RESEARCH ARTICLE

MANAGEMENT OF AFLATOXINE PRODUCING FUNGI IN GROUNDNUT (*ARACHIS HYPOGAEA* L.) VARIETIES IN CENTRAL TIGRAY THROUGH SOIL SOLARIZATION

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ARTICLE INFO	ABSTRACT
	Groundnut is the most important rich source of protein, minerals and vitamin. Pre-harvest contamination is influenced by soil moisture and temperature. Field experiments were conducted in northern Ethiopia, at two locations to determine the effect of soil solarization on yield and yield components of groundnut varieties, and to determine *Aspergillus spp*. inoculum in the soil. 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. Pod yields were found increased by 265.6 kg ha^{-1} and 182.22 kg ha^{-1}on solarized plots at Mayweyni and Ramma respectively. Increase in yield related parameters (14.8% increase in number of seed per plant and 71.4% increase in number of pods per plant) were found from early planting dates as compared to later planting time at Mayweyni. Generally, yields varied across locations; mean pod yield in Mayweyni was 360.9 kg ha^{-1}higher than the yield in Ramma.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an annual legume of the family *Fabaceae.* It is native to South America. Groundnut is produced in China, India, the United States of America and many Sub-Saharan African countries (FAO, 2004). Developing countries account for 92% of total global groundnut production (ICRISAT, 2005). 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). It is one of the leading agricultural crops of the world for production of high quality edible plant oil (36-54%), easily digestible protein (12-36%), fat (41-52%), and carbohydrate (11-27%) (Adegoke *et al*., 2004; 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). These characteristics led the groundnut to become sensitive to fungal contamination. 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). Aflatoxin contamination does not affect crop productivity but it makes the produce unfit for consumption as toxins are injurious to

human and animal health. The marketability of contaminated produce, particularly in international trade is considerably reduced due to stringent standards of permissible limits on aflatoxin contamination set by the importing countries (Klinger, 2001).

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 (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.

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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 pre-harvest 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 pre-harvest invasion of groundnut by aflatoxine producing 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 yield and yield components of groundnut varieties, and to determine *Aspergillus* spp. inoculum in the soil

MATERIALS AND METHODS

Description of experimental sites

The field experiments were 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°C, respectively. The laboratory experiments were conducted in Plant Pathology Laboratories at Haramaya University.

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 4°C until used for analyses.

Determining Aspergillus spp. Inoculum in the Soil

Soil samples were taken from two depths namely, 5 and 10 cm (Ahmad *et al*., 1996) in three rounds, namely just before soil solarization 12 soil samples ; after solarization of the soil 12 samples from main plots; and at harvest 108 soil samples from each sub- sub plots for both locations. Soil samples were subjected to mycological analyses to determine the effect of soil solarization in reducing the soil populations of *Aspergillus* spp. Mycological examination of soil samples were conducted as follows:

Suspension (= 10^{-1} dilution) of each sample was prepared by taking 1 g in 9 ml diluents (sterile distilled H_2O) in a sterile universal tube, capped tightly and shaken for 15 minutes. Then prepared second (= 10^{-2}) and third (= 10^{-3}) dilutions in the same manner by taking 1 ml of 10^{-1} and 10^{-2} dilution to 9 ml sterile distilled H_2O and mixed thoroughly. Thus, final suspension (= 10^{-3} dilution) of each soil sample was prepared and spread per sample on Rose Bengal Agar media (selective medium for A*spergillus* spp.) (Cotty, 1994) per sample. One plate for each sub-sub plot was used as a sample unit. After 7days incubation at 25 °C, colonies of *A. flavus* were counted (Cotty, 1994). Colony forming units (Cfu/g soil) per gram of soil was computed by the following formula (Klich, 2002).

$$\frac{Cfu}{g \; of \; soil} = \frac{ABC}{DE}$$

A =   number of *A. flavus* colonies
B =   volume of sterilized water added (ml)
C =   dilution factor
D =   weight of soil sub-sample (g)
E =   volume of soil suspension spread (ml)

Data Collection

For each location, the following data were recorded: Colony forming unit per gram of soil (Cfu/g soil): was calculated for individual and total fungi using the formula in section 2.3. Days to 50% flowering: the number of days from emergence to

the date on which 50% of the plants in each plot have at least the first flower.

