Chemistry International 7(4) (2021) 224-229

International Scientific Organization
http://iscientific.org/
Chemistry International
www.bosaljournals.com/chemint/

Quantification of aflatoxins in dry fruits using ELISA with reference to standards for human consumption

Arif Nazir[1*], Muhammad Asif[1], Imran Kalim[2], Naveed Ahmad[3] and Munawar Iqbal[1]

[1]Department of Chemistry, The University of Lahore, Lahore, Pakistan
[2]Food and Biotechnology Research Centre, PCSIR Complex Laboratories, Lahore, Pakistan
[3]Department of Chemistry, Division of Science and Technology, University of Education, Lahore, Pakistan
*Corresponding E. mail: anmalik77@gmail.com

ARTICLE INFO

Article type:
Research article
Article history:
Received March 2021
Accepted August 2021
October 2021 Issue
Keywords:
Aflatoxins
Dry fruits
Food safety
Fungal growth
ELISA

ABSTRACT

The present research work is being executed on account of detection of aflatoxins B1, AFB2, AFG1 and AFG2 in dry fruits. Total 50 samples of dry fruits including dried figs, dried apricots, peanuts, almonds and dried dates were collected from different areas of Punjab, Pakistan and were analyzed for aflatoxin detection (AFs) by enzyme linked immunoassay technique (ELISA). The results showed that AFs were detected in 33% (n = 17) samples having range from 2.8-14 µg/kg in dry fruits, with an average limit 9.12 µg/kg. In 59% (n=10) samples of dried fruits the AFs limit was above the maximum limits, i.e., ML = 10 µg/kg as given by European Union (EU). Moreover, 41% (n=7) of the total detected samples having AFs limits below the maximum limit of aflatoxins as purposed by the EU. It is hereby concluded that the need is to launch an uninterrupted and rigid national supervising architectural plan in order to raise the standard and safety of dry fruits for human consumption.

Capsule Summary: The aflatoxins (B1, AFB2, AFG1 and AFG2) were monitored in dry fruits by ELISA and AFTs levels detected in fruits revealed contamination, which need to be monitored regular for human consumption.

Cite This Article As: A. Nazir, M. Asif, I. Kalim, N. Ahmad and M. Iqbal. Quantification of aflatoxins in dry fruits using ELISA with reference to standards for human consumption. Chemistry International 7(4) (2021) 224-229.
https://doi.org/10.5281/zenodo.6801794

INTRODUCTION

Aflatoxin is a standout amongst the most lethal moieties found in nature that can be harmful to people and different creatures (Gratz et al., 2007). These are considered as carcinogenic, hepatogenic, mutagenic, immunosuppressing and neoplastic (Varga et al., 2011a). Aflatoxin is considered as cancer-causing agent (Humans, 2002) indicated by International Agency for Research on Cancer (IARC). Different kinds of staple food items which may had the chance of getting contamination with aflatoxin including oilseed, spices (chilies, black pepper) cereal, tree nut, maize, groundnut (peanut, pistachio, fig, dried fruits), cheese, milk and dairy goods. Contamination of food items due to aflatoxin is a great concern regarding food quality and security (Hassan et al., 2017a; Iqbal, M et al., 2019; Kamran et al., 2018).

Mycotoxins, secondary metabolites, are produced by fungus during chemical or enzymatic reactions and largely affects human beings, animals and plants (Binder et al. 2007). Owing to mycotoxins contamination huge amount of crop spoils per year (Jubeen et al., 2020). Unfortunately, Pakistan is present in the temperate region which is most favorable growth of AFs producing fungi. IARC ranked AFs as 1A class human carcinogen (Cancer, 2002). AFs are formed by a specific genus of molds named *Aspergillus* (Gerbaldo et al.,

2012; Varga et al., 2011b). During inadequate storage and pre-harvest condition, their spores can spread and contaminate crops (Pitt, 2000). Amongst the many types of AFs, *Aspergillus parasiticus and Aspergillus nomius* produces Aflatoxin G_1 and G_2 (Molla et al., 2021). Many countries including Pakistan are being affected by aflatoxin contamination of staple food.

