THE EFFECT OF TEMPERATURE AND DAY LENGTH ON THE CONCENTRATION OF FATTY ACIDS AND ORGANIC ACIDS IN LEAVES OF *PORTULACA OLERACEA*: EFFECT OF ORAL ADMINISTRATION OF THESE LEAVES ON ACUTE INTESTINAL INFLAMMATION INDUCED IN MICE.

Thesis

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by

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TABLE OF CONTENTS

CONTENT **PAGE**

3.0 RESULTS

ABBREVIATIONS

AA	Arachidonic acid
ALA	α-linolenic acid
ARO	Agricultural Research Institute
DAI	Disease activity index
DHA	Docosahexaenioc acid
DPA	Docosapentaenioc acid
DSS	Dextran sulphate sodium
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EPA	Eicosapentaenioc acid
FAME	Fatty acid methyl esters
GC	Gas chromatography
GLA	Gamma linolenic acid
H&E	Haematoxylin and eosin
HPLC	High performance liquid chromatography
HTAB	Hexadecyltrimethyl-ammonium bromide
IBD	Inflammatory bowel disease
ICAM-1	Intercellular adhesion molecule-1
IL	Interleukin
LA	Linoleic acid

LDL	Low Density Lipoproteins
LTB_4	Leukotriene B_4
MPO	Myeloperoxidase
NF-κB	Nuclear factor κB
OA	Oxalic acid
Pon	*Portulaca oleracea nitida*
Pop	*Portulaca oleracea papillato-stellulata*
Pos	*Portulaca oleracea sativa*
PPAR	Peroxisome proliferator-activated receptors
PUEFA	Polyunsaturated Essential Fatty Acids
RBC	Red blood cells
ROS	Reactive oxygen species
TFA	Total fatty acids
TNF-α	Tumour necrosis factor-α
TXA_2	Thromboxane A_2
UC	Ulcerative colitis
VCAM-1	Vascular cell adhesion molecule -1
W.H.O	World Health Organisation
WBC	White blood cells

LIST OF TABLES AND FIGURES

ABSTRACT

Puslane (*Portulaca oleracea*) has been identified for cultivation as a food crop since its identification as an exceptionally rich source of bioprotective substances (α-linolenic acid, linoleic acid, antioxidants, vitamins) which are considered essential for growth, development and disease prevention. Also its short vegetative period and almost all year round production makes it more favourable or cultivation.

Many studies have been conducted to show the effect of different plant nutrition on both crop production as well as the nutritional value of the plant. However little is known about the suitable temperature and day length conditions necessary for optimizing the nutritional value of the plants *i.e.* greater fatty acids concentrations and lesser organic acids, and improving upon its agronomic potentials. The aim of this study therefore was to determine the effects of temperature and day length on the concentration of fatty acid and organic acid in leaves of purslane and also its influence on crop production. Three microspecies *P. sativa*, *P. nitida*, *P. papillato-stellulata* from the *Portulaca* complex were used in this study. Plants were grown under four different day/night temperature conditions (34/28, 28/22, 22/16, 16/10^{0}C), long day (LD) and short day (SD) for 8 weeks. We determined plant height, leaf length, plant number of internodes, leaf and stem fresh weights and dry weights, moisture content, percentage leaf dry weight in plant fresh weight during the experiment. Plant leaves were also harvested at 8-true leaf stage for fatty acid analysis as well as the organic acid concentrations. We found that plant height, leaf length, number of plant internodes, average length of internodes, leaf and stem fresh and dry weight, net plant fresh and dry weights of all microspecies decreased as the temperature decreased from 34-16^{0}C in both day length regimes. The commercial green-leaf *P. sativa* recorded the highest measurements for all the parameters mentioned above (LD, SD) with the exception of the leave dry weight and percentage moisture content. With these measurements, *P. nitida* was similar to *P. sativa* especially within the 34-22^{0}C temperature condition, LD and SD. Percentage leaf dry weight in whole plant fresh weight was also significantly increased in *P. nitida* at temperatures 34-22^{0}C, LD and SD. All plants except *P. nitida* significantly increased in oxalic acid concentrations as the temperature decreased to 16/10^{0}C in both day length regimes. Significant decreasing trends in ascorbic acid and the

major fatty acids determined (α-linolenic acid, linoleic acid and palmitic acid) were also found as the temperature condition reduced to 16/10^{0}C especially with the *P. nitida* microspecies. There was however no significant differences in the major fatty acids determined among the three microspecies in the 34-22^{0}C temperature condition, long day and short day. Our results therefore conclude that decreasing the temperature for growth to as low as 16/10^{0}C reduced plant growth and performance (even death) and decreased the nutritional value of all plants microspecies. Our results also indicate potential agronomic traits of local microspecies *P. nitida*.

