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# RESEARCH ON EVOLUTION APHLATOXINS DEPENDING ON THE CONDITIONS OF TEMPERATURE

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***Abstract***

*After the integration in European Union in 2007, Romania will be standing at the European standards in what concerns the quality of the nutrition whit a great impact upon animals healthand as a result of people,and the usement of the animal powder will be ban in feeding the animals and olso there will be other ways to cover the necessity of fodder proteins.*

*This microorganisms have the capacity to produce in general an enzymatic hydrolytic complex that offers more advantages esides animal and vegetables enzyme,became the possibilities to develop and produce enzyme on cheap fields,and special outfit. Stalks of Aspergillus Flavus are producing alfatoxine. This have been cultivated for days on Wort field, at a temperature of 2, 7, 13, 18, 24, 35, 41, 49 and 520 degrees. Maximal production of alfatoxine had benn successful at 29 and 350 degees. The rote of alfatoxine B1 production besides G1 alfatoxine variated depending on temperature. Alfatoxine production haven to been in the same way as Aspergillus Flavus. One of the Aspergillus Flavus stalks didn t prodece alfatoxine at 410 degees, at the growing leavle almost maxime.After days alfatoxine haven t any results either lower temperature 180 degees or even* at high temperature 350 degees.

**Key words:** Mycotoxins, feed, metabolites, strains, isolation.

## INTRODUCTION

Aphlatoxin producing ability medium was tested distinguish aflatoxin – positive from aflatoxin – negative strains of Aspergillus Flavus in naturally occurring populations from corn at harvest. All of the aflatoxin – positive strains and some of the aflatoxin – negative strains produced aflatoxins when cultured on cracked corn. Although thr data indicate that aflatoxin producing ability medium is not entirely reliable in distinguishing potential aflatoxin producing strains of Aspergillus Flavus from nontoxigenic strains, it is significant that the medium did not yield false positive.

The aflatoxins are toxic metabolites of the fungi Aspergillus flavus and Aspergillus parasiticus. These fungi invade the forages and under suitable conditions produce aflatoxins (B1, B2, G1, G2). The consumption of aflatoxins by the animals results in various pathological conditions (aflatoxicoses), with symptoms that vary depending on the species of animal, the age, the degree of forage invasion by the fungi and the quantity of the consumed feed. Nowadays, researchers pay more attention to the aflatoxins, because the latter do occur in many parts of the world. The

regions more affected by aflatoxins are those with tropic or subtropic climate, since the levels of humidity and temperature play an important role in fungi's growth. The consumption of aflatoxin B1 results in the excretion of aflatoxin M1 into the milk. M1 is one of B1's metabolites and in some cases it is detected in concentrations higher than the maximum desirable limits fixed by each state, making the milk inappropriate for consumption. Furthermore, there are many reports about the effects of aflatoxins on the reproductive system. Sexual maturation, growth and maturation of the follicles, levels of hormones, gestation, growth of foetus are some of the parameters that are possibly influenced by aflatoxins. Regarding the genital system of male, most reports are related with the likely effect of aflatoxins on the size and weight of the genital organs, on spermatogenesis, on the number and morphology of spermatozoa, as well as on the levels of hormones.

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CHC13 extraction colour seems to be in correlation with alfatoxine concentration. Aspergillus Flavus stalks cultivated at 2,7 and 410 degees, 12 weeks time, didn t produce alfatoxine. At 130 degees both stalks produced alfatoxine after 3 weeks, and one of the stalk produced higher qualities in time. The second stalk produce bigger qualities in 6 week time, but in 12 weeks there were lower quality of alfatoxine than in 6 weeks.

Although there many articles published regarding alfatoxine, since Sargent and his colaboraters demonstrated the relation between Aspergillus fungus Link and Fries, there are a few number of investors in study the

elements which influence the producing the alfatoxine Aspergillus Flavus. Agnihotri affirmed that the temperature is the main element in physic ambient medium that influence methabolic activities of fungs. For researching this last affirmation, the study have been enterprise taking in consideration temperatue on growing and producing alfatoxine by Aspergillus Flavus. An preliminary report in regarding thistask have already been made.

**MATERIAL AND METHODS**

Two strains of Aspergillum flavus, production of aflatoxins have been cultivated for days on average Worth, a temperature 2, 7, 13, 18, 24, 35, 41, 46 and 520C. Maximum production of aflatoxins was held at 240C. Two strains of A. flavus were different aspects of the production of aflatoxins have been selected for this study. The two strains (M 93 - isolated from wheat and M 122 - isolated from raw peanuts) caused aflatoxin B and G in different quantities.

Previously, we did experiences of passages from each of the two strains of Aspergillum flavus, on average allocated Worth agar culture in Petri plates. After the development of the crop (the sparkler) were maintained at a temperature of 5oC. All spores that I used for inoculation were obtained from the two Petri plates.

