

Effect of Toxicity *Fusarium Sporotrichioides* Isolated From Wheat Crops and Extract *Eruca Sativa* on Oxidative Stress Factors in Male Adult Rabbits

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Abstract: The Fungus strains of wheat crops from the region Hawija district in Kirkuk city were collected and then isolated on PDA agar plates .the morphology and structure of the resulting fungus, accompanied by the formation of conidia , are all important factors that we put in perspective. As data collection trial , we purchased 16 rabbits , which were fully males and divided into four groups: in the first group as the control group, the second group infected and the third group infected and treated by 50 mg of plant extract, and the last group. The last one will be treated by 100 mg of Eruca Sativa extract. Similarly, we have taken the level of Oxidative marker factors after the treatment process. The MDA values which are representative. Of the lipid peroxidation in the infected rabbits. Were highly significant when compared to the normal group ($p < 0.05$). In the meantime the GSH and the catalase which scavenged the reactive oxygen species becoming very low were lower than the MDA values ($p < 0.05$). It was seen as slightly different between 100 and 50 mg leaves extract of the connected rabbit. Were found in one or more of the level of MDA, GSH, and catalase, or LCAT, and compared to the healthy rabbits that significant quantities of such variation ($p < 0.05$).

Keywords: *Fusarium sporotrichioides*; Eruca Sativa; Oxidative stress; Male Rabbits.

INTRODUCTION

Hence, the elucidation of sporotrichioides toxicity tolerance has the key role in regard to due to their influence on occurrence of human intoxication. *Fusarium* kinds would be famous for their hyphae infecting the cereal grains like wheat, barley, or maize and second consequence would be the reduction of the produce quality. For example, this fungi is also responsible for the production of mycotoxin like deoxynivalenol and nivalenol and fusarenone and T-2 toxin and HT-2 toxin at levels which can be too high and thus pose risks of toxicity to livestock (Ferrigo, D. *et al.*, 2016).

The role of reactive oxidative species in rabbits by *Fusarium sporotrichioides* toxicosis is a fundamental aspect of the investigation of the mechanisms of toxicity at resistance. Oxidative stress stress come as a direct consequence of the disproportion between microscaled ROS production and the antioxidant system of the body, which cannot subdue or scavenge the harmful effect of these compounds. In case of *Fusarium sporotrichioides* disease, the stressful situation is caused if mycotoxins which are T-2 and HT-2 toxins are detected. These agents act as the strong provocateur of oxidatative stress which adversely affects genes and interferes with DNA , RNA, protein synthesis and mitochondria function of mammalian cells (Omotayo, O. P., & Babalola, O. O. 2023).

studies on rabbits infected with red spot infection caused by *Fusarium sporotrichioides* pinpointed that T-1 and HT-2 toxins continued to upregulate oxidative stress markers. The diminution of the ceramide synthases as a consequence of these

toxins with the subsequent release of ROS inside cells with the consequent oxidative cellular harm. Production of free radicals is seen in response to oxidative stress which in turn induces lipid peroxidation and cell injury .As a result, this adversely affects rabbits health (Iqbal, N. *et al.*, 2024).

Similarly, the next event as a result of *Fusarium* exposure is acceleration of apoptosis and prevent operation of cell growth as a result of the increase in the sphinganine and the sphingosine. These effects are seen precisely in newly developing fast proliferating tissue such as intestine cells lining. The accumulation of these sphingoid bases is noted in documentation of fumonisin toxicity such as FBI which is well known to exhibit various harmful effects on animal life (Abramson, D. *et al.*, 2004). Overall, one of the major factors that is involved in the development of the *Fusarium* toxins resistance mechanisms in the rabbit species that are infected by the *Fusarium sporotrichioides* is the induction of oxidative stress by such T-2 and HT-2 mycotoxins. Measuring to what extent these mycotoxins cause disbalance of the oxide species in a rabbit could provide well-grounded options for combating aversive impacts on the animal welfare or general health (Mesterházy, Á. *et al.*, 2012).

The pivotal purpose of this research in the influence of oxidative stress on *Fusarium sporotrichioides* on rabbits is to explore the lengthening effects and the role of oxidative stress in these forms developing resistance against this fungus. The study will be conducted by isolating

the fungus and utilizing control experiments that comprise adult male rabbits. The key step in this study will be monitoring oxidative stress indicators in sick rabbits compared to healthy once. This will be a precious piece of knowledge as it will reveal the way *Fusarium sporotrichioides* affects cytokine response in rabbits and how important the amelioration of oxidative stress is for patients.

