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Research Article

Aflatoxin exploration in basmati brown rice collected from various locations of Lahore, Pakistan

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Abstract:

Aflatoxins are produced by various fungal species, the two primaries being Aspergillus flavus and Aspergillus parasiticus. Aflatoxins contaminate various food products, resulting in them being toxic and unsafe for consumption, hence, being classified as potent carcinogens. In this paper, samples of brown basmati rice were analyzed for contamination with aflatoxins using the ELISA method. 50 brown rice samples were collected from various shops in Lahore, Pakistan, in the year 2019-2020. During summer, 25 of the samples were collected from April-September whereas the rest 25 were collected in winter from October-March. The rice samples were in a poor condition because of high levels of aflatoxin contaminations. The percentage of aflatoxins contamination for summer and winter was 88% and 80% respectively. Further, 60% of summer collected samples and 44% of winter collected samples exceeded the permissible level allowed by European Union (10µg/kg for total aflatoxins). The samples with the highest aflatoxin for summer contained 49.60±0.45µg/kg whereas for winter the concentration was 25.07±0.04 µg/kg.

With regard to their toxic nature and harm to human health, these calculated levels were frightening. These show a dire need for the implementation of practices to monitor the quality of these rice by all the rice producers and storage holders in Pakistan.

Key words: aflatoxin; brown rice; basmati; elisa; quality management

Introduction

Aflatoxins are a species of naturally occurring toxins that are produced as a by-product of Aspergillus, primarily Aspergillus flavus and Aspergillus parasiticus. Around 18 different forms of aflatoxins are identified to date. However, the major types include B1, B2,, G1, G2, M1 and M2 (Bakirdere et al., 2012). All the listed types are based on fluorescent nature under UV light and the relative mobility of the chromatogram (Campone et al., 2011). Among the listed aflatoxins, B1 holds abundant importance in regard to its occurrence and toxicity. Aflatoxin B1 is termed carcinogenic and is further linked with liver cancer (Qureshi et al., 2015). Aflatoxicosis is the condition linked with excessive consumption of aflatoxins

Aflatoxins can be produced in various foods manufacturing stages such as; production, storage or processing if optimal conditions for growth are provided i.e., 30ºC and 90% humidity (Tavakoli et al., 2014). Previous research conducted by Reddy et al. in 2008 verified the contamination of aflatoxin B1 on rice samples in India. These toxins generally contaminate various crops causing great loss to the world economy (Shabeer et al., 2022).

Rice is at the second level of cereal crop production after wheat and consist of a major part of the human diet all around the world (Reiter et al., 2010). Rice is termed a staple food throughout Asia and Pakistan, having an ample supply due to the country being agriculture-based (Zahra et al., 2017). The consumption of white rice is more, however, demand for brown rice continues to grow due to its high nutritional value (Choi et al., 2015). Rice under standard circumstances does not provide a favorable environment for the growth and contamination of aflatoxins and Aspergillus, however, massive rainfall and extreme temperature may provide the required environment for contamination of aflatoxins (Siruguri et al., 2012).

Rice is mostly cultivated in subtropical areas having warm and humid environments i.e., 30ºC and 90% humidity. Rice is normally dried after harvesting and stored in a non-suitable storage environment. Rice is regarded to be a suitable substrate for the growth of fungus (Lai et al., 2015). According to FAO, due to inappropriate storage conditions, 15% of the rice harvest is destroyed every year as a result of fungal growth (Dors et al., 2011). Rice is often contaminated with aflatoxins which are basically mycotoxins. Factors like moisture content, activity of water, temperature, storage duration, early contamination levels and the toxigenic possibility of fungal strains. The prevailing condition such as moisture and temperature permute the aflatoxin production during storage and result in an annual loss of helpful food bio-sources i.e., rice, resulting to influence the economy vastly (Iqbal et al., 2012).

Analytical techniques used for the determination of aflatoxins and their level include High-Performance Liquid Chromatography (HPLC), Thin Layer Chromatography (TLC), Enzyme-Linked Immuno Sorbent Assay (ELISA) and Liquid Chromatography-Mass Spectroscopy (LCMS) (Matumba et al., 2015; Lin et al., 1998). In order to stay safe from the diverse effects of aflatoxin, it is important that steps are taken to curb its contamination. A number of pre-harvest and post-harvest management strategies (biological, physical, and chemical) are being practiced minimizing levels of aflatoxins in high-risk foods (Hell and Mutegi, 2011).

