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Research Article

Aspergillus Species Groundnut Seed Invasion as influenced by Soil Solarization and Time of Planting

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Abstract

Aflatoxin contamination of groundnut is a serious problem in most groundnut-producing countries where the crop is grown under rainfed conditions. Field experiments were conducted at two locations to determine the effect of soil solarization on *Aspergillus* spp. inocculum in the soil and to evaluate the effect of soil solarization and time of planting on Aspergillus spp. seed invasion and yield of groundnut varieties. Soil samples were taken in three rounds and analyzed for aflatoxigenic population. Soil solarization reduced fungal inoculum and increased groundnut yields. Individual and total cfu $g^{-1}$of soil was determined before, after solarization and at harvest. Four *Aspergillus* species namely, *A. flavus, A. parasiticus, A. niger and A. terreus* were identified and their densities were significantly ($P \leq 0.05$) reduced at after solarization. In the solarized plots, *A. flavus and A. parasiticus* were found reduced by 53.8 and 45% cfu g^{-1} at Ramma and 36.4 and 44% cfu g^{-1}at 5 and 10 cm soil depths at Mayweyni, respectively, after soil solarization in the solarized plots than the nonsolarized plots. At harvest, *Fusarium spp., A. flavus* and *A. terreus* were detected. Three *Aspergillus species* namely, *A. flavus, A. niger*, and *A. parasiticus* were isolated from seed samples plated on Czapek-Dox Agar medium. Early planting of the varieties showed the lowest level of seed infection by *A. flavus* (22.8%).

Keywords: *Aflatoxin,* Aspergillus *Spp, , groundnut, , planting time, soil solarization,.*

Introduction

Groundnut (*Arachis hypogaea* L.) is the 13th most important food crop of the world. It is the world's 4th most important source of edible oil and 3rd most important source of vegetable protein. Groundnut seeds contain high quality edible oil (50%), easily digestible protein (25%), and carbohydrate (20%). It is grown on 26.4 million ha worldwide with total production of 36.1 million metric tons, and an average productivity of 1.4 metric tons ha^{-1}. (FAO, 2004). It is also rich in Ca, K, P, Mg, vitamin E, vitamin B1, B2, B6, nicotinic acid and other vitamins (Atasie *et al*., 2009). Total land coverage of groundnut in Ethiopia is about 41,761 ha and the production is estimated to be 46,887.2 tones (EARO, 2009). These characteristics led the groundnut to become sensitive to fungal contamination.

Aflatoxin contamination of groundnut is a serious problem in most groundnut-producing countries where the crop is grown under rainfed conditions (Rao *et al*., 1995). Aflatoxins are a family of extremely toxic, mutagenic and carcinogenic compounds produced mainly by *Aspergillus flavus* and *A. parasiticus* Speare (Goto *et al.*, 1996; Peterson *et al*., 2001). Regulations will have limited effect on health protection, as the producers themselves mainly consume groundnut produced. The existing food shortage in Ethiopia forces people to consume what they might have even when the food is moldy otherwise rejected. This exposes at least some of the population to higher risk of consumption of aflatoxin-contaminated food (Amare, 2002).

Pre and postharvest aflatoxin management can be achieved by agronomic and cultural practices, such as soil amendment, date of planting, use of resistant varieties and appropriate harvesting and postharvest handling practices (drying and storage) (Waliyar *et al.*, 2008). There is a need for information on the occurrence of aflatoxins in groundnut produced at different agro ecological zones of Ethiopia. Such information will not only extend the data base on aflatoxin occurrence but also is useful in order to assess the public health hazard due to mycotoxin.

Aspergillus flavus has been reported as one of the major fungi that could limit the production of the crop in most groundnut-producing areas of Ethiopia (Teklemariam *et al*., 1986). The fungus has been implicated in storage disease of groundnut. Although systematic survey is lacking, studies that covered different agroecological regions in Ethiopia have highlighted that a vast majority (80%) of *A. flavus* isolates from Ethiopian cereal grains were capable of producing aflatoxin (Dawit and Brhanu, 1985). In eastern parts of Ethiopia, great majority (84.6%) of *A. flavus* isolates from groundnut seed were capable of producing aflatoxin (Amare *et al.*, 1995). Aflatoxin levels ranging from 5 to 250 ppb were detected in groundnut samples from eastern Ethiopia (Amare *et al*., 1995).