Days to 90 % maturity: the number of days from emergence to the date at which 75% of the plants in a plot have reached physiological maturity; pods were considered matured when the kernels were fully developed, testa assuming the varietal color, and the inside wall of pods darken to brown.

- Number of pods per plant; these were computed from counts carried on five randomly selected plants from middle rows of each sub-sub plot.
- Number of seeds per plant: this was computed from five randomly selected plants from middle rows of each plot
- Pod yield (kg) per plot: this was recorded after harvesting pods from the middle rows by and then converted to kg/ha.
- 100-seed weight (g): A sample of 100 seeds were taken from each sub-sub plot and weighed. Percentage of *A. flavus* invasion of seed (%): the amount of seed that was invaded by *A. flavus* from the total samples.
- Disease severity: leaf spot severity was recorded using the Florida peanut leaf spot rating scale (visual disease scoring) (1-9 scaling) (Chiteka *et al*., 1988).

RESULTS AND DISCUSSION

The Effect of Soil Solarization on *A. flavus* Inoculum in the Soil

At Mayweyni, solarization significantly reduced propagule densities (cfu g^{-1}) of soil fungi as compared to the control at both 5 and 10 cm soil depths (Table 1). The total cfu g^{-1} at 5 and 10 cm soil depth in solarized plots showed a 44.7 and 29.9% reduction over the control, respectively. In an earlier investigation, 30-70% reduction in fungal population was observed after 40 days of the end of the solarization process (Ahmad *et al*., 1996). Generally, considerable difference was observed between similar treatments within different soil depths at Mayweyni in the amount of total filamentous fungi (cfu g^{-1}), as illustrated in Table 1. This clearly showed that soil solarization is more pronounced at the upper soil depths. This is in full agreement with various reports (Ben-Yephet, 1988; Buercky, 1988; Kim and Han, 1988; Lazarovits *et al*., 1991; Ahmad *et al*., 1996) that soil solarization reduced the number of soil borne fungi and other microorganisms especially in the top 10 cm soil depth.

Four *Aspergillus* species namely, *A. flavus, A. niger, A. parasiticus* and *A.terreus* were detected both pre-treatment (before soil solarization) and after treatment (after soil solarization) and only *A. niger* and *Fusarium* spp. at harvest time in Mayweyni. All the detected *Aspergillus* species had shown significant (P≤ 0.05) reduction during the four weeks solarization period (Table 1). Number of cfu g^{-1} soil of *A. niger* at 5 cm soil depth was higher as compared to 10 cm at both sampling periods (Table 1). *Aspergillus flavus* had 53.8 and 45% reduction over the control at 5 and 10 cm soil depths, respectively (Table 1). On the other hand, *A. parasiticus* showed 36.4 and 44% cfu g^{-1} at 5 and 10 cm soil depth, respectively, at Mayweyni field experiments after treatment. At harvest, two *Aspergillus* spp. namely, *A. flavus*, *A. niger* and *Fusarium* spp. were detected from Mayweyni. Mean number of cfu g^{-1} showed significance (P≤0.05) reduction on *Fusarium* spp. at both soil depths and *A. flavus* and *A. niger* had only significant difference at 5 cm soil depth. *Aspergillus parasiticus* and *A. terreus* were detected during all sampling periods, i.e., before soil solarization, after soil solarization and at harvest in Ramma (Table 3). However, they were not detected at harvest from Mayweyni (Table 2). This could be due to the differences in soil type, previous field history, temperature and rainfall. *Fusarium* spp. were found to be the most dominant among the species isolated at 5 cm (1.5×10^3 cfu g^{-1}) and at 10 cm (1.4×10^3 cfu g^{-1}) during harvest time at Mayweyni (Table 2).

