

# Influence of Plant Population and Harvest Date on Peanut (*Arachis hypogaea*) Yield and Aflatoxin Contamination

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## ABSTRACT

Research was conducted in Malawi at Mpat-sanjoka farm in Salima district during the 2015-2016 and 2016-2017 growing cycles to determine interactions of plant population and harvest date on peanut (*Arachis hypogaea* L.) yield and aflatoxin concentration in peanut at harvest with the cultivar CG7. Peanut was seeded in raised beds spaced 75-cm apart with three different planting patterns to establish three final plant populations. A single row planting pattern consisted of one row of peanut on each center with seed spaced 15-cm apart was used to plant 89,000 seed/ha (low plant seeding rate). A twin row planting pattern included two rows of peanut spaced at 25 cm apart with 15 cm between seeds was used to plant 178,000 seed/ha (medium plant population). A triple row planting pattern consisted of three rows of peanut spaced 25 cm apart with 7 cm between seeds was used to plant 278,000 seed/ha (high density). Peanut was dug 10 days before physiological maturity, at physiological maturity, and at both 4 wk, and 6 wk after physiological maturity. Pod yield increased as seeding rate and subsequent plant population increased but decreased as harvesting was delayed past physiological maturity. Yield of peanut with the highest plant population exceeded that of low and medium populations; yield of the medium plant population was greater than the low population in one of two years. Aflatoxin concentration at harvest was not affected by plant population but increased as harvest was delayed past physiological maturity. Harvesting peanut 10 d prior to physiological maturity did not affect yield or aflatoxin contamination compared with harvesting at optimum maturity.

Key Words: cultural practices, seeding rate.

Peanut (*Arachis hypogaea* L.) is one of the most widely grown legumes by smallholder farmers in Malawi (Nyondo *et al.*, 2018). Peanut is a relatively inexpensive source of dietary protein and other essential nutrients for both urban and rural households (Okello *et al.*, 2010a 2010b). Peanut also serves as an important source of livestock and poultry feed (Kochhar 1986; Usman *et al.*, 2012). However, yield is low (700 kg/ha) in Malawi compared to other countries because most smallholder farmers in Malawi grow peanut with little or no inputs (Ngwira *et al.*, 2019). In addition to low yields, aflatoxin, a mycotoxin produced by *Aspergillus flavus* and *A. parasiticus* contaminates peanut-based food products and contributes to poor health (Bowen and Hagan, 2015). Peanut and food products that contain aflatoxin above established levels can limit marketing opportunities (FAO, 2001; Matumba *et al.*, 2015; Waliyar *et al.*, 2010). The European Union and Malawi Bureau of Standards accepts aflatoxin level of 4 µg/kg while the World Health Organization (WHO) standard is 20 µg/kg (Monyo *et al.*, 2012; Otsuki and Wilson, 2001). Aflatoxin contamination adversely affects human health in a number of ways including liver cancer and immunosuppressive effects (Guchi, 2015; WHO, 2006).

Aflatoxin can be present prior to harvest during the growing cycle when peanut is exposed to prolonged high day and night temperatures and drought during pod filling (Payne, 1998; Sanders *et al.*, 1984). Pre-harvest aflatoxin contamination in peanut is often associated with cracked pods that allow entry of soil that contains *A. flavus* (Craufurd *et al.*, 2006). High relative humidity and soil temperatures ranging from 25 to 35 C are favorable for *A. flavus* development (Bowen and Hagan, 2015; Cole *et al.*, 1984; Hill *et al.*, 1983). Weather conditions that result in delayed harvest increase the likelihood of greater damage from arthropods and vertebrates that increase pod damage and subsequent movement of soil into pods (Desai *et al.*, 2008). Minimizing stress associated with high temperatures, drought, and damage from other organisms can minimize aflatoxin contamination of peanut going into drying and storing steps in the supply chain (Torres *et al.*, 2014).