In several parts of Northern Ethiopia, groundnut is becoming more attractive to the farmers due to its higher net profit per unit area compared to other crops (Dereje Assefa, personal communication). However, there are constraints resulting in quality deterioration and health problems due to high aflatoxin contamination of the crop in the area. Recent studies covering three groundnuts producing Woredas of Tigray reported a contamination level ranging from 0.08 to 295 ppb (Assefa, 2010). The same study also indicated that *A. flavus* and *A. niger* were the dominant species detected from groundnut samples with a range of 10-100% and 0-90% incidences, respectively. Considering the widespread occurrence of aflatoxigenic fungi and prevalent conditions favoring preharvest aflatoxin contamination, such as moisture stress it is prudent to develop suitable measures for the management of the aflatoxin problem in Northern Ethiopia.

It is also well established that preharvest invasion of groundnut by aflatoxigenic fungi is greatly favored by drought stress. Planting time is an important production component that can be manipulated to counter the adverse effects of environmental stress. This is accomplished through shifting plantings (sowings) so that any stress caused by environment is avoided during the critical stage of plant growth. Thus, effective reduction of soil borne inoculum coupled with cropping practices, such as planting date and the use of suitable varieties could contribute towards reducing fungal invasion and subsequent aflatoxin contamination of groundnut. Thus, the specific objectives of the current study were to find out the effect of soil solarization on *Aspergillus* spp. inoccla in the soil, and evaluate the effect of soil solarization and time of planting on *Aspergillus* seed invasion.

Materials and Methods

Description of experimental sites

The field experiment was conducted in Central Zone of Tigray Region, at Ramma Farmers' Training Center (FTC) and Mayweyni Farmers' Training Center (FTC). Ramma FTC is located at 14'' 22.25'N latitude and 038'' 47.32'E longitude at an elevation of 1429 meters above sea level in the northern part of Ethiopia. It lies in the *Kola* agro- ecological zone. The soil type is sandy. Mayweyni FTC is located at 14'' 23.47'N latitude and 038'' 48.75'E longitude at an elevation of 1403 meters above sea level. The soil type is sandy loam. The average annual rainfall in these areas range from 400 to 600 mm and the rainfall distribution is erratic beginning in June and ending in August. The mean daily temperatures for Ramma and Mayweyni are 24 and 28^{o}C, respectively. The laboratory experiments were conducted in Plant pathology laboratory at Haramaya University, Ethiopia.

Treatments and experimental design

Three groundnut varieties namely, 'Sedi, 'Werer 961' and 'NC343' were obtained from Melka Werer Agriculture Research Center, treated with Mancozeb at rate of 3g/kg and planted in field experiments at the two locations. The treatments were laid out in split-split plot design with three replications. Main plots were assigned to soil solarization treatments (solarized and nonsolarized), sub plots to planting time (early, normal and later) and three groundnut varieties as sub-sub plots. The experimental field was thoroughly cultivated and then leveled so as to minimize such

protrusions as clods, stubble, and stones. It was divided into plots and the main plots were solarized by covering with transparent polyethylene plastic sheet for 28 days before planting. The edges of the plastic sheet were buried 15 cm into the soil in order to keep high moisture content and reduce heat escape throughout the experiment. The plastic sheet was removed one day before planting for every sowing date treatments. Three planting dates namely, normal planting (farmers' practice), ten days earlier and ten days later than the farmers practice were used in the experiments. The varieties were consisting of different maturity groups. Variety 'Sedi' is the earliest maturing, while 'NC343' is latest.