At Ramma, soil solarization significantly reduced propagule densities (cfu g^{-1}) of total soil fungi as compared to the nonsolarized plots (Table 3). The total Cfu g^{-1} at 5 and 10 cm soil depth at Ramma showed 60.9 and 44.4% of reduction respectively, after soil solarization (Table 3). Mean number of fungal cfu g^{-1} at different soil depths were higher in the nonsolarized plots i.e., 4.1×10^3 (5 cm) to 3.8×10^3 (10 cm) while the cfu g^{-1} in the solarized plots were 1.2×10^3 (5 cm) to 2×10^3 (10 cm) at Ramma (Table 3). Bacha *et al*. (2007) from Pakistan has reported 5×10^3 (20-30 cm) to 3×10^3 (0-10 cm) cfu g^{-1} in the nonsolarized soils while the cfu g^{-1} in the solarized plots were 1.5×10^3 (20-30 cm) to 18.5×10^3 (0-10 cm). At Ramma, *A.flavus, A. niger, A. parasiticus* and *A.terreus* were detected during all sampling periods and all the four *Aspergillus* species had significant (P≤0.05) difference among treatments after four weeks of soil solarization at both soil depths except *A. flavus* at 10 cm soil depth at Ramma. In the solarized plots, *A. flavus* was reduced by 55.3% of at 5 cm soil depth after the application of soil solarization (Table 3).

Table 1. Propagule densities of total soil fungi and *Aspergillus* spp. isolated from 5 and 10 cm soil depths before and after soil solarization at Mayweyni, North Ethiopia

	Total fungi [2]				*A. flavus* [2]				*A. niger* [2]				*A. parasiticus* [2]				*A. terreus* [2]			
	Before Treatment		After Treatment		Before Treatment		After Treatment		Before Treatment		After Treatment		Before Treatment		After Treatment		Before Treatment		After Treatment	
TRMTS[1]	D5	D10	D5	D10	D5	D10	D5	D10	D5	D10	D5	D10	D5	D10	D5	D10	D5	D10	D5	D10
Solarized	4.14	3.43	1.35	1.64	5.12	0.52	0.66	0.48	1.45	1.26	0.66	0.29	0.21	0.28	0.21	0.28	0.7	0.66	0.40	0.02
Control	4.29	3.47	2.44	2.34	5.12	0.56	0.50	0.51	2.47	2.38	0.61	0.33	0.33	0.50	0.33	0.50	0.9	1.22	0.37	0.41
LSD	NS[3]	NS	0.7	0.7	NS	0.08	NS	NS	0.8	1.8	0.05	0.04	0.1	0.1	0.1	0.1	0.05	NS	0.01	0.01
CV (%)	6.5	8.0	10	9.0	4.8	3.6	6.0	9.6	12	10	2.0	7.0	10.8	9.0	10.8	9.0	5.0	8.9	1.0	1.10

[1]TRMTS = Treatment
[2] = Fungal density expressed as Cfu x 10^3 g^{-1} soil
[3] NS = not significantly different at P≤0.05
[4]D5 and D10=5 and 10 cm soil depths, respectively

Table 2. Colony forming units of *A. flavus*, *A. niger* and *Fusarium* spp. isolated from 5 and 10 cm soil depths at harvest at Mayweyni, North Ethiopia

TRMTS[1]	Total fungi [2]		*A.flavus* [2]		*A.niger* [2]		*Fusarium* spp. [2]	
	D5	D10	D 5	D10	D5	D10	D5	D10
Solarized	3.78	3.75	0.62	0.60	0.36	0.33	1.52	1.40
Control	3.68	3.53	0.59	0.63	0.32	0.35	1.45	1.38
LSD	NS[3]	NS	0.03	NS	0.03	NS	0.1	0.1
CV (%)	14.4	24.4	10	12	15.7	15	18	15

[1]TRMTS = Treatments
[2] = Fungal density expressed as Cfu x 10^3 g^{-1} soil
[3] NS = not significantly different at P$\leq$0.05
  D5 and D10 cm = soil depth at 5 and 10 cm, respectively

Table 3. Propagule densities of total soil fungi and Aspergillus spp. isolated from 5 and 10 cm soil depths before and after soil solarization at Ramma, North Ethiopia

