IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 15, Issue 5 Ser. I (May 2021), PP 06-13
www.iosrjournals.org

Impact of different storage conditions on the level of aflatoxin contamination in wheat flour

Ankita Soni[1], Naresh K Nirmal[1]*
[1]*Department of Zoology, University of Rajasthan, Jaipur-302004*

Abstract
Aflatoxin contamination in wheat is greatly affected by storage conditions. Wheat, which is a staple food in Rajasthan, is readily contaminated by this toxin if stored in a hot and humid environment. This work is a preliminary and basic study that strives to analyze the impact of various storage conditions on aflatoxin levels in wheat flour samples with reference to the climate of Jaipur (Rajasthan). A local survey was conducted to know the prevalent storage practices. Flour samples were then stored in different ways (as revealed in the survey) for 30 days and the level of the aflatoxin was measured in each sample at different time points by ELISA tests. Results of the study indicated that aflatoxin contamination increases with time in open containers under ambient temperature and humidity conditions. However, further studies are required with a higher number of samples to obtain a statistically significant result.

Keywords: *Mycotoxins, Aflatoxins, Wheat flour, Aspergillus*

Date of Submission: 24-04-2021 Date of Acceptance: 08-05-2021

I. Introduction

Wheat is a staple cereal in India, especially, Rajasthan. Rajasthan is also a major producer of wheat in the country. In the year 2014-15, the state was awarded 'Krishi Karman Award' for being the highest wheat-producing state. However, the quality of wheat grains is greatly dependent upon various methods of harvesting, drying, preparation and marketing (including storage). A major concern worldwide is the contamination of the grain by fungi. Fungi during their development on various food items, produce extraordinary auxiliary metabolites which are called 'mycotoxins'. Mycotoxins not just lessen the healthy benefit of the food items yet additionally render them harmful for human utilization.

Mycotoxins: Mycotoxins are a group of toxic metabolites produced via secondary metabolic pathways in fungal cells which are the source of toxin contamination in various food grains. Most known mycotoxins are derived from acetate or amino acids that are produced by species belonging to the genus *Aspergillus, Penicillium, Fusarium* and *Alternaria* [1] **.** One-fourth of the cereals devoured on the planet are affected by mycotoxins. There are five mycotoxins that occur in food, namely, deoxynivalenol, zearalenone, ochratoxin, fumonisins and aflatoxins. Mycotoxin producing fungi broadly falls into two categories: - (a) that invade before harvest are field fungi and (b) that occur after harvest are called storage fungi [2]**.** Among the mycotoxins affecting food and feed, aflatoxin is the major one that ultimately harms human and animal health [3].

Aflatoxins: Aflatoxins are toxins produced by some fungi especially *Aspergillus flavus* and *A. parasiticus* which grow on improperly managed agricultural crops. Aflatoxins occur in various cereals, oilseeds, spices, and nut. Wheat, a staple food grain in Rajasthan, is highly susceptible to fungal contamination during its growth and further processing like harvesting, transport and storage. Various aflatoxins are conveyed by *Aspergillus* species of which Aflatoxin B1 (AFB1) is the most potent hepatocarcinogen in humans and animals. In a multicenter study drove by ICMR from different samples from Indian states found that AFB1 amount of > or = 5 µg/kg were recorded in 40.3% of the test samples, and concentrations above the Indian permissible regulatory limit of 30 µg/kg were found in 16% of the examples [4]**.**

To keep the public safe from aflatoxin danger, different countries have set various limits for the presence of aflatoxins in wheat and product of wheats, differing from 4 µg/kg (ppb) in the European Union to 30 µg/kg (ppb) in India [5]**.** Increasing temperature and climate change further complicate the situation because *Aspergillus* growth is greatly affected by temperature change [6].