The present study was also undertaken to explore the ameliorative effect of purslane on a mice model of human acute ulcerative colitis (UC). Acute colitis was induced by exposing female C57BL/6 mice to 3.5% DSS in drinking water for 8 days. Five groups of mice were used: three of them received in their chow 24% (6 g) powdered purslane leaves which contained (4800 mg ALA), 8% (2 g) leaves which contained (1600 mg ALA) and 0.8% (0.2 g) leaves which contained (160 mg ALA). The combined chow and leaves were given *ad libitum* for 10 days prior to DSS administration and this continued till the end of the experiment. DSS was also induced in one control group fed with standard diet. At the end of the experiment, mice were sacrificed and colonic damage was assessed both histologically and biochemically. Plasma fatty acids, colonic length, disease activity index (DAI) and MPO were determined. Administration of purslane leaves induced a significant increase in plasma DHA and in the 24% treated group and not 8% or 0.8% groups as opposed the normal control. EPA was not found in any of the groups. Administration of DSS resulted in a significant development of ulceration in the colon along with a rise disease activity and MPO. Dry powdered puslane leaves provided in the food at different concentrations did not significantly attenuate DSS-induced colonic inflammation along with MPO and DAI. Mild protectiveness was however seen among the 24% and 8% fed groups histologically. The reason behind the mild protectiveness observed histologically and not biochemically is unclear. The inability of purslane leaves to mitigate ulceration caused by DSS might have been related to the ingestion of other organic compounds (organic acids, coumarins, alkaloids, anthraquinones) in the leaves which have been suggested to have toxic effects in higher concentrations, low concentrations of ALA used or the non-conversion of ALA

to EPA. It is concluded that purslane may be a novel food factor for preventing experimental UC if the omega 3 active components are isolated from the leaves and purified devoid of other organic compounds. Further research in this plant is needed to identify its therapeutic effects on UC.

1.0 Introduction

Portulaca oleracea which is referred to as common purslane in English (**see appendix 1**) is a persistent annual herbaceous plant with fresh succulent leaves. It is a C4 plant which tastes like spinach (**see Fig. 1).** It has often also been described as a summer "weed with nutritive potentials" (Palaniswamy et al., 2002; Fontana et al., 2006). Purslane is one of the many species belonging to the family *Portulacaceae* which has been shown to have a high growth rate with a short vegetative period. It is self compatible and produces many small viable seeds (Liu et al., 2000). It comes in both ornamental and culinary cultivars which makes it suitable both as human food as well as animal fodder (Lim et al., 2007). Studies have shown its efficient water use hence its ability to survive in both droughts, saline soil conditions by way of enhancing their anti oxidative capacity and proline accumulation (Palaniswamy et al., 2001; Cros et al., 2007; Yazici et al., 2007; Kilic et al., 2008). Kilic et al., (2008) and Yazici et al.,(2007) demonstrated that its short vegetation period, all year production, its spreading habit as well as its ability to withstand salt stress makes it suitable to intercrop with fruit trees which are generally salt sensitive due to irrigation processes. This in effect will enable higher and continuous cumulative salt removal.

In a series of investigations of several large Mediterranean islands, following a study of *Portulaca* in the Canary Islands (Danin & Reyes- Betancort 2006), collections have also been made in Cyprus, Crete, Rhodes and Sicily. Purslane is considered here as an aggregate of several microspecies. Since no stable diagnostic characters may be seen on the vegetative parts of the various taxa (Danin et al., 1978) we keep using the seed morphology characters. The known and the more recently discovered taxa are documented with their typical seed-surface morphology, seed size, and chromosome number.

Although its origin is uncertain, purslane is a cosmopolitan plant growing in all continents and islands between the north and south latitudes of 40^0C (Danin et al., 1978). It grows in the wild, in flower beds, corn fields, waste lands and is cultivated in many parts of the world including cold areas such as Canada and the warm Caribbean islands (Fontana et al., 2006). In these temperate regions however, it is seen to flourish in the summers only and dies under conditions of shade and frost

(Singh, 1973; El-Keblawy and Al-Ansari., 2000). According to Fontana et al., (2006), purslane is seen to grow well at day /night temperatures of approximately 27/22^{o}C in long days of 16 hours. Seedlings are found throughout the year provided there is adequate moisture. But their density is much reduced in the winter (Singh, 1973). Even though purslane has a long history of use as human food, animal feed as well as for medicinal purposes, it is considered a minor crop in the United States of America (USA) (Liu et al., 2000; Cros et al., 2007; Kilic et al., 2008). The World Health Organization (WHO) listed it as one of the most used medicinal plants in the world, thus acquiring the name "global panacea" (Lim et al., 2007). Reports according to Palaniswamy et al., (2001) and Simopoulos, (2005) show that one hundred grams of fresh purslane leaves can supply up to 300-400 mg of α-linolenic acid (ALA) a polyunsaturated essential fatty acid (PUEFA) as well as uncommonly high concentrations of several antioxidants such as 12.2 mg α-tocopherol, 26.6 mg ascorbic acid, 1.9 mg β-carotene and 14.8 mg glutathione (which normally occurs in high amounts in fresh meat). This according to research has tagged purslane leaves as well as its seeds as the richest vegetable source of PUEFA especially α- linolenic acid yet examined (Ezekwe et al., 1999; Liu et al., 2000). It is also reported by Palaniswamy et al. (2000) and Liu et al., (2000) that the content of ALA in purslane is much higher than that found in other leafy vegetables such as spinach, lettuce, borage (*Borago officinalis* L),evening primrose (*Oenothera paradoxa Hudziok*) and even some fish species investigated to date.

