each columns are not significantly different from the control group (Dunnett’s test, $P < 0.05$). AGRP: Atractylis gummifera rhizome powder.

Gross pathology findings
At necropsy, gross pathological examination showed mild to moderate hypertrophy of kidneys and congestion with hemorrhage in the nasal cavity, the lungs, and the liver of dead rats in treatment groups.

Histopathological findings
No pathological changes were observed in untreated control animals (Fig. 1-3). Pathological changes induced by PAGR, MEAGR, AEAGR treatments were observed in kidneys, liver, lungs, and small intestine of male and female rats. Severe congestion and hemorrhage with mild hemosiderosis were dominant common pathological findings in all above-mentioned organs for both male and female rats. Liver tissue sections from both sexes showed hepatocyte degeneration, cytoplasmic vacuolation, centrolobular necrosis, and proliferation of Kupffer cells (Fig. 1-3). Histopathological examination of male and female rats revealed mild to moderate degeneration of the renal tubular lining epithelium, diffuse interstitial pneumonia, and edema in the lung (Fig. 1-3). Microscopic examination of the intestines of treated rats showed severe enteritis, sloughing of the epithelial lining of villi, and hyperplasia of goblet cells (Fig. 1-3). Although many organs showed pathological changes related to AGR toxicity, the liver was more affected in both male and female rats.
Acute toxicity of A. gummifera rhizome to Wistar rat

Figure 1: Histopathology of control group organs (C1: liver, C2: kidney, C3: lungs and C4: intestine) and histopathological changes of Wistar rats organs (A, B, C, D, E, F: lungs (G), and intestine (H, I) induced by AGR powder, H&E. A(x40): Severe centrolobular necrosis (↓); B(x40): Edema (★), congestion (+) and hemorrhage (↑) with prominent proliferation of Kupffer cells (◆); C (x 40): Severe periportal inflammatory cell infiltration (cholangitis) (◇); D(x 40): Degenerated and necrotic hepatocytes (◇); E(x10): Severe congestion (+) and hemorrhage (↑); F(x40): Degeneration and necrosis (◇) of tubular epithelial lining; G(x 40): Severe diffuse congestion (+), edema (★) and hemorrhage (◆) of lung parenchyma with mild inflammatory cell infiltration (◇); H(x10): Severe enteritis with erosion avitus epithelium (◇); I(x40): Eosinophilic infiltration (◇) of lamina propria (between crypts).

Figure 2: Histopathology of control group organs (C1: liver, C2: kidney, C3: lungs and C4: intestine) and histopathological changes of Wistar rats organs (A, B, C, D, E, F: liver (G), and intestine (H) induced by the methanolic extract (ME) of AGR. H&E. A(x40): Diffuse centrolobular coagulation necrosis (↓), vacuolated hepatocytes (◇) and severe congestion (+); B(x40): Mild inflammatory cell infiltration (◆); moderate hemorrhage (◇), with notable Kupffer cells hyperplasia (◇); C(x40): Severe tubular epithelial necrosis (◇) with glomerular dilation (◇); D(x40): Diffuse congestion (+) and hemorrhage (◆) of renal cortex and medulla; F(x10): Diffuse spinal congestion (↓); F(x10): Severe diffuse congestion (↓), edema (★) and hemorrhage (◆) of lung parenchyma with moderate inflammatory cell infiltrate; G(x40): Severe diffuse broncho-pneumonia (◇); H(x10): Severe acute enteritis with diffuse destruction of villi (◇).
Discussion

The extraction yields obtained in this study are in agreement with those reported by Bouabid et al. (2019) in an in vivo study of the anti-diabetic effect of aqueous and methanolic extracts of macerated A. gummifera, with 24.5% and 6.7%, respectively. Sifour and his collaborators, however, have found a 16.74% yield from aqueous extraction of A. gummifera rhizome (Sifour et al., 2012). Polar extracts are the richest in total phenolic and flavonoid compounds. This result is supported by several studies. For example, Moure et al. (2001) in their work on Gevuina avellana hulls found that the yield of polyphenols extraction was higher for most of the polar solvents. In a similar study conducted by Khadhri et al. (2013), quantitative determination of polyphenols, Flavonoids, and condensed tannins in the two aqueous and methanolic extracts of A. gummifera rhizome revealed different values: 7 mg Eq AG/g DM, 4 mg Eq AG/g DM, 0.5 Eq Qr/g DM, 0.7 Eq Qr/g DM and 1.7 mg Eq Cat/g DM, for polyphenols, Flavonoids and condensed tannins in the methanolic and aqueous extracts, respectively (khadhri et al., 2013), while Sifour et al. (2012) have reported that amounts of polyphenols and Flavonoids in the aqueous extract was 44.4 mg and 16.74 mg/ml of the extract. Bouabid et al. (2020) in an in vitro and in vivo study of antioxidant properties of aqueous and organic extracts of A. gummifera, reported that the methanolic and aqueous extract macerated tissue contains a rate of total polyphenols and flavonoids equal to 102.88 mg Eq AG/g DM, 49.59 mg Eq AG/g DM and 17.25 Eq Qr/g DM, 12.56 Eq Qr/g DM (Bouabid et al., 2020).

