

Floristic importance of *Phyllanthus amarus* Schumach & Thonn. in selected farming communities and its domestication in Oyo State, Nigeria

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Abstract

Phyllanthus amarus is a pantropic weed with numerous medicinal uses. It is collected in the wild, often in unwholesome environment and is hardly available all year round, which calls for its domestication for eventual cultivation. The distribution and seedling growth of *P. amarus* were studied in two seasons at two farming communities in Oyo State, Nigeria. A 25 x 25 m plot was marked out in Akufo and Fiditi. Fifteen quadrats (50 x 50 cm) were randomly placed using x-y ordinate system to enumerate and determine Relative Importance Value (RIV) for all low growing plants. Growth and dry matter accumulation of seedlings were monitored in a pot trial in clay and sandy-loam soils, combined with four watering intervals (1, 2, 3 and 7 days). Data were analysed using ANOVA and means separated by Least Significant Difference at 5% level of probability. Results showed that *Phyllanthus amarus* is more abundant at Fiditi (RIV=47.16%) than Akufo (RIV=9.15%) at the onset of wet season but reduced at the mid wet season. It performed best ($P < 0.05$) on sandy-loam soil and daily watering significantly effected its best growth and dry matter yield. *Phyllanthus amarus* responded positively to daily watering, but its importance value reduced with recruitment of other species as the wet season advanced, which presented it as an inferior competitor and explains its reduced availability in the wild at the peak of wet season and in the dry season. For cultivation purposes therefore, *Phyllanthus amarus* should be grown in well-drained soils with regular weeding carried out and with adequate moisture provided.

Keywords: Biomass, Community Structure, *Phyllanthus amarus*, Soil texture, Water stress

Introduction

A weed can be described as a plant whose virtues have not been discovered (Emerson, 1876 as cited by Zimdahl, 1994); once discovered, it becomes a resource (Claudius-Cole *et al.*, 2018). Many plants classified as weeds have culinary,

therapeutic, soil restorative and industrial purposes. Such plants that are generally rich sources of many natural therapeutic products, are mostly used for human welfare, especially to reduce human suffering from many diseases. The World Health Organization (WHO) has estimated that up to 80% of the world's population rely on

plants for their primary health care (Ramesh *et al.*, 2008).

Medicinal plants have been used in virtually all human cultures for management of many ailments. Traditional medicinal practices are an important part of primary health care delivery system in most of the developing world (Sheldon *et al.*, 1997). In Nigeria, the majority of citizens still use medicinal plants and visit traditional medicine practitioners for their health care needs (Odugbemi *et al.*, 2006). The use of traditional medicine and medicinal plants in most developing countries as a normative basis for the maintenance of good health has been observed (UNESCO, 1996). *Phyllanthus amarus* has been widely utilized for medical delivery in many cultures in Nigeria, Cote d'Ivoire, Sierra Leone, Trinidad, Congo, Kenya, etc (Ogazie *et al.*, 2017).

Phyllanthus amarus Schmach. and Thonn. is a pantropic weed whose **vernacular names include** Black catnip, Carry me seed, Child pick-a-back, Gale of wind, Gulf leaf flower, Hurricane weed, Shatter stone, stone breaker (English), Herbe au chagrin, Petit tamarind (French), Bhumi Amalaki (India), Dukung anak (Malaysia), Meniran (Indonesia), Zhen chu cao (China) (Burkill, 1994). In Nigeria, it has the following local names Ebe Benizo (Benin), Ehin olobe (Yoruba), miracle plant (Bayelsa) (Gill, 1992). It was formerly in the family Euphorbiaceae (Chaudhary and Rao, 2002), but now belongs to the family Phyllanthaceae based on molecular systematic studies over the years (Wurdack *et al.*, 2005). *Phyllanthus* is one of the 79 genera of Phyllanthaceae and all the genera are found in Nigeria (Wahab, 2019). *Phyllanthus amarus* grows in human

disturbed areas such as roadsides, yards, dumpsites and fields. The *P. amarus* is propagated by seeds and is well adapted to varieties of soils under tropical conditions. However, it rarely survives under dry or very low temperature conditions, but water logging does not show any lethal effects (Wahab, 2019).

In Nigeria, *Phyllanthus amarus* extract shows antimicrobial activity by inhibiting the growth of *Staphylococcus aureus*, *Vibrio cholerae*. and *Salmonella typhi.*, the causal agents of urinary tract infection, cholera and typhoid fever respectively (Ohalete *et al.*, 2013). Fresh roots are beneficial for managing jaundice (Yusuf *et al.*, 2009). *Phyllanthus amarus* has been pharmacologically certified to have the following activities: anti-hepatitis B virus (Thyagarajan *et al.*, 1988), anti-HIV (Notka *et al.*, 2004), anti-mutagenetic (Raphael *et al.*, 2002), anti-inflammatory (Raphael and Kuttan, 2003), anti-oxidant (Hari-Kumar and Kuttan, 2004), anti-carcinogenic (Rajeshkumar *et al.*, 2002), anti-microbial (Adegoke *et al.*, 2010), anti-malaria (Ajala *et al.*, 2011), anti-sickling (Gbadamosi *et al.*, 2012), and gastroprotective (Shokunbi and Odetola, 2013).

It has been observed that *Phyllanthus amarus* is not always readily available in the dry season, and at the peak of wet season. Also, when it is available for collection in the wet season, it is collected in the wild which often are at dumpsites, drainages, roadsides, and other unwholesome environments. Information on the cultivation requirements of *P. amarus* aimed at making sufficient quantity and quality of the plant available for direct use or conversion to some medicinal forms is highly desirable. Considering the importance of the plant and its increasing

demand, it is the aim of this study to determine the floristic importance of *P. amarus* in selected farming communities in Oyo State, Nigeria, and to determine some domestication/cultivation requirements of the plant.

Materials and Methods

Field distribution was carried out at Akufo in Ido local government area, Ibadan, Oyo State (latitude 7° 30' 43.68" N; longitude 3° 47' 37. 65" E and elevation 254 m above sea level) and Fiditi in Afijio local government area, Oyo State (latitude 7° 42' 912" N; longitude 3° 55' 243" E and an elevation of 279 m above sea level), Nigeria. Germination biology was conducted in the ecology laboratory and moisture stress study at the roof top screen house of the Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan, Nigeria. The Department is on latitude 07° 27.030' N; longitude 03° 53. 53.824'E; and an elevation of 218 m above sea level. Ibadan is located in the rain forest-savanna transition zone with a rainfall evaporation ratio of about 1.0 (Awodoyin *et al.*, 2013).