Other biologically active compounds or nutrients found in purslane for which it has been named the "power food" of the future include antioxidants such as vitamins A, C, essential amino acids, oxalic acids, alkaloids, coumarins, flavonoids, melatonin (which also has anti-inflammatory properties) together with others such as catecholamines, noradrenaline and dopamine, good amounts of protein as well as carbohydrates (Ezekwe et al., 1999; Palaniswamy et al., 2002; Cros et al., 2007). Antioxidants as documented in literature are good neutralizers of free radicals as well as decrease the oxidation of low density lipoproteins (LDL), which are potential contributors to atherosclerosis and other chronic infections.

The food intake of modern society is characterized by foods containing a high omega-6/ omega-3 ratio, usually high fat, low antioxidant/ high carbohydrate diet so

that wild plants consumption such as purslane are left under-utilized. Recent studies however suggest that there is a newer interest in cultivating purslane as a crop in many parts of the world (used as salad or a potherb) due to its good nutritional and health promoting properties (Liu et al., 2000). In many countries, people use purslane as a diuretic, febrifuge, antiseptic, antispasmodic or a vermifuge all in the name of folk medicine. Purslane has also been shown to have antibacterial, anti inflammatory, analgesic, skeletal muscle relaxant and wound healing effects (Xiang et al., 2005; Oliviera et al., 2009). In West Africa, juices from the leaves and stems have been effective when applied topically on swellings, bruises, abscesses and boils (Ezekwe et al., 1999).

Alpha-linolenic acid, an essential omega-3 fatty acid which accounts for about (60%) of the total fatty acid content in purslane leaves (Liu et al., 2000) has very beneficial role in human growth and in disease prevention (Fontana et al., 2006). It is the precursor of the longer-chain fatty acids, eicosapentaenioc acid (EPA 20:5, ω3), docosapentaenioc acid (DPA 22:5, ω3) and docosahexaenioc acid (DHA 22:6, ω3) **(see Fig. 2)** predominantly found in some marine fishes such as tuna and salmon and to which a wide range of health benefits have been attributed (Liu et al., 2000). These long chain fatty acids have been proven to have antiarrhythmic, anti-inflammatory, antithrombotic, hypolipidemic and vasodilatory properties which are beneficial in cases of coronary heart diseases, hypertension, type 2 diabetes mellitus, and patients with renal disease, rheumathiod arthritis and ulcerative colitis (Fontana et al., 2006; Cros et al., 2007). This makes omega 3 an important constituent of our diet.

Unlike linoleic acid (LA) an omega 6 fatty acid which is found in high amounts in almost all foods including domesticated animals and animal products as well as in most cereals and grains, ALA is also the precursor of a specific group of hormones (prostaglandins) which may offer a great deal of protection against human chronic disease conditions (Palaniswamy et al., 2002). Considering the essentiality of LA and ALA and their long chain derivatives as important components of animal and plant cell membranes, their ratio in human diet is of crucial importance since they belong to two different families which are not inter convertible nor are they metabolically or functionally the same (Palaniswamy et al., 2001; Simopoulos,

2002**b**). A high accumulation of LA in cells as documented in literature as opposed ALA is seen to have a negative metabolic effect since the eicosanoids from arachidonic acid (AA), the metabolites of LA are biologically active even in small quantities. Therefore a high dietary balance of ALA and LA in the ratio 1:1-2 as compared to that of 1:20-30 (which is typical of current western diet) is therefore desirable for homeostasis and of putative benefit on the promotion of health and disease prevention (Simopoulos, 2002). Because plant leaves are richer in ALA than LA, they provide a more desirable ratio of fatty acids than most animal sources. This in effect could be used effectively for improving the nutritional status of people especially in regions of the world where malnutrition is rampant (Ezekwe et al., 1999; Palaniswamy et al., 2001).

