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Quantitative estimation of aflatoxin and pesticide residues from turmeric (*curcuma longa*) as obtained in the selected area of Chamarajanagara and Mysuru districts, Karnataka

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Abstract
Chamarajanagar and Mysuru are the major turmeric (*curcuma longa*) cultivated districts in Karnataka state. The present study was carried out in the different villages such as Badanaguppe, Kariyanakatte, Doddarayapete, Jyothidowdanapura, and Hebbasuru villages of Chamarajanagar district and Nagawala, Kodi, Sannegowdacolony, Belikere and Yelwala villages of Mysore district, Karnataka. During the cultivation of turmeric the farmers are used the pesticides in each steps, for high yielding purpose. This study was revealed that to measure the amount of pesticides present in the final product. Aflatoxin is produced by the fungi aspergillusflavus and it having properties of nephrotoxic, teratogenic, carcinogenic, and immunosuppressive. It can be determined by the reliable method like HPLC (column symmetry C-18; 4.6X250 mm) used with detection by florescence (excitation 364 nm, emission 465 nm). Like that the organophosphors also determined by GC (column summery RTX-5; 30m) the level can be indicated in ppb and ppm respectively.

Keywords: Turmeric, aflatoxin, organophosphorous, HPLC, GC

Introduction
Turmeric *(Curcuma longa),* "golden spice" as well as the "spice of life." derived from the rhizomes of the plant cultivated and widely used as coloring agent is most extensively in India, followed by Bangladesh, China, Thailand, Cambodia, Malaysia, Indonesia, Philippines and South East Asia. India is the largest producer, consumer and exporter of turmeric. (Duggi, *et al*., 2013) [9].

Turmeric is a medicinal plant which belongs to family Zingiberacea (Chattopadhyan, *et al*., 2004)[6]. Curcuminoids component present in the turmeric include mainly curcumin (diferuloylmethane, demethoxycurcumin, and bismethoxycurcumin) (chainnawu, 2003)[5] it play an important role in the biological activity of turmeric. Curcumin, having medicinal properties such as antioxidant, anti - inflammatory, anti - platelet, cholesterol lowering, antibacterial and antifungal activity (Peter, 2000) [14] it can also act as a scavenger of oxygen free radicals it protect hemoglobin from oxidation (Unnikrishnan, 1995) [16] apart from that the turmeric used in Ayurveda, Unani, Siddha, and household remedies for the treatment of anorexia, cough, rheumatism and intestine disorder. Sprains and swelling caused by injury (Ammon, *et al*., 1991) [1].

The leaves are known as great source of vitamin and minerals (Chattopadhyan, *et al*., 2004) [6]. Turmeric is useful in the treatment of measles. Turmeric roots are dried in the sun and ground to a fine powder. Mixed with a few drops of honey and the juice of few bitter gourd leaves can be taken by those suffering from measles (Bhowmik, *et al*., 2009) [4]. Turmeric contains chemical substances such as curcuminoids altatone, bisdemethoxycurcumin, dimethoxycurcumin, diaryl heptanoids, and tumerone act as anticarcinogen (Guddadarangavvanahally, *et al*., 2000) [12] Several studies mentioning that the spices contain pesticide residues and their harmful effect on human health have been reported (C*haturvedi, et al., 2013*) [7]. Another contaminant associated with turmeric is aflatoxin. Aflatoxins are produced by organisms belonging to genus *aspergillus* (Yang, *et al.*, 2011) [18].

Methodology

Collection of samples

Samples were obtained from farms of different villages of Chamarajanagar and Mysuru district. The turmeric (*Curcuma longa*) samples were collected from the various locations and transferred immediately into zip polythene bags. Samples were stored in deep freezer (-20°C).

Chemicals/materials used

Acetone, petroleum ether, Dichloro methane. Diethyl ether, Sodiumsulphate, Florosil, Sodium chloride, Teen 20, Methanol, Potassium bromide, Nitric acid, Acetonitril, Butter paper, Flutted filter paper, Micro fiber filter paper, IAC (immune affinity column) and HPLC water were of HPLC grade and procured locally.