Sub-sub plot size was 3 m long by 2.4 m (four ridges) wide and seeds were sown singly at 10 cm spacing along the ridges. There was 1 m interval between sub-sub plots and between sub plots and 1.5 m between main plots. The earliest sowing was done on 7 June, and the normal and latest were carried on 14 and 24 June 2010, respectively. Weeding was undertaken as and when needed to keep the plots weed free. The plots were not irrigated during the growing season and the varieties were left exposed to direct sunlight with no form of shading during the growth period. At harvest, groundnuts from each variety were stored in the cold room at $4C^{o}$ until used for analyses.

Procedures for crop parameters and seed invasion data collection

To determine the natural seed infection by *Aspergillus* spp. and other fungi, undamaged pods from the middle two rows were carefully hand- shelled and 100-seed of each plot were surface sterilized by soaking in 5% aqueous solution of sodium hypochlorite (NaOCl) for 3 minutes. The seeds were immediately rinsed with sterile distilled water and plated on Czapek-Dox agar medium (Cz) (CM97, Oxoid, Bakingstone UK) (Klich, 2002). Fifteen seed each were plated on the medium in 14.5 cm diameter Petri dish. After incubation at room temperature for 7days the growing colonies of fungi were visually recorded. Finally,four *Aspergillus* species namely, *Aspergillus flavus*, *A. niger*, *A. parasiticus and A. terreus* were isolated from groundnut seed using czapek-Dox agar medium. fungi identification was carried out based on macro-morphological (reverse and surface coloration of colonies, presence of pigment, and colony texture) and micro- morphological characteristics (conidia size, conidial head, shape of vesicle). Standard text (Klich, 2002) was used in the identification process.

Data Collection

For each location, the following data were recorded:

Soil temperature

During the soil solarization period, the temperature of the solarized and nonsolarized plots were measured using digital thermometer, three times a day (morning, mid-day and evening).

Colony forming unit per gram of soil (Cfu/g soil) was calculated for individual and total fungi using the formula

$$\frac{Cfu}{g\,soil} = \text{average colonies per plate x dilution factor}$$

Percentage of *Aspergillus* invasion of seed (%)

The amount of seed that was invaded by *Aspergillus* spp. from the total samples

Data Analysis

The data were subjected to analysis of variance (ANOVA) using statistical analysis systems (SAS V. 9.0). Percentage data were square root transformed prior to statistical analysis. Least significance difference (LSD) was used for comparing treatment means.

Results and Discussion

The effect of soil solarization and planting time on groundnut seed invasion by *Aspergillus* Species

The three groundnut varieties were investigated for the presence of *Aspergillus* species and seed invasion. The laboratory analysis of seed invasion revealed that soil solarization significantly reduced *A. flavus* (5.9), *A. parasiticus* (3.1) and *Aspergillus niger* (2.6) as compared to nonsolarized plots at Mayweyni. Soil solarization showed significant ($P \leq 0.05$) difference only on *A. parasiticus* at Ramma. Planting time alone did not show any effect on seed invasion at both seed samples. *A. niger* was found to be the most dominant

(mean of 33.6) followed by *A. flavus* (22.9) and then *A. parasiticus* (14.3) at Mayweyni (Table 5). Generally, *A. niger* was found to be the most dominant (mean of 33.6) followed by *A. flavus* (22.9) and *A. parasiticus* (12.3), respectively, at Mayweyni

Interaction effects of groundnut varieties on seed invasion by *Aspergillus* Spp.

There was significant interaction between soil solarization and planting time treatment on seed invasion by *A. flavus* and *A. niger* at Ramma (Table 2). The interaction effect on *A. flavus* and *A. niger* was similar for the normal planting time (Pd2) at Ramma. Interaction between varieties and soil solarization was significant on seed invasion by *A. flavus* at Mayweyni. Greater percentage of seed invasion was obtained from "Werer 961" (20.6%) and "Sedi" (21.4%) than 'NC-343' (18.9%) at Mayweyni. Significant interaction between variety and planting time was obtained both at Ramma and Mayweyni on seed invasion by *A. flavus*. Generally, the variety 'Werer 961'was found the most susceptible to seed invasion by *A. flavus* during all planting times (Table 2). Moreover, greater percentage of seed invasion by *A. flavus* was obtained from normal planting time (pd2) at Mayweyni. Overall, the response of varieties to planting time on groundnut seed invasion by *Aspergillus* species was generally greater in Mayweyni than at Ramma. This could be due to the environmental factors such as temperature, rainfall, soil type and previous crop (maize). The overall interaction (Sol x Pd x V) was found significant ($P \leq 0.05$) on *A. niger* with mean of 33.6 at Mayweyni.

