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EVALUATION OF ASPERGILLUS SPP CONTAMINATION IN THE GILAN PROVINCE, IS STORED AND FRESH HARVESTED RICE CROPS

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ABSTRACT

Introduction: *World attention on food safety are increasingly developed. Polished rice grains as a staple food in terms of consumption may be contaminated to different types of fungi. This study aimed to investigate the incidence / prevalence of fungal pollutions in the rice cropped seeds in Gilan province agricultural area that they have been put argument to evaluate. Material and Methods: In this study randomized sampling of fresh rice crops and stored facilities were applied, cultured in a series of steps on Saboraud, s agar, Czapek agar medium are of choice for Aspergillus spp studies concomitantly macroscopic and microscopic compartments characteristics following diagnostic log with the keys to identify species designated as Aspergillus species were reported by ICPA. Results: According to three times enumeration the total number of colonies grown on agar plates containing specifications; The greatest surge number of colonies were observed in samples of 2013 by a week of incubation at 25°c (20×105),while of 2014 the largest number of colonies were on 2nd week day of incubation25°c (7×105), The resultant findings showed that 50% of the tested samples were contaminated to Aspergillus spp, only 10% were related to black and more belonging to subgenus circumdati and section nigri. Conclusion: Which indicate that recently taken and stored samples came into microbial incubation and counts must not neglect. The comparison ratio showed that the black colored ones were repeatedly more isolateable, especially A.niger (57.14% & 12.5%), A.carbonarus (21.42% & 18.75%) and A.foetides (3.16% & 37.5%).*

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KEY WORDS

Aspergillus section nigri, Rice grains, Pollutions, Gilan province

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INTRODUCTION

Mycotoxins are natural toxic secondary metabolites produced by fungi. For centuries, the quality of many products has been reduced by mycotoxins, toxins that are produced by molds in many agricultural products, but especially in grains are observed after harvest during storage even when processed, concentrate and feed the animals [1,2]. Fungal contamination of rice, directly used in human food, if there are suitable conditions of the possibility of producing mycotoxins, endanger human health even failures and pollution, of pollution fungal effect, is high and at least 20% cereals is corrupted and unusable [3]. Crops contamination, especially rice to mycotoxins encounter to the wide range of their effects on the human and animal body parts as well as economic losses, make important issues in the field of food hygiene and public health too [4]. Rice is the most important cereal food consume by the most people of the world, especially the Eastern even Middle east. Half of the world population, are dependent on rice as a main food. Iran with having rice cultivation and annual production of close to 2/5 million tons, occupies a global rank 23th in production and 13th in taking it to be allocated. In Iran, particularly in the northern provinces rice is the staple food of a million people, in the preparation of food Such as cooked rice, dried fruit, odor and Processed food made from rice flour along with its waste used in animal feed [5]. The rice three major fungi pollutants as follows: Aspergillus, Fusarium and Penicillium. These fungi with attacks on agricultural products cause discoloration, reduce marketablity, friability, vulnerability and pollution as they have toxins. One of the important factors for rice contamination, is moisture percent of the product, weather condition and storage quality are the most important and determinative factors for rice contamination [6].

MATERIALS AND METHODS

In this research randomly 30 samples of stored and freshly harvested rice (2013:14 and 2014:16 samples) from 9 cities in Gilan (Kelachay, Roodsar, Langerood, Lahijan, Rezvanshahr, Rasht, Anzali, Khomam, Khoshkebijar) for each 10 ton by 100 g sampling were obtained and performed with CBS standard sampling techniques, after sampling process and transfer the samples to the laboratory, were coded, weighing 20 gr of each sample divided in 2:10 gr preprations the first 10 gr milled for mycotoxins

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measurement the second transferred to 40 ml of saline solutionthen centrifuged for fifteen minutesat a speed of 2000 rpm for two time, after homogenization the falcon contents entered into the appropriate test tubes and centrifuged (15 minutes at 3000 rpm) till supernatants were evacuated tubes and the resulting residues to transfer, in microtubes after by above steps into the culture plates of sabouraud agar medium containing 2% malt be used.Using as teriles wab of samples subculture dinprepared agar media plates and the all were coded, incubated in 2 ± 25°C for a period of 3,7 and 14 days plates were evaluated for growth, colony countand the results were recorded. During the study, colonies grown on environment marked the days listed and transferred by sterile scalpel with a volume of 5mm^3 to micro tubes containing 1ml of saline solution and stored at 4-8°C for the second phase culture were kept. The second phase began cultivatingas follows: subroud agaris used for the first cultures and transfer to micro-tubes the plates were incubated, and according to plan cultures checked and likewise above transferred to the micro-tubes containing saline and were stored at 4-8°C. The third culture of stored colonies in the second stage was used in order to obtain a single colony using the needle and plates containing single colonies were separated for the next step into account for the final determination by slide culture used which considered selective culture for samples, to research the *Aspergillus* was the target respectively Czapek agar preparing slide culture and the designated culture time results were examined then slides prepared using lactophenol staining and the standard diagnostic method by ICPA rule using optical microscopy and identification of key reviews and the final result were recorded [7].

