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Jayarajavarma B
Centre for Plant Protection studies, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Kamalakannan A
Centre for Plant Protection studies, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Paranidharan V
Centre for Plant Protection studies, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Gopalakrishnan C
Centre for Plant Protection studies, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Sivakumar U
Centre for Plant Protection studies, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Uma D
Centre for Plant Protection studies, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Kannan K
Centre for Plant Protection studies, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
Jayarajavarma B
Centre for Plant Protection studies, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Detoxification of fumonisin by *Lippia nodiflora* (L.): An Indigenous medicinal plant

Jayarajavarma B, Kamalakannan A, Paranidharan V, Gopalakrishnan C, Sivakumar U, Uma D and Kannan K

Abstract
Fumonisins are the group of secondary metabolites produced by a fungi *Fusarium verticillioides* which often contaminate foods and most potent naturally occurring carcinogen. Fumonisins are highly stable molecules which are extremely difficult to remove of destroy once formed in a commodity. In the present study, to explored the potential of some indigenous medicinal plants in the detoxification of fumonisin in *vitro*. Of the various plants extracts tested, the methanolic extract (1%) obtained from the leaves of *Lippia nodiflora* (L.,) Verbenaceae showed the maximum degradation of fumonisin. The methanol extract of L.*nodiflora* showed significant levels of detoxification of other Fumonisins B2 also. Maximum detoxification decreased at pH7 and pH3. Time course study of fumonisin detoxification by *L.nodiflora* extract showed that degradation of fumonisin occurred within 10 min and the percentage of degradation gradually increased with increase in incubation time.

Keywords: Fumonisin, *Lippia nodiflora*, detoxification, medicinal plant

Introduction
Fumonisins are Mycotoxins produced mainly by the fungus *Fusarium verticillioides* a primary fungal contaminant of Maize and maize based products throughout the world. (Shephard *et al*., 1996) [10]. It was first discovered in South Africa in 1988 (Gelderblom *et al*., 1988; Marasas *et al.,* 2001) [5] FB1 is the most commonly found, not only in maize and maize-based foods, but also in beer, rice, sorghum, triticale, cowpea seeds, beans, soybeans and asparagus. FB1 can cause two diseases in farm animals. *i.e* leucoencephalomalacia and porcine pulmonary oedema in horses. It is also carcinogenic, hepatotoxic, nephrotoxic and embryotoxic in laboratory animals. In humans, fumonisins are associated with oesophageal cancer and neural tube defects based on studies conducted in Transkei and Texas (Marasas *et al.,* 2001) [10]. The International Agency for Research on Cancer (IARC) designated FB1 in Group 2B as 'possibly carcinogenic to humans' (IARC 1993).Till now, twenty-eight types of fumonisins have been isolated and they can be classified into A, B, C and P series. FB1, FB2 and FB3 are the principal fumonisins analyzed as natural contaminants of cereals (Soriano *et al.,* 2005; Wang *et al*., 2008) [16, 19].
Various Physical, chemical and biological methods have been described for detoxification of mycotoxins especially fumonisin and aflatoxins in food and feeds (Piva *et al*., 1995: Johanna *et al*.,2016) [12, 8] However, each treatment has its own limitations, since the products after treatment should be safe and the nutritive values of the treated product should not be altered. Many authors reported that the detoxification of fumonisin by ammoniation (Norred *et.al*., 1991) [11] Enzymatic transformation (Duvick *et.al*., 1998: Hartinger and Moll, 2011) [4] naturally occurring phenols (Beekrum *et al.,* 2003) [1]. This paper reports the potential of methanol extract from leaves of *Lippia nodiflora* (L) in the detoxification of fumonisin.

Material and Methods
Fumonisin Standard FB1and FB2 was obtained from Sigma-Aldrich Cheme, USA. Fumonisin FB1 was prepared in acetonitrile: water (1:1) and stored at 4ºC. Stock solution of individual fumonisin standards with a concentration of 250 µg/ml were used, from which a working standard was prepared containing 20µg/ml of each analog.