Analysis of Aflatoxin

Samples were weighed in to blender jar, add NaCl along with methanol and HPLC water in the ratio of 80:20, cap the blender jar with parafilm blend at high speed for one min. Then content was filtered it in to a beaker through fluted filter paper. Then 10 ml of filtrate was taken in to 50ml graduated cylinder and 40 ml of 20% tween20 solution was added. Again, the contents were filtered into graduated cylinder through a glass fiber filter in to 250ml beaker and this filtrate was used for the aflatest column.

Immuno-Affinity Column Clean-Up

The aflatest-P columns were attached to the pump stand and 10 ml of filtrate was pipetted to the syringe and passed through the immune-affinity column, the entire sample was passed through the column and the column was rinsed with 10 ml of HPLC water twice. And 20 ml stoppered test tube was placed under the tip of the column and 1 ml of methanol was added to the column. All the methanol eluent was collected in the test tube and transferred to a sample vial. Now the sample was ready for injection to the HPLC. The sample was eluted and chromatograms obtained.

Stock and working standard of organo phosphorous pesticides of appropriate concentration in acetone *(standard from EPA of equivalent)*were prepared. The standards were stored under refrigeration. *(Shelf life for stock standard is one year and that for working standard is six months)*

- All the glass wares were rinsed thoroughly first with acetone and then with petroleum ether before starting the analysis.
- A reagent blank along with sample solutions were prepared.
- **Sample preparation:** The entire sample was reduced by mixing and quartering and a subsample of 100g taken for crushing/blending. A representative sample of 20g (in duplicate) was taken for analysis.

Extraction

The weighed samples were transferred to a 500 ml stoppered conical flask and freshly prepared acetone-water mixture(225ml-125ml) was added and kept in orbital shaker for 1 hour with occasional shaking then the content were filtered through Whatman No.1 filter paper in a Buchner funnel, using vacuum pump. Solution was shaken well and 100 ml of filtrate was transferred to the 250ml separating funnel. Fifty ml of petroleum ether was added first and then 50ml methylene dichloride and content were shaken vigorously for about 2 minutes and then allowed to stand for the separation of layer. After clear separation of layer, the lower layer was transferred to another 250ml separating funnel. To this was added 10 ml MDC and shaken well and allowed to stand. Upper layer was transferred to a beaker after passing through a funnel containing anhydrous sodium sulphate which was supported by glass wool pre wetted with petroleum ether. After the separation of layer, the lower layer of the second separating funnel was collected to the same beaker by passing through the same funnel containing sodium sulphate. The prepared solution was transferred quantitatively to rotary evaporator flask/water bath and concentrate to minimum volume. This is the ready sample for clean-up.

Florosil clean-up for organo phosphorous

The activated florosil (about 4") were placed in the column and about 0.5" sodium sulphate wasadded. Pre wet the column with 50 ml petroleum ether. The concentrate is transferredto sintered column using 8ml HPLC grade acetone, and walls of chromatographic tube wererinsedwith additional small portion of acetone. This was drained with 100 ml of petroleum ether and collected in a 500 ml beaker. Column was elutedat about 5ml/minute with 200ml diethyl ether-petroleum ether (50:50). The eluentwas collectedin the beaker containing petroleum ether. The eluent is now ready for concentration step for organo phosphorous pesticides by rotary evaporator/water bath. The above extract was concentratedto minimum volume by using K.D. evaporator and the content rinsedwith acetone and transferred to a graduated stopped test tube and was again were made up with to 4ml of acetone (Fssai, 2015)[10]. This is ready for GC analysis. (FAO and EU, 2008)[2, 3].

Calculation

$$\text{Aflatoxin(Conc.)} = \frac{PG\ (Conc.)}{20\mu l} \times \frac{1000X1\mu l}{10ml} \times \frac{50ml}{10ml} \times \frac{100ml}{25mg \times 10^3}$$

$$\text{OP(Conc.)} = \frac{PG\ (Conc.)}{2\mu l} \times 1000 \times \frac{6.0\mu l}{100ml} \times \frac{350ml}{20\mu g} \times \frac{1}{10^6}$$

$$\text{OP(Conc.)} = \text{PG(Conc)} \times \frac{0.525}{10^3}$$

Analysis of aflatoxin by HPLC

Table 1: The retention time, area under curve and concentration of different types of aflatoxin in known standard