Components influencing the mycotoxin deterioration: Upon the improvement of precise and sensitive strategies for the examination of mycotoxins, scientists have discovered that different components work reliantly to affect fungal colonization and mycotoxins production. Components that influence mycotoxins production and deterioration are arranged as physical, chemical and biological. Physical factors include ecological conditions like temperature, relative humidity and pest infestation that prompt fungal colonization and mycotoxin production. Many fungi which produce mycotoxins are favored by hot and humid weather

conditions. Generally , seed grains humidity more than 13% and relative humidity more than 65% and also the temperature between 26◦C and 32◦C provide the conditions for microbial activity [7]**.** In an investigation, by utilizing RT-PCR reasoned that the production of fungal biomass and AFB1 were most elevated at 28◦C and 0.96 aw, while no contagious development or AFB1 production was found at 20◦C with aw estimations of 0.90 and 0.93 [8]. Depending upon the storage conditions, wheat flour experiences different temperature and humidity when stored for extended periods. This difference in environmental factors may cause changes in aflatoxin concentration.

II. Materials And Methods

Survey: A local survey was conducted to check the awareness regarding mycotoxins (Figure 1,2 &3), aflatoxins among the localities of Jaipur and nearby area.

University of Rajasthan
Department of Zoology
MSc Microbiology

SURVEY ON FOOD HABITS AND AWARENESS REGARDING MYCOTOXINS

*** Required**

Email address *

Name

Your Educational Qualification *

Below graduation

Graduate

Post graduate and above

Place of your stay: **Date:**

Questionnaire

Here we are conducting a survey about the awareness regarding mycotoxin contamination and food storage methods used in Rajasthan. Your response is highly appreciated and will help us to design safer and better storage practices and guidelines for common people. This survey is part of a student project. Your valuable time will motivate young students to work harder for scientific research and inquiry.The average time for completion of this survey is 2 minutes.

1. Are aware of Aflatoxins?
   Yes ☐ No ☐
2. Are you aware that mycotoxins contaminatemost of common foodgrains?
   Yes ☐ No ☐
3. Which cereal/sdo you consume? (Tick one or more)
   ☐ Wheat

Figure 1. Survey questionnaire

- ☐ Jowar
- ☐ Rice
- ☐ Barley
- ☐ Corn
- ☐ Any other (Please specify)

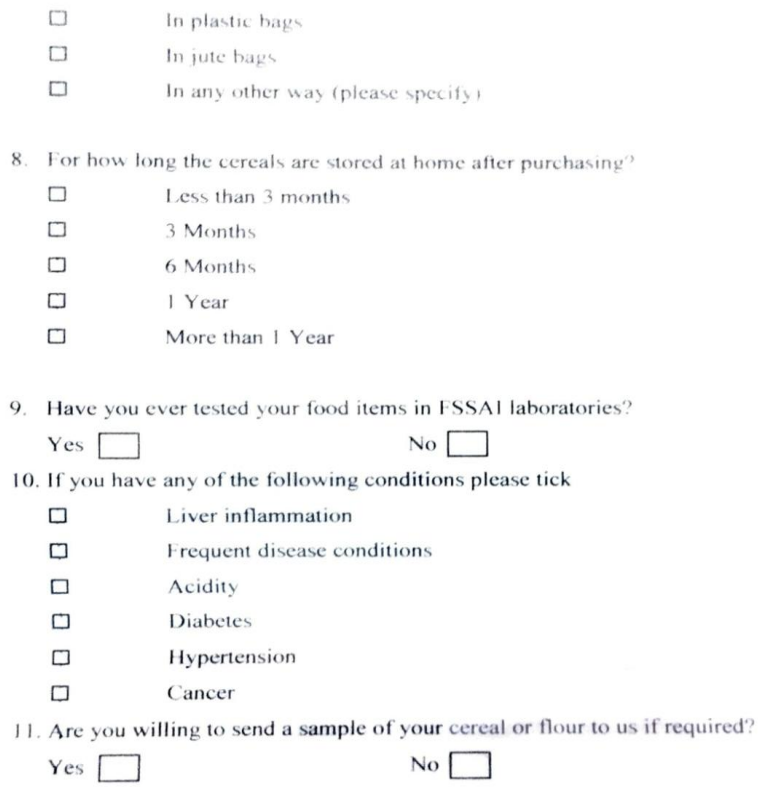
4. Which flour do you consume?
   - ☐ Commercially available flour
   - ☐ Cereal stored at your home and ground at home by electrical grinder machine
   - ☐ Cereal stored at your home and ground at flour mill
   - ☐ Cereal stored at your home and ground at home ground by hand grinder machine
   - ☐ Any other (Please mention)