Plant materials
The medicinal plants were collected from the herbal garden at the Horticulture College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Preparation of leaf extracts

The leaves of medicinal plants were dried under shade and ground to fine powders. One gram of the leaf powder was mixed with 100 ml of 10% methanol in a conical flask and kept overnight at room temperature (27±20C) in a shaker at 200 rpm. The mixture was filtered through two layers of muslin cloth and then centrifuged at 14000 rpm for 10 min. the supernatant was sterilized using 0.2 µl disposable syringe filters (Millipore, MA, USA) and used for further studies.

Test for fumonisin detoxification by medicinal plants.

Two hundred µl of plant leaf extracts was mixed with 0.1 ppm of FBI and FB2 incubates at room temperature (27±20C) for 24hrs. After incubation, the mixture was centrifuged at 14000 for 10 min and the supernatant was transferred to tube containing 300 mg Amberlite XAD-4 (37380-42-0, Sigma–Aldrich Co., USA) which had been activated with 2 ml methanol and washed with deionized water, and the tube was stirred for 5 h or overnight in an orbital shaker after adding 40 ml deionized water. The XAD-4 beads were then washed with 200 ml deionized water, then transferred the XAD-4 beads to a Bond Elute column without stuffing by deionized water and the toxins were eluted with 3 ml 100% methanol. The eluent was dried under vacuum with freezing at 65°C and dissolved in 200 µl deionized water. The solution was filtered through a 0.2 µm syringe-filter and 20 µl was injected directly into the HPLC–ELSD. All samples were analyzed in triplicate.

High performance Liquid Chromatography (HPLC) analysis of Fumonisin (FB1)

The HPLC system is an "Agilent 1200 series", consists of an isocratic unit with a quaternary pump capable of a flow rate of 0.2 - 10ml/min and a suitable manual injector with Rheodyne 7725i 7-port sample injection valve capable of 10µl injections where the sample is loaded into the external 20 µl sample loop through the injection port. A 15 cm long reversed-phase column containing C18 or C8 modified silica packing material of 3 to 5 µm particle size is present inside the system for the separation of the compounds. The HPLC system is equipped with an evaporative light scattering detector.

The HPLC–ELSD method conditions were performed according to procedures with some modifications (Bojja *et al.*, 2004). The mobile phases were (A) water– trifluoroacetic acid (TFA) (100:0.025, v/v) and (B) acetonitrile–TFA (100:0.025, v/v), with a gradient of 0–20% B in A in the first 5 min, 20–40% B from 5 to 10 min, 40–80% B from 10 to 15 min, 80% B from 15 to 20 min, and 80–0% B from 20 to 25 min. The flow rate was 1.0 ml/min.

The conditions set for ELSD (Agilent 1260 infinity ELSD) were 45 °C of drift tube temperature, 2.0 l/min nitrogen gas flow and gain value of 1 in the impactor-on mode.

Preparation of Standards

Fumonisin standards: Fumonisin standards were prepared in acetonitrile: water (1:1) and stored at 4°C. Standards stored for long periods in methanol undergo slow degradation. Stock solution of individual fumonisin standards of concentration 250 µg/ml were used, from which a working standard was prepared with a concentration of 10 µg /µl and diluted into four different concentration 0.15, 0.3, 0.6, 1.2 µg /µl. The calibrant stock solutions of individual FB1 acetonitrile-water (50+50, v/v) was prepared. Fumonisin calibrant solutions are stable for 6 months when stored at 4 °C. A calibration curve was prepared from the four different dilutions of 0.15, 0.3, 0.6, 1.2 µg /µl concentration of 10 µg /µl. 20 µl of dilution was directly injected to the HPLC system. Calibration plot was prepared and checked for linearity.

Determination

The established condition standard FB1 gave a peak at a retention time of 11.33 min. The peak areas for fumonisin in the sample chromatogram are determined and the amount of each fumonisin analogue injected is determined from the calibration plot. From the calibration curve the amount of Fumonisin level in nanogram in the aliquot of solution injected into the HPLC was read from the calibration curve.

Time course of fumosnisin detoxification by *Lippia nodiflora* extract

Two hundred µl of plant leaf extracts was mixed with 0.1 ppm of fumonisins and incubates at room temperature (27±20C). At different time intervals viz., 10 min, 30 min, 1 h, 3 hr and 6 hr the fumonisin content in the reaction mixture was determined as described above.