The results obtained showed that males are more sensitive than females in response to the various treatments of AGR. Shumake and Hakim (2000) reported that the smell of carbon disulfide in the presence of zinc phosphorus bait attracted male rats much more than female rats, which reflects their mortality rate, however, the reasons for the differences between the two sexes are unknown, so,
females may react differently than males to secondary plant metabolites (MSP) (Shumake and Hakim, 2000; Hansen et al., 2016). According to, Capasso et al. (1984), Hasani (2007), Rasekh et al. (2005), Al-Asheh et al. (2006); the administration of the plant *Teucrium polium* is influenced by the nature of the sex (Al-Asheh et al., 2006; Capasso et al., 1984; Hasani, 2007; Rasekh et al., 2005). Also, the AGRP treatment was very toxic compared to the methanolic (MEAGR) and aqueous (AEAGR) extracts of *A. gummifera* rhizome for both sexes. Differences in response to *A. gummifera* toxicity were reported by Luciani et al. (1978) who concluded that the toxicity also differs in male rats between the albino and Wistar strains: in albino rats, Atractylside (ATR) is not toxic at doses up to 200 mg/kg, while in Wistar strains 60 mg/kg can lead to death, while our results show that the powder causes significant toxicity with an LD$_{50}$ of 417 mg/kg/d and 500 mg/kg/d in male and female Wistar rats respectively. The application of a mixture of odors from different secondary metabolites of plants is a means of controlling rodents due to their effect which keeps them away from feeding the crops or causing their deterioration. Over the past 15 years, the scientific goal of using plant secondary metabolites in the management of rodent populations has been to develop suitable alternatives (Hansen et al., 2016).

Our study recorded the appearance of clinical signs with digestive, neurological, and respiratory disorders following the toxicity by AGR and their intensity led the rats to a state of coma after thirty-six hours of treatment before their death. According to Hammiche (2013), similar clinical signs have been reported in humans poisoned by *A. gummifera* after a latency period of a few hours, usually 8 to 12 hours, the first general signs including important digestive signs mainly hemorrhagic vomiting followed by neurological signs (coma with neurovegetative signs) and respiratory disorders (Hammiche et al., 2013). The poisoning by *A. gummifera* has several phases: the latency phase from six to twenty-four hours and even thirty-six hours, gastro hepatic disorders represented by nausea and vomiting observed in the symptomatic phase (Skalli S, 2002). Some complications cited by Ahid et al. (2012) include respiratory disorders with congestion, progressive dyspnea, asphyxia, and cardiovascular disorders with tachycardia. In his study on an Asteraceae: *Smallanthus sonchifolius*, De Oliveira (2011) observed clinical signs of renal toxicity, hypoactivity, difficulty in breathing, and respiratory arrest caused by oral administration of *S. sonchifolius* leaf-rinse extract and aqueous extract at doses of 10 mg/kg and 100 mg/kg respectively in rats (De Oliveira et al., 2011). Accordingly, in the present study, the treated rats were in severe respiratory distress before death which confirms the toxicity of the plant even if the mechanism is not clear.

