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EFFECT OF RICE FALSE SMUT DISEASE ON AMINO AND FATTY ACIDS CONTENTS OF RICE GRAINS

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ABSTRACT

False smut disease of rice, incited by *Ustilaginoidea virens* (Cooke) Takah. is regarded as new and important disease in the recent years in Egypt. Investigations were carried finely to find out its impact on grains components, as well as ustiloxin production, by the fungus. Reduction of both tested amino and fatty acids contents has been observed in the infected grains. Certain fatty acides i.e., myristic, stearic, arachidic, behenic, and linolenic were decreased by 9.06%, while palmitic, palmitoleic and oleic were increased by 8.21%. The most dramatic observation was the increase of four amino acids namely, glycine, alanine, valine and isoleucine contents by 15.38% in molded smutted grains compared with healthy ones, which showed its corelation with fungal ustiloxins formation.
Keywords: Rice, False smut, *Ustilaginoidea virens*, Amino and Fatty acids, Ustiloxin.

INTRODUCTION

Rice (*Oryzae sativa* L.) is an important cereal food in Egypt. The cultivated area was 1,519,665 feddans in 2003 produced 5,800,000 m.tons (FAO, 2033). Rice grains contains many carbohyrates, chiefly starch (up to 75%) but rather little protein (about 7.5%) as much as2.2% fat, 0.8%cellulose and 5.9% ash (El-Khishen *et al.*, 1992).Seeds play a vital role for healthy production of the crops. Seeds carry pathogenic organisms that cause heavy yield losses (IRRI, 1983, Ou, 1985 and Webster and Gunnell, 1992). False smut disease of rice incited by *Ustilaginoidea virens* is considered a newly destructive disease in the Northern Governorates of Egypt. This disease caused marked reduction in the total yield (Kamara and Saleh, 2002). The false smut balls are growing parasitically on panicles of rice plants .It has been reported that such panicles suffered from this disease have poor crop and rather feeding on such deteriorated seeds cause poisoning to the domestic animals, because of their toxic effect (Suwa 1915; Hu-Dj, 1986 and Nakamura *et al.*, 1993).

The present work was concerned with the impact of false smut disease of rice, on the quality and quantity of amino and fatty acids of certain local rice cultivars .Furthermore ustiloxin product formed by *Ustilaginoidea virens* has been extracted and determined from false smutted rice grains. Biological activities of ustiloxin derivatives that it induces abnormal swelling of rice seedling roots have been studied.

MATERIALS AND METHODS

1- Preparation of rice grains for analysis

Healthy and naturally infected rice grains of four commercial cultivars named Giza-171, Giza-181, Sakha-101 and Sakha-102 were collected from

rice fields located at Kafr-El-Sheikh, El-Sharkia, El-Gharbia and Dakahlia Governorates. Whole grains of each sample were grounded in the laboratory mill, 3100 (Parker Commercial Co., LTD.Tokyo, Japan).

1.1.1 Determination of Amino Acids

Qualitative and quantitative amino acids content were determined to each 50 mg grounded grain sample by employing the Beckman Amino Acid Analyzer (Model 119 CL). NHCL containing mercaptoethanol was used for hydrolysis according to the method described by Duranti & Cerletti, (1979). Each amino acid content was calculated by measuring the obtained area under each peak in the flow shart represented every amino acid and according to the following equation:

$$\text{Amino acid (g/loog protein)} = \frac{\text{Sample area x std. Conc.x dilution x100 x100}}{\text{Standard area x100x weight of sample x100x (\%protein on dry basis)}}$$

1.1.2 Determination of Fatty Acids

Fatty acids content of each 10 grams rice grain sample were extracted by chloroform-methanol according to the method suggested by Floch *et al.*, (1957).Methyl esters of fatty acids were prepared according to the procedures mentioned by Radwan, (1978). Final analysis of fatty acids was carried out by using Shimadzu – Gas –liquid chromatography (GLC; type GG 4 –CM, PFE, Kyoto, Japan), under condition of 0.5 mm wide bore chrome – packed glass column (Sp 2340 Silica), Flame Ionization Detector 270 °C, initial oven temperature 150°C rising to 240°C at 5°C/min. and the carrier gas was nitrogen at a flow – rate of 25 ml / min. A mixture of standard fatty acids was injected in the GLC prior to the running of tested samples to be used for comparison.

1.1.3 Extraction and Determination of Ustiloxin

False smut balls were isolated from the blackened rice smut naturally contaminated with *U. virens* and extracted with water at room temperature for 1 hour. Concentrated crude extract was future purified by a column chromatography with 20 % aqueous methanol .The yield of the pale yellowish-brown powder (U-3) '330 mg from 50g of the false smut balls' was spread on a silica gel TLC with n-button : methanol :water at 3:1:1 to separate Ustiloxin A.

