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# Journal of Horticulture and Forestry

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**African spider plant (*Cleome gynandra* L.) as biofumigant against weeds during Turfgrass (*Paspalum notatum*) establishment**

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## Full Length Research Paper

# African spider plant (*Cleome gynandra* L.) as biofumigant against weeds during Turfgrass (*Paspalum notatum*) establishment

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Weeds are the most common pests, which interfere with turfgrass establishment and uniformity lowering functional and aesthetic quality of lawns. Killing weed seeds or suppressing their germination is necessary before establishing new lawn to prevent weeds from gaining a foothold hence, giving turfgrass a competitive advantage. A field study was conducted at Bukura Agricultural College, Kenya, to explore the potential of biofumigation with African spider plant (*Cleome gynandra*) as an environmentally friendly alternative to use of synthetic herbicides for establishment of weed-free *Paspalum notatum* turfgrass. Biofumigation with chopped *C. gynandra* plants at flowering stage, incorporated into the soil at the rates of 4, 6 and 8 kg m<sup>-2</sup>, respectively, and was compared with Basamid<sup>®</sup> (97% Dazomet) at 0.029 kg m<sup>-2</sup> and untreated negative control in a randomized complete block design experiment. *C. gynandra* at rates of 6 or 8 kg m<sup>-2</sup> was as effective as Basamid<sup>®</sup> at 0.029 kg m<sup>-2</sup> in significantly suppressing *Galinsoga parviflora*, *Galinsoga ciliata* and *Bidens pilosa* weed populations and reducing weed infestation. Other weed species occurred in the experimental plots in insignificant populations. The results of this study demonstrated that *C. gynandra* has potential for use as a biofumigant against weeds during lawn establishment.

**Key words:** *Cleome gynandra*, biofumigation, environmentally friendly, weeds, herbicides, lawn.

## INTRODUCTION

Lawn turfgrass has become an integral part of most landscapes that are professionally established and maintained to provide functional, recreational, aesthetic and therapeutic benefits to users. *Paspalum notatum* turfgrass is desirable for sod production and makes good low-maintenance lawns (Newman et al., 2011; Trenholm

et al., 2011) with low to moderate fertility requirements, and tolerance to drought, insect pests and diseases, and close grazing by animals (Hancock et al., 2013). It is also useful in phytoremediation of phosphorus-impacted soils and integrated pest management of nematodes and fungal diseases when used in rotation with annual crops

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(Newman et al., 2011). However, weeds have been found to be the most common pests in turfgrass areas (Martin, 2012). These opportunistic plants quickly germinate and grow in the absence of turfgrass competition thereby marring the lawn appearance if left uncontrolled (Chalmers and McAfee, 2009). Weeds compete with turfgrass for water, nutrients, light and space, resulting in lawn deterioration and if allowed to dominate, they out do the turfgrass (Dernoeden, 2005). Weed invasion is a problem especially in the bare spaces between newly planted grasses (Cameron, 2006). Initial removal from the site before establishing lawns is therefore necessary in order to avoid persistent weed problems later (Turf and Landscape Digest, 2004; Landschoot, 2013). The quality of a new lawn is directly related to the success of its establishment as a well-established lawn is easier to maintain (Landschoot, 2013). Weeds increase lawn maintenance cost and some affect human health by causing allergy reactions (Zimdahl, 2007) and their control is one of the biggest frustrations in maintaining lawns (Burke, 2013).

Weed management strategies for landscape and turf settings include: chemical control, cultural practices, biological control, use of organic products and weed suppressive plant materials (Bertin and Weston, 2004). Weed control measures can be employed prior to planting or as a component of post establishment program. Key to avoiding persistent weed problems is the initial removal of the undesirable plants from the site before establishing the lawn (Thurn et al., 1994; Turf and Landscape Digest, 2004; Landschoot, 2013). During lawn site preparation, weeds are mainly controlled by cultivation (tillage) or use of herbicides. Herbicides however, contribute to environmental contamination and are hazardous to human health (Cole et al., 2011; Grey and McCullough, 2012) and cultivation only is not sufficient as there are usually many weed seeds in the soil.

