

Original Research Paper

Effects of Some Environmental Stresses on Bio Indicators and Population Densities of *Glomus mossae* Fungi Associated with Wheat (*Triticum aestivum* L.) Crop

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Abstract: Field experiment was conducted to determine the effect of soil salinity and *Fusarium germanium* on population of *Glomus mossae* Fungi within root of wheat as manifested by the fungal spore's number, density infection and the percentage of infected root by *Glomus mossae*. The results didn't show effect the environmental stresses on comparability reproductive for *Glomus mossae* fungi associated with wheat crop. The results significant increase ($P = 0.05$) in average of spores number, intensity infection and the percentage of infected root (43.00, 60.8 and 72.67) by *Glomus mossae* treatment. A significant increasing happened in the percentage of germination seed of wheat with *Glomus mossae* fungi in soil Electric conductivity 2.64 dS/m^{-1} was (75.7%, chlorophyll content 32.33 spad and plant height 40.00 cm) compared with treatment infection *Fusarium germanium* fungi in the soil with Electric conductivity 14.5 dS/m^{-1} since the ratio of germination was (24.3%, chlorophyll content in the plant 13.67 spad and height plant 12.00 cm). On the other hand the results recorded a significant increasing in macronutrient (N, P and K%) by *Glomus mossae* treatment which recorded (2.567, 0.272 and 2.283%) respectively compared with treatment infection *Fusarium germanium* fungi (1.7, 0.100 and 1.800%) respectively. Interaction treatment showed a significant increasing by *Glomus mossae* treatment with soil Electric conductivity 14.5 dS/m^{-1} in terms of fungal spores number, density infection and the percentage of infected root of *Glomus mossae* (55.00, 75.7 and 93.00) respectively compared with treatment infection *Fusarium germanium* fungi with soil Electric conductivity 2.64 dS/m^{-1} (19.33, 13.00 and 52.33) respectively. Finally the results showed a significant increasing interaction by *Glomus mossae* treatment with soil Electric conductivity 2.64 dS/m^{-1} in the (ratio of germination 75.7%, chlorophyll content in the plant 32.33 spad and height plant 40.00 cm) compared with treatment infection *Fusarium germanium* fungi with soil Electric conductivity 14.5 dS/m^{-1} (24.3, 13.67 and 12.00) respectively.

Keywords: Fungus *Glomus mossae*, *Triticum aestivum*, Salinity, *Fusarium germanium*

Introduction

Wheat (*Triticum aestivum* L) is belong to Cereals plants, which represent the most world's strategic crops in terms of its daily consumption and food importance (Hassan, 1999). During the growing season, this crop is exposed to many biotic and abiotic stresses (Levitt,

1980). The most important biotic stress is the pathogenesis of the *Fusarium* species which are considered to be one of the most common species of *Fusarium* wilt disease affecting vegetable plants, Crops and ornamental plants (Bogale, 2006). *Fusarium species* cause a significant decline in the productivity of crops in many countries of world (Agrios, 2005) which

is one of the most serious fungal diseases affecting plants due to suit their growth in environmental conditions that prevailing in the region where the farmers has to apply many chemical pesticides in order to reduce its severity. This work would be useful for revealing the numerous fungal strains that are not affected by chemical pesticides, although they are used at high concentrations and contribute to environmental pollution and human health (Alimi and Ayawale, 2014). Due to hazards use of chemical pesticides, Global attention has focused on the search for methods of biocontrol using bacteria and beneficial fungi as biocontrol agents. Several recent studies have indicated that *Glomus mossae* vaccines have a significant effect on the growth of wheat plant and in the control of fungal diseases that affect this crop. The *Glomus mossae* are characterized by the production of high plant growth promotion such as IAA, Cytokinin and GA3 which excreted in rhizosphere and transferred to plant tissues through the symbiotic relationship with microorganisms (Siddiqui *et al.*, 2006). As well as its ability to secrete Glomalinalin, which works to enhance soil structure, this improves the ability to hold water for a long time (Adeleke and Odedeji, 2010). It has been found that the *Glomus mossae* have significant impact in reducing the infection of endemic pathogens in soil (Naseby *et al.*, 2000). Salinity is one of the problems impediment agricultural production and salt-affected soils are widespread in large parts of the world, accounting for more than one-third of the world's irrigated land (Lauchli and Epstein, 1990) and studies have shown that plants infected with *Glomus mossae* are more resistant to abiotic stress conditions such as salinity, drought and toxicity with heavy metals (Miransari, 2011). Many studies confirm that plants infected with *Glomus mossae* under saline stress conditions increase the accumulation of amino acid prolin in maize and wheat yield due to the activation of the P5CS gene responsible for prolin production in cells as a response to stresses (Sheng *et al.*, 2008; Azad *et al.*, 2012; Salman, 2013). The aim of this study was to determine the effect of salinity and *Fusarium germinarium* on population of *Glomus mossae* associated with wheat crop.