5. How do you store wheat flour?
   - ☐ In stainless steel cans with lid
   - ☐ In stainless steel cans without lid
   - ☐ In iron cans with lid
   - ☐ In iron cans without lid
   - ☐ In plastic bags
   - ☐ In any other way (please specify)
   - ☐

6. In how much time you consume all the flour available at home?
   - ☐ 10 days
   - ☐ 20 days
   - ☐ 30 days
   - ☐ 45 days
   - ☐ 60 days
   - ☐ More than 60 days

7. How do you store wheat grain?
   - ☐ In stainless steel cans/drums with lid
   - ☐ In stainless steel cans/drums without lid
   - ☐ In iron cans/drums with lid
   - ☐ In iron cans/drums without lid

Figure 2. Survey questionnaire



☐ In plastic bags
☐ In jute bags
☐ In any other way (please specify)

8. For how long the cereals are stored at home after purchasing?
☐ Less than 3 months
☐ 3 Months
☐ 6 Months
☐ 1 Year
☐ More than 1 Year

9. Have you ever tested your food items in FSSAI laboratories?
Yes ☐ No ☐

10. If you have any of the following conditions please tick
☐ Liver inflammation
☐ Frequent disease conditions
☐ Acidity
☐ Diabetes
☐ Hypertension
☐ Cancer

11. Are you willing to send a sample of your cereal or flour to us if required?
Yes ☐ No ☐

Figure 3. Survey questionnaire

Storage: 2 Kg of home stored wheat grains were milled using electric mill to yield wheat flour. For this study, wheat flour was stored for the period of 30 days (October 2019) in different storage containers. According to the survey, the storage containers are as follows: a) Steel Container closed (SCC) and Steel container open (SCO), b) Iron container closed (ICC) and Iron container open (ICO), c) Polythene bag closed (PBC) and polythene container open (PBO), d) Plastic container closed (PCC) and plastic container open (PCO).
In each type of container 250 g of freshly milled wheat flour was stored for 30 days (from 5/10/19 to 3/11/19). To determine the effect of environmental factors on the aflatoxin formation, temperature and humidity of flour in different containers were recorded daily.

Measurement of temperature and humidity: Temperature and humidity of storage area and storage containers were recorded daily for 30 days using HTC-1 LCD Digital Thermometer and Hygrometer.
Total aflatoxin estimation: For the analysis of aflatoxin concentration, duplicates of sample from each container were collected at day 0, day 7 and day 30 respectively in sterilized plastic zip bags. The samples were kept refrigerated until analysis. The analysis of total aflatoxin was conducted using Celer AFLA ELISA Kit (Code: MA210) according the manufacturer's protocol. The assay had detection limit of 2µg/kg (ppb).

Figure 4 . (A) SCC& SCO, (B) ICC& ICO, (C) PBO& PBC AND (D) PCC & PCO.

Statistical analysis: All results were analyzed using One Way ANOVA Test followed by multiple comparison with GraphPad Prism 8 software. Significance was determined at $p<0.05$ for all analyses.

III. Results And Discussion

General survey: A general survey was conducted to check the awareness for mycotoxins among localities and M.Sc. students of Jaipur and nearby places. A total of 70 respondents were approached and out of which 48.57% of respondents were not aware of mycotoxins while only 37.14% of respondents were aware of aflatoxins. The survey was also conducted to know about the storage practices and storage duration of wheat flour. Out of 70 respondents mostly i.e. 71.42% use SCC to store the wheat flour while aluminium containers with lid were used by only 2.87%, respondents which use other containers are as follows 10% store wheat flour in an ICC, 8.57% in plastic bags or containers and only 7.14% stores wheat flour in SCO. Similarly, 35.71% of total respondent stores wheat flour for 20 days while 30% for 30 days, 20% for 10 days, 5.71% for 45 days, and 4.82% for more than 60 days and only 2.87% stores wheat flour for 60 days before consuming it fully.