	Total fungi [2]				*A. flavus* [2]				*A. niger* [2]				*A. parasiticus* [2]				*A. terreus* [2]			
	Before Treatment		After Treatment		Before Treatment		After Treatment		Before Treatment		After Treatment		Before Treatment		After Treatment		Before Treatment		After Treatment	
TRMTS[1]	D5	D10	D5	D10	D5	D10	D5	D10	D5	D10	D5	D10	D5	D10	D5	D10	D5	D10	D5	D10
Solorized	4.13	3.76	1.24	2.04	0.26	0.23	0.17	0.24	1.55	1.06	0.87	0.73	1.40	0.91	0.26	0.24	0.24	0.29	0.74	0.64
Control	3.97	3.69	3.17	3.67	0.26	0.18	0.38	0.46	1.54	1.14	1.49	1.22	1.49	0.91	0.36	0.36	0.28	0.22	1.64	1.47
LSD	NS[3]	NS	0.7	1.2	NS	NS	0.1	NS	NS	0.02	0.3	0.4	0.07	NS	0.09	0.06	NS	0.02	0.7	0.6
CV (%)	14.6	5	8.9	12.6	14	6	14.	21	5	3.7	8.7	12.7	1.5	7	8	5	8	2.0	16.9	17.5

[1]TRMTS = Treatments
[2] = Fungal density expressed as Cfu x 10^3 g^{-1} soil
[3] NS = not significantly different at P$\leq$0.05
[4] D5 and D10= soil depth at 5 and 10 cm, respectively

Table 4. Colony forming units of total fungi propagules and individual *Aspergillus* species isolated from 5 and 10 cm soil depths at harvest at Ramma, North Ethiopia

TRMTS[1]	Total fungi [2]		*A. flavus* [2]		*A. niger* [2]		*A. terreus* [2]		*Fusarium* spp.[2]	
	D5	D10	D 5	D10	D5	D10	D5	D10	D5	D10
Solarized	3.78	3.75	0.69	0.68	0.33	0.39	0.68	0.68	1.51	1.57
Control	3.68	3.53	0.68	0.69	0.33	0.33	0.69	0.68	1.46	1.59
LSD	NS[3]	NS	NS	NS	NS	0.04	NS	NS	NS	0.02
CV (%)	14.4	24.0	4.9	5.2	5.0	22.7	4.2	3.5	9.9	2.7

[1]TRMTS = Treatments
[2] = Fungal density expressed as Cfu x 10^3 g^{-1} soil
[3] NS = not significantly different at P$\leq$ 0.05
[4] D5 and D10 = soil depth at 5 and 10 cm, respectively

On the other hand, *A. niger* was reduced 41.6 and 40.2% at 5 and 10 cm, respectively. While *A. terreus* increased at both soil depths during the soil solarization period. Generally, *A. niger* was found the most dominant species before and after soil solarization at Ramma. It is one of the commonly reported soil fungi from warm areas and the black conidial color of *A. niger* provides protection from high temperature, thus providing a competitive advantage in warm habitats (Horn *et al.,* 1995; Pitt and Hocking, 1997).

Ahmad *et al*. (1996) suggested that *Penicillium* species and *A. flavus* were hard to control through soil solarization and their population quickly starts flourishing after the end of solarization. However, in the current study significant reduction was observed on *A. flavus* after solarization. The technique efficiently controlled *Aspergillus niger*, *Macrophomina phaseolina, Emericella, Fusarium, Helminthosporium,* and *Verticillium* spp. Bacha *et al.* (2007) isolated *A. flavus* from soils of Islamabad, Pakistan both at nonsolarized and solarized plots. Although it was detected both before and after solarization in the present study, solarized treatments resulted in considerable reduction at both soil depths that were sampled (5 and 10 cm).

The results of the current study revealed that A. terreus was showing significant (P$\leq$0.05) difference after soil solarization at both soil depths at Ramma field experiment. However, it was found that increased after four weeks of soil solarization. Counts of A. terreus were made and found that it can survive solarization and was detected in exceptionally high numbers both in solarized and nonsolarized plots. *A. terreus* is distributed worldwide in soils but more abundant in tropical and subtropical regions than in temperate regions and is more common in cultivated soils (Klich, 2002).