1-4 Rice Seedling Test

Effect of ustiloxin on rice seedlings cv. Giza-171 was tried. Rice seeds were germinated in distilled water at 27 °C, therafter, incubated in a water extract of the smut sample solutions with a given concentration 20, 40, 60, 80, and 100% of ustiloxin respectively. The shape of germs and roots of treated seedlings were observed gradually. Toxicity developed symptoms were recorded started from the 5th day of seedling up to the 20th day of the experiment. Seeds severed as check, germinated only in distilled water without any ustiloxin solution (Nakamura *et al.*, 1993).

RESULTS AND DISCSSION

Fatty acids are the integral constituents of every fat or oil. Complexity of their glycosides depends upon the number and amount of various fatty acids.

Fig. (1) Indicate that false smut disease of rice, reduced the amount of certain fatty acids namely: myristic, stearic, linoleic,arachidic and behenic from 47.37% in healthy grains, to 43.08% in infected ones.

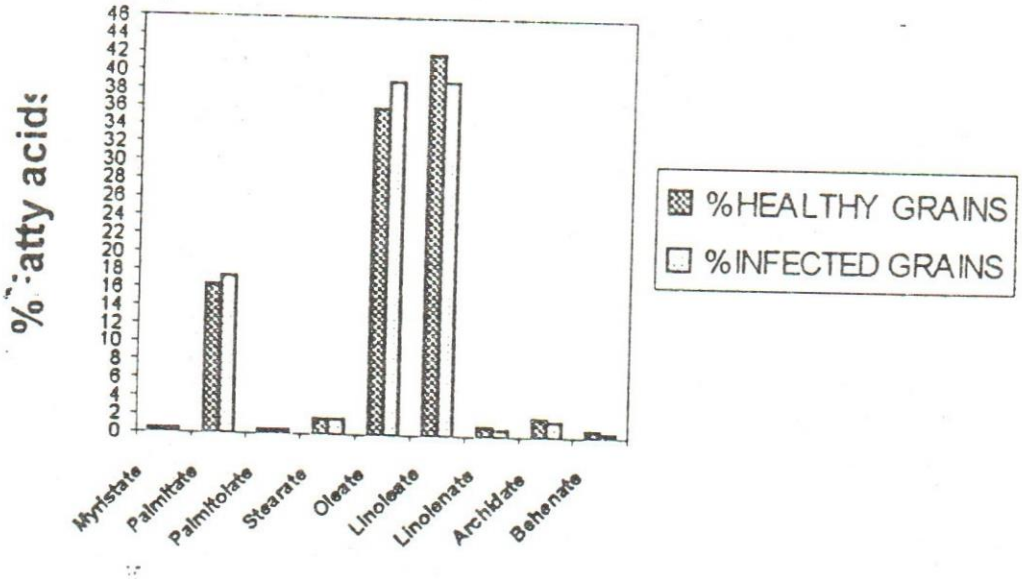

Fig. (1) Effect of *Ustilaginoidea virens* on fatty acids contents in naturally- infected rice grains.

Both types of saturated and unsaturated fatty acids were present in infected and healthy rice grains, with marked qualitative and quantitative variations among different cultivar. The most dominate fatty acids in healthy rice grains were the saturated Palmitic (C 16:0) and the two unsaturated oleic (C 18:1) and linoleic (C 18:2) which were detected a clear disturbed levels in the other infected grain samples. Even the non-dominant acids showed such decrease in their quantities as affected by infection. Results

also found in both Giza -171 and Giza- 181 infected grains showed more decrease in its fatty acids than those of Sakha -101 and Sakha- 102 infected grains according to their susceptibility to false smut disease (Kamara& Saleh, 2002). Therefore, as the fungal infection could affect the balance or the sequence of fatty acids within the grain contents, it might change the edibility of such grains. This result has been supported by Sakaguchi *et al.*,1984 and Abou-Elsoud *et al.*,1999 who mentioned that such decrease of fatty acids might be due to one or more of pathways which have been introduced by the fungus .It might explain the dehydrogenases activity of *Ustilaginoidea virens* or its necessary for mycotoxin formation by the fungus .Also the decrease in certain amino acids content in the infected rice grains might be due to its consumtion by the fungus to form ustiloxin . Ustiloxin is considered as an antimitotic tetrapeptides containing 13–membered rings, including an ether linkage. Their structures are closely related to that of phomopsin , a mycotoxic hexapeptide to cause lupinosis . Its NMR spectra indicated that it is a tetrapeptide consist of 3-(3, 4-oxyphenyl) - 3hydroxy - N – methyl – alanine, a valine, a 3 – oxyisoleucine and a glycine moieties.