Peak	Type of Aflatoxin	Ret. Time (min.)	Area under curve (mA)	Area%	Conc.
1	G2	7.067	264859	11.573	20.000
2	G1	8.114	660603	28.866	80.000
3	B2	8.916	445142	19.451	20.000
4	B1	10.402	917926	40.110	80.000
Total			2288527	100.000	

The aflatoxin content in turmeric samples was detected in the samples were collected from Badanaguppe, Doddarayapete, Jyothigowdanapura villages of chamarajanagar and Nagawala village of Mysuru districts as observed peaks in chromatogram and represented in tables 2 to 5.

Table 2: Retention time, area under curve and concentration of different types of aflatoxin in sample obtained from Badanaguppe

Peak	Name	Ret. Time (min.)	Area	Area%	Conc.
1	B1	10.721	222779	100.000	9.731 (0.97ppb)
Total			222779	100.000	

Table 3: Retention time, area under curve and concentration of aflatoxin in sample obtained from Doddarayapete

Peak	Type of Aflatoxin	Ret. Time (min.)	Area under curve (mA)	Area%	Conc.
1	B1	18.664	415043	100%	11.554 (1.15ppb)
Total			415043	100%	

Table 4: Retention time, area under curve and concentration of different types of aflatoxin in sample obtained from Jyothigowdanapura village

Peak	Type of Aflatoxin	Ret. Time (min.)	Area under curve (mA)	Area%	Conc.
1	G1	13.331	265074	41.226	7.562(0.75ppb)
2	B1	18.775	377898	58.774	10.511(1.05ppb)
Total			642972	100%	

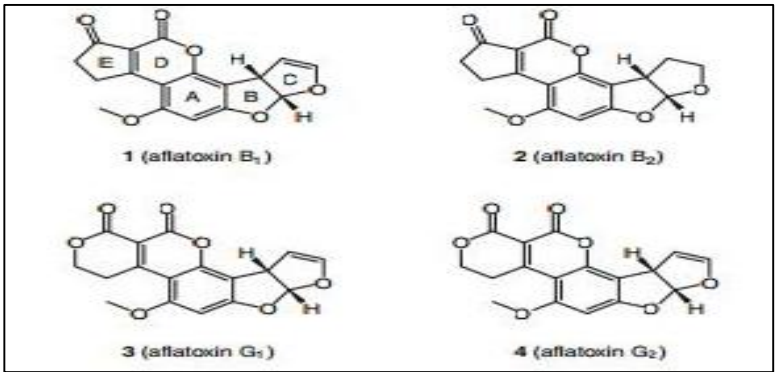
Table 5: Retention time, area under curve and concentration of aflatoxin in sample obtained from Nagawala village

Peak	Name	Ret. Time (min.)	Area	Area%	Conc.
1	B1	11.148	32548	100%	1.580(0.15PPb)
Total			32548	100%	

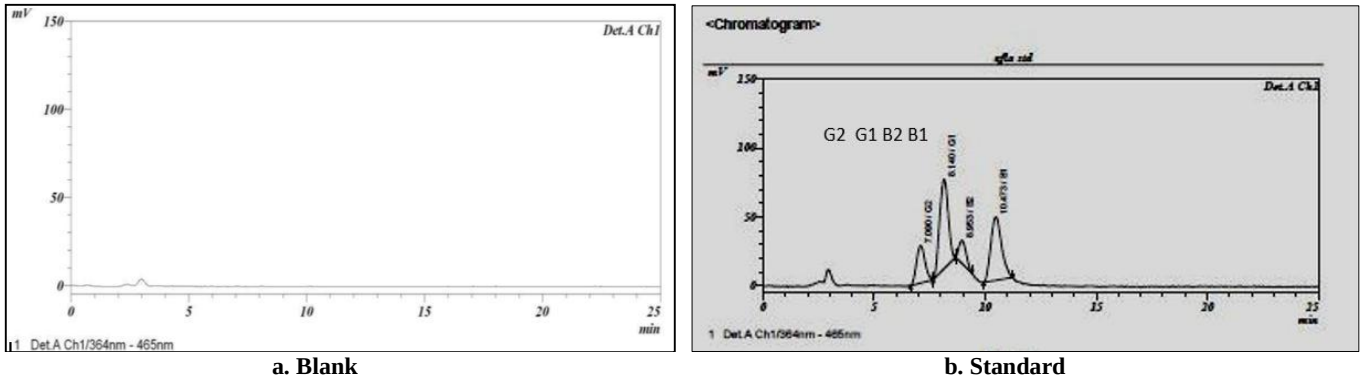
Table 6: Maximum residual level (MRLs) in spices for Aflatoxin suggested by EU standards