At harvest *A. flavus, A. niger, A. terreus,* and *Fusarium* spp**.** were detected in soil samples from Ramma. There was significant difference in *Fusarium* spp. among treatments at 10 cm soil depth. *Aspergillus parasiticus* was not detected at harvest from both soil depths at Ramma (Table 4). Long term effect of soil solarization over *Fusarium* spp. was reported by Katan *et al*. (1983) who suggested soil solarization had a pronounced and durable effect on the elimination of fungal propagules of *Fusarium* wilt of cotton. Although the major benefit of soil solarization was the reduction of the population of *Fusarium* species, solarization was also effective in controlling the population of weeds significantly in the standing crops, the reduction being greater in the

solarized plots. This could have been caused by thermal killing of weed seeds or germinating seedlings (Abdel-Rahim *et al*., 1988).

Soil solarization reduces population density or colony forming units (cfu g^{-1}) of *Fusarium* spp. were reduced by the soil solarization. Actually, some soil borne pathogens can be highly sensitive to soil solarization method (Gonzalez-Torres *et al*., 1993). Hossein *et al*. (2007) reported that the population density of *Fusarium* spp. was reduced from 300 cfu g^{-1} to 100 cfu g^{-1}soil after two weeks soil solarization. Population density of the pathogen also decreased to 100 cfu g^{-1}soil after 4 weeks of soil solarization. This finding is similar with the current observation. At harvest, the amount of cfu g^{-1} soil of *A. niger* was low at both field experiments as compared to the before and after soil solarization. However, the cause for the reduction was not clear. If soil solarization is done over large area, chances of windborne contamination from nearby untreated fields would be minimized. Spores of several terrestrial fungi, such as *Aspergillus*, are disseminated by air. Such terrestrial fungi sporulate only when exposed to air and not in submerged cultures (Vezina *et al*., 1965).

Although some studies have shown that *A. flavus* has been isolated from groundnut seed in Argentina (Novas and Cabral, 2002; Pildain *et al.,* 2004), there is scarce information about its soil populations. The soil densities of *Aspergillu*s species in the area under study were similar to those found in previous studies in the groundnut-growing region in Argentina (Barrosa *et al*., 2006) and in US (Horn and Dorner, 1999). Data were not encountered on thermal inactivation point of neither *A. flavus* nor the other species isolated in the present study.

The effect of soil solarization on yield and yield components of groundnut varieties

At Mayweyni, there were significant (P<0.05) differences between solarized and control plots in terms of stand count and pod yield. Pod yield increased from 2103.1 kgha^{-1} to 2285 kg ha $^{-1}$ in the solarized plots (Table 5). Cheong *et al* (1995) observed highly positive correlation of proportion of 100- seed weight and shelling ratio with seed yield of groundnut and record 3.2 t ha^{-1} with clear polythene while 2.3 t ha^{-1} from the bare (nonsolarized) treatments in Iri, while Choi and Chung (1997) recorded more flowers, pods and kernels and greater100-kernal mass in plastic sheet solarized plots than on the unsolarized plots in Suwon, Korea. Park *et al*. (1996) reported that seed yield increase in soybean by 18% with transparent plastic film and by 15% with black film. Previous studies revealed that soil solarisation reduces the number of fungi, weeds and other microorganisms. It often results in increased yield and plant growth response (Buercky, 1988; Kim and Han, 1988; Lazarovits *et al*., 1991) even in the absence of known pathogens.

Among the yield and yield component parameters recorded, number of pods per plant, number of seed per plant showed significant difference between planting times (Table 5). Only hundred seed weight and days to 50% flowering had significant (*P<0.05*) difference among the three varieties tested. In the present study, increase in number of seed per plant (14.8%) and

in number of pods per plant (7.4%) was found from early planting as compared to later planting at Mayweyni. The current study clearly showed that early planting at the beginning of June soon after onset of rain generates advantage over the later (end of July) planting in the study area. A previous study indicated that early planting produced 20% to 50% greater pod yields than late planting (Naab *et al.,* 2004). Still other earlier studies detected that the impact of planting time on pod yield was remarkable in all groundnut cultivars. The late planted groundnuts yielded considerably less than those planted earlier (Johnson, 2005).