Aflatoxin contamination can occur during harvesting, drying, packing, transportation and reposition. The humid conditions, high temperature and heavy rainfall provide most favorable conditions for AFs production (Asghar et al., 2016). The AFs are reported in order of toxicity as AFM2 < AFM1 < AFG2 < AFB2 < AFG1 < AFB1 (Kabak et al., 2017). In tropical regions, like Pakistan, the pre-harvest conditions i.e. moisture and high temperature are mainly responsible for aflatoxin development (Zahir et al., 2007). The possibility of risk of fungal growth may enhanced by increasing storage time (Hassan et al., 2017b).

The European Union (EU) food law proposed the mycotoxins permissible limit for all aflatoxin in food is 5-10µg/kg (Commission et al., 2010). According to FDA & FAO, the aflatoxin limit in food commodities is 2 µg/kg (Food and Administration, 2010). Quantitative determination of AFs in food commodities can be achieved by techniques like TLC, HPLC, spectrometry, florescence, biosensors and enzyme linked immunosorbent assay (ELISA) (Hassan et al., 2017a; Iqbal, Munawar et al., 2019; Nazir et al., 2021; Nazir et al., 2019).

Typically, all types of AFs showed maximum absorption at 360 nm (Akbas et al., 2006). Based on the fluorescent property of AFs, they have been classified as "B" for blue (425 nm) and "G" for green-blue (450 nm). G toxins are more fluorescent than B toxin (Alcaide-Molina et al., 2009). It is noteworthy to control the production of AFs by suppressing mycotoxins growth (Banerjee and Sarkar, 2003). The inculcation of AFs into the blood through various mechanisms causes severe effects on humans and animals (Qazi and Fayyaz, 2006). Dry fruits are always at more risk of microbe's attack due to of its production process, poor collecting conditions and inadequate drying times. These are produced mostly in states with humid conditions, very high temperature, and heavy rainfall, so, leading to the appropriate conditions for the production of toxigenic molds and finally aflatoxin productions (Iqbal, Munawar et al., 2019).

The objective of this research work is to quantify the aflatoxin contamination in dry fruits (peanuts, dried apricots, dried dates, peanuts and almonds) collected from northern areas of Punjab, Pakistan.

MATERIAL AND METHODS

Sample collection

All the reagents and chemicals employed in this study were of analytical grade (Merck, Darmstadt, Germany). Around 50 dry fruits samples, i.e., *Ficus carica* (Dried Figs) and *Prunus armeniaca* (Dried apricots), *Prunus dulcis* (Almond), *Arachis hypogaea* (Peanut), *Phoenix dactylifera* (Dried Dates) were collected from different areas of Punjab (Sialkot, Chawinda, Pasrur, Daska, Sambrial, Narowal, Gujrat, Jhelum, Gujranwala, Muridkay, Kamoki, Lahore, Rawalpindi, Taxila, Islamabad, Murree, Kharian).

Sample preparation

According to the sampling methods the samples were collected, grinded and mixed thoroughly for extraction. The samples are stored at 35-46 °F (2-8 °C). In order to grind all the samples a grinder was used to get a homogeneity and sample representative. About 50 g of each sample took for the determination of AFs. All the grinded samples were preserved in the air tight polyethylene bags. 5 g of powdered sample is mixed with 25 ml of 70% methanol and the mixture is shaken for 3 minutes. Extract is filtered and filtrate is collected.

Enzyme Linked Immunosorbent Assay (ELISA)

Preparation of samples was preformed according to directives of test kit manual. Twenty-five milliliter methanol, water (70:30) was added to the 5 g of sample, and then the samples were shaken robustly for 3 minutes. The extract was clarified through filter paper and after that diluted along with distilled water (1:1). At the end, 50 ml of the diluted remainders per well were used in the test. The quantitative examination of AFB1 in the samples was done, based on the competitive enzyme immunoassay via RIDASCREEN® Aflatoxin B_1 30/15 test kit (R-Biopharm, Darmstadt, Germany). Sample preparation and ELISA tests were executed according to the technique described by R-Biopharm (Nazir et al., 2019).