S. No	Chemical compounds	EU Standards
1	Afla –B1	< 5ppb
2	Afla –B2	Total MRLs level from all four compound is < 10ppb
3	Afla –G1	
4	Afla –G2	

Chemical structures of the different aflatoxins (Ismail, *et al*., 2015) [13]

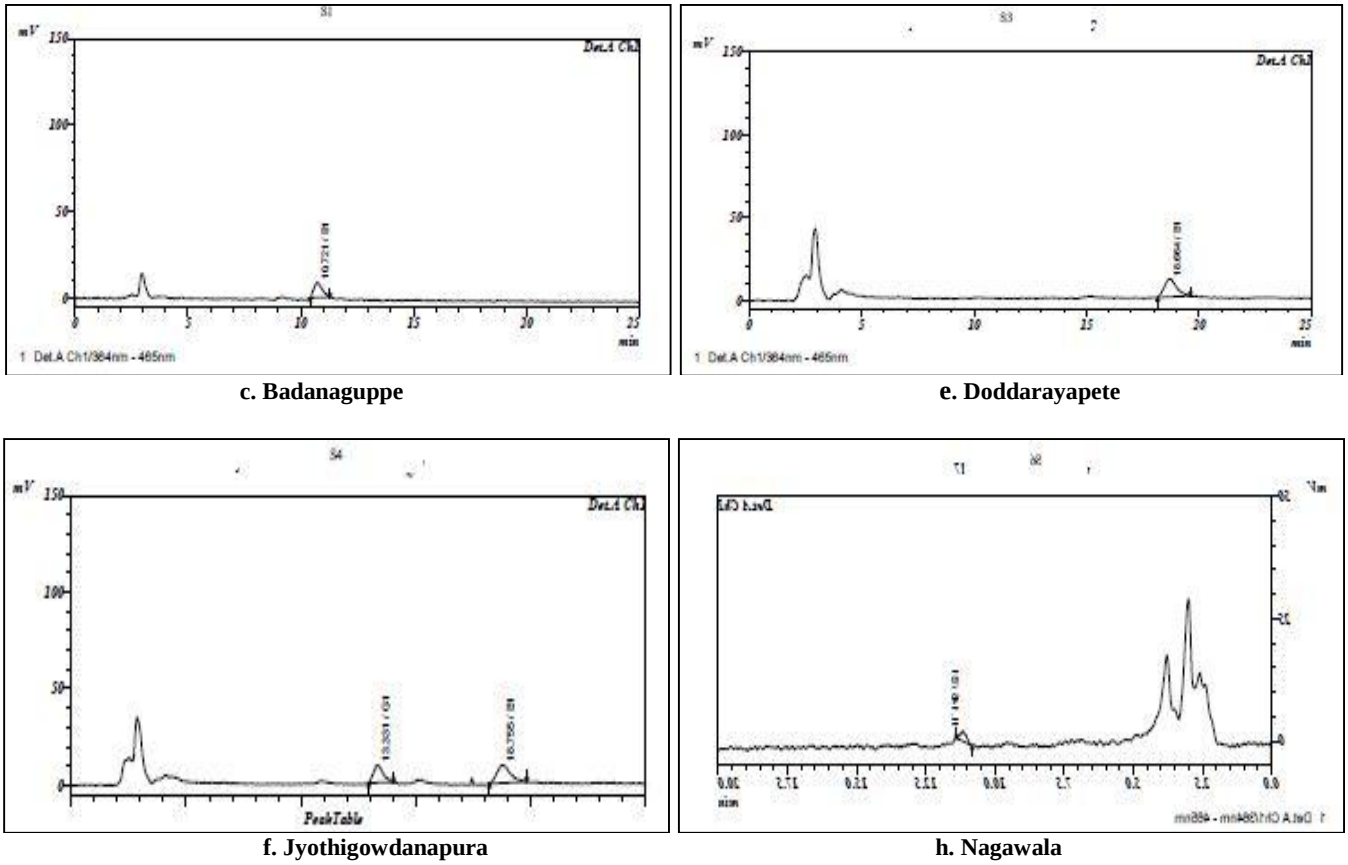


The following chromatograms depict the results obtained for the samples that were subjected to the test for aflatoxins. While the figure 2.1 shows the chromatograms for blank and the reference. Figures 2.2 shows the chromatograms for the test samples. Aflatoxin was detected in the samples collected from Badanaguppe, Doddarayapete, Jyothigowdanapura villages of chamarajanagar district and Nagawala village of Mysuru district evidenced by the presence of specific peaks in chromatogram.



a. Blank **b. Standard**

Fig 2.1: The chromatograms for Aflatoxin a) Blank, b) Standard showing peaks for different types of aflatoxins (G2, G1, B2, B1) employed in the study



c. Badanaguppe **e. Doddarayapete**

f. Jyothigowdanapura **h. Nagawala**