MATERIALS AND METHODS

To collect and isolate fungi from plant materials

The plants which presented the indications of the disease were harvested from the Hawija district of Kirkuk, Iraq and the material and fungus were further isolated. The sample collected in the field from a particular geographical area is transported in the laboratory; where it is subjected to an array of scientific practices to finally get exact results. Now, they will travel in a pipe water, standing even in sterile distilled water. In each case the sterile distilled water is used several times in order to completely remove the sterilizer. Hot water is used to break down the cell walls, with a filter paper next employed for drying the plant parts. This procedure consist in pouring 100ml of fresh culture medium into 500ml falcon flask followed by the placed of that flask into petri dish with 9cm diameter. We have four plants sections that run through each dish line, three of which supplies each plant part. Control on the sporulation of fungal growth over the culture medium is ensured by maintaining the dishes at particular temperature in an incubator at 25 ± 2 °C for five to seven days. If we want to get the fungal colonies with a certain phenotype, sterile – pickling can definitely be a way. The wanted procedure was done not by the cork borers being treated with flame and alcohol and then thrust through the plant parts, but rather by killing the surrounding tissue. In the next step, a particular region of the culture medium was randomly marked on the surface of a loop and then it was copulated on the plate that had media of PDA types. The plates were put in a biological as in their previous condition so that the co-cultivation with safe bacterial isolates could be successfully achieved as well.

Isolation and Morphological identification of the fungi species

The characteristics of my fungi morphology after they had been isolated were documented through cutting out a 5mm disk using aseptic techniques after 5 days of pure culture. Then later the peeling of these sections were put into the center of PDA – coated petri dish and incubated. The fungi were

viewed by using an electronic microscope and the information on their morphological traits was captured. The isotomy, dimensions, edges ranslumination, and color of the single fungal colony cultures were traced. I had to observe the color of the mycelium and the spore bodies of the fungi through the microscopic which had sufficient magnification so as to make the evaluation thorough .The specific task became easier by checking systematically the data on factors such as system , color , measure, and other microscopic characteristic . The field Characteristic and morphological features of the isolated and identified fungi were done to the great degree of detail. Instruction of designing of the section using the sterile loop carries and inoculate the solid medium slants is to inoculate. After that, the slopes are transferred to a fridge with the temperature of 4°C and –kept in it until it's needed for the future researches.

Conidial Suspension Preparation

To quantify the conidia, use a light microscope with the following the formula: $\text{Conidia concn} = Z \times 4 / n(10^6)$ where Z is the conidia count, and n the number of small squares (Leyva-Mir, S. G. *et al.*, 2022).

Choice of Animal Model

And a local market at the town Kirkuk picked up 16 males adult Male Rabbits the average were weight of 2 kg within the age limits of one year. The same food is given to each animal had water is supplied; there is a protocol for a uniform observation so that there is little or no use of machinery in newborn care.

Experimental Setup

The experimental setup, in this case, employed was that of using male rabbits divided into 4 groups, each group having 4 rabbits. The control group, the first group, received a normal diet and was used as a control in the study for experiment. The infection group (F. conidia) was second, which was injected intraperitoneally . As an unfortunate discovery, these rabbits lost the battle against the virus.

Rabbits from second group a 50 mg dose one - received conidia in their interior, then plant extracts and at the end were sacrificed .Likewise, fungal spores (*Fusarium conidia*) were given to the treatment group rabbits at 100 mg dosage through intraperitoneal injection. They were treated repeatedly with root extract for three weeks followed by sacrificing.

Preparation of plant extracts

To prepare the plant extracts, leaves were procured from a local market. These leaves were thoroughly washed with water and left to dry naturally at room temperature. Once completely dried, the plants were stored at -20°C until they were ready to be used. For the extraction process, 50 g of the dried leaves were combined with 500 ml of distilled water and boiled for duration of 30 minutes. Following this, the extract was filtered and subjected to lyophilization. Finally, the resulting extract was stored at -20°C for future use (Yarat, A. *et al.*, 2013).

Collection Sample

To collect a sample, the subject is anesthetized and then a hearts puncture is performed to obtain a blood in a test tube. Clotting the blood takes place after which the collection tubes are centrifuged for 10 minutes at 5000rpm, thus doing away with the serum. On the other hand, following the mechanical separation of the lungs, the process of homogenization with NaCl continues quickly. The final supernatant and serum will be subjected to critical analysis and differentiation, which will be conducted in a special icebox until ready for use (Razak, N. J., & Abass, M. H. 2023).

Oxidative stress factors

MDA level, an indicator of oxidative stress, was measured using TBA (thiobarbituric acid) method (Razak, N. J., & Abass, M. H. 2023). And glutathione (GSH) via (DTNB) as well as CAT, as suggested method (Wang, M. M. *et al.*, 2019).

Statistical Analysis

To compare the variation of the two groups this statistical analysis utilized analysis of variance (ANOVA) plots.