Source of seeds

The matured fruits of *Phyllanthus amarus* were collected from Araromi village in Ido local government area, Ibadan in April 2015. The mature fruits were cleaned and air-dried for two weeks then stored at room temperature (27±2 °C) until required.

Soil sampling and Physical and Chemical Analyses

Soil samples were collected at the locations and samples were pooled to get composite samples used for routine analyses in the Soil Laboratory, Department of Agronomy,

the University of Ibadan. The soil pH, particle size analysis, exchange acidity, exchangeable bases, effective cation exchange capacity (ECEC), percentage total nitrogen (%TN) and phosphorus were determined following Udo and Ogunwale (1986).

Field distribution and abundance of *Phyllanthus amarus*

The plot size surveyed was 25 m x 25 m in Akufo and Fiditi farming communities using 50 cm x 50 cm quadrat size. The random sampling method, using x-y ordinate system, was adopted for the enumeration. The field surveyed was marked out to have x and y baselines. The two baselines were pegged out at one meter interval. The x and y distances, as randomly selected, were marked out for each sampling point and the quadrat placed at the intersections with all plants rooting within each quadrat identified and counted for recording. Fifteen quadrats were enumerated in each site in the early wet season (April 2015) and mid wet season (July 2015).

Plants were identified using the handbook of West African weeds by Akobundu and Agyakwa (1987). Plants that could not be identified on the field were taken to the University of Ibadan herbarium in the Department of Botany for proper identification. The importance values (density, frequency and relative importance value) were determined for each species (Das, 2011; Awodoyin *et al.*, 2013). The community structure for each site and period was determined by the Species richness, Shannon-Wiener, Dominance and Evenness indices following Kent and Coker (1992).

The Relative Importance Value (RIV) for each species was calculated as:

$[(RD + RF)/2] \times 100$, where RD=Relative Density and RF= Relative Frequency

Absolute Density (D) is the number of individuals of a particular species per unit area.

Relative Density (RD) is the percentage value of density of a weed species relative to the total density of all species.

$RD = d/D \times 100$, where d = the density of species; D = total density of all species.

Absolute Frequency (F) is the measure of the chance of finding a species within a quadrat. That is, the number of quadrats that has a particular species in relation to the total number of quadrats laid.

Relative Frequency (RF): The frequency of a species relative to the total frequency of all species.

$RF = f/F \times 100$, where f = frequency of a species and F = total frequency of all species.

The community structure as informed by the ecological diversity of the weed species was determined by alpha diversity, which is the diversity of species within a particular community. It was determined by Taxa (S; number of species), Shannon-Wiener (H'), Evenness (J) and Dominance (D) indices using PAST software version 2.08 (Hammer, 2011; Awodoyin *et al.*, 2013).

Seed germination and seed treatment

Seed germination behaviour was studied on freshly harvested seeds and seeds air-dried for 15 days. Seeds were placed in sterilized Petri dishes lined with a single layer of Whatman No. 1 filter paper. The filter layer was moistened with distilled water and covered. The experimental Petri dishes

contained 50 seeds each and replicated three times.

In order to remove hard seed coat dormancy (Meenu and Pawan, 2012), seeds were pre-treated in hot water at varying durations 0, 20, 40, 60, 80, 100, 120 seconds (seven treatments) replicated three times. Twenty one strips (3 x 5 cm) of muslin cloth were made into which 50 seeds of *Phyllanthus amarus* were placed, wrapped and tied. All the 21 wrapped seed batches were dipped in boiling water at 100 °C. Three strips were randomly selected at each test duration (20 – 120 sec), withdrawn and immediately steeped in cold water. The contents of each treated seed batch were emptied each into a Petri dish separately. Germination was monitored in each Petri dish for 14 days from seeding in Petri dishes. The seeds in each Petri dish were watered when needed using 2 ml of distilled water. These tests were carried out twice on both fresh and air-dry seeds.

Effect of varying water regimes and soil types on performance of *Phyllanthus amarus* seedlings

Two soil types, from (clay and sandy-loam identified from previously conducted analysis) and four water regimes were combined in a 2 x 4 factorial fitted into completely randomized design to have eight treatment combinations with four replicates. The soil was sieved using 2 mm sieve. Nursery bags (30 x 19 cm) were filled with 4 kg of each soil type and arranged in the roof top screenhouse. The four watering regimes were every 1, 2, 3 and 7 days with 200 ml water at the specific watering time.

A nursery of *Phyllanthus amarus* was established with freshly collected mature seeds. At 30 days in the nursery, seedlings of uniform size (5±1 cm in height) were transplanted into the nursery bags at

two seedlings per bag. The seedlings were thinned to one stand per bag at one week after transplanting.

Growth data collected from one week after transplanting and every fortnight subsequently included plant height (cm) using meter rule, numbers of leaves by visual counting, numbers of branches per plant by visual counting **and stem diameter (cm) using vernier caliper**. At eight weeks after transplanting (WAT), content in each pot was carefully lifted out and root part lowered in a bowl of water for easy recovery of entire roots. Each plant was partitioned into shoot and root parts. The fresh root weight (g) and fresh shoot weight (g) were immediately taken using Mettler P1210 weighing balance. The plants were enveloped, labelled and oven-dried at 70 °C to a constant weight. The dry root weight and dry shoot weight were obtained as above.

Statistical analysis

The data from germination tests were square root transformed [$\sqrt{Y + 0.5}$] before analysis (Little and Hills, 1978). Analysis of Variance (ANOVA) was used to compare the treatments, based on the mean values of data obtained, and Least Significant Difference (LSD) was used to compare the treatments at 5% level of probability.

Results

Characteristics of the soil in the farming communities and the two soil types used in the growth study

Laboratory analyses of the soil sample in Fiditi (pH 6.55) and Akufo (pH 5.89) showed that the soils are mildly-acidic and are both loamy sand in texture. Also, they are grossly low in essential nutrients (Table 1). Analysis of the two soil types used for growth study also showed that both the soil in the clay textural class and the one in the sandy loam class were mildly-acidic. (Table 1).