RESULTS

Of rice samples harvested in 1392 (2013), the number of colonies counted on the fourteenth day has been 8×10^5. The tested samples produced in 1393(2014) showed a number of counted colonies 7×10^5. The samples grown colonies harvesting results as 3 different species of the black Aspergilli belonging to subgenus Circumdati section Nigri obtained as follows:

A.foetidus from rice samples obtained in 2013 and 2014 in Roodsar (6 &1), Rezvanshahr (10&0), Rasht (0&1), Anzali (3&0), Khomam (0&2), Jafarood (4&0) cities. *A.niger* in 2013 and 2014 rice samples in cities Roodsar (0&1), Rezvanshahr (0&5), Khoshkebijar (0&2), Anzali (1&0), Jafarood (1&0) and *A.carbonarus* of 2013 and 2014 rice in cities Roodsar (1&1), Rezvanshahr (1&0), Khoshkebijar (0&1), Rasht (1&0) and Anzali (1&0) were obtained as well as the other circumdati sections and species, **Figure-1**.

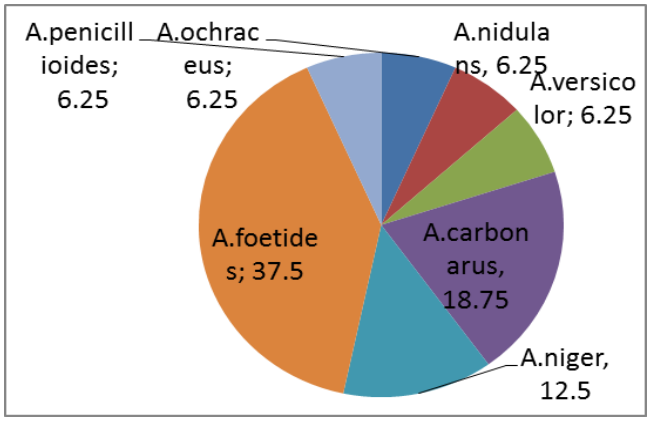

Fig:1. Frequency of Aspergillus species isolated from samples

DISCUSSION AND CONCLUSION

With regard to three times moulds grown colonies count averages on agar plates the greatest number of colonies could been harvested in seventh day of incubation (20×10^5) in comparison to on the third day of incubation (12×10^5) as on the fourteenth day (8×10^5) ves versa for the stored rice samples while of the fresh samples the greatest number of grown colonies count averages can be harvested on the fourteenth day of incubation of (7×10^5) and in the third day by 5×10^5 and the seventh day only 1×10^5 as colonies are recyclable which indicates that it should determine the microbial load of amples per incubation and counting procedures, recently obtained samples considered to have a priority to stored samples always be able to have more microbial load.

The sub-genus of the Aspergillus sector "Black" showed only 3 species *A.niger*, *A.foetidus* and *A.carbonarus*, *A.niger* (of storage to the 57.14%) in the most sampled cities found and its concentration on fresh samples were

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12.5%. *A.foetidus* have been obtained in the vast majority of collecting samples almost has been found repeatedly in a density of 3.16%, in each instance more than a colony has been harvested routinely. The fresh samples (37.5%) ability to separate more *A.niger* as the main species has been notified. Among the cities studied for the fresh rice samples Rezvanshahr and then Roodsar have the most grown appearance colonial seen. *A.carbonarus* in all stored samples (21.42%) more over than fresh samples (18.75%) have been found. *A.carbonarus* from the most cities were found, but lesser than *A.foetidus* and *A.niger* in terms of density, due to colonies have been identified. The resultant findings shows that 50% of examined samples contains Aspergillus pollution, while only 10% were Black Aspergillii and moreover were from subgenus circumdati and section nigri, although the black Aspergillii highly contamination and density ratio, especially *A.niger*, *A.carbonarus* and *A.foetidus*, after them non-black Aspergillii especially *A.versicolors*, *A.parasiticus*, *Aglaucus*, then finally *A.flavus* have often been identified.

