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Lantana camara photo by Ulrich Cziollek

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Antimicrobial effects of botanicals and biocontrol agents against *Phaeoisariopsis personata*, the causal agent of Tikka disease of groundnut

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Abstract. Ndifon EM. 2023. Antimicrobial effects of botanicals and biocontrol agents against *Phaeoisariopsis personata*, the causal agent of Tikka disease of groundnut. *Asian J Agric* 7: 71-75. Groundnut is highly susceptible to various pathogenic diseases, the most important of these is tikka. The aim of this study was to control the infection of groundnut caused by *Phaeoisariopsis personata* (Berk. & M.A.Curtis) Arx using two experiments. Each trial was set up using a completely randomized design, and each treatment was replicated thrice. Results show that *Eucalyptus globulus* Labill. resinous gum (at 50% and 100% concentrations) and *Terminalia catappa* L. resinous gum (at 50% concentration) were more effective in the inhibition of *P. personata*, followed by *Anacardium officinale* Pritz. resinous gum and *T. catappa* resinous gum (at 100% concentration). All the plant extracts significantly ($P \leq 0.05$) inhibited the pathogen. The percentage inhibition of *P. personata* by plant extracts ranged between 7.9-100%. All the isolates of *Trichoderma* were able to inhibit *P. personata* effectively, but the percentage inhibition of mycelial growth of *P. personata* reduced gradually with time. The percentage inhibition of *P. personata* by *Trichoderma* species ranged between 24-100%. The best biocontrol treatments were *T. virens* isolate BGMZ2, *T. harzianum* isolate BGMZ4, *T. hamatum* isolate ZXPB, and *T. harzianum* isolate ZXMZ. Using these biocontrol and botanical measures to manage the tikka leaf spot of groundnut is highly recommended.

Keywords: Leaf spots, peanuts, plant extract, *Trichoderma* species, yield loss

INTRODUCTION

Groundnut or peanut (*Arachis hypogaea* L. in the plant family Fabaceae) is a major oil crop rich in vitamins, protein, grease, oil, and fiber (Settaluri et al. 2012; Karmini et al. 2017). Peanuts are consumed worldwide in various forms, mostly in traditional cuisine. In addition, peanuts are widely used to produce peanut butter, confectionaries, roasted peanuts, snack products, soups, and desserts. Groundnut is essential in reducing malnutrition among the population in many African countries (Guimon and Guimon 2012).

Groundnut is cultivated to produce cooking oil, animal feed, groundnut flour, and for boiling/roasting as a snack (Patil and Pillai 2010; Awurum and Uwajimgba 2013). Groundnut is ranked sixth among the most important oil-seed crops globally (Koita et al. 2017). Groundnut seeds are rich in edible oil (50%), protein (25-30%), carbohydrate (20%), fiber, and ash (5%) (Awurum and Uwajimgba 2013). Groundnut is cultivated globally (including in Nigeria). Nigeria is among the first three groundnut-producing countries in the world. Groundnuts are cultivated throughout Africa, Asia, and the Americas, but the crop suffers severe yield loss due to diseases like Tikka disease. It is cultivated on a large scale in Abakaliki, Nigeria, despite being prone to leaf spot diseases. Like any other plant, groundnut is susceptible to more than 55 pathogens, including fungi, bacteria, nematodes, and viruses. Fungi are the causal agents of Tikka disease (Patil and Pillai 2010; Awurum and Uwajimgba 2013; Sangoyomi and Alabi 2016; Damicone 2017). Two fungi induce tikka leaf spot

disease, namely groundnut early leaf spot disease otherwise called *Cercospora* leaf spots or brown leaf spots or peanut cercosporiosis (induced by *Cercospora arachidicola* Hori. (Perfect stage - *Mycosphaerella arachidicola* W.A. Jenkins), and late leaf spot disease (caused by *Phaeoisariopsis personata* (Berk. & M.A.Curtis) Arx (Perfect stage - *Mycosphaerella berkeleyi* W.A. Jenkins).

The control of Tikka disease using biocontrol agents is in its rudimentary stage. Culture-filtrates of *Penicillium islandicum* Sopp. (50% concentration, applied at 70 DAS (i.e., days after sowing) proved potent against some pathogenic fungi of groundnut. It has been confirmed that groundnut seeds dressed with *Pseudomonas fluorescens* Rhodes formulation exhibited less disease severity after inoculation with late leaf spots. Moreover, *Trichoderma viride* Pers. (5% concentration) and *Verticillium lecanii* Zimm (5% concentration) were applied, significantly reducing the severity of leaf spot disease (Deyd and Dhutraj 2014).

Pal and Gardener (2006) emphasized that further development and effective adoption of available biocontrol agents require a greater understanding of the complex interactions among plants, people, and the environment. In addition, Yazdani et al. (2011) reiterated that using botanical fungicides (which are largely nonphytotoxic, nonsystemic, and easily biodegradable) can be an effective replacement for synthetic fungicides.

Studying the antimicrobial action of plant extracts and biocontrol fungi isolates is the first step to optimizing the use of these agents to protect groundnuts against Tikka disease and improve groundnut production. Therefore, the

aim of the present study was (i) to evaluate the antimicrobial activity of plant extracts and (ii) to assess the antimicrobial activity of some *Trichoderma* isolates against Tikka diseases of groundnut. The control of Tikka diseases of groundnut can be a determining factor in the production of groundnut.

MATERIALS AND METHODS

Study site

This study was conducted in the semi-humid tropics at the Faculty of Agriculture Laboratory complex, Alex Ekwueme Federal University Ndufu-Alike, Abakaliki, Nigeria (6.069°N by 8.199°E). Abakaliki experiences rainfall for more than nine months in a year. Therefore, the average relative humidity is usually high throughout the year. The mean relative humidity in the area remains above 70% for nine months per annum; thus, pathogenic propagules can easily survive in the environment. Abakaliki is located in the derived savannah ecological zone of Nigeria. It has well-drained iron-rich loamy soils.

Isolation and identification of the fungi

The infected groundnut leaves used for this research were obtained from the West Cameroons (05.120 - 06.220N by 09.180-10.220E). The groundnut leaves were collected from mature groundnut plants. *Trichoderma* isolates were obtained from groundnut seeds, mushrooms, crop seeds (i.e., maize, cowpea, pigeon pea, Bambara groundnuts, guava, mango, pawpaw, garden eggplant, orange, lemon, lime, eggplant, cacao, kidney beans, carrot, onions, sorghum, rice, millet, palm kernel nuts, roselle, date palm, cotton seeds, fig, lima bean, tomato, kola nut, and bitter kola). They were also obtained from farmland soils in the West Cameroons, as well as from northern (10.517N by 07.433E), north-central (10.376N by 07.709E), and south-eastern (09. 519N x 08.403E) Nigeria. The crop seeds were bought from markets and farmers/farms. The seeds were in a dehydrated state, and each seed was packaged in a separate envelope until it was processed later.

The fungi (*P. personata* and *Trichoderma* Pers.: Fr. spp.) were cultured on Acetate Differential Agar[®] enriched with dextrose and autoclaved at 120°C and 15 psi for 15 minutes according to the manufacturer's (Difco; USA) instructions. The isolated fungi were sub-cultured to obtain pure cultures (Ndifon 2022a) and identified with the aid of literature on fungi morphology (Narayanasamy 2011; Subrahmanyam et al. 2012; Fischbach and Dunning III 2015; Aglave 2019).

Experiment 1: Evaluation of the effects of plant extracts bio-assayed against tikka leaf spot disease

This poison food technique experiment was laid out in the laboratory using a Completely Randomized Design (CRD) with seven treatments, and each treatment was replicated three times. The treatment set consisted of the control, *Anacardium officinale* Pritz. resinous gum (50% and 100%), *Terminalia catappa* L. resinous gum (50% and

100%), and *Eucalyptus globulus* Labill. resinous gum at 50% and 100% concentrations.

The plant exudates (i.e., *E. globulus* resinous gum, *T. catappa* resinous gum, and *A. officinale* resinous gum) were utilized (at the rate of 166.7 g resinous gum per L of distilled water) to make 100% concentration of each plant extract. The plant extracts were strained through double-layered muslin clothes and filtered through Whatman's No. 1 filter paper. Each petri dish contained 15 mL of Acetate Differential Agar enriched with dextrose and the plant extract specified in the randomization/layout. The control received no plant extract and was inoculated in the same way with the Tikka disease pathogen (i.e., with a 2-mm disc of culture) as the other treated plots.

Experiment 2: Evaluation of the effects of *Trichoderma* isolates against tikka leaf-spot disease pathogen

This in vitro dual-culture experiment was laid out using CRD, and each treatment was replicated three times. The treatment set consisted of nine *Trichoderma* isolates (i.e., *T. harzianum* isolate BGMZA, *T. hamatum* (Bon.) Bain. isolate ZXPB, *T. harzianum* isolate ZXMZ, *T. harzianum* isolate BGMPP, *T. harzianum* isolate BGMZ3, *T. viride* Pers.: Fr. isolate AIBK, *T. harzianum* isolate AIBN, *T. harzianum* isolate ZXGV, and *T. virens* (J. Miller, Giddens & Foster) von Arx, Beih. isolate BGMZ2). The control was inoculated with *P. personata* only.

Each petri dish contained 15 mL of Acetate Differential Agar enriched with dextrose, which was autoclaved at 120°C and 15 psi for 15 minutes according to the manufacturer's instructions. Discs from the cultures of pathogen and *Trichoderma* isolates were cut using a 2-mm cork borer and placed at the edge of the Petri dishes. The control received no *Trichoderma* isolate and was inoculated the same way as other treated plots.

Data collection for both trials

The radius of the fungal colony was measured using a transparent ruler at 24-hour intervals starting from day 1 through day 7. The percentage inhibition was calculated using the following equation (Ndifon 2022b):

$$PI\% = ((C - T) / C) \times 100$$

Where:

PI : % inhibition of growth of the fungus

C : Perpendicular* radius of the fungal colony in the control plate

T : Perpendicular radius of the fungal colony in treated plate

*Perpendicular refers to the 'right angle' through the center of the Petri dish.

Data analysis

The data were subjected to an Analysis of Variance (ANOVA) procedure, and the means were separated using Duncan's Multiple Range Test (DMRT) at 5% probability level (as obtainable with Genstat[®] Discovery 2nd Edition statistical package). The descriptive statistics were assessed using IBM Statistical Package for Social Sciences (SPSS) version 25 and Microsoft Excel 365 procedure).

RESULTS AND DISCUSSION

The trend of percentage inhibition of radial growth of *P. personata* due to the application of plant extracts in vitro is presented in Table 1. The result shows that 50% and 100% concentrations of *E. globulus* resinous gum plant extract and 100% concentration of *T. catappa* resinous gum plant extract showed complete inhibition of *P. personata*. A 50% concentration of *A. officinale* resinous gum plant extract was the least effective treatment for managing *P. personata*. The percentage inhibition of *P. personata* by the plant extracts ranged between 7.9-100% inhibition. The results reveal that during the early stage of the trial (at 24 hours after inoculation (i.e., HAI)), a significantly different ($P \leq 0.05$) percentage inhibition existed between the treated experimental units and the control. *E. globulus* resinous gum plant extract at 100% concentration exhibited the highest inhibitory potential against the pathogenic species, followed by *E. globulus* resinous gum plant extract at 50% concentration.

This trend of inhibition was maintained (as at at previous data collection intervals) at 72 HAI, except that *A. officinale* resinous gum plant extract (at 50% concentration) showed dismal performance compared to the control. This treatment, however, performed significantly better than the control at 120 HAI. A clear pattern of inhibition was observed at 120 HAI, whereby *E. globulus* resinous gum plant extract treatments (at 50% and 100% concentrations) topped the charts, followed by *T. catappa* resinous gum plant extract at 50% concentration, *A. officinale* resinous gum plant extract (at 100% concentration) and *T. catappa* resinous gum plant extract (at 100% concentration). All the treated plots significantly inhibited the pathogen during the trial.

The percentage inhibition of mycelial growth of *P. personata* by applying biocontrol agents in vitro is presented in Table 2. The results show that at 24 HAI, all the isolates performed above average except for *T. harzianum* isolate AIBN. All the biocontrol agents effectively inhibited the radial growth of *P. personata* compared to the mean inhibition pattern. However, the percentage inhibition of the radial growth of *P. personata* reduced with time. The percentage inhibition of *P. personata* by *Trichoderma* species ranged between 24-100% inhibition, with the mean inhibition being 40%.

Therefore, to verify the probability of these observed differences being specious among the biocontrol treatments, a

representative extract of some intervals of the data was subjected to the ANOVA procedure, and the means were separated using the new Duncan's Multiple Range Test (DMRT) ($P \leq 0.05$) as publicized in Table 2. The results of statistical analysis revealed that during the mid-way stage of the trial (72 HAI), a significantly different ($P \leq 0.05$) percentage inhibition existed between the treated experimental units and the control. In addition, the results from the experimental unit - *T. harzianum* isolate AIBN was significantly different ($P \leq 0.05$) compared to the other treated experimental units.

At 120 HAI, all the treated experimental units showed significantly different ($P \leq 0.05$) percentage inhibition of the radial growth of the pathogen compared to the control. From the results of the last data collected at 168 HAI, a clearer percentage inhibition pattern was observed among the treatments. All the treated experimental units inhibited the growth of *P. personata* mycelia significantly compared to the control. The best treatments were *T. virens* isolate BGMZ2, *T. harzianum* isolate BGMZ4, *T. hamatum* isolate ZXPB and *T. harzianum* isolate ZXMZ followed by *T. harzianum* isolate BGMPP, *T. harzianum* isolate BGMZ3, *T. viride* isolate AIBK, *T. harzianum* isolate AIBN, and finally *T. harzianum* isolate ZXGV.

Discussion

In this study, all the plant extracts successfully inhibited the radial growth of *P. personata*. Ndifon and Lum (2021) reported that the leaves of *E. globulus* and four other botanicals (at 50 and 100% concentrations) inhibited the growth of *Aspergillus niger* van Tiegh. on white yams significantly compared to the negative control, and the level of inhibition was higher at the 100% concentration than at the 50% concentration. Apet et al. (2015) conveyed that *Ceratocystis paradoxa* (Dade) C. Moreau was effectively controlled by all the botanicals applied as follows: *Allium sativum* L. (63.9% inhibition), followed by *Zingiber officinale* Roscoe (61.5% concentration), and *Azadirachta indica* A.Juss. (59.8% concentration). These findings are in agreement with those of the current study. Moreover, Agbenin et al. (2010) publicized that neem seed powder significantly reduced the performance of *Fusarium* Syn. & Hans. and *Meloidogyne* Goeldi spp. in both the screen house and the field. Thus, plant extracts have a wide spectrum of microbes that they can control simultaneously.

Table 1. The effects of plant extracts on the radial growth of *Phaeoisariopsis personata*

Treatments	Percentage Inhibition (%)					Mean of Colony Radius (cm)		
	24h	48h	72h	96h	120h	24 h	72 h	120 h
<i>Eucalyptus globulus</i> resinous gum 100% concentration	100.0	100.0	95.2	96.2	96.7	0.0a	0.1a	0.1a
<i>Anacardium officinale</i> resinous gum 100% concentration	85.2	50.0	46.0	28.2	32.2	0.1a	1.1c	2.0c
<i>Terminalia catappa</i> resinous gum 100% concentration	80.3	47.5	42.9	29.5	27.8	0.1a	1.2c	2.2c
<i>Eucalyptus globulus</i> resinous gum 50% concentration	100.0	77.5	79.4	83.3	85.6	0.0a	0.4ab	0.4b
<i>Anacardium officinale</i> resinous gum 50% concentration	45.9	12.5	7.9	29.5	23.3	0.4b	1.9d	2.3d
<i>Terminalia catappa</i> resinous gum 50% concentration	100.0	57.5	52.4	33.3	33.3	0.0a	1.0bc	2.0c
Control**	0.0	0.0	0.0	0.0	0.0	0.7c	2.1d	3.0e
Mean	85.2	57.5	54.0	50.0	49.8	0.2	1.1	1.7
SED	-	-	-	-	-	0.06	0.28	0.10
FLSD ($P \leq 0.05$)	-	-	-	-	-	0.13	0.62	0.22

Note: **Percentage inhibition by control is zero based on the equation. Treatment means followed by the same letter(s) in a column are statistically similar using DMRT ($P \leq 0.05$)

Table 2. The effects of *Trichoderma* isolates on the radial growth of *Phaeoisariopsis personata*

Treatments	Percentage inhibition (%) of the radial growth of the fungus								Ranking of the means of radial growth of fungus		
	24 h	48 h	72 h	96 h	120 h	144 h	168 h	Area (%)	72 h	120 h	168 h
<i>T. harzianum</i> isolate AIBN	23.8	52.5	56.3	59.5	51.1	50.5	46.7	60.0	0.5b	1.0a	2.1ab
<i>T. viride</i> isolate AIBK	100.0	70.0	62.5	58.2	54.4	49.5	51.6	43.3	0.0a	1.1a	2.0ab
<i>T. virens</i> isolate BGMZ2	90.5	62.5	65.6	58.2	52.2	46.8	41.8	43.3	0.1a	1.1a	1.2a
<i>T. harzianum</i> isolate BGMZ4	85.7	62.5	65.6	62.0	55.6	55.0	57.4	43.3	0.1a	1.0a	1.7a
<i>T. hamatum</i> isolate ZXPB	100.0	80.0	70.3	58.2	50.0	52.3	57.4	40.0	0.0a	1.1a	1.7a
<i>T. harzianum</i> isolate ZXMZ	90.5	62.5	62.5	58.2	54.4	54.1	56.6	36.7	0.1a	1.1a	1.8a
<i>T. harzianum</i> isolate ZXGV	90.5	62.5	59.4	51.9	50.0	45.9	42.6	50.0	0.1a	1.3a	2.3b
<i>T. harzianum</i> isolate BGMPP	81.0	57.5	60.9	54.4	50.0	45.9	50.8	46.7	0.1a	1.2a	2.0ab
<i>T. harzianum</i> isolate BGMZ3	87.3	60.8	60.9	54.9	51.5	48.6	50.0	44.4	0.1a	1.2a	1.8ab
Control **	-	-	-	-	-	-	-	-	0.7c	2.6b	4.1c
Mean	83.2	63.4	62.7	57.3	52.1	49.8	50.5	45.3	0.2	1.1	1.7
SED	-	-	-	-	-	-	-	-	0.1	0.2	0.2
FLSD ($P \leq 0.05$)	-	-	-	-	-	-	-	-	0.2	0.3	0.5

Note: **Percentage inhibition by control is zero based on the equation. Treatment means followed by the same letter(s) in a column are statistically similar using DMRT ($P \leq 0.05$)

In this present study, all the *Trichoderma* isolates could effectively inhibit the radial growth of *P. personata*. The performance of the biocontrol agents revealed that their performance was isolate-dependent, and the best isolates were *T. virens* and some *T. harzianum* isolates, while *T. viride* isolate was the second-best biocontrol agent. Ndifon (2022a) affirmed these results with the findings that biocontrol agents (*Trichoderma* and *Cladosporium* Link Ex Fries spp.) significantly inhibited the radial growth of *Globisporangium ultimum* by 10-90% inhibition.

Verma et al. (2007) explained that *Trichoderma* spp. are antagonistic fungal agents usable against several pests and as plant growth enhancers. *Trichoderma* species exhibit faster metabolic rates, produce antimicrobial metabolites, carry out mycoparasitism, spatial and nutrient competition, antibiosis (by enzymes and secondary metabolites), and induction of plant defense system. These biocontrol agents can serve various purposes. That brings to mind the precaution requiring that we study these organisms more before utilizing them in new environments.

Kumar et al. (2014) reported that *T. harzianum* triggered maximum growth inhibition of *Sclerotium rolfsii* (Curzi) C.C. Tu & Kimbr) Sacc., *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris* (Frank) Donk, and *Sclerotinia sclerotiorum* (Lib.) de Bary (at 96 HAI: to the tone of 57.0%, 58.4%, and 64.8% inhibition respectively). Apet et al. (2015) reported that *C. paradoxa* was effectively controlled by *T. viride* (77.0% inhibition), followed by *T. harzianum* (70.7% inhibition) and *T. hamatum* (69.4% inhibition). These results are very encouraging because of the high percentage inhibition of fungal growth, and many species of fungi have biocontrol potentials.

Basumatary et al. (2015) perceived that the highest inhibition of the growth of *S. rolfsii* was caused by *T. harzianum* (77.4%), followed by *T. viride* (76.5%). Lower rates of inhibition were obtained when *Aspergillus niger* (30.5% inhibition), *Penicillium* sp. (29.1% inhibition), and *Curvularia* sp. (13.6% inhibition) were applied. Additionally, Gajera and Vakharia (2012) showed that 12

isolates of *Trichoderma* (i.e., six of *T. harzianum*, five of *T. viride*, and one of *T. virens*) effectively reduced the incidence of collar rot disease of groundnut caused by *A. niger*. The *T. viride* showed the highest inhibition of *A. niger* (86.2%), followed by *T. harzianum* isolate 2J (80.4%) 6 days after inoculation. These results are fully in accord with the current findings presented herein.

Using a combination of different methods of pathogen control has been attempted, and the combination treatments were effective. For example, Srinivas et al. (1997) sprayed mancozeb+carbendazim at 50 Days After Sowing (DAS), followed by spraying of *Calotropis procera* (Aiton) W.T. Aiton leaf extract at 70 DAS and observed a highly significant reduction in the leaf spot disease.

Deyd and Dhutraj (2014) reported that applying Bavistin (i.e., BAU-bio-fungicide) followed by applying neem leaf extract + K_2O significantly reduced the severity of leaf spot disease. Hasan et al. (2014) expounded that neem leaf (*A. indica*), debdaru leaf (*Polyalthia longifolia* (Sonn.) Thwaites.), *datura* leaf (*Datura metel* L.), and BAU-bio-fungicide (Bavistin) foliar spray significantly controlled leaf spot disease compared to the control in the field.

This study scrutinized the antimicrobial effect of botanicals and biocontrol agents against the tikka leaf spot disease of groundnut. Two experiments were set up to determine if the plant extracts or biocontrol agents can suppress the growth of *P. personata* on groundnut. It can be concluded from the present study that all the plant extracts from *E. globulus* resinous gum, *A. officinale* resinous gum, and *T. catappa* resinous gum effectively inhibited the growth of *P. personata* in vitro. Furthermore, the *Trichoderma* species trial (i.e., as a biocontrol agent) revealed all *Trichoderma* isolates: *T. virens* isolate BGMZ2, *T. harzianum* isolate BGMZ4, *T. hamatum* isolate ZXPB, *T. harzianum* isolate ZXMZ, *T. harzianum* isolate BGMPP, *T. harzianum* isolate BGMZ3, *T. viride* isolate AIBK, *T. harzianum* isolate AIBN, and *T. harzianum* isolate ZXGV, they have effectively controlled the tikka leaf spot pathogen.

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Value-chain analysis of the ginger sub-sector in Salyan District, Nepal

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Abstract. Acharya N, Chaurasia J, Kandel P. 2023. Value-chain analysis of the ginger sub-sector in Salyan District, Nepal. *Asian J Agric* 7: 76-82. A Value Chain (VC) strategy of market-driven firms is required to solve the present shortcomings in ginger's marketing channel and the ensuing value addition throughout the channel. The study examines ginger's producer share, pricing spread, marketing margin, and value-chain diagram. Furthermore, 50 producer families from each of Malneta of Sharada Municipality and Chande of Siddhakumakh Rural Municipality in Salyan District, Karnali Province, Nepal were selected for the household survey using purposive random sample based on the degree of production and producers' cultivation of ginger in more than one ropani, i.e., total 100 households, five collectors, five wholesalers, and five retailers were surveyed for analysis. The total ginger productivity at the research location was 15.43 Mt/ha. *Sutho* (dried ginger) earned an average of 25.81% of the earnings. Furthermore, 100% of producers said they were satisfied with the low price but higher than the farms' gate price by NPR 24. There was an NPR disparity of NPR 8 in retail marketing costs between retailers and end users, and NRs gap of NPR6 in marketing costs between wholesaler and retailer. While the difference in marketing costs between the collector and the distributor was, NPR 6. Retailers and consumers had the largest net margin (20%), which collectors and wholesalers followed. Consumer pricing was (8.3%), wholesalers and retailers (6.66%) and the producer share was calculated to be just 40%.

Keywords: Disparity, net margin, producer share, *sutho*, value chain

INTRODUCTION

Nepalese people depend heavily on agriculture, which generates 23.95% of the country's GDP (Gross Domestic Product). In Nepal, 400,000 farmers are employed in the ginger sector (CBS 2021). Regarding output, Nepal ranks fourth internationally, providing 9.2% of the total ginger in 2019 (CBS 2021). Ginger is planted on 325 hectares in the Salyan District, with a 3,217 Mt production and a 9.90 Mt/ha productivity (MoALD 2021) (Figure 1). India and China are the top two producers, accounting for 35.2% and 18.3% of the total, respectively (MoALD 2021). The Nepal Trade Integration Strategy-2017 designated ginger as one of the 12 priority export items due to its strength brought on by ideal geoclimatic conditions, excellent quality, and continuous rising demand in the worldwide market. The Nepal Trade Integration Strategy seeks to raise the export price for Nepalese ginger from 217 US\$/MT to 815 US\$/MT by 2020 through value addition within the nation (MOF 2016). The share of ginger, which has the HS code 91010, in overall exports was only 0.6%, and the RCA index for ginger in Nepal's NTIS 2010 export potential product list was 146.4 (Salike and Lu 2015). In the fiscal year 2020-2021, Nepal exported 6,065,622 kg of ginger for a cost of NPR 448,364,000 (TEPC 2021). According to MoALD (2021), Nepal exported neither crushed nor grounded fresh ginger of 7,656,835 kg worth of NPR

234,502,000 (1,776,800\$), *Sutho* (dried ginger) of 808,861 kg at NPR 123,519,000 (935,892\$) and other ginger crushed or grounded 381,235 kg at NPR 77,757,000 (589,158\$) NPR in the fiscal year 2019/2020. Ginger is typically exported from Nepal in huge quantities in its fresh and *sutho*, or traditionally dried forms. Recently, ginger with an organic certification has been transported to European markets in very small quantities (Acharya 2014).

Kaplinsky (2000) and Li et al. (2021) state that a value chain is a set of tasks and services necessary to move goods from manufacturing to consumption or final use. The actors in the value chain are the suppliers of the input, the producers, the processors, the traders (wholesalers, retailers), and the consumers (Kaplinsky 2000). According to Kaplinsky and Morris (2001), Value-Chain Analysis (VCA) is the full spectrum of tasks necessary to take a good or service from conception through the various stages of production (involving a combination of physical transformation and the input of different producer services), delivery to final consumers, and final disposal after use. Analysis of the institutional support for production at different phases is added to VCA in addition to the movement of a product from one stage to another and identification of the players, firms, and their services (Kaplinsky and Morris 2001). This occurs through process, product, functional, and chain upgrades.

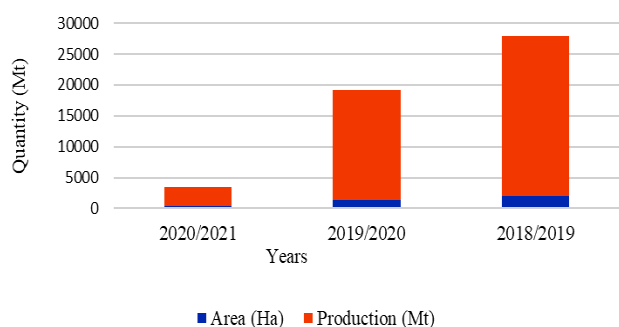


Figure 1. Area and production of ginger in three consecutive years in the Salyan District, Nepal

A group of vertically connected economic agents is called a "value chain" when each agent serves as both a supplier and a client of an upstream and downstream agency that are both members of the chain. These agents directly contribute to producing, processing, and delivering the goods through the various phases that increase the value of the nation's resources (Bellu 2013). The entire process from production to consumption, including harvesting, grading, packaging, storing, price fixing, selling, and buying, is called agricultural marketing (MDD 1999). The marketing margin, often called the retail farm gate margin, is the sum of the prices paid to farmers for their produce and the retail price of the commodity (Colman and Young 1989). Many studies have examined the socioeconomic analysis of the ginger value chain and the efficiency of ginger production or agricultural practices. Nonetheless, it isn't easy to calculate quantitative information on economic prospects in many sections of its value chain. Ginger's producers, processors, and marketers have gotten little attention regarding how they may boost profitability. This study aims to fill this gap by estimating actors' net margins in the channel, examining the economic relationships operating along the ginger value chain, and sketching a process value-chain diagram.

MATERIALS AND METHODS

Selection of study area

Based on their level of production potential, Malneta in the Sharada Municipality (28.38° N, 82.18° E) and Chande in the Siddhakumakh Rural Municipality (28.26° N, 82.11° E), Salyan District, Nepal were specifically chosen as research study locations (PMAMP 2018). The ginger growers who cultivated over or equal to 1 ropani (509 m²) were selected in a sampling frame.

Sources of information

During the research study, both primary and secondary information was gathered. All value chain participants, including input suppliers, producers, traders, and service providers, as well as important informants from related industries and household surveys, served as the main sources of information. Additionally, secondary data on ginger production, marketing, and the value chain approach

was gathered from various articles, reports, journals, books, and online resources, typically from the DFNCCI, zone office under the PMAMP, district ginger trader's association, etc.