Establishing adequate populations of peanut increases yield and uniformity of harvested peanut

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(Onat *et al.*, 2017; Okello *et al.*, 2010a 2010b). The current recommended density of peanut planted in Malawi is 89,000 plants/ha when established in rows spaced 75 cm apart at a distance of 15 cm between plants in each row (Ngwira *et al.*, 2019). However, Onat *et al.* (2017) reported that increasing the population to 133,000 plants/ha increased yield compared with lower densities in Turkey. Greater yields were also noted in the United States (Kvien *et al.*, 1987) and India (Rasekh *et al.*, 2010) when higher plant populations were established as compared with lower plant populations. As farmers consider increasing seeding rates to achieve higher plant populations, it is important to determine if this approach affects aflatoxin contamination. The impact of plant population on aflatoxin contamination has not been documented in the peer-reviewed literature.

Harvesting peanut when the kernels and pods reach physiological maturity often results in greater yields, higher market grade characteristics (Okello *et al.*, 2010a 2010b), and can minimize seed infection by *A. flavus* (Mehan *et al.*, 1986; Sanders *et al.*, 1985). Harvesting peanut prior to physiological maturity can result in a distribution of kernels that contain a higher percentage of smaller and less mature kernels as compared with harvesting at physiological maturity (Carter *et al.*, 2017). When peanut is harvested past physiological maturity, pods have greater exposure to pathogens and arthropods that can cause damage (Okello *et al.*, 2010a 2010b; Singh and Oswalt, 1995). Pods that are past physiological maturity for an extended period of time, especially if soil moisture is adequate, can sprout and result in lower yield and quality (Nautiyal *et al.*, 2001; Singh and Oswalt, 1995). Pods can also shed from plants and may not be harvestable (Kaba, 2014).

The majority of farmers in Malawi establish plant populations lower than what is needed to optimize yield because of seed quality issues and expense (Ngwira *et al.*, 2019). Farmers also do not fully appreciate the relationship of harvest date, physiological maturity, and the impact of pests on yield and quality, including the human health and marketing ramifications of contamination with aflatoxin (Kaba, 2014). The relationship between plant population and harvest date has not been evaluated in Malawi in a systematic way with respect to peanut yield and aflatoxin contamination. Therefore, research was conducted to determine the impact of plant population and harvest date on peanut yield and contamination by aflatoxin at harvest in Malawi.

**Table 1. Monthly rainfall recorded at Mpatsanjoka farm in Salima district of Malawi during cropping cycles of 2015-2016 and 2016-2017 from Nov through March. No measurable rainfall occurred after March during the peanut growing cycle.**

Month	2015-2016	2016-2017
	—mm—	
Nov	0	29
Dec	118	233
Jan	246	256
Feb	248	255
March	0	264
Total	612	1037

## Materials and Methods

The experiment was conducted at Mpatsanjoka farm in Salima district of Malawi (13°42.740'S, 034°28.879'E) during crop cycles in 2015-2016 and 2016-2017. Mpatsanjoka farm is located near Lake Malawi at an elevation of 550 m above sea level. Monthly average rainfall from Nov through March for each cropping cycle is presented in Table 1. No measurable rainfall was observed after March of each year. Soil was a sandy loam with pH 5 and organic matter content of 2.7%. Peanut was seeded on 13 December 2015 and 22 December 2016 in raised seedbeds with a height of 20 to 30 cm (Table 2). Immediately after planting, dimethenamid-*P* (Frontier-P herbicide, Australian Pesticides and Veterinary Medicine Authority, Kingston, Australia) was applied at 0.84 kg ai/ha to control weeds. Hand weeding was used throughout the season to minimize weed interference. No other pesticides were applied to control arthropods or pathogens.

Treatments consisted of the virginia market type cultivar CG7 (Ngwira *et al.*, 2019) seeded in single, twin, and triple row patterns at rates of 89,000, 178,000, and 285,000 seed/ha to establish low, medium, and high plant populations, respectively (Table 3). The seeding rate of 89,000 seeds/ha was

**Table 2. Planting and harvest dates for peanut during two growing cycles in Malawi.**

Field operation	2015-2016	2016-2017
Planting	Dec 13	Dec 18
Harvest 10 days before physiological maturity <sup>a</sup>	April 22	April 27
Harvest at physiological maturity	May 2	May 7
Harvest 4 week after physiological maturity	May 31	June 5
Harvest 6 weeks after physiological maturity	June 14	June 18

<sup>a</sup>Physiological maturity as described by Ngwira *et al.*, 2019.

