NITROUS ACID INDUCED MUTAGENESIS ON AGROMORPHOLOGICAL TRAITS, SHELF LIFE AND NUTRITIONAL COMPOSITIONS IN SELECTED INDIGENOUS LEAFY VEGETABLE GENOTYPES

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MTech/SLS/2018/7988

DEPARTMENT OF PLANT BIOLOGY

SCHOOL OF LIFE SCIENCE

FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGER STATE, NIGERIA.

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF TECHNOLOGY (MTECH) IN BOTANY (APPLIED PLANT GENETICS AND BREEDING)

SEPTEMBER, 2023

DECLARATION

I hereby declare that this Thesis **Titled "Nitrous Acid Induced Mutagenesis on Agromorphological traits, Shelf Life and Nutritional Compositions in Selected Indigenous Leafy Vegetable Genotypes"** is a collection of my original research work and it has not been presented for any other qualification anywhere, information from other sources (published or unpublished) has been duly acknowledged.

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SIGNATURE & DATE

CERTIFICATION

Thethesis Titled **"Nitrous Acid Induced Mutagenesis on Agromorphological traits, Shelf Life and Nutritional Compositions in Selected Indigenous Leafy Vegetable Genotypes"** by **JIYA, Sussan Adishetu(MTech/SLS/2018/7988)** meets the regulations governing the award of Master of Technology (MTech) in Botany (Applied plant Genetics and breeding, Federal University of Technology, Minna and its approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to the Almighty God for his Grace and Mercy during this period.

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ABSTRACT

The mutagenic effects of Nitrous acid on Agro-morphological traits, Shelf-life and Nutritional composition on three leafy vegetables were investigated. Dry seeds of *Celosia argentea* L. (NGB00023), *Solanum macrocarpon* L. (NHGB/09/133) and *Amaranthus hybridus* L. (NGB00077) were collected, pre-soaked in water for 1hour and treated with varying concentration of Nitrous acid (0.2 %, 0.4 %, 0.8 % and 1.0 %) for 1 hour, the untreated was designated as control. The treated seeds and control were planted in the experimental site in a completelyrandomised design (CRD) with four replicates during the 2020/2021 planting season. Agro-morphological data were taken, the proximate, minerals and vitamin contents were analysed using standard analytical procedures. The results of the quantitative analysis showed significant difference ($P<0.05$) among the treatment for all parameters observed. For NGB00023, NHGB/09/133 and NGB00077 the least seedling heightof 4.88 cm, 4.60 cm and 4.83 cm respectively were all recorded in the control plants. The least average number of leaves for NGB00023, NHGB/09/133 and NGB00077 as recorded in the control were 26.43, 15.32 and 35.00 respectively.The agromorphologicaldata showed significant increase as concentration increases. Also treated plants showed variations on phenotypic features of the leaves such as leaves with irregular shapes and serrated margins, bifurcated apex, chlorosis, broader leaves, blunt apex and acute apex. In addition,shelf-life determination showed thatthe control samples loss the highest weight in NGB00023 with (75 %) and the lesser weight with (30%) at 1.0% concentration, Similarly, in NHGB/09/133 the control loss the highest weight (64 %) while the lesser weight was at 0.2 % concentration treated leaves (32 %), in NGB00077, the 0.2 % concentration loss the highest weight (51 %) while 0.4 % and 0.8 % concentrations with (30 %) loss lesser weight. High variation in proximate, minerals and vitamin composition was recorded among the treatments as influenced by Nitrous acid. In NGB00023Nitrous acid significantly ($p<0.05$) effects all proximate composition except total ash, crude fibre, in NGB00077,crude fat and crude protein, the control values was higher than treated leaves,all minerals also varied significantly. InNGB00023, Vitamin A and B_2 records highest value in the control (10.517 mg/100gand 70.14 mg/1000g) while the least (9.561 and 5.69 mg/100g) was recorded at 0.2% concentration, Vitamin C contents ranges between (35.40-50.21 mg/100g) and is within the recommended dietary allowance per day at (45mg). Thus higher concentration (0.8 % and 1.0 %) of Nitrous acid is more suitable for improved agromorphological parameters, shelf life and nutritional compositions of the studied vegetables. Thus, Nitrous acid has differential influence in all the leafy vegetables and could be utilized for further improvement.

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ABBREVIATIONS, GLOSSARIES AND SYMBOLS

AOAC: Association of Official Analytical Chemists

FAO: Food and Agricultural Organization

IPGRI: International Plant Genetic Resources Institute

IITA: International Institute for Tropical Agriculture

MAP: Modified Atmosphere Packaging

CRD: Completely Randomized Design

WAP: Weeks After Planting

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Leafy vegetables are the edible parts of plant that are consumed wholly or in parts, raw or cooked as part of main dish or salad. They are widely designated as "protective foods" in human diet due to their varied health benefits attributable to the richness in vitamins, essential fatty acids, minerals, amino acids and dietary fibre (Shukla *et al*., 2016). They are an essential component of people's diet in Nigeria and other parts of West Africa. Regular consumption of leafy vegetable is increasing among the masses due to high awareness about the health benefits (Randhawa *et al*., 2015). They offer advantages because of low cost and wide availability.

Among the vast leafy vegetables is the genus *Amaranthus hybridus* belonging to the family Amaranthaceae, commonly known as Green or Chinese Spinach in English, 'EfoTete' or 'ArowoJeja' in Yoruba, 'Akwukwo' or 'Inene' in Igbo and 'Alayyafoo' in Hausa (Bensch *et al*., 2003). It is a cosmopolitan genus found both in Temperate and Tropical regions of the World, with 70 Species and 400 varieties including the cultivated grain Amaranths species and weedy types (Matthew, 2019). *Amaranthus* species are regarded as store house for vitamins such as vitamin C, B6, foliate and carotene. It has been shown to contain large amount of squalene, a compound that has both health and industrial benefits (He and Corke, 2003).

Celosia argentea L. is commonly called "Lagos Spinach" or "Sokoyokoto" in Yoruba and "FararAlayyafo" in Hausa. It is also known as Cockscomb which is derived from Greek word (keleos) meaning" burning'' and refers to the flame like flowers it produces, It belongs to the family Amaranthaceae. Two common varieties of this Genus

are common cockscomb (*Celosia argentea* var. *cristata*) which has a wide comb-shaped inflorescence, and feathered amaranth (*Celosia argentea* var. *plumosa*) with featherlike flowers (Cai *et al*., 2001). This plant has high protein and vitamin contents and is also a good source of calcium, iron, carbohydrates and phosphorus (Ayodele and Olajide, 2011).

Solanum macrocarpon L. commonly known as African Eggplant or Gboma(Hausa-'Dauty'; Igbo-'Anara', Yoruba-'Igbagba') is of the Solanaceae family. It is a tropical biennial plant that is closely related to the eggplant having a large cultivar and varieties which grows in areas of high rainfall found in the tropical and humid regions of West and Central Africa, South East, Asia, South America and the Caribbeans. Some cultivars can be found in the savanna and semi-arid region of Northern Ghana, Burkina Faso and their neighboring countries. (Schippers, 2000).*Solanum macrocarpon* L is generally known not to contain huge amount of protein and other nutrients. It is high in potassium, a necessary salt that helps in maintaining the function of the heart and regulate blood pressure. It is useful in weight reduction and also good for a diabetic patient because of very low calorie content. **(**Gbeyonron, 2015).

Lack of knowledge on appropriate quality preservation practice and technology results in high qualitative and quantitative loss in vegetables. Loss of quality and deterioration in harvested leafy vegetables is manifested through yellowing as a result of loss of chlorophyll, wilting, loss of textural properties and decay from pathological breakdown. Thus there is need to look into how these important traits could be enhanced through mutation breeding; similar traits in some crops had been improved using chemical mutagens.

Ferguson and Denny (1995) reported multiple groups of chemical compounds such as Nitrous Acid as an “underutilized resource” in plant chemical mutagenesis. This acid has been reported to promote seed germination, photomorphogenesis, mitochondrial activity, leaf expansion, root growth, stomatal closure, fruit maturation, senescence, iron metabolism and defense response, playing key roles in the activation of defense genes (Wendehenne *et al*., 2001). Although Nitrous acid mutagenesis is used for genetic improvement of virus, fungi and bacteria for multiple biotechnological purposes, its exploitation in plant genetic improvement has been lacking. The mutagenic effects of Nitrous Acid have also been reported in several plants such as *Citrullus lanatus* and *Moringa oleifera*, Foxtail Millet, *Digitaria* (Animasaun *et al*., 2014), *Vicia faba* (AL-Shamma and Sahib, 2014). This study therefore attempts to look into effects of Nitrous acid on leafy vegetables and the optimal concentrations that can improve Agro-morphological and qualitative characters in M_1 generation.

1.2 Statement of the Research Problem

The availability, affordability and consumption of leafy vegetables becomes a challenge during the dry season in poor household due to high cost of the vegetables, making it unlikely to meet the recommended dietary allowance (Akintayo *et al*., 2019). In Nigeria and many African countries, vegetables are very abundant during the rainy season and immediately after this season it becomes scarce leading to a considerable low consumption of vegetables, this is due to the shortage of water supply (Akintayo *et al*., 2019). Increase in demand and seasonality of production in leafy vegetables have resulted into high losses as a result of short shelf life.

High perishable and low storage capacity of leafy vegetable species in the fresh form are the main constraint to increased production, marketing and consumption of

traditional leafy vegetables (Onyango *et al*., 2008). In the market, sellers constantly apply water to leafy vegetables to make them look fresh otherwise they lose weight. There are number of measures in place to extend shelf life such as cold rooms and evaporative cooling methods (Basediya *et al*., 2013). These measure are still of high cost for small holder farmers due to the high electricity bills involved.

Ali *et al*. (2018) reported the use of certain chemicals such as sulphuric acid and chlorine as an antimicrobial and anti-browning agents in vegetables in enhancing post-harvest quality, however many of such chemicals pose serious threat to the consumers of such vegetables, chlorine solutions for example have been associated with the formation of carcinogenic compounds (Baskaran *et al*., 2013).

Despite all the vast benefits of these leafy vegetable, its consumption and uses are under threat in Nigeria due to low yield and neglect by the agrarian community, low leaf yield of vegetables could be attributed to several factors such as environmental, agronomical, low soil fertility and low yielding varieties which have short growth periods

Solanum macrocarpon L. has not attracted the interest of researchers compared to other Solanaceous species (Tomatoes and Peppers). As an important underutilized leaf and fruit vegetable in sub-Sahara Africa, production is hindered by dearth of improved varieties, susceptibility to biotic and abiotic stresses (Adeniji *et al*., 2018).

1.3 Aim and Objectives

The aim of this study was to determine the effects of Nitrous acid on Agro-morphological, Shelf life and Nutritional composition in selected indigenous genotypes of leafy vegetables.

The specific objectives of this research were to:

i. determine the effects of nitrous acid on the agro-morphological traits among the selected leafy vegetable genotypes
ii. determine the optimum concentration of nitrous acid for inducing beneficial mutations
iii. identify the effects of Nitrous acid on the shelf life of leafy vegetables in M_1 stored at room temperature
iv. quantify the nutritional composition of *Celosia*, *Amaranthus* and *Solanum* genotypes in M_1 generation

1.4 Justification for the Study

Abdulrahman *et al*. (2018) reported that various chemicals have positive or negative effects on living organisms. Among the popular chemo-mutagens are Sodium azide and Nitrous acid, their effects can occur both spontaneously and artificially following induction by mutagens creating a broad variety of morphological and yield structure parameters in comparison to normal plants.

The interest of this leafy vegetables has increased in recent years, because it plays an important role in human nutrition especially in the aspects of food security and micro nutrient deficiencies (Borokini *et al*., 2017). World Health Organization (WHO), (2002) reported that low vegetable intake causes about 31% of heart disease and 11% of stroke worldwide.

There is the need for a more consumer friendly and safer approach to prevent early post-harvest deterioration, thus increasing the shelf-life of these leafy vegetables. Concerted efforts towards improvement of these qualitative traits using Nitrous acid in the local genotypes of these leafy vegetables are yet to be documented and would constitute a major step towards the improvement of these crops.

The application of chemical mutagen such as Nitrous acid is reported to promote seed germination, stomatal closure, photo-morphogenesis, iron metabolism, defense response and has played significant role in plant breeding (Wendehenne *et al*., 2001). Such chemical could be exploited for improved yield and elongation of shelf life in leafy green vegetables.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and Domestication of *Celosia Argentea, Amaranthus Hybridus* and *Solanum Macrocapon*

2.1.1 Origin and domestication of *celosia argentea*

The genus *Celosia argentea* L. originates from the subtropical and temperate regions of Africa, South America, and South East Asia (Cai *et al*., 2001). They are classified as a member of the family Amaranthaceae. Two widespread ornamental varieties of this genus are common cockscomb (*Celosia argentea* var. *cristata*) which has a wide comb-shaped inflorescence, and feathered amaranth (*Celosia argentea var. plumosa*) with featherlike flowers (Cai *et al*., 2001). In Nigeria, and other parts of West Africa, it is widely grown in gardens.

2.1.2 Origin and domestication of *amaranthus hybridus*

Amaranths refers to plants of the genus *Amaranthus,* which contains 60-70 species of which 40 are native to the Americas and the rest to Africa, Asia, and Europe (Zhigila *et al*., 2014). Southern Asia, Central and South American are considered as Center of Origin for most species that has been held under cultivation since time immemorial. The word *Amaranthus* is basically derived from the Greek word "Anthos'' (flowers) which means everlasting or unwilting. The domestication of wild amaranths began over 8,000 years ago. Amaranth has a long history of cultivation in Nigeria likely to be introduced from America by early Europeans visitors (Matthew, 2019). Many more species are eaten globally than would be considered truly domesticated. Amaranths have not been the subject of modern intensive breeding efforts and frequent hybridization between cultivated and wild populations has led to the existence of many intermediate types (N.R.C., 2006). The two subfamilies, Amaranthoideae and Chenopodioideae, are

closely related, and for a long time were considered as one evolutionary line due to their morphological similarities. Separation of these types is not entirely distinct, taxonomically or functionally. Striking similarities has been observed among sizable number of Amaranthus species (Alege and Daudu, 2014).

2.1.3 Origin and domestication of *solanum macrocapon*

Solanum macrocarpon, also known as Gboma is a tropical biennial plant that is closely related to the eggplant, about 25 *Solanum* species are cultivated in Nigerian (Ibrahim *et al*., 2017). It is said to have African ancestry and its local cultivars grown for the leaves are commonly found in West and Central Africa (Bukenya and Bonsu, 2004). *Solanum* has a large cultivar and varieties which grows in areas of high rainfall found in the tropical and humid regions of West and Central Africa, South East Asia, South America and the Caribbeans. It is reported to be cultivated in many parts of Nigeria (Chinedu *et al*., 2011). The Gboma eggplant is distributed along the coast of West Africa (Ghana, Nigeria, Togo, Ivory Coast, Benin), East and South African countries (Malawi, Zambia, Zimbabwe and Mozambique). The species *macrocarpon* comprised four groups (*Solanum macrocarpon* L. Complex, *Solanum macrocarpon* L. 'semi wild', *Solanum macrocarpon* L. 'Mukono' and *Solanum macrocarpon* L. 'Nabingo') (Bukenya, and Bonsu, 2004).

2.2 Botanical Description of *Celosia Argentea, Amaranthus Hybridus* and *Solanum Macrocarpon*

2.2.1 Botanical description of *celosia argentea*

Celosia argentea is an erect, short-lived annual herb, which grows up to 150 cm – 200 cm in height with alternate leaves. The stem is ridged, glabrous, branches up to 25 per plant, ascending. Leaves are alternate, simple, without stipules and are light green to

dark green form; petiole indistinctly demarcated; blade ovate to lanceolate-oblong or narrowly linear, up to 15 (–20) cm × 7(–9), tapering at base, acute to obtuse and shortly mucronate at apex, entire, glabrous, pinnately veined (Grubben and Denton, 2004). Inflorescence is dense, many-flowered spike, at first conical but becoming cylindrical, up to 20 cm long, bracteate, silvery to pink, in ornamental forms completely or partly sterile and in many colours. The spikes are pink but later turn white when the seeds are mature. Flowers are small, bisexual, regular, penta-merous; Sepals free, narrowly elliptical-oblong, 6–10 mm long; stamens fused at base; ovary superior, 1-celled, style filiform, up to 7 mm long, stigmas 2–3, very short. Fruit is ovoid to globose capsule 3–4 mm long, circumscissile, few-seeded. Seeds lenticular, 1–1.5 mm long, black and shining, shallowly reticulate (Grubben and Denton, 2004).

2.2.2 Botanical description of *amaranthus hybridus*

Amaranthus hybridus L, is an annual herbaceous plant having a densely branching habit that are erect or postrate of 1- 6 feet high. The leaves are alternate petioled, 3 – 6 inches long, dull green, and rough, hairy, ovate or rhombic with wavy margins. The flowers are small, with greenish or red terminal panicles developing into a berry shape, fleshy red or pink. The seeds are small and lenticellular in shape; with each seed averaging 1 – 1.5 mm in diameter and 1000 seeds weighing 0.6 – 1.2 g, Taproot is long. It is rather a common species in waste places, cultivated fields and barnyards in Nigeria (Akubugwo *et al*., 2007)

2.2.3 Botanical description of *solanum macrocarpon*

Solanum macrocarpon L. is a perennial shrub and robust, it grows up to 1.5 meters tall, with glabrous or prickly stems. The leaves are simple, alternate and blades are large (15 – 46 cm × 8 – 30 cm), stipules are absent; and the petiole grows up to 7 cm long. The

fruits are large (ranged from 3 to 12 cm in diameter and 2 to 6 cm long) spherical or depressed, usually cream, green, whitish or purple or with lighter markings at harvest. At maturity, the fruits turn yellow or orange or brown in colour and the surface may crack (Adeniji, 2013).