A comparison is made with standard samples of aflatoxins. The ELISA detector was used to examine the samples. When the standard sample and test sample are overlying on one other it indicates the presence of aflatoxins in the sample. The intensity of standard sample is compared with fluorescent intensity of sample having aflatoxins. The volumes of the samples were noted. The sample amount should not be very small that it would create hindrance in order to obtain accuracy in reading. In case, if samples are so small, solution should be dilute and to get better results ELISA procedure should be repeated. The aflatoxins in the samples were estimated as shown in Eq. 1.

$$Estimation\ of\ Aflatoxin\,(mg/kg) = \frac{V_{stand} \times C_A \times V_{sol}}{W_{sample} \times V_{ext}} \quad (1)$$

Where, V_{stand} = Aflatoxins standard volume in ml of samples, C_A = Aflatoxins standard concentrations in mg/ml, $V_{ext.}$ = Sample extract volume in ml, V_{sol} = Solvents volume in mL need to dilute the final extracts, W_{sample} = The weight of original samples in 'g' having final extracts.

RESULTS AND DISCUSSION

Table 1: Summery of screening analysis of aflatoxin in dry fruits analyzed through ELISA

AFs Samples	Peanut	Almonds	Dried Figs	Dried Apricots	Dried Dates
AF Detected	B1	B1	B1	B1	B1
Samples	10	10	10	10	10
Contaminated Samples	4	3	3	4	3
Aflatoxins not Detected	6	7	7	6	7
Contamination Detected %age	40	30	30	40	30
Max (µg/kg)	13	12	12	13.5	14
Min (µg/kg)	5.2	4.5	3.8	2.8	4.3
EU Limits			10 µg/kg		

The samples of dry fruits were analyzed using Enzyme Linked Immunosorbent Assay (ELISA) for quantitative and qualitative analysis of aflatoxins. The ELISA technique was used to observe samples of dry fruits. Quantification of results represents the presence of AFs contamination in dry fruits samples including figs, apricot, almond, peanut and dates. About 34% of samples were found contaminated with Aflatoxin B1 (AFB1) whereas the remaining 66% samples of dry fruits were free from aflatoxin. The other types of AFs including AFB2, AFG1 and AFG2 was not observed in dry fruit samples during analysis. FDA allows the range 2-4 µg/kg in feed products and 2 µg/kg for all the food especially that is used for the human consumption. All samples exhibit aflatoxins contamination in the range of 2.8 µg/kg –14 µg/kg (Table 1). Fig. 1 shows the degree of aflatoxin contamination for dry fruits samples.

AFTs in peanuts

The samples of peanuts collected from different area of Punjab were subjected to analysis through ELISA. Table 2 shows the area from where the samples have been collected. Ten (10) samples of peanut were analyzed for AFs contamination. Only 4 samples were found contaminated with aflatoxin. The results signify that the peanut samples which were collected from Sambrial region having maximum value of aflatoxin, i.e., 13 µg/kg while the sample which was collected from Main Bazar Pasrur has minimum level of aflatoxin, i.e., 5.2 µg/kg. Out of the total four contaminated samples of peanut, 3 samples have aflatoxin beyond the permissible limit (10 µg/kg), while one sample has aflatoxin below the permissible limit of dry fruits.

AFTs almonds

The ELISA technique was applied to the samples of Almond collected from different areas of Punjab to investigate AFs contamination. Out of 10 samples of almond only three samples were found contaminated with aflatoxin. Two samples have aflatoxin beyond the permissible limit while one has below the permissible limit (Table 2). The sample which was collected from Satellite Town Gujranwala has maximum level of aflatoxin, i.e., 12 µg/kg and the one from Main Bazar Kamoke has minimum level of aflatoxin, i.e., 4.5 µg/kg.

AFTs in dried figs

Dried figs samples were collected from different areas of Punjab and were subjected to ELISA for analysis. Only three samples out of 10 were found contaminated with aflatoxin. Two samples have aflatoxin beyond the permissible limit 10 µg/kg whereas one has below the permissible limit. The samples of dried figs which was collected from Kahna Lahore has maximum level of aflatoxin, i.e., 12 µg/kg and the sample which was collected from Main Market Gulberg has minimum level of aflatoxin, i.e., 3.8 µg/kg (Table 2).

AFTs in dried apricot

For screening analysis of dried apricot, the samples were collected from different regions of Punjab, i.e., Lahore and Rawalpindi to detect aflatoxin. Only three samples showed aflatoxin in which showed beyond the permissible limit (10 µg/kg) while one has below the permissible limit. The samples of dried apricot which was collected from F-9 Market Islamabad has maximum level of aflatoxin i.e., 13.5 µg/kg and the sample which was collected from main bazar Taxila has minimum level of aflatoxin, i.e., 2.8 µg/kg.

