

## Article

# On-Farm Demonstrations with a Set of Good Agricultural Practices (GAPs) Proved Cost-Effective in Reducing Pre-Harvest Aflatoxin Contamination in Groundnut

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**Abstract:** Aflatoxin contamination in groundnut is an important qualitative issue posing a threat to food safety. In our present study, we have demonstrated the efficacy of certain good agricultural practices (GAPs) in groundnut, such as farmyard manure (5 t/ha), gypsum (500 kg/ha), a protective irrigation at 90 days after sowing (DAS), drying of pods on tarpaulins after harvest in farmers' fields. During 2013–2015, 89 on-farm demonstrations were conducted advocating GAPs, and compared with farmers' practices (FP) plots. Farmers' awareness of GAPs, and knowledge on important aspects of groundnut cultivation, were also assessed during our experimentation in the selected villages under study. Pre-harvest kernel infection by *Aspergillus flavus*, aflatoxin contamination, and pod yields were compared in GAPs plots, vis-à-vis FP plots. The cost of cultivation in both the plots was calculated and compared, based on farmer's opinion surveys. Results indicate kernel infections and aflatoxins were significantly lower, with 13–58% and 62–94% reduction, respectively, in GAPs plots over FP. Further, a net gain of around \$23 per acre was realized through adoption of GAPs by farmers besides quality improvement of groundnuts. Based on our results, it can be concluded that on-farm demonstrations were the best educative tool to convince the farmers about the cost-effectiveness, and adoptability of aflatoxin management technologies.

**Keywords:** groundnut; aflatoxins; good agricultural practices; on-farm demonstrations

## 1. Introduction

Mycotoxin contamination of agricultural commodities is a serious food safety issue worldwide. Of different mycotoxins, aflatoxins are very important, and are frequent contaminants of food crops, importantly, groundnut during pre- and post-harvest stages [1–3]. Aflatoxins are mainly produced by *Aspergillus flavus* and *A. parasiticus*. Major aflatoxins are B1, B2, G1, and G2 based on their fluorescence under UV light and chromatic mobility. Aflatoxins M1 and M2, metabolically biotransformed and hydroxylated forms of B1 and B2, respectively, are usually secreted in milk when lactating animals consume aflatoxin contaminated feed [4,5]. According to the Food and Agriculture Organization (FAO), 25% of the agricultural crops worldwide are affected by aflatoxins [6]. The affected crops include cereals, millets, oilseeds, spices, tree nuts, pulses, and figs [7]. Both human and animal health are affected, due to significant levels of exposure to aflatoxins [8–10]. Chronic exposure to aflatoxins can lead to hepatocellular carcinoma (HCC). Most of the HCC cases are reported to be in sub-Saharan

Africa and Southeast Asia, due to uncontrolled aflatoxin exposure through food and the prevalence of hepatitis B virus (HBV) infection [11].

Groundnut (peanut; *Arachis hypogaea* L.) is an important food legume that is affected with aflatoxins at both pre- and post-harvest stages [1,12,13]. Worldwide, groundnut is grown in an area of 25.4 million ha, with an annual production of 45.2 million tons and a productivity of 1.77 t/ha [14]. In India, groundnut is grown to an extent of 5.25 million ha, with a production of 9.47 million tons and a productivity of 1.80 t/ha [14]. Important groundnut growing states of India are Gujarat, Andhra Pradesh, Tamil Nadu, Odisha, Karnataka, Telangana, and Maharashtra. Anantapur district of Andhra Pradesh state is one of the largest groundnut growing districts in the country, with an area of 0.6 to 0.8 million ha grown consistently every year. Groundnut in Anantapur district is predominantly grown as a rainfed crop, and is often subjected to end-of-season dry spells, and so aflatoxin contamination levels are high [15]. Important predisposing factors for aflatoxin contamination in groundnut are soil *A. flavus* populations, susceptible cultivars, late season drought, mean soil temperatures of 28–31 °C in the pod zone, and injuries to pods due to varied reasons [16–18]. Post-harvest factors favoring aflatoxin contamination in storage are pod moisture levels of >10%, relative humidity of >65%, and insect damage.

Adoption of good agricultural practices is gaining paramount significance in groundnut production in view of their importance in minimizing aflatoxin contamination, as well as being environmentally sustainable. For example, soil moisture conservation through deep ploughing and cultivation across the slope, judicious application of potassic fertilizers, and gypsum ( $\text{CaSO}_4$ ) application at the time of flowering, reduce *A. flavus* invasion of pods and subsequent aflatoxin contamination [15]. Studies confirmed that gypsum, when applied at flowering, reduced aflatoxins by 40%, and enhanced yield, produced healthy kernels, and reduced *A. flavus* infection [19]. On-farm experiments in West Africa showed that farmyard manure (FYM), an organic soil amendment, contributes to reduction in pre-harvest aflatoxin contamination of groundnut [20]. Late season drought is another factor that contributes to higher aflatoxins in kernels during pre-harvest phase of groundnut [21,22]. It has been long known that irrigation, 3–4 weeks before harvesting, reduced *A. flavus* invasion and aflatoxin contamination in groundnuts [23]. Integrated aflatoxin management holds the key to addressing this complex problem. But the question lies with the economic feasibility of interventions, their availability to resource poor farmers and the willingness to spend extra dollars in adoption. Keeping this in view, we attempted to demonstrate the efficacy and cost-effectiveness of certain good agricultural practices (GAPs) in farmers' fields in reducing aflatoxin contamination of groundnut. Critical insight on the farmers' awareness levels on pre- and post-harvest aflatoxin contamination, and their adoption levels, is also essential to effectively manage this menace. Hence, we included a baseline survey with a specially designed questionnaire in our study. Our major objective was to showcase to the farmers the cost-effectiveness of the interventions, with on-farm demonstrations in farmers' fields comparing the efficacies of GAPs vis-à-vis farmers' practices (FP).

## 2. Results

### 2.1. Soil Chemical Properties

The selected locations are typified by shallow red soils, and the experimental fields have a normal pH, ranging from 7.03 (Dharmavaram) to 7.99 (Rapthadu). The soils from the other two locations have a pH of 7.11 (Kudaeru) and 7.16 (Atmakur) (Table 1). Further, the soil organic carbon levels for these selected locations were under the "low" category (up to 0.5%), ranging from 0.32 to 0.49% (Table 1). The soil calcium levels were medium in Rapthadu (1501 ppm); whereas high in Dharmavaram, Kudaeru, and Atmakur (1612, 2289, and 2039 ppm, respectively) (Table 1).