2.3 Cultivation of *Celosia Argentea*, *Amaranthus Hybridus* and *Solanum Macrocapon*

2.3.1 Cultivation of *celosia argentea*

Seed propagation is the conventional method for producing *Celosia argentea*. The seeds are sown directly and transplanted after seedling emergence (Ilodibia *et al*., 2016). Growth is possible in regions with a temperature range of 25 to 30 °C and in well-drained, slightly acidic soils (Ayorinde *et al*., 2017). However, it is affected by weeds (Omovbude *et al*., 2016), rainfall (Nedunchezhiyan *et al*., 2020). It is tolerant to a wide range of soil conditions. They grow best in full sunlight. A temperature ranges of 25 to 30^{o}C is suitable. Both green and red forms grow well at low altitudes, where temperature fluctuations are limited.

2.3.2 Cultivation of *amaranthus hybridus*

Amaranths seeds are often cultivated directly in the soil as sole crops on beds or intercropped with other crops initially on a nursery beds and later transplanted after 2-3 weeks (Achigan *et al*., 2014). Amaranths have a high capacity for osmotic adjustment and a C4 photosynthetic pathway that allows efficient use of carbondioxide in a large range of temperature and moisture stress environments, likely a major factor in their wide geographic distribution. *Amaranthus* species can grow up 15 to 150 cm and exhibits difficulties especially in the early stage of seedling growth. The plant begins to flower at 65 cm after approximately 57 days and is ready for harvest after 129 days (Paredes and Hernandez,1992). Most indigenous plants are adapted to the prevailing

conditions and require few agricultural inputs and perform well in areas unsuitable for introduced vegetables.

2.3.3 Cultivation of *solanum macrocarpon*

Solanum macrocarpon seeds are sown in a nursery. Presoaking of seeds in hydrogen peroxide for 24 hours generally improves germination. Germination typically begins a week after sowing (Ojo and Olufolaji, 1997). Seedlings of leafy cultivars are transplanted after 4–6 weeks at a spacing of 50 cm × 50 cm while the spacing for fruit cultivars is about 1 m × 1 m. Flowering starts 2–3 months after germination. The crop is mainly self-pollinated. It has low levels of out-crossing, which is mainly done by bees and other pollinating insects. Flowers open early in the morning when it is still dark. The stigma is receptive some hours before the flowers open and remains receptive for about two days. Leaf harvest starts 6–9 weeks after transplanting, usually about a week after flowers have appeared. The leaves are harvested periodically and depending on the availability of water the crop continues to produce for up to a year. Ripe fruits are harvested one month later for seed. Cultivars with early regrowth are most suitable for high yields as a leaf vegetable. The seeds are orthodox and if kept dry and cool will maintain a high viability for several years. However, if kept at room temperature and high air humidity, viability is lost in a few months.

2.4 Pest and Diseases in Leafy Vegetables

The pest and diseases of leafy green vegetables can be broadly divided into the following four groups: insects, fungi, bacteria and viruses. *Celosia argentea* is readily attacked by *Meloidogyne incognita* and *Meloidogyne javanica*, insect pests. Stem and leaf spot is a previously unreported severe disease of *Celosia argentea.* The pathogen was identified as *Gibberella baccata*.

Eggplant is rather resistant to diseases and pests compared to exotic vegetables like tomatoes. Many diseases and pests have been reported to attack the crop, but most are rarely very harmful: *Puccinia penniseti* (yellow rust), *Geotrichum candidum* (rusty brown leaf spot), *Fusarium* sp., *Rhizoctonia solani*, *Verticillium dahliae* (wilt), *Gloeosporium melongenae* (anthracnose), *Leveillu lataurica (*powdery mildew), *Phomopsis vexans* (phomopsis rot), *Phytophthora parasitica* (fruit rot), *Ralstonia solanacearum* (bacterial wilt) and mosaic virus.

Amaranth stem weevils: *Hypolixus runcatulus*, *Hypolixus nubilosus* (*Coleoptera*: *Curculionidae*) It is a major pest of the cultivated amaranthus, larvae tunnel the stems and adult feeds on tender leaves. Atanu, (2018).

Leaf miner: *Liriomyza huidobrensis (Diptera; Agromyzidae*) is a polyphagous pest and is known to attack the host plants from 14 different families, both cultivated and wild including amaranth. Other important hosts are faba beans, onions, melons, garlic and peas Mureithi, *et al*., 2017.

Diseases such as wet rot, anthracnose, white rust, leaf spots, root rot wilting and damping off on seed beds have been reported in amaranth fields in Africa and other continents (Nampeera *et al*., 2019). In Nigeria, Awurum and Uchegbu, (2013) reported of up to 50% yield loss of amaranth production in due to wet rot disease.

2.5. Proximate Composition of *Celosia Argentea, Amaranthus Hybridus* and *Solanum Macrocarpon*

2.5.1 Proximate composition of *celosia argentea*

Ayodele sand Olajide (2011), who worked on determination of the proximate, minerals and amino acids composition of *Celosia argentea*, reported that the crude fat, fibre and protein contents of the plant were moderate with 1.10, 3.53 and 5.17% respectively with

high ash content of 22.43%. They further stated that amino acid analysis revealed high contents of the essential amino acids with methionine as the limiting amino acid. This is quiet adequate when compared with essential amino acids recommended by World Health Organization (WHO) (2002). The nutritional composition of *Celosia argentea* per 100 g edible portion was determined as 83.8 g water; 185kj energy; 4.7 g protein; 0.7 g fat; 7.3 g carbohydrate;1.8 g fibre; 260 mg Calcium; 43 mg Phosphorus and 7.8 mg Iron(Grubben and Denton, 2004).

2.5.2 Proximate composition of a*maranthus hybridus*

The Proximate analysis showed the percentage moisture content, ash content, crude protein, crude lipid, crude fibre and carbohydrate of the leaves as 84.48, 13.80, 17.92, 4.62, 8.61 and 52.18%, respectively while its calorific value is 268.92 Kcal/100 g (Achigan *et al*., 2014). Elemental analysis indicated that the leaves contained sodium (7.43 mg/100 g), potassium (54.20 mg/100 g), calcium (44.15 mg/100 g), Magnesium (231.22 mg/100 g), Iron (13.58 mg/100 g), Zinc (3.80 mg/100 g) and phosphorus (34.91 mg/100 g). The vitamin composition of the leaves in mg/100 g was-carotene (3.29), ascorbic acids (25.40) (Akubugwo *et al*., 2007).

2.5.3 Proximate composition of *solanum macrocarpon*

Solanum macrocarpon L contains water (92.5 %), protein (1 %), fat (0.3 %), and carbohydrates (6 %). Edem, *et al* (2009) determined the proximate composition of two species of garden egg*; Solanum gilo* and *Solanum aubergine.* Analysis showed that *Solanum gilo*fruits had the following composition: moisture (74.80%), carbohydrate (52.13%), crude protein (14.87%), crude fibre (16%), crude fat (7%) and ash (10%). It also contained (93.7%) of ascorbic acid. The *Solanum aubergine* fruits on the other hand contained moisture (94.6%), carbohydrate (58.5%), crude protein (15.75%), crude fat (4%), crude fibre (11.75%) and ash (10%). It also contained 75.9% ascorbic acid. In

similar vein, Bukenya and Bonsu, (2004) reported on some nutrients of *Solanum macrocarpon* L,and the values were; moisture (89.0 %), crude protein(1.4 %), crude fat(1.0 %), crude fibre (1.5 %), ash(0.4 %), carbohydrate(8.0 %), vitamin C(5.0mg/100g), total carotenoid (80ug/100g), niacin(0.43mg/100g) and pantothenic(0.08mg/100g).

The proximate composition of *Solanum macrocarpon* was determined by Eletta *et al*. (2017), and the values were moisture content 89.80 %, crude fat 1.10 %, crude fibre 1.50 %, ash 0.72 % and carbohydrate 6.01 %. Chinedu *et al*. (2011) reported on some mineral composition of *Solanum macrocarpon*, the values range as follows; calcium 13 mg/100g, potassium 224 mg/100g, magnesium 12 mg/100g, iron 0.71 mg/100g, phosphorous 28 mg/100g, sodium 0.2 mg/100g, zinc 0.12 mg/100g and manganese 0.17 mg/100g. Ajayi *et al*. (2018) also reported on some minerals which the values were; calcium 9 mg/100g, potassium 230 mg/100g, magnesium 14 mg/100g, iron 0.24 mg/100g, phosphorus 25 mg/100g, sodium 0.8 mg/100g, zinc 0.16 mg/100g and manganese 0.25 mg/100g.Some minerals composition of *Solanum macrocarpon* were also determined by Ukwela, (2010) and was reported as follows; calcium 16.50 mg/100g, phosphorus 24.36 mg/100g, magnesium 13.04 mg/100g, iron 0.33 mg/100g, potassium 211 mg/100g and sodium 0.40 mg/100g.

2.6 Nutritional and Medicinal Value of *Celosia Argentea, Amaranthus Hybridus* and *Solanum Macrocapon*

2.6.1 Nutritional and medicinal value of *celosia argentea*

Food plants such as leafy vegetables have played an important role in human nutrition especially in the aspect of food security and micronutrient deficiencies (Borokini *et al*., 2017). They are usually picked fresh, used as greens in salads or blanched, steamed, boiled, fried in oil, and mixed with meat, fish, cucurbit seeds, groundnut or palm oil.

Daily consumption of leafy vegetable in diets improves vision, health of digestive system and reduce the risk of stroke, heart disease and chronic diseases (Boeing *et al*., 2012).*Celosia argentea* leaves and young shoots are used as avegetable, used in soups and stew. The leaves retain a pleasant green colour when cooked. An edible oil can be obtained from seed.(Gajanan *et al.,* 2020).The leaves of *Celosia argentea* is used in traditional medicine to cure several disorders such as fever, diarrhea, mouth sores, itching, wounds, jaundice, gonorrhea, inflammation, also various ethnopharmacological studies have reported its medicinal properties which includes anti-inflammatory, anti-diarrhea, anti-urolithiatic and wound healing properties (Varadharaj and Muniyappan, 2017).

2.6.2 Nutritional and medicinal value of *amaranthus hybridus*

The importance of *Amaranthus hybridus* in indigenous medicine range from weight reduction to treatment of several ailments including asthma, allergic rhinitis, nasal catarrh, skin infections, rheumatic disease and swollen joint pains, gastro-esophageal reflux disease, constipation and dyspepsia The leaf is used as soup condiment in Nigeria and the protein contained in the leaf and fruit is reported by Oboh *et al*. (2005) to be of high quality. In parts of Benin, vegetable Amaranths were recommended for young children, lactating mothers and patients with constipation, fever, hemorrhage, anemia or kidney complaints (Akubugwo *et al*., 2007). The folic acid in Amaranths reduces the risk of neural defects in pregnant women and their newborns (AVRDC, 2011). Laker (2007) observed that of more than 100 different indigenous leafy vegetables species in Africa, Amaranths is the most widely consumed and was declared as one of the future promising crops to feed the global population.

2.6.3 Nutritional and medicinal value of *solanum macrocarpon*

The leaf of *Solanum macrocarpon* plant is useful for tooth ache and as a remedy for snake bites (Grubben and Denton, 2004). They are used in traditional medicine for treatment of various ailments,ranging from weight reduction, asthma, skin infections, rheumatic disease, gastro oesophageal reflux diseases,constipation and diabetes. The protein contained in the leaf and fruit is reported by Oboh *et al*. (2005) to be of high quality. *Solanum macrocarpon L* has proven to be of immense benefits from patients suffering from raised intraocular pressure (glaucoma) as well as heart disease. It possesses ulcer protective properties which makes it cheap source of natural anti-ulcer remedy.

2.7 Mutagenicity of Nitrous Acid

Nitrous acid is a potent chemical mutagen which exerts its effect by the deamination of the amino groups of the adenine, cytosine and guanine residues of the nucleic acid causing chemical alterations as well as cross-links of undefined structures deletions. Although, Nitrous acid mutagenesis is used for genetic improvement of virus, fungi and bacteria for multiple biotechnological purposes, its exploitation in genetic improvement has been lacking. However genetic engineering has made a significant contribution to strain improvement, random mutagenesis is still a cost-effective procedure for reliable short-term strain development and its frequently the method of choice. Atmospheric Nitrous acid was observed to affect the physiological of pine trees,they found that exposure to Nitrous acid over two months affects photosynthesis and nutrient status of pine trees by increase in the carbon to nitrogen ratio(Sakugawa and Cape, 2007). Previous studies have described the growth-modulating properties of NO and its

interaction with auxin in modulating root growth and developmental processes (Shapiro, 2005).

Abdulrahaman *et al*. (2018) reported that the rate of germination, seedling height, number of leaves, tiller per plant, and leaf length in *Moringa oleifera* was significantly increased with time of exposure of Nitrous acid. All treatments induced early maturity but with low seed set and yield among 6 and 8-hour treatment time of 0.1% plants in Fonio millet. Generally, the performance of the treated plants was better with short exposure time and 4 hour of treatment (Animasaun *et al*., 2014). Similarly, significant increase was observed when *Vicia faba* was treated for 24 hours with Nitrous acid in number of branches/plant, pods/plant, seed weight rate, seed yield, and protein yield (AL-Shamma and Sahib, 2014). Nitrous acid has been reported to deaminate nucleobases S*in vivo* in DNA (Abdulrahaman *et al*., 2018)

2.8 Factors Influencing Shelf-Life of Leafy Vegetables

Shelf-life is the period of time during which the food product will remain safe, retaining the desired sensory, chemical, physical and microbiological characteristics, and complying with any label declaration of nutritional data when stored under recommended conditions (IFST, 1993). All vegetables are living plant parts containing 65 to 95 percent water, and they continue their metabolic activities even after harvest, vegetables once harvested has a very short shelf-life (Pinela *et al*., 2017). This makes farmers to sell them soon after harvest. Several factors affect the shelf-life of harvested vegetables. They include pre-harvest and post-harvest environment, genetic makeup of the crop or variety and the physiology of the harvested produce. The factors affecting quality of fruits and vegetables can be classified largely into three groups, i.e. (a) Pre-harvest factors, and (b) Harvest factors (c) Post-harvest factors.

Pre-Harvest Factors that are classified into environmental factors includes temperature, light, wind, humidity and rains (Srivastava and Kumar, 2002).Genetic makeup and mineral nutrition is an important factor that potentially affects both the quality and postharvest life of fruit. Optimum plant performance depends on a balanced availability of mineral nutrients that can be limited in many soils around the world.Harvest factors: Quality is dependents on timing of the harvest for most vegetables. Size, flavour, tenderness, texture and colour can all be influenced by harvest timing (Selvakumar, 2014).

2.9 Determination of Shelf-Life

Statistical approach has been outlined by (Gacula and Kubala, 1975), which describes a number of options for controlling the number of necessary measurements. In the most commonly operated type of test called a partially staggered design, a single batch of product (or replicate batches) is put on test at time zero, and samples are taken off for testing at intervals determined by the expectation of the probable shelf-life, if there is no prior knowledge of the shelf-life, it may be necessary to take sufficient samples at each time point, therefore requiring extensive experimentation. In a variant of this procedure (called a staggered design by Gacula and Kubala), the number of samples tested is increased up to the acceleration point, at which failure is expected, and after which a constant number of samples is tested. This basic type of design has the clear advantage that data related to shelf-life are generated at intervals and build up to give a moving picture of deteriorative Change, the criteria that can be used for interpreting sensory shelf-life data have been reviewed by (Dethmers, 1979) and fall into three categories: first detectable change, measured attribute change and change in consumer acceptability. The first detectable change (or just noticeable difference) in product quality can be measured using difference tests, assuming that a suitable reference

sample is available. Predictive modeling has been used to establish a theoretical shelf-life as a function of temperature for the microbial spoilage of packaged green asparagus (Garcia *et al*., 1998).

2.10 Measures to Improve Shelf-Life of Leafy Vegetables

Leafy green vegetables are unique among food products in that they remain as the living tissues up to the moment they are consumed, cooked or otherwise processed.

2.10.1 Chemical treatments

Postharvest quality can be maintained by using different anti-microbial and anti-browning agents. These agents include chlorine based solutions, hydrogen peroxide (H_2O_2), peroxy-acetic acid (PAA), organic acids, and electrolyzed water (Artes *et al*.,2009).Hydrogen peroxide (H_2O_2) is a compound which has bactericidal, sporicidal and inhibitory ability based on oxidation of fungi and bacteria, and it was successfully used to control vegetable pathogens during storage. Treatment with H_2O_2 can extend the shelf life and reduce natural and pathogenic microbial populations in melons, oranges, apples, prunes, tomatoes, whole grapes and fresh cut produce (Cengiz and Certel, 2014).

Chlorine based solutions are commonly used as a disinfectant due to its very strong oxidizing properties and cost effectiveness, but chlorine have been associated with the formation of carcinogenic compounds. In addition, Chlorine based compounds have a limited effectiveness in the reduction of microbial load on fresh produce. However, high levels may cause taste and odour defects on treated products (Baskaran *et al*., 2013).

Peroxy-acetic acid has not reported any harmful byproducts and it is very effective in controlling *Escherichia coli* O157:H7 and Listeria monocytogenes in apples, strawberries, lettuce and cantaloupe (Rodgers *et al*.,2004). Today, the use of reducing

compounds such as ascorbic acid and its derivatives, cysteine and glutathione, is most effective for controlling enzymatic browning (He and Luo, 2007).

Organic acids have been applied largely to slow down enzymatic and non-enzymatic browning, deterioration of texture and microbial growth on fresh produce (Aguayo, 2003). Chelating agents such ascorbic acid, polycarboxylic acids (citric, malic, tartaric, oxalic and succinic acids), polyphosphates (ATP and pyrophosphates), macromolecules (porphyrins, proteins) and ethylenediamine tetra-acetic acid (EDTA), which can inactivate enzymes by binding to transition metals in the metal-enzyme complex, have been used for a variety of food processing applications. A typical combination of anti-browning agents for fresh cut products may consist of a chemical reducing agent, an acidulant and a chelating agent. However, internalization of bacteria and inaccessible sites of fruits and vegetables are the major limitations of applying anti-microbial and anti-browning agents (Mahajan *et al*., 2014).