Since aflatoxins are such contaminants that are unavoidable, World Health Organization has asked to take all possible measures to ensure the lowest possible level for aflatoxins. Various countries have regulated specific tolerance limits in order to control aflatoxin levels in their food crops (Kumar et al., 2021). In accord with European Commission 2010, the maximum permissible level for aflatoxin B1 and total aflatoxin present in white rice is set at 2 µg/kg and 4 µg/kg whereas for brown rice the level is 5 µg/kg and 10 µg/kg respectively.

The prime objective of the current study was to determine the levels of aflatoxin in brown basmati rice collected from various locations of Lahore, Punjab in the seasons of summer and winter using methods of ELISA.

Material and Methods:

The current study and experimentation were performed at the Food and Biotechnology Research Centre, PCSIR, Lahore between the years 2019-2020.

Physical Testing

Homogenous rice samples were collected from each rice variety that was obtained from different rice shops, marked, and stored for analysis. The smashed, defective, and other contaminations of rice pieces were removed before sample preparation (Tahir et al., 2021).

Sample collection:

A total of 50 samples of brown basmati rice were collected from various local shops in Lahore, Pakistan. 25 of these samples were collected in summer from April-September whereas the remaining 25 were collected in winter from October-March. To ensure representative sampling, an appropriate plan was devised. All the samples were collected from the basements of the shops and placed in jute bags. 500 g of each brown rice sample was collected and stored in a cool dry place inside zipped plastic bags till the experiment was performed. Using the AOAC (2005) procedure, the rice samples were inspected for moisture.

Extraction

From each rice sample, 200 g of rice were mixed and grinded into a fine powder (passable through a 20-mesh sieve) to ease experimental analysis. Using AOAC 2005, method, 5 g of the grinded sample was mixed in 25 mL of 70% methanol followed by vigorously shaking for 3 minutes. The filtrate was extracted using a filter paper and used as a sample after dilution with distilled water in a 1/1 ratio.

Aflatoxin determination using ELISA:

The brown rice samples were tested using ELISA quantitative test kit (Veratox, Neogen, USA). 100 □L of the conjugate was placed into the red-marked mixing well. Using a new tip, 100 µL of the control solution and sample solution were transferred to the mixing wells. The solution was mixed by pipetting up and down 3-4 times followed by incubating for 20-30 minutes at room temperature. The solution from the wells was disposed of, followed by washing them with buffer solution twice. The wells were dried by patting them lightly on a paper towel. To the dried wells, 100 µL of the substrate was pipetted, mixed and incubated at room temperature for 20 minutes. Lastly, 100 µL of the red stop solution was pipetted to the wells, allowing the solution to sit for 10 minutes. The results were read at 450 nm using an ELISA reader (Nazir et al., 2020).

Results and Discussions:

Aflatoxin contamination in rice is a matter of great concern in terms of economy as well as human health. Aflatoxin contamination of rice and various food crops is the main concern of food safety in tropical and subtropical climates as high temperature and humidity favor the growth of mycotoxigenic fungi. Rice is cultivated at a moisture content between 16% and 37%, which favors the growth of fungus (Reiter et al., 2010).

In the current study samples of rice (n=50) were collected from Lahore, Pakistan and analyzed using methods of ELISA. The samples were collected during the course of two seasons i.e., 25 for each summer and winter. Out of the 25 brown rice samples collected and analyzed during summer, 22 were positive for aflatoxin contamination. As the permissible limit for aflatoxin contamination in brown rice is 10□g/kg, from the results we can see that, among the 22-aflatoxin positive brown rice samples, 15 were above the permissible levels and the rest 7 were within the permissible levels. During winter, 20 of the collected brown rice samples were positive for aflatoxin contamination. From these, 11 of the rice samples were found to be contaminated beyond the permissible levels and 9 were found to be contaminated within the permissible range.