The quantity of omega-3 fatty acid in purslane however depends on the growing conditions and the plant age. It is reported by Fontana et al., (2006) that purslane grows well and the ALA is optimized in leaves when plants are grown at day/night temperatures of 27/22^{0}C, in long days of (14-16 h) and when about 60% of the total nitrogen supply is in the form of ammonium. This led to several investigations which showed that the omega 3 fatty acids in purslane were enhanced when grown in nutrient solutions with approximately 65% of the total nitrogen supply provided as ammonium instead of nitrate and also when harvested at 14-16 true leaf stage Palaniswamy et al., (2000). This minimizes the oxalic acid content and the saturated fatty acid content thereby improving upon the nutritional content of the leaves. ALA usually occurs as two thirds of the total fatty acid (TFA) component in plant chloroplast. Thus the provision of ammonical nitrogen in the nutrient solution dramatically stimulates TFA accumulation in a relatively short time, thus improving upon the ALA concentration as compared to nitrate which proceeds too slowly to maximize chloroplast development and TFA accumulation in young leaves (Palaniswamy et al., 2000).

Even though common purslane has not been proven to have any cytotoxicity or genotoxicity from the consumption of its water extract, its acceptance as a green leafy vegetable ready for consumption is still hindered despite its nutritive value. As found in most plant species, purslane has been identified to contain high concentrations of oxalic acid (OA). OA is a widely occurring natural product in

animal tissues, as well as other organisms like fungi. It is mainly identified as a free acid or soluble sodium and potassium salts or as insoluble calcium salt (Caliskan, 2000). Research shows that its presence with essential minerals such as calcium and iron inhibits their absorption by forming insoluble salts of calcium oxalate which can be pathological to human health (Palaniswamy et al., 2004). This is seen to increase the risk of kidney stones, the occurrence of hyposideremia and hypocalcemia as well as reported cases of illness amongst vegetarians due to high consumption from its fresh leaves (Palaniswamy et al., 2002). High oxalate pasture grasses are also acutely toxic for grazing animals (Caliskan, 2000). Unlike animals, oxalic acid and oxalate accumulation in plants may be very tolerable. It has been implied that oxalic acid/ oxalate might be related to ionic balance in plants such that the concentration of both active oxalic acid and calcium may be reduced when in excess since both may have a toxic effect. On the other hand, calcium oxalate crystals may serve as a storage form for calcium for future use when they dissolve during calcium deficiency conditions to supply calcium for growth and cell maintenance (Caliskan, 2000). This is however not the case with humans and animals.

Upon this, Palaniswamy et al., (2002) and (2004) conducted an experiment to determine the amount of nitrogen in the form of nitrate to ammonium ratio (NO_3^- - N: NH_4^+ - N) that would minimize the oxalic acid content in purslane leaves. The results indicated that the concentration of oxalic acid decreased with 75% of total nitrogen provided as ammonium instead of nitrate in the nutrient solution and also with age. Ammonical nitrogen as reported can be directly used by plants in the synthesis of amides and amino acids, whereas when nitrogen is provided in the form of nitrate, the nitrate has to be reduced in the shoots before it can be used by the plant. In doing so, more OH^- ions are assimilated and in trying to neutralize this, greater oxalic acids are formed in the leaves and stems. Others also have it that nitrate ions inhibit the oxalic acid oxidase activity preventing the break down of oxalic acid, resulting in the high accumulation. There have been suggestions of a possible oxalic acid oxidase pathway in animals which could make use of oxalate to produce hydrogen peroxide that can promote the burst of phagocytes (Caliskan, 2000).

Previous studies have also demonstrated that cultural conditions can influence the omega 3 fatty acid concentrations as well as the oxalic acid concentrations in purslane leaves. In spite of all the intensive studies conducted on purslane with regards it s nutritive value (see above), the effect of environmental conditions such as different temperature, have not been studied in-depth. In this work, we grew three microspecies of purslane (in the sense of Danin and Reyes, (2006) and Danin et al., (2008) (*P. sativa*, the commercial type, *P. nitida* and *P. papillato-stellulata*) of purslane in an automated temperature control environment (phytotron) exposed to both short and long day conditions. Leaf samples were harvested at 8-true-leaf stage and analyzed for both fatty acid and oxalic acid profiles. This aimed to understand the effect of environmental conditions on the fatty acid as well as the oxalic acid in the *Portulaca* complex.

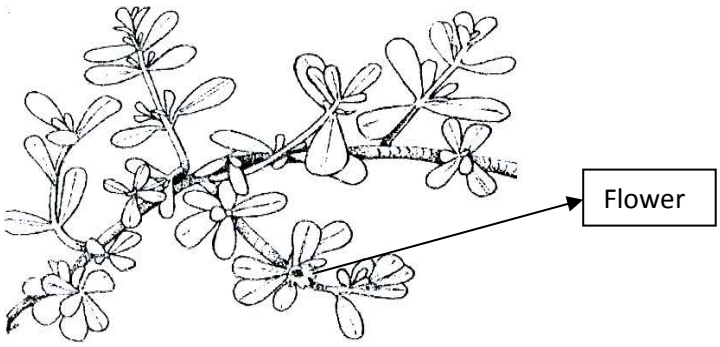


Fig 1: Branch of *Portulaca* with flower (adapted from Cudney et al., 1999)