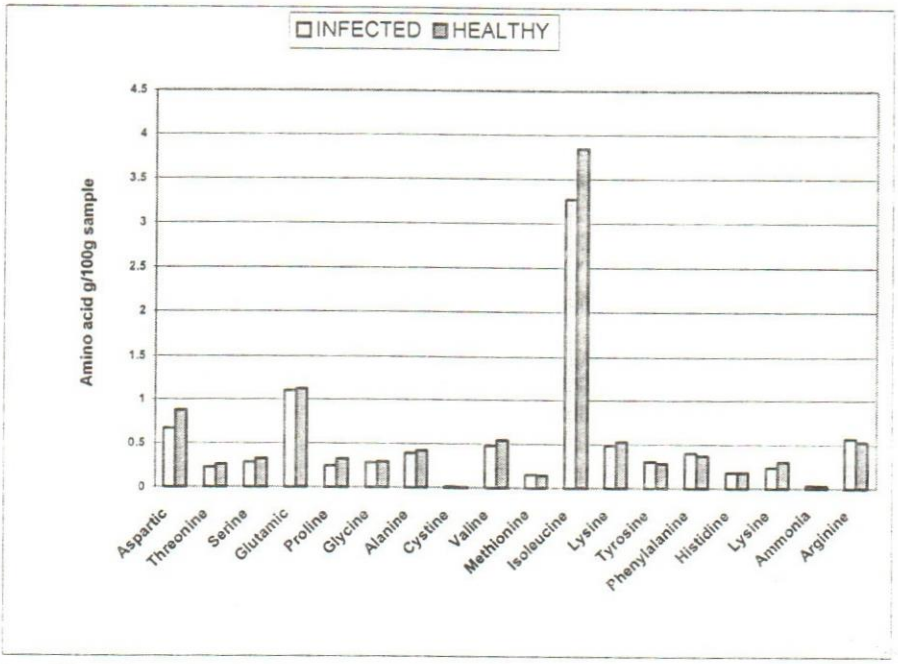

Fig. (2) Effect of *Ustilaginoidea virens* on amino acids contents in naturally infected rice grains.

Data in Fig (2) showed an observed increase of glycine, alanine, valine and isoleucin. The percentage of these four amino acids was increased from (4.42) in healthy grains to (5.1) in the infected .The percentage of increase was 15.38. This indicates that once the fungus attacks the grains, it begins to utilize its contents to form the specific toxin. The decrease in the other amino

acids levels could explain the ability of the fungus to consume them as they enter in the pathway of ustiloxin formation. Besides, such amino acids play an important role as nitrogen source for fungal growth and probably utilized for stimulating ustiloxin production.These findings agree with Ott *et al.*, (1962) and Mona Nour Eldine, (1995) who explained that the fungus use certain amino acids drived from its host as they enters in the pathway of its toxin formation. Mean- while, toxin production reversed the action of ustiloxin drugs, which could strongly inhibits microtubule assembly and show broad biological activity against plant and animal cells. This explains the result obtained from rice seedlings treatment with ustiloxin water extracted solution inducing the abnormal swellings (Hu-Dj, 1986).

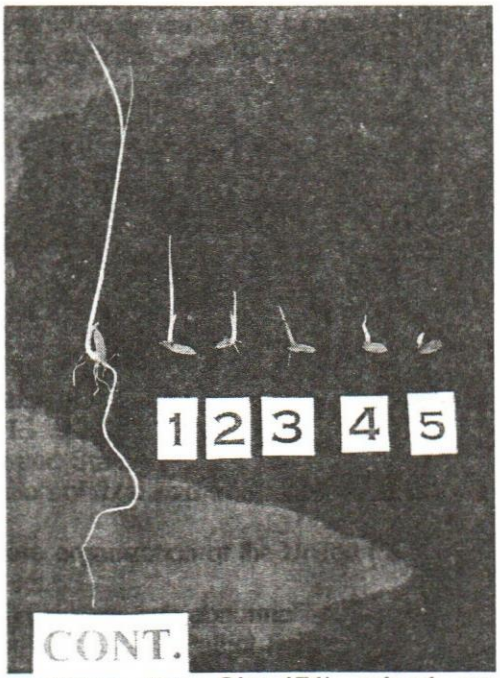

Fig (3) Rice seedlings (cv. Giza-171) raised on toxin medium supplmented with five concentrations 20,40,60,80 and100 %(1,2,3,4and 5 respectively) of ustiloxin, after 7 days of sowing, at room temperature (18 - 22°C).