Relative importance value (RIV%) and Community Structure of low growing plants in Akufo and Fiditi Study Sites

There were 31 and 19 plants species identified in Akufo and Fiditi, respectively. At the onset of wet season in Akufo, *Chromolaena odorata* had the highest (12.49%) RIV and six other weed species had the lowest (1.03%) RIV. However, *Phyllanthus amarus* had RIV 9.51%. In Fiditi, *Phyllanthus amarus* had the highest (47.16%) RIV while *Amaranthus spinosus* had the lowest RIV of 0.5% (Table 2). At the mid wet season in Akufo, *Chromolaena odorata* still had the highest RIV value of 15.66% while *Ageratum conyzoides* had the lowest (0.23%), with *Phyllanthus amarus* having RIV of 3.42%. In Fiditi, *Sida acuta* had the highest (19.34%) RIV in the mid wet season and *Tridax procumbens* had the lowest (0.21%), while *Phyllanthus amarus* had RIV 9.63% (Table 3).

Table 1. Physical and chemical characteristics of the soils in the farming communities and the two soil types used for growth study

Soil characteristics	Fiditi	Akufo	Soil Type A	Soil Type B
pH(1:1) H ₂ O	6.55	5.89	6.7	6.55
Organic carbon (%)	3.81	1.89	0.24	1.84
Total nitrogen (%)	0.40	0.20	0.03	0.16
Available phosphorus(mg/g)	47.42	5.84	11.97	56.07
Calcium (Cmol/kg)	7.51	3.31	2.43	10.25
Magnesium (Cmol/kg)	3.13	1.97	66.61	1.25
Potassium (Cmol/kg)	0.68	0.77	0.29	0.3
Sodium (Cmol/kg)	0.70	0.53	0.39	0.48
Acidity (Cmol/kg)	0.60	0.60	0.40	0.65
Aluminum (Cmol/kg)	44.01	46.41	46.43	0.06
ECEC (Cmol/kg)	57.53	54.26	125.08	12.38
Manganese (mg/g)	20.12	134.02	110.25	165.50
Iron (mg/g)	233.17	113.24	72.48	30.65
Copper (mg/g)	4.62	1.60	0.81	2.65
Zinc (mg/g)	36.14	14.62	6.23	6.10
Sand (g/cm ³)	0.842	0.842	0.168	0.65
Silt (g/cm ³)	0.124	0.101	0.234	0.324
Clay (g/cm ³)	0.34	0.54	0.598	0.35
Textural class	Loamy sand	Loamy sand	Clay	Sandy-loam

Soil Type A - First sample used for growth experiment; Soil Type B - Second soil sample used for growth experiment

At the onset of wet season, Akufo had 31 plants species, dominance value 0.08, Shannon-Wiener value 2.86, evenness value 0.56, while at the mid wet season, Akufo had 37 plants species, dominance value 0.07, Shannon-Wiener value 0.54 and evenness value 0.56. Also, at

the onset of wet season, Fiditi had 19 plants species, dominance value 0.3, Shannon-Wiener value 1.74 and evenness value 0.3, while at the mid wet season, Fiditi had 21 plant species, dominance value 0.12, Shannon-Wiener value 2.44 and evenness value 0.55 (Table 4).

Table 2: Relative importance value (RIV %) of low growing plants in Akufo and Fiditi Study Sites at the onset of Wet Season

Species	Akufo			Fiditi		
	RD	RF	RIV	RD	RF	RIV
<i>Acalypha segetalis</i>	-	-	-	3.94	5.48	4.71
<i>Acmelia brachyglossa</i>	-	-	-	3.66	9.59	6.63
<i>Agaricus</i> species	1.40	1.59	1.49	-	-	-
<i>Ageratum conyzoides</i>	0.47	1.59	1.03	-	-	-
<i>Alchornea cordifolia</i>	3.72	4.76	4.24	-	-	-
<i>Althernanthera repens</i>	-	-	-	1.13	4.11	2.62
<i>Amaranthus spinosus</i>	-	-	-	0.17	0.33	0.50
<i>Anthonontha macrophylla</i>	1.40	3.17	2.29	-	-	-
<i>Axonopus compressus</i>	0.93	1.59	1.26	-	-	-
<i>Azadirachta indica</i>	-	-	-	0.85	4.11	2.48
<i>Blighia sapida</i>	0.47	1.59	1.03	-	-	-
<i>Brophyllum pinnatum</i>	7.91	1.59	4.75	-	-	-
<i>Celosia leptostachya</i>	1.40	1.59	1.49	-	-	-
<i>Chloris pilosa</i>	0.49	1.57	1.03	-	-	-
<i>Chromolaena odorata</i>	10.70	14.29	12.49	1.69	5.48	3.58
<i>Combretum hispidum</i>	4.19	4.76	4.47	1.97	4.11	3.04
<i>Commelina benghalensis</i>	8.37	9.52	8.95	-	-	-
<i>Elaeis guineensis</i>	1.40	1.42	1.41	-	-	-
<i>Heliotropium indicum</i>	1.70	3.14	2.29	-	-	-
<i>Ipomoea aquatica</i>	1.40	1.56	1.48	-	-	-
<i>Laportea aestuans</i>	1.40	3.18	2.29	1.41	2.74	2.07
<i>Leptochloa filiformis</i>	0.95	1.56	1.26	-	-	-
<i>Mallotus oppositifolius</i>	17.68	6.35	12.01	-	-	-
<i>Mucuna</i> species	0.97	1.54	1.26	-	-	-
<i>Oldenlandia herbacea</i>	0.48	1.57	1.03	0.85	1.37	1.11
<i>Phyllanthus amarus</i>	2.76	6.75	9.51	20.23	26.93	47.16
<i>Pteridium aquilium</i>	12.09	11.11	11.11	-	-	-
<i>Pteris</i> species	12.21	12.08	12.15	-	-	-
<i>Schwenckia americana</i>	-	-	-	0.28	1.37	0.83
<i>Senna hirsuta</i>	1.40	1.59	1.49	-	-	-
<i>Senna obtusifolia</i>	0.47	1.59	1.03	1.97	4.11	3.04
<i>Senna occidentalis</i>	-	-	-	1.69	4.11	2.90
<i>Senna siamea</i>	-	-	-	0.85	4.11	2.48
<i>Setaria barbata</i>	0.93	1.59	1.96	-	-	-
<i>Sida acuta</i>	7.91	4.76	2.29	25.35	13.7	19.52
<i>Solenostemon monostachyus</i>	1.40	1.59	1.49	-	-	-
<i>Stachytarpheta cayennensis</i>	1.89	1.59	1.03	-	-	-
<i>Stachytarpheta jamaicensis</i>	-	-	-	0.56	1.37	0.97
<i>Talinum fruticosum</i>	1.89	1.59	1.03	-	-	-
<i>Trianthema portulacastrum</i>	-	-	-	-	4.11	2.90
<i>Tridax procumbens</i>	2.33	1.59	1.49	1.69	-	-
<i>Urena lobata</i>	-	-	-	2.25	5.48	3.87