According to Bello et al. (2007) biofumigation and biosolarization are alternatives to toxic herbicides. Biofumigation, a terminology coined during the ninth Australian research assembly on brassicas by Kirkegaard et al. (1993), is the practice of using chemicals released from decomposing plant material to suppress soil pathogens, insects and germinating weed seeds (Karavina and Mandumbu, 2012). Biofumigant effects are largely related to the high concentration of glucosinolates (GSLs) precursors to isothiocyanates (ITCs) which have broad biocidal activity (Johnstone et al., 2013). Isothiocyanates are sulfur containing compounds generated by the glucosinolate-myrosinase system in plants (Hara et al., 2009). The concentration of GSLs and ITCs in the soil is highest 30 minutes after incorporation of pulverized biofumigation crops and can still be detected for up to 8 and 12 days, respectively (Gimsing and Kirkegaard, 2006). At high concentrations ITCs are general biocides that act like some commercial pesticides

such as Vapam and Dazomet (University of Idaho, 2013). Incorporation of ITCs into the soil has been found to be effective in suppressing some weeds (Norsworthy and Meehan, 2005; Bello et al., 2007). Glucosinolate containing plant tissues may therefore contribute to reduction in use of synthetic herbicides if weed seeds are targeted (Brown and Morra 1996).

Spider plant (*C. gynandra* L. syn. *Gynandropsis gynandra* L.) is a common African leafy vegetable and medicinal plant belonging to the order brassicales as brassicas (Aparadh et al., 2012) with main secondary metabolites in it being alkaloids, cyanogenic glycosides, steroidal nucleus and anthraquinones (Ajaiyeoba, 2000). Glucosinolates in *C. gynandra* include methylglucosinolate, cleomin and glucocapparin which give rise to methyl isothiocyanates when hydrolyzed (Silué, 2009). Homogenized leaves of *C. gynandra* have been found to emit significant quantities of methyl-isothiocyanate, propyl-isothiocyanate, butyl-isothiocyanate and isobutyl-isothiocyanate plus a number of aldehydes, terpenes, alcohols, acetates and ketones (Nyalala et al., 2013). The current study therefore evaluated biofumigation potential of *C. gynandra* on weed control during lawn establishment.

## MATERIALS AND METHODS

### Experimental site

The study was conducted at Bukura Agricultural College in western Kenya which lies at longitude 0° 13' 15" North, latitude 34° 36' 44" East and altitude of 1474 m above sea level. The area has a daily mean temperature of about 22°C and annual rainfall range of about 1700 to 1800 mm distributed over two main cropping seasons; the long rainy season from March to July, and the short rainy season from September to December. The region is in the Lower Mid-land one agro-ecological zone (LM1), normally described as the sugar cane zone with soil classified as Orthic Ferralsol. The study comprised of two field experiments. The first trial was carried out from August 2013 to March 2014 during the short rainy season and the second one from March to October 2014 during the long rainy season.

### Plant materials used

African spider plant (*C. gynandra* L.) seeds were purchased from Kenya Seed Company Limited and directly drilled in rows spaced 0.3 m apart, prior to turfgrass establishment. Phosphorus at the rate of 40 kg ha<sup>-1</sup> plus nitrogen at the rate of 18 kg ha<sup>-1</sup> were applied during planting. Plants were thinned to intra row spacing of 0.2 m three weeks after planting and top-dressed with nitrogen at the rate of 52 kg ha<sup>-1</sup>. At initial flowering stage, the *C. gynandra* plants were uprooted, chopped into small pieces (≤ 0.3 m) and used for biofumigation.

### Experimental design and treatment application

The area for lawn establishment was cultivated four weeks, prior to treatment application. The experiment was laid out in a randomized complete block design with four replications with each plot measuring 4 m<sup>2</sup>. Blocks and plots were separated by 1 and 0.5 m