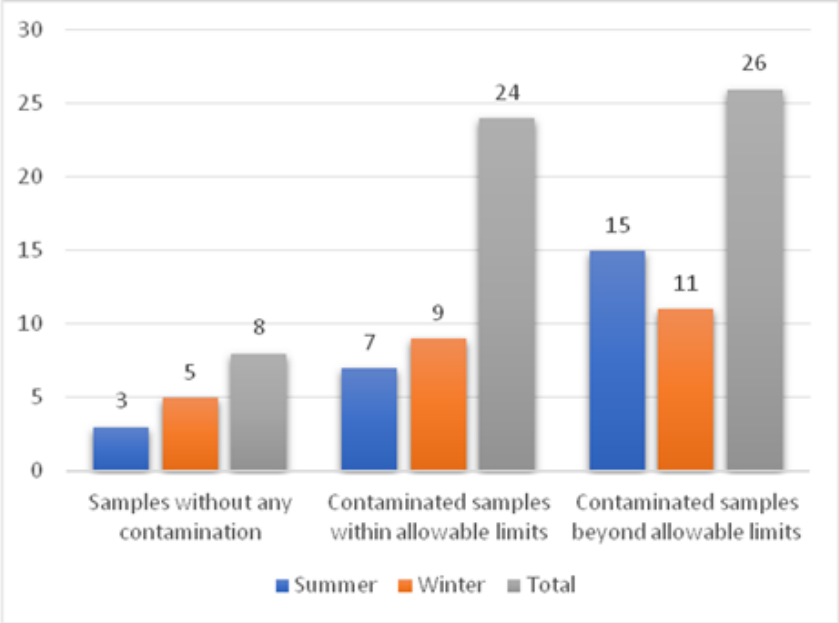

Figure 1: Comparison of Aflatoxins in Brown Rice with respect to seasons

On conducting the percentage analysis of the results, we see that for samples collected during summer (Figure 2), 88% of them were contaminated with aflatoxins. 60% of the contaminated samples had levels of aflatoxins beyond permissible levels whereas the remaining 28% had aflatoxins levels within the permissible level. On the other hand, for samples collected during winter (Figure 3), 80% of the tested samples were found to be contaminated with aflatoxins. Among the samples that were contaminated, 44% were beyond the permissible level of aflatoxin contamination whereas the remaining 36% were within the permissible level. These findings also correlate to similar findings in a study conducted in Vietnam by Nguyen et al., (2007), where more than 51% of the samples were contaminated with aflatoxins. Another research on aflatoxin contamination in rice by Nazir et al., (2021) showcased 35% of their rice samples to be contaminated with AFB1.