During the crop season there was well distributed rain fall in the study area, this might have contributed to reduced seed invasion by *Aspergillus* spp. recorded from late planted groundnuts. The seed invasion by *A. flavus* at the late planting time was relatively low. This is not in agreement with the findings of Stewart *et al*. (1997) who reported that late planted groundnuts had higher incidence of A. *flavus* than those early planted. In the current study late planted plots had lower seed invasion as compared to early planted ones. Barrosa *et al*. (2002) suggested that groundnut seeds may be colonized during their development by *A. flavus* and the soil is the main source of inoculum for this species before harvest. The present study is in conformity with Bowen *et al*. (1996) who reported from Alabama that the percentage of seed invaded by *A. flavus* fungi averaged 12.2% and ranged from 0 to 52.5% invasion of seed in the late planted varieties.

The current study is consistent with Pitt *et al*. (1993); Horn *et al*. (1995) and Mohale (2004). Horn *et al*. (1995) reported that *A. flavus* was the most abundant species isolated from soil populations of *Aspergillus flavus* and *A. parasiticus* in Southwestern Georgia, while the highest frequency of occurrence of both *Aspergillus flavus* and *A. niger* on Australian groundnuts was reported by Pitt *et al*. (1993). Mohale (2004) examined the distribution and occurrence of *Aspergillus* species on groundnut varieties and reported *A. flavus* (48%), followed by *A. nige*r (36%), *A. parasiticus* (7.8%), *A. terreus* and (5.7%), respectively. The present study is in conformity with the reports of ICRISAT (1992) which indicated that groundnut seed colonization by *A. flavus* ranged from 22.8 to 28.7% which was conducted on different types of freshly harvested groundnut varieties.

Shazia *et a*l. (2004) reported 10% pre-emergence and 3% post-emergence infection of groundnut by *A. flavus.* Hill *et al.* (1983) reported that *A. flavus* is a natural contaminant of groundnuts and is capable of infecting groundnut seeds prior to harvesting. Other studies have indicated that *A. flavus* may be parasitic on the groundnut plant (Horn and Dorner, 1999) and worldwide in distribution (Pitt and Hocking, 1997). *Aspergillus flavus* is indigenous to the soil of the groundnut field hence its dominance. Horn and Dorner (1999) suggested that groundnut cultivation selects for *A. flavus*. In addition, conditions of drought stress accompanied by elevated soil temperatures are conducive to *A. flavus* invasion of groundnuts (Cotty, 1997; Payne, 1998; Horn and Dorner, 1999). Barrosa *et al.* (2006) from Argentina have reported that *A. flavus* link as the dominant species from section *Flav*i both from soil and groundnut seeds in Argentina. Clinton (2007) has also reported that *A. flavus* as one of the fungus attacking germinating groundnut seed. Generally, in this study, *A. flavus* seed infection level was relatively low as compared to previous reports. This is attributed to the high and well distributed rainfall in the study area during the season.

Mean value of groundnut seed invasion by *A. niger* was 33.6, which was relatively high. *Aspergillus niger* is one of the most commonly reported fungi from warmer climates, indoor environments, i.e, both in the field situations and stored foods (Pitt and Hocking,

Appendix 1 Table 1. Weakly average temperature during soil solarization at Mayweyni FTC												
Average soil temperature at **control**							Average soil temperature **solarization**					
Soil depths	**morning**		**Mid day**		**Evening**		**morning**		**Midday**		**Evening**	
	5cm	10cm	5cm	10cm	5cm	10cm	5cm	10cm	5cm	10cm	5cm	10cm
1st week	18.89	17.77	27.16	27.64	26.59	26.9	54.01	54.94	61.6	61.66	58.93	58.27
2nd week	19.91	18.44	28.62	28.29	26.9	27.4	52.91	52.32	63.66	63.8	60.63	58.96
3rd week	19.11	18.2	28.22	28.45	28.18	29.35	53.14	53.41	64.25	64.2	60.72	60.07
4th week	20.92	20.62	33.17	32.95	30.82	30.39	44.66	43.63	57.21	56.83	53.58	53.19