Materials and Methods

Preparation of Fungi Disease Isolation

Fusarium graminearum has been isolated from the equipment of the Laboratory of Plant Diseases, which belong to the Integrated Control Center/directorate of Agricultural Research/Ministry of Science and Technology (formerly). The fungi were prepared using natural media of wheat seeds which were soaked for 24 h in tap water. The seeds were then distributed in 250 mL conical flask with 100 g per flask which was sterilized by autoclave with 20 min at 121°C and 1.5 bar pressure

and 2 for 2 days. It was then inoculated with 0.5 cm disease fungi tablets and incubated at 25°C for two weeks with daily manual shaking to avoid coagulation of mixture (Bovill, 2007).

Preparation of Glomus Mossae

The isolates of (*Glomus mossae*) were obtained from fungal parts (infected roots + spores + mycelium). These structures were mixed with a certain amount of sterilized compost and the fungal fungus was calculated according to the method described by (Kormanik *et al.*, 1980).

Field Experiment

A field experiment was carried out during 2014-2015 in the Agricultural Research Directorate-Ministry of Science and Technology (30 km southeast of Baghdad) where two equal plots of land were selected for cultivation with two meters separation between the two parts. Soil of first plot was replaced by alluvial soil with Electrical Conductivity (EC) 14.5 dS/m⁻¹ of 30 cm of upper soil layer. The (EC) of second plot soil was 2.64 dS/m⁻¹. Soil samples were taken for analysis in the laboratory (Table 1). The population density of *Glomus mossae* in soil was 10/gm soil. Experiment was plowed and grazed with disk compartments. The field was then settled and divided into 1×1 m² plots. Each plot consisted of five lines with one meter long for each treatment, the distance between the plots 20 cm. The land was divided into three main sectors, separated by a distance of 1 m. In order to reduce lateral water movement between the plots, polyethylene was inserted in soil around each separated plot for 0.25 m depth. The experimental were 48 units. Wheat seed (Cham type) planted at 160 kg/ha. The inoculum was added to seeds according to pad method. The following treatments were conducted in fields with three replicates: Wheat seeds without inoculums as a (control treatment), seeds inoculated with *Glomus mossae* (G), seeds infected with *Fusarium graminearum* (F), wheat seeds treated with *Glomus mossae* fungi + *Fusarium Graminearum* (GF) under two levels of soil salinity (2.64 dS/m⁻¹ and 14.5 dS/m⁻¹).

Calculation of Number of Spores, Intensity and Percentage of Infection of Glomus Mossae

The decanting wet sieving method described by Gerdeman and Nicolson (1963) was used. Samples were taken from soil around the roots of the wheat plant at 10 g at the end of experiment. The suspension containing spores were transferred to a glass baker, diluted to 250 mL was placed. The suspension in 50 mL plastic tubes and place in the centrifuge at 4000 rpm for 5 min. Minutes (without adding sucrose solution) to facilitate the collection of spores at surface of suspension and then withdraw 1 mL and transfer to the nematode count slice to calculate the spore rate in 1 mL of suspension and for the purpose of calculating the number of spores in 10 g soil followed the equation set by Gaur and Adholya (1994).