wide paths, respectively. The experiment compared untreated plots, fresh chopped *C. gynandra* plants incorporated into the soil at 4 kg m<sup>-2</sup>, 6 kg m<sup>-2</sup> and 8 kg m<sup>-2</sup> and Basamid® (97% Dazomet) at 0.029 kg m<sup>-2</sup>. Untreated and Basamid® at 0.029 kg m<sup>-2</sup> treatments served as the negative and positive controls, respectively. Fresh chopped *C. gynandra* plant materials were incorporated into the soil up to 0.3 m depth then covered with 0.14 mm thick clear polyethylene sheet. Plots treated with Basamid® were fumigated with the product at the rate of 0.029 kg m<sup>-2</sup> and also covered with polyethylene sheet while the untreated plots were left uncovered. The edges of covering polyethylene sheet were buried 0.15 m into the soil to ensure air tight conditions for four weeks. The treated plots were uncovered and left to aerate for 14 days to clear effects of the isothiocyanates, as recommended for Basamid®, before the turfgrass was planted. Crops with compounds inhibitory to weed seeds may also be phytotoxic to crop seeds (Ngouajio et al., 2014). Isothiocyanates from biofumigants have been detected in the soil for up to 12 days. Therefore, 14 days aeration period was applied for all the treatments after which all the plots were raked and leveled for lawn establishment.

### Turf grass establishment

*Paspalum notatum* turfgrass obtained from Bukura Agricultural College were dug up and cut into circular plugs of 0.15 m diameter using a fabricated plug-cutter and planted in holes spaced at 0.3 m by 0.3 m. The plugs were planted at ground level then watered immediately. During planting, P was applied to all plots at the rate of 40 kg ha<sup>-1</sup> plus N at 18 kg ha<sup>-1</sup>. One month later, the plots were top-dressed with N at 52 kg ha<sup>-1</sup> and maintained moist until the turfgrass fully established.

### Data collection

Data collected included: days to first emergence of weeds, species and number of weeds, and total fresh and dry weight of the weeds. Data collection was done from the inner rows of the experimental plots leaving a distance of 0.3 m from the plot margins, which served as guard rows. The plots were monitored daily and days to first emergence of weeds recorded. In order to determine the weed species and population, sampling areas were randomly selected using a quadrat (0.3 m × 0.3 m). The species and number of weeds were recorded every 7 days. At flowering stage, all the weed species within the sampling area of each plot were uprooted and weighed using electronic balance to determine fresh weed biomass. The weeds were sun dried and weighed to obtain dry biomass.

### Statistical analysis

Data on days to weed emergence and biomass were subjected to analysis of variance (ANOVA) at  $P \leq 0.05$  using R statistical software version 3.2.2 (R Project, 2015) while the numbers of each weed species were checked for normal distribution and log-transformed where necessary before analysis. Significantly, different means were separated using Tukey's honestly significant difference (Tukey's HSD) test at  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

### Effects on weed emergence

Weed species emerged 14 days after turfgrass

establishment in all the treatments during season one. In season two experiment, weeds emerged within 6 days in the cultivation only treatment while biofumigation with spider plant at 8 kg m<sup>-2</sup> and application of Basamid® at 0.029 kg m<sup>-2</sup> similarly resulted in the highest number of days (12.75) to first weed emergence (Table 1). Weeds easily invade bare spaces between newly planted grasses as pointed out by Cameron (2006).

Weed emergence was similar between treatments during trial one experiment but varied significantly in trial two. The difference in days to first weed emergence between the two trials can be attributed to soil moisture, which is one of the environmental factors that affect seed germination (Lu et al., 2006). Dry conditions limit moisture availability and seed germination (Dadach et al., 2015) resulting in delayed emergence of seedlings. Both experiments were carried out under rain fed conditions and supplemented with hand watering during dry weeks. Hand watering using a watering can, may have targeted the planted plugs leading to limited moisture availability to the weed seeds within the plots. Trial one experiment was conducted during the short rains (September to December) while trial two study was mainly carried out during long rains (March to July) in the area. Besides, treatments were applied before the onset of the short rains when the soils were drier in trial one while in trial two they were applied at the onset of long rains when the soil moisture content was high. This implied that the entire plot areas were exposed to sufficient moisture for germination and emergence of the weed seeds within the plots. *Oxalis latifolia* was the main species that emerged first in trial one probably due to its underground storage structure which enables it to sprout and grow even under limited soil moisture conditions.