Histopathology is considered a rapid and sensitive method frequently used to identify the effects of toxic substances in animal organs and tissues. Pathological findings showed multisystem organ injuries induced by AGR including congestion, hemorrhage, degeneration, and necrosis. AGR treatments caused severe hepatic, intestinal, respiratory, and renal damage in experimental rats leading to multisystem organ failure. Experimental toxicological studies of AGR in Wistar rats revealed hepatocellular necrosis and pericentrallobular, extensive sinus congestion. Also, biochemical and histological studies have shown that this toxic plant is of high hepatotoxicity and extensive sinus congestion in the kidney (Dahamna, 2018). Histopathology findings showed that AGR induced congestion and hemorrhage in most of the studied organs which seem to be similar to anticoagulant rodenticide effects. Anticoagulant rodenticides are inhibitors of reductase enzymes, mainly epoxide reductase therefore they are vitamin k antagonists by preventing the regeneration of vitamin k, they can cause hypocoagulation within 24 hours after intoxication which can worsen in 48 to 72 hours where the risk of hemorrhagic complication can be major (Gamelin and Harry, 2005).
Similar findings were described by Dapar et al. (2007) reporting that the *Securidaca longipedunculata* extract caused histopathological changes in the kidneys and liver characterized by acute tubular necrosis with interstitial spread and glomerular hemorrhage, hepatocytes presented early fatty degeneration in some foci with hemorrhage, while pulmonary toxicity was expressed by massive damage to the alveoli and bronchiolar epithelium (Dapar et al., 2007). Also, Pinto and Geuel have demonstrated that liver damage such as steatosis, necrosis, fibrosis, and cirrhosis can be caused by plants (Pinto Da Cunha and Geuel, 2002). Fall and his colleagues (2011) demonstrated that the leaves of *Aphania senegalensis* caused liver toxicity with a 2000 mg/kg dose in rats (Fall et al., 2011). Carlier et al. (2014) have cited that post mortem histopathological analysis of tissues can confirm poisoning at ATR by the presence of centrilobular and tubular renal hepatic necrosis, in particular in animal experiments (Carlier et al., 2014). According to De Berardinis et al. (2000), a mechanism of direct toxicity with a series of secondary immune reactions can cause hepatocyte necrosis (De Berardinis et al., 2000), however, Ingwale et al. (2009) have reported that liver damage is due in most cases to direct cytotoxicity, but this is not always the case as a mixture between cytotoxicity and immunogenicity may be involved.

Kidney changes observed in the present study are correlated with those obtained by De Oliveira et al. (2011), reporting kidney damage characterized by degeneration of the glomeruli as well as the proximal and distal tubules of rats treated with the leaf-rinse extract at a dose of 100 mg/kg. Similarly, kidney damage has been manifested by the use of the ethanolic extract of *Tithonia diversifolia* (Elufioye et al., 2009). Similar histopathological changes were described by Pramestuti et al. (2019) in a rodenticidal effect study of *Carica papaya* in albino mice; they have observed necrosis in the internal organs (stomach, kidney, liver, and intestine) and congestion in the kidney and liver of treated compared to control animals (Pramestuti et al., 2019).

It is well known that aqueous and organic extracts of the underground part of *A. G.* are rich in secondary metabolites; polyphenols, flavonoids, catechic tannins, and anthocyanins, which have important biological activities with an absence of alkaloids. Although, the powder is rich in tannins, flavonoids, and contains traces of saponins, quinones, and sterols, the toxicity of *A. gummifera* is not due to alkaloids but rather because of two glycosides: ATR and carboxyatractyloside (CATR) (Benbouziane and Beneddra, 2016; Bouabid et al., 2018). The toxicity of AGR plant was proven by the presence of the two active ingredients ATR and CATR which inhibit the transport of phosphorylated nucleotides through the mitochondrial membrane, which prevents mitochondrial oxidative phosphorylation and disrupts tissue respiration by blocking the cycle of tricarboxylic acids (Krebs cycle) (Obatomi and Bach, 1998, Hmaouda, 2004). Also (Ahid et al., 2012) stated that the role of metabolism is also unclear, but it is evident that ATR interacts with hepatic biotransformation systems. The liver is the primary target organ for active substances such as ATR and CATR which cause severe liver failure and hepatolobular necrosis. The toxic effects of *Atractylis gummifera* are owing to ATR, a powerful inhibitor of oxidative phosphorylation in mitochondria. This action is exerted especially in cells rich in mitochondria such as hepatocytes and in proximal tubular epithelial cells, which contain carriers that allow ATR to cross the cell membrane (Daniele et al., 2005).