RD = Relative Density; RF = Relative Frequency; RIV = Relative Importance Value

Table 3: Relative importance value (RIV, %) of low growin g plants in Akufo and Fiditi Study Sites at the mid wet season

Species	AKUFO			FIDITI		
	RD	RF	RIV	RD	RF	RIV
<i>Acalypha segitalis</i>	-	-	-	0.19	3.62	3.81
<i>Acmelia brachyglossa</i>	1.1	3.14	4.24	1.4	5.47	6.87
<i>Agaricus sp.</i>	1.65	0.21	3.72	-	-	-
<i>Ageratum conyzoides</i>	0.08	0.15	0.23	-	-	-
<i>Althernanthera repens</i>	-	-	-	0.13	0.84	0.97
<i>Anthonontha microphylla</i>	0.3	0.02	0.63	-	-	-
<i>Axonopus compressus</i>	0.33	0.42	0.75	-	-	-
<i>Azardiracchta indica</i>	-	-	-	0.29	0.03	0.32
<i>Blighia sapida</i>	0.27	0.55	0.82	-	-	-
<i>Boerhavia erecta</i>	-	-	-	0.33	1.48	1.81
<i>Bryophyllum pinnatum</i>	1.93	0.58	2.51	-	-	-
<i>Celosia leptostachya</i>	0.14	0.96	1.1	-	-	-
<i>Chloris pilosa</i>	0.1	1.62	1.72	-	-	-
<i>Chromolaena odorata</i>	5.84	9.82	15.66	1.31	6.12	7.43
<i>Cleome viscosa</i>	2.45	3.87	6.32	-	-	-
<i>Combretum hispidum</i>	0.17	0.74	0.91	1.43	0.2	1.63
<i>Commelina benghalensis</i>	1.47	5.12	6.59	-	-	-
<i>Elaeis guineensis</i>	0.94	0.16	1.1	-	-	-
<i>Heliotropium indicum</i>	0.86	1.85	2.71	-	-	-
<i>Imperata cylindrical</i>	0.13	0.53	0.66	-	-	-
<i>Ipomoea aquatic</i>	3.79	8.09	11.88	-	-	-
<i>Ipomoea triloba</i>	-	-	-	0.1	0.55	0.73
<i>Laportea aestuans</i>	0.06	0.67	0.73	2.29	4.44	6.73
<i>Leptochloa filiformis</i>	3.88	10.02	13.9	-	-	-
<i>Mallotus oppositifolius</i>	0.05	0.68	0.73	-	-	-
<i>Merremia aegyptia</i>	0.24	0.05	0.29	-	-	-
<i>Mimosa diplotricha</i>	-	-	-	0.31	1.61	1.92
<i>Mitracarpus villosus</i>	0.35	0.1	0.45	-	-	-
<i>Mucuna pruriens</i>	0.08	0.29	0.37	-	-	-
<i>Oldenlandia herbacea</i>	0.91	0.19	1.1	0.32	0.2	0.52
<i>Pennisetum purpureum</i>	0.14	1.33	1.47	-	-	-
<i>Phyllanthus amarus</i>	0.97	2.45	3.42	3.3	6.33	9.63
<i>Pteridium aquilium</i>	0.77	0.32	1.1	-	-	-
<i>Pteris spp</i>	0.39	1.93	2.32	-	-	-
<i>Schwenckia Americana</i>	-	-	-	0.64	0.07	0.71
<i>Senna hirsute</i>	0.15	0.86	1.01	-	-	-
<i>Senna obtusifolia</i>	0.03	2.37	2.4	-	-	-
<i>Senna occidentalis</i>	-	-	-	1.32	0.3	1.62
<i>Senna siamea</i>	0.09	1.14	1.23	-	-	-
<i>Setaria barbata</i>	0.05	0.27	0.32	-	-	-
<i>Sida acuta</i>	2.03	10.68	12.71	1.6	14.31	19.34
<i>Solenostemon monostachyus</i>	3.57	9.06	12.63	2.43	1.88	4.31
<i>Stachytarpheta cayennensis</i>	1.54	0.3	1.84	0.11	0.03	0.14
<i>Stachytarpheta jamaicensis</i>	-	-	-	0.24	0.28	0.52
<i>Talinum fruticosum</i>	0.43	2.03	2.46	-	-	-
<i>Trianthema portulacastrum</i>	-	-	-	0.41	2.14	2.55
<i>Tridax procumbens</i>	0.26	0.49	0.75	0.04	0.17	0.21
<i>Urena lobata</i>	-	-	-	0.38	1.96	2.34

RD = Relative Density; RF = Relative Frequency; RIV = Relative Importance Value

Seed germination as affected by hot water pre-treatment

In the fresh seeds, germination improved with hot water treatment for 80 seconds in hot water. The germination at 80 seconds treatment (6.69) was significantly

higher than treatments for 40 seconds but was similar to other treatments. In the dry seeds, non-treated seeds had the best germination (3.38) that was significantly higher than only the 120 seconds treatment that had the lowest (2.40) germination (Table 5).

Table 4: Diversity indices of the low growing plant species on the location surveyed in 2015

Diversity indices	Onset of wet season (April 2015)		Mid wet season (July 2015)	
	Akufo	Fiditi	Akufo	Fiditi
Taxa_S	31	19	37	21
Dominance_D	0.08	0.30	0.07	0.12
Shannon-Wiener_H	2.86	1.74	3.00	2.44
Evenness_H/lnS	0.56	0.30	0.54	0.55

Table 5: Mean cumulative percentage germination of *Phyllanthus amarus* seeds treated in boiling water.