1.1 Biology and Taxonomy of Purslane used.

Portulaca in its wide sense is considered to be a polyploidy complex which consists of more than 15 microspecies in its aggregate (Danin et al., 2008). Its polyploidy complex consists of 6 hexaploids (including the commercial *P. sativa* and *P. papillato-stellulata*) and 4 tetraploids (including *P. nitida*), diploid and pentaploid. Seed size and seed coat morphology have been the main measure used in taxon delimitation since the vegetative parts do not provide any good diagnostic feature (Danin et al., 1978; 2008)

Seed morphology and size

Seed diameter of most seeds of *P. sativa* is greater than 1.1 mm. This is the only diagnostic character by which *P. sativa* differs from the other wild types below.

P. papillato-stellulata: seed diameter of most seeds is less than 0.85 mm, testa cells star-shaped, and rays long, many of them with papillae at the end of the seed.

P. nitida: testa cells are isodiametric, with long rays, star-shaped too, flat and shiny surface with no papillae. Seed size is between 0.65-0.85 mm (Danin et al., 2006; 2008).

The above authors reported that germination of purslane takes place under high temperature as previously mentioned, in light and appropriate moisture conditions. Plants died off and degenerated in frost. In Israel, purslane can be found in the summer times mostly in irrigated fields, ornamental plots, moist abandoned areas and gardens. In a previous screening programme of the four main microspecies in Israel (*P. granulato-stellulata, P. papillato-stellulata, P. nitida and P. trituberculata*), it was found that differences existed in their morphological features. That is, *P. nitida* and *P. granulato-stellulata* grew in a recumbent form, while *P. papillato-stellulata* and *P. trituberculata* grew in an erect or decumbent form similar to the commercial green-leaf *P. sativa* (Radhakrishnan et al., 2001; Szalai et al., submitted).

1.2 Omega 3 fatty acids and acute inflammatory conditions

As mentioned above, omega-3 fatty acids have been suggested as a treatment for various chronic inflammatory diseases including inflammatory bowel disease (IBD) of which ulcerative colitis (UC) is a part. Sufficiently high intakes of omega 3 ALA decreases or better still modulates the production of inflammatory eicosanoids, cytokines, adhesion molecule expression such as intercellular adhesion molecule-1 and vascular cell adhesion molecule -1 (ICAM-1and VCAM-1), nitrogen metabolites and reactive oxygen species from arachidonic acid (AA) in most cell structures thereby inhibiting its metabolism and altering the expression of inflammatory genes (Calder, 2006). UC is a chronic and relapsing condition of which its etiology is poorly understood. It is characterized by colonic and rectal tissue edema, increased colonic epithelial permeability, haemorrhage and extensive infiltration of leukocytes in the mocusa layer of the colon (Zhang et al., 2009). It also involves the over expression and increased influx of neutrophils as well as of the most powerful eicosanoids from arachidonic acid (an omega 6 fatty acid) metabolism via the 5-lipoxygenase pathway,(leukotriene B_4 (LTB_4) , and the cyclooxygenase pathway (prostaglandin E_2 and thromboxane A_2(TXA_2) which are both released in the intestinal mucosa. These eicosanoids are involved in modulating the intensity and duration of the inflammation and can be seen during the induction of colitis (Calder, 2008).

Another beneficial effect of omega 3 PUEFA in UC management is its ability to inhibit proinflammatory cytokines (interleukin 1β (IL-1β), IL-6, IL-12 and tumour necrosis factor (TNF-α) (Belluzzi et al., 2000; Zhang et al., 2009) which are produced by monocytes and venous endothelial cells in the wake of this disease condition. Chronic over production of these cytokines are particularly destructive and are implicated in muscle wasting, loss of bone mass alterations in body composition (Calder, 2006). Although the mechanism for inhibiting TNF-α and IL-1β by dietary n-3 fatty acids is unknown, research shows an involvement of this fatty acid in inhibiting n-6 eicosaniod synthesis which has a positive correlation with cytokine synthesis (Caughey et al., 1996). Studies have also demonstrated that all these proinflammatory cytokine genes have nuclear factor IκB (NF-κB) binding sites which are transcriptionally regulated by the factor (Calder, 2006; Zhang et al., 2009; Calder, 2009). Omega 3 fatty acid consumption has been shown to enhance

peroxisome proliferator-activated receptors (PPAR-γ) (which regulate inflammation) in the intestines of UC patients, inhibiting NF-κB and attenuating cytokine gene expression (Wild et al., 2007; Calder, 2008). Several theories in literature have emerged pertaining to the development and pathogenesis of this disease (which is currently presumed to result from a complex interplay among genetic, environmental, microbial and immune factors). Total effect treatment until now remains to be a goal. There have been implications of persistent intestinal infection, a defective mucosal barrier to antigens and a dysfunctioning immune response to ubiquitous antigens (Wild et al., 2007; Singh et al., 2009). Another report by Barbosa et al., (2003) also shows that IBD results from an imbalance between prooxidant and antioxidant mechanisms resulting in oxidative stress caused by reactive oxygen species (ROS) created by the activation of phagocytic cells in UC. Thus, the intake of omega 3 fatty acid may act as a scavenger of free radicals. In all, UC patients have a 5-fold increased risk of developing colon cancer (Calder et al., 2009).