Temperature, humidity and aflatoxin concentration: Environmental factors such as temperature, humidity and organoleptic properties of grain and flour play an important role in their spoilage. In general, the relative humidity of more than 65% and a temperature between 25°C and 30°C are favorable conditions for the fungal growth. The climatic conditions of Rajasthan vary depending upon location and season. This study was conducted during the month of October in Jaipur. It is known that the item assortment, climate designs, temperature, moistness, water action, oxygen levels, low upkeep necessities, not dry enough, creepy-crawly or rat movement and different issues influence *Aspergillus* development and aflatoxin creation in putting away items [9]. In the present study, the average temperature and humidity were 29.7°C and 44.9%, respectively, during the experimental period which was less favorable for the growth of fungi. This can be a possible explanation for the lower concentration of aflatoxin observed in the study. During the storage period, the temperature of the study area decreased (Figure 5) while humidity varies. On Day 30 the temperature is slightly lower while humidity is slightly higher than the Day 7 which resulted in a higher amount of aflatoxin on Day 30. In a study, it was found that corn crops from a soggy territory contain higher aflatoxin contrasted with those that originate from a dry and warm zone [10]. Aflatoxin concentration in different storage containers on day 1, day 7 and day 30 are shown in table 1 and figure 6. On day 1, aflatoxin concentration was less than 2ppb (ND*) in all the storage containers. In the steel container with lid aflatoxin amount was less than 2ppb during the whole study period. In containers other than steel with lid, aflatoxin conc. increases during the study period. In an iron

container with lid and polythene bag closed, only one sample of day 30 has the aflatoxin conc. of 2.002 ppb and 2.1 ppb respectively. The highest amount of aflatoxin concentration was found in an open polythene bag on day 30 (3.503 ppb). In a study, it was revealed that the measure of AFB1 in wheat tests extended between the least qualities (2.4ppb) to the most elevated worth (45.26ppb). According to the study, the most elevated mean of AFB1 focus in wheat crop was on February 2015 (30.04 ppb), while the low were recorded on September and October 2014 (1.27-1.08ppb), separately [11]**.** Statistical analysis revealed that the observed difference was not significant at P value 0.05. However, if the concentration in fresh sample and not detected is taken 0, then the mean difference becomes highly significant. Therefore, sensitivity of the method used plays a great role in studying the aflatoxins. Studies with greater number of samples and extending for longer durations of time are required to further analyze the status of aflatoxin contamination in stored wheat in homes.