**Table 3. Planting pattern, seeding rate, and average plant population at harvest during two growing cycles in Malawi.**

Planting pattern	Plant population	Space between bed centers	Spacing of rows on each bed	Space between seed within a row	Plant population at harvest		
					Seeding rate	2015-2016	2016-2017
			cm		no./ha		
Single	Low	75	-	15	89,000	63,750	44,844
Twin	Medium	75	18	15	178,000	95,625	71,563
Triple	High	75	18	7	267,000	231,719	240,938

established on beds spaced 75 cm apart with one row of peanut with 15 cm between seeds. The seeding rate of 178,000 seeds/ha was established on beds with 75-cm centers with two rows of peanut spaced 18 cm apart with 15 cm between seeds. The seeding rate of 285,000 plants/ha was established on beds with 75-cm centers with three rows of peanut spaced at 18 cm with 7 cm between seeds. The final in-row spacings for these respective plant populations/ha during each cycle are presented in Table 3. Seeds were treated with thiram (Thiram 50WP, Bayer Crop Science, Research Triangle Park, NC) at 3g/kg of peanut seed and planted in furrows created manually at a depth of 3 cm. Within each plant population, peanut was harvested 10 d before physiological maturity, at physiological maturity, 4 wk after physiological maturity, and 6 wk after physiological maturity. The internal color of pods was used to determine maturity by collecting representative plants across the field. When 70% of pods visible darkening caused by removal of the endocarp as kernels increased in size and development, peanut was considered physiologically mature (Ngwira *et al.*, 2019). Approximately 50% of pods expressed this level of darkening 10 d prior to physiological maturity. After gently removing peanut pods from the soil, above-ground vegetation, pods, and remaining roots were placed on a Mandela cock (Ngwira *et al.*, 2019). When pod moisture was 8.5%, total plant biomass, pod weight, grain weight, and aflatoxin contamination in pods were determined.

Aflatoxin concentration was determined using 300 g of shelled peanut from each plot. The sample was weighed and homogenized using a hand grinder (Globe Trek, Navi Mumbai, Mumbai) and thoroughly mixed. A 10-g sub-sample was removed and agitated in 30 ml of ethanol (65%) and water (35%) for 1 min. The sub-sample was filtered using a 500  $\mu$ L pipette (Fisher Scientific, Pittsburg, PA) with the diluent. One hundred  $\mu$ L of the filtered liquid was inserted into the sampling cup and left in the cup for 6 min. Neogen Reveal Q+ lateral flow strips (Neogen Corp., Lansing, MI) were inserted into the strip holder (Neogen Corp., Lansing, MI) to determine aflatoxin concentration

using the Mobile Assay mReader software (Mobile Assay Inc., Boulder, CO).

Data for total biomass, pod weight, grain weight, and aflatoxin contamination were subjected to analysis of variance for the split-plot design using GENSTAT 18<sup>th</sup> edition computer package (VSN international, England, United Kingdom). Seeding density served as the whole plot unit and harvest date served as the sub-plot unit. Differences of main effects and interactions were separated using Fisher's Protected LSD test at  $p < 0.05$ .

## Results and Discussion

Interactions of growing cycle by seeding rate by harvest date were not significant for plant biomass at harvest, pod yield, and aflatoxin concentration ( $p > 0.05$ ). However, main effects of seeding rate and harvest date, the interaction of growing cycle and seeding rate, and the interaction of growing cycle and harvest date were significant. Data for plant biomass, pod yield, and aflatoxin concentration will be presented for these interactions combined over growing cycle and the other treatment factor (Tables 4 and 5). Oakes *et al.* (2020) reported that both seeding rate and harvest date affected pod yield but response to these treatment factors was independent.

Above-ground plant biomass increased as seeding rate and subsequent plant population increased in one of the two growing cycles (Table 4). In 2016-2017 growing cycle, plant biomass increased for each increase in plant density. Bell *et al.*, (1987) reported that peanut biomass increased from 12,600 kg/ha to 16,900 kg/ha with increasing plant density up to the maximum density of 588,000 plants/ha.