**Table 1.** Soil chemical characteristics in different locations of Anantapur district of Andhra Pradesh, India, as estimated during 2013 rainy season.

Mandal/Location	Soil Parameters		
	pH	Organic Carbon (%)	Calcium (ppm)
Rapthadu	7.9 ( $\pm 0.09$ )	0.32 ( $\pm 0.03$ )	1501 ( $\pm 116.4$ )
Dharmavaram	7.0 ( $\pm 0.22$ )	0.48 ( $\pm 0.04$ )	1612 ( $\pm 152.0$ )
Kudaeru	7.1 ( $\pm 0.17$ )	0.44 ( $\pm 0.02$ )	2289 ( $\pm 286.3$ )
Atmakur	7.2 ( $\pm 0.17$ )	0.49 ( $\pm 0.17$ )	2039 ( $\pm 170.7$ )

pH: Acidic  $\leq 6.5$ ; Normal = 6.5–8.5; Alkaline  $\geq 8.5$ ; Organic Carbon: Low = 0.5%; Medium = 0.5–0.75%; High  $\geq 0.75$ ; Calcium: Low  $\leq 1000$  ppm; Medium = 1000–1600 ppm; and High = 1600–2400 ppm; Values in parentheses are standard error of means.

## 2.2. Extent of Farmers' Awareness and Adoption of Good Agricultural Practices (GAPs) and Technologies in Groundnut

Our baseline survey, conducted with 280 farmers, on three aspects of groundnut cultivation indicated that the farmers' awareness was poor on technical know-how. Only 1.4% of the respondents were aware of the aflatoxin problem and perceived it as bitterness of the seed. However, none of them were aware of the associated health risks due to aflatoxin-contaminated grain consumption. None of the farmers were practicing crop rotation. Further, only 36.7% of the respondents were inclined to crop varietal preference. Of these, 44.3% were aware of varieties with higher yield potential. Similarly, 36.8% of the respondents were aware of drought resistant varieties and their role in mitigating drought stress effects. About 13.2% of the respondents among the farmers with varietal preference were using varieties with high oil content (Table 2). Regarding on-farm practices, all the respondents were aware of the importance of harvesting the crop at the right maturity stage (100% respondents). Further, only 7.1% of them were using FYM, 3% were applying gypsum, whereas 78.2% were using chemical fertilizers. The major abiotic stress, as perceived by all the respondents, was drought. In the surveyed areas, the major insect pests causing significant economic losses were lepidopteran pests, such as *Spodoptera* sp., *Helicoverpa* sp., red hairy caterpillar (RHC), and leaf folder. Among diseases, late leaf spot (LLS), collar rot, stem rot, and viral diseases are the main yield limiting factors. With regard to post-harvest practices, the farmers' awareness on separation of healthy pods from diseased/damaged pods for storing was 100% (Table 2). Further, all the farmers were using jute bags for storing groundnut pods. The major storage pest in groundnut was bruchid (*Caryodon serratus*). All the pod material produced in these four mandals is usually disposed to the local traders in the village (Table 2).

**Table 2.** Extent of adoption of Good Agricultural Practices (GAP) and other related information on groundnut cultivation in Anantapur district of Andhra Pradesh, India (as per January 2013 survey).

Serial No.	Parameter Assessed	Prevalence/Extent of Adoption among Farmers
<b>Awareness on Technical Aspects</b>		
1	Aflatoxin problem	1.4%
2	Crop rotation	Nil
3	Crop varietal preference	36.7%
4	Extent of adoption of resistant/tolerant/other improved varieties	36.8% (drought) 44.3% (high yields) 13.2% (high oil content)
<b>Adoption of On-Farm Practices</b>		
5	Application of Farm Yard Manure (FYM)	7.1%
6	Application of chemical fertilizers	78.2%
7	Gypsum application	3%

Table 2. Cont.

Serial No.	Parameter Assessed	Prevalence/Extent of Adoption among Farmers
10	Major insect pests	<i>Spodoptera</i> sp., <i>Helicoverpa</i> sp., RHC, leaf folder
11	Major plant diseases	Leaf spots, collar rot, stem rot, bud and stem necrosis diseases
8	Crop harvesting at right maturity	100%
9	Major abiotic stresses	Drought (100%)
<b>Adoption of Off-Farm/Post-Harvest Practices</b>		
12	Separation of healthy pods from diseased pods prior to storage	100%
13	Major storage pests	Bruchids
14	Storage system	Jute bags (Traditional storage)
15	Marketing	Local traders in village

### 2.3. On-Farm Demonstrations

A total of 89 on-farm demonstrations were conducted with a set of good agricultural practices (GAPs) and compared with farmers practices (FP) during the three years of experimentation. Both the fields with GAPs and FP during 2013–2015 were located adjacently. The groundnut kernel infection by *A. flavus* and subsequent aflatoxin contamination were significantly lower in plots followed with GAPs over that of farmers' practices (Table 3). In 2013, the range of kernel infection by *A. flavus* was 0–20.0% (with an average of 4.4%) in plots with GAPs as against 0–96.7% (with an average of 10.3%) in plots with farmers practices (FP). In 2014, GAPs plots recorded 0–66.7% (with an average of 9.8%), whereas in FPs, the kernel infections were in the range of 0–60.0% (with an average of 11.2%). In 2015, the kernel infections with *A. flavus* were in the range of 0–16.7% (with an average of 7.9%) in GAPs followed plots, as opposed to 3.3–53.3% (with an average of 15.4%).

The kernel aflatoxin levels as a measure of pre-harvest contamination was also lowest in GAPs followed plots over FP in all the three years. During 2013, plots with GAPs have recorded aflatoxin levels in the range of 0–301 µg/kg (with an average of 44 µg/kg) as opposed to 0–1772 µg/kg (with an average of 115 µg/kg) in plots with FP. During 2014, the aflatoxin levels were in the range of 0–71 µg/kg as opposed to 0–10,169 µg/kg in plots with FP (Table 3). During 2015, the aflatoxin levels in GAPs followed plots were in the range of 13–113 µg/kg, as opposed to 12–2282 µg/kg in FP plots.