Sample and sampling techniques

Therefore, to prepare the inventory of farmers in the study areas, farmers groups and cooperatives, PMAMP, and the Agriculture Knowledge Center (AKC) were consulted. From Sharada Municipality and Siddhakumakh Rural Municipality, a random sample of 100 producers (50 from each of two local levels), five collectors, five wholesalers, and five retailers were selected.

Data analysis

Various statistical programs, including MS Excel and SPSS, entered and analyzed the collected data and information.

Price spread

Price spread, often known as retailer price, is the distinction between the farm gate price farmers receive and the price consumers pay (Gardner 1975).

$$\text{Price Spread} = \text{Retailers } (P_r) - \text{Farm gate price } (P_f)$$

Producer's share

The price paid to producers is called their "producers' share," a percentage (%) of the retail price or the price consumers pay. The following formulas can be used to compute it (Mankiw 2020).

$$P_s = (P_f/p_r) * 100$$

Where,

P_s = Producers share

P_r = retailer's price

P_f = Producers price (farm gate price)

Indexing

The relative importance of the various production and marketing issues was examined using a 5-point scale of extremely important, highly important, moderately low, relatively low, and low problems: 5, 4, 3, 2, 1. The index of importance was computed using the following formula

$$I_{\text{imp}} = \sum (S_i * f_i / N)$$

Where,

I_{imp} = index of importance

\sum = Summation

S_i = scale value

F_i = Frequency of importance given by the respondents

N = Total number of respondents

Value-chain analysis

The price paid to the interested stakeholders (Producers, wholesalers, retailers, and consumers) and the production cost were calculated, as well as the margin due to each participant in the value chain. The producer's share and marketing margin spread were also computed for each distinct chain in the study area. Price spread was also examined at each stage of the value chain.

RESULTS AND DISCUSSION

Ginger production and productivity

The study site's overall ginger productivity has resulted at 15.43 Mt/ha (Table 1). This was discovered not aligned with the report of (MoALD 2021) because the survey was carried out in areas with high concentrations of ginger production (Malneta and Chande); the productivity of ginger in Salyan in 2021 was 9.90 Mt/ha while this study at 15.43 Mt/ha. Moreover, at a 1% significance level, the Productivity at Malneta (16.12) outperformed the Chande (14.75). The average household production of ginger was discovered to be 2,113.46 kg.

Price satisfaction level of respondents

Ginger producers were classified into low, medium, and high prices based on local collectors' prices. Table 2 shows that all responders (100%) fell into the low-price category. However, they didn't like the fee that was charged to them. Another impediment to manufacturers' comfort with low prices was a lack of market knowledge (Khanal 2018).

Quantity of ginger sold (fresh mature rhizome, *sutho*, and seed)

Three types of harvested ginger were offered for sale at the study site. They were seed, mature, and dried rhizomes (*sutho*). *Sutho* was more expensive per kilogram, followed by seed and freshly matured rhizome. Therefore, *sutho* was created to add value because it would generate more revenue than mature rhizomes (Acharya 2014). Moreover, 11,178.22 kg of fresh mature ginger was sold. It was discovered that Malneta (11,354.32) sold more fresh mature rhizomes than Chande (11,002.12). Malneta (772.20) sold more *sutho* than Chande (652.89), and the difference was significant at the 5% level. These results are consistent with those of (Acharya et al. 2019). This resulted from Malneta's high standards for and volume of ginger production (GRP 2017). Similar to this, Malneta (957.91) sold more seed rhizomes than Chande (114.867), which was significant at the 1% level (Table 3). Malneta is referred to as a "seed hub" since the highest-quality seed rhizomes are grown there (PMAMP 2018).

Revenue generated

The average gross profit from the sale of fresh ginger, dried ginger (*sutho*), and seed rhizome was discovered to be NPR 277,615.72. The income of farmers at Malneta (NPR 320,000.00) was 1% greater than that of farmers at Chande (NPR 235,231.45) (Table 4). These results concur with (Upadhyaya et al. 2020).

Revenue from *sutho* per hectare and contribution of *sutho* (in%) in total revenue

Sutho, a dried form of ginger, was created as a value addition to ginger because its price is much higher than that of fresh ginger and seeds. The revenue of farmers at Malneta (101,752.23=771\$) was substantially more from selling *sutho* than farmers at Chande (68,012.34=461\$). *Sutho* made an average contribution of 25.81% to the total revenue. Compared at Chande (23.76%), Malneta had a

higher percentage value (Table 5). The results of this investigation are consistent with those of (Upadhyaya et al. 2020).

Marketing of ginger, means of transport, packaging, and way of grading

Out of all ginger growers surveyed, 64% of respondents sold their produce directly to local collectors, 12% sold to collection centers within or outside the district, and 24% sold to cooperative-owned collection centers. Porters were the primary mode of transportation, followed by pickups and tractors, due to the research site's mostly rocky topography. At the 1% significance level, 78.4% of respondents used porters to move their products, compared to 12.2% and 9.54%, who used tractors and pickup trucks, respectively. Moreover, 83% of respondents used *doko* for ginger for material transportation, and 17% used jute bags. Similarly, all responders (100%) manually graded their ginger (Table 6). The results of this observation are consistent with those of (Neupane et al. 2019).

Value-chain of fresh ginger

The average cost to produce one kilogram of fresh ginger was NPR 11.51 (Figure 2), resonating with the findings of (Acharya et al. 2019). When compared to findings from Gurung et al. (2021), where the cost of production was 35.67 NPR per kilogram, the cost of production of the study site was low. Organic ginger was used instead of chemical pesticides, which was credited with the inexpensive cost. To local dealers, producers sold their produce for NPR 16 per kilogram. Local vendors charged wholesalers (inside and outside the district) NPR 24 per kg for their produce while incurring NPR 22 per kilogram in transportation, NPR 2.5 per kg in storage, NPR 1.5 per kg in standardization (drying, cleaning, and packing), and NPR 40 per kg in end-user prices. A similar value-addition cost was found in the findings of (Gurung et al. 2021). The average price paid by consumers (March/April) was found to be NPR40 per kg in Srinagar bazaar. The price was reasonable compared to the off-season, where end consumers have to pay NRs 60-80 in the local market (PMAMP 2018). Retailer and end consumers had a retail marketing cost difference of NPR 8, wholesaler and retailer had a retail marketing cost difference of NPR 6, and collector and wholesaler had a retail marketing cost difference of NPR 6, resulting in a net margin difference of NPR 2, NPR 2, and NPR 8 between the actors mentioned above. The highest net margin was found between retailers and consumers (20%), followed by wholesalers and retailers (6.66%) and collectors and wholesalers (8.3%). The findings of (Gurung et al. 2021) can be used to confirm similar proportional outcomes. However, this study's producer share was somewhat lower than that of (Gurung et al. 2021), which showed a share of 62.71%. Similarly, a producer share of 66.5% was determined by (Maharatha et al. 2019) in the study of price behavior and marketing of Tomatoes in the same district. This might be due to a good, controlled marketing route.

Thus, the price spread was = retailers' price – farm gate price.

$$= 40 - 16$$

$$= \text{NPR } 24$$

Similarly, producers share = $(16/40) * 100$
= 40%

Marketing chain

Farmers, local traders, wholesalers, retailers, and consumers comprised most of the study site's value chain actors (Figure 3). All the processes required to get farmers' fresh or processed products to customers nationally and worldwide are referred to as ginger marketing (ANSAB 2011).

Market functions

The main role in the value chain for ginger is the input function (supply), which helps farmers produce ginger.

Rhizome seed, fertilizer, bio-pesticides, and other inputs that are accessible locally, as well as through agro-vets, GOs, and NGOs, can be used. Following production, local traders, cooperative collection centers, within and outside the district performed the collection function. The gathered rhizome was subsequently sold to wholesalers (regional) to retailers and eventually to end consumers in Nepal. Produce was sold to customers by retailers in Salyan, Surkhet, Nepalgunj, and other nearby communities' Local traders were also engaged in selling the produce directly to exporters. The role commission agent in ginger value-chain is inevitable. Commission agents collect fresh ginger from exporters of Nepal and sell to wholesalers in India. The channeling is done through Rupadiya (Nepal-India border) (Adhikari 2019). Little amount of gingers are processed by processors of Surkhet and shipped to nations like UAE, Netherlands. The report (ANSAB 2011; Gurung et al. 2021) also revealed similar findings.

Table 1. Ginger production and productivity at the research location

Variable	Overall (N=100)	Sharada Municipality, Malneta (N=50)	Siddhakumakh Rural Municipality, Chande (N=50)	Mean difference	t value	p-value
Productivity of ginger (Mt/ha)	15.43	16.12	14.75	1.37***	3.75	0.000
Production of ginger(kg)	2,113.46	2,221.35	2,005.57	215.78	0.54	0.511

Note: *** represents a 1% level of significance

Table 2. Price satisfaction level of the ginger producer at the research location

Variable	Sharada Municipality, Malneta (N=50)	Siddhakumakh Rural Municipality, Chande (N=50)	Overall (N=100)	Chi-square	p-value
Price satisfaction range					
Low	50(100)	50(100)	100(100)		

Table 3. Quantity of ginger sold (fresh mature rhizome, *sutho* and seed)

Variable	Overall (N=100)	Sharada Municipality, Malneta (N=50)	Siddhakumakh Rural Municipality, Chande (N=50)	Mean difference	t- value	p-value
Quantity of fresh mature ginger sold(kg/ha)	11,178.22	11,354.32	11,002.12	352.2	-0.52	0.601
Quantity of <i>sutho</i> sold(kg/ha)	772.20	901.51	652.89	248.62**	2.01	0.041
Quantity of seed(rhizome) sold(kg/ha)	537.40	957.91	114.87	843.02***	2.24	0.001

Table 4. Revenue generated from ginger

Variable	Overall (N=100)	Sharada Municipality, Malneta (N=50)	Siddhakumakh Rural Municipality, Chande (N=50)	Mean difference	t-value	p-value
Revenue from selling ginger/ha	277,615.72	320,000.00	235,231.45	84,768.55	6.34***	0.001

Note: *** represents a 1% level of significance

Table 5. Revenue from *sutho* per hectare and contribution of *sutho* (in%) in total revenue

Variable	Sharada Municipality Malneta (N=50)	Siddhakumakh Rural Municipality Chande (N=50)	Overall (N=100)	Mean difference	t-value	p-value
Revenue from <i>sutho</i> /ha	101,752.23	68,012.34	84,882.28	33,739.89**	2.05	0.041
Contribution (in%) of <i>sutho</i> in total revenue	27.87	23.76	25.81	4.11	0.87	0.360

Note: ** represents a 5% level of significance

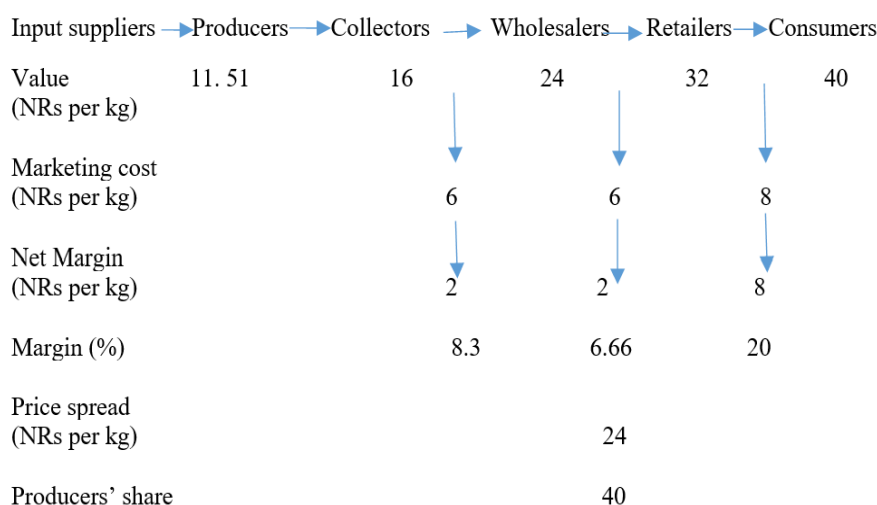
Table 6. Marketing of ginger, means of transport, packaging, and way of grading

Variable	Sharada Municipality, Malneta (N=50)	Siddhakumakh Rural Municipality, Chande (N=50)	Overall (N=100)	Chi-square	P-value
Where (ginger selling)					
Directly selling to traders	33(66)	31(62)	64(64)	0.67	0.42
Collection center within /outside districts	7(14)	5(10)	12(12)		
Cooperative's own- collection center	10(20)	14(28)	24(24)		
Means of transport					
Porters	22(44)	35(70)	58(78.4)	28.31***	0.000
Horse/Mule					
Pickup	9(18)	5(10)	14(9.5)		
Tractor	18(36)	10(20)	28(12.2)		
Transporting materials					
Doko	43(86)	40(80)	83(83)		
Jute bags	7(14)	10(20)	17(17)		
Way of grading					
Manually through hands	50(100)	50(100)	100(100)		
Through machines					

Note: *** represents a 1% level of significance. Figure in parenthesis indicates percentage

Table 7. Actors affecting the price of ginger in the research location

Factors	1	0.8	0.6	0.4	0.2	Rank
Local collectors	61	10	3	0	0	I
Wholesalers	0	27	43	3	1	III
Cooperatives	0	0	20	45	9	IV
Farmers	8	36	7	22	1	II
Exporters	5	0	0	7	62	V

**Figure 2.** Marketing margin and producers' share of ginger in the research location

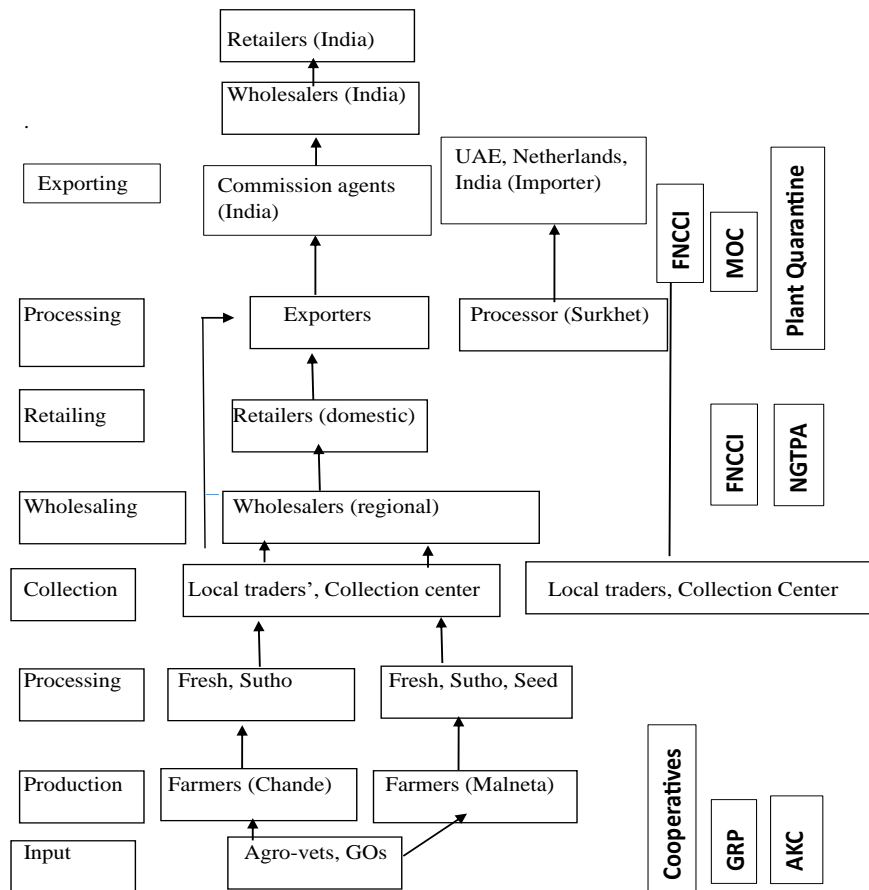


Figure 3. Value chain map of ginger in Malneta and Chande of Salyan District, Nepal (ANSAB 2011; Acharya 2014)

Actors

Producers

The producers of ginger are farmers who grow it for financial gain (income). They grow their farm by purchasing the appropriate materials from input providers.

Local traders/collectors

Local traders and collectors gather ginger for commercial purposes. Farmers immediately trade their produce for a set price with neighborhood collectors (Bellu 2013).

Wholesalers and retailers

The ginger was delivered to retailers or wholesalers from the collecting points. Many ginger products were sent to wholesalers of Salyan, Surket, and Nepalgunj by local traders and eventually India through commission agents to the wholesalers (India). This is aligned with the findings of (ANSAB 2011). Retailers are the players who eventually sell ginger to customers, whereas wholesalers are the ones who supply the stuff to them. Due to their involvement in numerous transactions, wholesalers provide retailers with poor profit margins per unit weight of the products. On the other hand, retailers transact little with a large profit margin for each product unit weight. These results are consistent with those from Chen et al. (2013).

Exporters

Local or international businesses that properly adhere to the quarantine can export ginger to markets outside Nepal.

Processors and end consumers

Moreover, less ginger gets processed in the research location due to insufficient processing factories and infrastructures (PMAMP 2018).

Enablers

Organizations, institutions, and other bodies that create an atmosphere beneficial to the value chain are enablers. Government agencies include the National Spices Development Program (NSDP), National Ginger Research Program (NGRP), Ministry of Agriculture and Livestock Development (MOALD), Department of Agriculture (DOA), Agriculture Knowledge Center (AKC), National Spices Development Program (NSDP), National Ginger Research Program (NGRP), Ministry of Industry, Plant Quarantine, Commerce, and Supplies (MOICS), etc.; the findings resonating with the value chain of orthodox tea of Illam enablers (Adhikari et al. 2017). The enablers mentioned above work for the value chain of ginger in the study site, and these results are consistent with those of (ANSAB 2011; Acharya 2014).

A project titled "Enhancing Sanity and Phyto-sanitary Capacity in Nepalese Ginger Exports through Public-Private Partnerships" is being launched by MOICS, MOALD, FAO, AEC, and FNCCI. Furthermore, the Nepal Ginger Production and Traders Association (NGPTA), a local partner, is helping to begin this project in Nepal.

Actors affecting the price of ginger

Several different parties influence the price of ginger (Table 7). Based on the farmers' perceptions, 5-point scale of extremely important, highly important, moderately low, relatively low, and low problems; 5, 4, 3, 2, 1 was used to rank the variables that influence the price of ginger. Local collectors were found to be the most significant and determining element, followed by farmers, wholesalers, cooperatives, and exporters, according to the value from the rank scale. This result is consistent with those of Khanal (2018).

In conclusion, ginger has huge potential in itself, and the Salyan district is a significant center for the production of ginger. Moreover, problems in price and marketing behavior problems dominate the ginger value chain in this district. Producers are unhappy with the price they are given, either. Due to a lack of industry and processors, dried ginger (*sutho*) commercial activity and revenue are deemed poor on the site. Increased *sutho* sales can lead to exponential growth in ginger's gross revenue. The value-chain analysis identifies five actors: producers, collectors, distributors, retailers, and consumers. Ineffective and lengthy marketing channels raise the cost of value chain operations, ultimately impacting consumers by increasing the marketing margin between producers and end consumers. Due to a bigger marketing margin that subsequently develops between the producer and end-consumer, the producer's share in income bargaining is minimal. Enablers that assist actors, including in exporting, must be aware of the prevailing problems and work effectively to boost the ginger sector's income.

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Allelopathic effect of *Lantana camara* aqueous leaf extract on the seedling germination of *Zea mays* (maize)

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Abstract. Mistica DJP, Magsino NJS, Tanjusay ZC, Paclibar GCB. 2023. Allelopathic effect of *Lantana camara* aqueous leaf extract on the seedling germination of *Zea mays* (maize). *Asian J Agric* 7: 83-87. *Lantana camara* L. is an invasive weed rampant in the Philippines and threatens agricultural crops. This study was conducted to evaluate the growth inhibitory effects of *L. camara* aqueous leaf extract in seedling germination of *Zea mays* L. Different concentration levels (25%, 50%, 75%, and 100%) of the *L. camara* leaf extracts as compared to the control were administered to the hypochlorite-treated seeds for two weeks. Results showed a significant difference among the treatments in the number of germinated seeds and main shoot and primary root elongation using ANOVA. Furthermore, the t-test revealed that the presence and absence of the aqueous leaf extract in seed germination are significantly different, showing significant inhibitory effects in the number of germinated seeds and the length of the shoot and root when the aqueous leaf extract was present. Moreover, in vitro assay also indicated that the concentration of the aqueous leaf extract is directly proportional to the growth inhibitory effects on seed germination and root and shoot elongation. Therefore, it is recommended to test the allelopathic effect of *L. camara* aqueous leaf extract in other agricultural crops and its effect on the soil and crop nutrition.

Keywords: Growth inhibitory effect, invasive weed species, *Lantana* bioassay, maize, root and shoot elongation

INTRODUCTION

One of the major threats to biodiversity is the invasive alien species. *Lantana camara* L. is one of the most widespread invasive weed species (Taylor et al. 2012; Enyew and Raja 2015) which is rampant in the Philippines and were already established in disturbed areas (Paclibar and Tadosa 2019; Paclibar and Tadosa 2020) and many agricultural areas. The *L. camara* is considered both a pest abs and an ornamental plant (Mishra 2014; Tadele 2014), while some consider it to help in bioremediation and for medication due to its medical properties (Arbiastutie et al. 2017; Ved et al. 2018). For example, lantana could be used to treat gastric and duodenal ulcers in rats (Sathish et al. 2011), as an agent for larvicidal activity (Chavan and Nikam 1982), as an antibacterial agent against Gram-negative and Gram-positive bacteria (Barreto et al. 2010).

Studies about the allelopathic effect of *L. camara* were recorded in agricultural crops (Sahid and Sugau 1993; Ahmed et al. 2007; Choyal and Sharma 2011; Gantayet et al. 2014; Kar et al. 2014; Mishra 2014; Tadele 2014; Enyew and Raja 2015; Rusdy and Ako 2017; Anwar et al. 2018; Mawal and Patil 2019; Gautam et al. 2021), bryophytes (Mishra 2014), forest crops (Hossain and Alam 2010) and communities (Gentle and Duggin 1997) and even to other weeds (Saxena 2000; Mishra 2014). In line with this, stimulatory effects of *L. camara* were also recorded in some agricultural crops (Tadele 2014; Enyew and Raja 2015; Ming et al. 2020).

Zea mays L. or corn, is one of the Philippines' most important food sources and agricultural crops. *Zea mays* is recognized as the second principal agricultural crop next to rice and play an important role as one of the major sources of livelihood for Filipino farmers (Gerpacio et al. 2004). Several studies have been conducted showing the significant inhibition of seed germination and seedling growth of *Z. mays* due to allelopathic effects of plants such as *Castanea henryi* (Skan) Rehder & E.H.Wilson (Ming et al. 2020), *Sonchus arvensis* L. (Bashir et al. 2018), *Ambrosia artemisiifolia* L. (Bonea et al. 2017), *Gliricidia sepium* (Jacq.) Kunth and *Acacia auriculiformis* A.Cunn. ex Benth. (Oyun 2006) among others. Furthermore, the allelopathic effect of *L. camara* in maize was studied by Tadele (2014), Enyew and Raja (2015), Mawal and Patil (2019), and Gautam et al. (2021). However, few accounts were available for full concentration, and few allelopathic effects study was conducted in the Philippines.

Weed infestations are recognized as a serious biological constraint in many different crop plantation types, either lowland or highland. The manual weeding is reduced because of high labor costs has been accompanied worldwide by the intensive use of synthetic herbicides (Tadele 2014). Since weed infestations are recognized as a serious biological constraint in crop plantations, further studies about the allelopathic effect on agricultural crops must be conducted. Therefore, this study was conducted to evaluate the allelopathic effect of *L. camara* in the seedling germination and root and shoot elongation of *Z. mays*; to determine the significant differences of the effect of

aqueous leaf extracts concentrations of *L. camara* to the seedling germination, and root and shoot elongation of *Z. mays*; identify the concentration with the most inhibitory activity; and determine the effect of the presence and absence of aqueous leaf extracts to the *Z. mays*. Farmers, governing bodies, and agriculture and weed management organizations can use the findings as a baseline.

MATERIALS AND METHODS

Extract preparation

Mature leaves were collected in Biluso, Silang, Cavite (14.2369° N, 121.0378° E), which had already proliferated in the wide farmland. After the authentication from a qualified botanist, the collected leaves were air-dried for 15 minutes to let the ethanol be off after disinfection. The leaves were then processed using the preparation performed by Tadele (2014) through immersion of 100 grams of leaves of *L. camara* into 1,000 mL (1 L) containers filled with 500 mL (0.5 L) distilled water, which were then sealed in an Erlenmeyer flask, and later shaken and stored at a temperature of 21°C for at least 24 hours. After storing, the aqueous extracts were filtered using filter paper and diluted into 25, 50, 75, and 100% concentrations. The extracts were stored in Erlenmeyer flasks sealed with parafilm and kept in the fridge (5°C).

Germination and growth records

The seeds of maize were selected through fractionation and their viability. Afterward, the maize seeds were washed with distilled water and surface sterilized with sodium hypochlorite aqueous solution (Muzzo et al. 2018) for at least 5 to 10 minutes to prevent contamination and were rinsed thoroughly with distilled water multiple times; while the controls were treated only with distilled water. Next, 15 seeds of maize were evenly placed in 9 cm-diameter petri dishes lined by Whatman No. 1 filter paper (Akpan et al. 2017) with three replicates per treatment. The seeds were then treated with 5 ml of 25%, 50%, 75%, and 100% aqueous extract concentrations and 5 ml of distilled water for the control, which were applied daily with one dosage applied from 8:00 to 9:00 AM and a second dosage at around 3:00 to 4:00 PM to keep the moisture within the filter paper; this allowed seedling development for 14 days (2 weeks) period. Finally, the setup was placed inside the laboratory, wherein the same amount of natural light was treated on all the treatments. The prerequisite for considering a germinated seed is the appearance of the radicle. Therefore, germinated seeds were counted and checked daily while the root and shoot lengths were measured after the experiment (Tadele 2014).

Data analysis

The Germination Capacity (GC) and the root and shoot Relative Elongation Ratio (RER) were calculated using the formula (Bewley and Black 1994; Saxena et al. 1996):

$$GC (\%) = \frac{(\text{No. of germinated seeds})}{(\text{Total No. of seeds sown})} \times 100$$

$$\text{RER of shoot} = \frac{(\text{Mean shoot length of tested plant})}{(\text{Mean shoot length of control})} \times 100$$

$$\text{RER of root} = \frac{(\text{Mean root length of tested plant})}{(\text{Mean root length of control})} \times 100$$

The significant differences among the treatments were evaluated using Analysis of Variance (ANOVA I) and further assessed using the post hoc Tukey's HSD (Honestly Significant Difference) test. The significant difference between the presence and absence of the aqueous leaf extracts of lantana was calculated using a t-test.

RESULTS AND DISCUSSION

Seed germination of *Zea mays*

The Germination Capacity (GC) of the *Z. mays* seeds was found to be highest at the control (56.67%), followed by 25% (53.33%), 100% (36.67%), and lowest at 50% and 75% concentrations with both GC of 26.67% (Figure 1). The number of seeds grown per treatment was also significantly different ($P < 0.05$), wherein the control has the highest number of seeds grown ($\bar{x} = 5.67$) and lowest at 50% ($\bar{x} = 2.67$) and 75% ($\bar{x} = 2.67$) concentrations (Table 1). It is noteworthy that a 25% concentration of lantana leaf extract has no significant difference from the control, then 50%, 75%, and 100% concentrations had no significant differences indicating that high concentrations of lantana aqueous leaf extracts negatively affect the seed germination, and GC of *Z. mays*. The results of the study were aligned with the study of Sahid and Sugau (1993), Ahmed et al. (2007), Hossain and Alam (2010), Kar et al. (2014), Mishra (2014), Rusdy and Ako (2017), Anwar et al. (2018), and Mawal and Patil (2019) stating that *L. camara* leaf extracts had inhibitory effects in the germination of several agricultural crops, forest crops, and other weeds. In line with this, a 75% concentration of *L. camara* leaf extracts had the most inhibitory effect on the germination of *Z. mays*.

Moreover, the highest concentration of the extract did not greatly inhibit the GC capacity. That aligns with the study of Sahid and Sugau (1993), wherein the full strength of *L. camara* extract did not significantly inhibit the germination of spinach and cucumber seeds. Also, Maharjan et al. (2007) revealed that the highest concentration of *Parthenium hysterophorus* L. had an inhibitory effect on the tested agricultural seeds except on *Z. mays*. Dzafic et al. (2013) discussed that maize has autotoxic effects and can decrease root biomass. Even though the active compounds of both the study plants are not within the scope of the study, it could be considered that the water-soluble compounds interacted with the leaf extract; thus, instead of inhibiting the growth of maize, it has a higher GC than at a lower concentration. As Dorning and Cipollini (2006) explained, allelopathy is most effective in inhibiting plant growth when the plant has resistance to autotoxic effects. It could be observed as well that the GC of all the treatments is low, which can be hypothesized that this is due to the thermal stress, which has been indicated by the study of Farooq et al. (2008), which revealed the optimum temperature for maize germination is between 25-28°C. In

addition, Farooq et al. (2008) also added that poor germination could occur under optimal temperatures.