In cases of isolation, identification and development, experiences in the production of aflatoxins, strains of A. flavus were grown on solid culture medium, namely Worth agar, because extracting the chemical from the mycelium and increase the surface agar is simpler and faster than extracting from a liquid medium. In its analysis of growth utilized medium culture, namely liquid broth Worth because of the weight of consecutive increases and the mycelium is simply on a liquid medium. We take this situation comparing the production of aflatoxins as the average liquid and solid on average only in terms of quality.

For the production of aflatoxins have inoculated increase both in bottles of 500 ml Erlenmeyer which contains 80 ml Worth agar (solid medium), as well as in bottles of 300 ml Erlenmeyer which contains 50 ml broth Worth (liquid medium). After inoculation all containers with media culture sown were incubated at a temperature of 22 ° C for 18-20 hours to ensure germination increased. At the end of the period of germination we executed a test to identify and see if the method of inoculation was done properly in order to increase the germination producers of aflatoxin. After germination period we inoculated in 10 containers of media culture agar Worth, increase from the two strains of A. flavus that we incubated at different temperatures, respectively: 2, 7, 13, 18, 24, 35, 41 , 46 and 520C.

After Incubation period we have Quantitative by mixing content replicated three containers, washing the nutrient solution (culture medium) and weighing mass mycelium combined dry.

Incubated in Aflatoxins containers representing each individual in temperatures that were incubated, were treated with CHCl3. Extract obtained I appreciated in terms of color, after which we concentrated. Aflatoxins were determined by weight, of each extract by submitting evidence of the glass plates with silicate gel. We have collected evidence and witnesses of aflatoxin B1 authentic and aflatoxin G1 were submitted evidence. Sheets were treated with solvent of methanol by thin-layer chromatography, the method that allows detection of any amounts of aflatoxin over 0, 0375 aflatoxin B1 and 0, 0225 aflatoxin G1 (microgram / vial). Extracts with less conclusive results have been dried and diluted again with 0.5 ml soil. of CHCl3.

In order to confirm this aflatoxins, extracts and standards we have made in the form of spots and we come into a system solvent benzene-alcohol-water (16/38/19 v / v). Values aflatoxins of RF were different in the two systems solvent for each system, but RF was identical to that of the standard respectively. This aflatoxins in both strains of A. flavus we have identified in the two containers and checked previously selected by biological methods.

All experiments that we executed media culture were incubated at temperatures between 2, 7, 13, 18, 24, 35, 41, 46 and 520C for 3, 6 and 12 weeks. At some temperatures aflatoxins were not directly produced by two strains of A. flavus. Each test has been replicated in four, after which we witness inoculated and containers that were incubated at 220C. In all cases stems from the witness containers were developed, they sporulating and produced large amounts of aflatoxin.

**RESULTS AND DISCUSSION**

Maximum total production of aflatoxin I got it from crops grown at 240C. At this temperature increases have led to the maximum of each type of aflatoxin B and G by both strains of A. flavus. We found that different ratio between the amount of aflatoxin B1 and aflatoxin G1 when the two strains of A. flavus were grown at different temperatures. Maximum increases were not correlate with peak production of aflatoxins but certain temperatures can be increased appreciably, but without production of aflatoxins. Production of a aflatoxins was null in both strains at temperatures of 2, 7, 13, 41, 46 and 520C. The intensity of the color yellow extracts CHCl3 was in good correlation with the concentration aflatoxins.

The results of long-term experiments show that aflatoxins not occur at 2, 7 and 410C at 3, 6 and 12 weeks. At 130C, but were obtained the following results:

• A. flavus M95 has produced more aflatoxin B1 and G1 route, with peak production of aflatoxins total to 12 weeks.

• A. flavus M122 produced high amounts of aflatoxin B1 and G1 during the first 6 weeks of Incubation, but were lower quantities and after 12 weeks.

Increase maximum total strain M95 and M122 I recorded it in a range of up to 15 days following the values of temperature, from 290C to 350C. Strains M122 has an area of higher temperatures in terms of production, compared with strains M95.

After these experiments, the results on the production of aflatoxins by the two strains of A. flavus, found that aflatoxins not occur at temperatures of 2, 7, 41, 46 and 520C. If the production in food, fodder and other consumer goods is similar to them on our culture should be possible to avoid contamination by aflatoxin these substrate by keeping them at appropriate temperatures. Following the study we have identified and field temperature that should be avoided for the preservation of food, etc. fodder. At 410C found an increased A. flavus without production of aflatoxins. Aflatoxin that does not occur even if the 410C A. flavus presents an intense increase in the temperature does not necessarily an indication of the production of aflatoxin. Differences between the optimal temperatures for growth and development of aflatoxins, compared with the optimal temperatures for the production of the two strains may indicate a difference in terms of inactivation of enzymes that help the growth and development of aflatoxins due to temperature. Modifications in the production of aflatoxin B1 and G1 at certain temperatures, and they indicate the influence of temperature on Biosynthesis of the two aflatoxins.