Nitric oxide (NO) is a highly reactive and acts as a multifunctional signaling molecule in various plant physiological processes, such as fruit ripening and senescence of fruits and vegetables (Wendehenne *et al*.,2001). Postharvest application of NO is a potential new technology to reduce post-harvest losses of fruits and vegetables during handling and marketing (Pristijonos *et al*., 2008). Exogenous application of NO by gas fumigation or dipping in a solution has demonstrated beneficial effects to reduce the production of ethylene, reduce rate of respiration and reduce ion leakage resulting from better maintenance of cellular integrity, reduction in oxidative stress through reduced lipid oxidation and enhanced activity of a range of antioxidant enzymes (Singh *et al*., 2013). Successful application of NO has been reported for apple, banana, kiwifruit, mango, peach, pear, plum, strawberry, tomato, papaya, loquat, Chinese winter jujube

fruit and Chinese bayberry (Manjunatha *et al*. 2012). It was found that treatments of strawberries with NO delayed the onset of senescence and extend postharvest life by inhibiting the action of ethylene (Wills *et al*., 1999). Nitric oxide has been combined with cold storage conditions and modified atmospheric conditions to improve the shelf life of fruits and vegetables such as Mango, green beans and broccoli (Zaharah and Singh, 2011).

Sulfur dioxide (SO_2) treatment is widely used due to its universal antiseptic action and economic application. SO_2 technology has been tested for control of postharvest decay on fruits such as table grapes, fig, banana, lemon or apple. However, the SO_2 concentration necessary to inhibit fungal growth may induce injuries in grape fruits and stems and sulfite residues pose a health risk for some individuals (Palou *et al*., 2010).

Calcium chloride is used to reduce chilling injuries, suppress senescence, enhance the storage and marketable life of fruits by maintaining their firmness and quality. Calcium application also delays aging or ripening, reduces postharvest decay, reduce the incidence of physiological disorders and increases resistance to diseases. It has been suggested that calcium treatment can increase tissue firmness and reduced the susceptibility to physiological disorders and reduced the risk of salt-related injuries in peaches (El-Ramady *et al*., 2015).

2.10.2 Gaseous treatment

Activated oxygen is the best available technology that can replace traditional sanitizing agents. It is a strong and ideal, germicide, sanitizer, sterilizer, anti-microbial, fungicide and deodorizer and detoxifying agent (Graham, 2000). It has been reported that shelf life of fruits and vegetables can be increased when they are subjected to ozonation (Perez *et al.,* 1999). Ozone oxidizes the metabolic products and neutralizes the odours

generated during the ripening stage in storage of fruits. This helps preserve and almost double the shelf life on fresh produce. It also enhances the taste by retaining the original flavour of the products. Its use does not leave any toxic byproducts or residues, does not affect healthy cells or alter its chemistry and is non-carcinogenic. The effectiveness of ozonation can be influenced by different factors such as presence of steam and humidity level (Kim and Yousef, 2000).

1-methylcyclopropene (1-MCP) is a synthetic cyclic olefin which can block the access to ethylene binding receptor there by inhibiting the action of ethylene. It has been found that avocado treated with 1-MCPshowed significantly less weight loss and retained greener colour than control fruit at the full ripe stage (Sisler and Serek, 1997). However, application of 1-methylcyclopropene to permit extended storage requires prior assessment of the appropriate concentration range and storage conditions for each type of produce at maturity (Cubells-Martinez *et al.*, 1999).

Modified atmosphere packaging known as MAP technology and controlled atmosphere storage (CAS) are novel techniques that are widely applied for preservation of agricultural products especially for fruits and vegetables. MAP technique is used to extend the shelf life of commodities by sealing them in polymeric film packages to modify the oxygen and carbon dioxide concentration levels within the package atmosphere (Mangaraj and Goswami, 2009). Composition of the air inside the package is changed due to the respiration and transfer of gases through the package. In contrast, it creates an atmosphere richer in carbon dioxide and lower in oxygen. Reduced oxygen and elevated carbon dioxide levels effectively reduce the rate of respiration of fruits and vegetables (Fonseca *et al*., 2002).

The use of MAP also reduces the incidence of decay, compositional changes and softening of tissues, Furthermore, it can retard the browning reactions and senescence thereby extending the shelf life (Rennie *et al.,* 2001). The first application of MAP was reported in extending the shelf life of apples in an atmosphere with reduced O_2 and increased CO_2 concentrations in 1927. The basic idea of the MAP technique of fresh fruits and vegetable is the replacement of the air of packaging head space with predetermined atmospheric gases different in proportion from that of air.

Cling film is a thin transparent plastic film adheres to surfaces and to itself, is used for sealing food items. Cling films are high-quality food wrap films, which prevent food from insects and microbial contamination, dust, keep it fresh, and also minimize the risk of wastage of food by increasing its shelf life (Bhanus *et al*.,2015).

2.10.3 Physical treatments

During the past few years there is a higher demand for heat treatments in post-harvest technology instead of chemicals. However, usage is limited due to the high cost (Mahajan *et al.,* 2014). There are different types of heat treatments including hot water dip, saturated water vapor heat, hot dry air and hot water rinse with brushing. Heat treatments have shown beneficial effects for insect control, prevention of fungal development, delayed ripening through inactivation of enzymes and prevention of postharvest storage disorders including chilling injury (Lurie and Pedreschi, 2014). Heat treatments have been used to preserve the colour of Asparagus to prevent the development of off-flavours, to prevent development of overripe flavours in cantaloupe and other melons, to the longevity of grapes, plums, bean sprouts and peaches, broccoli, green beans, kiwi fruits and celery (Fallik, 2004). Edible coatings are thin layer of material which provides a barrier to moisture, oxygen and solute movement for the

food. It can be a complete food coating or can be disposed as a continuous layer between food components (Gols *et al.,* 2013). They provide a barrier to moisture and minimize the loss of moisture during storage. However, thick coating on fruits and vegetables surface becomes an undesirable barrier between the external and internal atmosphere and restricts exchange of respiratory gases. It may result in anaerobic respiration, which produces much more carbon dioxide, acetaldehyde and ethanol. The acetaldehyde and ethanol results in fermentation and gives off-flavor to the product, which are detrimental to the perceived quality (Srinivasa *et al*., 2002).

Food irradiation is a process of exposing the produce to speed particles or rays for improving the shelf life (Ferrier, 2010). However, all fruits and vegetables are not appropriate for irradiation including cucumbers, grapes, and some tomatoes as they are sensitive to radiation (Mostafavi *et al.,* 2012). Most studies indicated that, the irradiation of fresh fruits led to a reduction in firmness and thus, it can be recommended for sprouting inhibition (in the range of 50-200 Gy) and disinfestations purposes in sprouting foods such as potatoes, garlic, onions and yams (Marcotte,2001). In contrast, people are very confused to distinguish irradiated foods from radioactive foods. Irradiation process is not possible to induce radioactivity in the food by using gamma rays or electron beams up to10 MeV (Farkas, 2004).

2.11 Mutation Breeding in Leafy Vegetables

Spontaneous and induced mutations may result to potentially novel genotypes with considerably higher shelf-life, improved physical performance and reduced risks of diseases (Daudu, 2011).Genetic variation is very important for any crop breeding program. The inbred nature of many leafy vegetables dictates the relatively limited genetic variability in the crops as compared to cross pollinated crops. Mutation is a valuable tool to create novel traits in plants and can be classified as natural mutations

and induced mutations. Natural mutations are still occurring in the crop and its wild relatives, though at a low rate, and resulting beneficial characters can be selected for human needs. Mutagenic agents, such as X-rays, ultraviolet radiation, neutrons, protons, alpha, beta, and gamma rays, or chemical mutagens like ethyl methane sulfonate (EMS), are used to either increase mutation rates or yield mutants unavailable from natural sources (Falusi *et al*., 2012). Also, mutations provide a valuable source of variation in plant material from which the breeder can make selection.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Field evaluation for the effects of nitrous acid on the agro-morphological characteristics was conducted at the Experimental Garden and Plant Biology laboratory, Federal University of Technology, Minna, Niger State, during the 2021 planting season. Geographically Minna is located in the North Central geopolitical zone of Nigeria, found within Latitude 9º3855.8 N and Longitude S6º3119.7 E. The climate has two different seasons, a wet season between May and October and dry season between November and April every year.

3.2 Collection and Identification of Seeds

Dry seeds of three leafy vegetable genotypes were obtained from the National Centre for Genetic Resources and Biotechnology (NACGRAB), Moore Plantation Ibadan, Oyo State, they were identified as *Celosia argentea* L. (NGB00023), *Solanum macrocarpon* L. (NHGB/09/133) and *Amaranthus hybridus* L. (NGB00077).

3.3 Seed Germination Test

This test was done before planting to show viability of seeds for each genotype, twenty (20) seeds each of the different genotypes were treated with different concentration of Nitrous acid and placed in a germination kits with a sterilized filter paper and distilled water was filled to water level to allow water reaching the top then placed in an incubator, percentage germination was checked after three, seven and ten days to observe the rates of germination. Seeds were considered as germinated when the radicle appeared from the seed coat and counted. Number of seeds showing germination were

counted and expressed in percentage. The percentage germination was calculated using the formula below.

Germination (%) = $\frac{MeanNumberofseedsGerminated}{TotalNumberofseedssown} \times 100$

3.4 Collection and Tagging of Soil Sample

A total of sixty (60) planting sack bags were properly labeled, Sandy loamy soil samples were obtained from the experimental garden of Department of Plant Biology, Federal University of Technology, Minna. The soil samples were filled in the labeled bagswith 20 litres of soil of bag length 22 cm by width 19 cm arranged in rows and kept in the open exposure of sunlight, soil samples were soaked with water and the bags perforated at the base to allow easy seepage and to disallow water-logging in the bags prior to planting.

3.5 Treatment of Seeds

The dry seeds of leafy vegetables were treated at the Departmental Laboratory of Plant Biology Federal University of Technology, Minna with freshly diluted Nitrous acid at different concentrations 0.2%, 0.4%, 0.8% and 1.0%.

3.5.1 Procedure for the Treatment of Seeds

A total of forty (40) dried seeds for each treatment were placed in labeled plastic containers and were presoaked in distilled water for one hour and then treated with different concentrations of freshly prepared solutions of Nitrous acid (NA) for one hour. In preparation of the different concentrations, firstly, stock solution is prepared by taking 1ml of Nitrous acid using a syringe in a beaker and then 99 ml of distilled water was added. Then to prepare 0.2 % concentration, 2ml of stock solution was added into 98 ml of distilled water, for 0.4% concentration, 4 ml of the stock solution was added to 96 ml of distilled water, for 0.8% concentration, 8ml of stock was diluted in 92 ml of

distilled water, for 1.0% concentration, 10 ml of stock solution was diluted in 90 ml of distilled water, untreated seeds were taken as control. After treatment, seeds were thoroughly washed in distilled water to leach the residue of the chemical.

3.6 Planting of Seeds

10 seeds were planted in each bags and lightly covered with the soil under the same environmental conditions. Harvesting was done by uprooting the whole plant at 9 weeks after planting (WAP). Data were recorded for various parameters at definite intervals

3. 7 Field Experimental Design

Twenty (20) planting bags were allocated for each genotype. This research was laid out in a Completely Randomized Design (CRD) with four replicates in planting bags in the experimental field. All agronomical and cultural practices such as removal of weeds and thinning as well as harvest were done manually to raise a good crop.

3.8 Data Collection

Data on vegetative and yield parameters was recorded during the planting period following the methods stated by International Board for Plant Genetic Resources (IBPGR).

3. 8.1 Measurement of vegetative parameters

The specific quantitative traits that were determined include:

Plant height (PH): The heights of 4 randomly selected plants were measured using metric tape from the base to the stem apex of the plant at 3, 5, 7 WAP and at maturity.

Number of leaves per plant (NL): Total number of leaves per plant for 4 selected plants from each concentration including control were counted and recorded at 2 weeks' interval of 3,5,7 and 9 weeks.

Leaf area (cm^2): Leaf length x Leaf width x 0.7489

Leaf length: This was measured from the base of the leaf through the leaf midrib to the leaf apex using a meter rule.

Leaf width: The width was measured from one end of the leaf to another horizontally across the leaf using a meter rule.

3. 8.2 Measurements of yield parameters

Yield parameters from the three genotypes of leafy vegetables were determined as a function of Number of spikes per plant (NS) and Length of spikes (LP) for NGB00023 and NGB00077 were recorded while for NHGB/09/133 Weight of fruits (WF) and Number of fruit is done by randomly selecting four fruits for all the treatment including the control using a weighing scale and by visual counting at maturity.

3.9 Determination of Shelf Life in *Celosia Argentea*, *Amaranthus Hybridus* and *Solanum Macrocarpon*

Leaves were tied in bundles (20) as each of the different concentration of the vegetables were harvested from the garden early in the morning and well labelled. They were taken to the laboratory and packed loosely in a well-ventilated environment at room temperature until deterioration was observed. This was followed by measuring the initial weight using the weighing balance at an interval of twelve hours and recorded. Yellowing percentage was calculated by recording the observations at every alternate day from the zero day of storage till the end of storage life. Slightly yellowed leaves were sorted from the samples and yellowing percentage was calculated. Shelf life was charged by wilting, discolouration and decay (Jackline, 2019).

3.10 Proximate, Minerals and Vitamins Composition

3. 10.1 Proximate composition evaluation

The proximate analyses (% dry matter, % moisture, % ash, % crude fat, % crude fibre and energy contents was analysed according to methods of the Association of Official Analytical Chemists (AOAC, 2010).

3. 10.1.1 *Determination of moisture content*

Moisture content was measured using air-oven following methods of Association of Official Analytical Chemists (AOAC,2010). A material test chamber M720 was used to dry an empty weighing vessel at 105 °C for 1 h (W1) and weighed (W2). The dry sample (5 g) was then poured in to the vessel, oven dried at 105 ± 1 °C until constant weight was attained. This was then cooled in a desiccator; after which it was weighed (W3). The percentage moisture was calculated as:

Moisture content (%) = $\frac{W2-W3}{W2-W1} \times 100$

where W1 = weight of the empty vessel. W2 = weight of the vessel + sample. W3 = weight of vessel + dried sample.

3. 10.1.2 *Determination of ash content*

The ash content was determined using a dry ashing method (Agrilasa, 2007). A porcelain crucible was dried at 105 °C for 1 h, after cooling in a desiccator, and then weighed (W1). The samples (2 g) were placed in the previously weighed crucible and reweighed (W2). The crucible with its content was then ashed first at 250 °C for 1 h at 550 °C for 5 h. (Furnace E-Range, E300-P4, MET-U-ED South Africa) and allowed to cool and the weight was taken (W3). The percentage ash was calculated as:

Moisture content (%) = $\frac{W2-W3}{W2-W1} \times 100$

where W1 = weight of a dried porcelain crucible. W2 = weight of the crucible + sample. W3 = weight of the crucible + ashed sample.

3. 10.1.3 *Determinationof crude lipid*

Crude lipid was determined using the Soxhlet extraction technique (AOAC, 2010). The lipid content of the sample (5 g) was extracted using 100 mL of petroleum ether. The mixture was filtered, and its lipid content was collected in a pre-weighed (W1) clean beaker. Thereafter exhaustive lipid extraction was done on the same sample with 100 mL of petroleum ether for 24 h. It was then filtered and decanted into (W1) beaker. The lipid content was concentrated to dryness in a steam bath and oven dried at 40–60 °C and the beaker was reweighed (W2). Percentage of lipid was calculated as;

$$\text{Moisture content (\%)} = \frac{W2-W3}{\text{Wieght of oriinal sample}} \times 100$$

3. 10.1.4 *Determination of crude fiber content*

A modification of the acid/base digestion method described by Aina*et al*., (2012) was used to determine the dietary fiber. A 5 g of sample was digested with 100 mL of 0.25 M sulfuric acid solution by boiling under reflux for 30 min and quickly filtered. The insoluble matter was rinsed four times with boiling water to remove the remaining acid. This process was repeated on the residue using 100 mL of 0.31 M sodium hydroxide solution. The final residue was washed with water until it was free of base. It was then oven-dried at 100 °C, cooled in a desiccator and weighed (C1). The weighed sample was incinerated in a muffle furnace at 550 °C for 5 h, transferred to cool in a desiccator and weighed (C2). The percentage crude fiber was calculated as:

$$\text{Moisture content (\%)} = \frac{C2-C3}{\text{Wieght of sample}} \times 100$$

3. 10.1.5 *Determination of crude protein*

The total nitrogen amount in the sample was determined following the micro Kjeldahl method (AOAC, 2010). Digestion of 2 g the sample was done in a Kjeldahl flask by boiling 20 mL of concentrated H_2SO_4 and a Kjeldahl digestion tablet until a clear mixture was obtained. The digest was filtered into 250 mL volumetric flask, made up to

mark with distilled water and set up for distillation. Ammonia was steam-distilled from the digest to which 50 mL of 45% NaOH solution has been added. The distillate (150 mL) was collected into a conical flask containing 100 mL 0.1 N HCl and methyl orange was used as an indicator. The ammonia reacted with the acid in the receiving flask and percentage nitrogen (N) was estimated by back titration against 2 M NaOH. Nitrogen calculated using the following equation.

Moisture content (%) = $\frac{W2-W3}{\text{Wieght of sample in grams}} \times 100$

where, N = normality, percentage crude protein was obtained by multiplying the nitrogen value by a factor of 6.25 % crude protein = Nitrogen in sample × 6.25.

3. 10.1.6 ***Determination of total carbohydrate content***

The carbohydrate content was estimated by deducting the total crude protein, crude fiber, ash and lipid from the total dry matter as: % Total carbohydrate = 100 – (% Moisture content + % Total Ash + % crude fat + % crude fiber +% crude protein).

3. 10.1.7 ***Determination of energy content***

Total energy of the samples was calculated by the difference method. The water factors: 4, 9 and 4 kcal were employed to calculate the caloric value by summing the multiplied values for crude protein, crude lipid and carbohydrate respectively as:

Energy value (kcal/100 g) = (crude protein × 4) + (crude lipid × 9) + (total carbohydrate × 4).