Number of Spores Per 10 g Soil = Rate of Spores Counting in 1 mL × Final Size of Suspension

The disease incidence and severity of *Glomus mossae* fungi was calculated according to the method described by Phillips and Hyman (1970) by taking 50 g of dried rizosphere soil. Hardness was resolved on the premise that the cells of the root cortex did not hold color, whereas fungal cell walls maintained the dye even after it was shortened. The method is to extract the root mass of the plants and gently wash them with running water to get rid of the suspended dust and then in a preservative solution made of formalin: Acetic acid: ethanol (13 mL of formalin, 5 of acetic acid and 200 mL/50% of ethanol). Cut the roots into little pieces (1 cm) by 10 pieces/plants placed in glass bottles size 20 mL and added a 10% of KOH solution and placed in a water bath at a temperature of 70°C for 15, the roots were extracted and washed with distilled water after placed in a sieve (60 mesh), the roots were then transferred by using forceps to Petri dishes with hydrogen peroxide solution treated 1% at room temperature for 10 minutes. The roots were then washed thoroughly with distilled water and then treated with 1% HCl solution for 3 min. The roots were transferred to 100 mL glass bottles supplied with Acid Fochsin, which was prepared according to the method described previously, by mixing 63 mL of Glycerol +875 mL of lactose +63 distilled water +0.1 g of Fochsin pigment, the roots were treated within 30 min heat 70 m in a water bath, the root pieces were then extracted and arranged on a glass slide of 10 pieces/slide where they were examined under the microscope (Kormanik *et al.*, 1980). The intensity of the infection according to (Dheyab, 2013) equation was calculated and the following gradient was used to measure the intensity of the injury; 0 = no injury, 1 = 25% of the root total is infected, 3 = 26-50% of the root total is infected, 3 = 51-75% of the root total is infected and 5 = 76-100% of the root total is infected.

The intensity of infection of *Glomus mossae* according to the equation (Mckinney, 1923) was calculated as follows:

$$\text{Intensity of infection} = \frac{\text{Total (number of infected pieces} \times \text{degree of injury)}}{\text{total number of examined} \times \text{Highest degree of injury}}$$

The infection percentage was calculated by the formula described by (Giovannetti and Mosse, 1980):

$$\text{Percentage of } Glomus \text{ mossae infection} = \frac{\text{Number of infected root pieces}}{\text{Total number of examined root parts}} \times 100$$

The rate of seedling germination was calculated on the surface land after 14 days of cultivation in the field. Chlorophyll content was measured at the time of spike production by a chlorophyll-based chlorophyll content control device (CCM-200) of US origin and OPTI-

SCIENCES manufacturer. The content of leaves randomly was measured for 3 plants in three replicates of each treatment. Plant height was measured at the end of the experiment using a measuring tape and wheat plants were cut off after four months from the date of cultivation. The leaf content of the nitrogen, phosphorus and potassium component was measured according to (Labetowicz, 1988).

The experiment was carried out in accordance with the RCBD design and three replicates for each treatment and the data was analyzed according to the program. Genstat was compared with the Least Significant Difference (L.S.D) at the probability level (0.05) (Mohammedi and Al-Mohammadi, 2012).

Results and Discussion

The results of Tables 1 to 3 showed that the number of fungal spores per gm soil and the intensity of infection by *Glomus mossae* and the percentage of infected roots varied with the variability of soil EC. The results showed a significant increase in number of *Glomus mossae* spores per gm soil and intensity of infection by *Glomus mossae* and percentage of infected roots in plants that inoculated with *Glomus mossae* (43.00 spores/gm soil, 60.8% and 72.67%) compared with the treatment of infection with *Fusarium* (22.67 spores/gm soil, 13.2% and 36.00%) respectively. The increase in both the number of *Glomus mossae* spores and the intensity of infection and the percentage of infection of the microorganisms in the inoculated wheat plants increases the density of the inoculate (spore, hypha, infected root) compared to the plants infected with *Fusarium*. There is a decrease in the percentage and intensity of infection of bean root with *Glomus mossae* this may be due to competition of microorganisms with the pathogenesis on root secretions in the razosphere and competition is significant when there is more than one type of fungus in the area of the razosphere. The results showed a significant increase in number of *Glomus mossae* spores/gm soil and intensity of infection by *Glomus mossae* and percentage of infected roots for soil with 14.5 dS.m⁻¹ by (35.5 spores/gm soil, 48.5% and 59.83%) respectively compared to plants grown in soil with 2.64 dS.m⁻¹ (23.83 spores/gm soil 29% and 39.92%) respectively. The treatment of interaction between plants infected with *Glomus mossae* and salinity showed the highest number of *Glomus mossae* spores, the intensity of infection by *Glomus mossae* and the percentage of infected roots (55.00 spores/gm soil, 75.7%, 93.00%) compared to the treatment of *Fusarium* infected plants and soil with EC 2.64 dS.m⁻¹ (19.33 spores/gm soil and 13.0%). The percentage of infected roots in soil with EC 14.5 dS.m⁻¹ was 32.67%. This may be attributed to the roots of plants that are subject to environmental stress such as drought and salinity. The fungus increases the internal hypha and the vesicular within the roots to cope with these conditions (Siddiqui *et al.*, 2006).