Frimpong (2004) reported that plant height, biomass, and pod yield were significantly affected by planting time and environmental factors. Due to the fact that the early and normal planting dates allow a long growth period, plants are exposed to suitable temperature regimes during the vegetative and reproductive growth stages for the entire growing period. In contrast, plant growth was negatively affected by late planting time due to the decreased vegetative and reproductive duration. The three varieties showed significant effect on 50% flowering date after emergence. 'Sedi' had shortest flowering dates (mean of 32.7 days) followed by 'Werer 961' (33.2 days) after emergence as compared to 'NC 343' which had 34.9 days. On the other hand, the three varieties had significant (*P<0.05*) difference in terms of 100- seed weight at Mayweyni. Greater 100-seed weight was recorded from 'Sedi' (mean of 41.4) as compared to 'Werer-961' (39.3) and 'NC-343' (38.6) (Table 5). However, at Ramma the varieties did not differ significantly in terms of 100-seed weight. Arıoğlu *et al*. (2001) suggested that the 100-seed weight was affected by environmental conditions, which is consistent with the present findings. EARO (2009) reported that 100-seed weight for 'Werer 961'was 40.9 on research fields.

There were significant effects of soil solarization on stand count at both field experiments with 15.9 and 15.97 plants per m^2 recorded in Mayweyni and Ramma respectively. Experiments were established at 16.7 plants m^2, but only those in the solarized plots were close to this value at both locations (Table 5 and 6). Generally, stand count at nonsolarzed plots were less than the solarized plots, this might be due to nematodes and root rot (Sharma and Waliyar, 1992) which have substantial effect on stand establishment. Previous studies suggested that the combination of drought, high soil temperature, water logging and nematodes caused variable stand counts and growth of groundnut (Sharma and Waliyar, 1992)

At Ramma, there was significant *(P<0.05)* difference between solarized and nonsolarized plots in terms of stand count, number of pods per plant, days to 90% maturity and pod yield (Table 6). Pod yield increased from 1700.5 kg ha^{-1} to 1966.1 ka ha^{-1} in the solarized plots. Generally, yields were varied across locations in the solarized plots; pod yield was low at Ramma as compared to similar treatments at Mayweyni. The difference in the same varieties in yield at the two locations might be attributed to complex genetic and environmental factors such as temperature and rainfall. Soil solarization caused physical, chemical and biological changes especially in the top 10 cm soil (Ahmad *et al*., 1996; Abdel-Rahim, 1998) and favored

yield. In the present study, observations on plant growth showed that the groundnut plants in the solarized plots were generally vigorous and reached 90% physiological maturity earlier than the unsolarized plots in Ramma field experiment. The more favorable soil environment under the solarization, during the early part of the growing season, might have resulted in increased number of pods per plant, pod yield and physiological maturity. This is in agreement with Ramakrishna *et al*. (2006) who observed polyethylene sheet treated plots increased groundnut yields.

yield for 'NC-343' and 'Sedi' 2.5 to 3.0 and 1.1 to 2.5 t/ha at rain fed areas respectively. Hiruy (2008) reported that 'NC-343'shown yield of 2.5 qt ha^{-1}at irrigated site of Bisidimo and Babile. Yields might be differing across location due to genetic and environmental factors. George (2002) from Florida reported that the main influence on the yield and quality of groundnut appears to be temperature during growth.