Duration of hot water treatment (seconds)	Fresh seeds	Dry seeds
0	3.86 ± 0.37 (1.46)	3.38 ± 0.14 (1.34)
20	4.97 ± 0.51 (1.73)	2.99 ± 0.32 (1.23)
40	5.37 ± 0.59 (1.82)	2.99 ± 0.32 (1.23)
60	5.84 ± 0.59 (1.92)	3.16 ± 0.33 (1.28)
80	6.69 ± 0.40 (2.09)	2.88 ± 0.51 (1.20)
100	5.99 ± 0.40 (1.95)	2.73 ± 0.18 (1.15)
120	5.75 ± 0.21 (1.90)	2.40 ± 0.18 (1.05)
LSD _{0.05}	1.23	0.94

Values are transformed means ($\bar{Y} + 0.5$) ± standard error. Percentage values are in parenthesis.

Effect of soil types and watering regimes on growth and biomass of *Phyllanthus amarus* seedlings at 8 WAT in Ibadan, Nigeria

Plant height (cm/plant)

Plants in the sandy-loam soil had better height than those in clay soil at all watering regimes (Table 6). *Phyllanthus amarus* in sandy-loam with daily watering had the greatest height (P < 0.05) (57.80 cm) and

clay watered every 7 days had the lowest height (32.68 cm). Plant height in the clay soil watered every 1, 2 and 3 days intervals were statistically similar but significantly taller than plants in clay soil watered every 7 days. Also, *P. amarus* in sandy-loam soil watered 2 and 3 day intervals were statistically similar in height but were significantly shorter than those in the same soil type watered daily and significantly

taller than plants watered only once in 7 days.

Number of Branches per plant

Phyllanthus amarus in sandy-loam soil with daily watering had the highest (P 0.05) number of branches (48.50) and those watered once in 7 days had the lowest (30.50) (Table 6). Plants in the clay and watered every 2 days had significantly more branches than the other clay treatments. Also, sandy-loam soil watered at 2 and 3 day intervals were similar but had significantly fewer branches than those watered daily and significantly more branches than plants watered at 7 day intervals.

Number of leaves per plant

The sandy-loam soil effected higher number of leaves compared to clay soil at all watering regimes in the *P. amarus* plants, except for those watered at 7 day intervals (Table 6). Plants in sandy-loam

soil watered daily had the highest (P 0.05) number of leaves (76.00) while those watered at 7 day intervals in the same soil had the fewest number of leaves (44.25). In the clay soil, plants watered at 2 day intervals had more leaves than those in the other Clay treatments, although the differences were not significant. Also, number of leaves of *P. amarus* in sandy-loam soil watered at 2 and 3 day intervals were similar but significantly fewer than those watered every day and significantly more than every 7 days.

Stem Diameter (mm/plant)

Sandy-loam at daily watering had the best stem diameter (3.16±0.30 mm) and clay at 7 day interval was the lowest (2.04±0.30 mm) (Table 6). The sandy loam at daily watering was higher than other sandy loam treatments but only significantly better than sandy loam at 7 day interval. Also, clay at daily watering was better than other clay treatments but the difference was significant with only clay at 7 day interval.

Table 6: Effect of soil types and watering regimes on the growth of *Phyllanthus amarus* seedlings at 8 WAT in Ibadan, Nigeria.

Treatments	Plant Height (cm/plant)	Number of Branches	Number of leaves	Stem Diameter (mm/plant)
Clay +watered daily	45.03±3.66	34.5±2.66	54.75±3.47	3.04±0.29
Clay +watered every 2 days	42.10±3.06	41.25±6.97	57.50±1.19	2.94±0.09
Clay + watered every 3 days	41.33±1.01	34.00±1.08	49.00±2.55	2.81±0.21
Clay + watered every 7 days	32.68±2.91	31.75±1.65	48.00±2.80	2.04±0.30
Sandy-loam + watered daily	57.80±1.05	48.50±1.44	76.00±1.58	3.16±0.30
Sandy-loam +watered every 2 days	46.90±0.88	39.75±1.49	64.75±2.43	2.74±0.22
Sandy-loam +watered every 3 days	44.15±2.33	38.50±1.04	64.50±3.97	2.61±0.10
Sandy-loam +watered every 7 days	33.40±1.04	30.50±0.96	44.25±0.85	2.19±0.22
LSD _{0.05}	5.74	6.12	6.73	0.64

Values are means ± standard error.

Root Weight (g/plant)

For both fresh and dry root weights, plants watered daily in sandy loam soil were the highest and those watered at 7 day intervals in the same soil were the lowest (Table 7). For the fresh root weight, plants watered 1, 2 and 3 day intervals in sandy-loam soil were similar and only the ones watered daily had significantly more weight than the ones watered every 7 days. Plants in the

clay soil treatments were not significantly different.

With increasing interval of watering the dry root weight decreased in the sandy-loam soil, with daily watered plants having the highest weight (1.97 g) and those watered at 7 day intervals having the lowest weight (0.23 g). Dry root weight in clay soil watered at 1, 2 and 3 day intervals were similar and all weighed significantly more than those watered every 7 days.

Table 7: Effect of soil types and watering regimes on fresh and dry root and shoot weight of *Phyllanthus amarus* seedlings at 8 WAT in Ibadan, Nigeria.

Treatments	Root weight (g/plant)		Shoot weight (g/plant)	
	Fresh	Dry	Fresh	Dry
Clay +watered daily	0.66±0.19	0.20±0.03	4.18±0.76	0.89±0.08
Clay +watered every 2 days	0.45±0.13	0.32±0.04	3.07±0.56	1.02±0.16
Clay + watered every 3 days	0.42±0.05	0.33±0.04	2.38±0.38	0.95±0.27
Clay + watered every 7 days	0.44±0.16	0.12±0.04	2.28±0.55	0.59±0.17
Sandy-loam + watered daily	1.17±0.22	0.45±0.08	6.89±0.36	1.97±0.42
Sandy-loam +watered every 2 days	0.68±0.14	0.37±0.07	5.18±0.75	1.58±0.10
Sandy-loam +watered every 3 days	0.70±0.05	0.28±0.01	3.81±1.30	1.53±0.10
Sandy-loam +watered every 7 days	0.26±0.01	0.07±0.03	1.49±0.28	0.23±0.08
LSD _{0.05}	0.58	0.12	1.74	0.52

Values are means ± standard error.

Shoot Weight (g/plant)

In both clay and sandy-loam, both fresh and dry shoot weight, decreased with increasing interval of watering. However, plants responded better in the sandy-loam treatments compared to the clay treatments at all watering regimes except 7-day interval with regards to both fresh and dry weights (Table 7).