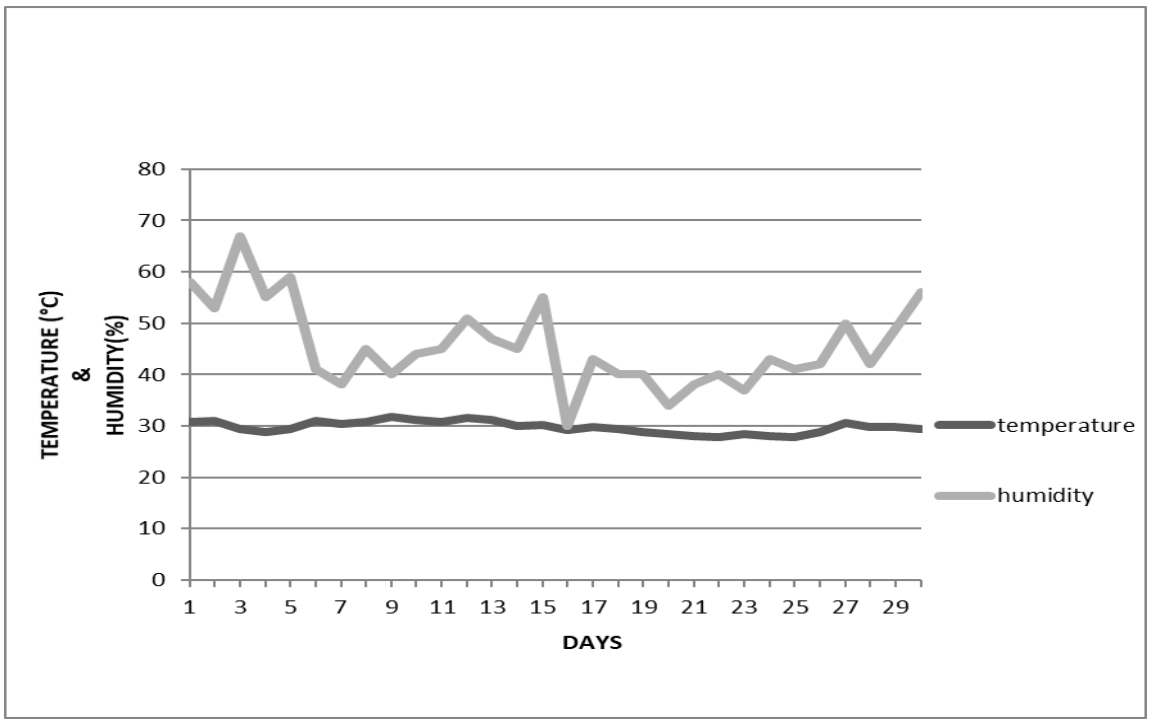


Figure 5. Temperature and humidity of storage area

Table 1. Aflatoxin contamination in wheat samples from the study.

	AFLATOXIN CONTAMINATION IN WHEAT SAMPLES					
S.NO.	**CONTAINER TYPE**	**DAY 1**	**DAY 7**		**DAY 30**	
			A	**B**	**A**	**B**
1	IRON CONTAINER LID CLOSED (ICC)	ND*	ND*	ND*	2.002	ND*
2	IRON CONTAINER LID OPEN (ICO)	ND*	2.006	2.126	3.004	3.126
3	STEEL CONTAINER LID CLOSED (SCC)	ND*	ND*	ND*	ND*	ND*
4	STEEL CONTAINER LID OPEN (SCO)	ND*	ND*	2	2.856	2.701
5	POLYTHENE BAG CLOSED (PBC)	ND*	ND*	ND*	ND*	2.1
6	POLYTHENE BAG OPEN (PBO)	ND*	2.704	2.821	3.503	3.212
7	PLASTIC CONTAINER CLOSED (PCC)	ND*	ND*	ND*	2.005	2.009
8	PLASTIC CONTAINER OPEN (PCO)	ND*	2.191	2.012	3.274	2.453

ND* The sample has lower aflatoxin than the detection limit of the system (>2.000ppb)

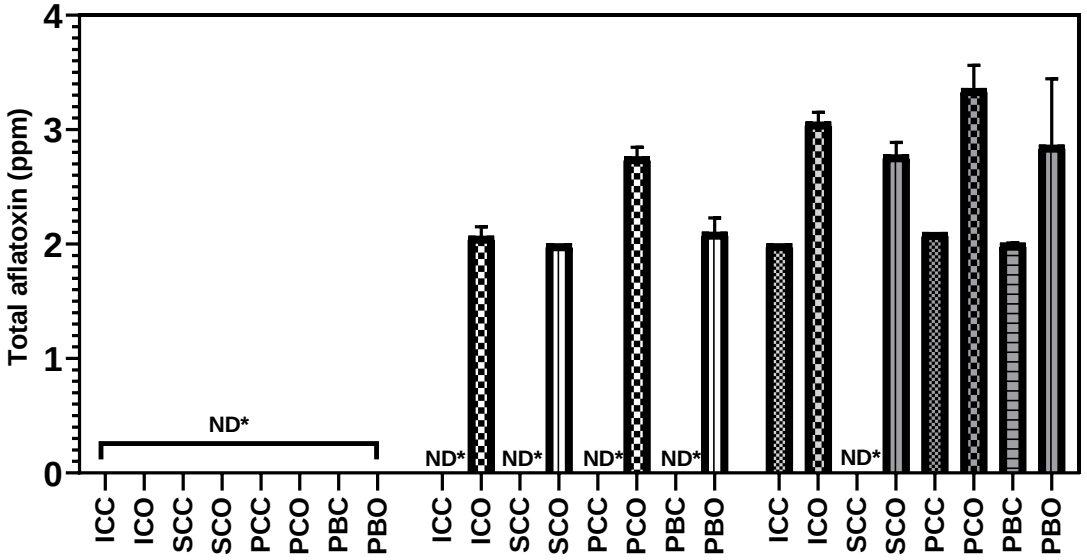


Figure 6. Aflatoxin concentration in various samples. ND* The sample has lower aflatoxin than the detection limit of the system (>2.000ppb).

