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EVALUATION OF ASPERGILLUS SPP REFLECTING TO SUBGENUS CIRCUMDATI SECTION FLAVI CONTAMINATION IN THE GILAN PROVINCE, IS STORED AND FRESH HARVESTED RICE CROPS

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ABSTRACT

Introduction: World attentions 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 incubation 25 °c(7×105), The resultant findings showed that 50% of the tested samples were contaminated to Aspergillus spp, 40% of this pollution were non-black and more belonging to the subgenus circumdati and section flavi. Conclusion: Which indicate that recently taken and stored samples came into microbial incubation and counts must not neglected, followed by non-black colored isolates, especially A.parasiticus (64.28%&6.25%), A.glaucus(57.1%&0), A.flavus (42.8%&0), A.ochraceus(14.28%&6.25%), A.penicillioides(14.28%&6.25%), A.terreus(7.14%&0) , A.nidulans (7.14% & 6.25%) , A.oryza (7.14% & 0) A.versicolor (3/16% & 6/25%).

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KEY WORDS

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INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by fungi. For centuries, the quality of many agricultural products has been reduced by mycotoxins, natural toxins that are produced by molds in many agricultural products, but especially in grains and oilseeds, and sometimes both, are observed after harvest during storage even when food is processed, concentrate and feed the animals [1]. Fungal contamination of rice directly, used in human food, if there are suitable conditions for the development or indirectly in effect, of the possibility of producing mycotoxins, endanger human health. Failures and corruption of grain and, products, of pollution fungal effect, is high and at least 20% cereals is corrupted and unusable [2,3]. Crops contamination, especially rice to mycotoxins by the wide range of their effects on the human and animal body parts as well as economic losses, this pollution make important issues in the field of food and public health as well [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 fungi major 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. Despite the inherent resistance of rice against fungal infections, this product in the course of cultivation, care, harvesting and first challenge exposure infection caused by fungi. One of the important factors for rice contamination, is the moisture percent of the product.Weather condition and storage quality are the two important and determinative factors for rice contamination by mycotoxins [6,7].

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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 gr sampling were obtained and performed with CBS standard sampling techniques, after sampling process and transfer the samples to the laboratory,were coded,weighing 20gr of each sample divided in 2:10 gr preprations the first 10 gr milled for mycotoxins measurement the second transferred to 40ml of saline solutionthen centrifuged for fifteen minutesat a speed of 2000 rpm for two time, after homogenization the falcon contents enter edin to the appropriatetest tubes and centrifuged (15 minutes at 3000rpm) 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, incubatedin 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 cultivating as follows: subroud agar is 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 results was recorded [8].

RESULTS

Of rice samples harvested in (2013), the number of colonies counted on the seventh day have been 20×10^5. The tested samples produced in (2014), showed a number of counted colonies 1×10^5. The samples grown colonies harvesting resulted as 12 different species of the genus Non-black *Aspergillus* obtained as follows:*A.versicolor* isolated from rice samples of 2013 and 2014 in cities Roodsar (9 &1), Rezvanshahr (9 & 0), Langerood (1 & 0), Khoshkebijar (1 & 0) and Anzali (1 & 0). *A.parasiticus* harvested from rice samples of 2013 and 2014 in cities Rezvanshahr (4 &0), Roodsar (3 & 1), Langerood (1 & 0) and Anzali (1 & 0) obtained. *A.glaucus* only obtained in the samples of rice belonging to 2013 in the cities; Rezvanshahr (4), Roodsar (2) and Anzali (2), *A.flavus* only rice of 2013 in the cities Roodsar (4) and Anzali (2) as well, *A.fumigatus* only in 2013 and obtained from the cities Roodsar (2) and Rezvanshahr (2) too. *A.penicillioides* reflected in the rice samples of 2013 and 2014 in cities Rezvanshahr (1 & 0), Khoshkebijar (1 & 0), Khomam (0 &1) and Anzali (0 &1) in contrast *A.oryza* only obtained in the rice samples of 2013 in the cities Langerood (1), Lahijan (1), Rezvanshahr (1) and Anzali (1) unlikewise *A.ochraceus* harvested from rice in 2013 and 2014 in cities Roodsar (0 &1), Rezvanshahr (1 & 0) and Khoshkebijar (1&0). *A.nidulans* isolated from samples of 2013 and 2014 in Roodsar (2 & 0), Langerood (1& 0), Khomam (0 &1) and Anzali (1&0), *A.terreus* only seen in the samples of rice belonging to 2013 in the Khoshkebijar (1), even *A.ostianous* only isolated in 2013 rice samples obtained in Rezvanshahr (1) city and *A.eurotium* seen only in 2013 Roodsar (1) rice samples (Figure 1) were obtained as well as another circumdati section Nigri's species. **Figure-1**