AFTs in dried dates

The ELISA technique was applied on the samples of dried dates collected from different locations of Punjab to detect the aflatoxins. Ten Samples of Dried Dates were collected from Murree, Kharian and Gujrat. Two samples have aflatoxin beyond the permissible limit (10 µg/kg) while two have below the permissible limit. The samples of dried dates which were collected from Al-Fareed Market Murree Cantt showed maximum aflatoxin, i.e., 14 µg/kg and the sample which was collected from Chinar Chowk Murree Cantt has minimum level of aflatoxin, i.e., 4.3 µg/kg. EU gave tolerant limit of aflatoxin 10 µg/kg for dried fruits, for spices 50 µg/kg and FDA permits 20-300 µg/kg for other processed commodities of corn used in feed and for all food 20 µg/kg for human consumption especially (FDA).

Table 2: Aflatoxins contamination in dried fruits collected from different regions

Sr. #	Samples Location	Samples	Aflatoxin concentration (µg/kg)
1	Lahai Bazar Sialkot	Peanut	10.7
2	Gohadpur Sialkot		Not Detected
3	Main Bazar Sialkot		Not Detected
4	Tehsil Bazar Sailkot		Not Detected
5	Cantt Sialkot		Not Detected
6	Main Bazar Chawinda		11.5
7	Main Bazar Pasrur		5.2
8	Fawara Chowk Daska		Not Detected
9	Main Bazar Daska		Not Detected
10	Sambrial		13
11	Narowal	Almond	Not Detected
12	Katchehri Road Wazirabad		Not Detected
13	Sheikhpur Gujrat		Not Detected
14	Raja Bazar Jehlum		10.5
15	Tehsil Bazar Gujranwala		Not Detected
16	Stalite Town Gujranwala		12
17	Main Bazar Kamonki		4.5
18	Salamatpura Muridke		Not Detected
19	Badami Bagh Lahore		Not Detected
20	Barket Market Lahore		Not Detected
21	Liberty Market Lahore		Not Detected
22	Main Market Lahore		3.8
23	Ichra Bazar Lahore	Dried figs	Not Detected
24	Moon Market Lahore		Not Detected
25	Township Lahore		Not Detected
26	Kahna Lahore		12
27	Sadar Rawalpindi		11.7
28	College Road Rawalpindi		Not Detected
29	Laiqat Bagh Rawalpindi		Not Detected
30	Faizabad Rawalpindi		Not Detected
31	Main Bazar Taxila		2.8
32	HMC Markets Taxila	Dried apricot	Not Detected
33	Aslam Market Wah Cantt		Not Detected
34	Jinnah Super Market Islamabad		Not Detected
35	Aabpara Market Islamabad		Not Detected
36	F-9 Market Islamabad		13.5
37	Main Stop Rawat		3.6
38	Kalar Syedan Rawalpindi		Not Detected
39	Khana Stop Market Islamabad		12.3
40	Mall Road Murree		Not Detected
41	Main Bazar Old Murree		Not Detected
42	Al-Fareed Market Murree Cantt		14
43	GPO Chowk Murree Cantt		Not Detected
44	Chinar Chowk Murree Cantt		4.3
45	Athal Chowk Islamabad	Dried dates	Not Detected
46	Taili Mohala Murree Road		Not Detected
47	Naseera Bazar Kharian		9.8
48	Mini Market Kharian		Not Detected
49	Zaib Market Gujrat		Not Detected
50	Allah Lok Market Gujrat		Not Detected

The results represent the contamination of dried Figs having values 3.8, 11.7 and 12 µg/kg. Aflatoxin B1 was found in dried apricot samples were 2.8, 3.6, 12.3 and 13.5 µg/kg in commodities. Findings revealed that the dry fruits stored for longer period should be monitored of aflatoxin contamination to avoid the health hazardous related to aflatoxins.