Peanut pod yield was greater in both growing cycles for the seeding rate of 285,000 seed/ha compared with the lower seeding rates (Table 4). In the 2015-2016 growing cycle, no difference in yield was observed for the low and medium seeding rates. However, in the 2016-2017 growing cycle, peanut yield for the medium seeding rate exceeded that of the low seeding rate. Aflatoxin contamina-

**Table 4. Influence of seeding rate on total peanut biomass, peanut grain yield, and aflatoxin contamination.<sup>a</sup>**

Plant population <sup>b</sup>	Total plant biomass		Grain yield		Aflatoxin contamination	
	2015-2016	2016-2017	2015-2016	2016-2017	2015-2016	2016-2017
	kg/ha				µg/kg	
Low	8240 a	5580 c	810 b	760 c	24.5 a	4.6 a
Medium	9460 a	7030 b	880 b	1020 b	23.2 a	9.0 a
High	10120 a	7500 a	1350 a	1330 a	37.3 a	6.3 a
P > F	0.141	<0.001	0.0050	<0.001	0.1190	0.5200

<sup>a</sup>Means within a cropping cycle for each measurement followed by the same letter are not significantly different according to Fisher's Protected LSD test. Data are combined over harvest dates.

<sup>b</sup>A seeding rate of 89,000 seeds/ha was used on beds spaced 75 cm apart with one row of peanut with 15 cm between seeds to establish a final plant population of 63,750 plants/ha (2015-2016) or 44,844 plants/ha (2016-2017) in the single row pattern. A seeding rate of 178,000 seeds/ha was used on beds with 75-cm centers with two rows of peanut spaced 18 cm between apart with 15 cm between seeds to establish a final plant population of 95,625 plants/ha (2015-2016) or 71,563 plants/ha (2016-2017) in the twin row pattern. A seeding rate of 267,000 seeds/ha was used on beds with 75-cm centers with three rows of peanut spaced 18 cm apart with 7 cm between seeds to establish a final plant population of 231,719 plants/ha (2015-2016) or 240,931 plants/ha (2016-2017) in the triple row pattern.

tion at harvest was not affected by seeding rate in either growing cycle.

Increasing the seeding rate often ensures greater uniformity of pod maturity, improved quality of grain for marketing, and maximum yield (Okello *et al.*, 2010a 2010b). However, variation in response to seed spacing has been observed. Konlan *et al.* (2013) in Ghana reported that decreasing spacing between seeds increased yield by 6.2% in one year and 16.0% in a second year of the study. Rasekh *et al.* (2010) reported higher yields when the in-row seeding rate was increased from 3 plant/m to 8.3 plants/m. Awal and Aktar (2015) and Gabisa *et al.* (2017) reported greater yield with a plant population of was increased. El Naim *et al.* (2011) reported that peanut plants spaced 10 cm apart yielded 40% less than peanut planted 40 cm apart under rain-fed conditions.

Above-ground peanut biomass was greater 10 d prior to physiological maturity compared to mass at physiological maturity (Table 5). When harvest was delayed past physiological maturity, biomass continued to decrease. This was due to significant

vegetative growth and by this time the crop did not lose its leaves while the soil was still moist. Peanut pod yield was similar when harvested at physiological maturity, or 10 d prior to physiological maturity (Table 5). In the first growing cycle, yield was similar when peanut was harvested at physiological maturity or 4 wk after physiological maturity. In contrast, in the second growing cycle, peanut yield was lower when harvested 4 wk after physiological maturity than harvest at physiological maturity. Delaying harvest to 6 wk after physiological maturity resulted in the lowest grain yield. The decrease in pod yield with the delayed harvesting was due to field losses as many remaining in the field due to natural pod shed. Young *et al.* (1982) estimated typical digging losses of 8% of total yield, but can reach 40% at dates when harvest is delayed past optimal maturity. Okello *et al.* (2010a 2010b) reported that delayed harvesting causes yield losses of greater than 400 kg/ha and kernel quality reduced by 3%.