3. 10.2 Vitamins analysis

3. 10.2.1 ***Vitamin A***

The retinol content in the samples was estimated using the method described by Onyesife*et al*. (2014). Briefly, 1 g of sample was macerated with 20 mL of petroleum ether. The solution was incubated for 2 h and filtered, evaporated to dryness and 0.2 mL

of chloroform-acetic anhydride (1:1 *v*/*v*) added to the residue. Thereafter, 2 mL of 30% TCA-chloroform was added to the mixture and absorbance was measured at 620 nm using a UV-3000PC spectrophotometer. Retinol standard was prepared in similar fashion. The concentration of vitamin A in the sample was extrapolated from the standard curve using the equation: $Y = 0.001x + 0.0008$, $R^2 = 0.9969$.

3. 10.2.2. *Vitamin C*

Ascorbic acid (Vitamin C) was quantified using the 2,6-Dichlorophenol-indo-phenol, sodium salt (DPIP) titration method as described by Adebooye (2008). The samples (1 g) were separately homogenized with 40 mL of a buffer solution made up of 1 g/L oxalic acid and 4 g/L sodium acetate anhydrous. The mixture was titrated against a solution containing 295 mg/L DPIP and 100 mg/L sodium bicarbonate. The end-point of the titration was identified by the disappearance of the initial blue color and results were expressed as mg/100g dry weight.

3. 10.2.3. *Vitamin B_2*

Thiswas calculated according to Okwu and Ndu (2006).

$$\text{Riboflavin mg/100g} = \frac{100}{W} \times \frac{Au}{As} \times \frac{C \times Vt}{Va}$$

Where:

W=weight of sample ash

Au=absorbance of test sample

As=absorbance of standard phosphorus solution

C=concentration of standard phosphorus solution

Vt=total extract volume

Va= volume of extract analyzer

3.11. Mineral Analysis

Leafy vegetable samples are spread on clean paper and then placed in the oven until complete desiccation. Samples were then crushed and ground into a mortar The minerals in the leafy vegetables analyzed were sodium, calcium, potassium, magnesium, phosphorus from solution obtained when 2.0 g of the samples were digested with concentrated nitric acid and concentrated perchloric acid in ratios 5:3, the mixtures were placed ona water bath for three hours at 80^{o}C. The resultant solution was cooled and filtered into 100 ml standard flask and made to mark with distilled water. filtered solution is used to assay minerals on Atomic Absorption Spectrophotometer (Buck scientific model200A) (Adegbaju *et al*.,2019).

3.12. Data Analysis

Analysis of Variance (ANOVA) using SPSS Microsoft Window System Software version 20.0 was used to determine the significance of variations due to the effect of different concentration of Nitrous acid on the quantitative data. The means were separated using Duncan Multiple Range Test (DMRT) was used to separate the treatment means at the probability level of $P \leq 0.05$. (George and Mallery, 2016). Experimental tests were conducted in triplicates and expressed as mean ± Standard error (S.E)**.**

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 Results

4.1.1. Effects of nitrous acid on leaf shape of *Celosia argentea*

The morphology of leaves in the control generally showed normal shapes with entire margin and acute apices, while the treated plant had distinct abnormalities (Plate 4.1). These changes developed in the leave morphology showed different characters like: indented apex at 0.2 concentrations, crinkled with acute apex at 0.4 concentration, bifurcated apex at 0.6 concentration and leaves with distinct acuminate apex at 0.8 concentration of Nitrous acid.

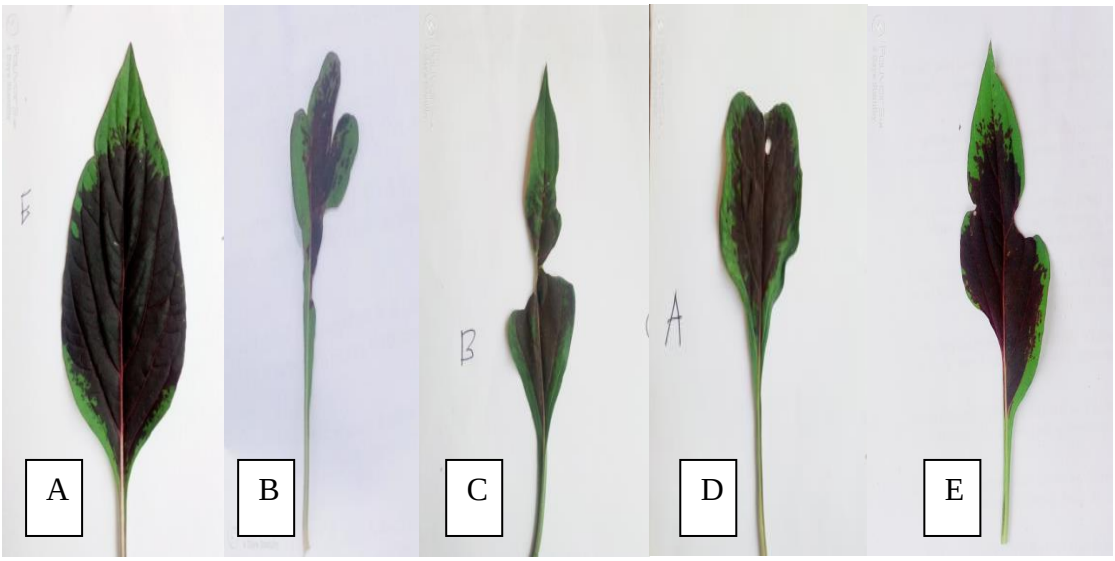

Plate 4.I: Leaf morphology of *Celosia argentea* (NGB00023):A. Normal leaf shape B. Leaf with indented apex C. Crinkled leaf with acute apex D. Leaf with bifurcated apex E. Leaf with distinct acuminate apex.

4.1.2 Effects of nitrous acid on leaf shape of *Solanum macrocarpon*

The morphology of leaves in the control generally showed normal shapes with entire margin while the treated plant had distinct abnormalities (Plate 4.2). These changes developed in the leave morphology showed different characters like: bifurcated apex at 0.2 concentration, leaves with blunt apex but oblique at one side at 0.4 concentration, leaves with broader width than length at 0.6 concentration and leaves with serrated margins at 0.8 and 1.0 concentrations of Nitrous acid.

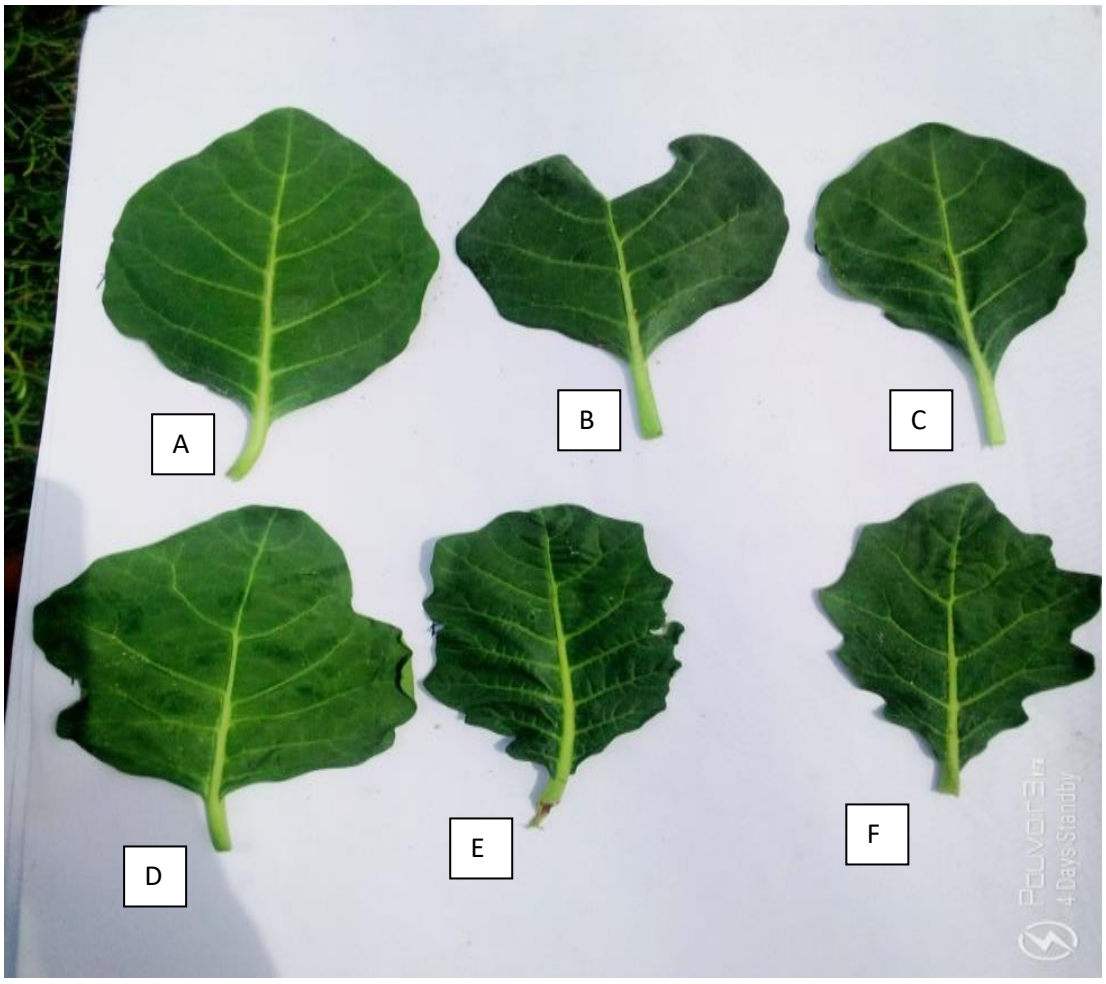

Plate 4.2: Leaf morphology of *Solanum macrocarpon* (NHGB/09/133): A. Normal leaf shape; B. Leaf with bifurcated apex; C. Leaf with blunt apex but oblique at one side; D: Broader leaf than length; E & F: Leaves with serrated margins

4.1.3 Effects of nitrous acid on leaf shape of *Amaranthus hybridus*

The morphology of leaves in the control generally showed normal shapes with entire margin while the treated plant had distinct abnormalities (Plate 4.3). These changes developed in the leave morphology showed different characters like: serrated margins at 0.2 concentration, leaves with bifurcated apex at 0.4 concentration, tapering leaves lamina with acute apex at 0.6 concentration, leaves with acuminate apex at 0.8 concentration, leaves with emarginated apex and bi-foliage apex 1.0 concentrations of Nitrous acid.

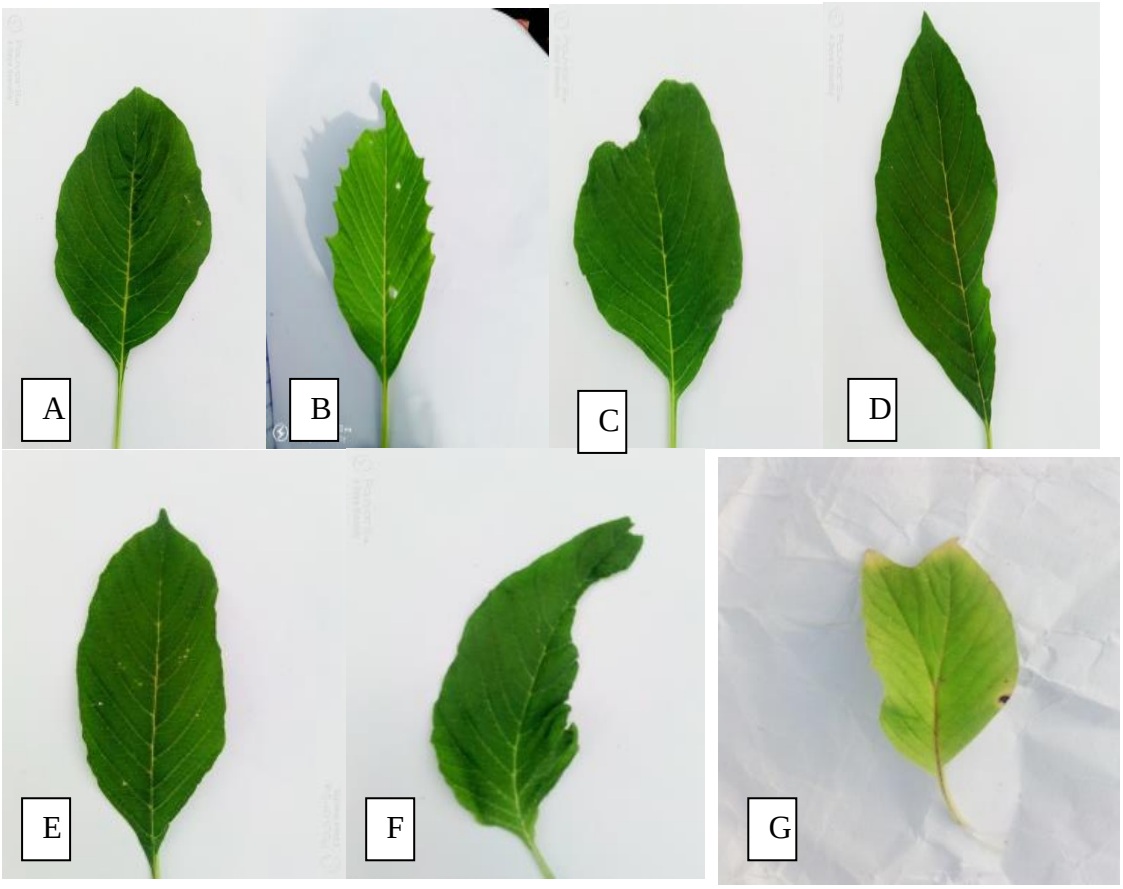

Plate 4.3: Leaf morphology of *Amaranthus hybridus* (NGB00077) A. Normal leaf; B. Leaf with serrated margins; C. Bifurcated Apex; D. Tapering leaf lamina with

acute apex; E. Leaf with acuminate Apex; F. Leaf with emarginated apex; G. Leaf showing chlorophyll deficiency with bifurcated apex

4.1.4 Effects of nitrous acid on the spike morphology of *Amaranthus hydridus*

The Spike morphology of *Amaranthus hydridus* (NGB00077) as shown in table 4.4 indicated that 0.8 % concentration of nitrous acid induced pink colouration of the spikes. At 0.4 % concentration of nitrous acid, the spikes were pinkish in colour but acute in shape. The control as well as lower nitrous acid concentrations produced greenish spikes while 1.0 % produced high yield with sparsely arranged spikes.

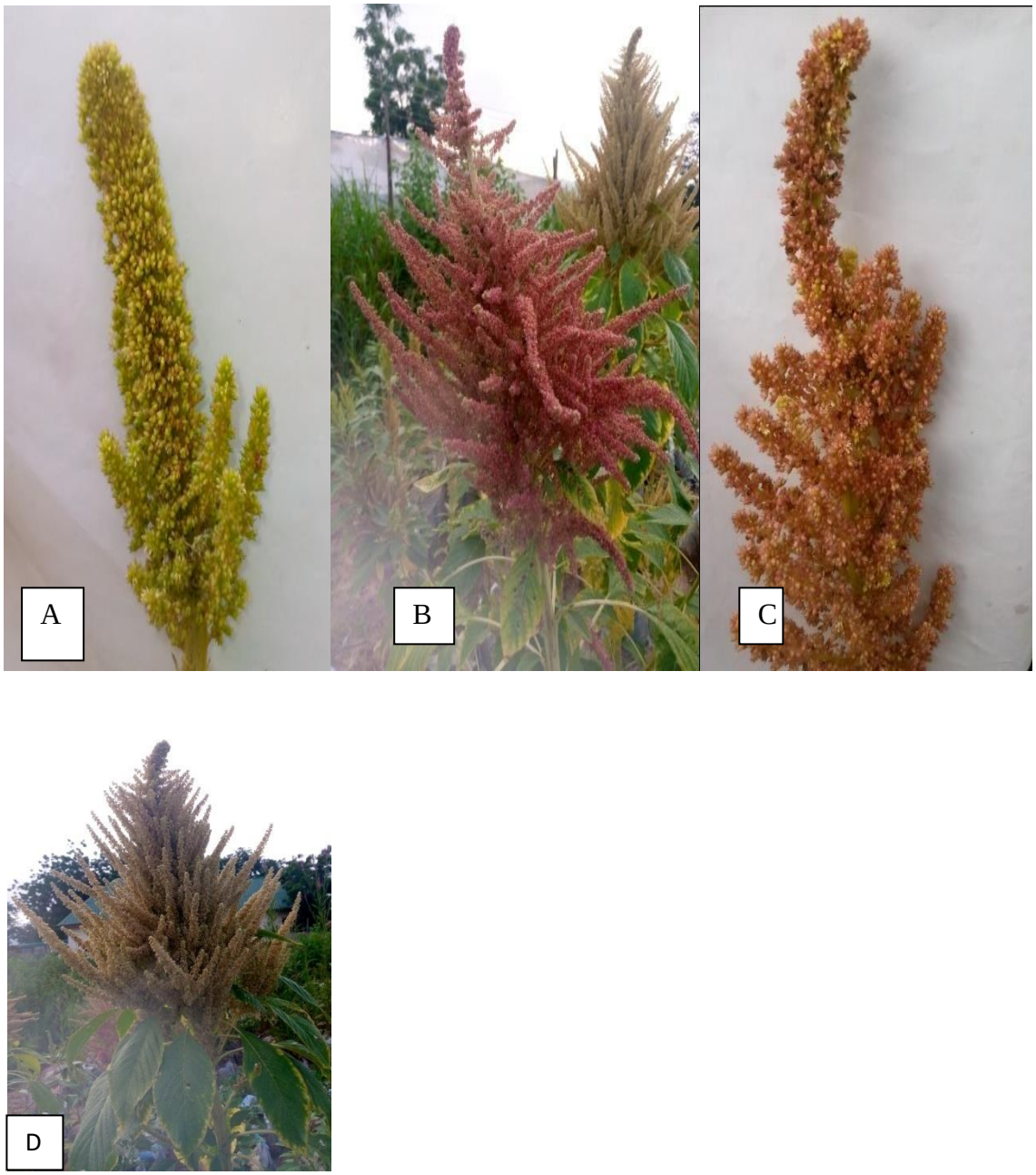

Plate 4.4: Spike morphology of NGB00077 (*Amaranthus hydridus*) A. Low yield Spikes with greenish colouration (control plant). B. Spikes with pink colouration(0.8 % of Nitrous Acid) C. Spikes with pink colouration and acute shape spike(0.4 % of Nitrous Acid) D. high yield Sparsely arranged spikes with greenish colouration(1.0 % of Nitrous Acid).