Root and shoot elongation of *Z. mays*

The root and shoot elongation of *Z. mays* were significantly different among the treatments ($P < 0.05$) (Table 2). The longest length of the root was observed in the control ($\bar{x} = 28.0$), while the shortest length of the root was observed in 75% concentration ($\bar{x} = 12.11$). The root's Relative Elongation Ratio (RER) reveals that *Z. mays* roots exposed to different aqueous concentrations of *L. camara* had relatively lower RER than the control. Furthermore, the 75% concentration was the lowest (43.25%), followed by 100% (47.82%) and 50% (59.35%), while the 25% concentration was the highest (81.75%) (Figure 2). Based on the findings, the control was significantly different among the treatments ($P < 0.05$) except in 25% concentration in terms of root elongation, wherein the higher the concentration, the inhibitory effect of *L. camara* aqueous leaf extracts was also higher, making the root shorter in length.

The longest length of the shoot was observed in the control ($\bar{x} = 37.45$), and the shortest was observed in the 75% concentration ($\bar{x} = 14.83$) (Table 2). The result shows that the shoot of *Z. mays* that were exposed to several concentrations had lower RER compared to the control (Figure 3), in which the 50% (65.13%) had the highest RER followed by 25% (60.16%), 100% (47.69%) and 75% (36.60%) had the lowest RER. That indicates *L. camara* concentrations inhibit the elongation of shoots of *Z. mays*. Based on the results, the control had significant differences among the treatments used, indicating that aqueous leaf extracts of *L. camara* can inhibit shoot elongation.

The *L. camara* leaf extracts significantly negatively affected the elongation of the root and shoot of *Z. mays*, wherein the higher concentrations had higher inhibitory effects. The proportionality of the concentration and the inhibitory effects were in line with the study of Ahmed et al. (2007), Hossain and Alam (2010), and Kar et al. (2014). It is also worth noting that the control differed from the other treatments except for 25% concentration in root elongation. Also, 75% concentration had the lowest length for both root and shoot, with RER of 43.25% and 39.6%, respectively. The inhibitory effects of *L. camara* leaf extracts on the root and shoot of other agricultural and forest crops were observed in several studies (Ahmed et al. 2007; Hossain and Alam 2010; Kar et al. 2014; Enyew and Raja 2015; Rusdy and Ako 2017; Anwar et al. 2018; Mawal and Patil 2019).

Presence of *L. camara* leaf extract

The *Z. mays* germination and root and shoot elongation were significantly different between the presence and absence of *L. camara* leaf extracts indicating that *L. camara* leaf extracts had inhibitory effects on the growth of *Z. mays*, as revealed in Table 3. Furthermore, the absence of *L. camara* leaf extracts had a higher mean (\bar{x}) as compared to the presence of *L. camara* leaf extracts. This result coincides with the study of Gentle and Duggin (1997), wherein the removal of *L. camara* significantly increased

the germination of *Alectryon subcinereus* (A.Gray) Radlk. and *Cryptocarya rigida* Meisn. in three Australian forest communities. Therefore, adding leaf extract of *L. camara* was found to have a maximum inhibitory effect compared to the stem and root extracts (Choyal and Sharma 2011).

Table 1. Germination of *Z. mays* seeds in response to *L. camara* aqueous leaf extracts at different concentrations after 2 weeks.

<i>Lantana camara</i> leaf extract concentrations (%)	Mean of germinated seeds of <i>Zea mays</i>
0	5.67 ^a
25	5.33 ^a
50	2.67 ^b
75	2.67 ^{bc}
100	3.67 ^{bcd}

Note: Different letters imply significant differences among the treatments ($P < 0.05$)

Table 2. Root and shoot elongation of *Z. mays* (mean) in response to *L. camara* aqueous leaf extracts in different concentrations after 2 weeks.

<i>Lantana camara</i> leaf extract concentrations (%)	Root elongation of <i>Z. mays</i> (\bar{x})	Shoot elongation of <i>Z. mays</i> (\bar{x})
0	28.00 ^a	37.45 ^a
25	22.89 ^{ab}	22.53 ^b
50	16.62 ^{bcd}	24.39 ^{bc}
75	12.11 ^c	14.83 ^{bcd}
100	13.39 ^{cd}	17.86 ^{bcd}

Note: Different letters imply significant differences among the treatments ($P < 0.05$) per variable

Table 3. The presence and absence of *L. camara* leaf extracts in response to the growth of *Z. mays* after two weeks

<i>Zea mays</i> growth	Presence of <i>L. camara</i> aqueous leaf extract (\bar{x})	Absence of <i>L. camara</i> aqueous leaf extract (\bar{x})
Germination	3.58 ^X	5.67 ^Y
Root elongation	16.25 ^X	28.00 ^Y
Shoot elongation	19.90 ^X	37.45 ^Y

Note: Different letters signify significant differences between rows

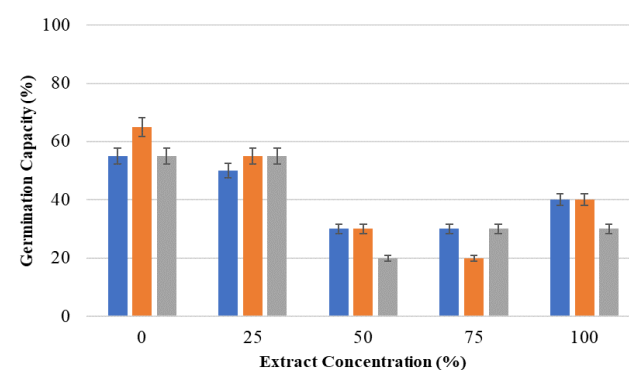


Figure 1. Germination Capacity (GC) of *Z. mays* in response to *L. camara* aqueous leaf extracts at different concentrations. Bars indicate GC with Standard Error (SE)

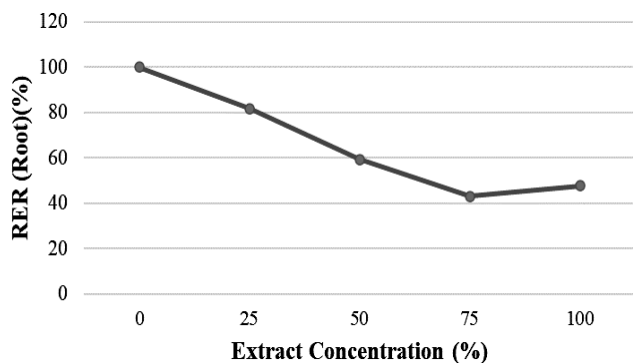


Figure 2. Relative elongation ratio of *Z. mays* roots in response to different extract concentrations of *L. camara* after 2 weeks

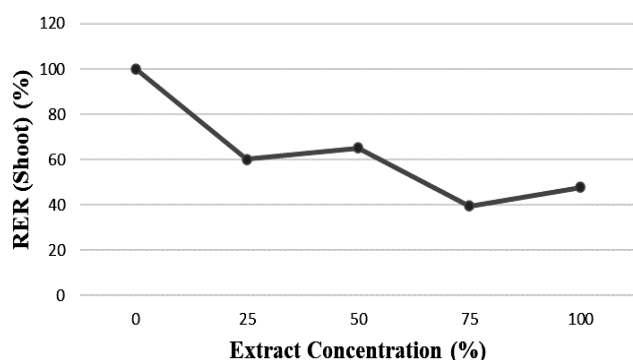


Figure 3. Relative elongation ratio of *Z. mays* shoots in response to different extract concentrations of *L. camara* after 2 weeks

In conclusion, the study concluded that the different concentrations of *L. camara* aqueous leaf extracts had inhibitory effects on the germination and root and shoot elongation of *Z. mays*, wherein the higher the concentration is, the higher the inhibitory effect. Since the thermal stress and autotoxicity affected the lantana's germination capacity, their association with the different extract concentrations should be further studied. Furthermore, though bioassay is deemed important in assessing the allelopathic potential of *L. camara*, however, field investigations should be done to relate and compare the in vitro study to the field results. Also, the effects of *L. camara* aqueous leaf extract on other agricultural crops in the Philippines should be investigated.

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Integrated nutrient management enhances the growth efficiency and productivity of *Tagetes erecta* cv. Sirakole

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Abstract. Mohanty A, Acharya S, Swain BB, Mishra S, Mohapatra D, Mohapatra PK. 2023. Integrated nutrient management enhances the growth efficiency and productivity of *Tagetes erecta* cv. Sirakole. *Asian J Agric* 7: 88-97. The African marigold (*Tagetes erecta* L. cv. Sirakole) is a highly significant plant because it can be cultivated year-round, produce large blooms, have a long post-harvest life, and compactness. To determine the optimal fertilizer and manure combination, we conducted a study to explore the plant's morphological, yield, and physiological characteristics under different combinations of fertilizers, Vermicompost (VC), Poultry Manure (PM), and biofertilizer (*Azospirillum* + phosphate solubilizing bacteria). The most successful combination for promoting vigorous growth, dense canopy, prolific branching, longer flowering duration, large-sized flowers, and higher yield was achieved with 50% Recommended Dose of Nitrogen and Phosphorus (RDNP) + VC equivalent to 25% Recommended Dose of Nitrogen (RDN) + PM equivalent to 25% RDN + biofertilizers (@400 gha⁻¹). Additionally, plants in this combination exhibited higher chlorophyll content (Chl) and photosynthesis (P_N) than those in plots with RDNP alone or other amendments. While photosynthetic fluorescence yield (φP₀) and energy dissipation (φD₀) did not vary, plots with RDNP had lower excitation (Ψ₀) and electron movement (φE₀), as well as lower photosynthesis performance indices (PIs), compared to the nutrient combinations. Additionally, the organically amended plots showed higher microbial density and soil enzyme activities than those with RDNP alone. Our findings indicate that using a combination of fertilizer, manure, and biofertilizers is the most effective way to improve the photosynthetic capacity and flower yield of *T. erecta* cv. Sirakole.

Keywords: Nutrient management, OJIP fluorescence rise, photosynthesis, productivity, *Tagetes erecta*

INTRODUCTION

In recent decades, commercial horticulture has emerged as a promising avenue for supplementing the income and livelihood of small and marginal farmers in India. Commercial flower cultivation, in particular, has developed due to its short crop duration, consistent income for an extended period, and higher returns than other non-conventional crops. This has led to a continuous increase in the area under floriculture and loose and cut flower production for export in India. One of India's most commercially exploited flower crops is the African marigold (*Tagetes erecta* L., Family: Asteraceae), with several varieties such as giant double yellow, giant double orange, early yellow, early orange, and Sirakole being grown. Sirakole is particularly significant due to its all-season cultivation, compact flower clusters, long post-harvest life, and large attractive blooms. The crop can be propagated through seeds and cuttings and grows well in all soil types. For the winter crop, seeds are sown from October to November, and the seedlings are transplanted when they reach a height of 5-7 cm or through transplanting of cuttings in the first week of November. For the summer crop, cuttings are transplanted in early February. In addition to being used as loose flowers, African marigolds are a source of xanthophylls and a major

pigment source for the poultry industry, food color, and dyeing of fabrics (Attokaran 2017).

Commercial marigold cultivation in India is more organized in Tamil Nadu, Karnataka, and West Bengal (Kaur et al. 2022). The quality of marigold flowers is strongly influenced by the nutrient levels in the soil, particularly nitrogen and phosphorus (Acharya and Dashora 2004). However, the selection and management of soil can be poor, and overuse of synthetic fertilizers and chemicals leads to environmental pollution and potential harm to soil microflora and fauna, affecting the overall benefit-cost ratio. Therefore, there is a need for combination farming, which involves using biological, organic, and balanced nutrient amendments for integrated soil fertility management and sustained commercial and profitable flower production.

Marigold is a high-demand crop for nitrogen and potassium, especially during its early growth stages. Farmers often use high nutrient dosages to improve growth and yield and separately apply inorganic fertilizers, organic manures, and biofertilizers. Previous studies have evaluated the effects of these nutrient regimes on marigold performance using growth and yield parameters (Borah et al. 2020; Choudhury et al. 2020; Reddy and Saravanan 2020). Foliar application of organic fertilizers has increased nutrient concentrations in marigolds. Furthermore,

integrated nutrient management, which combines inorganic nutrients, vermicompost, poultry manure, and biofertilizers, has also been tried on other varieties of *T. erecta* but not on Sirakole (Kaur et al. 2022). However, there is no report on intensive integrated nutrient management involving inorganic nutrients (nitrogen, phosphorus, and potassium), Vermicompost (VC), Poultry Manure (PM), and biofertilizers to evaluate the plant performance and the consistency in yield of *T. erecta* cv. Sirakole. The *T. erecta* cv. Sirakole is India's most preferred marigold variety for all-season cultivation. In addition, OJIP fluorescence and photosynthetic parameters have not yet been explored for evaluating the physiological efficiency and yield of horticultural crops under organic practice. On the other hand, in various crop species, OJIP fluorescence has been proven to be a valuable parameter for evaluating plant performance. Plant efficiency, particularly photosystem (PS) II behavior, is more accurately evaluated by prompt chlorophyll fluorescence (Strasser et al. 1995). The OJIP test and direct measurement of photosynthesis and other physiological processes give detailed information on the plants' physiological efficiency under different nutrient regimes (Chhotaray et al. 2014; Shasmita et al. 2022). This study reports the cultivation performance, growth, and physiological responses of *T. erecta* cv. Sirakole under different nutrient management and the results of this study provide recommendations for an ideal integrated nutrient combination for effective augmentation of flower quality and yield.

MATERIALS AND METHODS

Study area and treatment plot design

The experiment spanned a year and consisted of three crop phases – Kharif (July-October), Rabi (November-

February), and Summer (March-June) plantations, which were initiated in the first week of July, November, and March, respectively. The experimental plots were chosen from Krishi Vigyan Kendra, Jajpur of Odisha University of Agriculture and Technology, Bhubaneswar, India. These plots had been under nursery use since 2007 and followed conventional cultivation practices. Different plots from the same field were chosen for each crop phase to ensure statistical rigor, with each plot treated as a replicate.

Before the commencement of the experiments, the plots were tilled, divided into 2.1 m x 1.8 m subplots having a 0.6 m gap between two adjacent plots. The subplots were allotted randomly, in triplicates, for each nutrient treatment. Well-decomposed farm yard manure (FYM @9.5 kgplot⁻¹ corresponding to 25 tha⁻¹) was uniformly spread on the plots, lightly irrigation, and soils were mixed thoroughly. The soil (0-15 cm on top) was analyzed for N, P, and K contents before amendments were 251.8, 25.13, and 282.4 kgha⁻¹, respectively.

Six different treatment combinations of fertilizers and manures were made as per the proportions in Table 1. A common dose of K₂O (100 kgha⁻¹) was added to all the experimental plots except T1 three weeks before transplantation. This was followed by adding organic manures (VC and PM) in plots as per the proportions given in Table 1. Half the nitrogen, the full dose of phosphorus, and the remaining potash were added to plots at the time of transplantation. Biofertilizer preparation contains *Azospirillum* and PSB in a 1:1 ratio (400 gha⁻¹) in T5 and T6 at the time of transplantation. The other chemical fertilizer amendments were made per the proportion and schedule in Tables 1 and 2, respectively.

Table 1. The scheme of fertilizer and manure amendments of the experimental plots

Combination	Nutrient amendment
T1	No external nutrient addition
T2	The recommended dose of fertilizers [450 kg urea (209.79 kg nitrogen), 200 kg single super phosphate (13.97 kg phosphorus), and 200 kg mureate of potash (101.23 kg potassium)] per hectare
T3	VC equivalent to 50% of RDN + PM equivalent to 50% of RDN
T4	50% RDNP + VC equivalent to 25% of RDN + PM equivalent to 25% of RDN
T5	VC equivalent to 50% of RDN + PM equivalent to 50% of RDN + Biofertilizers (<i>Azospirillum</i> and PSB in equal proportion) @ 400 gha ⁻¹
T6	50% RDNP + VC equivalent to 25% of RDN + PM equivalent to 25% of RDN + Biofertilizers as in T5

Note: PM: Poultry Manure, RDN: Recommended Dose of Nitrogen, RDNP: Recommended Dose of Nitrogen and Phosphorus, VC: Vermicompost

Table 2. The scheme of amendments of manures and fertilizers in the plots during 120 days of the experiment

Schedule	Amendment
21 days before transplantation	FYM (25 tha ⁻¹) in all plots + mureate of potash (100 kgha ⁻¹) (except T1) + VC and PM
At the time of transplantation	Urea (225 gha ⁻¹) + mureate of potash (100 kgha ⁻¹) + single super phosphate (200 kgha ⁻¹)
30 days after transplantation	Urea (225 kgha ⁻¹)

Note: N, P, and K were applied as urea, single super phosphate, and mureate of potash, respectively. The amendments were made in proportion mentioned in Table 1

Each plot was prepared by transplanting 20 seedlings (21 days old; raised from stem cutting) during the evening hours of 4.00 PM to 6.00 PM. The newly transplanted seedlings were irrigated immediately and subsequently watered regularly. Thirty Days After Transplantation (DAT), the first earthing-up operation was performed, and the remaining half dose of nitrogen was added. Therefore, pinching of the apical meristem of primary branches was initiated 30 days after transplantation to stimulate secondary branching. Subsequent pinching operations were carried out weekly until the first flower bud was observed. Furthermore, earthing-up operations were performed every 15 days until 90 DAT to ensure optimal plant growth and development.

Experimental procedure

The growth parameters were recorded during 30-120 days of observation at 30 days intervals. Plant height (cm), stem girth (cm) at 5 cm above the soil level, the number of primary and secondary branches and leaves per plant, canopy diameter (cm), and canopy cover (%) were recorded as the morphological parameters. The yield parameters viz., duration of 50% flower bud initiation (days), first flowering (days) and flowering period of the crop (days), fresh and dry weight of flowers (g), flower diameter (cm), number of flowers per plant, flower yield (kg ha^{-1}), petal meal yield (g kg^{-1} flower) and xanthophyll yield (g kg^{-1} flower) were recorded during the cropping period following the methods described by Mohanty (2014). The Xanthophyll content of the flower was estimated by extraction with a combination extract (hexane: acetone: methanol: toluene: 10:7:6:7).

The physiological efficiency of the crop was measured between 8.00 AM-10 AM at 90 DAT from the fourth pair of fully opened leaves from the apex. The chlorophyll content of these leaves (rel units) was estimated by a SPAD meter (SPAD-502, Spectrum Technology Inc., USA). Photosynthesis (P_N), transpiration (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were measured in the field condition with the help of an Infrared Gas Analyzer (IRGA; CIRAS-2, PP-Systems) under ambient temperature and CO_2 concentration following the procedure described by Chhotaray et al. (2014). The range of leaf surface temperature, relative humidity, and ambient irradiance at the time of measurement during the year was 24.3-36.8°C, 50.4-74.5%, and 785-1140 $\mu\text{E m}^{-2}\text{s}^{-1}$, respectively. For measuring respiration, the leaves were darkly adopted for 10 min in the cuvette (auto PLC 6, PP Systems), after which respiratory CO_2 evolution was measured in the dark using IRGA.

The OJIP fluorescence rise was measured using a plant efficiency analyzer (Handy PEA, Hansatech Instruments, UK) following the procedure described by Chhotaray et al. (2014). The fluorescence rise of O, J, I, and P peaks were taken after 20 μs ($F_{20\mu\text{s}}$), 2 ms ($F_{2\text{ms}}$), 20 ms ($F_{20\text{ms}}$), and at F_M (F_M), respectively. The fluorescence parameters, viz., 2 ms relative variable fluorescence (V_j), the net rate of PS II closure (M_0), the quantum yield of primary photochemistry (ϕP_0), rate of trapped exciton movement

beyond Q_A (Ψ_0), the quantum yield of electron transport (ϕE_0), the quantum yield of energy dissipation (ϕD_0), the performance index of primary photochemistry (PI_{ϕ}), performance index of exciton movement beyond Q_A (PI_{Ψ}), performance index on absorption basis (PI_{ABS}) and performance index of reduction of end electron acceptor of PS I (PI_{TOTAL}) were calculated using the fluorescence equations of Stirbet and Govindjee (2011).

The plant samples for uptake of N, P, and K were made by collecting the sample at 120 DAT. The whole plant was uprooted, washed thoroughly, and dried in a hot air oven for 48 h at 70°C. The dried plant parts were ground to a fine powder in a 'Willey Mill.' This fine powder was again dried in an oven at 60°C for 24 h before estimating total N, P, and K. Total nitrogen was measured by digesting the samples with concentrated H_2SO_4 and digestion mixture in Microkjeldhal assembly. For phosphorus and potassium determination, powdered plant samples were digested with a diacid mixture ($\text{HNO}_3 + \text{HClO}_4$ in a 3:2 ratio) after predigesting with concentrated nitric acid.

Moreover, a soil sample from the top 15 cm of each experimental plot was collected using a sterile steel corer, aseptically sealed in a polyethylene bag for the enzyme and microbial analysis. The samples were homogenized aseptically in the laboratory and were used for analysis. The microbial populations were estimated by the dilution plate method. The inoculated plates were incubated for 24 h at 37°C for bacteria and at 25°C for 72 h in the case of fungi, after which the colony-forming unit (CFU g^{-1} dry soil) was counted. Nutrient agar and potato dextrose agar media were used to grow bacteria and fungi. The activities of amylase, invertase, and cellulase were measured spectrophotometrically (Systronic, India) by taking starch, sucrose, and carboxymethyl cellulose as substrates, respectively. The absorbance values were quantified into enzyme activity as glucose produced ($\text{mg g}^{-1}\text{soil h}^{-1}$) using the regression equation of a glucose standard. The activities of protease and dehydrogenase were measured using sodium caseinate and trichloro-Triphenyl Tetrazolium Chloride (TTC) as substrates, and tyrosine and triphenyl formazan as standards, respectively, as described by Chhotaray et al. (2014). Phosphatase was extracted and quantified spectrofluorimetrically (excitation at 323 nm and emission at 452 nm) with the help of a Varian spectrofluorometer (Cary-eclipse, Varian) (Chhotaray et al. 2014).

Data analysis

Samples were collected from 5 plots of each amendment. Samples of in vivo observations were collected from 10 plot plants and averaged. Further, the data of five season plots were pooled together to serve as a replicate. The experiments were conducted in three crops grown in Kharif, Rabi, and Summer seasons in an equal number of plots and were repeated for two years. The values presented as tables and figures are thus pooled by six replicates (three seasons x two years). That means each parameter was separated using Duncan's Multiple Range Test (DMRT) tests at $P=0.05$.

RESULTS AND DISCUSSION

Growth and yield attributes

The morphological attributes of *T. erecta*, grown in different experiment plots, were measured for 120 d at 30 d intervals. No significant difference in plant height, stem girth, and number of branches was reported among T2 through T6 after 30 d of transplantation, though in T1, these attributes were significantly low. After 60 d of transplantation, however, all the mentioned morphological parameters were the highest in T6 and the lowest in T1. Minimum variation among the treatments was observed in the number of leaves per plant, whereas maximum variation was observed concerning canopy cover on all observation days (Table 3). Canopy diameter and cover density showed the most remarkable variation among the treatments indicating that the amendments appreciably encouraged the growth of plants. A comparison of T2 with T5 and T6 showed that T5 and T6 (biofertilizer amended) had better growth performance during 60 days than T2 (with RDNP). Consistently T6 showed the highest performance of all selected morphological attributes, followed by T5, T4, T2>T3>T1. Significant variation among the treatments was observed in the prolongation of the crop to > 90 d (Table 3). The morphological parameters

followed the pattern T6>T5>T4>T2>T3>T1. T5 and T6 showed significantly high values of the morphological attributes during 120 days of observations compared to T2.

The yield characteristics of the crop were measured after 120 d of transplantation. No significant variation from T1 through T5 was observed concerning the initiation of flowering. In contrast, in T6, the duration for initiation of flowering (50% bud initiation and first flowering) was slightly prolonged compared to other treatments. However, significant variations among treatments were observed concerning flowering duration (d), flower diameter (cm), fresh wt and dry wt of flowers (g), number of flowers per plant, flower yield (kg ha⁻¹), petal yield (g kg⁻¹ flower) and xanthophylls yield (g kg⁻¹ flower) (Table 4). All these parameters were found to be the highest in T6, whereas the lowest was recorded in T1, as expected. The flowering duration continuously increased from T1 through T6 and could be almost doubled in T6 compared to T1. Consequently, T5 and T6 had significantly bigger flowers, more flowers per plant, and higher yields than T1 and other nutrient combinations. However, the petal meal yield (g kg⁻¹ flower) did not vary significantly among treatments, whereas the xanthophyll yield (g kg⁻¹ flower) of T1 was significantly lower than any of the nutrient combinations.

Table 3. The morphological attributes of *T. erecta* grown in different experimental plots at mentioned Days After Transplantation (DAT) during 120 d of cropping

DAT	Parameters	T1	T2	T3	T4	T5	T6
30	Plant height (cm)	12.83 ^b	27.30 ^a	28.36 ^a	28.11 ^a	26.91 ^a	27.53 ^a
	Stem girth (cm)	0.63 ^b	1.09 ^a	1.08 ^a	0.96 ^a	0.99 ^a	0.98 ^a
	Primary branches (no. plant ⁻¹)	3.46 ^c	8.42 ^a	8.36 ^a	6.76 ^b	7.83 ^a	8.03 ^a
	Secondary branches (no. plant ⁻¹)	10.43 ^b	17.33 ^a	17.73 ^a	16.06 ^a	16.93 ^a	17.82 ^a
	Leaves (no. plant ⁻¹)	27 ^a	71 ^a	70 ^a	70 ^a	69 ^a	73 ^a
	Canopy diameter (cm)	8.67 ^c	23.15 ^a	21.72 ^a	18.85 ^b	22.24 ^a	22.63 ^a
	Canopy cover (%)	3.12 ^c	22.28 ^a	19.61 ^a	14.77 ^b	20.56 ^a	21.29 ^a
60	Plant height (cm)	25.56 ^d	43.06 ^c	43.9 ^c	45.54 ^b	44.83 ^b	48.84 ^a
	Stem girth (cm)	0.86 ^c	1.05 ^b	1.26 ^a	1.25 ^a	1.28 ^a	1.32 ^a
	Primary branches (no. plant ⁻¹)	5.96 ^c	10.66 ^b	13.76 ^a	12.91 ^a	12.23 ^a	12.76 ^a
	Secondary branches (no. plant ⁻¹)	16.36 ^c	31.43 ^b	35.16 ^a	35.32 ^a	34.43 ^a	33.96 ^a
	Leaves (no. plant ⁻¹)	106 ^b	161 ^a	159 ^a	164 ^a	163 ^a	167 ^a
	Canopy diameter (cm)	12.84 ^d	29.37 ^{bc}	28.51 ^c	30.69 ^{ab}	29.48 ^{bc}	31.8 ^a
	Canopy cover (%)	6.85 ^d	26.13 ^c	31.28 ^b	39.16 ^{ab}	36.13 ^b	42.04 ^a
90	Plant height (cm)	34.36 ^e	48.36 ^c	44.13 ^d	51.43 ^b	53.66 ^b	57.6 ^a
	Stem girth (cm)	0.99 ^e	1.29 ^c	1.13 ^d	1.31 ^c	1.51 ^b	1.72 ^a
	Primary branches (no. plant ⁻¹)	8.9 ^e	14.27 ^c	13.03 ^d	15.01 ^c	16.93 ^b	19.21 ^a
	Secondary branches (no. plant ⁻¹)	24.03 ^e	39.22 ^c	36.06 ^d	41.26 ^c	45.13 ^b	50.16 ^a
	Leaves (no. plant ⁻¹)	180 ^e	215 ^c	204 ^d	219 ^c	237 ^b	259 ^a
	Canopy diameter (cm)	16.55 ^f	33.94 ^{de}	32.47 ^e	34.58 ^{cd}	38.18 ^b	43.17 ^a
	Canopy cover (%)	11.39 ^f	42.41 ^d	29.13 ^e	49.71 ^c	60.60 ^b	77.48 ^a
120	Plant height (cm)	38.83 ^e	53.73 ^c	49.4 ^d	54.57 ^c	59.03 ^b	63.47 ^a
	Stem girth (cm)	1.05 ^e	1.32 ^{cd}	1.21 ^d	1.35 ^c	1.59 ^b	1.83 ^a
	Primary branches (no. plant ⁻¹)	10.42 ^e	15.87 ^{cd}	14.73 ^d	16.75 ^c	18.77 ^b	21.37 ^a
	Secondary branches (no. plant ⁻¹)	25.87 ^e	41.3 ^c	37.43 ^d	42.74 ^c	47.79 ^b	52.77 ^a
	Leaves (no. plant ⁻¹)	203 ^e	240 ^c	226 ^d	246 ^c	265 ^b	281 ^a
	Canopy diameter (cm)	19.17 ^e	36.75 ^c	33.5 ^d	36.52 ^c	40.82 ^b	45.45 ^a
	Canopy cover (%)	15.28 ^f	47.35 ^d	33.77 ^e	55.45 ^c	69.27 ^b	85.88 ^a

Note: The means indicated by the same letter(s) in the superscript in a row are not significantly different from each other at P = 0.05 and n = 36. DMRT made the mean comparison

Photosynthesis and transpiration

A significant change in the chlorophyll content of *T. erecta* leaves, measured at 90 DAT, was observed with various treatments. The leaf chlorophyll content (in SPAD units) of T5 and T6 differed significantly from other amended and unamended plots (Table 5). Correspondingly, there was a great variation in P_N among treatments, which increased continuously from T1 through T6. Nevertheless, the plants in plots only with organic amendment (T3) had lower photosynthetic performance than in T2. However, E and g_s were not significantly influenced by the nutrient or organic amendments though there was a marginal increase in water loss from T1 through T6. The value of C_i was lower in T3 through T6 than in T1 and T2, though a corresponding pattern of change in g_s was not observed.