Table 1: Some chemical and physical properties of the studied soil samples

Property	Value		Unit	Texture gm.kg ⁻¹			
	Soil with EC 2.64 dS.m ⁻¹	Soil with EC 14.5 dS.m ⁻¹		Soil with EC 2.64 dS.m ⁻¹	Soil with EC 14.5 dS.m ⁻¹		
PH (1:1)	7.2	7	-----	600	sand	424	sand
N	9.5	9	mg.Kg ⁻¹	200	caly	288	caly
Ava. P	30.62	25.82		100	silt	288	silt
Ava. K	210.46	260.48		Sand		Clay loam	
Ca	226.11	543.66					
Cl	274.27	2938.59					
SO ₄	1200.03	3271.14					
Mg	76.73	266.3					
Na	292.05	3526.47					

Table 2: Effect of *Fusarium* and *Glomus mossae* infection and soil salinity on average number of *Glomus mossae* spores/gm soil

Treatments\Soil EC (dS.m ⁻¹)	2.64 dS/m ⁻¹	14.5 dS/m ⁻¹	Mean treatments
Control	21	28.00	24.50
F	19.33	26.00	22.67
G	31	55.00	43.00
FG	24	31.67	27.83
Mean soil salinity	23.83	35.17	

F = *Fusarium graminearum* G = *Glomus mossae* fungi

FG = *Fusarium graminearum* + *Glomus mossae* fungi

L.S.D treatment = 1.218 L.S.D soil salinity = 1.722

2.436 =D interactions .S.L

Table 3: Effect of *Fusarium* and *Glomus mossae* infection and soil salinity on *Glomus mossae* infection intensity

Treatments\Soil EC (dS.m ⁻¹)	2.64 dS/m ⁻¹	14.5 dS/m ⁻¹	Mean treatments
Control	31.0	52.7	41.80
F	13.0	13.3	13.20
G	46.0	75.7	60.80
FG	25.7	52.7	39.20
Mean soil salinity	29.0	48.5	38.75

F = *Fusarium graminearum* G = *Glomus mossae* fungi

FG = *Fusarium graminearum* + *Glomus mossae* fungi

L.S.D treatment = 6.64 L.S.D soil salinity = 4.69

L.S.D interaction = 9.38

The results in Tables 5 to 7 show refers to the significant superiority of plant that inoculated with *Glomus mossae* in percentage of seed germination, chlorophyll content and plant height (73.3%, 28.17 spad and 32.50 cm) respectively, compared to the treatment of infection with *Fusarium* (26.3%, 14.00 spad and 12.33 cm) respectively. Above showed significant superiority in the studied parameters of plants growing in soil with a EC 2.64 dS.m⁻¹ (56.2%, 21.11 spad and 24.00 cm) respectively compared to soil with EC 14.5 dS.m⁻¹ (52.7%, 18.25 spad and 18.25 cm) for percentage of seed germination, chlorophyll content and plant height respectively. The treatment of interaction between *Glomus mossae* and soil EC 2.64 dS.m⁻¹ were significantly higher with percentage of seed germination, chlorophyll content and plant height of (75.7%, 32.33 spad and 40.00 cm) respectively compared to the interaction between the *Fusarium* and soil with EC 14.5 dS.m⁻¹ of (24.3%, 13.67 spad and 12.00 cm) for seed germination, chlorophyll content and plant height respectively, which did