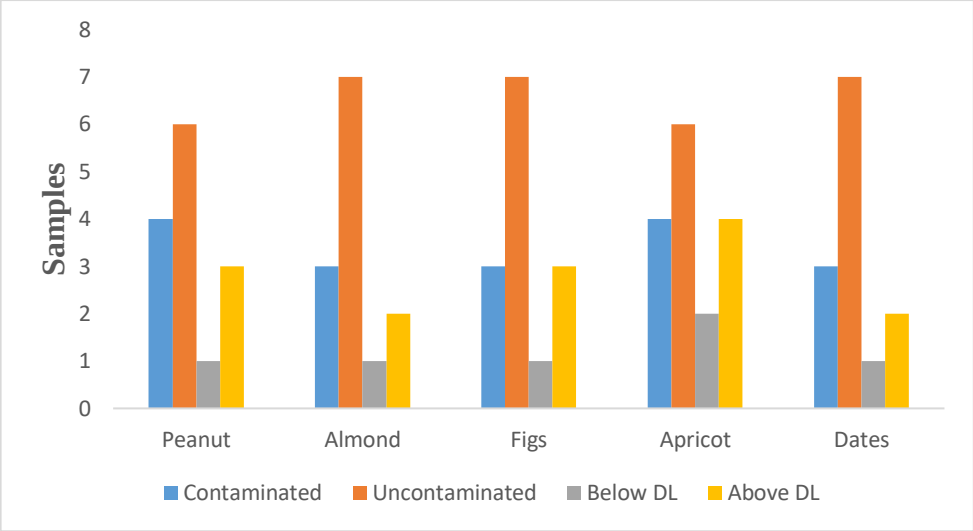

Fig. 1: Aflatoxin contamination in dry fruit samples

which 12.3 and 13.5 are beyond the European standard limits. AFBI was found in almond samples were 4.5, 10.5 and 12 µg/kg, among these values 10.5 and 12 µg/kg were exceeded the limit purposed by European standard. In peanut samples AFBI was found 5.2, 10.7, 11.5 and 13 µg/kg, in which three values are beyond the European limit. 10 µg/kg in food is the permissible limit according to FDA. In dried dates detection of aflatoxin was found having range 4.3-14 µg/kg. Although, many samples of dry fruit containing aflatoxin within the range given by FDA and U.S. About 33 samples of dry fruits have no aflatoxin at all. Only 17 samples of dry fruits contain aflatoxin. Among all the contaminated sample, there were 10 samples having aflatoxins beyond the permissible limits and there were 07 samples having aflatoxins within limits given by EU for human being that are below 10 µg/kg for dry fruits.

CONCLUSION

Following conclusions have been drawn. Fifty samples of dry fruits were collected from different areas of Punjab (Pakistan) and were analyzed through ELISA technique. Seventeen samples were found contaminated with aflatoxin having limit from 2.8-14 µg/kg µg.kg^{1} in the samples of dry fruits while 33 samples are free from aflatoxin. Ten samples of dry fruits have aflatoxin beyond the permissible limit, i.e., 10 µg/kg which is given by EU while seven samples have aflatoxin below the permissible level. Moreover, need of hour is that more and more measures should be conducted on to avoid AFs contamination and research should be carried on to find the detoxification methods for AFs present in different food

REFERENCES

Akbas, M.Y., Ozdemir, M.J., 2006. Effect of different ozone treatments on aflatoxin degradation and physicochemical properties of pistachios. Journal of the Science and Food Agriculture 86(13), 2099-2104.

Alcaide-Molina, M., Ruiz-Jiménez, J., Mata-Granados, J., de Castro, M.L.J., 2009. High through-put aflatoxin determination in plant material by automated solid-phase extraction on-line coupled to laser-induced fluorescence screening and determination by liquid chromatography–triple quadrupole mass spectrometry. Journal of Chromatography A. 1216(7), 1115-1125.

Asghar, M.A., Zahir, E., Rantilal, S., Ahmed, A., Iqbal, J., 2016. Aflatoxins in composite spices collected from local markets of Karachi, Pakistan. Food Additives and Contaminants. 9(2), 113-119.

Banerjee, M., Sarkar, P.K., 2003. Microbiological quality of some retail spices in India. Food Research International 36(5), 469-474.

Cancer, I.A.R., 2002. IARC handbooks of cancer prevention. The Agency.

Commission, E.P., Medicines, E.D., Healthcare, 2010. European pharmacopoeia. Council of Europe.

Food, U., Administration, D., 2010. Code of federal regulations.