Consequently, the E/ P_N showed a significant decreasing trend from T1 through T6. On the other hand, P_N/Chl

showed an increasing trend, which was significant when compared to T1 but insignificant among T2 through T4 and between T5 and T6. Because of a continuous improvement of P_N , the WUE showed an increasing trend. In addition, T6 showed the highest WUE, 63% and 20% higher than T1 and T2, respectively. Similarly, the respiration rate of all amended plots was high, with T5 and T6 showing significantly higher respiration than other treatments.

The crop's biomass's total nutrient uptake ($kg\ ha^{-1}$) during cropping time varied significantly among treatments (Table 6). Higher plant growth and improved crop yield resulted in a significantly high nutrient removal rate in the plots amended with manures or biofertilizers. The removal of N, P, and K was the highest in T6 and the lowest in T1. The nutrient accumulation by the plant biomass followed the pattern $T6 > T5 > T4 = T3 > T2 > T1$.

Table 4. The yield characteristics of *T. erecta* grown in different experimental plots during 120 d of cultivation

Yield parameters	T1	T2	T3	T4	T5	T6
50% flower bud initiation (d)	50.77 ^b	49.63 ^b	50.53 ^b	48.93 ^b	51.47 ^{ab}	53.13 ^a
First flowering (d)	60.97 ^{ab}	59.14 ^b	59.73 ^b	58.92 ^b	60.27 ^{ab}	63.31 ^a
Flowering duration (d)	36.63 ^e	57.35 ^c	47.97 ^d	57.38 ^c	63.93 ^b	69.71 ^a
Flower diameter (cm)	3.52 ^e	4.77 ^c	4.46 ^d	4.85 ^c	5.32 ^b	5.76 ^a
Flower fresh weight (g)	4.13 ^e	5.43 ^c	5.12 ^d	5.52 ^c	5.95 ^b	6.37 ^a
Flower dry weight (g)	0.202 ^e	0.282 ^c	0.258 ^d	0.282 ^c	0.332 ^b	0.361 ^a
Flowers per plant (no.)	49.16 ^e	67.17 ^c	64.44 ^d	68.27 ^c	72.73 ^b	77.63 ^a
Flower yield ($kg\ ha^{-1}$)	9901 ^e	16001 ^c	14501 ^d	16400 ^e	17301 ^b	18201 ^a
Petal meal ($g\ kg^{-1}$ flower)	78.97 ^b	79.51 ^{ab}	78.63 ^b	80.15 ^a	79.34 ^{ab}	80.67 ^a
Xanthophyll yield ($g\ kg^{-1}$ flower)	1.873 ^b	2.188 ^a	2.126 ^a	2.217 ^a	2.211 ^a	2.266 ^a

Note: Same as for Table 3

Table 5. The photosynthetic and physiological performance of *T. erecta* grown in different experimental plots was measured at 90 DAT

Parameter	T1	T2	T3	T4	T5	T6
Chl	31.35 ^d	40.57 ^b	38.21 ^c	41.11 ^b	44.77 ^a	46.26 ^a
P_N	9.09 ^d	12.35 ^b	11.19 ^c	12.26 ^b	14.52 ^a	15.47 ^a
E	6.74 ^a	6.78 ^a	6.79 ^a	6.82 ^a	6.95 ^a	7.06 ^a
g_s	569 ^a	576 ^a	572 ^a	581 ^a	588 ^a	584 ^a
C_i	369 ^a	327 ^b	357 ^a	319 ^b	316 ^b	323 ^b
E/ P_N	741 ^a	543 ^c	607 ^b	556 ^c	485 ^d	458 ^d
P_N/Chl	0.290 ^c	0.304 ^b	0.293 ^{bc}	0.298 ^{bc}	0.320 ^a	0.334 ^a
WUE	0.899 ^e	1.214 ^c	1.099 ^d	1.198 ^c	1.393 ^b	1.461 ^a
R	3.483 ^e	3.947 ^c	3.788 ^d	3.895 ^c	4.245 ^b	4.369 ^a

Note: Chl: Chlorophyll content (SPAD units); P_N : Net photosynthesis ($\mu\text{mol CO}_2\ \text{m}^{-2}\ \text{s}^{-1}$); E: Transpiration ($\text{mmol H}_2\text{O}\ \text{m}^{-2}\ \text{s}^{-1}$); g_s : Stomatal conductance ($\text{mmol H}_2\text{O}\ \text{m}^{-2}\ \text{s}^{-1}$); C_i : Intercellular CO_2 concentration ($\mu\text{mol m}^{-2}\ \text{s}^{-1}$); WUE: Water Use Efficiency ($\text{gCkg}^{-1}\ \text{H}_2\text{O}$); R: Respiration ($\mu\text{mol CO}_2\ \text{m}^{-2}\ \text{s}^{-1}$)

Table 6. The total nutrient uptake ($kg\ ha^{-1}$) by *T. erecta* grown in different experimental plots during the cropping period

Nutrient	T1	T2	T3	T4	T5	T6
Nitrogen	35.87 ^e	47.63 ^c	41.24 ^d	48.56 ^c	56.28 ^b	64.09 ^a
Phosphorus	8.25 ^e	12.59 ^c	10.34 ^d	12.74 ^c	14.98 ^b	17.56 ^a
Potassium	36.92 ^e	48.44 ^c	41.01 ^d	49.52 ^c	55.28 ^b	61.32 ^a

The pigment fluorescence

The OJIP fluorescence transient of *T. erecta*, measured at 90 DAT, showed significant variation among treatments. With the amendments from T1 through T6, the fluorescence showed a continuous and significant increase at the peak and intermediate inflections. Such an increase in any of the amendments was significantly higher than in T1. The variations were more in I and P levels of the transient, though only T1 showed a very low level of P fluorescence as compared to other amendments (Figure 1.A). Nevertheless, P rise increased with the amendments from T2 through T6, and the difference in F_M among treatment plots was significant except between T3 and T4. There was also a continuous increase of F_V from T1 through T6 but no appreciable variation of tF_M was noted (tF_M ranged between 240–260 ms). The highest P rise was observed in T6, followed by T5, and the variations were insignificant between the two treatments. The variable fluorescence spectra, calculated by normalizing the F_0 (to zero) and F_M (to one), showed a variation among treatments at J inflection. As expected, the highest J rise was seen in the T1 plot, followed by T2 (Figure 1.B). The difference among the fluorescence rise at J inflection in other treatment plots was insignificant. To measure the rate of change in the fluorescence at intermediate inflections and verify the hidden peaks in the fluorescence rise, the differential variable fluorescence spectra were determined by normalizing the variable fluorescence spectra with T6 (Figure 1.C). It was observed that T1 had significantly high fluorescence (12.84%) at J (2 ms). However, there was no indication of any fluorescence change at ≤ 2 ms, but in T1, a higher rise was seen at 5 ms.

Q_A reoxidation and the rate of electron movement beyond Q_A were determined by calculating ϕP_0 , ϕE_0 , and ψ_0 . ϕP_0 ranged between 0.712 to 0.768 in the selected six treatments, with a variation of only 8% among the treatments (Figure 2). However, there was significant variation in ϕE_0 and ψ_0 , which continuously and significantly increased from T1 through T6, showing a difference of 37% and 28%, respectively. Consequently, energy dissipation (ϕD_0) showed the reverse trend among the treatments from T1 through T6. There was nearly 24% higher dissipation in T1 when compared to that of T6. Similarly, the M_0 of T1 was significantly higher than any other treatment, and a continuous increase of M_0 from T1 to T6 was also recorded. The highest variation was observed concerning V_j , about 51% of the total absorbed energy in T1, and decreased to 38% in T6. The performance indicating bioenergetic parameters, viz., PI_ϕ , PI_ψ , PI_{ABS} , and PI_{TOTAL} , showed a similar pattern of increase with the amendments (Figure 3). While T1 showed the minimum values of these indices, the maximum values were reported in T6. The increase of the photosynthetic performance was continuous from T1 through T6, though the differences were not always significant. Nevertheless, there was a significant correlation between the performance indices and the organic amendments, alone or in combination with the fertilizers ($R^2 = 0.89, 0.92, 0.87,$ and 0.83 for $PI_\phi, PI_\psi, PI_{ABS},$ and PI_{TOTAL} , respectively).

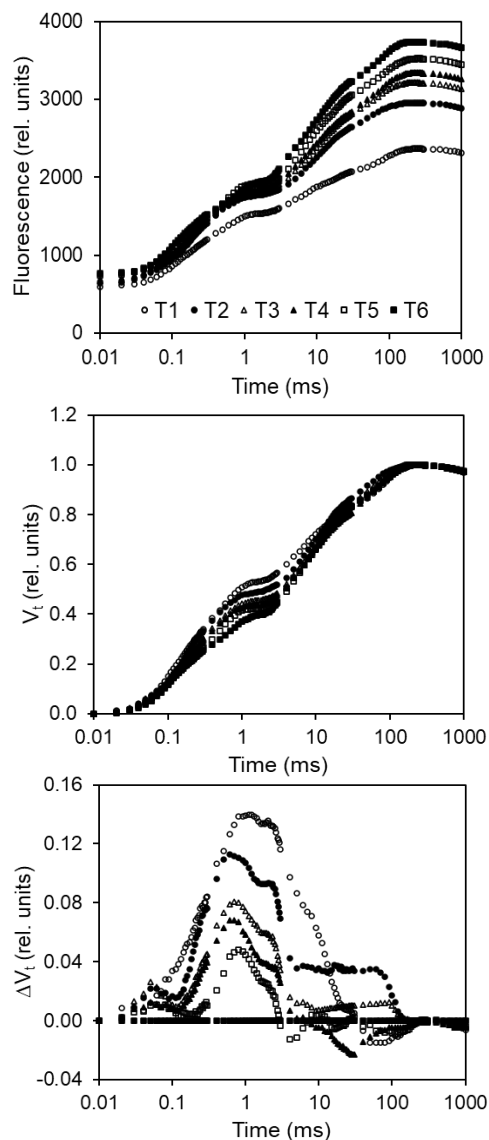


Figure 1. A. The OJIP, B. Variable (V_t), C. Relative variable (ΔV_t) fluorescence transients of *T. erecta* grown in plots with different amendments. The transients were measured at 90 DAT. Legends: ○- T1; ●- ϕ T2; △-T3; ▲-T4; □-T5; ■-T6

The soil enzyme activities

The microbiological analyses of soil were made to determine the dynamics of change in the soil properties of the plots at 90 DAT. The unamended and the fertilizer (RDNP) amended plots showed the minimum levels of bacterial and fungal population counts, even though T2 had a comparable (and, in some cases, superior) performance concerning the crop's growth and yield attributes. All organically amended plots (T3 through T6) showed significantly high microbial count and soil enzyme activities. Furthermore, T5 showed the highest population count of bacteria and fungi, significantly higher than any other nutrient and fertilizer combinations (Figure 4). Correspondingly these plots also showed the highest rate of soil enzyme activities, which was significantly higher than of T6 concerning carbohydrate enzymes and phosphatases, but insignificantly higher in the case of protease and dehydrogenases (Table 7).

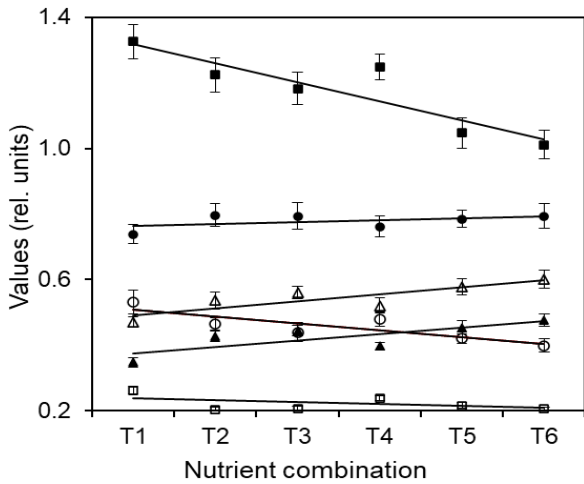


Figure 2. The derived parameters of OJIP fluorescence transients *T. erecta* were measured at 90 DAT. Legends: o- V_j ; Δ - Ψ_0 ; \square - ϕD_0 ; \bullet - ϕP_0 ; \blacktriangle - ϕE_0 ; \blacksquare - M_0

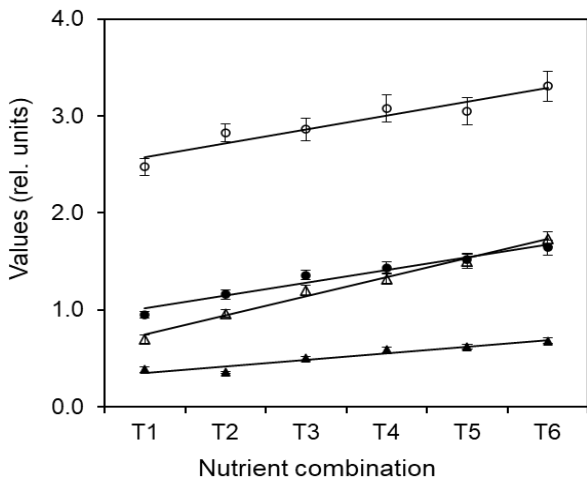


Figure 3. The performance indices (PI) of photosynthetic activity in *T. erecta* were measured at 90 DAT. The PIs were derived from the OJIP fluorescence transients. Legends: o- PI_{ϕ} ; \bullet - PI_{Ψ} ; Δ - PI_{ABS} ; \blacktriangle - PI_{TOTAL}

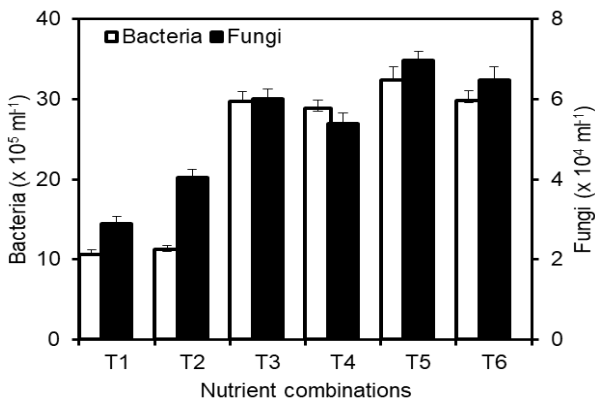


Figure 4. The density of bacteria and fungi in the top 15 cm soil of the experimental plots was measured after 90 days of transplantation

Discussion

Marigold is one of the most common loose flowers covering the flower market in every state of India and is in demand throughout the year. In this study, most of the morphological attributes of *T. erecta* did not significantly vary up to 60 DAT, but after that, remarkable differences among the treatments were observed. As expected, plants of unamended plots showed the slowest growth rate than those of other amended plots. However, plots with only organic amendments had lower growth attributes than plots with fertilizer, manure, or biofertilizer amendments. This indicated that only organic amendment failed to keep the plants photosynthetically active and supply nutrients required for plant growth, like RDNP and the combination treatments. Further, it was observed that a combination of fertilizers (NPK), manures (VC+PM), and biofertilizers (*Azospirillum* and PSB) significantly aided the growth of the plants, which was the highest among the treatments tried (Table 3).

The fertilizer, manure, and biofertilizer amendments highly influenced the yield characteristics. Irrespective of the amendment, the plants have a higher yield, bigger flowers, greater flower diameter, and more flower dry weight than the unamended field. When T2 (RDNP) and T4 (VC+PM) were compared, there was no significant difference between the two treatments concerning the yield parameters, which indicated that the organic amendments with the VC+PM corresponding to the recommended dose of nitrogen made in the present experiment, was enough to satisfy the nutrient requirement of the crop and to maintain the crop yield, even though there were lower values of morphological attributes than in T2. However, Alvarez (2021) observed that plots amended only with organic manures create a yield gap, as observed in the present case between T2 and T3. On the other hand, Mockeviciene et al. (2021) reported stabilization of the production potential of soil in long-term organic practice. Moreover, T5 (VC+PM+biofertilizers) and T6 (fertilizers and manures along with the biofertilizer application) have shown the best performance concerning the flower quality, quantity, pigment yield, and flowering duration. Thus, the biofertilizer amendment, in this case, caused better nutrient mobilization and supported plant growth, translating into a longer flowering period and better yield. Choudhury et al. (2020) have reported that a combination of RDF+VC and biofertilizer (*Azotobacter* + *Azospirillum* + PSB@ 4 kg ha^{-1}) enhanced plant growth and significantly improved flower yield of *T. erecta* cv. Pusa. It has been proposed that biofertilizer application with a healthy organic base in soil stimulated growth promoting substance in soil (IAA and Gibberellins), resulting in more vigorous plant growth, which is essential for higher crop yield (Dalawai and Naik 2014) and integration of organic manure with inorganic fertilizers builds a robust Soil Organic Carbon (SOC) base for consistent nutrient mobilization (Gao et al. 2022).

Similarly, by testing eight different biofertilizers separately and in combination, Reddy and Saravanan (2020) observed enhanced plant growth and qualitative and quantitative improvement of flowers. The authors have recommended the combination of *Pseudomonas*

fluorescence + Trichoderma viride as the best to improve yield, though; however, these do not have any role in nutrient mobilization. A combination of 75% recommended dose of NPK per hectare + 25% of RDN of VC and AM + *Azotobacter* has been reported to have increased flowering and flower diameter in *T. erecta* cv. Poornima yellow (Sivasankar et al. 2020).

It has already been reported that P_N is adversely affected by nutrient stress due to poor nutrient mobilization or nutrient deficiency in soil (Geider et al. 1998; Chhotaray et al. 2014; Shi et al. 2020). This study observed that Chl content and P_N were consistently higher in combination amendments than in separate amendments with RDNP or manure (VC+PM). The best rate of P_N and pigmentation was observed in T5 and T6, which were amended with biofertilizers. Further, these combinations also had higher P_N /Chl ratios indicating a high photosynthetic rate on a pigment basis compared to other amended plots. However, E and g_s remained unchanged as the plants were adequately irrigated and were not water stressed, but C_i decreased significantly in T5 and T6, which was thus of non-stomatal type and was due to efficient assimilation of CO_2 for photosynthesis with these treatments. This is supported by higher P_N and P_N /Chl in T5 and T6 compared to other treatments and has been reported by earlier workers (Mohapatra et al. 2010; Chhotaray et al. 2014; Shi et al. 2020).

Because the combination positively influenced metabolic efficiencies, the plants in plots with amendments supporting better growth also had higher rates of respiratory oxygen consumption. Elanchezhian and Panwar (1997) reported that *Azospirillum* treatment stimulated lateral roots' development and growth, improving the rhizosphere zone, nutrient utilization efficiency, and plant metabolic performance. This is supported by the present work's high nutrient utilization rate in biofertilizer-amended plots compared to corresponding plots without biofertilizer (T6 over T4; T5 over T3). It may be noted that N and P limitations cause a reduction in P_N , higher C_i , more NPQ, and low $\phi PS II$ (Shi et al. 2020). The limited conditions reduce photosynthesis-irradiance response due to the low rate of reductant supply (or electron transport) via light reaction (Geider et al. 1998).

We observed no significant change in F_0 but a significant improvement in F_m , which correlated well with the crop's photosynthetic efficiency and yield characteristics. A higher J rise was reported from the variable fluorescence spectra of plants without nutrient amendment or RDNP amendment. The comparative J rise (as seen in the differential variable spectra; Figure 1C) of all other amendments was, however, insignificant ($\leq 7.38\%$ higher than of T6). Nevertheless, the plants in T5 and T6 showed the minimum J variation (4.38%) between them and were proportionately lower than any other amendment. The mentioned two treatments have higher F_m and F_v , indicating a better PS II photochemical activity. No remarkable difference in the shape of the OJIP transient during the initial farming period indicated that the plants remained metabolically active during this period in all

treatments, but at 90 DAT, the change in shape in T1 indicates nutrient deficiency. This is supported by the ϕP_0 level, which remains high in all treatments and indicates photosynthetic performance (Stirbet and Govindjee 2011).

Consequently, the plants grown in the treated plots also had low dissipation indicating that the plants were physiologically in a good state. However, significant variations in ϕE_0 and Ψ_0 among treatments showed that at 90 DAT, the metabolic state of the crop varied with the treatments (Figure 2). As the availability of basic nutrients determines the metabolic state (Chhotaray et al. 2014; Shi et al. 2020; Gao et al. 2022), the electron transport rate, photosynthesis, and the translation of the photosynthates to yield varied accordingly. A significant positive correlation between amendment (T1 through T6) and the magnitude of the photosynthetic efficiency of the crop was improved with the amendment, and the intensity of activity was amendment dependent.

There are reports that organic amendments significantly enhanced the density of bacteria and fungi in agricultural soil (Chhotaray et al. 2014; Cao et al. 2021; Wu et al. 2021). In the present case, all organically amended plots, with or without fertilizer application, had significantly higher levels of bacteria and fungi than the unamended or fertilizer-amended ones. Further, T5, which was amended with VC+PM+biofertilizer, showed the highest microbial density among the treatments indicating that applied biofertilizers could build a syntrophy to affect an increase in soil microbial density. Since biofertilizers are known to enhance rhizospheric surface (Elanchezhian and Panwar 1997; Dalawai and Naik 2014; Choudhury et al. 2020), the healthy microbial population in T5 was due to the availability of surface as well as substrates for microbial growth. This agrees with the findings in other cropping systems that organic amendments build a healthy soil organic carbon base required to grow and maintain soil microflora (Chhotaray et al. 2014; Cao et al. 2021; Gao et al. 2022). Mockeviciene et al. (2021) reported that long-term application of organic manure maintained favorable conditions for soil microbes and carbon sequestration.

The soil enzyme activities have been used as a measure of the quality of agricultural soil. We observed that all the organic amended plots (T3 through T6) had significantly high activities of all the selected soil enzymes, and the enzyme activities were in significant positive correlation with soil bacteria and fungi. Each soil enzyme activity was proportional to microbial density, and, as expected, biofertilizer application encouraged soil microbial population; these plots showed high soil enzyme activities. Soil enzymes are an early marker of soil SOC load and microbial richness. Significantly low enzyme activity and microbial count in T2 showed that even though good yield could be achieved, cultivation practice only with RDNP is unsustainable for continued production. On the other hand, in T6, soil nutrient level, microbial health, crop health, and yield were not only maximum, but the nutrient uptake by the crop was the highest, thus confirming efficient nutrient mobilization.

Table 7. The activity ($\text{mgg}^{-1} \text{soil h}^{-1}$) of key enzymes of the top 15 cm soil of experimental plots was measured at 90 DAT of the crop in the field

Enzyme	T1	T2	T3	T4	T5	T6
Amylase	1.344 ^e	1.685 ^d	2.586 ^b	2.073 ^c	2.878 ^a	2.608 ^b
Invertase	2.186 ^c	2.375 ^c	3.289 ^{ab}	2.868	3.485 ^a	3.219 ^b
Cellulase	8.218 ^f	9.684 ^e	14.57 ^b	13.43 ^d	15.88 ^a	14.19 ^c
Phosphatase	3.185 ^e	4.425 ^d	9.926 ^b	9.376 ^c	10.66 ^a	9.718 ^b
Protease	0.274 ^c	0.439 ^c	0.804 ^{ab}	0.685 ^b	0.886 ^a	0.829 ^a
Dehydrogenase	0.034 ^d	0.065 ^c	0.092 ^a	0.077 ^b	0.098 ^a	0.088 ^{ab}

In conclusion, in the present study, the morphological attributes did not significantly vary among treatments up to 60 DAT. Still, significant variations were noted after that, showing that the treatment variations made in this experiment significantly impact plant growth performances. The improved growth performances and yield attributes in T6 than in other treatments proved that the combination is suitable for enhancing yield and giving the farmers more profit. Lower growth performance and yield in T3, as compared to T2, showed that organic amendment alone is not suitable to satisfy the immediate nutrient demand of the crop, but there was significant growth and yield enhancement with biofertilizer addition in soil. All the organically amended plots, solo or combined with fertilizer (50% RDNP), had physiologically active plants, high photosynthetic activities, and plant performances. These plots had healthy microbial populations and activities, thus indicating sustainability. Further work and detailed investigation on fine-tuning the combinations of amendments may determine a more viable treatment for more profitable and sustainable crop yield.

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Effects of climate change and adaptation strategies on urban crop production in Kinondoni City, Tanzania

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Abstract. Kifunda CF. 2023. *Effects of climate change and adaptation strategies on urban crop production in Kinondoni City, Tanzania. Asian J Agric 7: 98-107.* Many countries worldwide, including Tanzania, have felt the effects of climate change on the productivity of major economic sectors, such as agriculture. This study assessed climate change's effects on urban agriculture's sustainability in Kinondoni City, Tanzania. Specifically, the study aimed to determine: i) the effects of climate change on crop production in Kinondoni City over the last 30 years, ii) other challenges of urban agriculture in Kinondoni City, and iii) adaptation strategies employed by smallholder farmers in sustainable urban agriculture in Kinondoni City. Data collection involves accessing temperature and rainfall data from Tanzania Meteorological Agency (TMA) headquarters and administering household questionnaires to 386 respondents who engaged in urban agriculture in Kinondoni City. Five wards of Bunju (118), Kunduchi (42), Mabwepande (102), Mzimuni (28) and Wazo (96) were involved. Focused group discussion, key informant interviews, and direct observation were also employed. Data were analyzed for temperature and rainfall variations over the last 30 years and people's knowledge of climate change indicators. The adaptation strategies employed in sustaining crop production in the study area were also quantified. The study reveals that most smallholder farmers (>70%) are aware of the indicators of climate change, which correlate with the data obtained from TMA. Among the indicators include an increase in temperature (76%) and a decrease in rainfall (73%). Pest and diseases were ranked higher (88%) among the significant effects of climate change on urban agriculture. Land scarcity was also a significant challenge in urban agriculture. The use of pesticides and fertilizers was ranked higher (65%) among the adaptation strategies employed by smallholder farmers. It is recommended that building capacity for smallholder farmers through getting access to micro-credits at low-interest rates and government support may contribute significantly to sustainable urban agriculture in Kinondoni City. Also, farmers in Kinondoni City can adopt sustainable agriculture practices such as Soil and Water Conservation practices and the use of Drought-Resistant Crop varieties to improve their resilience to future climate risks caused by climate change.

Keywords: Agriculture, crop productivity, smallholder farmers, sustainability, urban agriculture

INTRODUCTION

Climate change is the greatest environmental problem of the 21st century affecting many people's lives worldwide (Intergovernmental Panel on Climate Change (IPCC) 2021; Mazhin 2020). All nations worldwide are already experiencing temperature changes, shifts in the rainy seasons, and an increasing frequency of extreme weather events and other climate change impacts (IPCC 2014; Omowunmi and Michael 2016). Cities are exposed to global climate change, and the impacts range from sea level rise, floods, droughts, and damage to infrastructure (Rosenzweig et al. 2014; IPCC 2023). Climate change severely threatens sustainable economic growth for Africa and may lead to extended poverty, particularly in Sub-Saharan Africa (IPCC 2014). The areas that are highly vulnerable to the impact of climate change are food production, the health sector, biodiversity, rangelands, and water resources (Kimani et al. 2014). Crop production is one of the specific areas that are greatly affected by climate change, where the variation in temperature and rainfall patterns and associated extreme weather events can profoundly affect the livelihoods of urban farmers and the availability of fresh produce for urban populations. To

avoid the costs that could result from delaying, adaption measures must be made faster as the climate changes.

Urban dwellers areas is food produced in rural areas to meet their dietary needs. Growing and producing food within urban areas is gaining increasing recognition for its numerous benefits and contributions to sustainable development. Urban agriculture is considered to be one of the ways to improve food security and household income of urban dwellers under conditions of persistent economic uncertainty and threats of climate change (Poulsen et al. 2015; Duží et al. 2017; Siegner et al. 2018; Bennedetti 2023). Farming in urban areas could offer a promising opportunity for low-income residents to improve their financial situation, as urban farming often requires a much lower initial investment than other business ventures. By utilizing small plots of land in the city, individuals or groups can grow fresh produce and generate income through sales or local partnerships. Urban agriculture can reduce urban poverty across Sub-Sahara Africa (Akinagbe and Irohibe 2014; Mudzengerere 2014). However, in many cities, it is more difficult for the urban poor to access the land needed for urban agriculture than for rich people (Mhache and Lyamuya 2019). Urban agriculture enhances food security and household income and contributes to

biodiversity conservation in urban areas by providing habitats for pollinators and reducing urban heat island effects, which are caused by the absorption and retention of heat by urban surfaces like buildings and roads. Urban agriculture can increase vegetation cover in urban areas, which helps to absorb and shade the sun's energy. This leads to a reduction in the amount of heat absorbed by urban surfaces and can mitigate the urban heat island effect (Abdul-Rahman et al. 2021; Hanson et al. 2021). It also helps preserve traditional crop varieties and local food cultures, promoting the conservation of agricultural biodiversity (Siegner et al. 2018). By promoting sustainable and equitable urban agriculture practices, cities can protect biodiversity, build resilient and sustainable food systems, and address climate change. However, it is important to consider potential impacts on local ecosystems, manage resources efficiently, and ensure equitable access to land and financial resource for sustainable urban agriculture.

Whereas many efforts and measures like the promotion of urban agriculture by the government and introduction of new technologies such as hydroponics and vertical agriculture are taken towards the intensification of the contribution of urban agriculture to food security, little attention has been put on assessing the negative impact of climate change on urban agriculture and quantifying the adaptation strategies employed by smallholder farmers in sustainable urban agriculture. This study therefore aimed to assess the effects of climate change on the sustainability of urban agriculture in Kinondoni City, Tanzania. The Kinondoni City was chosen for this study due to its large population size and because a significant portion of the population relies on agriculture as their primary source of

income (URT 2013). Specifically, the study aimed to assess: i) the effects of changes in rainfall and temperature on crop production in Kinondoni City over the last 30 years, ii) challenges of urban agriculture, and iii) adaptation strategies employed by smallholder farmers in sustaining urban agriculture in Kinondoni City. The study provides valuable insights into the support needed to develop effective adaptation strategies to overcome the effects of climate change and enhance food security and resilience to climate change in urban areas.