Effect of pH on detoxification of fumonisin by medicinal plants

Leaf powder (1g) was added to a 125 –ml conical flask containing 100 ml of 10% methanol and mixed for 30 min with a magnetic stirrer. The pH 3, pH 7 and pH 10 with 2N NaOH or HCL and stirred overnight. The following day the pH values of the solution were checked and adjusted. Two hundred µl of plant extract was mixed with 0.1 ppm of fumonisin and incubates at room temperature (27±20C) for 24hrs. After incubation, the mixture was centrifuged at 14000 for 10 min and the supernatant was analyzed for fumonisin by HPLC as described earlier.

Results and discussion

In the present study, methanolic extracts (1%) obtained from leaves of 16 plants evaluated for their ability to detoxify fumonisins. Of the various plant extracts tested, the leaf extract of *Lippia nodiflora (L)*showed the maximum degradation of FB1 and FB2 after incubation at room temperature and recorded degradation of 50.47%, 46.56% (Table1). Studies on the effect on pH of *L. nodiflora* extract on detoxification of fumonisin revealed that the highest percentage detoxification values of 94.4% and 71.43% (Table 2). The percentage of detoxification decreased as the pH decreased to neutral or acidic range. Time course study of fumonisin detoxification by *L. nodiflora* extract showed that degradation of fumonisin occurred within 10 min and the percentage of degradation gradually increased with increase in incubation time. (Table 3)

Contamination of agricultural commodities with is often unavoidable, even with good agricultural practices. Several surveys have been conducted concerning the natural occurrence of fumonisin in corn samples collected from households in Linxian County. Chu and Li (1994) detected high level (18–155 ppm; mean, 74 ppm) and lower level (20–60 ppm; mean, 35.3 ppm) of fumonisin in household moldy samples. However, lower incidence and level of FB1 were also reported in other surveys conducted in Linxian County by (Yoshizawa *et al*., 1994) [21] and (Wang and Zhu, 2002) [20]. The 91 percent maize samples contains the fumonisin level 0.1 ppm to 87.0 ppm and 84 percent of poultry feeds contain 0.1 ppm to 87.0 ppm level of fumonisin could be observed in Hariyana, India by Jindal N, *et.al*., 1999) [7]. The high level FB1 (0.30–3.20 µg/ g; mean, 1.42 µg/g) in samples from the granary, followed by household (0.25–1.80 ppm; mean, 0.73

ppm), central market (0.25–1.10 ppm; mean, 0.51 ppm), and store (0.22–0.34 ppm; mean, 0.28 ppm) in china could be detected by (Wang. J *et al*., 2008) [19]

The detoxifications of fumonisn studies are very limited. (Duvick *et al*., 1998) [4] demonstrated the Microorganisms capable of transforming FB1 to less toxic end products include *Exophiala spinifera* ATCC74269, *Rhinocladiellaatro virens* ATCC 74270, Bacterium ATCC55552, and *Sphingopyxis macrogoltabida* MTA144. (Beekrum *et al*., 2003) [1] reported that the naturally occurring phenol compound Chlorophorin was inhibiting the *Fusarium verticillioides* effectively followed by iroko, maakianin, vanillic acid and caffeic acid. Chlorophorin also was the most effective compound in reducing toxin production (94% reduction), followed by caffeic acid, ferulic acid, vanillic acid and iroko, which reduced FB1 levels by 90–91%. Detoxification of aflatoxin by plant products have been reported by several workers (Hajare *et al*., 2005; Sapcota *et al*., 2005; Sandosskumar *et al*., 2007; Velazhahan *et al*., 2010: Vijayanandaraj et al., 2014) [14, 13, 17]

(Sandoskumar *et al*., 2007) demonstrated the potential of leaf extract from zimmu plant to degrade the aflatoxin *Invitro.* (Vijayanandaraj *et al.,* 2014) demonstrated the potential of *Adhatoda vasica* leaf extract to degrade aflatoxin.

The reduction in the percentage of fumonisin after incubation with *Lipia nodiflora* extract in the present study suggests detoxification of fumonisin and the mode of action may be due to either adsorption or degradation. *Lippia nodiflora* Linn. (Verbenaceae) is a small creeping herb, commonly known as Jalpapli found in South Asia. The methanolic extract of leaves has been found to posses Anti-diuretic activity (Shukla *et al.*, 2009) Antitumor activity (Durairaj *et al.,* 2009) [3] Antimicrobial activity (Malathi *et al.*, 2011). In the present study dramatic reduction in the level of fumonisin was observed after incubation with *L.nodiflora* extract within 10 min and maximum percent degradation occurred at alkaline pH. The high effectiveness of the *L.nodiflora* extract in detoxification of fumonsisns suggest that this medicinal plant may be an ideal choice for development of functional foods or biologically safe herbal feed additives to reduce the fumonisin effects in animals.