RESULTS AND DISCUSSION

Fungal Culture and Microscopic Examination

Strains isolated and pure cultured in PDA agar medium may vary in color from white to brown with irregular cottony edges (Figure 1). After 5 days, the plates are completely darkened which now look cream colored from the back of the plate. Three types of spores were observed: microconidia and macroconidia, macroconidia that have $16.86 - 25.36 \times 4.34 - 4.40 \mu\text{m}$ and macroconidia that have $7.58 - 12.72 \times 2.47 - 4.22 \mu\text{m}$ (Fig 1). For instance, our outcomes align with the studies conducted by (Faraj, M. K. 1990; Comlekcioglu, N. *et al.*, 2021).



Figure 1. *Fusarium sporotrichioides* macroconidia and the larger macroconidia

Oxidative stress factors

The injection of *Fusarium sporotrichioides* in rabbits resulted in a significant increase in MDA values (serum: In subjects with hyperthyroidism the medians of lang and serum accessible, respectively, were 3.01 ± 0.93 and 2.79 ± 0.65 and

those of normal subjects were 1.88 ± 0.25 and 1.5 ± 0.99 . Nevertheless, MDA values had not shown any considerable differences towards that rabbits group which received *Eruca Sativa* extract (50 mg and 100 mg) by that one of the control specimen. On the other hand, the injection of *Fusarium*

sporotrichioides in rabbits led to significant increase in GSH value (serum: Reading from this , the carbon dioxide levels of the of the serum upper and lower data in contrast to normal rabbits 0.89 ± 0.22 and 0.81 ± 0.42 were equal to 0.339 ± 0.0053 and lungs (for the sewer : 0.222 ± 0.686) . Rabbits were divided into 3 study groups namely, the once that were not injected with *Eruca Sativa* leaf extract (50 mg and 100 mg), and the once that were not injected (normal rabbits) .What could be added is that the levels of catalase were

significantly higher in rabbits injected with *Fusarium sporotrichioides* . (Serum: Across tissue catalase activity was measured (kidney: 0.77 ± 0.87 and lung: 0.7 ± 0.072) showing a depression in the catalase activity which means damage to the cardiomyocytes occurred. The catalase activities in all the rabbits treated with *Eruca Sativa* extract (50 mg and 100 mg) group showed no significant difference ($p < 0.05$) contra healthy rabbits in both Fdp.2 and 3.