Pod yields were higher in GAPs plots over FP. During 2013, average pod yields in GAPs plots were 6.7 Q/acre (1 Q = 100 kg), whereas in FP, yields were only 4.7 Q/acre. During 2014, GAPs plots recorded an average of 6.2 Q/acre, whereas in FP, it was 4.3 Q/acre. Similarly, in 2015, the average yields in GAPs plots were 6.7 Q/acre, as opposed to 5.5 Q/acre in FP plots (Table 3).

The Mann–Whitney U test indicated that the pre-harvest aflatoxin contamination had shown significant variation between the practices followed by farmers in all the years (Table 4). Subsequently, aflatoxin levels were significant ( $p < 0.05$ ) between seasons/years and their interactions. The pod yield was also significant ( $p < 0.05$ ). However, the seed infection between seasons/years and their interactions was non-significant ( $p < 0.05$ ) in 2013 and 2014 seasons, whereas it was significant in 2015 ( $p < 0.05$ ) (Table 4).

**Table 3.** Range and mean of pre-harvest aflatoxin contamination, seed infection with *Aspergillus flavus*, and pod yield in groundnut plots with good agricultural practices (GAPs) and farmers' practices (FP), as evaluated during the rainy seasons of 2013, 2014, and 2015 in different villages of Anantapur district of Andhra Pradesh, India.

Practice/Characteristic	2013			2014			2015			Pooled (2013–2015)		
	Aflatoxins (µg/kg)	Seed Infection with <i>A. flavus</i> (%)	Pod Yield (Q/acre)	Aflatoxins (µg/kg)	Seed Infection with <i>A. flavus</i> (%)	Pod Yield (Q/acre)	Aflatoxins (µg/kg)	Seed Infection with <i>A. flavus</i> (%)	Pod Yield (Q/acre)	Aflatoxins (µg/kg)	Seed Infection with <i>A. flavus</i> (%)	Pod Yield (Q/acre)
FP	0.0–1772.1	0.0–96.7	3.0–6.5	0.0–10,169.3	0.0–60.0	3.0–6.0	12.2–2282.2	3.3–53.3	4.0–7.0	0.0–10,169.3	0.0–96.7	3.0–7.0
GAPs	0.0–301.3	0.0–20.0	6.0–7.0	0.0–71.5	0.0–66.7	5.0–7.0	12.6–113.5	0.0–16.7	5.0–7.5	0.0–301.3	0.0–66.7	5.0–7.5
FP <sup>a</sup>	114.8	10.3	4.7	388.3	11.2	4.3	326.1	15.4	5.5	276.4	12.3	4.8
GAPs <sup>b</sup>	43.9	4.4	6.7	24.7	9.8	6.2	27.1	7.9	6.7	31.9	7.4	6.5
Percent reduction/gain over FP	61.8	57.6	30.0	93.6	12.6	30.4	91.7	48.7	18.6	88.5	40.1	26.3

FP = farmers' practice; GAPs = good agricultural practices; 1 Q = 100 kg; <sup>a</sup> = average of 81, 80 and 80 farmers' fields; <sup>b</sup> = average of 9, 40 and 40 demonstration fields in 2013, 2014 and 2015 respectively.

**Table 4.** Non-parametric test (Mann–Whitney U test) for comparison of FP and GAPs data of 2013–2015.

Non-Parametric Test	2013			2014			2015		
	% Seed Infection	Aflatoxins (µg/kg)	Pod Yield (Q/acre)	% Seed Infection	Aflatoxins (µg/kg)	Pod Yield (Q/acre)	% Seed Infection	Aflatoxins (µg/kg)	Pod Yield (Q/acre)
Mann–Whitney U test	29.5 **	5 *	0 *	648 **	384.5 *	58 *	415.5 *	90 *	119 *
Z scores	0.93	3.1	−3.53	1.46	3.99	−7.13	3.7	6.83	−6.55

\* Significant at  $p < 0.05$  (two-tailed hypothesis); \*\* Non-significant (N.S.) at  $p < 0.05$ .

#### 2.4. Cost Economics of GAPs Vis-à-Vis Farmers' Practices

Costs of cultivation in plots with GAPs versus FP indicated that on per acre basis, farmers in Anantapur district incur costs of approximately \$230.71 for groundnut production (Table 5). Besides these costs, in the GAPs demonstration plots, additional costs of \$46.15 (FYM); \$19.23 (gypsum); \$7.69 (protective irrigation); and \$1.15 (tarpaulin sheet) were incurred. Thus, in GAPs plots, the total cost of cultivation was \$304.93. Pod and haulm yields were more in GAPs plots over that of FP. Overall, the net return in plots with GAPs were \$144.24 per acre, compared to \$121.55 in plots with FP (Table 5).

**Table 5.** Comparative costs of cultivation in plots with “Farmers’ Practices (FP)” and “Good Agricultural Practices (GAPs)”.

Agronomic Practices/Field Operations	Expenses Incurred (USD/acre)	
	Plots with FP	Plots with GAP
Ploughing	\$41.53	\$41.53
Basal applications		
(a) Fertilizers	\$23.07	\$23.07
(b) Seed cost	\$83.07	\$83.07
(c) Seed treatment (ST)	\$3.84	\$3.84
Weed management (Post-emergence)		
(a) 20 DAS	\$7.69	\$7.69
(b) 40 DAS	\$11.53	\$11.53
Plant protection costs	\$23.07	\$23.07
Harvesting & Drying	\$21.53	\$21.53
Stripping	\$15.38	\$15.38
GAP		
(a) Farmyard manure (FYM)	-	\$46.15
(b) Gypsum application	-	\$19.23
(c) Protective irrigation	-	\$7.69
(d) Tarpaulin sheet for pod drying	-	\$1.15
Total cost	\$230.71	\$304.93
Average pod yield	5.5 Q (at the rate of \$61.53/Q) = \$338.41	7 Q (at the rate of \$61.53/Q) = \$430.71
Haulm yield (t)	1.5 t (at the rate of \$9.23/t) = \$13.8	2 t (at the rate of \$9.23/t) = \$18.46
Total income	=\$352.26	=\$449.17
Net gain	\$121.55	\$144.24

DAS is days after sowing; ST with fungicide (Thiram at the rate of 3 g/kg seed); Plant protection costs are incurred on pest and disease management; pod harvesting manually at 120 DAS; FYM as basal application at the rate of 5 t/ha (at the rate of \$23.07/t); Gypsum application at the rate of 500 kg/ha (\$0.1/kg including labor charges) at 30–35 DAS; Protective irrigation at 90 DAS to prevent end of season drought; Costs incurred are means of 241 plots with farmers practices and 89 plots with GAPs respectively on per acre basis; One USD is equivalent to approximately 65 INR (Indian National Rupee).