MATERIALS AND METHODS

Study area

Kinondoni City is one of the four municipalities in Dar es Salaam City, Tanzania. The municipality is bordered by the Indian Ocean to the North East, Ilala District to the South, and Ubungo District to the North (Figure 1). Climatically, Kinondoni City experiences a modified type of equatorial climate. It is generally hot and humid throughout the year, with an average temperature of 29°C. The hottest season is from October to March, while it is relatively cool between May and August, with temperatures around 25°C. There are two rain seasons: a short rainy season from October to December and a long rainy season between March and May. The average annual rainfall is 1,300 mm. The climate is also influenced by the Southwest monsoon winds from April to October and Northeast monsoon winds between November and March (URT 2017).

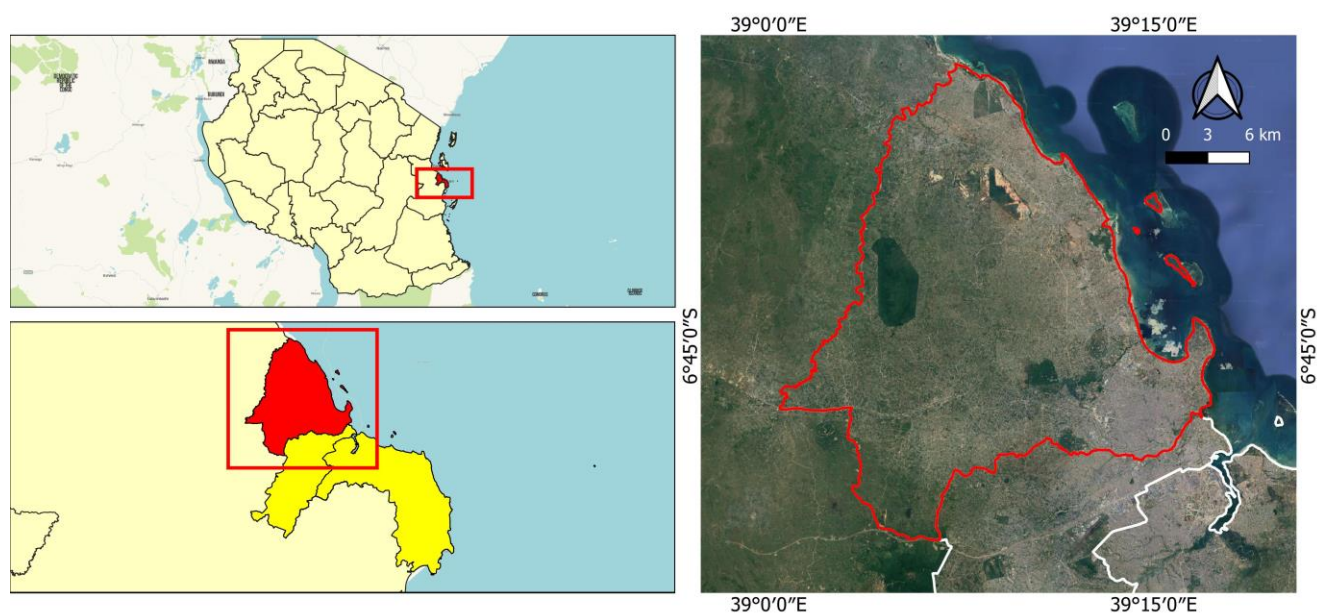


Figure 1. Map of Tanzania showing the location of Kinondoni City, Dar es Salaam Region, Tanzania

Kinondoni City was selected because it had the highest population, with some of the population engaged in agriculture (about 10%) as their main economic activity (URT 2013; Mlozi et al. 2017). Five wards were selected for the study: Bunju, Kunduchi, Mabwepande, Mzimuni, and Wazo. These wards had the majority of people practicing urban agriculture compared to the rest of the 20 wards found in Kinondoni City.

Data collection

The field survey was conducted from October 2018 to January 2019 and, among other activities, involved accessing temperature and rainfall data from Tanzania Meteorological Agency (TMA) headquarters in Dar es Salaam. Semi-structured questionnaires were administered to a total of 386 respondents from the five wards of Bunju (118), Kunduchi (42), Mabwepande (102), Mzimuni (28), and Wazo (96) who engaged in urban agriculture. The questionnaires provided the respondents' socio-economic characteristics, knowledge of climate change indicators, perceptions of climate change's impact on crop production, and the adaptation strategies employed by the farmers. A separate set of questions was addressed to key informants such as agricultural extension officers, leaders of the local markets, leaders of agricultural groups, and a few selected farmers from the five wards. Furthermore, focus group discussions were conducted using 5-10 group people. In addition, Direct observation was also conducted to cross-check the reliability of the information gathered from the techniques mentioned above.

Data analysis

Quantitative data from household interviews were compiled, summarized, and analyzed by using Statistical Package for Social Sciences (SPSS) software version 20

and Excel spreadsheets. Qualitative data from key informants and focus group discussions were captured through content analysis with the assistance of the Maxqda program. Both descriptive and inferential statistics were used. Data were presented using tables and figures.

RESULTS AND DISCUSSION

General characteristics of the respondents

The general characteristics of the respondents are presented in Table 1. Concerning gender, 44.8% of respondents were males, and 55.2% were females. People of middle age between 41-60 accounted for the largest portion of the studied population (44.6%). The majority of the households (53.4%) showed to have an average of 4 to 6 members per household. Migrants from different regions of Tanzania and respondents with primary education accounted for the majority of the respondents, 85.5% and 73.1%, respectively.

The dominance of females found in this study is a phenomenon that was expected. In many studies, females have been found to engage more in urban agriculture than males (Sebata et al. 2014; Mwangi 2015; Malekela and Nyomora 2019). In these studies, it has been shown that females participated in urban agriculture to supplement their household income and ensure food security for their families. Other studies noted that the participation of females in urban agriculture could also be due to gender roles and responsibilities in a particular society, as women traditionally have responsibilities related to food production and household management (Cele and Mudhara 2022; FAO 2023; Mwale 2023).

Table 1. Socio-economic characteristics of the respondents in Kinondoni City, Dar es Salaam Region, Tanzania

Respondents Characteristics	Name of the Ward					Total (%)
	Bunju	Kunduchi	Mabwepande	Mzimuni	Wazo	
<i>Gender</i>						
Males	13.0	6.1	10.1	3.9	11.7	44.8
Females	17.6	4.7	16.3	3.4	13.2	55.2
<i>Age</i>						
17-30	1.8	3.6	7.8	4.2	1.0	18.4
31-40	3.4	6.5	9.8	7.0	1.8	28.5
41-60	4.9	12.4	10.6	13.5	3.2	44.6
61+	0.8	2.3	2.3	1.8	1.3	8.5
<i>Origin</i>						
Natives	4.9	1.0	3.9	1.0	3.7	14.5
Migrants	25.7	9.8	22.5	6.3	21.2	85.5
<i>Education</i>						
Informal	1.3	1.6	2.8	0.8	3.1	9.6
Primary	21.5	7.5	20.5	6.2	17.3	73.0
Secondary	5.2	1.3	2.3	0.3	3.4	12.4
Tertiary	2.6	0.5	0.8	0.0	1.0	4.9
<i>Household size</i>						
1-3	10.1	1.8	5.4	1.6	6.5	25.4
4-6	15.5	5.7	16.8	3.7	11.7	53.4
7-9	4.1	2.3	3.4	1.6	5.7	17.1
10+	0.8	1.0	0.8	0.5	1.0	4.1

Urban agriculture, which often involves small-scale gardening and animal husbandry, aligns with these roles and can be seen as an extension of women's domestic responsibilities. The predominance of females in urban agriculture highlights their crucial role in ensuring food production, promoting local food security, and contributing to the socio-economic development of urban areas. Empowering and supporting women in urban agriculture can enhance livelihoods, gender equality, and sustainable urban food systems.

Most respondents under the middle age group of 41-60 years old indicate a potential working force useful for urban agriculture activities in the study area. The age group is in line with the age structure of the Tanzanian working population, which ranges between 15 to 64 years of age (URT 2014). According to Malekela and Nyomora (2019), most people who participate in urban agriculture are of middle age because they play an important role in various aspects of the production system. This group may also have valuable skills and experience that contribute to the development and implementation of sustainable production practices and the ability to manage and operate production facilities. Moreover, the middle age group may be more likely to establish networks and connections within the communities or industries, which help facilitate cooperation and knowledge sharing between different stakeholders. The dominant age of the respondents in this study, between 41 and 60, is consistent with other studies in different countries (Sebata et al. 2014; Fry 2018; OECD 2023). The participation of the middle age group in agriculture is vital as their skills, experience, and connections contribute significantly to the development, implementation, and sustainability of agricultural practices while fostering cooperation and knowledge sharing among stakeholders. Their presence in the agricultural sector ensures a strong workforce and promotes agriculture's continued growth and success.

Most of the respondents (73%) in the study area attained a primary level of education. Education is a key element in the recovery strategies to counter the various crises (Omoniyi 2013). Aduke (2011) asserts that in many developing countries, education is the primary means of breaking the cycle of poverty. Education is crucial in advocating for sustainable agricultural practices that minimize environmental damage and bolster long-term food security. It can raise awareness about climate change, biodiversity conservation, soil health, water management, and agroecology, enabling farmers to make deliberate and thoughtful choices of using sustainable farming methods

(Belay and Araya 2015; Pauw et al. 2015; Maini et al. 2021). Education is a transformative force that cultivates innovation, fosters sustainable practices, and paves the way for a prosperous future in agriculture. It serves as the cornerstone of agricultural development, enabling farmers to acquire the knowledge, skills, and awareness necessary for sustainable practices to increase the productivity of urban agriculture.

Changes in rainfall and temperature in the study area

Indicators of changes in climate in the study area are presented in Table 2. Based on multiple responses from the household survey, most respondents (76%) had experienced an increase in temperature followed by a decrease in rainfall (73%) in the study area. These claims were supported by the data obtained from the Tanzania Meteorological Agency (TMA). The data shows that there has been a decrease in rainfall in the last 30 years, from 1,430.9 mm in 1986 to 782.9 mm in 2016 (Figure 2). An abnormal increase in rainfall has also occurred, i.e., up to 1,500 mm/year, resulting in flooding. In April 2010, Dar es Salaam received unprecedented rainfall above 400 mm, resulting in widespread flooding (TMA 2017). In addition, there has been increasing in both minimum and maximum temperatures in the study area in the last 30 years, i.e., from 20.7°C in 1986 to 23.2°C in 2016 (Figure 3) and from 30.9 in 1990 to 32.1°C in 2015 (Figure 4) respectively.

According to farmers' point of view and the data from TMA, this study shows climate change is a reality in Kinondoni City. The study has revealed the presence of unreliable rainfall patterns and an increase in maximum temperatures in the study area. Many other studies have documented similar observations in parts of Tanzania, like Dodoma, Dar es Salaam, and Singida (Mlozi et al. 2014; Mwamfupe 2014; Myeya 2021). The reliance on rainfall makes rain-fed agriculture vulnerable to climate change. In addition, increased rainfall variability has affected the agricultural calendar and decisions over important farming activities (Mwamfupe 2014). The changes in crop production-related climatic variables will possibly have major influences on regional and global food production.

Moreover, the study by Malekela and Nyomora (2019) investigated the local indicators of climate change in Dar es Salaam City, Tanzania. Among the indicators mentioned were increased temperatures, changes in rainfall patterns, and a decline in rainfall. These findings are worrying as they indicate that climate change could affect the study area and progressively hinder agricultural activities.

Table 2. People's perceptions of the changes in rainfall and temperature in the study area

Indicators of climatic changes	Number of respondents (N=386)	Percent
Increase in temperature	293	76
Decrease in rainfall	283	73
Occurrence of floods	178	46
Shifting of the rain season	152	39
Occurrence of drought	126	33

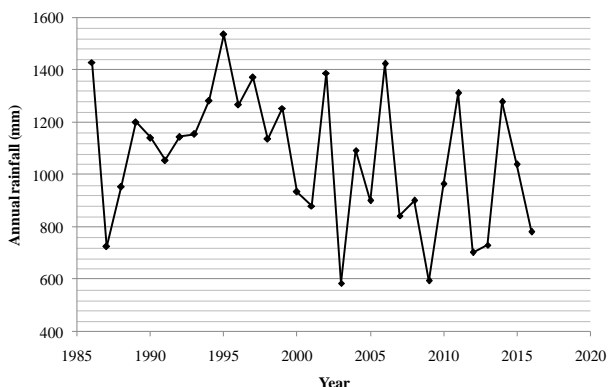


Figure 2. The general trend of rainfall pattern from 1986 to 2016 in Kinondoni City, Dar es Salaam Region, Tanzania

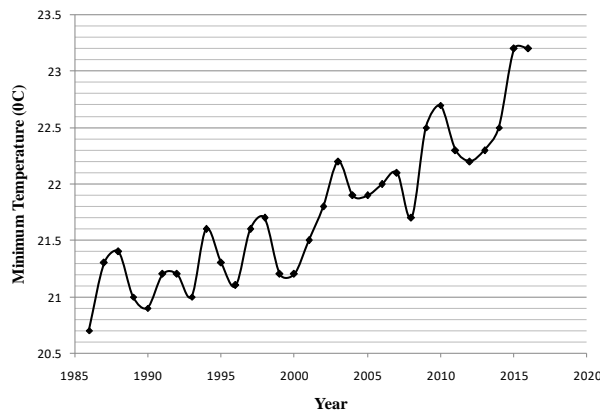


Figure 3. The general trend of Minimum Temperature from 1986 to 2016 in Kinondoni City, Dar es Salaam Region, Tanzania

Effects of climate change on urban agriculture

Indicators of the effects of change in climate in urban agriculture in the study area are presented in Table 3. Based on multiple responses from the household survey, smallholder farmers revealed to know the effects of climate change on crop production. Most respondents (88%) reported increased pests and diseases due to climate change in recent years. Temperature change and unreliable rainfall were said to cause water shortage in the study area (67%). The occurrence of floods was reported by very few respondents (27%). These farmers claimed to have floods due to unpredictable, heavy rainfall, which brought large crop losses. Especially for those farms located in lowland areas, such as river valleys, are frequently affected by floods. Farmers witnessed the effects of this erratic occurrence of rainfall in April 2018. The running water destroyed many farms along the Msimbazi and Mpiji river valleys and others covered by mud (Figure 5). This leads to a reduction in the size of the farms.

The results of this study concurred with studies done elsewhere which showed that an increase in temperature and unreliable rainfall lead to crop diseases, which in turn leads to low harvests hence extreme poverty to the smallholder farmers (Baker 2013; Kifunda 2014; Smith 2015). The rising temperatures are adversely affecting farmers, causing heat-related illnesses, exacerbation of pre-existing health conditions, psychological impacts, and significant financial losses due to crop damage and reduced yields, as well as the inability to work during extreme heat

events (El Khayat 2022; IPCC 2023). Hatfield and Prueger (2015) showed that an increase in temperature adversely affects crops as excessive heat is a limiting factor of production. Kasimba (2012) from Zimbabwe reaffirmed that an increase in temperature threatens crop growth as they would dry. The increased prevalence of pests and diseases poses a significant challenge to crop production in urban areas. Addressing this challenge through adaptive strategies for sustainable urban agriculture is imperative.

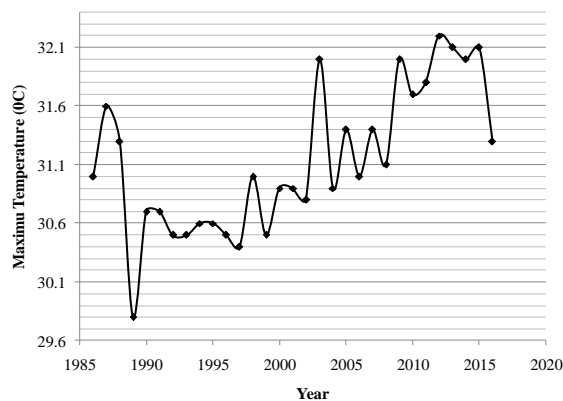


Figure 4. The general trend of Maximum Temperature from 1986 to 2016 in Kinondoni City, Dar es Salaam Region, Tanzania

Table 3. The effects of climate change on urban agriculture in Kinondoni City, Dar es Salaam Region, Tanzania

Indicators	Ward					Total (%) N=386
	Bunju	Kunduchi	Mabwepande	Mzimuni	Wazo	
Increase in pests and diseases	29.0	10.4	22.5	5.7	20.7	88
Shortage of water	20.5	9.1	18.8	3.4	15.0	67
Decline in crops quality	22.3	9.3	15.3	3.6	14.2	65
Low harvests	17.9	9.8	19.2	3.6	12.7	63
Seeds failing to germinate	8.3	3.6	10.1	1.8	6.5	30
Drying of crops	11.1	2.9	6.2	4.1	8.3	33
Occurrence of floods	8.0	2.6	5.2	7.3	4.1	27



Figure 5. Farms destroyed by running water along Msimbazi River Valley as witnessed by farmers in April 2018: A. A farm destroyed by running water in Mzimuni ward, B. A farm covered with mud in Mzimuni ward

Climate change leads to drought and declining crop yields, negatively impacting crop quality. Various studies have documented the impacts of climate change on water availability, crop yields, and quality. The increased frequency of droughts and water scarcity leads to reduced crop production. Similarly, research in different parts of the world has also demonstrated the negative effects of climate change on crop yields and quality (Challinor et al. 2014; FAO 2015; Pareek et al. 2020). Global efforts to mitigate climate change and reduce greenhouse gas emissions are crucial to safeguarding agricultural systems and ensuring future food security.

Other challenges of urban agriculture

Other challenges besides climate change affecting urban agriculture are presented in Table 4. According to the survey, the majority of respondents reported inadequate farm inputs as a major challenge (81.3%), while market problems were faced by only 47.4% of respondents (Table 4). Land scarcity was also a significant challenge, with 74% of all respondents indicating this as an issue. Urban agriculture was conducted in various locations, including backyards, open spaces such as river valleys and road reserves, building plots, and some respondents' farms. One extension officer noted, "Farming in her ward primarily occurs in open spaces and people's plots that the owners do not yet develop. This is because much of the land in the city is occupied by other things, such as houses and industries. For instance, some farmers in Kilongawima Street cultivate on road reserve areas, while others in Ununio Street farm in government open spaces, including a plot owned by the Daily News magazine".

Most urban farmers (38.9% of respondents) cultivate on freehold land given to them by the owners for free with the intention of taking care of the land (Table 5). However, due to the temporary nature of this land ownership, cultivation in the study area tends to shift from one place to another. Moreover, 85% of respondents reported conducting farming activities temporarily because they lack permanent

land in the city. Only 15% of all respondents conduct permanent farming, indicating that a small minority has secured more stable land tenure for their agricultural activities (Table 5).

The highlighted challenges in this study are not unique to Kinondoni City but are prevalent in many African cities, where urban agriculture is critical in providing food and income for urban residents. Limited access to appropriate agricultural inputs, including certified seeds for improved crop varieties, pesticides, and fertilizers, is a significant challenge for urban farmers (FAO 2017; FAO 2022). This is particularly concerning as it can lead to low yields, decreased profitability, and even the discontinuation of farming activities. Therefore, sustainable agricultural practices such as seed saving, organic farming, and agroecology should be promoted to address this challenge.

The scarcity of land in urban areas has resulted in many urban farmers relying on temporary land tenure arrangements, leaving them vulnerable to displacement and limiting their capacity to make long-term investments in their farming activities. Findings from this study resemble studies by Kiduanga and Shomari (2017) which show that most of the vegetable growers in Dar es Salaam city use land temporarily, which makes them ready to move out at any time when needed by the land owners. Also, Schmidt (2011) and Malekela and Nyomora (2019) conducted a study in Dar es Salaam revealing various farm location changes due to urban sprawl leading to farmland scarcity. Land competition has become the undermining factor for sustainable urban agriculture in cities; land is scarce and expensive in urban centers (Githugunyi 2014; Tuffour 2023). Land competition with other urban land uses, such as residential, commercial, and industrial, further exacerbates this challenge (Kuang et al. 2022; Tornaghi 2014). Addressing land scarcity requires innovative land management practices to maximize productivity and ensure sustainable crop production in the face of limited available land resources.

Table 4. Challenges facing urban agriculture in Kinondoni City, Dar es Salaam Region, Tanzania

Challenges facing urban agriculture	Ranking	Frequency n = 386	Percent
Inadequate farm inputs	I	314	81.3
Land scarcity	II	288	74.6
Inadequate capital	III	274	71.0
Insufficient extension services	IV	270	69.9
Limited irrigation technologies	V	203	52.6
Market problem	VI	183	47.4

Table 5. Nature of land ownership in Kinondoni City, Dar es Salaam Region, Tanzania

Land ownership	Wards					Total%
	Bunju	Kunduchi	Mabwepande	Mzimuni	Wazo	
Freehold land	16.1	2.3	13.2	0.8	6.5	38.9
Leasehold	9.1	4.1	7.5	1.3	4.9	26.9
Own land	5.2	1.0	4.7	2.6	5.7	19.2
Communal land	0.3	3.4	1.0	2.5	7.8	15.0

Inadequate capital is a significant constraint that impedes the growth and expansion of urban agriculture, resulting in low production levels and posing a threat to food security and livelihood enhancement in urban areas. Similar findings were observed in various studies by the World Bank (2013) and Mhache and Lyamuya (2015), which also identified lack of capital as a constraint for urban farmers, leading to low production. Insufficient capital hinders the development and expansion of urban agriculture and threatens food security and livelihood improvement in urban areas; the study highlights the need for increased investment in urban agriculture to overcome this challenge. In addition, inadequate irrigation technologies further exacerbate the challenges urban farmers face in Kinondoni City. Using poor facilities, such as buckets and hands, to irrigate crops is time-consuming and inefficient (Figure 6). Therefore, to improve production and efficiency in urban agriculture, there is a need for investment in appropriate irrigation technologies.

Effective extension services are critical for agricultural development and productivity. Inadequate extension services pose a significant challenge to agricultural development and productivity. Studies have shown that inadequate extension services are a significant challenge facing farmers in many regions. For example, Danso et al. (2018) highlight the critical role that extension services play in disseminating knowledge and providing technical

support to farmers. However, when these services are limited or unavailable, farmers are disadvantaged. Farmers may lack access to vital information, skills, and resources needed to enhance their agricultural practices when insufficient extension services exist. This can result in lower yields, decreased profitability, and limited new technologies or practices adoption.

Adaptation strategies employed by smallholder farmers

In response to rainfall and temperature changes in Kinondoni City, urban farmers have adopted several strategies to cope with climate changes in their area, as shown in Table 6. The majority of the respondents (about 65%) reported using pesticides and fertilizers as one way of adapting to the effects of climate change. However, some respondents (3.6%) admitted using mulching to cope with the situations. About 53.8% of the respondents use irrigation systems to overcome the problem of drying crops during the dry season. Farmers use water from boreholes, and others construct dams in the riverbed to increase the amount of water for irrigation activities (Figure 7). However, some farmers claim to stop farming during the dry season because their farms are far from water sources. Digging up a deep well appears to be challenging to them and not rewarding.

Table 6. Adaptation strategies employed by smallholder farmers to cope with the effects of climate change on crop production in Kinondoni City, Tanzania

Adaptation strategies	Ward					Total (%) N=386
	Bunju	Kunduchi	Mabwepande	Mzimuni	Wazo	
Use of pesticides and fertilizers	24.1	9.6	12.2	3.6	15.5	65.0
Use of irrigation	13.1	13.8	11.7	4.9	10.4	53.9
Stop cultivating	10.9	2.3	16.8	4.1	9.3	43.4
Application of mulching	1.3	0.5	1.5	0	0.3	3.6



Figure 6. The irrigation facilities used by urban farmers in Kinondoni City, Tanzania: A. Irrigation facilities used at Wazo Ward, B. Irrigation facilities used at Kunduchi Ward



Figure 7. A dam used by farmers for irrigation in the Mabwepande ward in Kinondoni City, Tanzania

The adaptation strategies practiced by smallholder farmers in the study area are more or less similar to those reported by several authors which were adopted by people for the sustainable production of various crops in different areas (e.g., Challinor et al. 2014; Kifunda 2014; Lipper et al. 2014; Malhi et al. 2021; Koné and Galiegue 2023). Altering amounts and timing of irrigation and other water management practices like drip irrigation have been suggested to be good adaptation strategies for smallholder

farmers (Ndamani and Watanabe 2015; Ogundeji 2022). However, the use of irrigation systems was observed to be practiced by very few smallholder farmers. In contrast, due to a lack of capital, others could not buy irrigation facilities like water pumps and pipes for carrying water from the rivers or dams (Kifunda 2014; Mkuna and Wale 2023; Nzeyimana et al. 2023). The findings also align with Odewumi et al. (2013), who reported using irrigation, fertilizers, mulching, and chemicals to enhance crop yields in the changing climate. Some Kinondoni City farmers stopped farming for sometimes waiting for rainfall as they cannot irrigate during the dry season. The results are supported by the study conducted in Dodoma City by Namwata et al. (2015), who reported that access to quality water is expensive and therefore unaffordable by many farmers. Other studies, such as the research of Koné and Galiegue (2023), have recommended the combination of improved seed varieties, soil and water conservation practices, and the application of Biochar as one of the best innovations to adapt to climate change in agriculture.

In conclusion, the study revealed that changes in rainfall and temperature in the last 30 years have also affected activities related to urban agriculture in Kinondoni City. Farmers have witnessed an increase in pests and diseases which proves to be challenging, leading to a decline in crop quality and total harvest. Farmers who appeared to access agricultural inputs, including irrigation facilities, were seen to be better off than those who didn't. Lack of enough capital was noted to be a big challenge to most of the farmers. Thus, assisting farmers in accessing

micro-credits that are provided at low-interest rates is necessary for the commercialization of their farming activities. It will be helpful to assist them in establishing their own Servings and Credit Cooperation Societies (SACCOS). Financial assurance to farmers will enhance access to farm inputs and use of irrigation systems which are essentials in sustainable urban agriculture, especially in changing climate. The use of other crops, such as drought and salt-resistant crops, and the adoption of innovative farming techniques such as drip irrigation, rainwater harvesting, and smart agriculture practices should be encouraged to ensure the sustainability of urban farming in the changing climate. Next, research may focus on the impact of adaptation strategies on the welfare of urban farmers.

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The effect of interaction between salicylic acid and drought stress on growth and photosynthetic rate of *Basella alba* and *B. rubra*

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Abstract. Ayuningtias AW, Solichatun, Pangastuti A. 2023. The effect of interaction between salicylic acid and drought stress on growth and photosynthetic rate of *Basella alba* and *B. rubra*. *Asian J Agric* 7: 108-115. *Basella alba* L and *B. rubra* L are two species of potential drugs and have very potential to develop because of the secondary metabolic content. The change of climate, which is uncertain and prolongs the dry season, is a problem in the cultivation of these plants. Drought stress affects the aspect in growth and photosynthesis rate. The application of salicylic acid tolerated plants due to drought stress and gave a physiologically different response from both plants. This study aimed to determine the interaction effect of Salicylic Acid (SA) on growth and photosynthesis rate in *B. alba* and *B. rubra*. A completely random design was used. Two factors of this study were salicylic acid and drought stress. The dosage of salicylic acid were 0 mM, 2 mM, 4 mM, and 6 mM, and field capacities were set by 100%, 75%, 50%, and 25%. Observed data in this study include plant height, leaf area, photosynthesis rate, stomata conductance, and transpiration rate at *B. alba* and *B. rubra*. The result showed in *B. alba* KL 50% and SA 4 mM (50.1 cm²), in *B. rubra* KL 25% and SA 4 mM (52.9 cm²) were the best treatment in plant high. The highest values of leaf area in *B. alba* are KL 75% and SA 2mM (91.72 cm²), and in *B. rubra*, are KL 50% and SA 4 mM (122.67 cm²). The treatment KL 50% and SA 6 mM on *B. alba* showed the highest result in photosynthesis rate, stomata conductance, and transpiration rate (0.8663 $\mu\text{mol m}^{-2} \text{s}^{-1}$; 0.9468 $\mu\text{mol m}^{-2} \text{s}^{-1}$; 0.1890 $\mu\text{mol m}^{-2} \text{s}^{-1}$), in *B. rubra* the highest value are KL 75% and SA 6mM (0.9202 $\mu\text{mol m}^{-2} \text{s}^{-1}$; 0.9468 $\mu\text{mol m}^{-2} \text{s}^{-1}$; 0,105 $\mu\text{mol m}^{-2} \text{s}^{-1}$). This study concludes that the interaction between salicylic acid and field capacity significantly affects growth and photosynthesis rate.

Keywords: *Basella*, drought stress, growth, photosynthesis, salicylic acid

INTRODUCTION

The pharmaceutical Indonesia industry can export 90% of domestic medicinal products, but almost 95% of production depends on imported raw materials (BPPT 2021). Imported raw materials cause a deficit and affect the whole of Indonesia's economy. Indonesia's high number of raw materials and drug additives encouraged researchers to research the production of pharmaceutical-grade active compounds with existing natural resources.