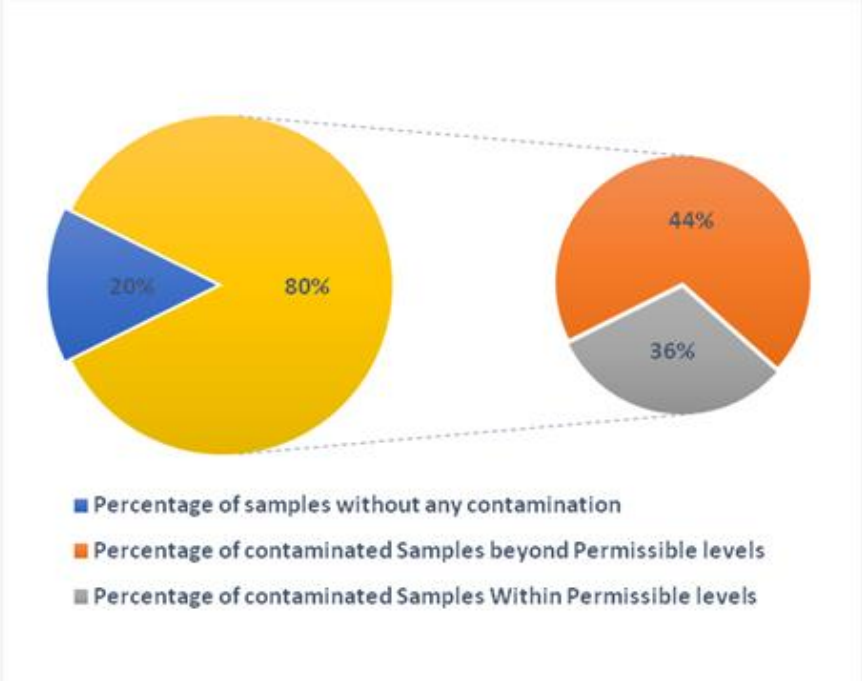

Figure 2: Percentage analysis of brown rice in summer

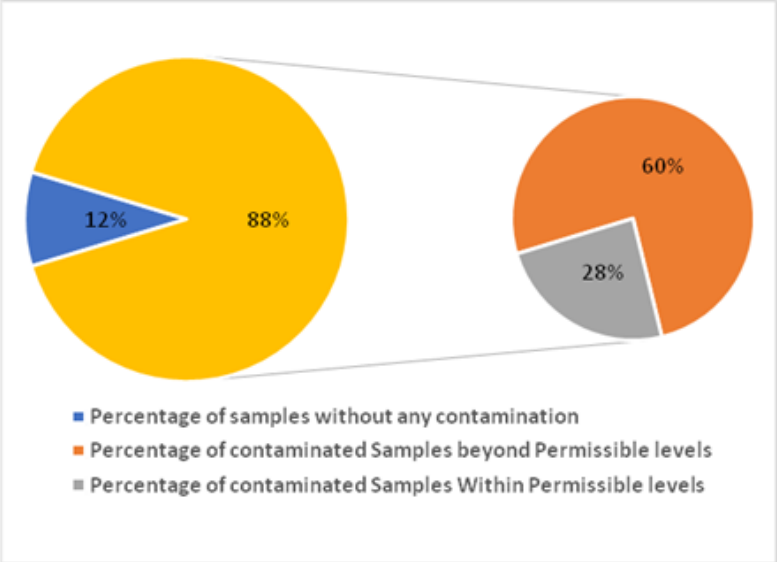

Figure 3: Percentage analysis of brown rice in winter

The results have shown us that the probability of brown rice aflatoxin contamination during the season of summer is more than that in winter. This can be because of the high temperatures and content of moisture during the season of summer, which serves as optimal conditions for aflatoxin growth (Cotty and Jamie-Garcia, 2007). From the tested samples, we can see that some samples of brown rice detected high levels of aflatoxin contamination. However, in previous studies conducted by Zahra et al., (2017) brown rice showed no affect to variation of temperature as compared to white rice. In summer collected samples, the highest levels of aflatoxin were 49.60 µg/kg, 17.66 µg/kg, 17.27 µg/kg and 17.23 µg/kg (Table 1). Alternatively, in winter collected samples, the highest level of aflatoxin detected were 25.07 µg/kg, 24.34 µg/kg, 17.17 µg/kg and 16.98 µg/kg (Table 2).

Sample Lab ID	Results Aflatoxins (µg/kg)	Fit/Unfit for Human Consumption
Rice-01-19- SUM	10.25±0.05	Unfit
Rice-02-19- SUM	12.34±1.02	Unfit
Rice-03-19- SUM	11.92±0.04	Unfit
Rice-04-19- SUM	9.43±0.10	Fit
Rice-05-19- SUM	5.25±0.35	Fit
Rice-06-19- SUM	17.27±0.21	Unfit
Rice-07-19- SUM	10.95±0.10	Unfit
Rice-08-19- SUM	15.31±0.45	Unfit
Rice-09-19- SUM	3.91±0.04	Fit
Rice-10-19- SUM	16.93±0.05	Unfit
Rice-11-19- SUM	14.29±0.15	Unfit
Rice-12-19- SUM	11.59±0.05	Unfit
Rice-13-19- SUM	17.68±0.31	Unfit
Rice-14-19- SUM	N.D.	Fit
Rice-15-19- SUM	N.D.	Fit
Rice-16-19- SUM	17.66±0.24	Unfit
Rice-17-19- SUM	49.60±0.45	Unfit
Rice-18-19- SUM	2.42±0.02	Fit
Rice-19-19- SUM	15.30±1.00	Unfit
Rice-20-19- SUM	N.D.	Fit
Rice-21-19- SUM	1.70±0.05	Fit
Rice-22-19- SUM	17.23±0.26	Unfit
Rice-23-19-SUM	1.92±0.02	Fit
Rice-24-19- SUM	2.93±0.05	Fit
Rice-25-19- SUM	11.43±0.05	Unfit