In a study in rats, Hopps et al. (1997) described the effects of the ATR and some of its derivatives on renal function; these experiments showed that ATR was highly toxic to the kidney, as shown by enzymuria and reduction of creatinine clearance, whereas atracylygenin leaves renal function almost unchanged. ATR toxicity is related to its chemical structure and increases when the hydroxyl groups in C-4 are esterified with isovaleric acid or when the hydroxyl groups in C-3 and C-4 are esterified.
with sulphuric acid. The toxic effects due to the inhibition of mitochondrial phosphorylation are hepatic necrosis and renal failure in animals and humans. The acute toxicity of ATR nevertheless differs according to the animal species and route of administration. Toxicity is higher in dogs than in mice and rats, suggesting even higher toxicity in humans (Luciani et al., 1978).

In the light of the results obtained in the field, the AGRP rat poison efficacy was demonstrated by a highly significant reduction of R. norvegicus population compared to the commercial chemical rodenticides. Abou-Hashem (2012) have shown that the ethanolic extracts of Eucalyptus globulus, Rhus continus, and Calendula aegyptiaca resulted in a reduction of the R. norvegicus population by 67.7%, 64.8 %, and 42.7% respectively, in the field conditions of the governorate of Menoufia, Egypt (Abou-Hashem, 2012). Eisa and Yassin (2016) reported that the extract of Oshar caused a 66% reduction in the R. norvegicus population compared to 78.40% reduction induced by zinc phosphide in the region of Qaha, Governorate of Qalyubia, Egypt. Also, Khidr et al. (2018) have found that the organic extracts of the solvents: ethanol, hexane, and ether oil from the Argel plant Gomphocarpus sinaicus Boiss recorded reduction percentages in rodent populations under field conditions of 68%, 37.7%, and 19% respectively. Based on these data, it can be deduced that the rhizome AGRP is a natural rodenticide candidate ready to be considered as an alternative compound for cereal crop protection.

**Conclusion**

Despite its use in phytotherapy, A. gummifera is a toxic plant to animals. Our results revealed that the rhizome of the plant is toxic in different forms (aqueous and organic extracts and as a powder), however, the powder was very toxic in acute toxicity studies for Wistar rats. Plant toxicity is clearly expressed by clinical signs and organ pathology (liver, lungs, kidney, and intestine) and is mainly due to the two active components of the plant; ATR and CATR according to previous studies. Promising results obtained with AGRP under laboratory conditions in Wistar rats prompted us to assess rat poison effect in field conditions in cereal storage stores that were confirmed by a significant (60%) reduction of R. norvegicus population. These results proved that the rhizome of the plant has a good rat poisoning effect and could be suggested as a strong candidate natural alternative to rat poison chemicals. As an anticoagulant rodenticide, it is strongly recommended for use because it is cheaper (economic) and easy to produce. Planting around silos and warehouses remains a proposal to control these rodents and protect stored grains and prevent transmission of diseases to consumers. Therefore, more research is needed to use the rhizome of A. gummifera as a natural rat poison with an adequate proportion of the active ingredients Atractyloside and carboxyatractylloside to control rodents.

**Conflicts of interest**

The authors declare no conflict of interest associated with this publication and there has been no financial support for this work that could have influenced its outcome.

**Acknowledgments**

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بررسی مشخصات فیتوشیمیایی و ستیت حاد ریزوم Kاراپی کنترل کننده آن روي موش نروژی در انبار

مختصرای چلفت،* هواری همیدا،** عبدالله حسینی،*** کلمنو مازو،*** سهاری میرا و مصطفی آبدی**

چکیده: این مطالعه با هدف بررسی میزان ترکیبات فن انجام کرد. در این پژوهش تأثیر بسیاری از عوامل مختلف و عصاره Rattus norvegicus بر روی رژیم غذایی و تربیت موش Atractylis gummifera در انبار بررسی شد. عصاره‌های مستقیم و آبی به ترتیب 90±0 و 60±0 درصد میزان بود. مقادیر کل پلی فنل‌ها (میلی گرم/مدال) گاکی اسید آزمایشگاهی ماده خشک، فلاح‌شناسی خشک (میلی گرم/مدال) کنارگیری گرم‌بندی گردشگری و محصول ماده خشک، نان (میلی گرم/مدال) کنارگیری گرم‌بندی گردشگری به ترتیب 15±0/0 و 27±0/0 و 10±0/0 و 9±0/0 و 17/14±0/0 و 7/70±0/0 و 1/17±0/0 و 7/74±0/0 و 1/17±0/0 درصد می‌باشد. 

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