Animal models of IBD which involve chemically induced colitis show that diet rich in ALA decreases colonic damage and inflammation as compared to omega 6 rich diets. This was shown in a study conducted by Calder, (2008) which investigated dextran sulphate sodium (DSS) -induced colitis in fat-1 mice (mice expressing C-ellegan desaturase gene, which have the property of converting omega-6 to omega -3 fatty acids) as opposed their wild-type counterparts. The results of this study proved the ability of the fat mice to produce ALA from LA due to the presence of an 'omega 3 desaturase gene'. They showed much less colonic damage and inflammation than the wild –type who did not have this gene.

IBD itself is associated with nutritional deficiencies. Some of these include anemia, hypoalbuminemia, hypomagnesia, hypocalcemia and hypophosphalemia as well as deficiencies in folic acid, niacin, vitamins A, B12, C, D, deficiencies in iron, zinc and copper. Several mechanisms however have been proposed to contribute to the malnutrition observed in IBD patients. Reports show decreased in oral intake of nutrients because of abdominal pains, anorexia, and mucosal inflammation with associated diarrhea leading to protein, blood, mineral and trace element loss. Also there is bacterial overgrowth which may have an adverse nutritional effect and most importantly, the drugs (sulfasalazine) used to manage the condition may lead to

malnutrition (Wild et al., 2007). Thus nutritional intake of omega 3 fatty acids either as an alternative or adjunct therapy is potentially important, especially since current management with drugs has many side effects.

Terrestrial or plant sources of omega 3 fatty acids until recently have not been exploited and most studies conducted on IBD have used fish oil. This may be in part due to the presence of other organic compounds such as oxalic acid which can be found in larger quantities in almost all plant tissues and its inhibition of some essential nutrients if consumed in higher amounts freshly. Nevertheless, as reported by Simopoulos,(1999), ALA found in green leafy vegetables, desaturates and elongates in human body to EPA and DHA (**Fig.2**) and by itself may have beneficial effects in health and in the control of chronic diseases. It is also important to know that ALA from plant sources has no problem of insufficient vitamin E intake as is seen with omega 3 from fish source (Simopoulos, 1999). This is because as an antioxidant it prevents oxidative stress caused by reactive oxygen species (ROS) (which has been proposed to play a key role in IBD) when omega 3 fatty acids are used (Bays, 2007).

In this study, we also investigated the effect of *Portulaca* on mice model of dextran sulphate sodium (DSS) - induced colitis which resembles human UC to provide evidence of the ameliorative potential of *Portulaca* leaves and its omega 3 fatty acid rich fractions in dextran sulphate sodium induced ulcerative colitis in mice.

DSS model of colitis

The mouse model of dextran sulphate sodium induced colitis is an experimental model that resembles acute human IBD. It shows a preclinical system involving the molecular events required for tumour formation in the presence of inflammation and assesses the ability of select agents to inhibit this process (Clapper et al., 2007). In this study, it was evaluated by the disease activity index (DAI). This was done by scoring body weight, gross bleeding and stool consistency. Histological injury scores were also taken after the induction of colitis by the administration of DSS orally in drinking water for eight days (Zhang et al., 2009).