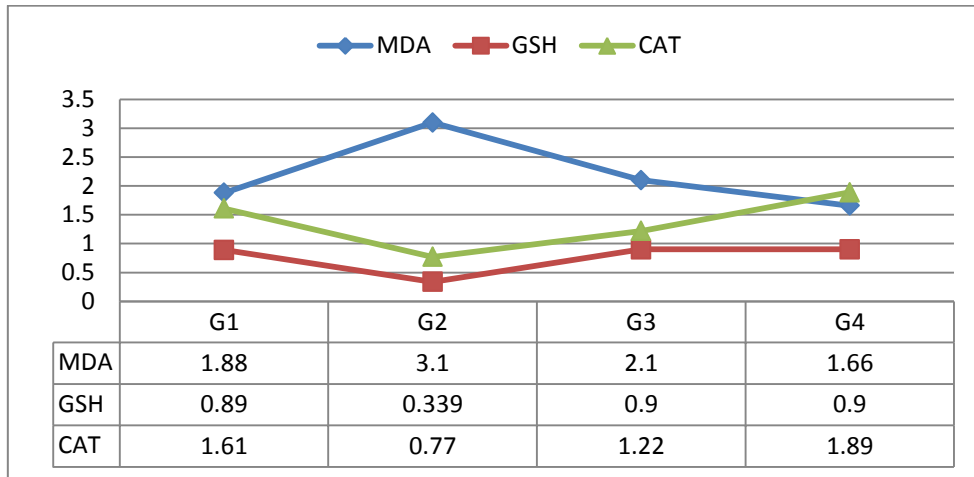


Figure 2: The level Oxidative stress factors in the Rabbits blood serum

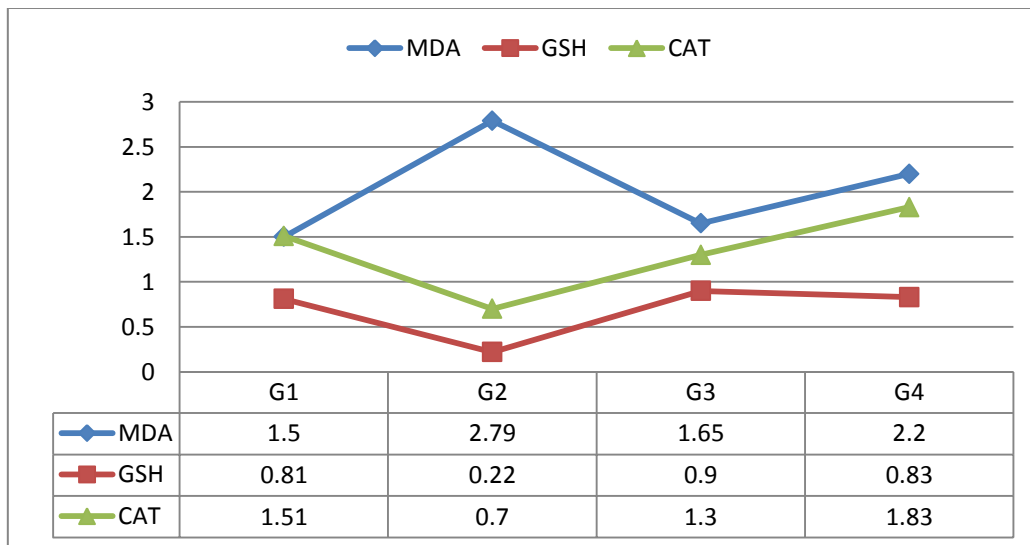


Figure 3: The level Oxidative stress factors in the Rabbits lung

Fusarium genus, which is widely inhabited globally, is implicated in mediating infection in numerous host plant species. These hyphae fungi are wide and thin and usually developed in sand, soil or air which can be found in all tropical and temperate parts of the globe. Although main focus lies to plant, *Fusarium* can also tigger serious organisms in the animals. Lung rejection patients are primary gate ways for fungal infection (*Fusarium* conidia) via the respiratory route, which

causes skin and mucosal skin lesion. Although *Fusarium* is seen everywhere, conidia sampling from the outdoor air is often positive of the presence of *Fusarium* . Also, some of the studies surprisingly had *Fusarium* more frequently than *Aspergillus* in these slightly atypical samples. Firstly, *Fusarium* has been cultured from indoor water storage devices and outdoor water places. Outdoor sports like the swim or merely water can spread the *Fusarium* conidia into the air.

Consequently, the airborne particles can find an easy entry and transmission to the respiratory tract, (Nucci, M., & Anaissie, E. 2022). While fumarium is the most common cause of infection in lung transplant patients, most frequently it strikes the lungs (Nucci, M., & Anaissie, E. 2007). Moreover, there is evidence to suggest that pulmonary features such as immunity become associated with higher case fatality rate even when taking into consideration the immune status. Fusarium pneumonia could be identified as nodular or activated lesion or alveolar or activated lesion because of their characteristic interstitial penetration. (Mac Tavish *et al.*, 2022) Symptoms frequently experienced by the patients are shortness of breath, dry cough and sharp chest discomfort. The appearance of mycotoxins can lead to gallantry reaction which in turn causes OS and free radical city. UB contrast it is stated that (Adhikari, M. *et al.*, 2017). The malfunctioning of the antioxidant system leads to an increase in the level of free radicals which subsequently leads to the damage of DNA, proteins and lipids (Wang, X. *et al.*, 2016). It is usually the case that a balance exists between the reactive species and the antioxidants. Over and above this, external factors may result in the generation of excess oxidative stress and wide production of free radicals (Assi, M. 2017), which in turn leads to an imbalance in mechanisms control the cellular homeostasis, oxidative stress is another instance of such damage. It occurs when the antioxidant system is destroyed and there ia a large amount of free radicals formed (Halliwell, B., & Whiteman, M. 2004). Many research papers have been written to investigate how antioxidant enzymes in poultry and pigs are impaired following exposure to mycotoxins of different types. The outcome of one of the (study30) consisted of signs of oxidative stress in the spleen, the reduced activity of antioxidant enzymes such as GSH-PX, GR and CAT, together with malondialdehyde (MDA) and GSH (Rajput, S. A. *et al.*, 2017). MDA plays the role of the critical indicator for the assessment of state of oxidative stress, as well as the oxidative damage of tissues by reactive oxygen species through lipid peroxidation (ROS) (Namik, M. F. *et al.*, 2018). The diminishing levels of glutathione can be caused by many factors that result in more fast break down and absorption. Glutathione is a very significant non-enzymatic antioxidant which rapidly deals with free radicals and their products, such radicals and products are rendered weak when Glutathione in an active state gets converted to an inactive state, Glutathione disulfide. It should

be noted that sulfur within GSH acts as a powerful reduction, inciting readily release of a hydrogen atom. This is possible by the relatively weak bond between sulfur and hydrogen (S-H) instead of the relatively strong bond between carbon and hydrogen that is shown as free radicals (C-H). Therefore, this mechanism prevents the cell membranes from being marred by naturally occurring free radicals. A crucial factor that can determine GSH depletion is related to a deficit of the constituents, and particularly coenzyme NADPH, which is responsible for its production. The pent phosphate sugar shares the function of the catalyst with the GS red enzyme. Thus, it takes part in the restoration of glutathione from its inactive state to its active form (Ali, J. M. *et al.*, 2017).

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