not differ significantly from control treatment with EC 2.64 dS.m⁻¹. The results in Tables 8 to 10 show a significant superiority for plants inoculated with *Glomus mossae* in parameters of plant concentration of nitrogen, phosphorus and potassium (2.567%, 0.272% and 2.833%) respectively compared with treatment of inoculate with *Fusarium* by (1.7, 0.10 and 1.80) respectively. The plant grown in soil with EC 2.64 dS.m⁻¹ showed significant superiority by (2.442%, 0.236% and 2.700%) respectively compared to soil with EC 14.5 dS.m⁻¹ (1.700, 0.136 and 1.867%) for plant concentration of nitrogen, phosphorus and potassium respectively. The interaction between *Glomus mossae* and soil with EC 2.64 dS.m⁻¹ showed significant superiority by (3.233%, 0.326% and 3.500%) while interaction between *Fusarium* and the soil with EC 14.5 dS.m⁻¹ was lowest value by (1.367%, 0.046% and 1.333%) for plant concentration of nitrogen, phosphorus and potassium respectively. The increase in the number of *Glomus mossae* spores in Tables 2 to 4 leads to an increase in the number of

root hairs, which has helped to spread the root mass and produce growth hormones such as Gibberellin, Auxins, Cytokines, Amino acids and Hormones, which increase the growth and expansion of cells and tissues, which reflected positively on the percentage of germination (Table 5), especially Gibberellin, which causes the important act of Gibberellic acid in formation of hydrolysis enzymes (amylase, protease and lipase) in seeds which leads to form the simple materials of sugars, amino acid and lipids that would release required energy to emerge the seeds (Bharathi *et al.*, 2004) encourage the production of soluble phenols and carbohydrates that have a role in maintaining the integrity of the chloroplast and thus increasing the absorption of the elements (Table 8), which enters in the structure of chlorophyll (Table 6). In vitro fertilization with *Glomus mossae* increases the effectiveness of photosynthesis due to symbiotic symbiosis of fungi that have the ability to convert unavailable phosphorus to the available phosphorus (Table 9). This will increase the growth of the host plant, which is provide *Glomus mossae* with carbon compounds and increasing the effectiveness of

these *Glomus mossae* (Demir, 2004). Moreover, increasing the concentration of potassium (Table 10) in the plant is necessary to obtain the phytochemical response in the plant, which is positively reflected on the indicators of vegetative growth such as plant height (Table 7). The results are corresponding with previous studies (Hamdia and Shaddad, 2010; Joachim *et al.*, 2009; Wang *et al.*, 2008). The decrease in the studied indicators in Table 5 to 10 in the presence of the pathogen *Fusarium* may be due to the consumption of nutrients in the plant to compensate for the damage caused by the presence of fungus or decrease the efficiency of the *Glomus mossae* in release and availability of plant nutrients in soil or perhaps the plant has induced several mechanisms, including increased activity of defense enzymes and salicylic acid and increase in total phenols as well as mechanical resistance by increasing the thickness of the walls of cells with lignin. The mechanization by which the plant can grow under saline conditions to coexist with the *Glomus mossae* may be by reduced sodium absorption by the plant while increasing absorption of K, N, P and high chlorophyll content (Giri and Mukerji, 2004).

Table 4: Effect of *Fusarium* and *Glomus mossae* infection and soil salinity on percentage of infected root by *Glomus mossae*

Treatments\Soil EC (dS.m ⁻¹)	2.64 dS/m ⁻¹	14.5 dS/m ⁻¹	Mean treatments
Control	23.00	53.00	38.000
F	39.33	32.67	36.000
G	52.33	93.00	72.670
FG	45.00	60.67	52.830
Mean soil salinity	39.92	59.83	49.875

F = *Fusarium graminearum* G = *Glomus mossae* fungi

FG = *Fusarium graminearum* + *Glomus mossae* fungi

L.S.D treatment = 2.931 L.S.D soil salinity = 4.145

2.072 = interaction D.S.L

Table 5: Effect of *Fusarium* and *Glomus mossae* infection and soil salinity on percentage of plant germination

Treatments\Soil EC (dS.m ⁻¹)	2.64 dS/m	14.5 dS/m	Mean treatments
Control	72.3	62.3	67.30
F	28.0	24.3	26.20
G	75.7	71.0	73.30
FG	53.0	49.0	51.00
Mean soil salinity	56.2	52.7	54.45

F = *Fusarium graminearum* G = *Glomus mossae* fungi

FG = *Fusarium graminearum* + *Glomus mossae* fungi

L.S.D treatment = 6.44 L.S.D soil salinity = 3.55

L.S.D interactions = 9.10

Table 6: Effect of *Fusarium* and *Glomus mossae* infection and soil salinity on chlorophyll content (spad)