The daily-watered sandy loam soil produced the highest fresh shoot weight (6.89 g) in *P. amarus* and the ones watered at 7-day interval were the lowest (1.49 g). In the sandy-loam soil, plants watered daily had significantly more shoot weight than plants watered at 3- and 7-day intervals, while plants watered at 2-day intervals were

similar to those watered every 3 days. Among clay treatments, daily watered plants had the greatest shoot weight although there were no significant differences among the watering regimes.

Dry shoot weight of *P. amarus* (1.97 g) in sandy-loam soil and watered daily was the highest while those watered every 7 days had the lowest (0.23 g) dry shoot weight. Plants watered daily in the sandy loam soil produced plants with better dry shoot weight than all other watering regimes but were however only significantly different from those watered every 7 days. Dry shoot weight of *P. amarus* watered every 1, 2 and 3 days were significantly more than shoot weight in all the clay treatments.

Discussion

Data obtained from this study showed that *Phyllanthus amarus* is more abundant in Fiditi than Akufo at the onset of wet season (April 2015). This spatial variation in the distribution may be due to competition among various weed species in the two study sites. It was observed that *P. amarus* importance value reduced drastically from 9.51% and 47.16% at the onset of wet season to 3.42% and to 9.63% at the mid of wet season in Akufo and Fiditi, respectively. With adequate moisture at the mid of wet season, more plant species had established to out-compete *P. amarus* in the ecosystems. This may explain the low population of *P. amarus* at the peak of rainy season and that competition is the major factor limiting its all year round availability as also reported by Radosevich *et al.* (1996). The higher (31) taxa in Akufo than Fiditi (19) may further explain the more intense interspecific competition that resulted in low population of *P. amarus* in Akufo than Fiditi. The high diversity ($H' = 2.86$) at Akufo implies random distribution of the species with none dominating, which was further proved by the low (0.08) Dominance value compared to Fiditi with low diversity ($H' = 1.74$) and high dominance ($D = 0.3$). The higher the Shannon-Wiener value, the higher the species diversity (Kent and Coker, 1992). At the mid wet season, the taxa increased to 37 and 21 weed species at Akufo and Fiditi, respectively, indicating that increased moisture availability at the mid wet season provided favourable conditions for germination of more seeds in the soil seed bank, and recruitment and establishment of seedlings of more weed species. The enumeration of more weed species was

confirmed by the increased species diversity and reduced dominance in the two study sites at the mid wet season. The reduced water stress in the mid wet season resulted in more random distribution than the onset of wet season.

The improvement of seed germination by hot water treatment confirmed dormancy mechanism. This is in agreement with Meenu and Pawan (2012) who reported hard seed coat dormancy in *Phyllanthus amarus*. The implication of the result is that fresh fruits of *Phyllanthus amarus* should be treated for 80 seconds in hot water while dry *Phyllanthus amarus* should be treated for 60 seconds in hot water for even germination in nursery production of seedlings.

The results obtained from the effect of varying water regimes and soil types on performance of *P. amarus* showed that the sandy-loam soil, with better drainage characteristics, aided growth better than the poor drainage observed in the clay soil. The poor drainage in the clay soil will result in poor aeration, which might explain the poor growth of *P. amarus* in clay soil when water was supplied to the plants at 1, 2 and 3 days intervals. Root system may penetrate deeper and extend wider in sandy-loam soil compared to clay soils and eventually allow better establishment of the plant on sandy-loam soil (Azza *et al.*, 2010). However, water supply to the seedlings at 7 days interval became an advantage to the plants in clay soil compared to sandy loam soil. This may explain the higher biomass accumulation by seedlings in clay watered every seven days compared to its counterpart sandy Loam soil in the present study. Water molecules are held longer by the small particles of clay soil compared to the sandy loam soil with big soil particles. Hence,

water availability to *P. amarus* was longer in Clay watered every seven days than Sandy-loam treatment. Hsiao and Acevedo (1979) reported that the reduction in plant growth under low soil moisture is as a result of water stress which cause losses in tissue water which reduced the turgor pressure in the cell, thereby inhibiting the enlargement, division of cells and subsequently cause a reduction in the uptake of nutrient elements causing a disturbance in the physiological process needed for plant growth.

Generally, *P. amarus* performed better on Sandy-loam soil than on clay soil. The result is in line with those obtained by Abou-Leila *et al.* (1993). This effect may be attributed to the physical properties of the soil, where sandy-loam soil is well aerated, porous and the ions absorption is easier while some ions adhere on the clay soil particles because of its compactness and low percolation. Also, the performance of *P. amarus* on sandy-loam soil compared to clay soil might be as a result of high organic matter (humus) contained in sandy-loam soil. Daily watering regime had the highest significant effect on the growth and biomass accumulation of *P. amarus* when compared to other watering regimes.

Conclusion and Recommendations

The floristic survey revealed that competition from other weed species was a major factor limiting the availability of *Phyllanthus amarus* at the mid or peak wet season which is as a result of favourable condition provided by abundance of moisture. The spatial and temporal variation in weed species diversity affected the population of *P. amarus*. The reduction in the importance value of *P. amarus* with recruitment of other species may present it as an inferior competitor. Brief steeping of

the seeds of *P. amarus* in hot water will break the hard seed coat dormancy and ensure uniform seed germination and uniform age of seedlings at harvest. *Phyllanthus amarus* performed best on well-drained sandy-loam soil and daily watering favoured best its growth and biomass accumulation. It is clear from the study that *P. amarus* will not be at its best performance under space and water stresses, and hence will be less available for collection in the wild at the peak of wet season and in the dry season. Therefore, it can be recommended that *Phyllanthus amarus* should be cultivated in well-drained soils with regular weeding carried out to reduce competition from other weed species and with adequate moisture provided. Also, cultivating it will ensure its availability all year round and its collection in wholesome environment.