4.1.4 Effects of nitrous acid on Fruit size and number of fruits *Solanum macrocarpon*

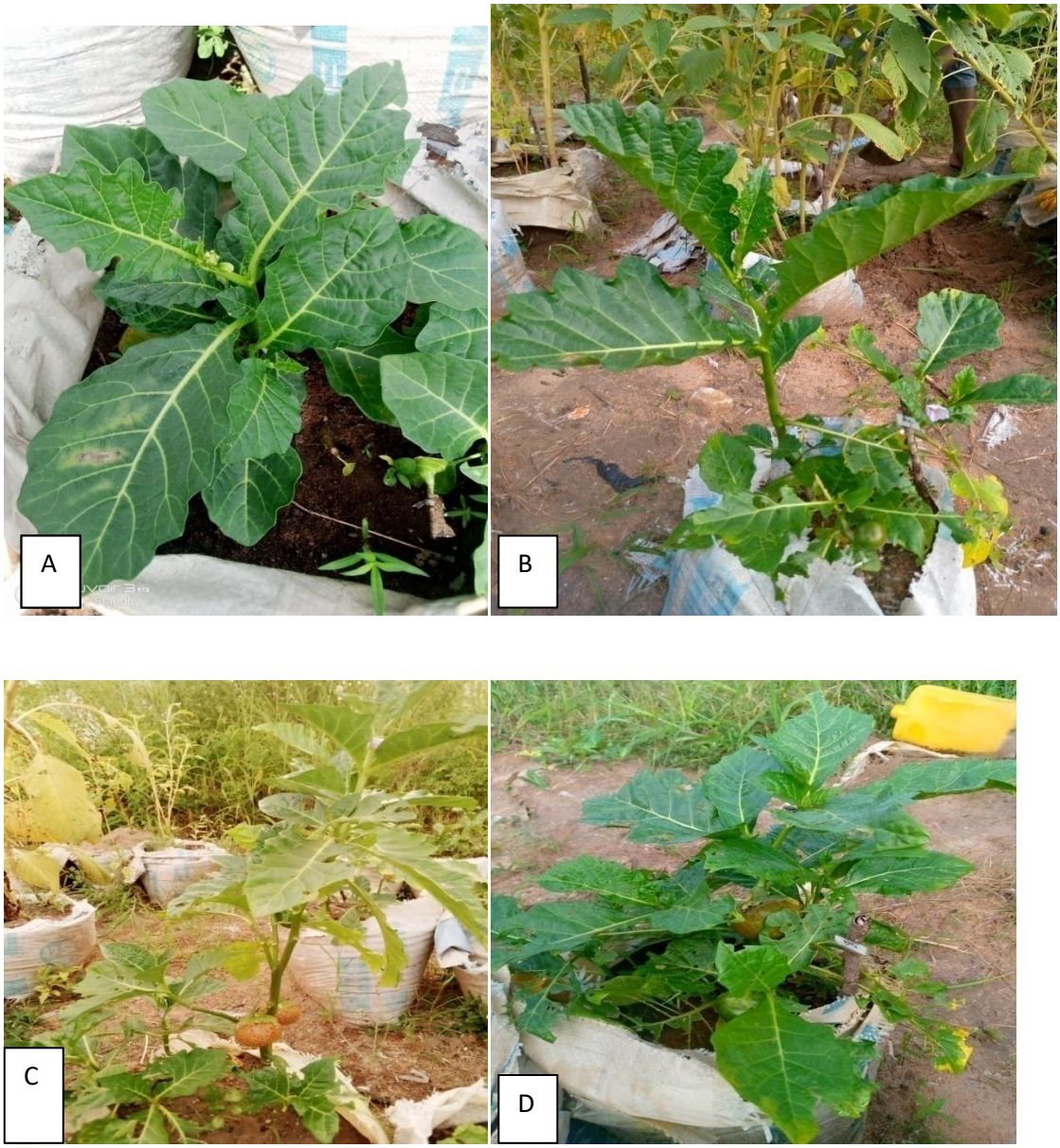

Plate 4.5: Fruit sizes and numbers of NHGB/09/133 (*Solanum macrocarpon*) A. Plant without fruits (0.0% control) B. Highest Plant with two fruits (0.4 % of Nitrous Acid) C. A more branched longer plant with highest number of fruits (0.8 % of nitrous acid). D. Broader leaves plant with biggest fruits size (1.0% of nitrous acid).

Plate 4.6: Spike morphology of NGB00023 (*Celosia argentea*) A. Lowest plant and light pinkish with less spikes (0.0 % control) B. An outgrowth of another spike with a more darker pinkish spikes on the Highest Plant (0.8 % of Nitrous Acid)

4.1.5. Effects of nitrous acid on plant height of M_1 generation

The quantitative morphological data collected for plant height is shown in table 4.1. In NGB00023 at week three after planting, the least plant height (4.88 cm) was recorded in the control, and this was significantly different ($p < 0.05$) from the heights recorded in the other treated plants. At five weeks after planting, the minimum heights (9.38 cm) was recorded in the control plants, and this was significant different ($p < 0.05$) from the heights recorded in the other treated plants. Similarly, at seven weeks after planting, the minimum heights (18.00 cm) was recorded in the control plants, and this was significantly different ($p < 0.05$) from the heights recorded in the other treated plants. At week nine, the shortest plant (28.75 cm) was found in plants treated with 0.4 % nitrous acid, and was significantly different ($p < 0.05$) from the heights recorded in the control and other treated plants.

In NHGB/09/133 at week three after planting, 5.33 cm was recorded as the highest height, this value was produced by plants treated with 0.4 % concentration of nitrous acid, and was significantly different ($p < 0.05$) from the heights recorded in the control and other treated plants. The least plant height (4.60 cm) was recorded in the control and was not significantly different ($p > 0.05$) from the values recorded in other treatment doses. Similar trend was also observed at week five after planting, here, 5.83 cm was observed in the 0.2 % concentration of nitrous acid treated plant while the lowest (4.93 cm) was observed in 1.0 % concentration of the Nitrous acid treated plants. At week nine the highest mean value (22.00 cm) was observed in 0.4 % treated plant, while the shortest (12.88 cm) was found in the control.

Similarly, in NGB00077, at week three the highest (6.65 cm) was found in 0.4 % concentration and was not significantly different ($p > 0.05$) from the value recorded at 0.8 % concentration, but was significantly different ($p < 0.05$) from the heights recorded

in the control and other treated plants. At week nine, the longest stem (120.63 cm) was observed at 0.2 % and was significantly different ($p < 0.05$) from the heights recorded in the control and other treated plants. The shortest stem (67.75 cm) was in the control and was significantly different ($p < 0.05$) from the heights recorded in the other treated plants.

Table 4.1: Effects of Nitrous acid on Plant Heights of Leafy Vegetables

TREATMENT (%)	3WAP (cm)	5 WAP (cm)	7 WAP (cm)	9 WAP (cm)
NGB00023				
Control	4.88±0.17^{a}	9.38±0.85^{a}	18.00±1.78^{a}	54.00±4.42^{ab}
0.2	5.63±0.27^{ab}	10.88±1.05^{ab}	23.80±2.11^{b}	84.63±5.44^{bc}
0.4	5.53±0.53^{ab}	13.75±0.48^{b}	24.75±0.92^{c}	28.75±0.92^{a}
0.8	6.50±0.19^{b}	13.13±1.23^{b}	22.63±3.75^{ab}	80.00±16.68^{b}
1.0	6.68±0.83^{b}	17.13±1.56^{c}	24.98±6.66^{c}	90.50±23.53^{c}
NHGB/09/133				
Control	4.60±0.18^{a}	5.13±013^{ab}	7.50±0.54^{a}	12.88±2.57^{a}
0.2	5.05±0.25^{ab}	5.83±0.39^{b}	10.38±0.75^{ab}	14.88±2.64^{ab}
0.4	5.33±0.38^{b}	5.50±0.54^{b}	9.63±1.59^{ab}	20.38±4.96^{bc}
0.8	4.68±0.20^{a}	5.10±0.32^{ab}	11.08±1.45^{b}	19.63±1.46^{b}
1.0	4.64±0.15^{a}	4.93±0.12^{a}	11.18±1.0^{b}	22.00±7.36^{c}
NGB00077				
Control	4.83±0.58^{a}	13.60±0.67^{a}	35.75±1.93^{a}	67.75±8.43^{a}
0.2	5.35±0.39^{ab}	16.15±2.11^{ab}	55.75±5.17^{b}	120.63±16.48^{c}
0.4	6.65±0.50^{b}	21.30±1.88^{b}	59.00±14.01^{b}	82.50±30.09^{ab}
0.8	6.05±0.31^{b}	14.80±1.60^{a}	38.50±4.37^{a}	97.75±17.58^{b}
1.0	5.63±0.36^{ab}	16.38±1.84^{ab}	40.08±3.68^{ab}	97.75±17.59^{b}

Values are means ± standard error of means. Values followed by the same letter(s) along the column are not significantly different at $p < 0.05$ as tested by DMRT.

WAP= Weeks after planting.

4.1.6 Effects of nitrous acid on number of leaves per plant of M1 generation

The quantitative studies showed that the mean number of leaves were highest in all treated plants when compared with the control (Table 4.2). In NGB00023, at three weeks the highest number of leaves (7.03) was recorded at 1.0 % concentration, and was significantly different ($p < 0.05$) from the values recorded in the control and other treated plants. At five weeks after planting, the least number of leaves (12.80) was recorded in the control, and was significantly different ($p < 0.05$) from the values recorded in other treated plants. At seven weeks after planting, the least number of leaves (16.03) was recorded in the control, and was significantly different ($p < 0.05$) from the values recorded in other treated plants. At nine weeks after planting, the highest value (60.50) was recorded in 1.0 % and the lowest value (26.43) was found in the control, and were significantly difference ($p < 0.05$) from the values recorded in other treated plants.

For NHGB/09/133, the control had the least number of leaves for all the time-line in the study and were significantly difference ($p < 0.05$) from the values recorded in other treated plants. At week three and five, the highest values (4.80 and 12.75 respectively) recorded at 0.4 % nitrous acid concentration were significantly difference ($p < 0.05$) from the values recorded in the controls and other treated plants. At seven weeks after planting, the highest value (17.18) was recorded in 1.0 % concentration and was significantly difference ($p < 0.05$) from the values recorded in the controls and other treated plants. At nine weeks after planting, the highest value (33.00) was recorded in 0.4 % and 0.8 % concentrations and was significantly difference ($p < 0.05$) from the values recorded in the controls and other treated plants and were significantly different ($p<0.05$) from the values recorded

In other treated plants .At 0.2% nitrous acid concentration, the highest number of leaves for all the time-line in the study were recorded and were significantly different ($p<0.05$)from the values recorded in the control and other treatments. For NGB00077, the control had the least number of leaves for all the time-line in treated plants.

Table 4.2: Effects of Nitrous acid on leaves number of the leafy vegetables

TREATMENT (%)	3WAP	5WAP	7WAP	9WAP
NGB00023				
Control	5.02±0.13^{a}	12.80±0.67^{a}	16.03±0.90^{a}	26.43±2.20^{a}
0.2	5.15±0.03^{a}	15.28±1.82^{ab}	32.08±5.13^{ab}	46.55±4.60^{b}
0.4	6.80±0.33^{ab}	19.00±2.33^{b}	34.81±6.40^{b}	44.36±3.80^{ab}
0.8	6.80±0.52^{ab}	19.05±3.00^{b}	33.51±7.28^{ab}	47.30±7.22^{b}
1.0	7.03±0.32^{b}	24.00±3.50^{bc}	34.82±10.20^{b}	60.50±13.30^{c}
NHGB/09/133				
Control	4.00±0.01^{a}	10.00±1.33^{a}	10.55±1.29^{a}	15.32±2.52^{a}
0.2	4.75±0.25^{ab}	13.30±1.60^{bc}	14.00±1.29^{ab}	25.50±2.02^{ab}
0.4	4.80±0.31^{b}	12.75±1.25^{b}	16.00±2.40^{bc}	33.00±2.55^{bc}
0.8	4.03±0.32^{a}	11.30±1.11^{ab}	15.25±3.62^{b}	33.00±2.65^{bc}
1.0	4.51±0.13^{ab}	11.50±1.28^{ab}	17.18±3.25^{c}	30.30±1.60^{b}
NGB00077				
Control	4.50±0.05^{a}	14.00±1.42^{a}	22.00±1.12^{a}	35.00±5.12^{a}
0.2	6.50±0.65^{b}	16.25±1.11^{b}	33.83±1.25^{c}	50.04±6.32^{c}
0.4	5.82±0.85^{ab}	19.30±1.82^{c}	27.31±6.76^{bc}	44.00±17.90^{ab}
0.8	5.54±0.29^{ab}	15.75±1.00^{ab}	23.88±2.11^{ab}	48.08±5.51^{b}
1.0	5.15±0.53^{ab}	17.00±0.82^{bc}	24.50±2.40^{b}	48.28±4.67^{b}

Values are means ± standard error of means. Values followed by the same letter(s) along the column are not significantly different at $p < 0.05$ as tested by DMRT.

WAP= Weeks after planting.

4.1.7 Effects of nitrous acid on yield parameters of M_1 generation.

The result of the effects of nitrous acid on the yield parameters of the M1 generation of the selected leafy vegetables are presented in Table 4.3. Data analysis showed that in most cases, the control were significantly different ($p < 0.05$) from the values recorded in the other treated plants. In NGB00023, the longest spike (14.25 cm) was recorded in 0.2 % nitrous acid concentration. This was not significantly different ($p > 0.05$) from the value recorded in 1.0 %, but was significantly different ($p < 0.05$) from the values recorded in the control and other treated plants. The highest mean number of spike (15.22) and the lowest number (12.85) at 0.8 % and 1.0 % nitrous acid concentration respectively, were significantly different ($p < 0.05$) from the values recorded in the control and other treated plants.

For NHGB/09/133, the number of fruit per plant ranged from 7.45 to 4.36. The highest weight of fruit per plant (10.38 g) was recorded in 1.0 % nitrous acid concentration, and this was significantly different ($p < 0.05$) from the values recorded in the control and other treated plants.

In NGB00077, the longest spike (9.37 cm) was recorded in the control. This was not significantly different ($p > 0.05$) from the value recorded in 0.2 %, but was significantly different ($p < 0.05$) from the values recorded in other treated plants. The highest number of spike (16.08) and the lowest number (11.79) at 0.4 % and 1.0 % nitrous acid concentration was significantly different ($p < 0.05$) from the values recorded in the other treated plants.

Table 4.3: Effects of Nitrous Acid on yield parameters of the Leafy Vegetables

TREATMENT	NGB00023		NHGB/09/133		NGB00077	
(%)	LS (cm)	NS	NFP	WFP (g)	LS (cm)	NS
Control	8.21±2.23^{a}	13.47±0.25^{ab}	4.36±0.06^{a}	7.15±0.13^{a}	9.37±0.50^{b}	15.18±0.09^{b}
0.2	9.14±0.05^{ab}	14.93±0.37^{b}	6.98±0.04^{ab}	7.88±0.13^{a}	9.15±0.22^{b}	15.84±0.44^{b}
0.4	14.25±0.28^{c}	14.52±0.13^{b}	6.96±0.06^{ab}	8.85±0.23^{ab}	8.56±0.39^{ab}	16.08±0.30^{bc}
0.8	11.14±0.37^{b}	15.22±0.36^{c}	6.73±0.12^{ab}	9.70±0.11^{b}	8.33±0.41^{a}	14.74±0.14^{ab}
1	14.00±2.17^{c}	12.85±0.28^{a}	7.45±0.11^{b}	10.38±0.15^{c}	8.24±0.42^{a}	11.79±0.15^{a}

Values are means ±S.E, values followed by the same letter(s) along the column are not significantly different at $p>0.05$ as tested by DMRT. LS: Length of Spike; NS: Number of Spike; NFP: Number of Fruit per plant; WFP: Weight of Fruit per plant.

4.1.8 Effects of Nitrous acid on Shelf Life Determination of the leafy vegetables

The leave samples of the controls of NGB00023, NHBG/09/133 and NGB00077 at room temperature (Plate: 5 to 7), showed gradual deterioration of the leave samples. The highest loss of weight was observed in the control.

PLATE 5. NGB00023(*Celosia argentea*) leaves sample at room temperature

PLATE 6.NHBG/09/133(*Solanum macrocarpon*) leaves sample at room temperature

PLATE 7. NGB00077(*Amaranthus hybridus*) leaves sample at room temperature

4.1.9 Decline in physiological weight of leafy vegetables

The results of the decline in the fresh weight of the leafy vegetables are presented in table 4.4. In NGB00023, the control loss the highest weight (75 %) after 84 hours. However, all the nitrous acid treated plants loss lesser weight during the same period. The 1.0 % nitrous acid concentration retained most water (30 %) hence lost lesser weight. This was followed by 0.2 % concentration treated plants (32 %), 0.4 % concentration (42 %) and then 0.8 % concentration (43%).