#### 2.5. Post-Harvest Losses in Groundnut

Farmers in the surveyed areas indicated that storage losses in groundnut are economically significant. The majority of the farmers were storing groundnuts in un-shelled form (pods) in jute bags. The general length of storage ranges from 0 to 6 months prior to disposal at local markets and/or to use them as seed in the next season. Mandal-wise, the post-harvest pod losses were up to 56% (Rapthadu), 50% (Dharmavaram), 48% (Kudaeru), and 47% (Atmakur) at six months after storage. These post-harvest losses were attributed by the majority of respondents to bruchids.

### 3. Discussion

Identifying production practices and reaching out to farmers so that they adopt them in reducing aflatoxin contamination are key in areas where groundnut is more prone to aflatoxin contamination. An attempt was made in result demonstration mode, to better inform the farming community about the efficacy and cost-effectiveness of certain good agricultural practices (GAPs). For instance, our on-farm demonstrations in farmers' fields are in support of several published reports on gypsum supplementation and aflatoxin reduction in groundnut [24,25]. Our results clearly indicate lesser kernel *A. flavus* infection and aflatoxin contamination during the three years of demonstrations (2013–2015) in fields that observed GAPs over those of farmer practices (FP). Soil amendments, like organic manures, favor beneficial microbial populations, and ultimately control plant pathogens [26,27]. Soils at the study experimental sites are characterized by low organic carbon, and medium to high calcium levels with neutral pH. Earlier studies have indicated the gypsum (source of calcium) application to soil reduced *A. flavus* infection, and thereby, resulted in less aflatoxin accumulation in groundnut [19,24,25,28]. Gypsum application reduced aflatoxin contamination by 40% in groundnut crop when applied at flowering. Besides yield enhancement, high quality kernels have been reported with gypsum application [19]. Further, both gypsum and FYM application were found to be synergistic with bio agents in reducing aflatoxin contamination [25,29].

In our studies, protective irrigation given at 90 DAS might have also been responsible for reduced aflatoxin contamination in GAPs plots over plots with FP. Average rainfall during the rainy seasons of 2013, 2014, and 2015 in Anantapur district of Andhra Pradesh was about 338 mm, which was much lower than the mean rainy season rainfall in Andhra Pradesh state (554 mm). Uneven distribution of rainfall in the rainy season during the three years of on-farm demonstrations and lack of or insufficient rain during the last 3–4 weeks of the standing crop might have further contributed to increased aflatoxin levels, especially in FP plots. This observation is in line with several published reports that have proven that drought stress and soil temperatures at 29 °C during 85–100 DAS result in high aflatoxin levels in kernels [17,30,31]. The reason for drought stress induced aflatoxin contamination is the high level of proline in plants that enhances aflatoxin production [32]. Since end-of-season drought is a common phenomenon in Anantapur district, the higher levels of aflatoxin in FP plots in our study are also attributed to the abiotic stress. The total rainfall in Anantapur district in October 2013, 2014, and 2015 was 40 mm, 94 mm, and 80 mm respectively, insufficient for pod development and to mitigate aflatoxin contamination. Lower aflatoxin levels in kernels from plots with GAPs are attributed to the protective irrigation at 90 DAS to prevent the end-of-season drought. This is due to this fact that the aflatoxin problem is widespread in groundnuts grown under rain-fed conditions compared to irrigated conditions [15]. Further, proper drying of pods on tarpaulins would enable cut-off of direct contact with the soil after harvest. This practice might also contribute to mitigating aflatoxin contamination in kernels in fields with GAPs compared to FP.

Our studies also revealed consistent mono-cropping of groundnut over the years in the surveyed areas of Anantapur. Crop rotation, however, lowers the rate of survival of different soilborne *Aspergilli*, especially when non-host crops are grown [33,34]. Awareness campaigns are therefore necessary in these crop-growing areas, pertaining to the factors favoring aflatoxin build up, their associated health hazards, and the methods to overcome these problems using GAPs. In our studies, GAPs plots have shown more net income per acre over plots with FPs. Coupled with this, the aflatoxin levels were also lower in the kernels of these GAPs plots. This is clearly a sign of spillover benefit, where yield, as well as quality, improved with the adoption of certain GAPs. Most importantly, we have shown the farmers cost-effectiveness of these technologies which usually drives the adoption rate at farmers' level [35], and continuous adoption of such agronomic practices will further improve soil health. Popularization of these GAPs technologies is therefore essential to overcome the aflatoxin related trade and health risks in groundnut. Sizeable post-harvest losses of groundnut, as per the farmers' opinion in the surveyed areas, are mainly attributed to the storage in jute bags. Grains stored in jute bags are more prone to bruchid attack, *A. flavus* attack, and aflatoxin contamination. However, the use

of high density polyethylene bags, such as Purdue Improved Crop Storage (PICS) bags that work on the principle of hermetic storage, is gaining wide acceptance among farmers to store groundnut. Viability of groundnut seeds stored in polyethylene bags is also retained for longer periods (up to 7 months) when compared to jute bags [36]. Similarly, our recent research on PICS bags demonstrated the efficacy of groundnut storage over long periods without quality deterioration [37]. Hence, wide publicity should be done on the use of these bags for storing groundnut in crop growing areas of Andhra Pradesh.

Our baseline survey results indicate poor technical awareness among groundnut farmers of Anantapur district of Andhra Pradesh state, India. Only 1.4% of the respondents ( $n = 280$ ) were aware of the aflatoxin problem. Creating awareness among them on pre-harvest and post-harvest aflatoxin contamination in groundnut is important, since aflatoxins are highly toxic to both humans and animals. Especially among the rural poor, aflatoxin exposure is likely higher due to their dependency on unprocessed foods. In children, aflatoxin exposure often causes stunting and growth retardation [38]. Further, only 7.1% and 3% of farmers were applying FYM and gypsum, respectively, according to our survey. Awareness campaigns are therefore critical to enlighten farmers on the economic and health issues associated with aflatoxin contamination in groundnut and its management using soil amendments, such as FYM and gypsum. Establishing partnerships between research institutions, agriculture departments, Non-Government Organizations (NGOs), farmers' groups, consumer groups, agrochemical manufacturers, and other stakeholders are essential to address this problem and its management [39–41]. Our survey results showed certain gaps that need immediate attention. For example, crop rotation was not in practice, which indicates the prevalence of mono-cropping in the surveyed areas. On the brighter side, the survey revealed that crop varietal preferences were made according to the type of biotic and abiotic stresses, crop harvest at maturity stage, and the practice of separating healthy pods from diseased pods prior to storage (Table 2).