Appendix II Table2. Weakly average temperature during soil solarization at Ramma FTC												
Average soil temperature at **control**							**Average daily soil temperature during solarization**					
Soil depths	morning		Mid day		Evening		Morning		Midday		Evening	
	5cm	10cm	5cm	10cm	5cm	10cm	5cm	10cm	5cm	10cm	5cm	10cm
1st week	19.38	17.78	27.16	27.03	27	27.04	47.71	46.27	59	56.7	53.6	52.36
2nd week	21.56	23.31	30.36	30.44	28.8	28.65	49.36	49.54	59.61	59.64	59.8	57.24
3rd week	19.1	18.37	28.3	28.7	28.76	28.99	53.14	53.41	60.24	64.2	60.79	60
4th week	23.53	22.52	33.49	33.16	32.37	31.75	40.2	44.56	58.47	57	55.88	53.34

Table 1. Percentage of groundnut seed invasion by *Aspergillus* species at Mayweyni and Ramma, North Ethiopia'

	Ramma				**Mayweyni**		
Treatments	*A. flavus*	*A. parasiticus*	*A. niger*	*A. terreus*	*A.niger*	*A. parasi*ticus	*A. flavus*
Solarized	21.1	11.2	17.6	0.344	32.3	10.9	19.9
Control	21.4	11.9	17.5	0.339	34.9	13.8	25.9
CV% (Sol)	2.6	4.4	6.5	5	1.4	2.6	8.9
LSD (0.05)	NS	0.62	NS	NS	1.91	0.97	0.98
Pd1	21.3	11.4	17.5	0.33	32.8	12.4	22.8
Pd2	21.5	11.6	17.4	0.339	34.2	11.9	23.3
Pd3	21.0	11.7	17.8	0.355	33.8	12.6	22.7
CV % (Pd)	4.0	5.1	7.8	3.4	2.0	2.2	12.1
LSD (0.05)	NS	NS	NS	0.019	NS	NS	NS
Werer961	21.0	11.4	18.1	0.347	33.8	12.2	23.2
Sedi	21.3	11.7	17.2	0.344	33.6	12.7	23.1
NC-343	21.5	11.6	17.3	0.333	33.4	11.9	22.5
CV % (V)	12.4	15.5	6.5	4.0	9.4	7.3	5.7
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS
Mean	21.27	11.56	17.55	0.341	33.60	12.30	22.90
R^2	49.48	40	40.10	58.29	48.29	61.66	84.8

Pd= planting date, Ns=not significant at $P \leq 0.05$

1997). The black conidial color of *Aspergillus niger* provides protection from sunlight and UV light, thus providing a competitive advantage in warm habitats (Horn *et al.,* 1995; Pitt and Hocking, 1997), thus, its dominance in the current study. The present report is in agreement with Horn (2005) who reported *A.niger* as the dominant colonizer of wounded groundnut seeds outside *Aspergillus* section *Flavi*. This species has moisture and temperature requirement that are slightly broader than those of *A. flavus* and grows faster (Ayerst, 1969; Marin *et al*., 1998). Neema (1992) reported that *A. niger* produced circular brownish spot on the cotyledon and this discoloured area rapidly rotted and spread to the stem and hypocotyls. The fungus attacks the plant soon after germination and apparently exerts its effect in substantial part by production of oxalic acid. In the present study, *A. niger* was found together with other *Aspergillus* species at both locations. This is similar with the work of Abdulla (1984); Heseltine *et al*. (1981); Hill *et al*. (1983) and Bayman *et al.* (2002) who reported that *A.niger* and *A. flavus* commonly co-occurred on agricultural commodities, such as groundnut and cotton seed.