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Adapting to Water Scarcity under Climate Change: Management to Enhance Water Productivity in Irrigated Rice

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Abstract

Decreasing water availability threatens the productivity of irrigated rice systems and strategies must be sought to save water. This paper reports on experiments conducted to access the potential for saving irrigation water. The field experiment was designed to evaluate rice water use under continuous flooding (CF) and alternate wetting and drying (AWD) under 45 and 90 kg N ha⁻¹ using three seedling ages (20, 30 and 40 days). The pot experiment treatments included: saturation (T1), continuous flooding to 3 cm (T2), continuous flooding to 5 cm (T3), irrigated to 5 cm 2 days after water depth dropped to 25 cm (T4), irrigated to 5 cm after water depth dropped to 25 cm but flooded to 5 cm from flowering stage (T5), irrigating to 5 cm 5 days after water depth dropped to 25 cm below surface (T6). The field study showed that, grain yield was not significantly different between CF and AWD, and that AWD saved between 4 to 10 % water. Water productivity in the field was higher for AWD compared with CF and water productivity increased with increasing nitrogen level. For the pot experiment, all treatments saved some amount of water relative to T3. Water saved ranged from 11 mm (1.2 %) for T2 to 303 mm (33.4 %) for T5. Treatment T5 recorded the lowest grain yield of 60.2 g/pot and this represents a yield loss of 23.2 % relative to T3. Therefore, mild AWD can increase water productivity and save water without reducing yield.

Keywords: Alternate wetting and drying, Climate impact adaptation, Rice irrigation, Water saving.

Introduction

Rice feeds more than 3 billion people worldwide and receives some 34–43% of the total world's irrigation water (Bouman *et al.*, 2007). Growing a water-intensive crop such as rice presents important future challenges in the face of projected climate change and variability in water supply. Climate change, along with the potential of decreasing water availability for agriculture, threatens the productivity of

irrigated rice ecosystems and ways must be sought to save the situation.

Conventional water management in lowland rice systems tends to continuously submerge (CS) fields for most part of the season leading to high water demand. With growing irrigation water demand and increasing competition across water-using sectors, the world now faces a challenge to produce more food with less water. One way of reducing water use in crop production is to increase water productivity, defined as the

amount or value of product per volume or value of water used (Molden *et al.*, 2001).

Water inputs can be reduced and water productivity increased by introducing periods of non-submerged conditions (Bouman and Tuong, 2001). One widely promoted water-saving technique is alternate wetting and drying (AWD) irrigation (Chu *et al.*, 2014), where irrigation water is applied to achieve intermittent flooded and non-flooded soil conditions. Alternate wetting and drying (AWD) has been reported to maintain or even increase yield and to be widely adopted by farmers (Yao *et al.*, 2012). However, when AWD systems were tested in tropical areas in Asia, such as in India and the Philippines, yields often decreased compared with CS conditions (Tabbal *et al.*, 2002). According to Belder *et al.* (2004) AWD most often reduces yield and the amount of Irrigation water that can be saved in alternate wetting and drying compared with continuous flooding without any yield loss ranged between 6 to 14 %. Belder *et al.* (2004) also observed water productivity under AWD to be 5-35 % higher than CS conditions, although characterized by 1-7 % decline in yield. Factors such as soil type, duration and level of water stress during the non-submerged periods, depth of water table, rice variety, life cycle stage of crop when water stress is imposed and many more factors could account for different results under AWD.

However, little information exists in Ghana on how AWD affects rice growth and yield. This study was therefore undertaken to (i) experimentally quantify how varying water use affects rice growth and yield, and (ii) determine the irrigation strategy that can increase water productivity while maintaining or

improving yield of rice.

M a t e r i a l s a n d M e t h o d s

Field experiment

Field experiments were also conducted from April to July in 2014 (major season) and from December 2015 to March 2016 (minor season) at the Soil and Irrigation Research Centre, Kpong Kpong (6° 9' N, 0° 4' E), University of Ghana. A split-split plot design was used with two water regimes: W1 (continuous flooding to 5 cm water head above the soil surface) and W2 (irrigated to 5 cm water head when the water depth dropped to 25 cm below the soil surface) as main plot treatment, two nitrogen rates (45 and 90 kg N ha⁻¹) as subplot and three seedling ages (20, 30 and 40 days old) as sub-subplots. Three replicates were used and the rice was grown for 120 days. Prior to the start of the experiment, core soil samples from the field were taken at four depths (0-7 cm, 7-14 cm and 14-21 cm, 21-28 cm) for determination of bulk density and saturated soil moisture content. A preliminary study was conducted to ensure that a water depth of 25 cm below the soil surface was “safe” for rice. This was achieved by perforating PVC pipes (50 cm in length and 15 cm in diameter) at 1 cm spacing along the entire length. The perforated pipes (piezometers) were pushed into the soil in a rice field so that 25 cm protruded above the soil surface. The soil from the inside of the PVC tube was removed so that the bottom of the tube was visible. This allowed monitoring of the drop in water level below the soil surface by dipping a stick into the pipe and measuring the drop in water level on a ruler (IRRI, 2009). The closer spacing ensured that the level of water in the rice basin was the same as the water level in the tube. (IRRI, 2009).

The plants were monitored during the tillering, panicle initiation and flowering stages, while allowing the water level to drop to 25 cm below the soil surface. The rice plants at these stages did not show any signs of moisture stress or wilting and flood water was re-established each time to 5 cm height. The 25 cm water depth was therefore considered to be “safe”.

For the main experiment, the perforated pipes were placed in plots as described earlier. Nitrogen (90 kg N ha^{-1}), phosphorus ($45 \text{ kg P}_2\text{O}_5$) and potassium ($45 \text{ kg K}_2\text{O}$) was applied using urea, triple superphosphate and muriate of potash, respectively. A medium duration rice variety, *Baika*, was used for this study. Plot sizes measured 3 m by 4 m and main plots (water treatments) were separated by a distance of 2 m. Water was delivered to plots with a small motorized pump using the velocity volume approach (Trimmer, 1994). The pump was set to the same speed of delivery each time it was used. Data was collected on rainfall from a meteorological station on site. At maturity, data was also collected on Harvest Index (ratio of grain weight to total biomass), filled grains per panicle, 1000-grain weight, tillers per hill and grain yield. Water saved in the field study was estimated as the difference in water input between AWD and CF. Water productivity was estimated as grain yield per unit water used.

Pot experiment

The experiment was conducted at the Soil and Irrigation Research Centre, Kpong ($6^\circ 9' \text{ N}$, $0^\circ 4' \text{ E}$), University of Ghana, during the major season (April to July) of 2014 and the minor season (December, 2015 to March, 2016). It was carried out using pots with diameter of 30 cm and height of 26 cm

under a rain-out shelter. The soil used was a Calcic Vertisol (FAO, 2001). Bulk density and saturated soil water content were determined. Water content of the soil was determined by following a similar procedure for the field trial. This was achieved by perforating polyvinyl chloride (PVC) pipes (30 cm in length and 2 cm in diameter) at 1 cm spacing along the entire length. The pipes were placed upright in the pots before filling the pots with 10 kg of soil. This ensured that the columns of the pipes were not filled with soil. The pots were irrigated to 5 cm water head and left to stand. The drop in water level in the pipe was monitored twice daily. As soon as the water level “disappeared” at the base of the pot (approximately 25 cm from the soil surface) the soil in the pot was mixed thoroughly and samples were taken for moisture content determination. A medium duration rice variety, *Baika*, was used for this study.