Similarly, in NHGB/09/133 the control loss the highest weight (64 %) after 84 hours. However, all the nitrous acid treated plants loss lesser weight during the same period. The 0.2 % nitrous acid concentration retained most water (24 %) hence lost lesser weight. This was followed by 1.0 % concentration treated plants (32 %), 0.8 % concentration (34 %) and then 0.4 % concentration (36 %). In NGB00077, the 0.2 % nitrous acid concentration loss the highest weight (51 %) after 84 hours. This was followed by 1.0 % concentration treated plants (42 %), control plants (39 %) and then 0.4 % and 0.8 % concentrations with 30 % weight loss each.

Table 4.4: Change in physiological weight loss between treatments in the three leafy vegetables

Conc. (%)	Initial (g)	12 Hours (g)	24 Hours (g)	36 Hours (g)	48 Hours (g)	60 Hours (g)	72 Hours (g)	84 Hours (g)	Weight loss (%)
NGB00023									
Control	14.11±0.28^{ab}	11.60±0.04^{ab}	11.41±0.00^{ab}	9.70±0.41^{a}	8.53±0.33^{a}	7.50±0.21^{a}	6.82±0.33^{a}	3.50±0.33^{a}	75 %
0.2	17.12±0.22^{b}	16.68±0.22^{b}	15.26±0.20^{b}	14.42±0.21^{ab}	13.71±0.35^{ab}	12.64±0.33^{ab}	12.22±0.11^{ab}	11.92±0.77^{b}	32 %
0.4	21.40±0.33^{bc}	19.20±0.35^{bc}	18.44±1.51^{bc}	18.10±2.20^{b}	17.02±0.88^{b}	15.93±0.20^{b}	13.80±3.32^{ab}	12.34±0.71^{b}	42 %
0.8	11.43±0.05^{a}	10.12±0.28^{a}	9.53±0.44^{a}	9.00±0.20^{a}	8.30±0.03^{a}	7.40±0.38^{a}	6.90±0.35^{a}	6.54±0.23^{ab}	43 %
1.0	22.15±0.21^{c}	20.19±0.76^{c}	20.12±0.81^{c}	18.90±0.49^{b}	17.70±1.02^{b}	16.84±0.03^{c}	16.16±0.37^{b}	15.40±0.01^{c}	30 %
NHBG/09/133									
Control	18.61±1.24^{a}	16.90±0.28^{a}	15.26±0.20^{a}	14.60±0.23^{a}	12.98±0.67^{a}	11.46±1.33^{a}	10.56±0.27^{a}	6.76±0.31^{a}	64 %
0.2	25.22±0.82^{c}	25.10±0.20^{c}	23.85±0.01^{c}	23.20±0.01^{c}	22.57±0.20^{c}	20.57±0.24^{c}	19.57±0.31^{c}	19.17±3.33^{c}	24 %
0.4	19.31±0.11^{ab}	17.58±0.18^{ab}	17.02±0.21^{ab}	16.76±0.23^{ab}	15.66±0.71^{ab}	14.58±0.34^{ab}	14.38±0.72^{ab}	12.28±0.01^{ab}	36 %
0.8	22.00±0.23^{b}	21.58±2.08^{bc}	20.00±7.20^{bc}	18.60±5.20^{b}	17.55±7.22^{b}	17.26±0.33^{bc}	16.58±0.97^{b}	14.59±0.22^{b}	34 %
1.0	20.60±0.28^{ab}	20.27±1.02^{b}	18.88±0.33^{b}	17.38±0.20^{ab}	16.84±0.33^{ab}	15.54±1.66^{b}	14.20±2.31^{ab}	13.95±1.30^{ab}	32 %
NGB00077									
Control	19.00±0.01^{a}	17.26±0.21^{a}	15.80±0.20^{a}	14.50±0.40^{a}	13.95±0.33^{a}	13.10±0.37^{a}	12.58±0.31^{a}	11.53±0.67^{a}	39 %
0.2	27.10±0.20^{c}	24.25±0.30^{c}	23.65±2.22^{c}	19.69±0.21^{b}	15.76±0.11^{ab}	14.89±0.02^{ab}	15.70±0.33^{b}	13.24±0.33^{ab}	51 %
0.4	22.77±0.76^{b}	20.88±0.44^{b}	20.34±0.10^{bc}	19.50±0.64^{b}	18.48±0.76^{b}	17.98±0.33^{c}	16.29±0.27^{c}	15.85±1.01^{b}	30 %
0.8	19.56±0.01^{a}	18.89±0.54^{ab}	17.23±0.73^{b}	16.58±0.23^{ab}	15.98±1.33^{ab}	15.27±0.22^{b}	14.50±0.73^{ab}	13.73±0.88^{ab}	30 %
1.0	20.20±0.91^{ab}	19.27±0.01^{ab}	16.78±0.51^{ab}	16.27±0.22^{ab}	15.57±0.31^{ab}	14.35±0.11^{ab}	12.50±1.45^{a}	11.80±0.72^{a}	42 %

Values are means±S.E, values followed by the same letter(s) along the column are not significantly different at p>0.05 as tested by DMRT.

4.1.10 Yellowing of leafy vegetables

The result of the yellowing of leaves was recorded in table 4.5. The control leaves showed more yellowing and the severity increased with increasing storage days for all the studied samples. For NGB00023, no leaf showed yellowing at the first day, but in the control, the number of yellow leaves became 4, 6, 7, 9 and 11 at day 2, 3, 4, 5 and 6 respectively. At day 6, 0.8 % treatment showed the least number of yellow leaves (4), this value was significantly different ($p < 0.05$) from the values recorded in the control and other treatments. For NHBG/0/133, no leaf showed yellowing at the first day, but the number of yellow leaves became 1, 3, 10, 15 and 16 at day 2, 3, 4, 5 and 6 respectively in the control. At day 6, 0.8 % treatment showed the least number of yellow leaves (11), this value was significantly different ($p < 0.05$) from the values recorded in the control and other treatments. For NGB00077, no leaf showed yellowing at the first day, but the number of yellow leaves became 4, 6, 8, 9 and 13 at day 2, 3, 4, 5 and 6 respectively in the control. At day 6, 1.0 % treatment showed the least number of yellow leaves (7), this value was significantly different ($p < 0.05$) from the values recorded in the control and other treatments.

Table 4.5: Yellowing of leaves between treatments at interval of days

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
NGB00023						
Control	0.00±0.05^{a}	4.00±0.04^{b}	6.00±0.25^{bc}	7.00±0.40^{b}	9.00±0.33^{bc}	11.00±1.25^{bc}
0.2	0.00±0.05^{a}	1.00±0.20^{ab}	2.00±0.05^{b}	3.00±0.15^{ab}	5.00±0.26^{b}	7.00±0.05^{b}
0.4	0.00±0.05^{a}	1.00±0.20^{ab}	1.00±0.20^{ab}	1.00±0.20^{a}	2.00±0.05^{a}	5.00±0.26^{ab}
0.8	0.00±0.05^{a}	0.00±0.05^{a}	1.00±0.20^{ab}	1.00±0.20^{a}	4.00±0.04^{ab}	4.00±0.04^{a}
1.0	0.00±0.05^{a}	0.00±0.05^{a}	0.00±0.05^{a}	3.00±0.05^{ab}	4.00±0.04^{ab}	5.00±0.26^{ab}
NHBG/0/133						
Control	0.00±0.05^{a}	1.00±0.20^{a}	3.00±0.15^{ab}	10.00±0.55^{b}	15.00±2.05^{c}	16.00±4.15^{bc}
0.2	0.00±0.05^{a}	2.00±0.05^{ab}	2.00±0.05^{a}	7.00±0.40^{a}	8.00±0.50^{a}	12.00±2.00^{ab}
0.4	0.00±0.05^{a}	2.00±0.05^{ab}	3.00±0.15^{ab}	11.00±1.25^{bc}	12.00±0.06^{bc}	13.00±0.05^{b}
0.8	0.00±0.05^{a}	1.00±0.20^{a}	2.00±0.05^{a}	7.00±0.40^{a}	9.00±0.33^{ab}	11.00±1.25^{a}
1.0	0.00±0.05^{a}	4.00±0.04^{b}	5.00±0.26^{b}	9.00±0.33^{ab}	11.00±1.25^{b}	12.00±2.00^{ab}
NGB00077						
Control	0.00±0.05^{a}	4.00±0.04^{b}	6.00±0.25^{b}	8.00±0.50^{bc}	9.00±0.33^{b}	13.00±0.05^{bc}
0.2	0.00±0.05^{a}	1.00±0.20^{ab}	2.00±0.05^{ab}	9.00±0.33^{c}	10.00±0.55^{bc}	10.00±0.55^{b}
0.4	0.00±0.05^{a}	0.00±0.05^{a}	1.00±0.20^{a}	4.00±0.04^{ab}	6.00±0.25^{a}	9.00±0.05^{a}
0.8	0.00±0.05^{a}	0.00±0.05^{a}	1.00±0.20^{a}	7.00±0.0^{b}	8.00±0.50^{ab}	8.00±0.50^{ab}
1.0	0.00±0.05^{a}	1.00±0.20^{ab}	1.00±0.20^{a}	3.00±0.15^{a}	6.00±0.25^{a}	7.00±0.40^{a}

Values are means ± S.E, values followed by the same letter(s) along the column are not significantly different at p>0.05 as tested byDMRT

4.1.11 Effects of nitrous acid on proximate composition of M_1 generation

4.1.11.1*Effects of nitrous acid on dry matter content of M_1 generation*

The results of the proximate composition of leafy vegetables treated with Nitrous acid are presented in table 4.6. In NGB00023 (*Celosia argentea*), the highest (12.98 %) and the least (9.28 %) values of dry matter were recorded in leaves treated with 0.2 % and 0.4 % nitrous acid respectively, these values were significantly different ($p < 0.05$) from the values recorded in the other treatments. In NHGB/09/133 (*Solanum macrocarpon*), The highest dry matter (13.05 %) was obtained from the leaves treated with 0.8 % Nitrous acid, this value was significantly different ($p < 0.05$) from the values recorded in the control and other treatments. The least value (11.29 %) recorded in 0.2 % was not significantly different ($p > 0.05$) from the values recorded in the control and 0.4 % nitrous acid concentration. In NGB00077 (*Amaranthus hybridus*), 0.4 % concentration of Nitrous acid produced the leaves with highest dry matter (14.78 %), this value was significantly different ($p < 0.05$) from all other treatments. Also, the least dry matter (11.83 %) obtained in 0.8 % treatment was significantly different ($p < 0.05$) from all other treatments.

4.1.11.2 *Effects of nitrous acid on moisture content of M_1 generation*

The results of the moisture composition of leafy vegetables treated with Nitrous acid are presented in table 4.6. In NGB00023 (*Celosia argentea*), the highest (91.41 %) and the least (84.03 %) values of moisture content were recorded in leaves treated with 0.8 % and 0.2 % nitrous acid respectively, these values were significantly different ($p < 0.05$) from the values recorded in the other treatments. In NHGB/09/133 (*Solanum macrocarpon*), the highest moisture content (90.20 %) was obtained from the leaves treated with 0.4 % Nitrous acid, this value was significantly different ($p < 0.05$) from the values recorded in the control and other treatments, while the least moisture content

(86.65 %) recorded at 0.8 % nitrous acid concentration was not significantly different ($p > 0.05$) from the values recorded in other treatments. In NGB00077 (*Amaranthus hybridus*), 0.8 % concentration of Nitrous acid produced the leaves with highest moisture content (89.84 %), this value was significantly different ($p < 0.05$) from all other treatments. Also, the least moisture content (84.43 %) obtained in 0.4 % treatment was significantly different ($p < 0.05$) from all other treatments.

4.1.11.3 *Effects of nitrous acid on total ash content of M_1 generation*

The results of the total ash content of leafy vegetables treated with Nitrous acid are presented in table 4.6. In NGB00023 (*Celosia argentea*), the highest ash content (1.80 %) was recorded at 1.0 % nitrous acid concentration, this value was significantly different ($p < 0.05$) from the values recorded in the other treatments while the least (1.22%) was recorded at 0.2% concentration and was significantly different ($p < 0.05$) from the values recorded in the other treatments. In NHGB/09/133 (*Solanum macrocarpon*), the highest ash content (2.89 %) was obtained from the leaves treated with 0.4 % Nitrous acid, this value was not significantly different ($p > 0.05$) from the value (2.54 %) recorded in the control, but significantly different ($p < 0.05$) from the values recorded in the other treatments while the least value (1.70%) was at1.0%.

In NGB00077 (*Amaranthus hybridus*), 0.2 % concentration of Nitrous acid produced the leaves with highest ash content (2.50 %), this value was significantly different ($p < 0.05$) from all other treatments. Also, the least value (1.78 %) obtained in 0.4 % treatment, was not significantly different ($p > 0.05$) from the value (1.80 %) recorded in the control, but was significantly different ($p < 0.05$) from all other treatments.

4.1.11.4 *Effects of nitrous acid on crude fat content of M_1 generation*

The results of the crude fat content of leafy vegetables treated with Nitrous acid are presented in table 4.6. In NGB00023 (*Celosia argentea*), the highest crude fat content (0.89 %) was recorded at 1.0 % nitrous acid concentration, this value was not significantly different ($p > 0.05$) from the least value (0.80 %) which was recorded in 0.8 % treatment. In NHGB/09/133 (*Solanum macrocarpon*), the highest crude fat content (2.07 %) was obtained from the leaves treated with 0.4 % Nitrous acid, this value was significantly different ($p < 0.05$) from the values recorded in the control and other treatments while the least (1.35 %) is recorded in the control.

In NGB00077 (*Amaranthus hybridus*), the control produced the leaves with the highest crude fat content (2.21 %), this value was significantly different ($p < 0.05$) from all other treatments while the least was recorded at 0.2% concentration and was not significantly different ($p > 0.05$) from the values recorded in the other treatments.

4.1.11.5 *Effects of nitrous acid on crude protein content of M_1 generation*

The results of the crude protein content of leafy vegetables treated with Nitrous acid are presented in table 4.6. In NGB00023 (*Celosia argentea*), the least crude protein content (1.34 %) was recorded in 0.8 % nitrous acid concentration, this value was significantly different ($p < 0.05$) from the highest value (3.78 %) recorded in the 0.2 % concentration. In NHGB/09/133 (*Solanum macrocarpon*), the least crude protein content (0.85 %) was obtained from the leaves treated with 0.2 % Nitrous acid, this value was significantly different ($p < 0.05$) from the values recorded in the control and other treatments.

While the highest value (4.62%) was obtained at 0.4% was significantly different ($p < 0.05$) from the values recorded in the other treatments.

In NGB00077 (*Amaranthus hybridus*), the highest crude protein content (5.04 %) recorded at the control, and the least value (2.96 %) recorded at 0.8 % treatment are significantly different ($p < 0.05$) from the values recorded in other treatments.

4.1.11.6 *Effects of nitrous acid on crude fibre content of M_1 generation*

The results of the crude fibre content of leafy vegetables treated with Nitrous acid are presented in table 4.6. In NGB00023 (*Celosia argentea*), the least crude fibre content (0.80 %) was recorded in 0.2 % nitrous acid concentration, this value was significantly different ($p < 0.05$) from the values recorded in the control and other treatments while the highest value (1.43 %) was obtained at the control and was significantly different ($p < 0.05$) from the values recorded in the other treatments. In NHGB/09/133 (*Solanum macrocarpon*), the highest crude fibre content (1.90 %) was obtained from the leaves treated with 0.4 % Nitrous acid, this value was not significantly different ($p > 0.05$) from the value (1.81 %) recorded in the control, but was significantly different ($p < 0.05$) from the values recorded in the other treatments. In NGB00077 (*Amaranthus hybridus*), the highest crude fibre content (1.65 %) recorded in 0.2 treatment was significantly different ($p < 0.05$) from the values recorded in the control and other treatments while the least value (1.18 %) was recorded at 0.4% concentration was significantly different ($p < 0.05$) from the values recorded in the other treatments.

4.1.11.7 *Effects of nitrous acid on carbohydrate content of M_1 generation*

The results of the carbohydrate content of leafy vegetables treated with Nitrous acid are presented in table 4.6. In NGB00023 (*Celosia argentea*), the highest carbohydrate content (9.27 %) was recorded in 0.2 % nitrous acid concentration, this value was significantly different ($p < 0.05$) from the values recorded in the control and other treatments while the least value (3.86 %) was obtained in the control was significantly

different ($p < 0.05$) from the values recorded in the other treatments. In NHGB/09/133 (*Solanum macrocarpon*), the highest carbohydrate content (5.51 %) was obtained from the leaves treated with 0.8 % Nitrous acid, this value was significantly different ($p < 0.05$) from the values recorded in the other treatments while the least value (1.01 %) was at 0.4 % concentration was significantly different ($p < 0.05$) from the values recorded in the other treatments. In NGB00077 (*Amaranthus hybridus*), the highest value (7.14 %) recorded in 0.4% treatment and was significantly different ($p < 0.05$) from the values recorded in other treatments while the least value (1.79 %) was at 0.8 % concentration was significantly different ($p < 0.05$) from the values recorded in the other treatments.

4.1.11.8 *Effects of nitrous acid on energy value of M_1 generation*

The results of the energy value of leafy vegetables treated with Nitrous acid are presented in table 4.6. In NGB00023 (*Celosia argentea*), the highest energy value (36.27 kcal/g) was recorded in 0.2 % nitrous acid concentration, this value was significantly different ($p < 0.05$) from the values recorded in the control and other treatments while the least (21.45 %) was at 0.8 % was significantly different ($p < 0.05$) from the values recorded in the other treatments. In NHGB/09/133 (*Solanum macrocarpon*), the least energy value (20.60kcal/g) was obtained from the control, this value was significantly different ($p < 0.05$) from the values recorded in the other treatments while the highest (37.67 %) was obtained at 0.8% concentration was significantly different ($p < 0.05$) from the values recorded in the other treatments except in 1.0% concentration with (37.53 %). .In NGB00077 (*Amaranthus hybridus*), the least energy value (27.88 kcal/g) recorded in 0.8 % treatment was significantly different ($p < 0.05$) from the values recorded in the control and other treatments while the highest value (36.07 %) was at 0.4 %.