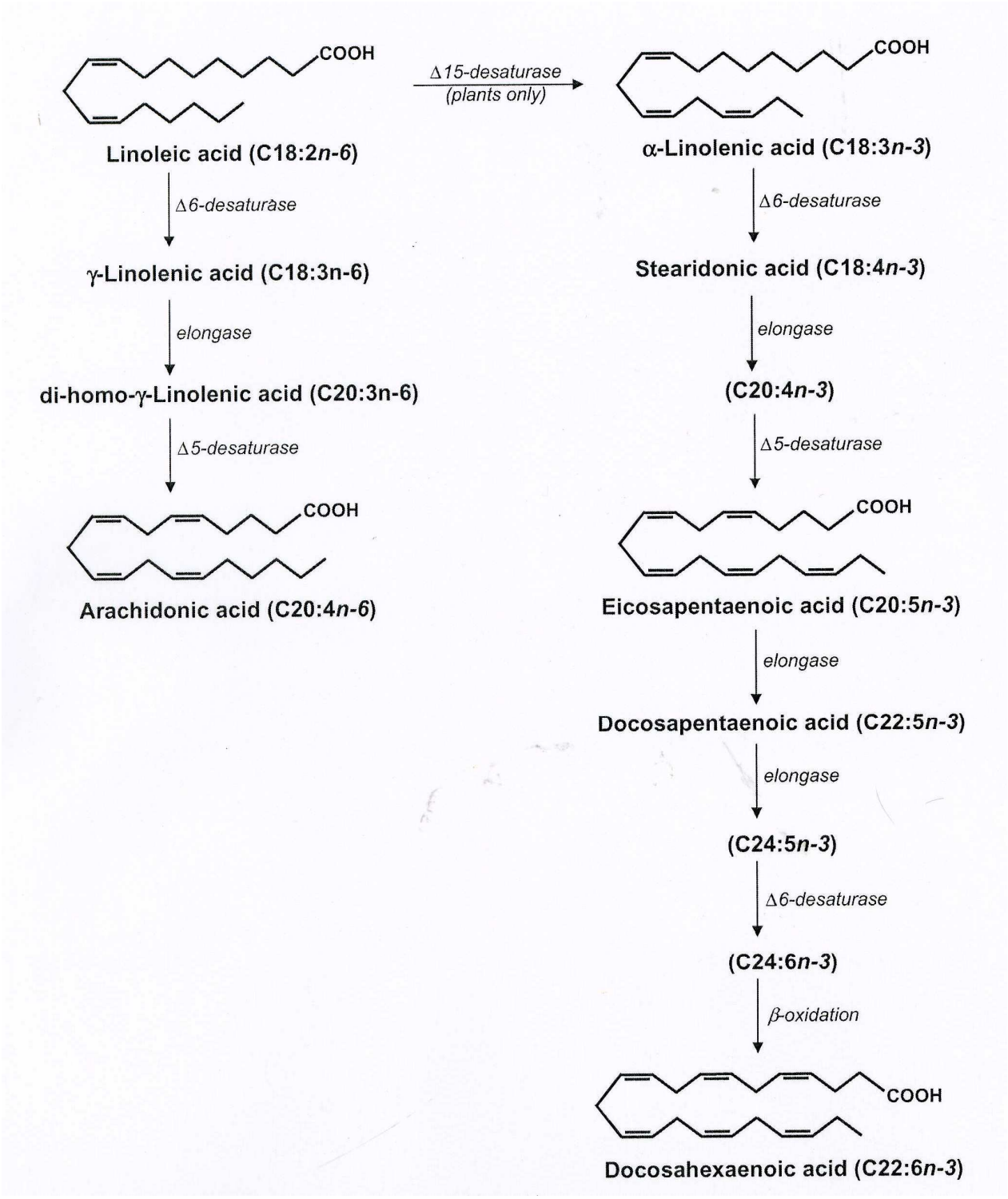


Fig.2. Biosynthesis pathways of n-6 and n-3 polyunsaturated fatty acids (adapted from de Roos et al., 2009)

1.3 Objectives

In the phytotron experiment, we monitored and evaluated plant performance and phenotype under different conditions. Under different day/night and temperatures regimes, we pursued the following specific objectives:

1. Analysis of morphological, phenological and biomass production.
2. Analysis of fatty acid and organic acid concentrations.

From the above experiment, we also:

3. Assessed of the role of *Portulaca* leaves on acute inflammation in Dextran sulphate sodium (DSS)-induced experimental colitis.

2.0 Materials and Methods

2.1 Plant materials

Seeds for all the three purslane microspecies were available at the Agricultural Research Institute (ARO), Department of Vegetable Research, Israel. These included *Portulaca nitida*, *Portulaca Sativa* and *Portulaca Papillato-stellulata*. The experiment was conducted in November 2008 at the Hebrew University of Jerusalem, Israel in the phytotron provided with automatic environmental control conditions for temperature, short and long day regimes. The experiment consisted of sowing 5-8 seeds each of the three microspecies of purslane in 240 plastic pots each filled with approximately 200 g of the growing medium (mixture of peat , perlite and vermiculite in the ratio of 1:1:1). This was left under a temperature of $34/28^{0}$C day/night provided with 16 hrs daylight regime to germinate. This was also bottom irrigated to prevent dispersal of seeds when irrigated above. After 7 days of germination and thinning to 3-5 seedlings per pot, the plants were transferred to the four growth chambers of different day /night temperature regimes, long day (16 hrs) and short day (9 hrs). In each chamber, 10 pots of each of the three different microspecies were assigned to both short and long day conditions. The accumulative plant density in each growth chamber was 180 plants. In total, 80 pots for each of the three microspecies (for both short and long day) were assigned to the four growth chamber labelled A, B, C, D as shown in the table below (**Table 2).** Initially during plant growth, irrigation was done every two days and later to every day as the plants gained anchor. Basic nutrient solution consisted of the following as shown in the table below (**Table 1**). This was the same for all the temperature rooms.