Table 1: Aflatoxin analysis of brown rice in summer

Sample Lab ID	Results Aflatoxins (µg/kg)	Fit/Unfit for Human Consumption
Rice-01-20-WIN	N.D.	Fit
Rice-02-20- WIN	N.D.	Fit
Rice-03-20- WIN	2.44±0.20	Fit
Rice-04-20- WIN	1.73±0.04	Fit
Rice-05-20- WIN	4.56±0.05	Fit
Rice-06-20- WIN	16.98±0.20	Unfit
Rice-07-20- WIN	24.34±0.08	Unfit
Rice-08-20- WIN	12.13±0.05	Unfit
Rice-09-20- WIN	11.34±0.10	Unfit
Rice-10-20- WIN	N.D.	Fit
Rice-11-20- WIN	N.D.	Fit
Rice-12-20- WIN	5.00±0.05	Fit
Rice-13-20- WIN	9.46±0.10	Fit
Rice-14-20- WIN	11.52±0.12	Unfit
Rice-15-20- WIN	14.28±0.02	Unfit
Rice-16-20- WIN	13.29±0.05	Unfit
Rice-17-20- WIN	N.D.	Fit
Rice-18-20- WIN	17.17±0.10	Unfit
Rice-19-20- WIN	25.07±0.04	Unfit
Rice-20-20- WIN	2.90±0.05	Fit
Rice-21-20- WIN	15.23±0.20	Unfit
Rice-22-20- WIN	2.54±0.50	Fit
Rice-23-20- WIN	5.12±0.05	Fit
Rice-24-20- WIN	1.33±0.02	Fit
Rice-25-20- WIN	17.15±0.20	Unfit

Table 2: Aflatoxin analysis of brown Rice in winters

These results are alarming as the presence of aflatoxins in food products is extremely dangerous to human health and well-being. With regard to their toxic nature, aflatoxins detectable by any technique should not be present in food products (Leszczynksa et al., 2001). Because of their threat to both human health and the economy, a number of techniques are adapted to limit their damage. Improved harvesting, packaging, storing, and distribution procedures are required to control aflatoxin contamination (Asghar et al., 2014).

Conclusion:

In conclusion, rice samples collected from various locations of Lahore during the season of summer and winter were contaminated with aflatoxin B1. These samples calculated 88% and 80% of aflatoxin contamination respectively using the methodology of ELISA. Excessive consumption of aflatoxin is a leading cause of liver cancer. Improper procedures before and after harvesting contribute to the contaminations. Extra caution should be implemented during the handling of these food products to ensure safety.

References

1. AOAC, Official Methods of Analysis. 2005. Association of Official Analytical Chemists, 18th Edition, Washington DC, USA.
2. Asghar, M. A., Iqbal, J., Ahmed, A., & Khan, M. A. (2014). Occurrence of aflatoxins contamination in brown rice from Pakistan. Iranian journal of public health, 43(3), 291.
3. Bakırdere, S., Bora, S., Bakırdere, E. G., Aydın, F., Arslan, Y., Komesli, O. T., ... & Yıldırım, E. (2012). Aflatoxin species: their health effects and determination methods in different foodstuffs. Central European journal of chemistry, 10(3), 675-685.
4. Campone, L., Piccinelli, A. L., Celano, R., & Rastrelli, L. (2011). Application of dispersive liquid–liquid microextraction for the determination of aflatoxins B1, B2, G1 and G2 in cereal products. Journal of chromatography A, 1218(42), 7648-7654.
5. Choi, S., Jun, H., Bang, J., Chung, S. H., Kim, Y., Kim, B. S., ... & Ryu, J. H. (2015). Behaviour of Aspergillus flavus and Fusarium graminearum on rice as affected by degree of milling, temperature, and relative humidity during storage. Food Microbiology, 46, 307-313.
6. Cotty, P. J., & Jaime-Garcia, R. (2007). Influences of climate on aflatoxin producing fungi and aflatoxin contamination. International journal of food microbiology, 119(1-2), 109-115.
7. E.C., European Commission Regulation No 165/2010 of 26 February, 2010. Amending No. 1881/2006 as regards aflatoxins. European Commission, Off. J. Europ. Union, L50: 8.
8. Iqbal, S. Z., Asi, M. R., Ariño, A., Akram, N., & Zuber, M. (2012). Aflatoxin contamination in different fractions of rice from Pakistan and estimation of dietary intakes. Mycotoxin research, 28(3), 175-180.
9. Kerstin, H., & Charity, M. (2011). Aflatoxin control and prevention strategies in key crops of Sub-Saharan Africa. African Journal of Microbiology Research, 5(5), 459-466.
10. Kumar, A., Pathak, H., Bhadauria, S., & Sudan, J. (2021). Aflatoxin contamination in food crops: causes, detection, and management: a review. Food Production, Processing and Nutrition, 3(1), 1-9.
11. Lai, X., Zhang, H., Liu, R., & Liu, C. (2015). Potential for aflatoxin B1 and B2 production by Aspergillus flavus strains isolated from rice samples. Saudi Journal of Biological Sciences, 22(2), 176-180.