### Effects on weed populations

*Galinsoga parviflora*, prevailed insignificantly ( $p \leq 0.001$ ) lower numbers in plots where *C. gynandra* was incorporated at 6 kg m<sup>-2</sup> or 8 kg m<sup>-2</sup> and those treated with Basamid® at 0.029 kg m<sup>-2</sup> than in the cultivation only plots after establishment of the lawn in both trials (Table 2). Furthermore, incorporation of *C. gynandra* at 4 kg m<sup>-2</sup> recorded significantly lower *G. parviflora* prevalence 35 days after establishment of paspalum turfgrass in trial one and throughout trial two. Cultivation only treatment recorded the highest number of *G. ciliata* plants at 14, 21, 28 and 35 days after establishment of paspalum turfgrass while the lowest number of the weeds species was obtained in plots treated with Basamid® at 0.029 kg m<sup>-2</sup> which was not significantly different from those of *C. gynandra* at 6 kg m<sup>-2</sup> or 8 kg m<sup>-2</sup> during both trials (Table 2). Similarly, cultivation of only treatment in both trials had the highest number of *B. pilosa* as application of Basamid® at 0.029 kg m<sup>-2</sup> or *C. gynandra* at 6 or 8 kg m<sup>-2</sup> resulted in the least prevalence of the weed throughout



**Table 1.** Effect of biofumigation with African spider plant (*Cleome gynandra*) on number of days to emergence of weeds after establishment of *Paspalum turfgrass*.

Treatment	Time to weed emergence (Days)	
	Trial 1	Trial 2
Untreated	14	6 <sup>c</sup>
Basamid (0.029 kg m <sup>-2</sup> )	14	8 <sup>b</sup>
<i>Cleome gynandra</i> (4kg m <sup>-2</sup> )	14	11.5 <sup>a</sup>
<i>Cleome gynandra</i> (6kg m <sup>-2</sup> )	14	12.75 <sup>a</sup>
<i>Cleome gynandra</i> (8kg m <sup>-2</sup> )	14	12.75 <sup>a</sup>

Means followed by the same letter in a season are not significantly different at  $P \leq 0.05$  according to Tukey's HSD.

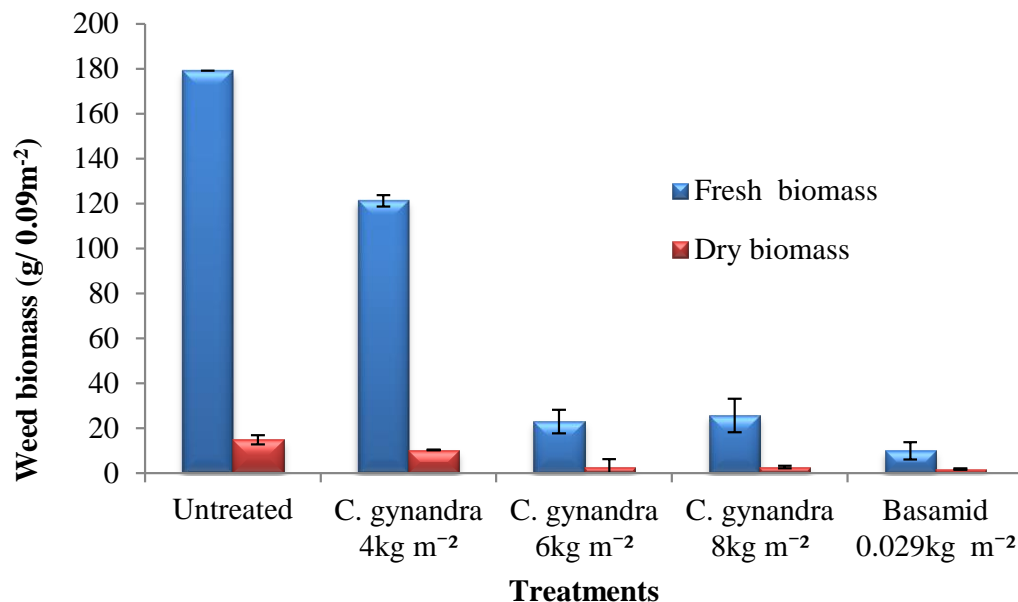
**Table 2.** Effect of biofumigation with African spider plant (*Cleome gynandra*) on mean number of *Galinsoga parviflora*, *G. ciliata*, *Bidens pilosa* and *Oxalis latifolia* weed species.