abundance fungal polution in the rice sampels of Mazandaran province although only from 7 semples fungal elements in PDA were isolated and from100 tested samples and 93% fora while at least one plate from one of samplified grain contamination has been shown of which Aspergillus (43.96%), Cladosporium (13.96%), Alternaria (10.21%), Penicillium (4.79%) were the most reliable pollutants.The most common agents isolated (Aspergillus, Cladosporium, Alternaria and Penicillium) including toxins production potential to food and in case of inaccuracies in terms of maintenance Perhaps it will rise more and more by the time pass. reporting among isolated Aspergillii *A.flavus* and *A.fumigatus* were the most frequent and some fungal genera isolated as rice pollutants in this study as well as in other studies in Iran in other vegetable seeds including Wheat, Sorghum and Maize have also been reported repeatedly. Several studies in other countries in the field of rice fungal contamination indicating in a lot of results the study are consistent to us likely the other Iranian or international researchers [7], a survey on Thai's polished rice microflora reported genus Aspergillus, Penicillium and mucoral fungi as the most common have been identified as he same way by [8], Aspergillus and Penicillium for the rice most common storage fungi counts [8], reviewed Vietnamese rice and showed Aspergillus (43.8%), Fusarium (21.9%) and Penicillium (10.9%) as the most common isolates a above mentioned [9], in their study on 196 moldy rices sample reported of the genus Aspergillus, Penicillium, Alternaria, Mucor separation. In their study on the stored rice for a year long, from 18 different ecosystems in India, stated that Aspergillus basically causes rice surface fungal contamination reflecting grains surface sterilization influences on lesser and very limited separating them. Taligoola et al (2004), also reviewed imported rice grains and ground rice, 60 fungal species in 30 different genus were separated that *A.candidus*, *A.flavus*, *A.niger* ,*Urotium amsteloidami*, *U.rubrum*, *Penicillium citrinum* etc. As the most common isolated agents. The mean of various fungi, basically Penicillium, Aspergillus, Mucor and Rhizopus species are the rice grains, main pollutant fungi whereas kept in step in case of high humidity, their penetration into grains and in areas with higher rainfall whit more moisture was seen too much more. in areas of the Mazandaran province west region also found out similarities in the case of above and oue findings for the east areas of the Gilan province especially reasonably due to the rainfall and higher humidity similarities thus genus Aspergillus and Penicillium have been the main causes of pollution likewise in this study also separated and probably indicate of seeds contamination to dust and also inappropriate storage conditions thus increasing influence of these elements in rice and products food consumption [8]. Despite the importance of fresh, stored and ready to use rice pollution because that almost all harvested and stored rice in Iran purchase and sale of small batches in the country as well as and local consumers, performs and used, state rice fungal contamination study is very little are more about toxcicogen set fungi and mycotoxins is focused. Based on existing standards the maximum allowed rice moisture is 14% [8,9], proved the relationship between insect invasion and especially rice infection increase to *A.flavus*, this type of pollution can because of long time improper rice storage have been shown [9]. In stored rice conditions of high humidity Aspergillii can cause the most contamination, however, a major share of contamination areas surface colonization introduced. Rice deep tissue infection can be evidence of surface colonization, rice well cooking before consumption, risks caused by the colonization will be be resolved, but if proper storage conditions were optimum for fungal invasiveness, provide rice inner tissue mycotoxin production Possibility and increases the risk of complications. However, a lot of studies have been yelled comprehensive study of the various components of the rice fungal flora and ecosystems of infection has not been done, findings show that the fungal pollution cases, contamination with mycotoxins in all samples is zero or close to zero. These results confirm the national standards country reports on the lack of mycotoxin contamination of imported rice complies given that some species are able to produce mycotoxins, these studies in examining the quality of the food in the country is necessary, to determine the types of fungi that infect and the ability to produce toxins by the fungi and measuring mycotoxins in food is too much important while rice production quantity is important but health and maintain quality, especially before consumption are a more important issue. Many fungal elements isolated from consuming rice, can directly play a role in the pathogenesis

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of human and on the other hand, some of them if you do not care when storing at home and of conditions, is capable of producing mycotoxins what have adverse effects on human health and well being. Therefore, identification of fungal contamination as a first step to determine the health of the consume prepared rice is recommended and must also be assessed as a grain health standard.

CONFLICT OF INTEREST

None declared.

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None.

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