Gerbaldo, G.A., Barberis, C., Pascual, L., Dalcero, A., Barberis, L., 2012. Antifungal activity of two Lactobacillus strains with potential probiotic properties. FEMS Microbiology Letters 332(1), 27-33.

Gratz, S., Wu, Q., El-Nezami, H., Juvonen, R., Mykkänen, H., Turner, P., 2007. Lactobacillus rhamnosus strain GG

reduces aflatoxin B1 transport, metabolism, and toxicity in Caco-2 cells. Applied and Environmental Microbiology 73(12), 3958-3964.

Hassan, S.M., Iqbal, M., Bokhari, T.H., Nisar, N., Tahir, M.A., Abbas, M., Kanwal, Q., Iqbal, D.N., Nazir, A., 2017a. Fungal infestation and aflatoxins synthesis control in stored poultry feed using medicinal plants. Environmental Technology and Innovation 7, 194-202.

Hassan, S.M., Sultana, B., Iqbal, M., Naz, S., Abbas, M., 2017b. Anti-aflatoxigenic activity of Punica granatum and Ziziphus jujuba leaves against Aspergillus paraciticus inoculated poultry feed: Effect of storage conditions. Biocatalysis and Agricultural Biotechnology 10, 104-112.

Humans, IARC, 2002. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC monographs on the evaluation of carcinogenic risks to humans 82, Lyon France.

Iqbal, M., Abbas, M., Adil, M., Nazir, A., Ahmad, I., 2019. Aflatoxins biosynthesis, toxicity and intervention strategies: A review. Chemistry International 5(3), 168-191.

Iqbal, M., Abbas, M., Nisar, J., Nazir, A., 2019. Bioassays based on higher plants as excellent dosimeters for ecotoxicity monitoring: A review. Chemistry International 5(1), 1-80.

Jubeen, F., Sher, F., Hazafa, A., Zafar, F., Ameen, M., Rasheed, T., 2020. Evaluation and detoxification of aflatoxins in ground and tree nuts using food grade organic acids. Biocatalysis and Agricultural Biotechnology 29, 101749.

Kabak, B., Dobson, A.D.J. 2017. Mycotoxins in spices and herbs–an update. Critical Reviews in Food Science and Nutrition 57(1), 18-34.

Kamran, M., Khan, M.R., Khan, H.U., Abbas, M., Iqbal, M., Nazir, A., 2018. Phytochemical and cytotoxic evaluation of Medicago monantha: In vivo protective potential in rats. Biomedicine and Pharmacotherapy 102, 1052-1063.

Molla, M.M., Kamal, M.M., Sabuz, A.A., Chowdhury, M.G.F., Haque Khan, M.H., Khatun, A., Miaruddin, M., Zashimuddin, M., Islam, M.M., 2021. Chemical composition, bioactive compounds, antioxidants potential and mycotoxin of minor exotic archidendron pauciflorum fruit with the focus to Bangladesh. Biocatalysis and Agricultural Biotechnology 34, 102039.

Nazir, A., Kalim, I., Imran, M., Bilal, M.A., Zahra, N., Ahmad, A., Iqbal, M., Fazal, U., 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), 1949-1954.

Nazir, A., Kalim, I., Sajjad, M., Usman, M., Iqbal, M., 2019. Prevalence of aflatoxin contamination in pulses and spices in different regions of Punjab. Chemistry International 5(4), 274-280.

Pitt, J.J. 2000. Toxigenic fungi and mycotoxins. British Medical Bulletin 56(1), 184-192.

Qazi, J.I., Fayyaz, Z.J.M., 2006. Aflatoxin contaminated foods and health risk perspective for Pakistani population. Mycopath 4(2), 27-34.

Varga, J., Frisvad, J.C., Samson, R., 2011a. Two new aflatoxin producing species, and an overview of Aspergillus section Flavi. Studies in Mycology 69, 57-80.

Varga, J., Frisvad, J.C., Samson, R.J. 2011b. Two new aflatoxin producing species, and an overview of Aspergillus section Flavi. Studies in Mycology 69, 57-80.

Zahir, Z., Naveed, M., Zafar, M., Rehman, H., Arshad, M., Khalid, M., 2007. Evaluation of composted organic waste enriched with nitrogen and L-tryptophan for improving growth and yield of wheat (Triticum aestivum L.). Pakistan Journal of Botany 39(5), 1739-1749.