Weed species	Treatment	Weeds / 0.09 m <sup>2</sup> on various days after establishment of paspalum turfgrass							
		Trial 1				Trial 2			
		Day 14	21	28	35	Day 14	21	28	35
<i>Galinsoga parviflora</i>	Untreated	2.00 <sup>a</sup>	6.75 <sup>a</sup>	8.75 <sup>a</sup>	18.25 <sup>a</sup>	9.50 <sup>a</sup>	18.25 <sup>a</sup>	19.50 <sup>a</sup>	19.50 <sup>a</sup>
	Basamid (0.029 kg m <sup>-2</sup> )	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.25 <sup>c</sup>	0.50 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>d</sup>	0.00 <sup>c</sup>	0.25 <sup>c</sup>
	<i>Cleome gynandra</i> (4 kg m <sup>-2</sup> )	0.75 <sup>ab</sup>	5.75 <sup>a</sup>	6.00 <sup>ab</sup>	11.75 <sup>b</sup>	5.75 <sup>b</sup>	9.50 <sup>b</sup>	12.75 <sup>b</sup>	13.25 <sup>b</sup>
	<i>Cleome gynandra</i> (6 kg m <sup>-2</sup> )	0.00 <sup>b</sup>	1.25 <sup>b</sup>	3.5 <sup>b</sup>	5.25 <sup>c</sup>	0.75 <sup>c</sup>	2.25 <sup>c</sup>	2.00 <sup>c</sup>	2.25 <sup>c</sup>
	<i>Cleome gynandra</i> (8 kg m <sup>-2</sup> )	0.00 <sup>b</sup>	1.75 <sup>b</sup>	1.75 <sup>c</sup>	3.75 <sup>c</sup>	0.00 <sup>c</sup>	0.50 <sup>d</sup>	0.50 <sup>c</sup>	0.50 <sup>c</sup>
<i>Galinsoga ciliata</i>	Untreated	1.00 <sup>a</sup>	5.50 <sup>a</sup>	8.25 <sup>a</sup>	17.00 <sup>a</sup>	9.00 <sup>a</sup>	18.50 <sup>a</sup>	19.50 <sup>a</sup>	19.00 <sup>a</sup>
	Basamid (0.029 kg m <sup>-2</sup> )	0.00 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.50 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>d</sup>	0.00 <sup>d</sup>	0.50 <sup>c</sup>
	<i>Cleome gynandra</i> (4 kg m <sup>-2</sup> )	0.25 <sup>a</sup>	4.25 <sup>a</sup>	7.25 <sup>a</sup>	15.00 <sup>a</sup>	5.50 <sup>a</sup>	10.00 <sup>b</sup>	12.00 <sup>b</sup>	12.00 <sup>b</sup>
	<i>Cleome gynandra</i> (6 kg m <sup>-2</sup> )	0.00 <sup>a</sup>	0.75 <sup>b</sup>	1.50 <sup>b</sup>	6.25 <sup>b</sup>	1.00 <sup>c</sup>	3.50 <sup>c</sup>	2.75 <sup>c</sup>	2.50 <sup>c</sup>
	<i>Cleome gynandra</i> (8 kg m <sup>-2</sup> )	0.00 <sup>a</sup>	0.25 <sup>b</sup>	1.75 <sup>b</sup>	6.75 <sup>b</sup>	0.00 <sup>c</sup>	0.75 <sup>d</sup>	0.50 <sup>d</sup>	1.25 <sup>c</sup>
<i>Bidens pilosa</i>	Untreated	0.00	2.00 <sup>a</sup>	2.00 <sup>a</sup>	3.00 <sup>a</sup>	0.00	2.50 <sup>a</sup>	1.75 <sup>a</sup>	3.00 <sup>a</sup>
	Basamid (0.029 kg m <sup>-2</sup> )	0.00	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>c</sup>
	<i>Cleome gynandra</i> (4 kg m <sup>-2</sup> )	0.00	0.50 <sup>b</sup>	0.2 <sup>b</sup>	0.25 <sup>b</sup>	0.25	0.00 <sup>b</sup>	0.00 <sup>b</sup>	1.00 <sup>b</sup>
	<i>Cleome gynandra</i> (6 kg m <sup>-2</sup> )	0.00	0.00 <sup>b</sup>	0.25 <sup>b</sup>	0.00 <sup>b</sup>	0.00	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>c</sup>
	<i>Cleome gynandra</i> (8 kg m <sup>-2</sup> )	0.00	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00	0.00 <sup>b</sup>	0.00 <sup>b</sup>	0.00 <sup>c</sup>
<i>Oxalis latifolia</i>	Untreated	1.50	2.50	4.25	2.75	2.50	5.00 <sup>a</sup>	5.00 <sup>a</sup>	5.00
	Basamid (0.029 kg m <sup>-2</sup> )	1.50	2.25	2.00	4.00	0.75	2.50 <sup>c</sup>	2.50 <sup>b</sup>	3.25
	<i>Cleome gynandra</i> (4 kg m <sup>-2</sup> )	2.25	3.50	5.00	2.75	2.75	4.25 <sup>ab</sup>	4.50 <sup>a</sup>	4.50
	<i>Cleome gynandra</i> (6 kg m <sup>-2</sup> )	1.25	2.50	3.75	3.50	2.50	4.00 <sup>abc</sup>	3.00 <sup>b</sup>	3.75
	<i>Cleome gynandra</i> (8 kg m <sup>-2</sup> )	1.50	1.75	2.75	2.75	1.50	3.25 <sup>bc</sup>	2.50 <sup>b</sup>	3.75