Among the yield and yield component parameters tested, only days to 90% maturity and number of pods per plant had significant (*P<0.05*) difference among planting time treatments

Table 5. The effect of soil solarization and planting time on groundnut yield and yield components at Mayweyni, North Ethiopia

Treatments	DEM	SNDC	50%FD	90%MT	PYKgha	PPP	SPP	HSWT
Solarized	6.8	15.9	33.7	111.7	2285.4	30.2	62.9	39.9
Control	6.5	15.4	33. 6	112.8	2103.2	30.9	66.4	39.7
CV (%)	1.8	1.2	1.30	0.80	8.0	10.8	9.6	2.3
LSD(0.05)	Ns	0.5	Ns	Ns	151.5	Ns	Ns	Ns
Pd1	6.6	15.8	33.5	115	2091.44	34.2	72.8	40.4
Pd2	6.5	15.5	33.5	110.4	2254.2	30.5	63.0	39.6
Pd3	6.8	15.5	33.9	111.39	2237.1	26.9	58.0	39.3
CV (%)	9.8	1.2	1.34	2.70	56.6	20.2	17.5	18.30
LSD(0.05)	Ns	Ns	Ns	Ns	Ns	3.98	8.8	Ns
Werer 961	6.50	15.7	33.2	112.7	2226.5	32.23	66. 9	39.3
Sedi	6.667	15.7	32.7	109.3	2233	28.3	66.9	41.4
NC-343	6.72	15.6	34.9	114.83	2123.3	31.0	66.9	38.6
CV (%)	11.4	1.22	9.0	13.50	29.7	19.2	21.5	11.9
LSD(0.05)	Ns	Ns	1.858	Ns	Ns	Ns	Ns	2.0134
Mean	6.63	15.6	33.629	112.26	2194.259	30.53	64.627	39.77
R^2	35	34.48	49.518	32	50.73	55.399	57.3	37.56

[1] Pd1=first planting date, Pd2= second planting date and Pd3= third planting date; no= not significant at P<0.05
[2] PPP= pod per plant, PYKgha= pod yield kg/ha, SPP =seed per plant, SNDTC= stand count, 50%FD= days to 50% flowering

Table 6. The effect of soil solarization, planting time on groundnut yield and yield components at Ramma, North Ethiopia

Treatments	DEM	SNDT	50%FD	90% MT	PYKg ha^{-1}	PPP	SPP	HSWT
Solarized	7.1	15.97	36.5	110.6	1966.1	33.1	53.2	34.4
Control	7.1	15.2	35.3	111.3	1700.5	30.3	53.2	35.9
CV (%)	1.0	0.5	3.1	0.8	11	0.9	6.5	3.8
LSD(0.05)	Ns	0.41	Ns	0.383	234.86	1.3543	Ns	Ns
Pd1	7.1	15.5	34.9	111.3	1907.2	31.6	52.9	36.5
Pd2	7.10	15.6	36.4	110.3	1800.9	32.8	53.7	33.6
Pd3	7.1	15.7	36.4	111.3	1791.9	30.7	52.9	35.5
CV (%)	2.8	0.8	2.1	1.3	15	2.1	5	11.6
LSD(0.05)	Ns	Ns	Ns	0.469	Ns	1.6587	Ns	Ns
Worer 961	6.97	15.7	36.1	111.6	1934.7	31.4	53.7	35.8
Sedi	7.11	15.5	35.9	108	1749.9	32.2	56.6	34.6
NC-343	7.11	15.6	35.8	113.3	1815.3	31.6	49.2	35.2
CV (%)	5.9	1.8	4.0	12.4	20.7	4.3	8.2	11.4
LSD(0.05)	0.02	Ns	0.07	0.52	N s	Ns	3.3	Ns
Mean	7.06	15.59	35.90	110.98	1833.31	31.71	53.16	35
R^2	42.48	51	37	27.55	36.04	46.64	62	39.34

Pd1= planting date; ns= not significant at P<0.05, PYKg ha[-1]= pod yield kg/ha, SPP =seed per plant, SNDT= stand count,
50%FD= days to 50% flowering 90%MT= 90% maturity date, HSWT=hundred seed weight, DEM= days to 50% emergence; PPP= pod per plant.