Table1: Detoxification of Fumonisins by methanolic extract from various medicinal plants *in vitro*

S. No.	Medicinal plants	Family	% reduction over control *	
			FBI	FB2
1.	*Adhatoda vasica* (L.) Nees.	Acanthaceae	45.26^{b} (42.28)	43.39^{b} (41.20)
2.	*Andrographis paniculata (Burm. f)* wall.ex.Nees	Acanthaceae	39.21^{d} (38.76)	36.54^{cd} (37.19)
3.	*Zimmu sp. (Allium cepa* L. *x. A. sativum)*	Alliacea	43.56^{bc} (41.3)	42.28^{bc} (40.56)
4.	*Tribulus terrestris* (L.)	Zygophyllaceae	25.51^{g} (30.33)	26.19^{gh} (30.78)
5.	*Blepharis maderaspatensis* (L.)	Acanthaceae	30.62^{ef} (33.59)	34.57^{e} (36.01)
6.	*Gymnema* sylvestre R.Br	Asclepiadaceae	34.01^{e} (35.67)	24.74^{hi} (29.82)
7.	*Calotropis gigantean* (L.) W.T. Aiton	Apocynaceae	12.63^{l} (20.81)	11.4^{l} (19.72)
8.	*Cassia angustifolia* M Vahl	Fabaceae	20.29^{hi} (26.77)	21.05^{j} (27.31)
9.	*Catharanthus reseus* (L.) G.Don	Apocynaceae	36.25^{de} (37.02)	35.8^{d} (36.75)
10.	*Alternanthera paranchoides* A.St. - Hil	Amaranthaceae	28.35^{f} (32.17)	27.51^{g} (31.63)
11.	*Vitex negundo* (L.)	Lamiaceae	41.93^{c} (40.35)	37.27^{c} (37.62)
12.	Coleus forskohli Andr	Lamiaceae	18.95^{ij} (25.80)	20.96^{j} (27.24)
13.	*Lippia nodiflora* (L.)	Verbenaceae	50.47^{a} (45.27)	46.56^{a} (43.02)
14.	*Aloe vera* (L.)	Xanthorhoeaceae	33.68^{e} (35.47)	32.1^{f} (34.51)
15.	*Cynodon dactylon* (L.) pers	Poaceae	21.47^{h} (27.60)	24.58^{hi} (29.72)
16.	*Gymnema* sylvestre R.Br	Asclepiadaceae	17.56^{ik} (24.77)	19.62^{ik} (26.29)

*Mean of three replications

Figures in the parenthesis are arc sine transformed values

Means followed by the same letter are not significantly different (p=0.05) by DMRT

Table 2: Effect of pH of *L. nodiflora* leaf extract on detoxification of fumonisins

pH	Percent reduction over control*	
	FB1	FB2
3	47.05^{c} (43.31)	35.71^{c} (36.69)
7	64.7^{b} (53.55)	55.5^{b} (48.18)
10	94.4^{a} (76.36)	71.43^{a} (57.69)

*Mean of four replications

Figures in the parenthesis are arc sine transformed values

Means followed by the same letter are not significantly different (p=0.05) by DMRT

Table 3: Time course of fumonisins detoxification by *L. nodiflora* leaf extract

Time intervals	Percent reduction over control*	
	FB1	FB2
10 min	57.29^{c} (49.19)	56.02^{d} (48.45)
30 min	58.49^{c} (49.88)	59.9^{c} (50.71)
1 h	60.78^{bc} (51.22)	72.16^{b} (58.15)
3 h	74.27^{b} (59.52)	83.97^{ab} (66.40)
6 h	80.45^{a} (63.76)	85.32^{a} (67.47)

*Mean of four replications

Figures in the parenthesis are arc sine transformed values

Means followed by the same letter are not significantly different (p=0.05) by DMRT

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