Aflatoxin contamination was similar when peanut was harvested 10 d prior to physiological

**Table 5. Influence of harvest date on total peanut biomass, peanut grain yield, and aflatoxin contamination.<sup>a</sup>**

Harvest date <sup>b</sup>	Total plant biomass		Grain yield		Aflatoxin contamination	
	2015-2016	2016-2017	2015-2016	2016-2017	2015-2016	2016-2017
	kg/ha				µg/kg	
10 days before physiological maturity	11820 a	9140 a	1030 ab	1100 ab	13.9 b	1.9 b
Physiological maturity	9580 b	6330 b	1180 a	1140 a	10.8 b	2.6 b
4 week after physiological maturity	8070 c	5990 c	980 ab	980 bc	40.0 a	8.6 ab
6 weeks after physiological maturity	7610 d	5350 d	850 b	920 c	48.6 a	13.4 a
P > F	<0.001	<0.001	0.066	0.014	<0.001	0.025

<sup>a</sup>Means within a cropping cycle for each measurement followed by the same letter are not significantly different according to Fisher's Protected LSD test. Data are combined over plant populations.

<sup>b</sup>Physiological maturity as described by Ngwira *et al.*, 2019.

maturity or at physiological maturity (Table 5). In the first growing cycle, aflatoxin contamination was greater when harvest was delayed by 4 and 6 wk past physiological maturity compared with harvest 10 d prior to physiological maturity or at physiological maturity. By 4 and 6 wk after physiological maturity, aflatoxin contamination was 40.0 to 48.6 µg/kg. During the second growing cycle, aflatoxin contamination did not exceed 13.6 µg/kg. The difference in aflatoxin due to harvest date most likely was associated with rainfall patterns during the latter part of the growing cycle in March. Rainfall ceased during the 2015-2016 growing cycle in March while rainfall during March 2017 was during 264 mm and just under twice the total rainfall during the growing cycle (Table 1). Adequate rainfall which reduces aflatoxin contamination in peanut because it increases tissue integrity hence reduce invasion of *A. flavus* (Diao *et al.*, 2015). The lower concentration of aflatoxin most likely was associated with complete maturation of pods when rainfall was more abundant in March which minimized infection when soil moisture in soil was higher. Sanders *et al.* (1984) reported that peanut pods developed without damage to shells and limit entry of *Aspergillus spp.* to colonize pods. Okello *et al.* (2010a 2010b) reported that harvesting peanut at optimum maturity reduces incidences of aflatoxin contamination. Peanut harvested after physiological maturity had high aflatoxin contamination because of over maturity and delayed harvesting which increases aflatoxin contamination (Diener and Davis, 1977; Okello *et al.*, 2010a 2010b).

In summary, increasing seeding rates and subsequent plant populations up to 285,000 seed/ha resulted in the greatest yield across the two growing cycles. However, seeding rate had no effect on aflatoxin contamination at harvest. It is postulated that higher plant populations likely shade soil and create cooler soil environment that is less conducive to *A. flavus* growth and infection. However, our results did not support that hypothesis. It was also postulated that harvesting peanut prior to physiological maturity would result in less aflatoxin compared with harvesting at physiological maturity or past physiological maturity. Our results are in contrast to those by Bowen and Hagan (2015) who reported less aflatoxin when peanut was harvested earlier than recommended to optimize pod yield when conditions were favorable for *A. flavus* development. However, our results were consistent with those of Young *et al.*, (1982) demonstrating that delaying harvest past physiological maturity can result in lower yield due to leaf and pod shed due to disease and natural processes

and sprouting of seed or damage caused by arthropods in soil. Pod damage caused by arthropods can allow soil to enter pods and increase the amount of *A. flavus* in pods and subsequently cause greater aflatoxin contamination.

This is the first experiment in the peer-reviewed literature that has addressed the impact of seeding rate and subsequent contamination by aflatoxin. Our results indicate that plant population and harvest date do not interact for pod yield or aflatoxin contamination. Oakes *et al.* (2020) also reported that both seeding rate and harvest date affected peanut pod yield independently. It is important to note that plant populations were established using three different planting patterns with peanut plants distributed differently across beds. Additional research is needed to determine the impact of a more dense population using a similar planting pattern to determine the impact of plant population on yield and aflatoxin contamination.

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