The three varieties tested showed significant *(P<0.05)* difference on days to 50% emergence, days to 50% flowering, days to 90% maturity and number of seed per plant at Ramma and 'NC-343' was found with 113.3 and 34.9 followed by 'Werer 961' 111.6 and 33.2; and 'Sedi' 108 an 32.7 days to 90% maturity and days to 50% flowering, respectively (Table 6). 'Sedi' was found the earliest with days to 90% maturity among the three varieties. The present finding is similar with the reports of EARO (2009) which suggests that 50% days to flowering, 90% days to maturity and yield on research field were 34, 127days and 2645 kg ha^{-1}, respectively for 'Werer 961'.the cultivar 'Sedi' is previously reported by EARO (2009) as the earliest one. Abdi (2004) reported that

at Ramma and number of pods per plant increased from 31.8 at early planting to 32.8 in normal planting. 'Sedi' was found as the earliest among the tested varieties at Ramma. This is similar with the findings of Abdi (2004). Maturity has a significant effect on flavor potential in groundnuts (Mozingo *et al*., 1991; Pattee *et al*., 1995; Sanders and Bett, 1995; McNeill and Sanders, 1996). Harvest and planting time studies show strong relationships in maturity and overall groundnut quality, with no significant relationship to pod size (McNeill and Sanders, 1996). McNeill and Sanders (1996) noted that groundnut from mature pods have greater flavor potential than those from immature pods.

Assessment of Disease Severity

The three varieties tested had significant (*P< 0.05*) difference in terms of late leaf spot disease severity both at Mayweyni and Ramma. Planting time and solarization did not differ significantly in terms of disease severity at both locations. The interaction between planting time and variety (Pd x V) was significant only at Ramma. Late leaf spot (caused by the fungus *Cercosporidium personatum*) severity on the test varieties during the crop season was assessed based on the symptoms (smaller lesions, more nearly circular and darker in colour than those of early leaf spot) by visual estimates of the percentage of leaf area diseased (Phipps and Powell, 1984) both at Mayweyni and at Ramma.

At both locations, 'Werer 961' was found to be susceptible variety to late leaf spot. In the present study, there was variability among varieties across locations to late leaf spot disease severity. 'Werer 961' had high severity (22.8%) followed by 'Sedi' (22.2%) and 'NC-343' (15.4%) at Ramma. Moreover, 'Werer 961' late leaf spot severity of 18.8% followed by 'Sedi' (15.1%) and 'NC 343' (13.6%) at Mayweyni. Varietal screening experiments for leaf spot and rust resistance was done at Werer, Babile and Loko and 'Werer 961' was found susceptible to leaf spot and rust (IAR, 1997). The variety 'NC 343' showed resistance to late leaf spot disease severity percentage at both locations. Late leaf spot appeared at relatively later (after two months) growth stage. Generally, there was a difference of disease severity across location and among varieties. The mean late leaf spot severity percentage recorded at Ramma was higher (22.8%) as compared to Mayweyni (18.8%). The difference in disease severity in the tested varieties could be due to infested crop debris under the soil, genetic and environmental factors such as temperature and rain fall between the two sites. Disease incidence and severity vary in location and governed by a complex set of genetic and environmental circumstances (ICRISAT, 1992), at low plant population the microclimate within the crop canopy does not favor diseases development, and agronomic evidence suggested that yields are maximal.

Late leaf spot diseases can reduce groundnut yields by 10-70% and are reported as the most important and widespread foliar disease of groundnut at rainy season (ICRISAT 1982). When not controlled, both late and early leaf spot diseases can defoliate groundnut and reduce anticipated yield by 50 percent (Shokes and Culbreath, 1997). The results of the current study revealed that early planted varieties had slightly lower disease severity percentage when compared to later planting times. This is in agreement with Adomou *et al.* (2000) who reported that groundnuts planted later in the season had greater leaf spot infection and progress of disease is faster and more severe compared to early sowing; early sown groundnut crops or cultivars in the region also serve as a major source of inoculum for late sown crops leading to greater disease incidence and progress; environmental conditions like higher temperature and greater humidity may also contribute to greater disease progress in late sown crops; and in addition later sown crops have higher probability of experiencing water stress during the critical pod filling phase resulting in lower yields.

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