The relationship apparent production of aflatoxin that the color of $CHCl_3$ extract, may serve in some cases to estimate the quantity of fast aflatoxin produced by fungi culture. Optimum temperature for the production of aflatoxins, and production of aflatoxin B1 maximum is 24 0C, and at 18 0C is increasing the maximum. 36 0C has been no production of aflatoxin B1. Aflatoxins G1 not occur at 18 0C, and between 18 0C and 24 0C stop production for this type of aflatoxins G1.

I've found a maximum growth of the two strains in the temperature between 29 and 35 0C. Optimum temperature for the production of aflatoxin B1 is 35 0C, and for aflatoxins G1este 18 0C. Most differences between the results of which appear due to the strain variability. After experiments we've done, evidence that did not put in evidence the production of aflatoxins after an interval of 5 days of Incubation, which were extended to 12 weeks, and

production of aflatoxins was held at a temperature of in advance were negative. Thus, I found that aflatoxins can be metabolized by some strains after several weeks.

**CONCLUSIONS**

Aflatoxins are the most dangerous biological contaminants. Ontogeny action is synergy with other oncoinductori.

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Regarding strain of Aspergillus flavus, after isolation in conditions of temperature and humidity can see that this is a species with only multiplication neuter, all populations are monoclonal

The consumption of food contaminated with aflatoxin on human health are estimated based on the indirect effect observed in animals. In the short term ingestion of a quantity of brown aflatoxins lead to acute poisoning. Target organ suffering serious injuries in such cases is the liver. Aflatoxicoza failure can occur through bleeding, acute liver failure and even death. Lethal dose varies from animal to animal and depends on many factors such as the amount of aflatoxin ingested, the age of the animal, health and nutrition status.

Mycotoxins naturally present in various food products constitutes a serious problem of food safety, especially in certain regions of the world where weather conditions or standards in agriculture are poor. There is convincing evidence that shows an association between exposure to aflatoxin and primary liver cancer. Mycotoxins produced serious damage and Animal Husbandry.

Mycotoxins are chemicals produced by certain species of molds (Aspergillus, Fusarium, Penicillium, Trichothecium etc.).. They may be emphasized and sporii or in the substrate on which increase fungii. There are a variety of mycotoxins very but not all are important in terms of food safety. Mycotoxins most important risk seminificative for food safety are aflatoxins, fumonisinele, ocratoxinele, patulina, trichotecina and ergotoxina.

Organization for Food and Agriculture Organization (FAO) estimates that worldwide up to 25% of food crops are significantly

contaminated with mycotoxins. The most important mycotoxins which presents significant risks to human health are synthesized by molds that grow on grain, particularly maize and wheat, barley, oats and oret. Rye may be parazitul the fungus Claviceps purpurea called popular horn rye that produces toxic alkaloids. Another category of crops that present a risk of contamination with mycotoxins are the nuts from the ground (peanuts), nuts, oilseeds and animal feed. Mycotoxins contaminate agricultural products before or after harvest. In general humidity and high temperatures favor the growth of fungi and production of mycotoxins. Bad conditions during harvest, storage, transportation and marketing, and they contribute to the growth of molds and to an increased risk of producing mycotoxins.

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Experts consider that the most effective method of avoiding the follow fungal intoxication is preventing infester with fungi to the crops. Measures to provide animal health surveillance of food at risk of contamination mycotoxică (cereals, nuts, etc.). There are numerous methods of biochemical analysis of foods with the aim of detecting mycotoxins in food. Europe has the most rigorous regulatory system of the presence of mycotoxins in food and many of the candidate countries have an even more detailed legislation regarding contamination with mycotoxins than the community. In Romania, the maximum permitted levels of aflatoxin B1 allowed in food is 5 micrograms / kg. Studies have shown that the effect

among others are due to carcinogenic compounds that occur in the body as a consequence of aflatoxin during its metabolism. Research by the American Veterinary suggests that some food additives such as BHT (butyl hydroxytoluene, or E 321) could be used in future as a measure of protection against unwanted effects of food contaminated with aflatoxin.
Intoxicated and contamination by aflatoxin are difficult diagnosed due to a large variety of atypical symptoms, especially if it is not known exposure to them. For example, an attack in the city would be difficult to detect, but could rather be seen in agricultural areas, where known diseases such specific farms. Aflatoxins are a public health problem because they can enter the food supplies of natural processes and thus to be observed by testing the samples by rapid methods. Once an attack is suspected, should be relatively easy to monitor and detect possible recidive immediately.

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