Data Table 1 and Fig.3 showed that rice seedlings raised on toxic medium supplemented with different concentrations of ustiloxin i.e., 20, 40, 60, 80 and 100%. All the tested concentrations of ustiloxin induced different toxic effects on rice seedlings. The toxic effect of ustiloxin on rice seedlings varied according to the concentration used. At the lowest concentration (20%), symptoms appeared as slight chlorosis. At (40%), seedlings became chlorotic and stunted. While at the higher concentrations i, e., 60, 80, and 100 % seedlings wilted and eventually died Fig (3).

Table (1): Effect of five tested concentrations of ustiloxin on symptoms developed on rice seedlings raised on toxin medium at room temperature (18 - 22) ˚C.

TIME (DAYS)	CONTROL	USTILOXIN CONCENTRATIONS (%)				
		20	40	60	80	100
5	Radical emergence Normal seedlings 1.5 cm long	Radical emergence Normal seedlings 1.5 cm long	Radical emergence Normal seedlings 1.5 cm long	Radical emergence Normal seedlings 1.5 cm long	Radical emergence Normal seedlings 1.5 cm long	Radical emergence Normal seedlings 1 cm long
10	Normal seedlings 4 cm long	Normal seedlings 3 cm long	Normal seedlings 2.5 cm long	Slight radical browing,leaf twisting seedlings became chlorotic 2 cm long	Radical browning became seedlings chlorotic 2 cm long	Radical and seedlings browning 1.5 cm long
15	Normal seedlings 6 cm long	Normal seedlings 4 cm long	Normal seedlings but stunted,3 cm long	Radical browning shriveled browning leaves,seedlings became stunted 3 cm long	Dead seedlings 2 cm long	Dead seedlings 1.5 cm long
20	Normal seedlings 10cm long	Normal seedlings with slight chlorotic 6 cm long	Slight chlorotic 4 cm long	Dead seedlings 3 cm long	Dead seedlings 2.5 cm long	Dead seedlings 1.5 cm long

This also implies the carcinogenic effect of ustiloxin koiso *et al.*, (1992). In the beginning of this century, Suwa, (1915) found a toxic effect of a water extracted from smut balls of *Ustilaginoidea* on rabbits. Marasas, (1974); and Gardiner, (1967), who observed the toxicity effects of smut rice balls in sheep and cattle, have reported similar effects. Finally, the conclusion can be drawn from the results of false smut balls feeding and the repeated administration of ustiloxins, which induces fatty changes, and local necrosis of liver in experimental animals (stein and stein, 1973). These findings led us to study their essential structural factors to interact with tubulin, which can lead to a variety of derivatives for biochemical purposes. The recent discovery of ustiloxin, a phytotoxin and mycotoxin, produced by *U. virens* on diseased tissues suggests that the fungus may be of concern as a contaminant on rice products consumed by livestock and humans (Koiso *et al.*, 1992, 1994).This increases the need to monitor the incidence of rice false smut disease.

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تأثير الإصابة بمرض التفحم الكاذب على مكونات الأحماض الأمينية والدهنية لحبوب الأرز

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أجريت الدراسة على مرض التفحم الكاذب الذي يصيب نباتات الأرز والمتسبب عن الفطر *Ustilaginoidea virens* والذي سجل في السنوات الأخيرة في مصر . وقد تم فحص للعينات المصابة بالمرض لمعرفة مدى تأثر محتوي حبوب الأرز من الأحماض الأمينية و الدهنية علاوة على تقدير مستويات السم الفطري المنتج بواسطة هذا الفطر في تلك الحبوب المصابة بالتفحم الكاذب .

أثبتت الدراسة أن هناك نقص ملحوظ لكميات الأحماض الأمينية والدهنية لحبوب الأرز المصابة بهذا المرض ، حيث وصل النقص لخمسة أحماض دهنية هي ميرستيك و ستياريك و اراكيدك و بهينيك و لينولينيك آلي ٠٦و٩% بينما زاد محتوى أحماض دهنية أخرى كالبالمتيك و البالميتوليك و الأوليك إلى ٢١و٨ % .

وأوضحت النتائج المتحصل عليها زيادة قدرها ٣٨و١٥% لأربعة أحماض أمينية هي الجليسين و الالنين و الفالين و الايزوليوسين في الحبوب المصابة بمرض التفحم مقارنة بالحبوب السليمة و يرجع ذلك آلي تخليق السم الفطري يوستيلوكسين الذي ينتجه الفطر المسبب لمرض التفحم الكاذب لحبوب الأرز .