Basella is a genus of potential drugs to develop because of its secondary metabolic content. There are two species, *Basella alba* L, and *B. rubra* L. In recent decades, the public has been interested in secondary metabolites of medicinal plants because of their therapeutic compounds. The secondary metabolites in plants are varied. The *B. alba* contains acacetin, rutin, betacyanin, saponins, kaempferol, and ferulic acid. The *B. rubra* contains tannins, alkaloids, saponins, steroids, and flavonoids. Secondary metabolic production by direct extraction must be accompanied by proper cultivation to prevent over-exploitation. Therefore, to avoid over-exploitation, the cultivation must use the right method to increase the yield and quality of production.

Water is an important environmental factor that affects crop productivity. Physiologically, Reactive Oxygen

Species (ROS) produced by plants under stress conditions often cause oxidative stress. Serious drought stress conditions result in stunted plant growth, low production yields, and poor dry matter accumulation. Therefore, water must be used wisely to maintain sustainable crop productivity. Red and white gondolas are plants that are easy to grow in both highland and lowland areas. But, NASA (2022) said that the earth's temperature in 2021 increased by 0.85% compared to the average temperature in 1951-1980's. The uncertain climate change and prolonged dry season were the problems for cultivating these plants. Extreme climate change causes plants to experience abiotic stress. Fresh water will be scarce if global changes are not controlled. Water is a molecule key in plant physiological activity; it helps transport metabolites and nutrients to all plant parts. In the long term, severe drought stress results in stunted plant growth, low yields, and decreased productivity. The bad impact is decreased plant size, leaf area, and yield. The other bad impact is stomata closing to reduce CO₂ binding, thus inhibiting photosynthesis. Cultivation of plants to optimize secondary metabolite levels can be carried out with various treatments, such as giving hormones and stress relievers (Ahmad et al. 2017; Movahedi et al. 2021; Simbeye et al. 2023)

The application of growth regulators plays an important role in the plant's response to stress. Plant hormones must be right so that gene activity and plant regulation in their life cycle are also good. Both plants, *B. alba* and *B. rubra* have different responses in dealing with stress. Applying phytohormones is an effort to increase plant growth due to drought stress. The salicylic acid hormone increases plant tolerance when dry stress occurs because it's effective for target cells even though produced in small amounts. In addition, salicylic acid plays an important role in increasing physiological processes, stomata conductance, photosynthetic rate, and chlorophyll content under stress. Salicylic acid activates the gene expression that responds to abiotic stress and induces enzyme expression and protein biosynthesis. The exogenous application of salicylic acid hormone upregulates dehydrin-like protein synthesis, chlorophyll content, and rubisco (Li et al. 2013; Nazar et al. 2015; Wiratmaja 2017). Najafabadi and Ehsanzadeh (2016) stated that applying salicylic acid to the leaves increased the photosynthesis rate in sesame under drought stress. This study aimed to determine the effect of interaction between salicylic acid and drought stress on growth and photosynthetic rate of *B. alba* and *B. Rubra* cultivated in Assalaam Islamic Modern Boarding School green house (used polybag), Pabelan, Kartasura, Sukoharjo, Central Java, Indonesia.

MATERIALS AND METHODS

Study area

This study was conducted from December 2022 to April 2023 and located in Assalaam Islamic Modern Boarding School green house, Pabelan, Kartasura, Sukoharjo, Central Java, Indonesia (7°3'11"S 110° 46'15"E). The temperature was captured between 23 to 31°C, and the humidity was around 64%. The ratio of media-fertilizer used per polybag is 2:1.

Procedures

Collection of seeds and preparation of seedlings

The *B. alba* and *B. rubra* seeds were bought from traditional markets with the *Ninu Farm* brand. Seeds are selected based on size, color and contain embryos. Then put the seeds in the seedling container and cover it with planting media mixed with manure. Regularly water every morning until the seeds are 8 weeks, then choose seeds of the same height.

Planting and maintenance

Seedlings were planted in polybags with 2 factors and 3 replications in a greenhouse. The first factor was Salicylic Acid (SA) doses of 0 mM (control), 2 mM, 4 mM, and 6 mM, and the second factor was field capacity (FC) 100% FC (control), 75% FC, 50% FC and 25% FC. Control treatment is the treatment that is not given salicylic acid and dry stress. There were 45 *B. rubra* and 45 *B. alba* with 15 treatment combinations, each repeated thrice.

Salicylic acid treatment was given using the method by Sanyari et al. (2013). It is sprayed on plants that already

have 3-6 true leaves, spraying salicylic with slight modifications every two days with an interval of 2 weeks for 2 months. Drought stress based on Field Capacity was given to plants 3 days after the first spraying of salicylic acid. The plant's watering was done every day with a watering quantity of 100% field capacity. After the age of the plants 60 days after planting, the watering volume was changed based on Field Capacity (FC) treatment (100, 75, 50, 25%). The initial process for determining Field Capacity (FC) was that polybags with 3 kg of planting media were doused with water and waited for the first drops to come out. Next, pour water until the first drop was 1,000 mL as a field capacity; then, calculate the groundwater level by taking 10 grams soil sample, letting it stand for 24 hours, and drying the sample in the oven at 60°C for 24 hours. The oven-dried soil samples were dried, and calculations were made for water concentrations; the formula for calculating soil water content by Saputra (2015). The weight of the soil obtained after baking is 6 g with 3 repetitions, then soil water content is determined by the following formula. Treatment of drought stress by looking at the condition, the amount of watering is adjusted to the level of water loss in treatment. Hence, the polybag conditions are within field capacity. The harvesting stage of *B. alba* and *B. rubra* was carried out when the plants were 90 days after planting or 4 weeks after treatment.

$$\text{GWL (\%)} = \frac{A-B}{A} \times 100\%$$

Where:

- A : Initial weight of soil sample before drying (g)
- B : Final soil weight after drying (g)

Observation

Plant height, leaf number, and leaf area were observed when the plants were 12 weeks after transplanting in the greenhouse. Photosynthesis measurements were carried out directly on the third leaf from the shoot. Photosynthesis and transpiration rate also stomatal conductance were observed with natural light 8.30 am-11.30 am with Plant Photosynthetic Meter NY-1020 (Zhengzhou Nanbei Instrument Equipment Co., Ltd.) measured on the leaf of *B. alba* dan *B. rubra*.

The leaf area measurement was done by the constant method. The leaves have fully opened, and the data is presented in cm² units. Measurement of leaf surface using millimeter paper with approximation, measured the length with (LA= L x W x K). Constant value on *B. alba* is 0.8934 and constant value on *B. rubra* is 0.9736

Data analysis

Normality and homogeneity tests were carried out at the beginning and then continued with a two-way Analysis of Variance (ANOVA) and continued with Duncan's multiple range test at a 5% level. DMRT test aims to determine the real effect of every treatment. If there is a significantly different effect with p-value < 0.05 or F count > F table, then H₀ is rejected.

The statistical test used the Pearson matrix correlation to measure the linear relationship between variables.

Pearson's correlation coefficients range from -1 (perfect negative correlation) to +1 (perfect positive correlation), with 0 indicating no correlation. The data were analyzed with SPSS 26.0 software.

RESULTS AND DISCUSSION

From 48 plants of *B. alba* and 48 plants of *B. rubra*, the growth rate parameters are height, leaf number, and leaf surface area (Table 1). The photosynthetic rate parameters are photosynthetic, transpiration, and stomata conductance (Table 2). The morphological appearance of *B. alba* and *B. rubra* plants can be seen in Figures 1 and 2.

Growth parameters

Plant height

Plant height is a variable that determines the effect between plant and plant growth parameters. The plant height measurement can determine the physiological function of plants. The DMRT test at the 5% level showed that the interaction of SA and DS had a slightly significant effect on *B. alba* and *B. rubra* (Table 1). The treatment between SA 6mM and FC 25% was the highest result in *B. alba* (52.90 cm), and between SA 4mM and FC 25% was the highest in *B. rubra* (52.70 cm).

Leaf area

Plant leaf area is a parameter directly related to photosynthesis and transpiration. The result showed (Table 1) that the treatment between SA 2 mM and FC 75% was the highest result in *B. alba* (91.72 cm²), and treatment between SA 4 mM and FC 50% was the highest result in *B.*

rubra (122.67 cm²). The lowest plant leaf area is in plants that were exposed to severe drought stress (25% FC), both *B. alba* (57.55 cm²) and *B. rubra* (57.59 cm²).

Leaves number

The result showed that the treatment between SA and FC did not affect the number of leaves of *B. alba* and *B. rubra*. Table 1 shows that the DMRT test at a 5% level in *B. alba* had the highest yield, namely 18 leaves in the treatment (25% FC and 2 mM SA) compared to the control, which totaled 11 leaves. In *B. rubra*, the highest yield interaction was in the 50% FC treatment and 6 mM SA with 23 leaves compared to the control, which totaled 12 leaves. The values between treatments in the graph are not different enough. The lowest number of leaves of *B. alba* is in several interactions (100% FC, 2 mM SA; 100% FC, 6 mM SA; 75% FC, 2 mM SA; 50% FC, 0 mM SA; and 25% FC, 0 mM SA) that is 10 leaves, in *B. rubra* the lowest number of leaves in several interactions (50% FC, 0 mM SA; 25% FC, 6 mM SA; and 25% FC, 0 mM SA) with 11 leaves.

Photosynthetic parameters

Photosynthetic rate

The result indicates (Table 2) the treatment between SA 6mM and FC 50% was the highest result in *B. alba* (0.8663 $\mu\text{mol m}^{-2} \text{s}^{-1}$), while the lowest value is the interaction between FC 25% and SA 0 mM (0.1144 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Treatment between SA 6mM and FC 75% was the lowest result in *B. rubra* (0.9202 $\mu\text{mol m}^{-2} \text{s}^{-1}$), and the highest is FC 25% and 0 mM SA (0.1406 $\mu\text{mol m}^{-2} \text{s}^{-1}$).

Table 1. The effect of interaction between Salicylic Acid (SA) and Drought Stress (DS) on *Basella alba* and *B. rubra* growth

Treatment	Height		Leaf area		Leaves number	
	<i>B. alba</i>	<i>B. rubra</i>	<i>B. alba</i>	<i>B. rubra</i>	<i>B. alba</i>	<i>B. rubra</i>
SA 0 Mm x FC 100%	48.20±2.27 D a	48.80±1.75 C ab	65.85±0.22 A a	58.75±0.23 A a	11±1.0 A a	12±2.51 A b
SA 2 Mm x FC 100%	35.13±4.35 D a	44.30±4.71 C a	65.11±0.77 A b	67.95±0.88 A d	10±1.73 A ab	15±3.51 A b
SA 4 Mm x FC 100%	43.16±1.79 D a	51.43±3.49 C a	66.63±0.48 A d	81.98±0.28 A c	12±2.51 A bc	12±4.35 A b
SA 6 Mm x FC 100%	27.90±1.35 D b	26.20±2.08 C b	74.86±0.78 A c	83.18±0.49 A b	10±1.0 A c	14±2.64 A b
SA 0 Mm x FC 75%	41.73±3.81 B a	51.93±4.16 B ab	74.61±0.43 D a	78.88±0.15 C a	8±2.08 B a	12±1.73 B b
SA 2 Mm x FC 75%	39.06±2.95 B a	33.23±2.66 B a	91.72±0.65 D b	87.45±0.07 C d	10±2.0 B ab	14±1.73 B b
SA 4 Mm x FC 75%	42.33±5.96 B a	49.50±1.05 B a	71.00±0.85 D d	122.67±0.23 C c	15±4.35 B bc	18±2.51 B b
SA 6 Mm x FC 75%	28.60±1.84 B b	25.56±5.12 B b	64.89±0.87 D c	66.67±0.33 C b	16±5.03 B c	21±3.78 B b
SA 0 Mm x FC 50%	44.13±5.25 C a	48.63±6.76 C ab	74.58±1.00 C a	76.90±0.87 D a	10±0.01 B a	11±1.00 BC b
SA 2 Mm x FC 50%	39.56±0.61 C a	42.20±4.45 C a	81.20±1.31 C b	68.76±0.64 D d	14±2.08 B ab	16±2.51 BC b
SA 4 Mm x FC 50%	50.16±2.65 C a	47.80±3.72 C a	82.8±1.18 C d	122.67±0.32 D c	16±5.03 B bc	17±4.35 BC b
SA 6 Mm x FC 50%	26.73±1.85 C b	25.56±1.96 C b	70.82±0.57 C c	74.64±0.48 D b	15±2.51 B c	23±3.78 BC b
SA 0 Mm x FC 25%	26.72±5.25 A a	44.06±10.79 A ab	57.55±0.24 B a	57.59±0.62 B a	10±0.57 B a	11±5.50 C b
SA 2 Mm x FC 25%	47.86±5.40 A a	50.50±2.69 A ab	91.67±0.85 B b	110.23±0.81 B d	18±2.0 B ab	12±3.24 C b
SA 4 Mm x FC 25%	47.86±2.02 A a	52.70±3.25 A ab	91.71±0.43 B d	68.100±1.11 B c	16±3.51 B bc	12±3.21 C b
SA 6 Mm x FC 25%	52.90±1.20 A b	32.43±1.91 A b	64.50±0.39 B c	58.75±0.43 B b	16±2.08 Bc	11±5.03 C b

Note: Different capital letters mean SA and DS levels, with each SA level marked in upper case letters significantly different, and DS marked in lower case based on the DMRT test at 5% level



Figure 1. All parts of *Basella rubra* plant. Note: a1. Control (not given stress and salicylic acid), b1. SA 0mM+FC 75%, c1. SA 0mM+FC50%, d1. SA 0mM+FC 25%, e1.SA 2mM+FC 50%, f1. SA 4mM+ FC 50%, g1. SA 6mM+FC 50%, h1. SA 2mM+FC 100%, i1. SA 4mM+FC 100%, j1. SA 6mM+FC 100%, k1. SA 2mM+FC 75%, l1. SA 4mM+ FC 75%, m1.SA 6mM+FC 75%, n1. SA 2 mM+FC 25%, o1. SA 4mM+FC 25%, p1. SA 2mM+FC 25%

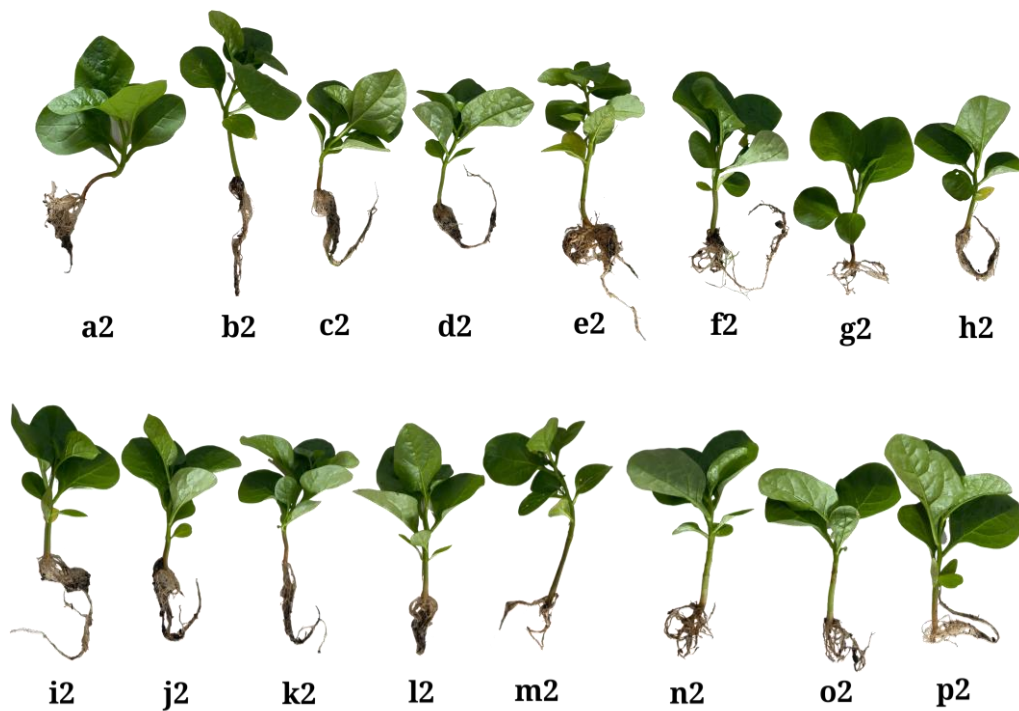


Figure 2. All parts of *Basella alba* plant. Note: a2. Control (not given stress and salicylic acid), b2. SA 0mM+FC 75%, c2. SA 0mM+FC50%, d2. SA 0mM+FC 25%, e2.SA 2mM+FC 50%, f2. SA 4mM+ FC 50%, g2. SA 6mM+FC 50%, h2. SA 2mM+FC 100%, i2. SA 4mM+FC 100%, j2. SA 6mM+FC 100%, k2. SA 2mM+FC 75%, l2. SA 4mM+ FC 75%, m2.SA 6mM+FC 75%, n2. SA 2 mM+FC 25%, o2. SA 4mM+FC 25%, p2. SA 2mM+FC 25%

Table 2. The effect of interaction between salicylic acid and drought stress on *Basella alba* and *B. rubra* photosynthesis rate

Treatment	Transpiration rate		Photosynthesis rate		Stomata conductance	
	<i>B. alba</i>	<i>B. rubra</i>	<i>B. alba</i>	<i>B. rubra</i>	<i>B. alba</i>	<i>B. rubra</i>
SA 0 Mm x FC 100%	0.012±0.0001 A c	0.0465±0.06 A a	0.2674±0.0001 B a	0.2133±0.0001 B a	0.1888±0.0001 B a	0.1615±0.004 A a
SA 2 Mm x FC 100%	0.0291±0.006 A a	0.0169±0.0002 A b	0.1084±0.002 B b	0.2424±0.002 B b	0.1084±0.002 B b	0.1885±0.0017 A b
SA 4 Mm x FC 100%	0.037± 0.002 Ad	0.018±0.001 A a	0.2636±0.002 B c	0.2423±0.002 B c	0.2636±0.002 B c	0.3869±0.004 A c
SA 6 Mm x FC 100%	0.0501±0.002 A b	0.0189±0.0001 A a	0.3962±0.0005 B d	0.1341±0.0005 B d	0.3962±0.0005 B d	0.1965±0.004 A d
SA 0 Mm x FC 75%	0.0197±0.0003 C c	0.0155±0.001 A a	0.1144±0.002 A a	0.2442±0.002 D a	0.1144±0.002 A a	0.326±0.006 C a
SA 2 Mm x FC 75%	0.0191±0.0002 C a	0.0425±0.0001 A b	0.2145±0.003 A b	0.5059±0.003 D b	0.2145±0.003 A b	0.1857±0.001 C b
SA 4 Mm x FC 75%	0.0108±0.0002 C d	0.0196±0.001 A a	0.5852±0.001 A c	0.2944±0.001 D c	0.5852±0.001 A c	0.5865±0.002 C c
SA 6 Mm x FC 75%	0.0643±0.0001 C b	0.0475±0.003 A a	0.6367±0.003 A d	0.9202±0.003 D d	0.6367±0.003 A d	0.9736±0.0005 C d
SA 0 Mm x FC 50%	0.0104±0.0007 B c	0.0105±0.0009 A a	0.3735±0.001 D a	0.464±0.001 A a	0.3735±0.001 D a	0.3368±0.004 D a
SA 2 Mm x FC 50%	0.0108±0.001 B a	0.0196±0.0006 A b	0.2446±0.003 D b	0.7448±0.003 A b	0.2446±0.003 D b	0.7258±0.003 D b
SA 4 Mm x FC 50%	0.0128± 0.0003 B d	0.024±0.0007 A a	0.8365±0.004 D c	0.8828±0.004 A c	0.8365±0.004 D c	0.9552±0.003 D c
SA 6 Mm x FC 50%	0.189±0.007 B b	0.0386±0.0008 A a	0.8663±0.002 D d	0.507±0.002 A d	0.9468±0.002 D d	0.8934±0.0007 D d
SA 0 Mm x FC 25%	0.0097± 0.001 D c	0.0121±0.002 B a	0.1144±0.007 C a	0.1406±0.0007 C a	0.1889±0.0007 C a	0.1857±0.001 B a
SA 2 Mm x FC 25%	0.0643±0.001 D a	0.0137±0.0004 B b	0.8044±0.002 C b	0.9075±0.002 C b	0.8044±0.002 C b	0.9546±0.003 B b
SA 4 Mm x FC 25%	0.0195±0.0001 D d	0.0192±0.004 B a	0.6716±0.003 C c	0.3773±0.003 C c	0.6716±0.003 C c	0.9736±0.005 B c
SA 6 Mm x FC 25%	0.011±0.001 D b	0.0285±0.0007 B a	0.2375±0.002 C d	0.5366±0.002 C d	0.2375±0.002 C d	0.3842±0.0005 B d

Note: Different capital letters mean SA and DS levels, with each SA level marked in upper case letters significantly different, and DS marked in lower case based on the DMRT test at 5% level

Transpiration rate

The results showed (Table 2) the highest value of *B. alba* plants at 50% FC and 6 mM salicylic acid ($0.1890 \mu\text{mol m}^{-2} \text{s}^{-1}$) compared to the control ($0.012 \mu\text{mol m}^{-2} \text{s}^{-1}$), while the lowest value was in the FC treatment 25% and 0 mM salicylic acid ($0.0097 \mu\text{mol m}^{-2} \text{s}^{-1}$). In *B. rubra*, the highest value was 75% FC and 6 mM salicylic acid ($0.0475 \mu\text{mol m}^{-2} \text{s}^{-1}$) compared to control ($0.0465 \mu\text{mol m}^{-2} \text{s}^{-1}$), the lowest value was 25% FC, 0 mM salicylic acid ($0.0121 \mu\text{mol m}^{-2} \text{s}^{-1}$).

Stomata conductance

The highest stomatal conductance (Table 2) of *B. alba* was at 50% FC and 6 mM salicylic acid ($0.9468 \mu\text{mol m}^{-2} \text{s}^{-1}$) compared to control ($0.1888 \mu\text{mol m}^{-2} \text{s}^{-1}$), while the lowest value was in the FC 25% and 0 mM salicylic acid ($0.1889 \mu\text{mol m}^{-2} \text{s}^{-1}$). In *B. rubra*, the highest value was at 75% FC and 6 mM salicylic acid ($0.9736 \mu\text{mol m}^{-2} \text{s}^{-1}$) compared to the control ($0.1615 \mu\text{mol m}^{-2} \text{s}^{-1}$), the lowest value was at 25% FC and 0 mM salicylic acid ($0.1857 \mu\text{mol m}^{-2} \text{s}^{-1}$).

Correlation between growth parameters and photosynthesis

The value of the correlation coefficient between parameters in *Basella* plants is shown in Table 3. Significant correlations were obtained between several growth parameters and the photosynthetic rate of *B. alba*. This shows that several growth parameters significantly contribute to the rate of photosynthesis and vice versa for the plants studied. As shown in Table 3 there is a positive linear correlation between height and leaf area ($r^2=0.247^*$), number of leaves and photosynthetic rate ($r^2=0.370^{**}$) and transpiration rate ($r^2=0.258^*$). Furthermore, leaf area is positively correlated with photosynthetic rate ($r^2=0.258^*$), photosynthetic rate is positively correlated with transpiration rate ($r^2=0.203^*$). For parameters that have a negative correlation only between plant height and transpiration rate ($r^2=-0.344^{**}$).

Discussion

Interaction effect between DS and SA on growth and photosynthesis rate

Drought stress greatly affects plant's physiological and biochemical activities, including photosynthesis, respiration, transpiration, hormone metabolism, and enzyme activity. ROS increases during drought stress, causing plant toxicity due to reduced electron transport activity. Excessive ROS Destroys Nucleic Acids (DNA), proteins, photosynthetic pigments, and membrane lipids and causes inactivation of enzymes involved in metabolism. Salicylic acid in plants experiencing drought stress inhibits catalase (CAT) activity; hence H_2O_2 increases. Furthermore, H_2O_2 helps

increase the activity of antioxidant enzymes to increase resistance in activating ROS. Plant responses to drought stress are varied, involving various mechanisms such as defense or modification of physiology, morphology, anatomy, and biochemistry, as well as adaptation processes related to short-term and long-term development and growth (Kordi and Fardin 2013; Hossain et al. 2015; Okunlola et al. 2017; Abobatta 2019; Hasanuzzaman et al. 2020; Zulfiqar and Ashraf 2021;)

Plants at 100% field capacity are in optimal growth conditions; water availability below field capacities inhibits plant metabolism. Under drought stress, plants are generally smaller because their vegetative growth is stunted. Salicylic acid and its derivatives sprayed on the leaves increased drought tolerance in *Basella* experiencing dry stress. Research shows that the application of salicylic acid (Morovvat et al. 2021) reported treatment of potatoes showed a significant effect on the water supply status of the plants; the effect of salicylic acid on irrigation treatment 60 and 80% showed an increase in potato yields under severe drought stress conditions. The best yield treatment was salicylic acid treatment with 100% irrigation. The reduction in length and weight caused by drought stress can be tolerated by applying Salicylic Acid (SA), which helps maintain internal water balance and protein synthesis during drought stress. Therefore, reducing leaf area during drought stress is an adaptation to reduce water loss through transpiration. Some plants can't maintain growth from drought stress when the field capacity is 100-50%. The turgor pressure of plants cannot be maintained if drought stress is severe (Yang et al. 2022).

Plants at various growth phases recognize drought stress and depend on the sensitivity or variety of the plant. For example, in this study, the two types of *Basella* showed that the growth percentage was not significantly different (Table 1). That shows *Basella* plants are not too sensitive to dry stress ranging from mild to severe intensity during the vegetative stage (growth of roots, stems, and leaves). This can be related to the synthesis of carbohydrates for cell division and growth due to the closure of stomata and considered to be repaired. Dry stress during the vegetative phase causes plant degeneration and poor and late germination, which affects plants' proper development; the responses of these two form *Basella* plants to drought through a tolerance mechanism. Drought avoidance describes the capacity of plants to maintain high water levels even with adequate humidity. This drought tolerance is the plant's ability to maintain a relatively high tissue water potential (Barnabás et al. 2008; Kumar et al. 2017; Yang et al. 2019)

Table 3. Pearson correlation matrix between growth parameters and photosynthesis

Parameters	Height	Leaves number	Leaf area	Photosynthesis	Transpiration	Stomata
Height	1					
Leaves number	-0,136	1				
Leaf area	.247*	0,190	1			
Photosynthesis	0.038	.370**	.258*	1		
Transpiration	-.344**	.258*	0,044	.203*	1	
N	96	96	96	96	96	96

Note: * Correlation is significant at the 0.05 (2-tailed), **Correlation is significant at the 0.01 level (2-tailed)

Photosynthesis is the main metabolic process that regulates plant growth and yield; this process is strongly influenced by water availability and nutrients in the soil. In dry stress, closing stomata reduces the amount of carbon dioxide reaching the leaves, causing the formation of ROS. Phytohormones such as salicylic acid are important in physiological and biochemical changes. A very important role of SA physiological response changes leading to plant adaptations to unfavorable environments has been reported. Although water shortages are harmful, plants can respond to varying degrees of water shortages; avoidance and tolerance mechanisms exist. The ability of plants to adapt to dry stress affects their biochemistry, physiology, and growth, especially photosynthesis (Nazar et al. 2015; Mishra et al. 2018; Sharma et al. 2022).

Drought stress (abiotic stress) decreases the photosynthesis rate (A), but applying salicylic acid sprayed on the leaves can reduce the impact. Dry stress with a 25% FC concentration is a severe condition for plants; a very low water deficit affects the photosynthetic rate, transpiration, and stomatal conductance (Table 2). During dry stress, stomata close progressively, followed by a decrease in photosynthesis. Closing of stomata significantly reduces the rate of photosynthesis and transpiration and negatively impacts plant growth and productivity. During drought stress, stomata limit gas exchange by closing stomata, which affects photosynthesis due to the limited CO₂ absorbed from the air. The stomata are closed to prevent excessive water loss from transpiration. Closing of stomata results in reduced NADPH during the Calvin cycle, so electron transport during photosynthesis decreases. During dry stress, photosynthetic activity is disrupted due to the energy balance between absorption capacity and energy uses. The rate of transpiration is greatly reduced under conditions of severe dry stress, but salicylic acid presumably can fix it (Pinheiro and Chaves 2011; Pirasteh-Anosheh et al. 2016; Liang et al. 2020; Rehschuh et al. 2020; Khalvandi et al. 2021; Alam et al. 2023).

This process begins by accumulating Abscisic Acid (ABA), closing stomata, and forming ROS. Furthermore, there was a decrease in the activity of carboxylase/oxygenase (Rubisco), NADP-malic enzyme, ribulose-1, 5-biphosphate, phosphoenolpyruvate carboxylase, pyruvate orthophosphate dikinase (PPDK) and fructose-1, 6-biphosphate (FEBase). Then the activity of rubisco is inhibited, causing downregulation of non-cyclic electron transfer, thereby inhibiting energy biosynthesis (ATP) and then a decrease in the rate of photosynthesis (Farooq et al. 2009). This study observed that the exogenous application of SA enhanced growth and photosynthesis and reduced the harmful caused by dry stress. SA-induced increase in photosynthesis, transpiration, and stomatal conductance during drought stress is caused by changes in physiological and biochemical processes due to SA induction. In our experiment, the photosynthesis rate increased significantly in drought-treated two species of *Basella* plant, and SA application enhanced the rate levels in these plants. The results of our research are SA can increase the rate of

photosynthesis in plants experiencing drought following research by Khalvandi et al. (2021) that the rate of photosynthesis increased by 28%, 48%, and 25% in winter-wheat given control and dry stress treatments.