Means followed by the same letter within a column are not significantly different at  $P \leq 0.05$  according to Tukey's HSD.

the period of the experiment (Table 2). There were no significant differences in prevalence of *Oxalis latifolia* weed species across the treatments during season one. However, in season two, biofumigation with *C. gynandra* at 8 kg m<sup>-2</sup> was similar to application of Basamid® at 0.029 kg m<sup>-2</sup> in recording significantly lower population of *O. latifolia* than the cultivation in only treatment 21 days

after establishment of paspalum turfgrass. Plots treated with *C. gynandra* at 6 kg m<sup>-2</sup> achieved the same effect 28 days after establishment of paspalum turfgrass (Table 2). Other weed species: *Amaranthus sp.*, *Euphorbia hirta*, *Cynodon dactylon*, *Cyperus rotundas*, *Phyllanthus urinaria* and *Xanthium occidentale* occurred in the experimental plots in insignificant populations.



**Figure 1.** Effect of biofumigant plant (*Cleome gynandra*), positive control Basamid on fresh and dry weed biomass in trial one.

Incorporation of glucosinolate- containing plant material into the soil results in degradation products was highly toxic to soil borne pathogens and weeds (D'Addabbo et al., 2014). Glucosinolates break down to form isothiocyanates (ITCs) which have been found to be effective in suppressing some weeds (Norsworthy and Meehan, 2005). In the current study suppression of germination of *G. parviflora*, *G. ciliata* and *B. pilosa* by chopped *C. gynandra* plant incorporated into the soil was likely due to ITCs introduced to the soil. Homogenized leaves of *C. gynandra* have been found to emit significant quantities of biologically active ITCs (Nyalala et al., 2013).

At low concentrations ITCs are beneficial to human health and at high concentrations they are general biocides that act like some commercial pesticides such as Vapam and Dazomet (University of Idaho, 2013). This explains why biofumigation with African spider plant, *Cleome gynandra*, at rates of 6 and 8 kg m<sup>-2</sup> was as effective as Basamid<sup>®</sup> 0.029 kg m<sup>-2</sup> in significantly suppressing *Galinsoga parviflora* and *G. ciliata* weed populations while at 4 kg m<sup>-2</sup> it had no significant effect on the same weed species although it was significantly effective than the untreated negative control in controlling *Bidens pilosa*. Weeds with underground structures, like *O. latifolia* which form bulbs, are difficult to manage, hence, it is persistence even under fumigation with Basamid<sup>®</sup> 0.029 kg m<sup>-2</sup>.