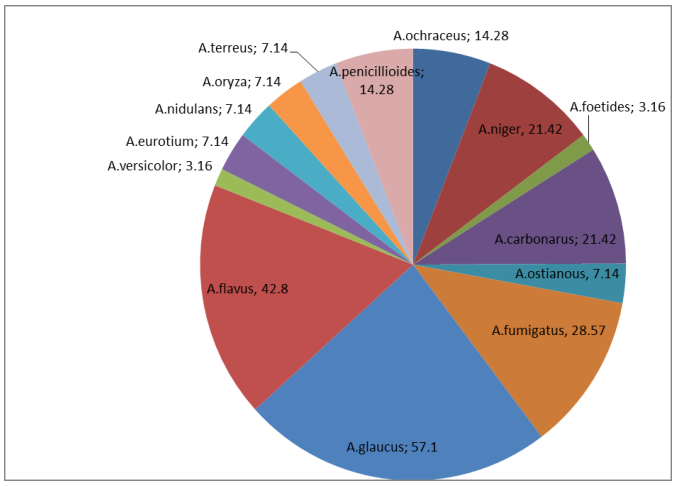

Fig:1. Frequency of Aspergillus species isolated from samples

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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) incomparison 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 (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 stored samples survey the most *A.versicolor* with frequency 3.16 % and fresh samples up to 6.25%, because more than a colony there have been isolated in any of these plates. The most of these isolates reserved by the Rezvanshahr and Roodsar cities and gained have seen about most conducted cities sampled. *A.flavus* only isolated in store samples with a frequency of 42.8 % and the Roodsar city showed more than other cities have been founded the mould. *A.glucus* (57.1%) obtained from Rezvanshahr then Roodsar more than other cities. *A.fumigatus* (28.57 %) of the city Rezvanshahr and Roodsar *were* more than the other cities. *A.ostianous* (7.14%) only from the city Rezvanshahr and *A.terreus* (7.14%) only in the city Khoshkebijar, *A.oryzae* (7.14%) from the four cities Langerood, Lahijan, Rezvanshahr and Anzali have been found. *A.eurotium* with frequency 7.14% only seen in the city Roodsar and the above list did not get and were not identified of fresh rice samples.

In contrast *A.parasiticus* (of the storage samples 64.28% and in fresh samples 6.25%) in the four cities Rezvanshahr, Roodsar, Langerood and Anzali where have been found. Resulted frequency for *A.penicillioides* (in the storage samples14.28% and in fresh samples 6.25%) noticed by the four cities khomam, Rezvanshahr, Anzali and Khoshkebijar. *A.ochraceus* frequency in the storage samples14.28% in comparison to fresh samples 6.25% calculated for the three cities Roodsar, Khoshkebijar and Rezvanshahr and been found. *A.nidulans* (of storage7.14% and fresh samples 6.25%) isolated from cities Roodsar, Langerood, khomam and Anzali.

The resultant findings show that 50% of examining samples contains Aspergillus pollution, which Non-black Aspergillii have been 40% of this pollution as causative agents, and more belonging to subgenus circumdati and section flavi have been the most species population. non-black Aspergillii especially *A.versicolors, A.parasiticus, Aglaucus*, then finally *A.flavus* often were identifiable.showed an abundance fungal polution in the rice sampels of Mazandaran province although only from 7 semples fungal elements in PDA were isolated and from 100 tested samples and 93% for a while at least one plate from one of samplified grains 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 ability 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 polutants 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. By 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. [9], reviewed Vietnamese rice and showed Aspergillus (43.8%), Fusarium (21.9%) and Penicillium (10.9%) as the most common isolates a above mentioned. 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 ecosystem in India, stated that Aspergillus basically causes rice surface fungal contamination reflecting grains surface sterilization influences on lesser and very limited separating them also reviewed imported rice graines 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. 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 like wise 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, 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 country as well as and local consumers perform and used, state rice fungal contamination study is very

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little are more about toxcicogen set fungi and mycotoxins is focused. Based on existing standards the maximum allowed rice moisture is 14%. In study indicating the average samples analyzed moisture content were in the standard range this amount can be used as a key factor effective to the reduction of fungal contamination and mycotoxins produced in product and the results of our recent study showed that *A.parasiticus* prevalence is more than *A.flavus* or *A.niger* perhaps this disorder is attributed to high moisture samples, in other words ideal conditions for *A.parasiticus* have been met while more Aspergillus contamination during rice storage, have been reported [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. In stored rice conditions of high humidity Aspergillii can cause the most contamination however, a major share of contaminatins are as 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 be optimum for fungal invasiveness provide rice inner tissue mycotoxin production Possibility and increases the risk of complications.However, a lot of studies have been yeald comprehensive study on the various components of rice fungal flora and ecosystems of infection has not been done, findings show that the fungal polutions 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 issues.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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FINANCIAL DISCLOSURE

None.

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