Six water treatments were used which included: saturation (T1), continuous flooding to 3 cm (T2), continuous flooding to 5 cm (T3), irrigated to 5 cm 2 days after water depth dropped to 25 cm (T4), irrigated to 5 cm after water depth dropped to 25 cm but flooded from flowering stage to 5 cm (T5), irrigating to 5 cm 5 days after water depth dropped to 25 cm below surface (T6). Twenty day old seedlings were transplanted with 2 seedlings per pot in a completely randomized design with ten replicates. The compound fertilizer NPK (15:15:15) was applied one week after transplanting as basal application at 300 kg ha^{-1} . Urea was applied at panicle initiation as top-dress at 100 kg ha^{-1} . At maturity, data was collected on filled grains per panicle, 1000-grain weight, number of tillers per hill and grain yield and water used. Water saved was estimated as the difference in water use for water treatments

using T3 (continuous flooding at 5cm) as the reference as it consumed the highest amount of water.

Statistical analysis

The data collected from the field study was analyzed as split-split plot, while that from the pot experiment was analyzed as completely randomized design using Analysis of Variance. Where significant differences were detected, least significant difference (LSD) test ($p = 0.05$) was used to test the differences between means.

Results

Field experiment

Rainfall and physical and chemical properties of the soil

Rainfall (Appendix 1) during the 2014 major season was 483 mm which occurred in 34 rainfall events. Total rainfall during the 2015-2016 minor season was 182.8 mm and this occurred in 14 rainfall events.

The characteristics of the soil is presented in Table (1) and Table (2). The soil has a pH of 6.7 with organic carbon content of 0.81 %. Total nitrogen, available phosphorus and cation exchange capacity values were 0.1 %, 5.6 mg kg⁻¹ and 34.4

cmol (+) kg⁻¹, respectively. Bulk density ranged between 1.41 to 1.55 Mg m⁻³ with a clay content of between 57 and 62 %. Saturated water varied between 32 and 40 %.

Irrigation water input

Seedling age had no significant effect ($p = 0.178$) on the amount of irrigation water applied. Irrigation water input for CF ranged between 631 to 643 mm while that for AWD ranged between 574 and 585 mm in the 2014 rainy season (Table 3). Irrigation water input during the minor season (2015-2016) season ranged between 761 and 866 mm. Total water input for both continuous flooding (CF) and *alternate wetting and drying* (AWD) was higher in the 2014 major season with values ranging between 1057 and 1126 mm compared to a range of 944 to 1049 mm for the 2015-2016 minor season.

Table 1. Chemical properties of the soil at the experimental site at Kpong, Ghana

Property	Value
pH in water	6.70
Organic C (%)	0.81
Total N (%)	0.10
Olsen P, mg kg ⁻¹	5.60
CEC, cmol kg ⁻¹	34.2

Table 2. Physical properties of the soil at the experimental site at Kpong, Ghana

Soil depth (cm)	Bulk density (Mg m ⁻³)	Sand	Silt	Clay	Textural class	Saturated water (% w/w)
		%				
0-10	1.41	40.5	2.5	57.0	Clay	32.4
10-20	1.48	38.9	2.7	58.4	Clay	34.6
20-30	1.55	36.0	2.4	61.6	Clay	39.7

Table 3. Water supply and averaged nitrogen rates over seedling ages in the field

Year	Rainfall (mm)	Irrigation (mm)		LSD	Total input (mm)		Difference (%)
		AWD	CF		AWD	CF	
<u>2014</u>							
90 N	483	574 ^a	643 ^b	16.7	1057	1126	6.1
45 N	483	585 ^a	631 ^b	25.4	1068	1114	4.1
<u>2015-2016</u>							
90 N	183	772 ^a	840 ^b	14.3	955	1023	6.6
45 N	183	761 ^a	866 ^b	19.0	944	1049	10.0

*AWD = Alternate wetting and drying, CF = Continuous flooding. *Means followed by the same letters in a row are not significantly different ($p < 0.05$) using LSD

Yield components

Results on yield components is presented in Table (4 and 5). Apart from CF for the 2015-2016 minor season where seedling age had no effect, panicle bearing tillers per hill for 40-day old seedlings was significantly lower ($p < 0.05$) than that for 20 and 30 day old seedlings for both CF and AWD. However, there was no difference in tillers per hill between CF and AWD. There

was no difference in harvest index (HI) between CF and AWD. Filled grains per panicle ranged from 96 to 103. During the 2014 major season, filled grains per panicle was not significantly affected ($p = 0.136$) by seedling age. However, during the 2015-2016 minor season, 40-day old seedlings had significantly lower filled grains per panicle. The 1000-grain averaged 26.7 g across all treatments.

Table 4. Number of Tillers per hill and Harvest Index of rice with two N rates in 2014 and 2015-2016

Seedling age (DAE)	Tillers per hill		Harvest Index	
	CF	AWD	CF	AWD
<u>2014</u>				
20	12.4±0.72 ^a	12.3±0.17 ^a	0.42±0.04 ^a	0.43±0.02 ^a
30	12.1±0.75 ^a	12.3±0.44 ^a	0.46±0.05 ^a	0.43±0.05 ^a
40	10.0±0.53 ^b	10.3±0.46 ^b	0.45±0.03 ^a	0.41±0.03 ^a
LSD	0.45	0.38	0.03	0.05
<u>2015-2016</u>				
20	11.2±1.01 ^a	11.8±0.42 ^a	0.48±0.03 ^a	0.47±0.03 ^{ab}
30	11.5±0.98 ^a	11.9±0.30 ^a	0.45±0.06 ^a	0.45±0.05 ^a
40	10.3±0.20 ^a	10.7±0.44 ^b	0.46±0.06 ^a	0.48±0.05 ^b
LSD	1.60	0.45	0.06	0.02

Values are means ± Standard deviation, DAE = days after emergenc e. Means followed by the same letters in a column are not significantly different ($p < 0.05$) using LSD.