Treatments\Soil EC (dS.m ⁻¹)	2.64 dS/m	14.5 dS/m	Mean treatments
Control	18.78	16.33	17.55
F	14.33	13.67	14.00
G	32.33	24.00	28.17
FG	19.67	18.33	19.00
Mean soil salinity	21.11	18.25	19.68

F = *Fusarium graminearum* G = *Glomus mossae* fungi

FG = *Fusarium graminearum* + *Glomus mossae* fungi

L.S.D treatment = 4.70 L.S.D soli salinity = 3.324

L.S.D interactions = 6.649

Table 7: Effect of *Fusarium* and *Glomus mossae* infection and soil salinity on plant height (cm)

Treatments\Soil EC (dS.m ⁻¹)	2.64 dS/m	14.5dS/m	Mean treatments
Control	25.00	20.00	22.50
F	12.67	12.00	12.33
G	40.00	25.00	32.50
FG	18.33	16.00	17.17
Mean soil salinity	24.00	18.25	21.125

F = *Fusarium graminearum* G = *Glomus mossae* fungi
 FG = *Fusarium graminearum* + *Glomus mossae* fungi
 L.S.D treatment = 4.60 L.S.D soil salinity = 3.256
 L.S.D interactions = 6.51

Table 8: Effect of *Fusarium* and *Glomus mossae* infection and soil salinity on N%

Treatments\Soil EC (dS.m ⁻¹)	2.64 dS/m	14.5 dS/m	Mean treatments
Control	2.067	1.833	1.950
F	2.033	1.367	1.700
G	3.233	1.900	2.567
FG	2.433	1.700	2.067
Mean soil salinity	2.442	1.700	2.071

F = *Fusarium graminearum* G = *Glomus mossae* fungi
 FG = *Fusarium graminearum* + *Glomus mossae* fungi
 L.S.D treatment = 0.3250 L.S.D soil salinity = 0.2298
 L.S.D interactions = 0.4596

Table 9: Effect of *Fusarium* and *Glomus mossae* infection and soil salinity on P%

Treatments\Soil EC (dS.m ⁻¹)	2.64 dS/m	14.5 dS/m	Mean treatments
Control	0.230	0.150	0.190
F	0.153	0.046	0.100
G	0.326	0.216	0.272
FG	0.233	0.116	0.175
Mean soil salinity	0.236	0.136	0.184

F = *Fusarium graminearum* G = *Glomus mossae* fungi
 FG = *Fusarium graminearum* + *Glomus mossae* fungi
 L.S.D treatment = 0.035 L.S.D soil salinity = 0.025
 L.S.D interactions = 0.050

Table 10: Effect of *Fusarium* and *Glomus mossae* infection and soil salinity on K%

Treatments\ Soil EC (dS.m ⁻¹)	2.64 dS/m	14.5 dS/m	Mean treatments
Control	2.533	1.867	2.200
F	2.267	1.333	1.800
G	3.500	2.167	2.833
FG	2.500	2.100	2.300
Mean soil salinity	2.700	1.867	2.283

F = *Fusarium graminearum* G = *Glomus mossae* fungi
 FG = *Fusarium graminearum* + *Glomus mossae* fungi
 L.S.D treatment = 0.338 L.S.D soil salinity = 0.239
 L.S.D interactions = 0.479

Conclusion

The use of bio-fertilizers, especially the fungus of *Glomus mossae*, improves the nutritional status of plants, which is reflected positively on growth indicators such as germination percentage, plant content of chlorophyll, plant height and nutrient content (N, P and K). Furthermore the inoculum with *Glomus mossae* is help plant to resistant to salt stress. The soil of 14.5 dS.m⁻¹ has reduced the germination percentage and content of chlorophyll, plant height and nutrients. The triple

interference between the studied factors did not show any increase in growth and nutrients. Further studies are recommended to understand the mechanisms of environmental stress conditions in the plant and to conduct studies on resistance mechanisms induced to resist pathogens (pathogens) using biotic agents.

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Authors Contributions

Ali Jabbar Abdulsada: Participated in all experiment.

Sadeq J.H. Dwenee: Coordinated the data analysis.

Naeem Saeed Dheyab, Doaa Abass Hanon and Bara Hassan Hamza: Coordinated the mouse work.

Wafaa Hadi Hasoon: Designed the research plan.

Hadi Mahdi Aboud: Supervision this work.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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