Fig 2.2: The chromatograms for Aflatoxin for samples collected from the Badanaguppe, Doddarayanapete, Jyothigowdanapura villages of Chamarajanagar district and Nagawala village of Mysuru district

Organo phosphorous analysis by GC

Majority of organophosphorus pesticides are liquids and have different vapor pressures at room temperature. The compounds used for agricultural purposes are available mainly as emulsifiable concentrates or wettable powder formulations for reconstitution as liquid sprays, and also as granules for soil applications. Use of organophosphorous during the agricultural practices leads to the negative effect on human health (WHO 2003)[17]. Therefore, it is important to use accurate and effective method for quantification of organophosphorous.

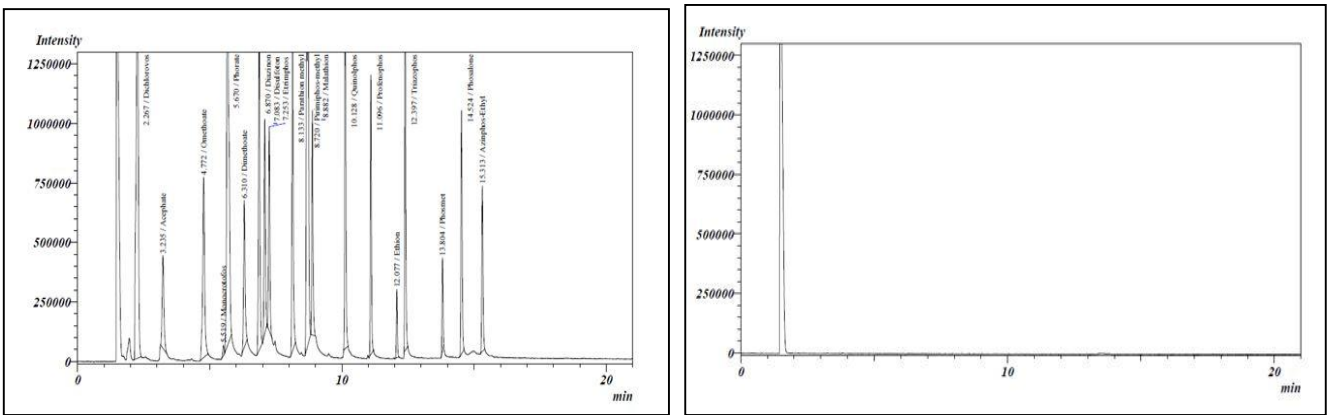
The standards of known concentrations, as per the ASTA method were run which served as the reference to compute the concentrations of the organophosphorous (Fukoto 1990) [11] in samples. Table 7 depicts the standard used in the study

Table 7: The retention time, area under curve and concentration of organophosphorous in known standard