Table: **4.6 Effects of Nitrous acid on proximate composition of M_1 generation**

Treatment (%)	Dry matter (%)	Moisture content (%)	Total ash (%)	Crude fat (%)	Crude protein (%)	Crude fibre (%)	Carbohydrate (%)	Energy value (kcal/g)
NGB00023								
Control	10.95±0.17^{ab}	88.26±0.50^{ab}	1.80±0.10^{b}	0.85±0.20^{a}	3.66±0.09^{b}	1.43±0.24^{b}	3.86±0.03^{a}	29.65±0.07^{bc}
0.2	12.98±1.73^{bc}	84.03±0.00^{a}	1.22±0.02^{a}	0.86±0.13^{a}	3.78±0.11^{b}	0.80±0.01^{a}	9.27±0.01^{bc}	36.27±0.06^{c}
0.4	9.28±0.38^{a}	90.05±0.01^{b}	1.40±0.40^{ab}	0.87±0.01^{a}	2.34±0.04^{ab}	0.92±0.01^{ab}	4.43±0.04^{ab}	25.59±0.11^{b}
0.8	10.08±0.87^{ab}	91.41±0.01^{c}	1.41±0.00^{ab}	0.80±0.67^{a}	1.34±0.03^{a}	0.93±0.00^{ab}	4.00±0.05^{ab}	21.45±0.10^{a}
1.0	11.41±0.10^{b}	88.41±0.00^{ab}	1.80±0.33^{b}	0.89±0.04^{a}	3.55±0.03^{b}	1.19±0.00^{b}	6.36±0.03^{b}	23.75±0.08^{ab}
NHGB/09/133								
Control	11.67±0.58^{a}	89.34±0.00^{a}	2.54±0.15^{b}	1.35±0.00^{a}	2.67±0.03^{ab}	1.81±0.07^{ab}	1.96±0.27^{a}	20.60±8.84^{a}
0.2	11.29±0.80^{a}	87.33±0.01^{a}	2.09±0.02^{ab}	1.55±0.00^{a}	0.85±0.03^{a}	1.30±0.08^{a}	3.18±1.87^{ab}	28.13±0.11^{ab}
0.4	11.66±0.97^{a}	90.20±0.15^{ab}	2.89±0.01^{b}	2.07±0.52^{ab}	4.62±0.06^{bc}	1.90±0.01^{ab}	1.01±0.10^{a}	35.43±0.15^{b}
0.8	13.05±0.27^{b}	86.65±0.03^{a}	1.72±0.06^{a}	1.57±0.00^{a}	3.50±0.00^{b}	1.13±0.01^{a}	5.51±0.04^{bc}	37.67±0.02^{bc}
1.0	12.98±0.21^{ab}	87.37±0.06^{a}	1.70±0.01^{a}	1.58±0.00^{a}	3.70±0.01^{b}	1.12±0.00^{a}	4.51±0.00^{b}	37.53±0.05^{bc}
NGB00077								
Control	13.62±0.20^{ab}	86.69±0.20^{ab}	1.80±0.00^{a}	2.21±1.66^{b}	5.04 ±0.08^{b}	1.19±0.00^{a}	4.74 ±0.06^{ab}	33.85 ±0.25^{b}
0.2	14.78±0.45^{b}	86.02±0.60^{ab}	2.50±0.00^{b}	0.57±0.00^{a}	4.82±0.00^{ab}	1.65±0.00^{b}	4.44±0.01^{ab}	32.88±0.01^{ab}
0.4	13.08±1.43^{ab}	84.43±0.33^{a}	1.78±0.01^{a}	0.61±0.00^{a}	4.86±0.02^{ab}	1.18±0.01^{a}	7.14±0.04^{bc}	36.07±0.07^{b}
0.8	11.83±1.06^{a}	89.84±0.30^{b}	2.23±0.02^{ab}	0.58±0.00^{a}	2.96±1.27^{a}	1.48±0.01^{ab}	1.79±0.22^{a}	27.88±0.17^{a}
1.0	13.69±0.01^{ab}	86.32±0.04^{ab}	2.13±0.01^{ab}	0.67±0.00^{a}	4.20±0.00^{ab}	1.41±0.00^{ab}	5.28±0.00^{b}	32.07±0.02^{ab}

Values are means±S.E, values followed by the same letter(s) along the column are not significantly different at p>0.05 as tested by DMRT.

4.1.12. Effects of nitrous acid on mineral composition of M_1 generations

4.1.12.1 *Effects of nitrous acid on sodium (Na) content of M_1 generations*

The results of the sodium content of leafy vegetables treated with Nitrous acid are presented in table 4.7. In NGB00023 (*Celosia argentea*), the control showed the least sodium content (16.0 mg/100g), this value was significantly different ($p < 0.05$) from the values recorded in other treatments while the highest (23.0 mg/100g) was at 1.0% and was significantly different ($p < 0.05$) from the values recorded in the other treatments.

In NHGB/09/133 (*Solanum macrocarpon*), the least sodium content (15.0mg/100g) was obtained from 0.2 % and 1.0 % treatments, this value was significantly different ($p < 0.05$) from the values recorded in the control and other treatments the highest value (20.0 mg/100g) was however obtained from 0.8 % concentration, this value was significantly different ($p < 0.05$) from the values recorded in the control and other treatments. In NGB00077 (*Amaranthus hybridus*), the highest sodium content (67.0 mg/100g) was recorded in 1.0 % treatment was significantly different ($p < 0.05$) from the values recorded in the control and other treatments while the least value (14.0 mg/100g) was not significantly different ($p > 0.05$) from the control value.

4.1.12.2 *Effects of nitrous acid on potassium (K) content of M_1 generation*

The results of the potassium content of leafy vegetables treated with Nitrous acid are presented in table 4.7. In NGB00023 (*Celosia argentea*), 1.0 % treatment showed the least potassium content (76.0 mg/100g), this value was not significantly different ($p > 0.05$) from the value (78.0 mg/100g) recorded at the control, but was significantly different ($p < 0.05$) from the values recorded in other treatments. In NHGB/09/133 (*Solanum macrocarpon*), the highest potassium content (80.0 mg/100g) was obtained from 0.2 % treatment, this value was significantly different ($p < 0.05$) from the values

recorded in the control and other treatments. In NGB00077 (*Amaranthus hybridus*), the highest potassium content (120.0 mg/100g) recorded in 0.8 % treatment was significantly different ($p < 0.05$) from the values recorded in the control and other treatments.

4.1.12.3 *Effects of nitrous acid on calcium(Ca) content of M_1 generation*

The results of the calcium content of leafy vegetables treated with Nitrous acid are presented in table 4.7. In NGB00023 (*Celosia argentea*), 1.0 % treatment showed the least calcium content (39.0mg/100g), this value was significantly different ($p < 0.05$) from the values recorded in the control and other treatments. In NHGB/09/133 (*Solanum macrocarpon*), the highest calcium content (79.0mg/100g) was obtained in the control, this value was significantly different ($p < 0.05$) from the values recorded in the other treatments. In NGB00077 (*Amaranthus hybridus*), the highest (73.0 mg/100g) and the least (30.0 mg/100g) calcium contents were recorded in 0.2 % treatment and control respectively, these values were significantly different ($p < 0.05$) from the values recorded in the other treatments.

4.1.12.4 *Effects of nitrous acid on magnesium (Mg) content of M_1 generation*

The results of the magnesium content of leafy vegetables treated with Nitrous acid are presented in table 4.7. In NGB00023 (*Celosia argentea*), 1.0 % treatment showed the least magnesium content (155.0 mg/100g), this value was significantly different ($p < 0.05$) from the values recorded in the control and other treatments. In NHGB/09/133 (*Solanum macrocarpon*), the least magnesium content (145.0 mg/100g) was obtained in 1.0 % treatment, this value was significantly different ($p < 0.05$) from the values recorded in the control and other treatments. In NGB00077 (*Amaranthus hybridus*), the highest (219.0mg/100g) and the least (118.0 mg/100g) magnesium contents were

recorded in 1.0 % treatment and control respectively, these values were significantly different ($p < 0.05$) from the values recorded in the other treatments.

4.1.12.5 *Effects of nitrous acid on phosphorus (P) content of M_1 generation*

The results of the phosphorus content of leafy vegetables treated with Nitrous acid are presented in table 4.7. In NGB00023 (*Celosia argentea*), 0.2 % treatment showed the least phosphorus content (79.79 mg/100g), this value was significantly different ($p < 0.05$) from the values recorded in the control (240.53 mg/100g) and other treatments. In NHGB/09/133 (*Solanum macrocarpon*), the least phosphorus content (144.04 mg/100g) was obtained in 0.2 % treatment, this value was significantly different ($p < 0.05$) from the values recorded in the control and other treatments. However, the highest value of 431.66 mg/100g was obtained from 0.8 % nitrous acid treatment; the value was significantly different ($p < 0.05$) from the values recorded in the other treatments. In NGB00077 (*Amaranthus hybridus*), the highest (21.94 mg/100g) and the least (10.56 mg/100g) magnesium contents were recorded in the control and 0.4 % treatment respectively, these values were significantly different ($p < 0.05$) from the values recorded in the other treatments.

Table: 4.7 Effects of nitrous acid on mineral composition of M_1 generation

Concentration	Na (mg/100g)	K (mg/100g)	Ca (mg/100g)	Mg (mg/100g)	P (mg/100g)
NGB00023					
Control	16.00±0.00^{a}	78.00±0.00^{a}	44.00±0.00^{b}	177.00±0.00^{b}	240.53±0.20^{b}
0.2	21.00±0.00^{b}	100.00±0.00^{b}	43.00±0.00^{ab}	173.00±0.00^{ab}	79.79±0.20^{a}
0.4	20.00±0.00^{ab}	107.00±0.04^{b}	46.00±0.00^{b}	185.00±0.01^{c}	173.25±0.07^{ab}
0.8	21.00±0.01^{b}	91.00±0.10^{ab}	45.00±0.00^{b}	179.00±0.01^{b}	431.66±0.07^{c}
1.0	23.00±0.00^{c}	76.00±0.00^{a}	39.00±0.00^{a}	155.00±0.01^{a}	257.24±0.27^{c}
(USDA,2008 mg/100g)	35.25	62.34	39.64	178.08	380.01
NHGB/09/133					
Control	18.00±0.01^{ab}	37.00±0.00^{a}	79.00±0.00^{c}	318.00±0.01^{b}	162.38±0.27^{ab}
0.2	15.00±0.00^{a}	80.00±0.00^{b}	47.00±0.01^{ab}	193.00±0.00^{ab}	144.04±0.07^{a}
0.4	18.00±0.01^{ab}	57.00±0.12^{ab}	63.00±0.09^{b}	318.00±0.01^{b}	162.38±0.27^{ab}
0.8	20.00±0.20^{b}	61.00±0.00^{ab}	49.00±0.00^{ab}	197.00±0.00^{ab}	180.69±3.64^{b}
1.0	15.00±0.56^{a}	36.00±0.00^{a}	36.00±0.00^{a}	145.00±0.00^{a}	349.06±0.40^{c}
(USDA,2019 mg/100g)	20.20	24.00	90.15	140.00	229.00
NGB00077					
control	14.00±0.00^{a}	47.00±0.08^{ab}	30.00 ±0.00^{a}	118.00 ±0.01^{a}	219.40±0.15^{c}
0.2	15.00±0.00^{a}	79.00±0.00^{b}	73.00±0.13^{b}	190.00±0.00^{ab}	187.20±0.02^{c}
0.4	14.00±0.00^{a}	94.00±0.01^{c}	86.00±0.01^{c}	343.00±0.00^{c}	105.60±0.01^{a}
0.8	37.00±0.00^{ab}	120.00±0.00^{c}	87.00±0.00^{c}	349.00±0.00^{c}	171.30±0.07^{b}
1.0	67.00±0.29^{b}	29.00±0.00^{a}	55.00±0.00^{ab}	219.00±0.00^{b}	159.80±0.03^{ab}
(USDA ,2010 mg/100g)	22.60	112.00	59.00	248.00	157.00

Values are means±S.E, values followed by the same letter(s) along the column are not significantly different at p>0.05 as tested by DMRT.

4.1.13. Effects of nitrous acid on vitamin composition of M_1 generations

4.1.13.1 *Effects of nitrous acid on vitamin A content of M_1 generation*

The results of the vitamin A content of leafy vegetables treated with Nitrous acid are presented in table 4.8. In NGB00023 (*Celosia argentea*), 0.2 % treatment showed the least vitamin A content (9.56 mg/100g), this value was significantly different ($p < 0.05$) from the values recorded in the control and other treatments. In NHGB/09/133 (*Solanum macrocarpon*), the least vitamin A content (5.61 mg/100g) was obtained in 0.4 % treatment while the highest (10.52 mg/100g) is at 0.2 % this value was significantly different ($p < 0.05$) from the values recorded in the control and other treatments.

In NGB00077 (*Amaranthus hybridus*), the highest vitamin A content (0.99 mg/100g) was recorded in the control and this value was significantly different ($p < 0.05$) from the values recorded in the other treatments.

4.1.13.2 *Effects of nitrous acid on vitamin C of M_1 generation*

The results of the vitamin C content of leafy vegetables treated with Nitrous acid are presented in table 4.8. In NGB00023 (*Celosia argentea*), 0.4 % treatment showed the highest vitamin C content (48.32 mg/100g), this value was significantly different ($p < 0.05$) from the values recorded in the control and other treatments. In NHGB/09/133 (*Solanum macrocarpon*), the least vitamin C content (42.37 mg/100g) was obtained in the control, this value was significantly different ($p < 0.05$) from the values recorded in other treatments.In NGB00077 (*Amaranthus hybridus*), the highest vitamin C content (4.90 mg/100g) was recorded in 0.4 % treatment, this value was significantly different ($p < 0.05$) from the values recorded in the control and other treatments.

4.1.13.3 *Effects of nitrous acid on vitamin B_2 of M_1 generations*

The results of the vitamin B_2 content of leafy vegetables treated with Nitrous acid are presented in table 4.8. In NGB00023 (*Celosia argentea*), the control showed the highest vitamin B_2 content (70.14 mg/100g), this value was significantly different ($p < 0.05$) from the values recorded in other treatments while the least (5.69 mg/100g) was obtained at 0.2 % concentration. In NHGB/09/133 (*Solanum macrocarpon*), the least vitamin B_2 content (10.66 mg/100g) was obtained in the control, this value was significantly different ($p < 0.05$) from the values recorded in other treatments while the highest (149.61 mg/100g) was obtained at 0.8 % concentration. In NGB00077 (*Amaranthus hybridus*), the highest vitamin B_2 content (0.23 mg/100g) was recorded in 0.2 % treatment, this value was significantly different ($p < 0.05$) from the values recorded in the control and other treatments while the least (0.19 mg/100g) was obtained with the control.

Table: 4.8 Effects of nitrous acid on Vitamin composition of M_1 generations

Concentration	Vitamin A (mg/100g)	VitaminC (mg/100g)	VitaminB2 (mg/100g)
NGB00023			
Control	10.17±2.11^{c}	43.26±0.04^{a}	70.14±0.06^{c}
0.2	9.61±0.09^{a}	43.26±0.04^{a}	5.69±0.00^{a}
0.4	9.41±0.04^{ab}	48.32±0.55^{b}	7.14±0.04^{ab}
0.8	10.15±0.09^{b}	43.52±1.11^{a}	37.73±0.06^{bc}
1.0	10.23±0.23^{b}	45.97±0.27^{ab}	8.70±0.06^{b}
(USDA, 2008 mg/100g)	9.00	59.00	28.30
NHGB/09/133			
Control	9.401±0.04^{b}	42.37±0.07^{a}	10.66±0.05^{a}
0.2	9.561±0.09^{b}	43.74±0.46^{ab}	62.08±4.66^{ab}
0.4	5.606±0.00^{a}	43.68±0.06^{ab}	96.69±0.00^{b}
0.8	10.189±0.22^{c}	50.06±0.04^{b}	149.61±0.14^{c}
1.0	9.561±0.09^{b}	50.21±0.13^{b}	149.21±0.04^{c}
(USDA,2019 mg/100g)	5.30	22.00	37.00
NGB00077			
control	0.99±0.44^{c}	4.29 ±0.04^{c}	0.19 ±0.02^{ab}
0.2	0.96±0.00^{b}	3.61±0.08^{b}	0.23±1.67^{b}
0.4	0.81±0.87^{a}	4.90±0.04^{d}	0.20±0.19^{a}
0.8	0.91±0.44^{ab}	4.39±0.10^{c}	0.20±0.08^{a}
1.0	0.81±0.87^{a}	3.54±0.06^{a}	0.20±0.02^{a}
(USDA,2010 mg/100g)	0.11	4.2	0.2

Values are means ±S.E, values followed by the same letter(s) along the column are not significantly different at $p>0.05$ as tested by DMRT.

4.2 Discussions

4.2.1 Effects of nitrous acid on leaf and spike morphology

The chemical mutagen, nitrous acid, induced mutation in the plants resulted into the morphological changes in the leaves and spikes. The variations were observed in the leaf margin, leaf lamina, leaf size, leaf apex and leaf shape. The normal leaf was with the acute apex but variation was observed in the leaf apex with bifurcated, emarginated and acuminate apex. Leaf abnormalities were attributed to the chromosomal breakage, disturbed auxin synthesis, disruption of mineral metabolism and accumulation of free amino acids (Salim, 2009).Higher concentrations (0.4 %, 0.8 % and 1.0 % concentrations of nitrous acid) in this study induced mutations in the leaves and yield parameters of the vegetables. This is similar to the work of More and Jagtap (2016) who reported bifurcated leaf, and lunate, elongated leaf lamina in the induction of morphological leaf mutations in *Lablab purpureus* L. It was also in conformity with the studies on the mutagenic effects of fast-neutron irradiation on selected morphological and yield trait of *Capsicum annum* and *Capsicum frutescens* by Daudu (2011).