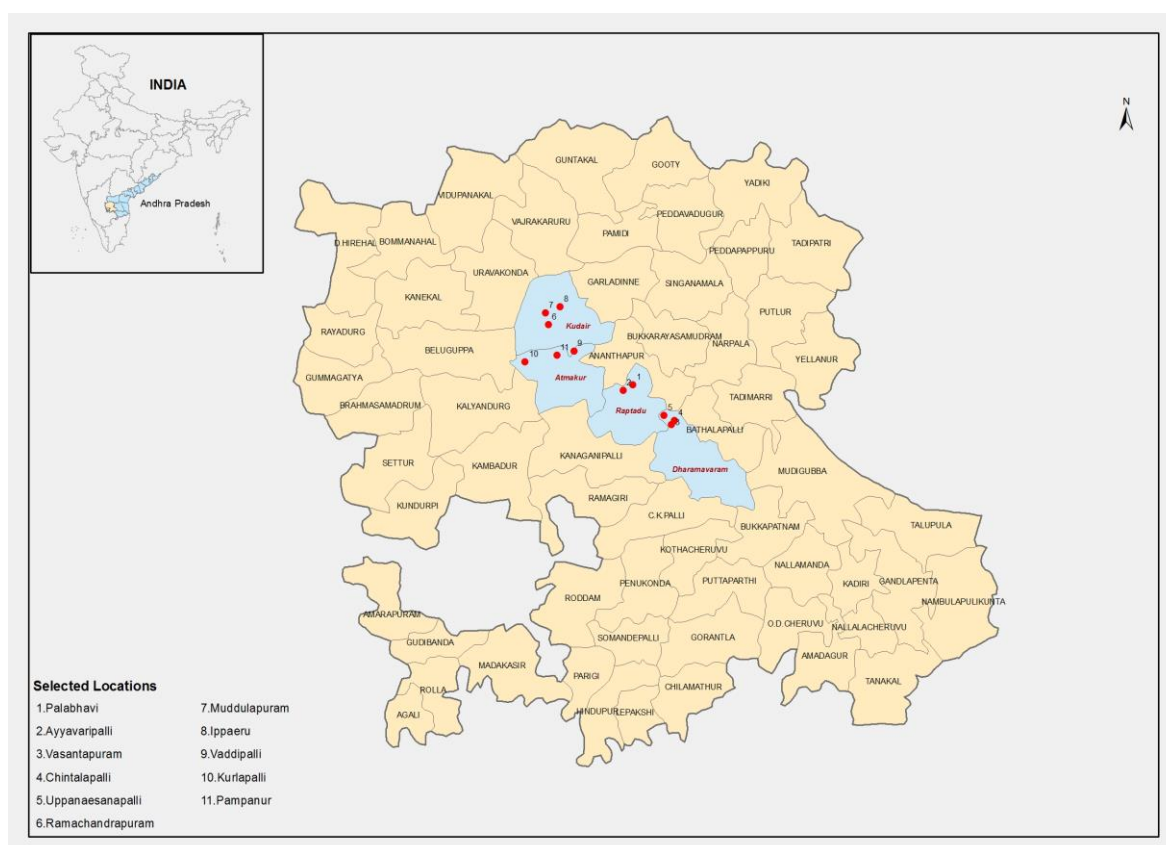
Aflatoxin mitigation strategies must be adopted at both pre-and post-harvest stages to better protect food crops from quality losses, more so in groundnut, which is mainly cultivated under rain-fed conditions, and is subject to drought in several parts of the world, especially in Asia and sub-Saharan Africa. Moreover, farmers' awareness and use of soil amendments, such as gypsum and FYM, are negligible. In this direction, a multi-pronged approach involving extension agents, researchers, NGOs should be encouraged to mobilize and enlighten farmers on a community basis on ways to overcome the ill-effects of aflatoxin contamination in groundnut, and also, the trade related losses.

## 4. Materials and Methods

### 4.1. Selection and Details of Sites for On-Farm Demonstrations

Anantapur district in Andhra Pradesh, a southern India state, was selected for the present study to demonstrate the cost-effectiveness and efficacy of certain GAPs in reducing aflatoxin contamination. This district was primarily selected because groundnut is the principal rainy season (Kharif) crop, with acreage of 5–6 lakh ha annually. The district is in the rain shadow zone of Indian plateau. The mean annual rainfall of the district for the previous seven years of experimentation (2007–2012) was 568 mm, which is slightly above the normal rainfall (553 mm) of this district. Other factors of consideration were frequent end of season drought spells in groundnut, high soil populations of *A. flavus*, and prevalence of aflatoxin contamination in kernels at above permissible levels [15,42].

The geographical locations of all the villages where on-farm demonstrations were taken up during 2013, 2014, and 2015, are shown in Figure 1. Soil chemical characteristics, such as soil pH, organic carbon content, and calcium levels, were estimated in these soils according to the standard protocols [43–45]. The groundnut variety used in the demonstrations is K-6. It is very popular in the region, and released by the state agricultural university in the year 2005.



**Figure 1.** Geographical locations of different villages in the selected mandals of Anantapur district of Andhra Pradesh, India, where on-farm demonstrations were taken up on aflatoxin management in groundnut during rainy seasons of 2013, 2014, and 2015.

#### 4.2. Extent of Farmers' Awareness and Adoption of Good Agricultural Practices (GAPs) during Pre- and Post-Harvest Stages of Groundnut

A preliminary survey was carried out in the selected mandals of Anantapur district during January 2013, to assess the extent of awareness and adoption of technologies in groundnut cultivation. A total of 280 respondents (farmers), who are in groundnut cultivation continuously for more than 10 years, were selected. Information pertaining to awareness on aflatoxin problem, crop rotation, groundnut varieties, farmyard manure (FYM), fertilizer application, major biotic and abiotic stresses, stage of harvesting, and marketing, were collected. Information on post-harvest losses, nature of damage, type of material used for pod/kernel storage, and period of storage, were also gathered. The collected information was compiled into three categories, such as awareness on technical aspects, adoption of “on-farm practices”, and adoption of off-farm/post-harvest practices.