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Assessment of genetic diversity among sweet potato varieties through RAPD markers in the Southern coastal region of Bangladesh

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Abstract. Saimon AH, Sultana S, Mannan MA, Mamun AA. 2023. Assessment of genetic diversity among sweet potato varieties through RAPD markers in the Southern coastal region of Bangladesh. *Asian J Agric* 7: 116-121. Sweet potato (*Ipomoea batatas* (L.) Lam.) is the sixth most significant food crop. Evaluating this crop's genetic diversity is crucial for food security and preserving agricultural genetic resources. Bangladesh is South Asia's second-largest sweet potato producer, but little is known about the genetic diversity of this crop there. The study aimed to assess the genetic diversity among six sweet potato varieties (Five BARI-released sweet potato varieties: BARI Misty Alu-10, BARI Misty Alu-11, BARI Misty Alu-12, BARI Misty Alu-14, BARI Misty Alu-15, and a local cultivar) using RAPD marker in the Southern coastal region of Bangladesh. Six primers were utilized to determine the polymorphic and monomorphic bands. Data was analyzed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram and Principal Component Analysis (PCA). Thirty-seven polymorphic bands were found, with an average of 3 polymorphic and 3.17 monomorphic bands. Primer OPM-02 showed the highest polymorphic bands (6). The results showed that BARI Misty Alu-10 and BARI Misty Alu-15 had the most genetic diversity, at 37%. The average polymorphism percentage was 44.88%. The dendrogram featured two distinct clusters that showed BARI Misty Alu-12, the most distant variety. The clustering pattern corresponded with PCA, demonstrating that BARI Misty Alu-12 had the most genetic variation (81.36%).

Keywords: Genetic diversity, molecular marker, polymorphism, RAPD, sweet potato

INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam.) is considered one of the most significant root crops in tropical and subtropical areas belonging to the Convolvulaceae family. It became sixth among the world's primary food crops (CIP 2019). It is considered one of the food security crops for its several resilient, vital features such as versatility, vigorous growth, drought and salt tolerance, cold tolerance, high productivity, and high adaptability with minimum inputs. (Lebot 2010; Sun et al. 2014; Ahmed et al. 2015). According to FAOSTAT (2020), Bangladesh is the second largest producer of sweet potatoes (27%) after India (68%) in South Asia. Sweet potato is a rich superfood with nutrients, carbohydrates, vitamins, and minerals, low fat and protein (Mahmud et al. 2021). It contains vitamin A, which helps alleviate vitamin A deficiency in children. Consuming sweet potatoes can also provide essential vitamins, minerals, and fibers, making it an ideal meal based on calories (Bovell-Benjamin 2007; Lebot 2010; Sun et al. 2019).

Genetic diversity is crucial for species continuity and adaptation to biotic and abiotic stress (Muhammed et al. 2012). It measures genetic variance and is a primary source of biodiversity (Hughes et al. 2008). Crop improvement programs require sufficient genetic diversity for successful hybridization. Primary and secondary centers of gene diversity occur in domesticated and introduced areas, with understanding this diversity essential for developing

effective measures for plant genetic resources (Roullier et al. 2013).

Agronomic, morphological, molecular, biochemical, physiological, and other traits can be used to assess genetic diversity. Among them, molecular markers manifest the presence of more polymorphic loci, making it possible to recognize separate accessions that might have identical morphological and agronomical characteristics (Goncalves et al. 2008); molecular markers aid in clarifying the crops' genetic framework, which supports successful breeding programs. Random Amplified Polymorphic DNA (RAPD) markers are frequently employed to determine the genetic connection between sweet potato cultivars (Tosti and Negri 2002), because the primers are in an arbitrary sequence, RAPDs can be created relatively quickly and easily (Verma et al. 2017). For assessing diversity and identifying germplasm in various plant species, RAPD is reasonably effective at detecting genetic variants (Moghaieb et al. 2017). On the other hand, the RAPD marker has some limitations, such as band profiles cannot be explained in terms of loci and alleles since the markers are not locus-specific (Tikendra et al. 2021). RAPDs have low reproducibility; hence, highly standardized laboratory techniques and purified DNA are essential (Reshma and Das 2021).

Many studies have assessed the genetic variability among different sweet potato varieties. Samiyarsih et al. (2020) investigated the genetic diversity among eight sweet potato cultivars in Central Java, Indonesia, and reported a

range of genetic similarities (37-93%) among the cultivars by RAPD markers. Shah et al. (2018) observed genetic distinctiveness among 92 sweet potato accessions collected from different locations in Malaysia and Indonesia through RAPD markers. However, no similar study of sweet potato varieties in Bangladesh's coastal region prioritized this experiment. Among the 17 sweet potato varieties released by Bangladesh Agricultural Research Institute (BARI), 5 sweet potato varieties recommended for saline areas (BARI Misty Alu -10, BARI Misty Alu -11, BARI Misty Alu -12, BARI Misty Alu -14, and BARI Misty Alu -15) and a local cultivar were used in this study. The study aimed to assess the genetic diversity among the 5 sweet potato-released varieties of BARI and a local cultivar through RAPD markers.

MATERIALS AND METHODS

Research site

The experiment was conducted at the Plant Protection Laboratory, Molecular, and Horticulture Laboratory of Agrotechnology Discipline, Khulna University, Khulna District, Bangladesh, from January 2022 to June 2022.

Plant materials

Moreover, 6 sweet potato varieties leaf samples were collected from two locations in the Khulna region: Germplasm Center of Khulna University and farmer's fields in Batiaghata Upazila of Khulna District (Table 1). The zone is within AEZ-11, which stands for 'High Ganga River Flood Plain. The maximum annual mean temperature of the region is 35.5°C, and the minimum is 12.5°C, whereas the mean annual rainfall is 1,710 mm (Rashid et al. 2014).

Procedures

DNA isolation

Six varieties of sweet potato leaf samples (each 3 g) were powdered with liquid nitrogen with a sterilized mortar and pestle and were taken in a 1.5 mL Eppendorf tube. Then, those were stored at -86°C ultra-low freezer (Thermo Scientific, USA). DNA was isolated using GeneDireX, Inc. Plant genomic DNA extraction kit followed the manufacturer's protocol. The genomic DNA of six varieties was extracted, and the Eppendorf tube's DNA solution was stored at -20°C. An electrophoresis test in 1% agarose gel was run to confirm the presence of DNA materials. The Gel documentation system showed a picture of DNA residues under UV light. Multiskin GO Microplate Spectrophotometer (Thermo Fisher Scientific, Germany) was used to quantify purified DNA at a wavelength of 260 nm. The final concentration of the template DNA for PCR

was adjusted to 50 ng (μL^{-1}) and stored at -20°C (Kandan et al. 2013).

PCR amplification and electrophoresis

A Polymerase Chain Reaction (PCR) was performed in a 0.2 mL thin-walled PCR tube. Amplifications were performed in a thermocycler (Biometra 4, Germany) utilizing the protocols: initial denaturation at 95°C for 4 min, 40 cycles of denaturation at 94°C for 30 s, following annealing at 55°C for 60 s, and the final extension at 72°C for 5 min. Then cool down to 8°C. Six Primers were utilized to screen out the DNA banding pattern (OPM-02, OPM-03, OPM-04, OPM-05, OPM-10, and OPM-12). The product amplifications were separated by electrophoresis using 1% agarose gels with TAE buffer and ethidium bromide (at 0.5 $\mu\text{g mL}^{-1}$) for staining. Then, high-voltage electricity (90 V) was supplied to the Tris Acetate EDTA buffer solution (1.0 X TAE) for 45 minutes.

A Bioneer gel electrophoresis machine was used. The electrophoresis gels were scanned, and photographs were taken with Biodoc Analyze Computer software related to the Gel documentation system, version 2.2 (Biometra gel documentation, A Biodoc Analyze 2.2 version, Germany). Molecular weight indicator 1kbp (Direct load, Sigma Aldrich, USA) was used to size the amplicons.

Data analysis

RAPD-PCR bands were detected using the gel documentation technique. Bands were scored according to absence (0) or presence (1). All scored band was considered as single allele/locus. The band sizes were determined using a standard 1kb ladder/marker. Some parameters such as polymorphism percentage, polymorphic information content, resolving power of the marker (R_p), and marker index were calculated to evaluate the RAPD primers utilized in this study's informativeness and discriminating power. Then binary data were subjected to similarity correlation analysis by simple matching coefficient, and then an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram was constructed.

The UPGMA is a clustering method used to analyze and represent the genetic relationships between different samples based on their genetic data. Moreover, Principal Component Analysis was conducted to calculate the eigenvalue and eigenvector, and then a 3D plot was made using Numerical Taxonomy and Multivariate Analysis System version 2.01e (NTSYSpc) portable software (Rohlf 2002). The Eigenvalues are used to determine how many factors to retain. The sum of the Eigenvalues usually equals the number of variables (Bhanupriya et al. 2014).

Table 1. Sweet potato variety name, label, and collecting site

Variety	Sample Labeling	Collecting Site
BARI Misty Alu-12	SP1	Germplasm Center of Khulna University
BARI Misty Alu-10	SP2	Germplasm Center of Khulna University
BARI Misty Alu-11	SP3	Germplasm Center of Khulna University
BARI Misty Alu-14	SP4	Germplasm Center of Khulna University
BARI Misty Alu-15	SP5	Germplasm Center of Khulna University
Local cultivar	SP6	Farmer's field in Batiaghata Upazila of Khulna District

RESULTS AND DISCUSSION

Genetic variation generated by RAPD markers:

Genetic variation was detected among six sweet potato varieties using RAPD markers. Polymorphic and monomorphic bands were detected among the amplified DNA products. Six RAPD primers produced 37 bands. Among them, 18 were polymorphic bands, and 19 were monomorphic bands. Primer OPM-2 generated the highest

polymorphism percentage (75%). Primers OPM-03, OPM-04, OPM-05, OPM-10, and OPM-12 showed 40%, 20%, 20%, 71.42%, and 42.86% polymorphism percentage, respectively, with an average of 44.88% polymorphism percentage per primer (Table 2).

RAPD patterns of the amplified bands with sizes between 500 to 3000 bp for all primers are shown in Figure 1.

Table 2. RAPD primer sequences with the number of polymorphic and monomorphic bands

Primer	Primer Sequence (5' to 3')	GC Content (%)	NPB	NMB	PP (%)
OPM-02	ACAACGCCTC	60	6	2	75
OPM-03	GGGGGATGAG	70	2	3	40
OPM-04	GGCGGTGTC	70	1	4	20
OPM-05	GGGAACGTGT	60	1	4	20
OPM-10	TCTGGCGCAC	70	5	2	71.42
OPM-12	GGGACGTTGG	70	3	4	42.86
Total number of amplified bands			18	19	
Average amplified bands			3	3.17	
Total Number of amplified bands (Monomorphic and Polymorphic)				37	
Average polymorphism percentage				44.88	

Note: NPB: Number of Polymorphic Bands; NMB: Number of Monomorphic Bands; PP: Polymorphism Percentage

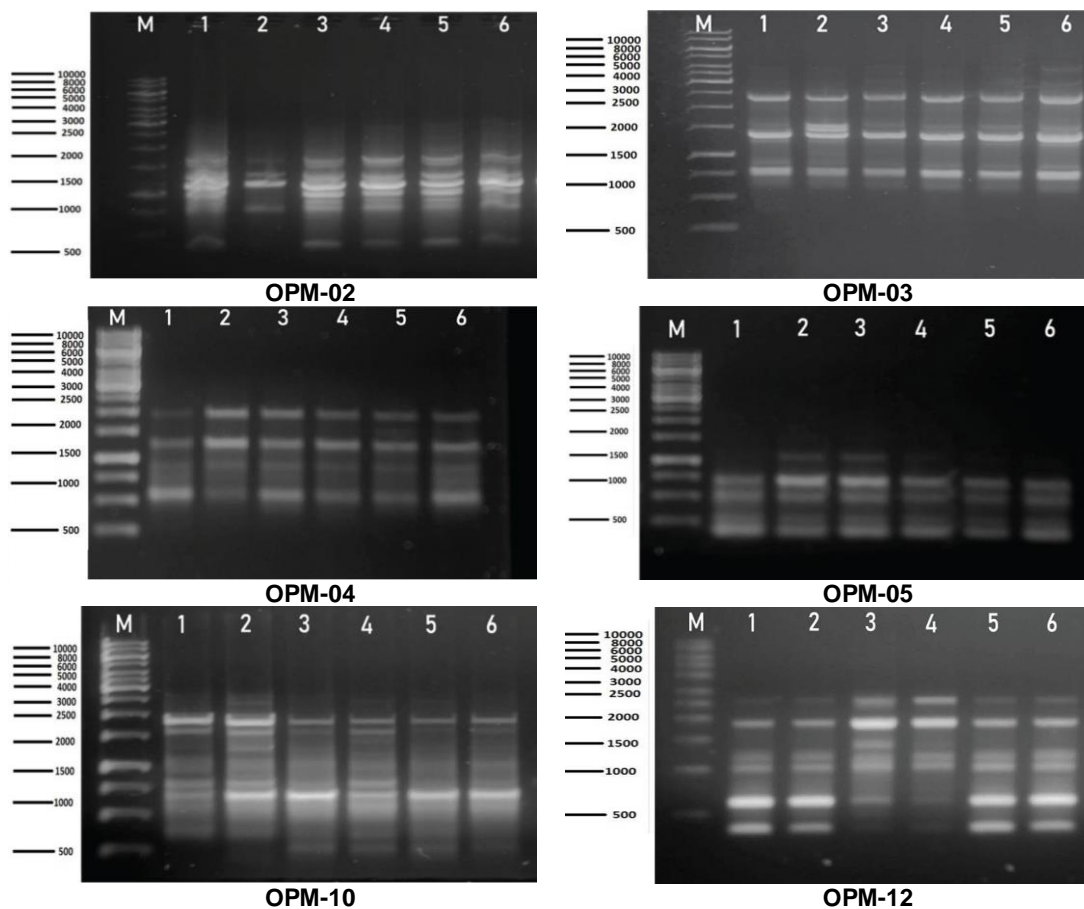


Figure 1. RAPD banding patterns of six sweet potato varieties generated by: A. OPM-02, B. OPM-03, C. OPM-04, D. OPM-05, E. OPM-10, F. OPM-12. Note: M: Ladder, 1: SP1, 2: SP2, 3: SP3, 4: SP4, 5: SP5, 6: SP6

Genetic similarity among varieties of sweet potato

The genetic similarity among six sweet potato varieties is shown in Table 3. According to Jaccard's coefficient, the genetic similarity of each variety ranged from 0.63 to 0.90. The SP5 and SP6 varieties showed the highest genetic similarity of 0.9, whereas SP2 and SP5 showed the lowest genetic similarity of 0.63.

Cluster analysis

The UPGMA cluster analysis constructed a dendrogram among six sweet potato varieties presented in Figure 2. All varieties studied were separated into two distinct clusters. Cluster I contained only one variety, BARI Misty Alu-10 (SP2), indicating its distant characteristic. Cluster II was divided into two subclusters (II A and II B), where subcluster IIA had only one variety, BARI Misty Alu-12 (SP1), and subcluster II B constituted SP3, SP4, SP5, and SP6 varieties.

Principal Component Analysis (PCA)

The Principal Component Analysis (PCA) mostly confirms the cluster analysis. The initial step is to compute eigenvalues, which specify how much overall variation is shown on the Principal Component (PC) axes. The first PC describes most of the variability in the original data compared to the subsequent PCs. Furthermore, the second PC explains most of the variability not described by the first PC and is unrelated to the first, and so on (Jolliffe 1986). Here, the first PC (SP2) accounted for about 81.36% of the total variation for all genetic traits. In comparison, the second PC (SP1) accounted for 7.85% of the total variation (excluding SP1), and so on, indicating genetic variability among the six sweet potato varieties (Table 4).

Based on the three-dimensional plot of PCA, the most genetically identical varieties were discovered in BARI Misty Alu-15 (SP5) and local cultivars (SP6). The distant relationship among the BARI Misty Alu-10 (SP2) and BARI Misty Alu-15 (SP5) was detected (Figure 3). SP2 exhibited a distant connection with the other varieties.

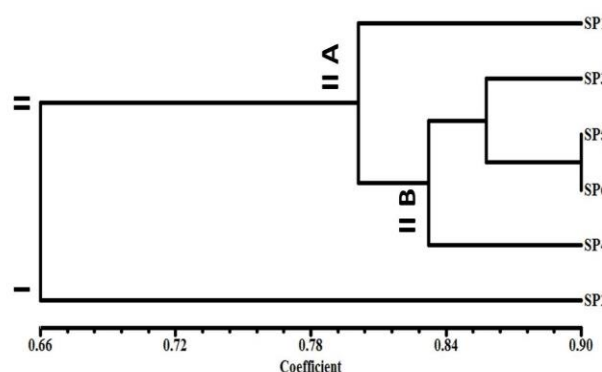


Figure 2. A dendrogram among six varieties of sweet potato generated by the UPGMA cluster analysis

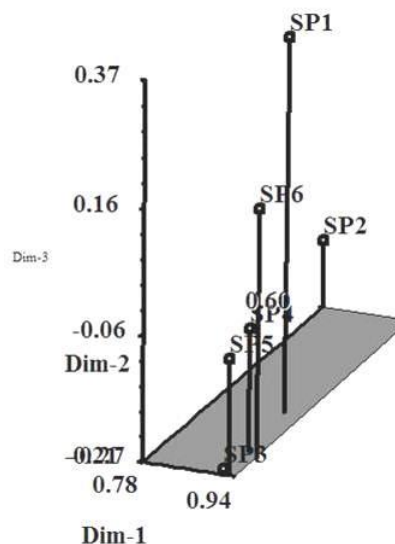


Figure 3. Three-dimensional plot of PCA showing relationships between six sweet potato varieties using genetic traits

Table 3. Genetic similarity among six sweet potato varieties

Variety	SP1	SP2	SP3	SP4	SP5	SP6
SP1	1.00					
SP2	0.71	1.00				
SP3	0.72	0.64	1.00			
SP4	0.81	0.66	0.84	1.00		
SP5	0.87	0.63	0.87	0.84	1.00	
SP6	0.87	0.66	0.84	0.81	0.90	1.00

Table 4. PCA analysis of the six (sweet potato varieties) principal components

PC components	Eigenvalues	Percent of variation	Cumulative variation
SP2	4.88	81.36	81.36
SP1	0.47	7.85	89.22
SP3	0.26	4.47	93.69
SP4	0.19	3.28	96.98
SP5	0.11	1.93	98.91
SP6	0.06	1.08	100.00
Sum of Eigenvalue= 06			

Discussion

Six primers amplified 37 polymorphic (18) and monomorphic (19) bands. Primer OPM-2 generated the highest number of amplified bands (8) and polymorphism percentage (75%) (Table 2). However, polymorphic bands generated by primer OPM-2 are lower than in the study conducted by Nusifera and Alia (2019). They reported that

primer OPM-2 generated 13 polymorphic bands on the cinnamon plant. Based on our results, the average polymorphism percentage was 44.88% (Table 2). These findings demonstrated the applicability of random PCR primers to characterize and evaluate intra-specific polymorphisms among sweet potato varieties. The higher the proportion of polymorphism, the more likely two or

more traits will be located on a single gene. Moulin et al. (2012) found a 42.8% polymorphism percentage compared to other studies using Egyptian sweet potato germplasm when tested on Brazilian sweet potato landraces. Lee et al. (2019) studied Korean sweet potatoes and found that the highest polymorphism percentage was 83.3%. The RAPD marker's high level of polymorphism detection and the ability to screen a larger number of anonymous loci suggest that this marker can effectively discover sweet potato germplasm.

Understanding the genetic diversity within and between populations is critical to creating efficient and cost-effective conservation methods for plant genetic resources (Cadima et al. 2017). Based on our study, the highest genetic similarity presented by BARI Misty Alu-15 and Local cultivar varieties is 0.90. This similarity indicates that they had a common parent, and their traits are remarkably similar. At the same time, BARI Misty Alu-10 (SP2) and BARI Misty Alu-15 (SP5) had the lowest genetic similarity of 0.63. This finding suggests that sweet potato germplasm represents diversity in genetic characters. This distinctiveness will help with environmental adaptation to different circumstances. As a result, such variety may provide helpful features to overcome the problems with food security (Onda and Mochida 2016). Soegianto et al. (2011) observed 0.78 genetic similarity between the *Biru Ungu* and *Bestak* clones utilizing RAPD markers. Veasey et al. (2008) investigated the genetic differences of 78 Brazilian sweet potatoes and revealed that 0.418 similarity was spread in households.

Cluster analysis was done by UPGMA dendrogram using the TREE program. Six sweet potato varieties were clustered into two main clusters using a cutoff point at a distance of 0.06. Cluster I featured only one variety, BARI Misty Alu-10 (SP2), while cluster II had the remaining varieties, indicating a distant relationship between SP2 and the other varieties (Figure 2). Because of the creation of these two clusters, the distribution pattern may contain two gene pools. Singh et al. (2017) and Bhadauriya et al. (2018) also reported a distant relationship during the clustering of sweet potato varieties.

PCA demonstrated the genotypic relationship among the varieties. Cluster analysis corresponds with PCA, indicating comparable results. According to PCA, the first four PCs comprised 96.96% of the total variation, whereas the first PC (SP2) accounted for 81.36% of the total variation and exhibited a high distant correlation among the varieties analyzed. There was a clear distribution pattern of sweet potato varieties based on their genetic variation. A possible explanation for the distribution pattern in this study would be farmer's preferences and genetic adaptation. Farmers often determine their variety selection by taste, texture or color, and yield. A farmer's decision to select a particular cultivar is based on a combination of technical and socioeconomic factors (Sthapit et al. 2008). Crop adaptation may involve natural processes like mutation and continuous outcrossing between genotypes (Flint-Garcia 2013). Thus, these processes may create variations in sweet potato germplasm. Special further assessment should be focused to characterize these varieties.

In conclusion, the study showed that genetic diversity among six sweet potato varieties was detected by RAPD markers in the Southern coastal region of Bangladesh. All varieties studied were separated into two distinct clusters based on cluster analysis. Cluster I contained only one variety, BARI Misty Alu-10 (SP2), indicating its distant characteristic. Cluster II was divided into two subclusters (II A and II B), where subcluster IIA had only one variety, BARI Misty Alu-12 (SP1), and subcluster II B constituted SP3, SP4, SP5, and SP6 varieties. Our result was confirmed by Principal Component Analysis (PCA).

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Evaluation of the effect of nicosulfuron and bentazone herbicides on growth and yield performance of two maize varieties in Mubi, Nigeria

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Abstract. Tizhe TD, Alonge SO, Adekpe DI, Ioortsuun DN. 2023. Evaluation of the effect of nicosulfuron and bentazone herbicides on growth and yield performance of two maize varieties in Mubi, Nigeria. *Asian J Agric* 7: 122-130. This study aimed to determine the effect of nicosulfuron and bentazone herbicides on the growth and yield performance of maize (*Zea mays* L.) varieties SAMMAZ 17 and SAMMAZ 37 in Mubi, Nigeria. The study was carried out between 2019 and 2020 wet seasons. Nicosulfuron and bentazone herbicides at 50, 100, 150, and 200 g/ha and 0.5, 1.0, 1.5, and 2.0 kg a.i/ha concentrations, respectively, along with hoe weeded and weedy check controls were applied in the field of the two maize varieties at 3, 5 and 7 Weeks after Sowing (WAS). The maize growth parameters were determined two weeks after treatment at three different times, while the yield parameters were determined using standard procedures. The results showed that nicosulfuron and bentazone had no significant effect on the Crop Growth Rate (CGR), Net Assimilation Rate (NAR), days to 50% silking (except Relative Growth Rate and days to 50% tasselling), and plant height, leaf area index and total biomass of the two maize varieties, when sampled at 2 and 4 (except at 6 weeks) weeks sampling after herbicides application at 3, 5 and 7 WAS. The application at 3 WAS resulted in lower values for virtually all the growth parameters than that at 5 and 7 WAS. The two herbicides, however, significantly affected most of the maize yield parameters. The maize plants on plots treated at 3 WAS had the highest values for all the yield parameters (except kernel depth) than those treated at other periods. The study concluded that the post-emergence application of nicosulfuron and bentazone herbicides had no significant influence on the growth parameters of SAMMAZ 17 and SAMMAZ 37; however, there was a significant effect on the yield parameters.

Keywords: Bentazone, herbicides effect, maize, nicosulfuron effect, yield parameters

INTRODUCTION

Maize (*Zea mays* L.) is an annual cereal crop belonging to the Poaceae family. It can be cultivated in every continent except Antarctica due to the lack of good environmental conditions favorable for its growth. Based on days to maturity, the crop is divided into four (4) categories, namely late, medium, early, and extra early maturing varieties that take 100-120, 91-95, 86-90, and 80-85 days to mature respectively (Ibrahim and Sunusi 2019). It is the most widely distributed crop grown globally in tropical and sub-tropical regions in not less than 166 countries. In 2020, the global production rate of maize was 1.16 billion metric tonnes cultivated on 197 million hectares (FAO 2014), with Nigeria having 12.8 million metric tonnes that however, rose to 13.94 million metric tonnes in 2021, thus placing Nigeria the 11th producer globally, and the second largest in Africa after South Africa (ThriveAgric 2022).

Maize is consumed by people of different food preferences and socio-economic backgrounds in Africa (Olaniyan 2015). Its consumption rate is estimated to be more than 116 million metric tonnes, with about 30% and 21% of the consumption rates being globally and in Sub-Saharan Africa, respectively (Knoema 2021). The entire parts of the plant can be used for food and non-food products. It could be consumed as a vegetable because of

its richness in dietary fiber and nutrients or used as feed for poultry and livestock, for extraction of edible oil, and for the starch and glucose industry (Hawaladar and Agasimani 2012).

The cultivation of maize is faced with the challenge of competition with weeds over growth resources that include soil nutrients, water, sunlight, and space during vegetative growth and reproduction, eventually leading to very poor grain yield (Haji et al. 2012). Considering the increase in demand for food by humans, the use of hand hoe weeding as a means to curtail crop/weeds competition to increase crop yield has been very costly and sometimes not available in the required quantity; this thus culminated in the introduction and use of herbicides (Haji et al. 2012). Nicosulfuron herbicides control broad leaves, sedges, and most grasses in maize fields post-emergence. It has general selectivity that ensures that all plants, including closely related plants growing near maize, are killed. The selectivity of the nicosulfuron herbicides is brought about by the ability of the maize plant to metabolize the nicosulfuron into harmless compounds (ThriveAgric 2022). Therefore, due to effective weed control and the use of nicosulfuron in maize fields, a significant increase in some yield parameters of the maize was recorded (Akadiri et al. 2017). Although nicosulfuron herbicides are made to control and kill plants other than maize, about 30% of visible injuries, reduction in height, and even death of some

varieties of maize when applied as post-emergence were reported (Robinson et al. 2017). Tianjun et al. (2018) reported that the significant reduction in growth parameters of maize due to the post-emergence application of nicosulfuron depended upon the maize variety and concentration used. Bentazone herbicides are selective contact herbicides that could be applied post-emergence in maize fields to control mostly broadleaf weeds, sedges, and a few grasses (Eastmond and Balakrishnan 2010). Using herbicides significantly increases maize plants' yield due to effective weed control (Sharara et al. 2005) and wheat (El-Rokiek et al. 2022). Although bentazone herbicides are selective for maize, rice, cowpea, sorghum, soybeans, and peanut, however, its application at the different developmental stages of soybeans resulted in some visual injuries that impacted negatively on the growth of the plant (Ali et al. 2021). The application of herbicides in crop fields, however, must be done at the appropriate time and dosage, as wrong timing for the application of post-emergence herbicides, even at the recommended doses, could inflict some injuries on plants that might affect the grain size and yield or even kill the desirable plants (Legleiter and Johnson 2012; Varshney et al. 2012). The period for application of post-emergence herbicides could also determine how effective the herbicides could be on different weed species available in the crop fields (Mayerová et al. 2018).

SAMMAZ 17 and SAMMAZ 37 are improved medium and late-maturing maize varieties developed by the Institute for Agricultural Research (IAR) Samaru, Zaria, in collaboration with the International Institute of Tropical Agriculture (IITA). The SAMMAZ 17 possesses a white color kernel and has tolerance to *Striga hermonthica*. The SAMMAZ 37 also has tolerance to *S. hermonthica* but possesses a yellow kernel and resistance to streak virus disease and drought. Therefore, because of the qualities these two maize varieties possess, farmers in areas with a high infestation of *S. hermonthica* and low annual rainfall are found to cultivate these maize varieties. However, there is a shortage of literature on the effect of nicosulfuron and bentazone herbicides on the growth and yield parameters of the two maize varieties. Also, farmers in the Adamawa State region are found to use nicosulfuron and bentazone-containing herbicides for the post-emergence control of weeds in maize fields because of their effectiveness in controlling narrow and broadleaved weeds. Therefore, because the effect of herbicides on plants depended upon some factors like time of application, the species and variety of plant, and concentrations of herbicide, this study was deemed necessary to assess the effect of nicosulfuron and bentazone herbicides on SAMMAZ 17 and SAMMAZ 37 growth and yield parameters.

MATERIALS AND METHODS

Study area

The study was conducted during the 2019 and 2020 wet seasons in the research farm of the Department of Crop Science, Adamawa State University, Mubi, Nigeria. The

location of the research farm falls within the North Eastern region of Nigeria, between Latitude 10°16' 06" N and Longitude 13°16' 01" E, and has an elevation of 582 m above sea level. The area has a tropical climate with an average annual temperature of 32°C, and lies within Nigeria's Sudan Savannah vegetation zone. The area has an average relative humidity of 28-45% and an average annual rainfall of about 1,056 mm.