Mean value of groundnut seed invasion by *A. parasiticus* was 11.6 at Mayweyni. Previous studies revealed that *A. parasiticus* is more prevalent in groundnut seeds than in other crops (Diener *et al*., 1987). Groundnut seeds, like corn ears, are more susceptible to infection by *A. flavus* than *A. parasiticus* (Horne *et al*., 1995). However, unlike corn ears which are infected almost exclusively by *A. flavus*, groundnut seed infection by aflatoxigenic fungi typically is made up of 10 to 30% *A. parasiticus* (Hill *et al*., 1985). This is in full agreement with the current study which revealed the percentage of *A. parasiticus* seed invasion that ranged from 11.6% at Mayweyni to 12.3% at Ramma.

Table 2. Significance interaction effects between planting time (Pd), Variety (V) and soil solarization on *A. flavus*, *A. niger* and *A. parasiticus* (percentage) at harvest of groundnut grown at Ramma and Mayweyni, North Ethiopia

		Ramma			Mayweyni		
Solarization x Pd		***A.flavus***	***A.niger***	***A. parasiticus***	***A.flavus***	***A.niger***	***A. parasiticus***
Solarized	Pd1	19.2	19.2	12.1	29.3	26.5	15.6
	Pd2	18.1	18.1	11.8	29.4	25.9	13.6
	Pd3	19.7	19.7	11.8	29.4	26.4	14.1
Control	Pd1	18.8	18.8	11.3	22.1	20.8	11.1
	Pd2	21.6	21.6	11.4	31.9	20.5	10.4
	Pd3	20.8	20.8	10.9	28.3	19.7	11.1
LSD (0.05)		0.94	2.486	NS	NS	NS	NS
Solarization x Variety							
Solarized	Werer 961	20.9	11.8	21.2	20.6	26.4	14.3
	Sedi	21.4	12.1	20.2	21.4	25.9	14.0
	NC-343	21.9	11.8	19.8	18.9	26.5	12.9
Control	Werer 961	21.1	10.9	18.8	26.4	20.6	10.1
	Sedi	21.2	11.4	18.7	25.9	21.4	11.4
	NC-343	21.0	11.3	19.5	26.5	18.9	11.1
LSD (0.05)		NS	NS	NS	3.118	NS	NS
Variety x Planting Date							
Werer 961	Pd 1	21.1	11.2	19.2	22.4	23.2	11.5
	Pd 2	22.2	11.5	20.3	23.2	24.8	12.6
	Pd 3	19.9	11.4	20.4	24.9	24.6	12.6
Sedi	Pd 1	21.2	12.5	19.4	24.6	25.2	13.7
	Pd 2	21.3	11.5	19.2	25.2	21.4	11.8
	Pd 3	21.4	11.3	19.9	21.4	23.9	12.6
NC-343	Pd 1	20.9	11.6	18.6	23.9	22.2	12.6
	Pd 2	20.8	11.9	20.2	22.18	22.1	11.4
	Pd 3	22.6	11.4	20.5	22.1	20.1	11.8
LSD (0.05)		2.214	NS	NS	2.300	NS	NS

NS= interactions not significant; Pd= planting date

Conclusion

The natural seed invasion by *Aspergillus* spp. was determined by plating seeds on Czapek-Dox agar medium and four *Aspergillus* spp. namely; *A. flavus, A. parasiticus, A. niger* and *A. terreus* were isolated from both sites. Seed invasion by *Aspergillus* spp. was reduced on the solarized plots than the control plots. In the interaction between solarization and variety (Sol x V) and interaction between planting time and variety (Pd x V), 'Werer 961' was susceptible to *A. flavus* and

late leaf spot, while 'NC-343'was moderately resistant at both sites. It can safely be concluded that *A. flavus* is more prevalent in groundnut seed in northern Ethiopia than *A. parasiticus*.

Preharvest seed invasion of groundnuts could be minimized by using soil solarisation and appropriate agronomic practices, *i.e*. planting times and planting suitable varieties. The varietal trial should be conducted over several seasons and locations to draw definite conclusions.

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