#### 4.3. On-Farm Demonstrations

A total of 89 on-farm demonstrations were conducted in different fields during 2013, 2014, and 2015 rainy seasons (June/July–September/October) to assess the cost-effectiveness and efficacy of certain “GAPs” vis-à-vis “FP” in reducing aflatoxin contamination in groundnut. Both the fields with GAPs and FP were located adjacently, and were of 1 acre (approximately 4000 m<sup>2</sup>) each, spread across eleven villages. The dates of sowing were in the first half of July month in all the three years. A seed rate of 60 kg/acre (treated with thiram at 3 g/kg seed), as per the farmers' practice, was adopted in both the fields. Additionally, the plots with GAPs included (1) FYM application; (2) gypsum application; (3) one protective irrigation; (4) pod drying using tarpaulins. FYM was applied during the last plough

stage at the rate of 2 tons per acre size plot (5 t/ha), gypsum was applied at the rate of 200 kg per acre (500 kg/ha) at the time of flowering (30–35 DAS), whereas the protective irrigation was given at 90 DAS to overcome the ill-effects of end-of-season drought on rainy season crop. Other crop management practices, in both the plots of GAPs and FP, were according to the farmers' routine practices. The crops in both the plots of GAPs and FP were harvested at around 120 DAS manually, and dried under the sun. In GAPs plots, tarpaulins were used to avoid direct contact with soil while drying the pods, and the final pod moisture was ensured at 8 to 9%. Pods were separated manually after drying; pod and haulm yields were calculated separately.

#### 4.4. Cost Economics of GAPs Vis-à-Vis Farmers' Practices

A total of 280 farmer respondents from the eleven targeted villages were interviewed on the actual costs incurred, and the split-up cost of groundnut cultivation. Similarly, the additional costs incurred on the implementation of GAPs were also calculated separately. The total cost of cultivation in plots of "FP" and "GAPs" was then arrived at, and the net returns in each plot were calculated based on the actuals provided by the farmer respondents.

#### 4.5. Estimation of Kernel *A. flavus* Infection and Aflatoxin Contamination

Groundnut pod samples were collected along a "W" pattern across each field, with five sampling points at mid- and end-points of each leg of the "W". At each sampling point, ten plants were uprooted, and pods were removed from branches and from the crown area of the plants. An estimated 100 to 150 pods were collected at each sampling point. Pods were bulked and thoroughly mixed from all sampling points for each field. Pods were dried placing them on the tarpaulin sheet to avoid direct contact with the soil after harvest. For ascertaining natural seed infection by *A. flavus*, pods were shelled, and 100 seeds/plot were selected. Seeds from each replication were surface sterilized by immersion for 3 min in a 0.2% aqueous solution of sodium hypochlorite. After three rinses in sterile distilled water, the seeds were transferred aseptically onto moistened filter paper in sterile Petri dishes. The Petri dishes were later incubated at room temperature ( $25 \pm 1$  °C) for a maximum of 7 days, and fungi growing from the seeds were identified and the percent *A. flavus* infection was enumerated [46]. The *A. flavus* isolates were confirmed based on the microscopic and macroscopic characteristics using standard procedures [47]. For aflatoxin estimation (AFB1), 500 g of pods were shelled, the seeds were powdered, and 20 g was titrated in 70% methanol (*v/v*; 70 mL absolute methanol in 30 mL distilled water) containing 0.5% KCl in a blender, until the seed powder was thoroughly homogenized. The extract was transferred to a conical flask and shaken for 30 min at 300 rpm in a mechanical shaker. The extract was filtered through Whatman No. 4 filter paper and the filtrate stored at 4 °C until needed for analysis using indirect competitive ELISA [48].

#### 4.6. Data Analysis

The data on rainfall and temperature during the complete crop growing period of the three years (2013–2015) were captured from a meteorological station located in one of the locations (Raphthadu) under study. The Mann–Whitney U test (non-parametric test) was performed using GENSTAT statistical package (version 14.0; Rothamsted Experiment Station, Harpenden, Hertfordshire, UK) for the comparison between GAPs and FP.

### 5. Conclusions

The results of our multiple on-farm demonstrations in farmers' fields provide very valuable information to groundnut producers on the management practices that are essential in containing aflatoxin contamination and improving the quality of groundnuts. Further, the GAPs that were demonstrated are cost-effective and easily adoptable. Overall on-farm demonstrations with best bet technologies serve as an important educative tool in addressing complex agricultural problems, such as aflatoxin contamination.