Treatments and experimental design

The study consisted of two herbicides (nicosulfuron and bentazone), each at 4 different concentrations (i.e., nicosulfuron: 50.0, 100.0, 150.0, and 200.0 g/ha; Bentazone: 0.50, 1.00, 1.50 and 2.00 kg a.i/ha), hoe weeded and weedy check, two maize varieties (SAMMAZ 17 and SAMMAZ 37) and 3 times of application (i.e 3, 5 and 7 WAS). A split plot design that was replicated three times was used for the study. The maize varieties and period of herbicide applications were assigned to the main plot, while the herbicide concentrations were in the sub-plots.

Source of seeds for planting

The seeds of the SAMMAZ 17 and SAMMAZ 37 maize varieties were obtained from the Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Land preparation

The experimental field, which was 90.5 x 54.5 m² in size, was after being cleared of thorns and any other plant regrowth ridged with an inter-row spacing of 75 cm apart using an animal-drawn ridger. It was then divided into 60 plots and replicated three times, thus giving a total of 180 plots.

Seed treatment and planting

The maize seeds for the study were treated with Momtaz 45 WS seed dressing chemical, then planted at three in a hole of about 2 cm deep at an intra-row spacing of 25 cm and inter-row spacing of 75 cm. The seedlings were thinned to one per stand at 2 WAS, thus giving a total of 120 seedlings/plot.

Herbicides application

The herbicides were applied at different concentrations using a back-mounted 16-liter Knapsack sprayer at 3, 5, and 7 WAS.

Fertilizer application

The maize plants in all the plots were supplied with a dose of NPK (15:15:15) fertilizer at the rate of 400 kg/ha at 2 WAS, while at 5 WAS, a dose of urea fertilizer (46% N) was applied at the rate of 130.43 kg N/ha.

Data collection

Measurement of maize growth parameters

The determination of the maize growth parameters that include plant height, leaf area index, total dry matter, crop growth rate, relative growth rate, net assimilation rate,

number of days to 50% tasseling, and number of days to 50% silking was carried out according to the methods described by Wood and Roper (2000) and Matusso (2016). As mentioned earlier, the sampling for the growth parameters of the maize plants treated with herbicides at 3, 5, and 7 WAS was carried out at 5, 7, and 9 WAS, 7, 9, and 11 WAS, and 9, 11, and 13 WAS, respectively.

Determination of yield parameters

Cob weight (g). The maize plant cobs from each plot were harvested when fully dried. Furthermore, 5 matured cobs randomly selected were de-husked and weighed from each treatment plot, and the average was recorded and expressed in grams.

Cob length (cm). The meter rule was used to measure the length of each of the 5 sampled cobs and the average taken and expressed in centimeters.

Cob diameter before shelling (cm). The diameter for each of the 5 cobs was measured with a Vanier caliper measured at the middle of the Cob. The average was determined and expressed in centimeters.

Cob diameter after shelling (cm). The diameter of each of the 5 cobs in 2.8.2.3 above was measured after shelling at the middle of the shelled Cob with a Vanier caliper, and the average values were taken and expressed in centimeters.

Kernel depth. The kernel depth was determined by subtracting the values of the cob diameter after shelling from the cob diameter before shelling using the values of cob diameter before and after shelling.

Number of kernel rows per Cob. The number of rows for each of the five cobs randomly selected was counted, and the average was recorded.

Number of kernels per row. The number of grains for each row of the three randomly selected maize cobs was counted, added, and divided by the total number of rows of the cobs, and the average was obtained.

Weight of kernels per Cob. The weight of kernels on each of the sampled 5 maize cobs was taken, and the average was recorded and expressed in grams.

Grain yield (kg) per hectare. The maize plants' cobs were harvested when the plants were matured and fully dried. The cobs harvested on each net plot were threshed, cleaned, weighed, and expressed in kg per hectare.

100 – grain weight (g). The 100 grain weight was determined by randomly selecting 100 grains from each plot's total grains and was weighed and recorded as 100-grain weight.

Threshing percentage (%). This is the percentage of the weight of kernels per cob weight concerning total cob weight. It was calculated using the equation below:

$$\text{Threshing percentage} = \frac{\text{Weight of kernels}}{\text{Cob weight}} \times 100$$

Cob yield per hectare. The harvested cobs of each net plot were dried, de-husked, weighed, and expressed in kg per hectare.

Data analysis

The data generated from this study were subjected to a two-way Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 26.0. Where there was a significant difference, Duncan's Multiple Range Test (DMRT) was used in separating means.

RESULTS AND DISCUSSION

Effect of nicosulfuron and bentazone on the growth parameters of SAMMAZ 17 and SAMMAZ 37

The results showed that the post-emergence application of nicosulfuron and bentazone herbicides at different concentrations in the field of SAMMAZ 17 and SAMMAZ 37 had no significant effect on the plant height of the two (2) maize varieties sampled at 2 and 4 weeks after herbicides application. Although, the herbicides resulted in comparable values for the plant height of the 2 maize varieties, but at each of the sampling weeks after the herbicides application, the different nicosulfuron treatments resulted in lower values for the plant height of the 2 maize varieties than did the different bentazone treatments and also the hoe weeded and unweeded controls. The 3, 5 and 7 WAS times of the herbicides application on the other hand, had significant effect on the plant height of the 2 maize varieties at all the sampling weeks after application. The 3 WAS application time led to significant reduction in plant height than did the 5 and 7 WAS applications when the plants were sampled at 2 and 4 weeks sampling after application and in the overall combined data (Table 1). Similarly, it was observed that at each of the sampling weeks after the application of the 2 herbicides and at the overall combined data, the different nicosulfuron treatments led to lower leaf area index in the 2 maize varieties than bentazone treatments and hoe weeded and unweeded control treatments. The leaf area index of the 2 maize varieties due to hoe weeded and unweeded control treatments were comparable at all the sampling weeks after application. The herbicides application at 3 WAS led to lower values for the leaf area index at all the sampling weeks and overall combined data compared with that due to 5 and 7 WAS application (Table 2). Also, the values for total biomass of the 2 maize varieties due to the varied treatments of nicosulfuron were significantly similar with that due to the different treatments of bentazone as well as that of the hoe weeded and unweeded control treatments at all the sampling weeks and the overall combined data. The herbicides application at 3, 5 and 7 WAS, however, had significant effect on the 2 maize varieties total biomass at all the sampling weeks after application. The 3 WAS application led to significant reduction in the maize plants total biomass when compared with that due to 5 and 7 WAS application at all the sampling weeks after application and the overall combined data (Table 3).

The reduction in values for those growth parameters as a result of nicosulfuron treatments could be linked to the temporary interference to DNA synthesis and stoppage of cell growth caused possibly by inhibition of branched-chain amino acids as a result of stoppage to the activity of

enzyme acetolactate synthase as attested to by Silva (2007). This explains why there was an increase in values for the growth parameters with an increase in sampling age. Meng et al. (2019) further reported the reduction in plant height and fresh weight of maize plants due to the application of nicosulfuron. Tianjun et al. (2018) reported that the reduction in values for plant growth parameters like leaf area, root length, root surface area, and root volume depended upon the variety of the plant, the herbicide types, and concentrations. Therefore, this could be responsible for the higher values of the growth parameters mentioned above of SAMMAZ 17 than that of SAMMAZ 37. The higher values for most of the SAMMAZ 17 and SAMMAZ 37 growth parameters as a result of bentazone treatments than that of nicosulfuron could be because nicosulfuron, especially at higher concentrations, affect some essential physiological plant processes that are involved in protein synthesis and cell growth which eventually affect normal plant growth (Agchemaccess 2022) than bentazone which only affect plants' photosynthetic rate.

Also, on the other growth parameters (except relative growth rate and days to 50% tasselling) of maize variety SAMMAZ 17 and 37, the nicosulfuron and bentazone treatments showed no significant effect (Table 4). The 2.0 kg a.i./ha bentazone treatment resulted in the higher values for the SAMMAZ 17 and SAMMAZ 37 growth parameters like Crop Growth Rate (CGR) and Relative Growth Rate

(RGR). In contrast, the 100 g/ha nicosulfuron treatment had higher values for Net Assimilation Rate (NAR) and silking than other treatments (Table 4). Similar findings were reported by Sharara et al. (2005), Sharma et al. (2018), and El-Rokiek et al. (2022), whose studies recorded a significant increase in growth parameters of maize, rice, and wheat, respectively. They associated the increase in the growth parameters to bentazone application. Also, Akadiri et al. (2017) assessed the effect of nicosulfuron, 2,4-D, and atrazine on the growth parameters of maize plants. They discovered that nicosulfuron treatments had higher values for some of the maize growth parameters like leaves per plant and stem girth than that due to the other herbicide treatments.

Also, the interaction effect of herbicides and time of application, herbicides and variety, time of application and variety and herbicides, time of application and variety was not significant on the plant height, leaf area index and total biomass of the 2 maize varieties at all sampling time (Tables 1-3). This was similarly observed in Table 4 especially on NAR, 50% silking and tasselling. This was an indication that the interaction of herbicides, time of application and variety of the maize plants was not such that they would significantly affect the plant height, leaf area index, total biomass and some of the other growth parameters of the 2 maize varieties.

Table 1. Effect of post-emergence application of nicosulfuron and bentazone on the plant height of maize varieties SAMMAZ 17 and SAMMAZ 37 in 2019 and 2020 wet seasons in Mubi, Nigeria

Treatment	Rate	Plant height (cm)			
		Sampling week			Mean
		2	4	6	
Herbicide – (H)					
Nicosulfuron	50 g/ha	86.09a	134.02a	203.08abc	141.07a
Nicosulfuron	100 g/ha	78.88a	130.70a	201.00bc	136.86a
Nicosulfuron	150 g/ha	78.27a	127.97a	197.45bc	134.56a
Nicosulfuron	200 g/ha	77.59a	121.20a	191.96c	130.25a
Bentazone	0.5 kg a.i./ha	90.63a	151.27a	215.36ab	152.42a
Bentazone	1.0 kg a.i./ha	89.06a	152.71a	200.64bc	147.47a
Bentazone	1.5 kg a.i./ha	85.16a	150.06a	220.63a	151.95a
Bentazone	2.0 kg a.i./ha	89.72a	145.37a	215.45ab	150.18a
Hoe weeded	-	91.79a	140.28a	213.10ab	148.39a
Unweeded	-	87.81a	139.99a	202.61abc	143.47a
SE±		10.65	10.36	5.79	7.15
TA (WAS) –(T)					
3		45.42c	115.49b	194.77b	118.56c
5		82.84b	149.77a	214.22a	148.94b
7		128.24a	152.82a	209.39a	163.48a
SE±		5.83	5.67	3.17	3.91
Variety (V)					
Sammaz 17		87.57a	141.81a	215.75a	148.37a
Sammaz 37		83.43a	136.91a	196.51b	138.95b
SE±		4.76	4.63	2.59	3.20
Interaction					
H x T		NS	NS	NS	NS
H x V		NS	NS	NS	NS
T x V		NS	NS	NS	NS
H x T x V		NS	NS	NS	NS

Note: TA: Time of application, NS: Not significant. Means under each column with the same letter(s) are not significantly different at $p \leq 0.05$ using DMRT

Table 2. Effect of post-emergence application of nicosulfuron and bentazone on the leaf area index of maize varieties SAMMAZ 17 and SAMMAZ 37 in 2019 and 2020 wet seasons in Mubi, Nigeria

Treatment	Rate	Leaf Area Index (cm)			
		Sampling week			Mean
		2	4	6	
Herbicide – (H)					
Nicosulfuron	50 g/ha	0.63a	0.73a	1.12cd	0.83bc
Nicosulfuron	100 g/ha	0.57a	0.71a	1.09d	0.79c
Nicosulfuron	150 g/ha	0.57a	0.75a	1.19cd	0.84bc
Nicosulfuron	200 g/ha	0.61a	0.73a	1.14cd	0.83bc
Bentazone	0.5 kg a.i/ha	0.59a	0.82a	1.56a	0.99ab
Bentazone	1.0 kg a.i/ha	0.62a	0.90a	1.40abc	0.97abc
Bentazone	1.5 kg a.i/ha	0.60a	0.85a	1.52ab	0.99ab
Bentazone	2.0 kg a.i/ha	0.71a	0.92a	1.59a	1.07a
Hoe weeded	-	0.59a	0.83a	1.40bcd	0.94abc
Unweeded	-	0.72a	0.77a	1.26abc	0.92abc
SE±		0.08	0.07	0.10	0.06
TA (WAS) –(T)					
3		0.21c	0.38b	1.24b	0.61c
5		0.39b	0.99a	1.43a	0.94b
7		1.26a	1.03a	1.31ab	1.20a
SE±		0.05	0.04	0.05	0.03
Variety (V)					
Sammaz 17		0.66a	0.87a	1.40a	0.98a
Sammaz 37		0.58a	0.73b	1.25b	0.86b
SE±		0.04	0.04	0.04	0.03
Interaction					
H x T		NS	NS	NS	NS
H x V		NS	NS	NS	NS
T x V		NS	NS	NS	NS
H x T x V		NS	NS	NS	NS

Note: TA: Time of application, NS: Not significant. Means under each column with the same letter(s) are not significantly different at $p \leq 0.05$ using DMRT

The 3, 5, and 7 WAS application times of nicosulfuron and bentazone herbicides had a significant effect on the plant height, leaf area index, total biomass, and other growth parameters like CGR, RGR, NAR and days to 50% tasselling and silking of both the SAMMAZ 17 and SAMMAZ 37 (Tables 1-4). The 3 WAS application resulted in the significantly lowest values for the plant height, leaf area index, and total biomass of the two maize varieties than the 5 and 7 WAS applications, resulting in higher values for the growth parameters (Tables 1-3). It was also observed that applying the herbicide at 3 WAS inflicted some physical injuries on the maize plants, especially the higher concentrations of the herbicides than applications at 5 and 7 WAS. On the other growth parameters, however, the application at 5 and 7 WAS resulted in the lowest days to 50% tasselling and silking and RGR and NAR, respectively, while that at 3 WAS led to the lowest value for CGR compared to that due to other periods of application (Table 4). The reduction in values for most of the maize growth parameters due to herbicide application at 3 WAS could be associated with the physical injuries sustained by the plants due to the herbicide application at that age. It could still be linked to the interference of DNA synthesis and cell growth caused by the inhibition of branched chained amino acids biosynthesis due to the stoppage of the activity of the enzyme acetolactate synthase as a result of the nicosulfuron application (Silva 2007). The higher values for some of the

SAMMAZ 17 and SAMMAZ 37 growth parameters at especially 5 WAS application than that due to 3 WAS application could be due to the ability of the maize plant to tolerate and convert the higher concentrations of the herbicides into a harmless compound at that stage than at 3 WAS application. A study by Eynollahi et al. (2017) reported a significant increase in values for maize growth parameters like plant height, leaf area index, and total biomass due to the application of 60 g a.i/ha of nicosulfuron at 2-4 leaf stage of maize than that treated with 40, 80 and 100 g a.i/ha of nicosulfuron at 4-6 and 6-8 leaf stages of the maize plant. Therefore, the age of the plant at which herbicides are applied determines the magnitude of the effect of the herbicides on the plant's growth parameters. Ali et al. (2021) agreed with this when they reported the effect of bentazone application at different developmental stages on the growth parameters of soybean cultivars.

The varietal data showed that SAMMAZ 17 and SAMMAZ 37 treated with nicosulfuron and bentazone were not significantly different in plant height, leaf area index, and total biomass, especially at 2 and 4 weeks of sampling after application (Tables 1-3). Similarly, on the other growth parameters, the two maize varieties did not differ significantly except on the days to 50% tasselling and silking. However, the maize variety SAMMAZ 17 had higher values for most growth parameters than SAMMAZ 37 (Table 4). This might be due to varietal differences as plant variety affects its response to herbicides, as Robinson et al. (2017) reported.

Table 3. Effect of post-emergence application of nicosulfuron and bentazone on the total biomass of maize varieties SAMMAZ 17 and SAMMAZ 37 in 2019 and 2020 wet seasons in Mubi, Nigeria

Treatment	Rate	Total biomass (g)			
		Sampling week			Mean
		2	4	6	
Herbicide – (H)					
Nicosulfuron	50 g/ha	32.17ab	49.68a	107.76a	63.20a
Nicosulfuron	100 g/ha	25.06ab	55.73a	84.17b	54.98a
Nicosulfuron	150 g/ha	30.16ab	54.36a	94.84ab	59.79a
Nicosulfuron	200 g/ha	27.57ab	50.82a	86.96b	55.12a
Bentazone	0.5 kg a.i/ha	28.68ab	55.17a	89.12ab	57.66a
Bentazone	1.0 kg a.i/ha	34.60a	54.85a	104.32ab	64.59a
Bentazone	1.5 kg a.i/ha	31.94ab	54.09a	92.73ab	59.59a
Bentazone	2.0 kg a.i/ha	34.00ab	44.93a	91.96ab	56.96a
Hoe weeded	-	33.86ab	60.49a	98.23ab	64.19a
Unweeded	-	24.06b	56.74a	87.92ab	56.24a
SE±		3.08	4.99	6.33	4.02
TA (WAS) –(T)					
3		9.88c	21.30b	68.22c	33.14c
5		19.11b	71.15a	115.72a	68.66b
7		61.64a	68.60a	97.46b	75.90a
SE±		1.68	2.73	3.47	2.20
Variety (V)					
Sammaz 17		30.90a	55.12a	95.25a	60.42a
Sammaz 37		29.52a	52.26a	92.35a	58.04a
SE±		1.38	2.23	2.83	1.80
Interaction					
H x T		NS	NS	NS	NS
H x V		NS	NS	NS	NS
T x V		NS	NS	NS	NS
H x T x V		NS	NS	NS	NS

Note: TA: Time of application, NS: Not significant. Means under each column with the same letter(s) are not significantly different at $p \leq 0.05$ using DMRT

Effect of nicosulfuron and bentazone on the yield parameters of SAMMAZ 17 and SAMMAZ 37

Table 5 shows the effect of nicosulfuron and bentazone treatments on SAMMAZ 17 and SAMMAZ 37 yield parameters significantly affected most yield parameters. It was observed that although the nicosulfuron treatments had higher values for most of the SAMMAZ 17 and SAMMAZ 37 yield parameters, the values for almost all of the yield parameters due to the two herbicide concentrations were comparable. The hoe-weeded control treatment had higher values for yield parameters like cob weight [CW (148.00 g)], cob length [CL (17.75 cm)], cob yield/hectare [CY/H (921.08 kg/ha)], kernel depth [KD (1.17)], and grain yield/hectare [GY/H (809.90 kg/ha)], which were significantly similar with that due to the two herbicides treatments, but significantly higher than that due to unweeded control treatment. However, the two control treatments (hoe weed and unweeded) had a comparable value for most other yield parameters (Table 5). The higher values for most of the yield parameters of SAMMAZ 17 and SAMMAZ 37 as a result of the nicosulfuron treatments than that due to bentazone treatments could be attributed to the effective weed control recorded that eventually led to less weed competition for available soil nutrients, water, and space. Akadiri et al. (2017) also found an increase in yield parameters of maize plants due to nicosulfuron application. The highest values for almost all the yield

parameters (including CY/H and GY/H) of the two maize varieties due to hoe-weeded control treatment could be a result of the effective weed control with less phytotoxicity compared with that due to the herbicides treatments. Nosratti et al. (2007) reported similar significantly higher values for GY/H and other yield parameters due to hoe-weeded control compared to nicosulfuron concentrations and other herbicides. Felix et al. (2019) also found that the highest GY/H due to hoe-weeded control was comparable to pre and post-emergence herbicides studied. The lowest values for most of the yield parameters (including CY/H, GY/H, HGW, WKC) due to unweeded control treatment was a result of high weed infestation/density that led to high maize/weeds competition for available essential environmental resources required for plant growth and development thus causing a reduction in values for most of the yield parameters of the two maize varieties.

The 3, 5, and 7 WAS application times of nicosulfuron and bentazone treatments were also significant on the yield parameters (except CL and KD) of the SAMMAZ 17 and SAMMAZ 37. The 3 WAS application resulted in higher values for all the yield parameters (except KD and HGW) of the maize varieties compared with that due to 5 and 7 WAS applications (Table 5). This confirmed that the time herbicide is applied determines its effect on the plant's yield or yield attributes (Soltani et al. 2007). Hence, the highest values for virtually all the maize variety SAMMAZ

17 and SAMMAZ 37 yield parameters due to 3 WAS application might be due to the early and effective weed control recorded at that stage. A similar finding was reported by Anderson et al. (1974) when they applied bentazone at the unifoliate, first trifoliate, and second trifoliate stages of soybean development and thus discovered that the bentazone application resulted in effective weed control and an increase in soybean yield at the first trifoliate stage of growth than it did at other stages of development. The finding also agreed with that of Sharara et al. (2005) who found that the single application of bentazone and other herbicides like fluroxypyr at two weeks after sowing maize plant effectively control weeds and thus significantly increase the yield parameters of the maize plant by about 52-74% when compared with that due to other period of application.

The varietal data showed that the maize varieties SAMMAZ 17 and SAMMAZ 37 treated with nicosulfuron and bentazone treatments significantly differed in yield parameters, including GY/H, CY/H, WKC, and CW (Table 5). SAMMAZ 17 had higher values for all the yield

parameters (except NKRC, KD, T% and HGW) than SAMMAZ 37. This could be due to varietal differences or the effect of the herbicides. Wilson et al. (2010) attested to these findings when they reported that the tolerance of maize to herbicides was based on factors like variety type, herbicide application dose, and environmental factors. Also, Soltani et al. (2007) observed the sensitivity of some sweet corn hybrids to herbicides that involved nicosulfuron, bentazone, mesotrione, primisulfuron, foramsulfuron, and isoxaflutole and associated their response to herbicides and variety of the maize.

The study concluded that the nicosulfuron treatments resulted in reduced values for most growth parameters of SAMMAZ 17 and SAMMAZ 37 than bentazone treatments. On the yield parameters of the 2 maize varieties, however, the 2 herbicides treatments had a comparable values for majority of the yield parameters. For higher yield performance of SAMMAZ 17 and SAMMAZ 37, the application of nicosulfuron and bentazone treatments should be at 3 WAS than at 5 and 7 WAS.

Table 4. Effect of post-emergence application of nicosulfuron and bentazone on the CGR, RGR, NAR, days to 50% tasselling and silking of maize varieties SAMMAZ 17 and SAMMAZ 37 in 2019 and 2020 wet seasons in Mubi, Nigeria

Treatment	Rate	Growth parameters				
		CGR (g/m ² /wk)	RGR (g/g/wk)	NAR (gcm ² /wk)	50% Tasselling	50% Silking
Herbicide (H)						
Nicosulfuron	50 g/ha	0.52a	0.061ab	0.003a	60.25cd	63.39a
Nicosulfuron	100 g/ha	0.48a	0.075ab	0.004a	61.22abc	64.28a
Nicosulfuron	150 g/ha	0.46a	0.061ab	0.003a	61.58ab	63.89a
Nicosulfuron	200 g/ha	0.47a	0.067ab	0.002a	61.72a	64.19a
Bentazone	0.5 kg a.i/ha	0.56a	0.046b	0.002a	60.17cd	63.33a
Bentazone	1.0 kg a.i/ha	0.50a	0.057ab	0.003a	60.69abcd	63.81a
Bentazone	1.5 kg a.i/ha	0.51a	0.068ab	0.003a	60.33cd	63.19a
Bentazone	2.0 kg a.i/ha	0.56a	0.100a	0.002a	59.81d	62.97a
Hoe weeded	-	0.48a	0.068ab	0.003a	60.64abcd	63.75a
Uweeded	-	0.52a	0.093a	0.003a	60.50bcd	63.69a
SE±		0.07	0.014	0.001	0.38	0.44
TA (WAS) (T)						
3		0.31c	0.069ab	0.003b	60.94a	63.46b
5		0.74a	0.089a	0.004a	60.08b	62.98b
7		0.47b	0.051b	0.002c	61.06a	64.52a
SE±		0.04	0.008	0.00	0.21	0.24
Variety (V)						
Sammaz 17		0.51a	0.062a	0.003a	61.84a	64.70a
Sammaz 37		0.50a	0.078a	0.003a	59.54b	62.60b
SE±		0.03	0.006	0.000	0.17	0.20
Interaction						
HxT		*	*	NS	NS	NS
HxV		NS	*	NS	NS	NS
TxV		NS	NS	NS	NS	NS
HxTxV		NS	NS	NS	NS	NS

Note: TA: Time of application, WAS: Week after Sowing, NS: Not significant, *: Statistically significantly different. Means under each column with the same letter(s) are not significantly different at $p \leq 0.05$ using DMR T

Table 5. Effect of post-emergence application of nicosulfuron and bentazone on the yield parameters of maize varieties SAMMAZ 17 and SAMMAZ 37 in 2019 and 2020 wet seasons in Mubi, Nigeria

Treat.	Rate	CW (g)	CL (cm)	CDBS (cm)	NKRC	NKC	Yield parameter						
							WKC (g)	CDAS (cm)	CY/H (Kg/ha)	KD	T%	GY/H (Kg/ha)	HGW (g)
Her (H)													
Nico.	50 g/ha	139.83ab	15.66b	5.31a	13.93abcd	472.59a	115.01bc	3.05ab	62.22ab	1.12ab	81.34a	50.83b	25.07b
Nico.	100 g/ha	151.79a	15.60b	5.25ab	13.60cd	448.00ab	115.91bc	3.19a	66.23a	1.17a	78.10ab	51.65b	26.10b
Nico.	150 g/ha	137.72ab	15.65b	5.30a	13.68bcd	470.48a	109.05c	3.01abc	61.21ab	1.18a	79.16ab	50.44b	33.75a
Nico.	200 g/ha	136.61ab	15.30b	5.23ab	13.65bcd	428.64b	109.92c	2.75d	61.51ab	1.27a	80.14ab	48.66b	26.67b
Bent.	0.5 kg a.i/ha	143.49ab	15.57b	5.19ab	14.20ab	472.76a	118.63abc	2.86bcd	65.10a	1.13ab	76.30b	52.06b	24.38b
Bent.	1.0 kg a.i/ha	139.82ab	15.63b	5.18ab	13.85bcd	456.79ab	114.92bc	2.84bcd	63.31ab	1.16a	82.88a	51.94b	25.15b
Bent.	1.5 kg a.i/ha	139.09ab	15.24b	5.18ab	14.03abc	478.49a	143.10a	2.80cd	60.50ab	1.19a	80.98ab	48.65b	25.57b
Bent.	2.0 kg a.i/ha	142.99ab	15.06b	5.25ab	13.36d	455.12ab	141.31ab	2.80cd	63.14ab	1.11ab	79.40ab	49.58b	25.68b
Hoe W.	-	148.00a	17.75a	5.15ab	14.47a	473.33a	118.02abc	2.93bcd	65.28a	1.17a	81.51a	58.60a	25.89b
Uweeded	-	127.67b	15.09b	4.95b	14.23ab	441.41ab	104.96c	2.96abcd	56.76b	1.00b	82.10a	46.65b	24.13b
SE±		5.33	0.64	0.10	0.18	12.62	8.46	0.08	2.37	0.05	1.54	2.16	1.29
TA (T)													
3 WAS		151.94a	15.93a	5.35a	14.12a	488.11a	140.68a	3.00a	67.42a	1.16a	81.97a	55.01a	25.85ab
5 WAS		133.61b	15.75a	5.10b	13.56b	442.96b	106.50b	2.87b	58.71b	1.13a	80.42ab	47.46b	27.71a
7 WAS		136.55b	15.29a	5.14b	14.01a	448.21b	110.07b	2.89ab	61.45b	1.16a	78.18b	50.25b	25.16b
SE±		2.92	0.35	0.05	0.10	6.91	4.64	0.04	1.30	0.03	0.84	1.18	0.71
Vari (V)													
Sam. 17		148.23a	15.79a	5.29a	13.86a	462.71a	130.09a	2.99a	65.43a	1.14a	79.36a	53.05a	25.74a
Sam. 37		133.17b	15.52a	5.11b	13.94a	456.81a	108.07b	2.85b	59.62b	1.16a	81.02a	48.77b	26.73a
SE±		2.39	0.29	0.04	0.08	5.64	3.79	0.03	1.06	0.02	0.69	0.97	0.58
Inter.													
HxT		NS	NS	NS	NS	NS	*	*	NS	NS	*	NS	NS
HxV		NS	NS	NS	*	NS	*	NS	*	NS	*	NS	*
TxV		NS	NS	NS	*	NS	*	NS	NS	NS	*	NS	NS
HxTxV		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Note: TA: Time of application, WAS: Week after Sowing, NS: Not significant, *: Statistically significantly different, Her: Herbicide, Nico.: Nicosulfuron, Bent.: Bentazone, Hoe W: Hoe weeded, Vari: Variety, Treat.: Treatment, Inter.: Interaction, CW: Cob weight, CL: Cob length, CDBS: Cob diameter before shelling, NKRC: Number of kernel row/cob, NKC: Number of kernel/cob, WKC: Weight of kernel/cob, CDAS: Cob diameter after shelling, CY/H: Cob yield/hectare, KD: Kernel depth, T%: Threshing percentage, GY/H: Grain yield/hectare, HGW: Hundred grain weight, Sam.: Sammaz. Means under each column with the same letter(s) are not significantly different at $p \leq 0.05$ using DMR T

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