#### Effects on fresh weed biomass

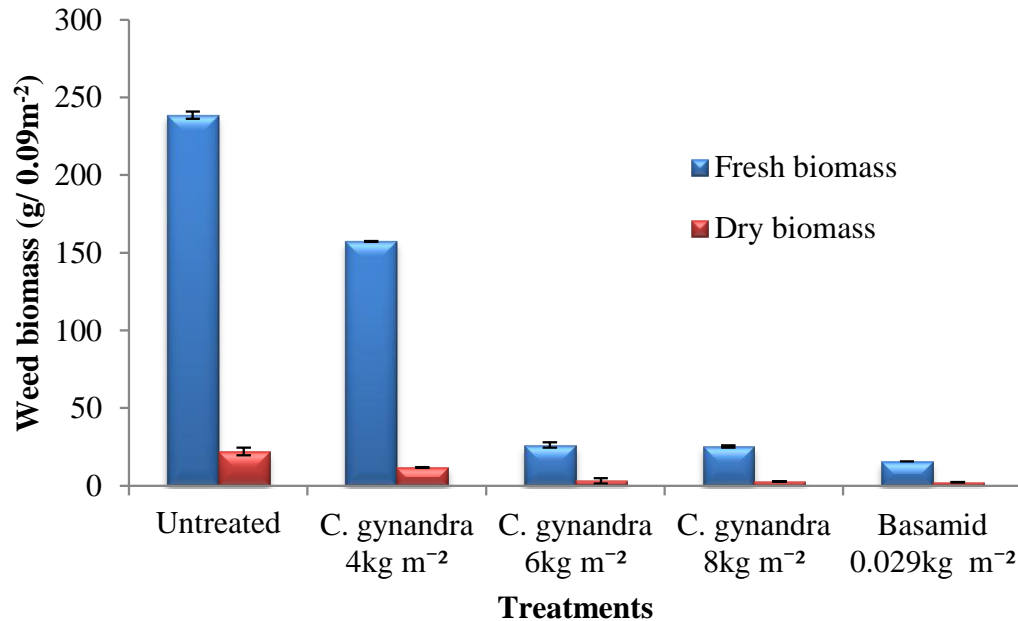
The highest fresh weed biomass was realized in

untreated plots during both trials one and two. Biofumigation with *C. gynandra* at 4 kg m<sup>-2</sup> had no significant effect on fresh weed biomass during trial one, but recorded significantly lower fresh weed biomass than the untreated in trial two. Incorporation of *C. gynandra* plants at 6 kg m<sup>-2</sup> or 8 kg m<sup>-2</sup> significantly reduced the total fresh weight of weeds to similar levels as Basamid<sup>®</sup> at 0.029 kg m<sup>-2</sup> (Figures 1 and 2).

#### Effects on dry weed biomass

Similar trend was observed in dry weed biomass with the untreated and *C. gynandra* at 4 kg m<sup>-2</sup> treatments resulting in the highest dry weed biomass. Biofumigation with spider plant at 6 kg m<sup>-2</sup> or 8 kg m<sup>-2</sup> was statistically similar to Basamid<sup>®</sup> 0.029 kg m<sup>-2</sup> in significantly reducing the total dry biomass of weeds (Figures 1 and 2). Plants compete for space by occupying space, the first plant that occupies an area tends to exclude all the others and have a competitive advantage (Zimdahl, 2007). The suppressive treatment effects on the weeds gave the turfgrass first priority to occupy the area, hence, outdoing the weeds and therefore contributed to the reduction of the total fresh and dry weed biomass.

Prevention is the best weed control strategy when establishing new lawn; weeds should be prevented from getting a foothold (Thurn et al., 1994). Biofumigation with African spider plant, *C. gynandra*, at rates 6 kg m<sup>-2</sup> or 8 kg m<sup>-2</sup> was as effective as fumigation with Basamid<sup>®</sup> at 0.029 kg m<sup>-2</sup> in significantly suppressing growth of some weeds. *C. gynandra*, at rates 6 or 8 kg m<sup>-2</sup> therefore has potential of being used as a biofumigant during lawn



**Figure 2.** Effect of biofumigant plant (*Cleome gynandra*), positive control Basamid on fresh and dry weed biomass in trial two.

establishment to reduce weed populations.

### Conflict of Interests

The authors have not declared any conflict of interests.


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