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## References

1. Waliyar, F.; Kumar, P.L.; Traore, A.; Ntare, B.R.; Diarra, B.; Kodio, O. Pre and post-harvest management of aflatoxin contamination in peanuts. In *Mycotoxins Detection Methods, Management, Public Health and Agricultural Trade*; Leslie, J., Bandyopadhyay, R., Visconti, A., Eds.; CABI: Waringford, UK, 2008; pp. 209–218.
2. Monyo, E.S.; Njoroge, S.M.C.; Coe, R.; Osiru, M.; Madinda, F.; Waliyar, F.; Thakur, R.P.; Chilunjika, T.; Anitha, S. Occurrence and distribution of aflatoxin contamination in groundnuts (*Arachis hypogaea* L.) and population density of Aflatoxigenic *Aspergilli* in Malawi. *Crop Prot.* **2012**, *42*, 149–155. [[CrossRef](#)]
3. Waliyar, F.; Umeh, V.C.; Traore, A.; Osiru, M.; Ntare, B.R.; Diarra, B.; Kodio, O.; Vijay Krishna Kumar, K.; Sudini, H. Prevalence and distribution of aflatoxin contamination in groundnut (*Arachis hypogaea* L.) in Mali, West Africa. *Crop Prot.* **2015**, *70*, 1–7. [[CrossRef](#)]
4. VanEgmond, H.P. (Ed.) Aflatoxin M1: Occurrence, toxicity, regulation. In *Mycotoxins in Dairy Products*; Elsevier Applied Science: London, UK, 1989; pp. 11–55.
5. D'Mello, J.P.F.; MacDonald, A.M.C. Mycotoxins. *Anim. Feed Sci. Technol.* **1997**, *69*, 155–166. [[CrossRef](#)]
6. Boutrif, E.; Canet, C. Mycotoxin prevention and control: FAO Programmes. *Revue de Médecine Vétérinaire* **1998**, *149*, 681–694.
7. Abidin, M.Z.; Ahmad, M.M.; Javed, S. Advances in molecular detection of *Aspergillus*: An update. *Arch. Microbiol.* **2010**, *192*, 409–425. [[CrossRef](#)] [[PubMed](#)]
8. Shephard, G.S. Aflatoxin and food safety: Recent African Perspectives. *J. Toxicol. Toxin Rev.* **2003**, *22*, 267–286. [[CrossRef](#)]
9. Williams, J.H.; Phillips, T.D.; Jolly, P.E.; Stiles, J.K.; Jolly, C.M.; Aggarwal, D. Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences, and interventions. *Am. J. Clin. Nutr.* **2004**, *80*, 1106–1122. [[PubMed](#)]
10. Strosnider, H.; Azziz-Baumgartner, E.; Banziger, M.; Bhat, R.V.; Breiman, R.; Brune, M.N.; DeCock, K.; Dilley, A.; Groopman, J.; Hell, K.; et al. Workgroup report: Public health strategies for reducing aflatoxin exposure in developing countries. *Environ. Health Perspect.* **2006**, *114*, 1898–1903. [[CrossRef](#)] [[PubMed](#)]
11. Liu, Y.; Wu, F. Global burden of aflatoxin-induced hepatocellular carcinoma: A risk assessment. *Environ. Health Perspect.* **2010**, *118*, 818–824. [[CrossRef](#)] [[PubMed](#)]
12. Diener, U.L.; Cole, R.J.; Sanders, T.H.; Payne, G.A.; Lee, L.S.; Klich, M.A. Epidemiology of aflatoxin formation by *Aspergillus flavus*. *Annu. Rev. Phytopathol.* **1987**, *25*, 249–270. [[CrossRef](#)]
13. Torres, A.M.; Barros, G.G.; Palacios, S.A.; Chulze, S.N.; Battilani, P. Review on pre- and post-harvest management of peanuts to minimize aflatoxin contamination. *Food Res. Int.* **2014**, *62*, 11–19. [[CrossRef](#)]
14. FAOSTAT. Food and Agricultural Organization Statistics Database. 2013. (FAOSTAT). Available online: <http://faostat3.fao.org/home/E> (accessed on 29 January 2016).
15. Yellamanda Reddy, T.; Sulochanamma, B.N.; Subramanyam, A.; Balaguravaiah, D. Influence of weather, dry spells and management practices on aflatoxin contamination in groundnut. *Indian Phytopathol.* **2003**, *56*, 262–265.
16. Hill, R.A.; Blankenship, P.D.; Cole, R.J.; Sanders, T.H. Effects of soil moisture and temperature on pre harvest invasion of peanuts by the *Aspergillus flavus* group and subsequent aflatoxin development. *Appl. Environ. Microbiol.* **1983**, *45*, 628–633. [[PubMed](#)]
17. Cole, R.J.; Sanders, T.H.; Hill, R.A.; Blankenship, P.D. Mean geocarposphere temperatures that induce pre-harvest aflatoxin contamination of peanuts under drought stress. *Mycopathologia* **1985**, *91*, 41–46. [[CrossRef](#)] [[PubMed](#)]