Peak	Ret. Time (min)	Name	Area (mA)	Height	Conc.	Units
1	2.267	Dichlorovos	9179844	1568984	2000.00	pG
2	3.235	Acephate	1816787	389424	4000.00	pG
3	4.772	Omethoate	3891511	756206	4000.00	pG
4	5.519	Monocrotofos	141960	44365	4000.00	pG
5	5.670	Phorate	10316861	1888548	2000.00	pG
6	6.310	Dimethoate	2394844	611262	2000.00	pG
7	6.870	Diazinon	5129908	1414095	2000.00	pG
8	7.083	Disulfoton	3150731	900220	2000.00	pG
9	7.253	Etrimphos	2821356	850826	2000.00	pG
10	8.133	Parathion methyl	525406	1626396	2000.00	pG
11	8.720	Pirimiphos methyl	9816919	1781049	2000.00	pG
12	8.882	Malalthion	2870442	938102	2000.00	pG
13	10.128	Quinolphos	5806111	2071751	2000.00	pG
14	11.096	Profenophos	3184702	1170602	2000.00	pG
15	12.077	Ethion	749567	284785	1000.00	pG
16	12.397	Triazophos	4486193	1655339	2000.00	pG
17	13.804	Phosmet	1026677	389577	2000.00	pG
18	14.524	Phosalone	3009899	1017151	2000.00	pG
19	15.313	Azinphosethyl	2254325	691752	2000.00	pG
Total			77302702	20050434		

The organophosphorous content in turmeric (*Curcuma longa*) samples was not detected as evidence by the absence of peaks in chromatogram.

The following figure 3.1 shows the chromatograms for blank and the reference of organo phosphorous compounds.



a. Standard **b. Blank**

Fig 3.1: The chromatograms for organo phosphorous a) Blank, b) Standard showing peaks for different types of organo phosphorous employed in the study

Plate 1: Disease affected leaf of turmeric plant observed in one of the farms visited

Plate 2: Common pesticides used for turmeric crop by the farmers in Mysuru and Chamrajnagar districts

Plate 3: Turmeric rhizomes being boiled on farm after harvest

Plate 5: HPLC equipment employed for the estimation of aflatoxin content in turmeric crop in the study

Plate 4: Turmeric samples used for estimating the pesticide residues in the study

Plate 6: Gas Chromatography equipment used for analyzing organophosphorous content of turmeric samples in the study

Results and Discussion

Aflatoxins are toxic and cancer causing chemical that are produced by certain molds such as *Aspergillus flavus* and *Aspergillus parasiticus* and cause acute and chronic diseases in humans and other animals. The international agencies have now permitted the presence of 20ppb of aflatoxin in food materials as the maximum permissible level (Dhanasekekarn, *et al.*, 2011)[8]. Therefore, the aflatoxin (G2, G1, and B2, B1) green and blue fluorescence content in the collected samples were analyzed.

The result in the present study shows the presence of B1 in the samples collected from Badanaguppe, Doddarayapete, Jyothigowdanapura villages of Chamarajanagar districts and G1 present in sample collected from jyothi gowdanapura village, based on the retention time. Even as per the European standard the presence of aflatoxin in the respective samples was in acceptable range. Hence, the collected samples from the different villages of Chamarajanagar and Mysore district were considered safe with respect to their aflatoxin content.

Organophosphorus (OP) is the general name for organic derivatives of phosphorus. OP compounds are usually esters, amides, or thiol derivatives of phosphoric, phosphonic, phosphinic, or thiophosphoric acids with two organic and additional side chains such as cyanide, thiocyanate, and phenoxy group (Soltaninejad, *et.al.*,2014)[15].

In this study nineteen chemical compounds of organophosphorous were quantitatively estimated in each sample by ASTA method. Samples were collected from different villages of Chamarajanagar and Mysore district. In all samples the results showed absence of peaks representing different organophosphorous in chromatograms. Thus, samples were considered to be free from the harmful organophosphorous chemicals.

Conclusion

The quantitative estimation of aflatoxins and pesticide (Organo phosphorous) of samples collected from different villages of Chamarajanagar and Mysore district, Karnataka revealed that the pesticides and aflatoxin studied were within the EU limits. Hence, it is indicative of good agricultural, storage and marketing practices.

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