IV. Conclusion

A total of 33 samples (1 fresh sample at day 1, 2 samples from each container on day 7 and day 30) were analyzed using the ELISA test. It is concluded from the results of ELISA test that SCC is the most reliable container to store the wheat flour for 30 days since it contains the aflatoxin less than 2 ppb during the study period while the PBO is most unsafe container to store the wheat flour as it contains the highest amount of aflatoxin contamination. However, none of the samples contains aflatoxin concentration more than the regulatory limits set by Indian standard. Statistical analysis revealed that the observed difference was not significant at P-value 0.05. Due to the important role of aflatoxin contamination, especially the negative impact of aflatoxin B1 on public health, efforts should be done to prevent the contamination, since prevention is the most economical and practical approach. In this study, humidity, temperature and storage duration were the most important factors associated with the aflatoxin contamination of the wheat flour. Hence, controlling environmental factors such as storage duration, temperature and humidity can help us with achieving this goal.

V. Limitations Of The Study

Findings of this study had some limitations. The first is the sample size and time limit. Further studies are required to analyze the effect of different seasons of Rajasthan on aflatoxin concentration with a greater number of samples to achieve statistically significant results. The second is the detection limit of kit because if the concentration in the fresh sample and not detected (ND) is taken 0, the mean difference becomes highly significant. Therefore, the sensitivity of the method used plays a great role in studying the aflatoxins.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

Acknowledgement

The authors are eternally grateful to the Head, Department of Zoology, University of Rajasthan (Jaipur) for providing all necessary support.

References

[1]. GAREIS M. Collection of occurrence data of Fusarium toxins in food and assessment of dietary intake by the population of EU member states. Rep Expert Particip SCOOP Task 3210-Part A Trichothecene [Internet]. 2003;13–235. Available from: https://ci.nii.ac.jp/naid/20001046845/en/

[2]. Ayalew A. Mycotoxins and surface and internal fungi of maize from Ethiopia. African J Food, Agric Nutr Dev (ISSN 1684-5358) Vol 10 Num 9. 2010;10.

[3]. Boutrif E. Prevention of aflatoxin in pistachios. Food, Nutr Agric. 1998;

[4]. Toteja GS, Mukherjee A, Diwakar S, Singh P, Saxena BN, Sinha KK, et al. Aflatoxin B 1 contamination in wheat grain samples

collected from different geographical regions of India: A multicenter study. J Food Prot. 2006;69(6):1463–7.
[5]. Trombete F, Moraes D, Porto Y, Santos T, Direito G, Fraga M, et al. Determination of Aflatoxins in Wheat and Wheat by-products Intended for Human Consumption, Marketed in Rio de Janeiro, Brazil. J Food Nutr Res. 2014;2:671–4.
[6]. Kumar P, Mahato DK, Kamle M, Mohanta TK, Kang SG. Aflatoxins: A Global Concern for Food Safety, Human Health and Their Management. Front Microbiol [Internet]. 2017 Jan 17;7:2170. Available from: https://pubmed.ncbi.nlm.nih.gov/28144235
[7]. An investigation on aflatoxin quantities in the prepared and processed autumn and spring wheat flour samples collected from 5 superior provinces of iran. 2014;4731:40–8.
[8]. Gallo A, Solfrizzo M, Epifani F, Panzarini G, Perrone G. Effect of temperature and water activity on gene expression and aflatoxin biosynthesis in Aspergillus flavus on almond medium. Int J Food Microbiol. 2015;217.
[9]. O'Brian G, Georgianna DR, Wilkinson JR, Yu jj, Abbas H, Bhatnagar D, et al. The effect of elevated temperature on gene transcription and aflatoxin biosynthesis. Mycologia. 2007;99:232–9.
[10]. Atehnkeng J, Ojiambo PS, Ikotun T, Sikora RA, Cotty PJ, Bandyopadhyay R. Evaluation of atoxigenic isolates of Aspergillus flavus as potential biocontrol agents for aflatoxin in maize. Food Addit Contam - Part A Chem Anal Control Expo Risk Assess. 2008;
[11]. Abdulmajeed A, Talib A, Bedair H. Detection of the Presence of Aflatoxins Type (B1) in Three Types of Stored Grain: Maize, Wheat, and Rice in Silos of Baghdad, Iraq. Egypt Acad J Biol Sci H Bot. 2015;6:1–7.

Ankita Soni, et. al. "Impact of different storage conditions on the level of aflatoxin contamination in wheat flour." *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, 14(5), (2021): pp 06-13.