18. Cole, R.J.; Sanders, T.H.; Dorner, J.W.; Blankenship, P.D. Environmental conditions required to induce pre harvest aflatoxin contamination of groundnuts: Summary of six years' research. In *Aflatoxin Contamination of Groundnut: Proceedings of the International Workshop, 6–9 October 1987*. ICRISAT Center, India; Hall, S.D., Ed.; ICRISAT: Patancelu, India, 1989; pp. 279–287.
19. Wilson, D.M.; Walker, M.E. Effects of gypsum and irrigation on *Aspergillus flavus* group colonization of peanuts. In *Proceedings of the American Peanut Research and Education Society, Tulsa, OK, USA, 12–15 July 1988*; p. 24.
20. Waliyar, F.; Ntare, B.R.; Diallo, A.T.; Kodio, O.; Diarra, B. *On-Farm Management of Aflatoxin Contamination of Groundnut in West Africa: A Synthesis Report*; International Crops Research Institute for the Semi-Arid Tropics: Patancelu, India, 2007; 24p.
21. Arunyanark, A.; Jogloy, S.; Wongkaew, S.; Akkasaeng, C.; Vorasoot, N.; Kesmala, T.; Patanothai, A. Heritability of aflatoxin resistance traits and correlation with drought tolerance. *Field Crops Res.* **2010**, *117*, 258–264. [[CrossRef](#)]
22. Hamidou, F.; Rathore, A.; Waliyar, F.; Vadez, V. Although drought intensity increases aflatoxin contamination, drought tolerance does not lead to less aflatoxin contamination. *Field Crops Res.* **2014**, *156*, 103–110. [[CrossRef](#)]
23. Wilson, D.M.; Stansell, J.R. Effect of irrigation regimes on aflatoxin contamination of peanut pods. *Peanut Sci.* **1983**, *10*, 54–56. [[CrossRef](#)]
24. Mixon, A.C.; Bell, D.K.; Wilson, D.M. Effect of chemical and biological agents on the incidence of *Aspergillus flavus* and aflatoxin contamination of peanut seed. *Phytopathology* **1984**, *74*, 1440–1444. [[CrossRef](#)]
25. Reding, C.L.C.; Harrison, M.A.; Kvien, C.K. *Aspergillus parasiticus* growth and aflatoxin synthesis on florunner peanuts grown in gypsum-supplemented soil. *J. Food Prot.* **1993**, *56*, 593–611. [[CrossRef](#)]
26. Lazarovits, G.; Tenuta, M.; Conn, K.L. Organic amendments as a disease control strategy for soilborne diseases of high-value agricultural crops. *Australas. Plant Pathol.* **2001**, *30*, 111–117. [[CrossRef](#)]
27. Chhetry, G.K.N.; Mangang, H.C. Effect of soil amendments on soil borne pathogens of French bean (*Phaseolus vulgaris* L.) in organic farming system in Manipur. *J. Agric. Sci. Technol.* **2011**, *1*, 68–72.
28. Wilson, D.M.; Walker, M.E. Calcium potential aflatoxin foe. *South-East. Peanut Farmer* **1981**, *19*, 6B.
29. Thakur, R.P.; Rao, V.P.; Subramanyam, K. Influence of biocontrol agents on population density of *Aspergillus flavus* as well as kernel infection in groundnut. *Indian Phytopathol.* **2003**, *56*, 408–412.
30. Sanders, T.H.; Cole, R.J.; Blankenship, P.D.; Dorner, J.W. Aflatoxin concentration of peanuts from plants drought stressed in pod or root zones. *Peanut Sci.* **1993**, *20*, 5–8. [[CrossRef](#)]
31. Craufurd, P.Q.; Prasad, P.V.V.; Waliyar, F.; Taheri, A. Drought, pod yield, pre-harvest *Aspergillus* infection and aflatoxin contamination on peanut in Niger. *Field Crops Res.* **2006**, *98*, 20–29. [[CrossRef](#)]
32. Payne, G.A.; Hagler, W.M., Jr. Effect of specific amino acids on growth and aflatoxin production by *Aspergillus parasiticus* and *Aspergillus flavus* in defined media. *Appl. Environ. Microbiol.* **1983**, *46*, 805–812. [[PubMed](#)]
33. Middleton, K.J.; Pande, S.S.; Sharma, S.B.; Smith, D.H. Diseases. In *Groundnut Crop: A Scientific Basis for Improvement*; Smart, J.S., Ed.; Chapman and Hall: London, UK, 1994; pp. 336–378.
34. Mutegi, C.K.; Ngugi, H.K.; Hendriks, S.L.; Jones, R.B. Factors associated with the incidence of *Aspergillus* section *Flavi* and aflatoxin contamination of peanuts in the Busia and Homa bay districts of western Kenya. *Plant Pathol.* **2012**, *61*, 1143–1153. [[CrossRef](#)]
35. Wu, F.; Liu, Y.; Bhatnagar, D. Cost-effectiveness of aflatoxin control methods: Economic Incentives. *Toxin Rev.* **2008**, *27*, 203–225. [[CrossRef](#)]
36. Reddy, P.S.; Basu, M.S.; Khaleque, M.A.; Haque, M.S.; Ali, A.; Malek, H.; Than, H.; Soe, T.; Regunathan, B.; Mishra, B.; et al. Status of groundnut research and production in South Asia. In *Groundnut—A Global Perspective. Proceedings of an International Workshop 25–29 November 1991*, ICRIST Center; Nigam, S.N., Ed.; ICRISAT: Patancelu, India, 1992; pp. 133–147.
37. Sudini, H.; Ranga Rao, G.V.; Gowda, C.L.L.; Chandrika, R.; Margam, V.; Rathore, A.; Murdock, L.L. Purdue Improved Crop Storage (PICS) bags for safe storage of groundnuts. *J. Stored Prod. Res.* **2015**, *64*, 133–138. [[CrossRef](#)]
38. Gong, Y.Y.; Hounsa, A.; Egal, S.; Turner, P.C.; Sutcliffe, A.E.; Hall, A.J.; Cardwell, K.; Wild, C.P. Post-weaning exposure to aflatoxin results in impaired child growth: A longitudinal study in Benin, West Africa. *Environ. Health Perspect.* **2004**, *112*, 1334–1338. [[CrossRef](#)] [[PubMed](#)]
39. Kumar, G.D.S.; Popat, M.N. Assessment of adoption gaps in management of aflatoxin contamination of groundnut (*Arachis hypogaea* L.). *J. Agric. Educ. Ext.* **2010**, *16*, 309–319. [[CrossRef](#)]

40. Kumar, G.D.S.; Popat, M.N. Farmers' perceptions, knowledge and management of aflatoxins in groundnuts (*Arachis hypogaea* L.) in India. *Crop Prot.* **2010**, *29*, 1534–1541. [[CrossRef](#)]
41. Kumar, G.D.S.; Popat, M.N. Factors influencing the adoption of aflatoxin management practices in groundnut (*Arachis hypogaea* L.). *Int. J. Pest Manag.* **2010**, *56*, 165–171. [[CrossRef](#)]
42. Kishore, G.K.; Pande, S.; Manjula, K.; Narayana Rao, J.; Thomas, D. Occurrence of mycotoxins and toxigenic fungi in groundnut (*Arachis hypogaea* L.) seeds in Andhra Pradesh, India. *Plant Pathol. J.* **2002**, *18*, 204–209. [[CrossRef](#)]
43. Okalebo, J.R.; Gathua, K.W.; Woomer, P.L. Exchangeable bases in soils. In *Laboratory Methods of Soil and Plant Analysis: A Working Manual*; Tropical Soil Biology and Fertility Programme: Nairobi, Kenya, 1993; pp. 52–54.
44. Nelson, D.W.; Sommers, L.E. Total carbon, Organic carbon and organic matter. In *Methods of Soil Analysis, Part 3: Chemical Methods*; Sparks, D.L., Page, A.L., Eds.; Soil Science Society of America & American Society of Agronomy: Madison, WI, USA, 1996; pp. 961–1010.
45. Thomas, G.W. Soil pH and soil acidity. In *Methods of Soil Analysis, Part 3: Chemical Methods*; Sparks, D.L., Page, A.L., Eds.; Soil Science Society of America & American Society of Agronomy: Madison, WI, USA, 1996; pp. 475–490.
46. Waliyar, F.; Bockelee-Morvan, A. Resistance of groundnut varieties to *Aspergillus flavus* in Senegal. In Proceedings of the International Workshop on Aflatoxin Contamination Groundnut, Patancheru, India, 6–9 October 1987; pp. 305–310.
47. Raper, K.B.; Fennell, D.I. *The Genus Aspergillus*; Williams and Wilkins: Baltimore, MD, USA, 1965; p. 686.
48. Reddy, S.V.; Kiran Mayi, D.; Uma Reddy, M.; Thirumala Devi, K.; Reddy, D.V.R. Aflatoxin B<sub>1</sub> in different grades of chilies (*Capsicum annum*) as determined by indirect competitive-ELISA. *Food Addit. Contam.* **2001**, *18*, 553–558. [[CrossRef](#)] [[PubMed](#)]



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