The occurrence of chimeric phenotypes at seedling stage clearly indicated the impact of mutagen and is in conformity with the study by Prasanth *et al*. (2020), who highlighted on expression of moringa phenotypes and it was speculated that there could be a desirable leafy mutant among the mutagenized population. Many researchers have observed the same results in different plants like alfalfa (More and Jagtap, 2016) and cowpea (Gaikwad, 2013). The mutagen stimulated growth of the cells of the lamina causing its remarkable expansion, increase in leaf area and provides large surface area for gaseous exchange (Nura *et al*., 2011). Leaf and spike irregularities might be due to the fact that mutation increases the genetic capacity for the utilization of photosynthates, due to the ability of plants to sustain increased photosynthesis.

4.2.2 Effects of mutagens on plant height

Plant height is a fundamental trait for breeding of crop plants, Significant variations in high mean of plant height recorded in this study is in agreement with the work of Esson *et al.* (2017) who reported that Plant height increased significantly at 0.1 % concentration of Nitrous acid in Fox tail Millet. This is also in conformity with the work by Falusi *et al*. (2012) who observed highly significant differences in plant height of irradiated *Capsicum annum* and *Capsicum frutescens*. This result is in contrast with the proportional reduction in plant height with increase in concentration of Sodium azide obtained in the work of Muhamune and Kothekar (2012) who also reported gradual decrease in seedling height of french bean (*Phaseolus vulgaris* L.) treated with mutagens as compared with the control, he stated further that reduced seedling height might be due to gross injury caused at cellular level, reduced mitotic cell division, chromosomal damage or loss of proliferation capacity of the cells. Nitrous acid induced optimal vegetative growth as similar increase in *Sesamun indicum* using sodium azide has been reported by Mensah *et al*. (2007). This relative increase in plant height in the leafy vegetables could be attributed to stimulatory effects of the hormones that are responsible for increased plant height in the vegetables.

4.2.3 Effects of mutagens on number of leaves

Leave number in this study is of immense importance as they are good characters in breeding of improved leafy vegetables. Maximum mean value recorded in this study is in agreement with similar results obtained by Nura *et al*. (2011) who reported increased number of leaves in the treated plants and their proportionate distance from one another in mutagenised Jute (*Corchorus olitorious* L.) with chemical mutagen increases under different concentrations.

Furthermore, the results is also in agreement with the work of Prasanth *et al*. (2020) who reported improved yield components traits for leaf biomass such as more and broader leaves with enhanced fresh and dry weight production in *Moringa oleracea with* induction with E.M.S in the leaf area and also among *Zea mays* mutants obtained due to irradiation in enhancing formation of more leaves.

4.2.4 Effects of mutagens on yield parameters

Increase in yield parameters is essential for selection. The number of spike, length of spike, number of fruit and weight of fruit increases as the treatment increases when compared to the control plants. Optimum yield were recorded in 0.8 % and 1.0 % treatment the least yield was recorded among control plants. Significant early maturity was observed in chemically treated plants where all treatment exposure time achieved maturity earlier than the control and is in conformity with the report of Animasaun *et al.* (2014) but in contrast with the experiment of Danish *et al.* (2018) who reported that the mutagenic effectiveness generally decreased with increasing doses/concentrations of gamma ray, DMS and DES, but it was higher in lower and intermediate concentrations of MMS. The increase in fruit number, size and weight could be due to the influence of available nutrients like nitrogen in Nitrous acid treatments and is supported with the claims by Wani (2017) who stated that the higher efficiency of a mutagen indicates relatively less biological damage (i.e. seedling injury, sterility) in relation to the Mutation induced.

4.2.5 Effects of mutagens on shelf life of leafy vegetables

There was a drastic drop in fresh weight of the control samples and highest leaves yellowing in the control, this could be due to loss of moisture during the experiment and can be attributed to leaf drying effect and high rate of senescence regulated by ethylene hormone produced by the plant during stress. This is in agreement with Juneau and

Tarasoff, (2012) who stated that control leaves at room temperature favours ethylene production in storage.

Increase in shelf life and slow decline in the treated leaves could be the reduction of ethylene gas which increases the shelf life and reduces shrinkage. It changes the chemicals complex molecular structure back to its safe and original basic elements and is in conformity with Jowkar *et al*. (2012) using citric acid to address microbial load which are responsible for rots of plant tissues in vase.

The result in this study confirmed that treated leaves after storage have low respiration rate and was affected by induced treatment, this is in agreement with the work of Jackline (2019) where chlorine was used to prolonged shelf life, also the respiration rate of a product strongly determines its shelf life, the higher the storage temperature the higher will be the respiration and transpiration. According to Ahmad and Siddqui (2015), when the shelf life of a crop decreases and many undesirable change take place such as decay and if not governed ultimately affects the nutritional quality of the crop. The mutational effects of EMS in the biometric parameters of plants were reported in several investigations, such as reduction in fresh and dry weights in *Zea mays* (.Gnanamurthy *et al*., 2012; in fenugreek (Hussein and Safinaz, 2013).

4.2.6 Effects of mutagens on the nutritional composition of the leafy vegetables

4.2.6.1 Effects of mutagens on the proximate composition of the leafy vegetables

The result on the effects of Nitrous acid on the proximate compositions of *Celosia argentea*, *Solanum macrocarpon* and *Amaranthus hybridus* varied significantly with treatment. The proximate components from this study were observed to have decreased with an increase in treatment concentration on protein, fat, dry matter and energy value. However, the reverse was in the trend in moisture, ash, carbohydrate and fibre as they increased with increase in the treatment concentration. Increase in moisture content in

the treated leaves as compared with the control (84.03 -91.41 %) is within reported values in some Nigerian green leafy vegetables Akubugwo *et al*. (2007). Decrease in protein contents in treated leaves when compared with control might be due to oxidative damage in mRNA resulting in inhibition of protein synthesis and protein degradation that caused protein function disruption due to modification of the enzymatic and binding properties, other multiple causes such as mitochondrial damage, destruction or decrease gas, auxin or cytokine level could also be responsible for the decrease in the protein level. this result is in agreement with Victor *et al*., 2019 on the effects of on *phaseolus lunatus* seeds. Similarly, Karim *et al*. (2008) also reported significant differences in protein percentages of chickpea seeds at various doses of gamma irradiation from (19.68 to 22.48%). The moisture content of *Celosia argentea* sample was(84.03 – 91.41 %) and is within the acceptable range for good keeping (Ayodele and Olajide, 2011). The fat content ranged from 0.80 to 0.89 %, this value is within the range reported by Sheela *et al*. 2004 in leafy green vegetables.Similar results as observed by (Reddi and Suneetha, 1992) in rice who noted that with MMS the effectiveness did not necessarily increase linearly with increasing concentrations; rather every concentration had its own effectiveness, independent of the other lower and higher concentrations.

4.2.6.2 Effects of mutagens on the mineral composition of the leafy vegetables

The mineral contents of the leafy vegetables treated alongside control showed an increase trend of sodium, calcium, phosphorus, potassium, sodium and magnesium in NGB00023 and NGB00077 while a decrease trend was recorded in calcium and magnesium, In NHGB/09/133 and these values were similar to the values reported by Ayodele and Olajide (2011). The mineral compositions for each leafy vegetable indicated that the values obtained were similar to the United State Department of

Agriculture (USDA) standards. The result in this study is in contrast with the work of (Adie and Ibeabuchi, 2022) who stated that fluted pumpkin leaves whose seeds were treated with different doses of x-ray showed a decreasing trend in the calcium, phosphorus and nitrogen contents with increased treatment doses in M_1 and M_2 generations while the iron content was significantly enhanced by 18.75 mGy in M_1 generation. Sodium and potassium are important intracellular and extracellular cations respectively. Sodium is involved in the regulation of plasma volume, acid-base balance and nerve and muscle contraction. Increase in calcium content in this study is in agreement with the work of Manganaris *et al*. (2007) who suggested that calcium treatment can increase tissue firmness and reduced the susceptibility to physiological disorders and reduced the risk of salt-related injuries in peaches. Also the post-harvest application of calcium chloride ($CaCl_2$) extend the storage life of pear up to 2 months, plum up to 4 weeks and apple up to 6 months at 0 - 2 ºC with excellent colour and quality. The increase in the longevity and shelf life of the leaves of the treated seeds could be attributed to increase in the calcium content. Interestingly, higher result was associated with higher concentrations. In the opinion of Talame *et al*. (2008), mutagenic chemicals affect mitochondrial membrane potentials of plant; as such it has the ability to induce mutation of any kind in plant cells and organs, and even the amino acid sequence. However, in the present investigations, concentration ranges from 0.2 - 1.0 % Nitrous acid induced better growth and higher yield compared to the control showed this indicates that concentrations were able to activate optimally the phyto-hormones and growth regulators in the plant.

4.2.6.3 Effects of mutagens on the vitamin composition of the leafy vegetables

There was variations in Vitamin A, B_2 and C contents in the leaves samples analysed. Nitrous acid was sensitive to increase vitamin C contents. In *Celosia argentea, Solanum macrocarpon and Amaranthus hybridus.*In NGB00023, Vitamin A and B_2 recorded highest value in the control (105.17 mg/kg and 70.14 mg/1000g) respectively while the least (95.61 and 5.69 mg/100 g) was recorded at 0.2 % concentration respectively and with a significant difference ($p<0.05$) from other treatments. The vitamin compositions for each leafy vegetable indicated that the values obtained were similar to the United State Department of Agriculture (USDA) standards.

Vitamin C contents showed that increase in Nitrous acid treatments increases the content of Vitamin C in the leaves and is within the recommended dietary allowance per day (45 mg).The result in this study is in line with the work of Abu *et al*. (2019) who reported similar results among two varieties of Pepper where highest vitamin C contents was 26.88mg/100g recorded in the treatment with 0.03% Sodium azide. The data obtained from USDA, 2010 and USDA, 2019 report clearly indicate that vitamin C content is high for *Solanum macrocarpon* and *Amaranthus hybridus* and is in agreement with the study of Oladejo (2019) who investigated four leafy vegetables from two different zones and stated that leafy vegetables are good source of vitamin C. This result is also in conformity with the study by Settaluri *et al*. (2015) who found that among five green leafy vegetables ascorbic acid content was highest in lettuce.

CHAPTER FIVE

5.0CONCLUSION, RECOMMENDATIONS AND CONTRIBUTION TO KWOWLEGDE

5.1 Conclusion

This study highlighted the effects of induced mutation of Nitrous acid on the agro-morphological, shelf-life and nutritional compositions of three leafy vegetable genotypes. Abnormalities in leaf shapes, spikes and increase fruits size in this study indicates that genetic variability was created in the treated mutant lines. Generally, the effectiveness of Nitrous acid increases with increasing concentration, inNGB00023 (0.2 %, 0.8 % and 1.0 %); NHGB/09/133 (0.4 %, 0.8 % and 1.0 %) and NGB00077 (0.4 %, 0.8 and 1.0 %) from this study showed a more promising mutants on the agro-morphological traits such as plant heights, number of leaves, number of spikes, length and weight of spikes and fruiting characters could be a good selling traits to farmers.

Shelf life by induction with Nitrous acid was extended as treated leaves showed very slow deterioration in fresh weight and rate of discolouration was more in the control leaves. NGB00023 recorded highest loss in weight with (75 %), NHGB/09/133 leaves samples loss (64 %) in the control while in NGB00077 (51 %) weight loss was recorded at 0.2 % Nitrous acid treatment, hence, Nitrous acid has effects on the gradual decline in weight at the end of 84hours storage at room temperature and could retain loss of moisture in the vegetable genotypes.

The study further affirms that the values of proximate, mineral and vitamin composition of the treated plants differs from the values of the control and effectiveness did not necessarily increase linearly with increasing concentrations rather every dose had its own effectiveness with treatment of Nitrous acid. Hence, it could be concluded that

higher concentration of Nitrous acid was the most efficient dosage for producing beneficial yield and prevent quantitative and qualitative losses. These valuable agronomic traits could be explored by breeder for the improvement of the vegetables.

5.2 Recommendations

I. It is therefore recommended that, higher concentrations in NGB00023 (0.8 % and 1.0 %), NHGB/09/133 (0.8 %, 0.4 % and 1.0 %) and NGB00077 (0.4 % and 0.8 %) of nitrous acid should be employed for mutagenesis as it has high physiological and genetic effects.

II. Further research should be carried out on higher doses of Nitrous acid to determine the effects of these mutagens on the plants as compared to the control.

III. Also, studies on diversity of pollen sizes and restitution should be done to establish its frequencies and effects of the mutagens on the reproductive capacity of the plant.

IV. Other chemical and physical mutagens should be employed in modifying these plants for human needs by researchers in the future.

5.3 Contribution to Knowledge

The study established that Nitrous acid is a potent mutagen for inducing beneficial traits such as plant height at maturity. In NGB00023 (*Celosia argentea)* highest plant was 90.50 cm, NHGB/09/133 (*Solanum macrocarpon*) was 22 cm with 1.0 % and NGB00077 (*Amaranthus hybridus*) 120 cm with 0.2 % concentration.

Number of leaves per plant (60 with 0.8 % and 33 with 1.0 % concentration of Nitrous acid), also 0.8 and 1.0 % concentration induced leaves abnormalities such as Bifurcated apex,serrated margins chlorophyll deficient shapes.

Shelf life of treated leaves was extended as gradual decline in weight loss percentage was obtained (30 % with 1.0 %, 24 % with 0.2 % and 30 % with 1.0 % concentrations of Nitrous acid, Also at the end of 6 days the severity of yellowed leaves was lesser in treated leaves compared to the control (4, 11 leaves with 0.8 % and 7 leaves at 1.0 %) respectively.

NGB00023 exposed to 0.8 % concentration had lowest protein and fat content (1.34 and 0.80 %) respectively, Nitrous acid was sensitive to increasing vitamin C in all the leafy vegetables (48.32 mg/100g with 0.4 % in NGB00023, 50.21 mg/100 with 1.0 % in NHGB/09/133 and 49.07 mg/100g with 0.4 % in NGB00077).

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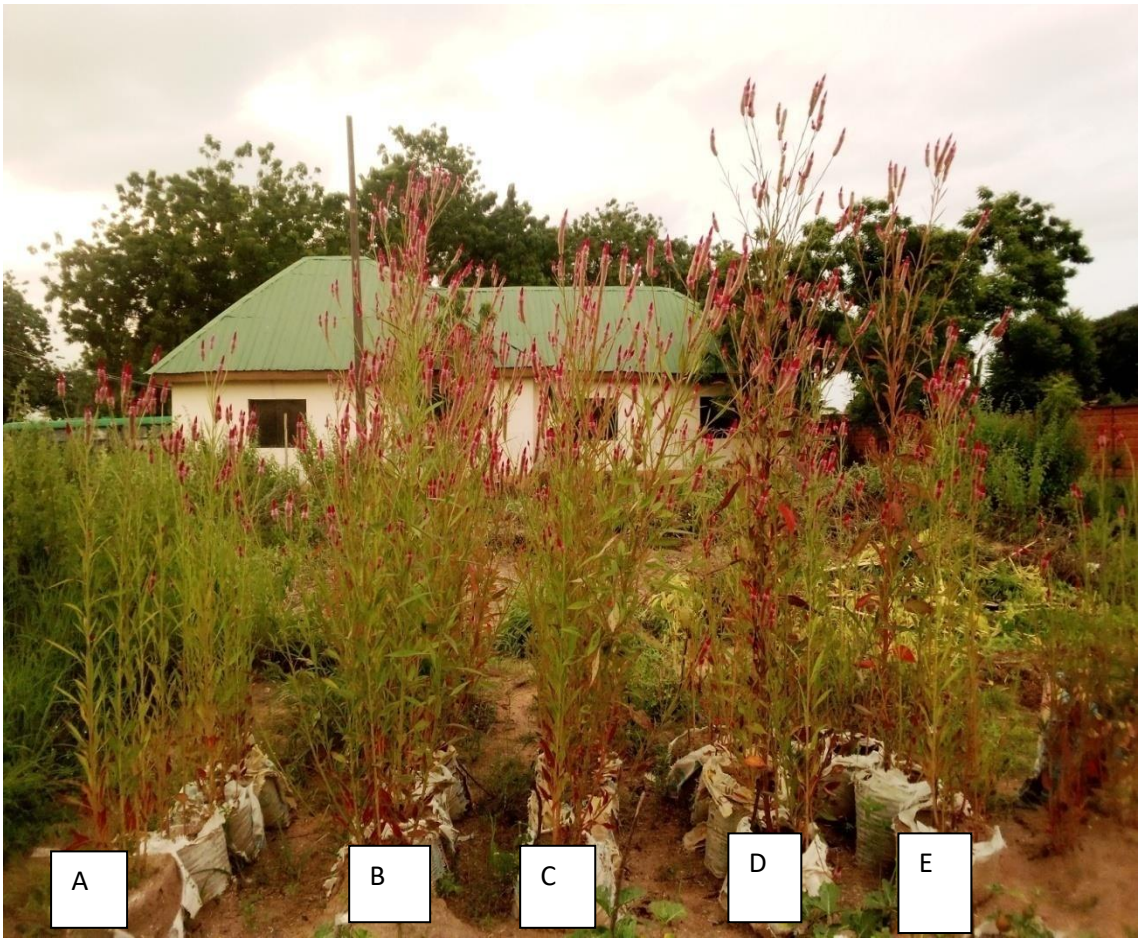
APPENDIX

Appendix A



Differences in Plant Height of M_1 Generation of the control and treated *Celosia argentea plants* with Nitrous acid

(A) Control

(B) 0.2% Concentration

(C) 0.4 % Concentration

(D) 0.8% Concentration

(E) 1.0% Concentration**Source: Field Photograph**

Appendix B

Differences in Plant Height of M_1 Generation of the control and treated *Amaranthus hybridus plants* with Nitrous acid

(A) Control

(B) 0.2% Concentration

(C) 0.4 % Concentration

(D) 0.8% Concentration

(E) 1.0% Concentration

Source:Field Photograph

Appendix C

Differences in Plant Height at Maturity of M_1 Generation of the control and treated *Solanum macrocarpon plants* with Nitrous acid

(A) Control

(B) 0.2% Concentration

(C) 0.4 % Concentration

(D) 0.8% Concentration

(E) 1.0% Concentration

Source:Field Photograph