12. Leszczyńska, J., Masłowska, J., Owczarek, A. L. I. N. A., & Kucharska, U. R. S. Z. U. L. A. (2001). Determination of aflatoxins in food products by the ELISA method. Czech Journal of Food Science, 19(1), 8-12.
13. Matumba, L., Sulyok, M., Njoroge, S., Njumbe Ediage, E., Van Poucke, C., De Saeger, S., & Krska, R. (2015). Uncommon occurrence ratios of aflatoxin B1, B2, G1, and G2 in maize and groundnuts from Malawi. Mycotoxin research, 31(1), 57-62.
14. Nazir, A., Kalim, I., Imran, M., Bilal, M. A., Zahra, N., Ahmad, A., ... & Ehtisham-ul-Haque, S. (2021). Incidences and Bio-Detoxification of Aflatoxins in Rice and Cattle Feed Crops under Different Agro-Ecological Zones. Polish Journal of Environmental Studies, 30(2).
15. Nguyen, M. T., Tozlovanu, M., Tran, T. L., & Pfohl-Leszkowicz, A. (2007). Occurrence of aflatoxin B1, citrinin and ochratoxin A in rice in five provinces of the central region of Vietnam. Food chemistry, 105(1), 42-47.
16. Qureshi, H., Hamid, S. S., Ali, S. S., Anwar, J., Siddiqui, A. A., & Khan, N. A. (2015). Cytotoxic effects of aflatoxin B1 on human brain microvascular endothelial cells of the blood-brain barrier. Medical mycology, 53(4), 409-416.
17. Reiter, E. V., Vouk, F., Böhm, J., & Razzazi-Fazeli, E. (2010). Aflatoxins in rice–a limited survey of products marketed in Austria. Food Control, 21(7), 988-991.
18. Shabeer, S., Asad, S., Jamal, A., & Ali, A. (2022). Aflatoxin Contamination, Its Impact and Management Strategies: An Updated Review. Toxins, 14(5), 307.
19. Siruguri, V., Kumar, P. U., Raghu, P., Rao, M. V. V., Sesikeran, B., Toteja, G. S., ... & Rani, S. (2012). Aflatoxin contamination in stored rice variety PAU 201 collected from Punjab, India. The Indian journal of medical research, 136(1), 89.
20. Tahir, N. I., Hussain, S., Kalim, I., Asghar, A., Syed, S. K., Idrees, M. A., & Ahmad, M. A. (2021). Aflatoxin Concentrations in Various Rice Varieties of Punjab, Pakistan. RADS Journal of Biological Research & Applied Sciences, 12(1), 39-53.
21. Tavakoli, H., Rostami, H., Zabihi, A., & Bitarafan, Z. (2014). A survey of aflatoxins, ochratoxin and Zearalenone Contamination in Imported and Iranian Rice in Iran. Agricultural Engineering Research Journal, 4(3), 61-64.
22. Trucksess, M.W. 2005. Natural Toxins. In: W. Horwitz, G.W., Latimer (Eds.), Official Methods of Analysis of AOAC International, AOAC International, Gaithersburg, MD, USA. pp. 1-85.
23. Zahra, N., Jamil, N., Ahmad, S. R., Munir, S., Saeed, M. K., & Kalim, I. (2017). Comparative analysis of brown and white rice samples for the presence of Aflatoxin B1 contamination and effect of environment temperature on Aflatoxin concentration during storage. Transylvanian Review, 25, 4507-4515.

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