Table 1: Composition of basic nutrient solution used in irrigation

Element	Concentration (mg/L)
N	140
P	16.3
K	139
B	0.06
Ca	129
Cu	0.03
Fe	9.3
Mg	56
Mn	0.27
Mo	0.01
Na	74
S	27
Zn	0.29
pH	**7.5**

Experimental design

Table 2: Growth conditions at the phytotron.

Growth chamber	**A**	**B**	**C**	**D**
Day temp.^{0}C	34	28	22	16
Night temp.^{0}C	28	22	16	10

On the ninth day after transfer to the various growth chambers, five pots of each microspecies in both short and long day in each room was observed for phenotypic characteristics including growth rate measured by height, number of internodes and leaf length. A meter rule was used to take height and leaf length measurements. This measurement was done on the same day for 8 weeks continuously in the four growth chambers. The rest of the other pots were harvested by cutting from the base at 8-true –leaf stage, 21days from the day of transfer to the growth chambers. This was done before flowering and leaf samples were used for fatty acid and organic acid extraction (see below). The harvested plants were immediately transported to the laboratory where 200 mg leaves were picked into microcentrifuge tubes under liquid

nitrogen and stored in -80^{0}C for the oxalic acid determination. Also Plant fresh weight (FW) and dry weight (DW) were determined for both stems and leaves in each of the temperature rooms for each treatment replication. For the rest of the plants, leaves and stems were separated and frozen at -80^{0}C, prior to their Lyophilization in a freeze dried apparatus (Labconco Corporation, Kansas City Missouri). Freeze-dried samples were used for the fatty acid determination.

2.2 Fatty acid extraction and methylation

The standard procedure used for analyzing the fatty acid contents of the plants was as follows. Fatty acids were extracted by a method modified from that of Yaniv et al., (1997). 10 mg to 20 mg of the freeze dried leave samples were placed in 10 ml glass screw-cap test tubes. In this, 1ml of 2.5 % (v/v) H_2SO_4 in methanol was added and tightly sealed with Teflon thread liners. This was kept in a heating module for 90 mins at 80^{0}C in a water bath. The test tubes were allowed to cool. After which 10 µl of internal standard (2 mg/ml, heptadecanoic acid, a $C_{17:0}$ fatty acid) was added gently. This was followed by the addition of 1.5 ml of 0.9 % NaCl solution and 1 ml of hexane. The mixture was vortex-mixed for 10 sec and then centrifuged (Hermle Z320 D-7209 Gosheim, Germany) for 5mins at a speed of 6000 rpm. 400- 600 µl of the upper phase was picked into glass vials sealed with Teflon liners to prevent evaporation. This was stored in -20^{0}C until injection for gas chromatography (GC) analysis.

Gas chromatography

Samples (1µl) were injected via an auto sampler onto a Supelco capillary column (SP 2560, 0.2 µm film thickness, 100 m x 0.25 mm) in a Agilent model gas chromatography system fitted with a flame ionization detector (Agilent model,6890N) and eluted with helium gas (carrier gas) at 30/ min. The injector and detector were heated to 260^{0}C. The column was temperature programmed from 140^{0}C in 5 min to 240^{0}C at a rate of 4^{0}C/ min. Fatty acid methyl esters were identified by comparing GC retention times with those of a mixture of standard fatty acids (Supelco, Sigma

Israeli Chemicals Ltd., Rehovot, Israel). Fatty acids were quantified using peak areas, against internal standards.

2.3 Organic acid extraction

The procedure used for the following extraction was that developed by Szalai et al., (submitted). Plant material (200 mg) was ground in liquid nitrogen and 0.5 ml of 4.5% (w/v) metaphosphoric acid (diluted in double distilled water) was then added and vortex-mixed. This was then centrifuged (Jouan SAMR 23i, France) for 10 min. at a speed of 12000xg after which the upper phase was transferred into small glass vials, and 500 µl toluene added and vortex-mixed again. The lower phase was then picked and filtered with PVDF filter fitted with a syringe (PVDF, 0.22 µm, Millipore, Billerica, MA) into glass vials with inserts. The filtrate was then injected and measured by High Performance Liquid Chromatograph, HPLC (Varian model ProStar 240).

The organic acids were separated on a reverse-phase HPLC column (250 x 4.6mm, 5µm, Merk, Darmstadt, Germany) using a Varian HPLC model ProStar 240 (Varian, Palo Alto, CA). The running solvent (double distilled water at pH 2.2 set with 80% H_2SO_4) had a flow rate of 1ml/min during the isocratic run. It was used for the detection and separation of oxalic acid, at 215 nm and ascorbic acid at 245 nm. Identification and quantification of organic acids in comparison with commercial standards was performed in a UV range of 200-300 nm (ProStar 335 photodiode array detector, Varian).

2.4 DSS-induced experimental colitis

Colitis was induced essentially as previously described by Zhang et al., (2009) with minor modifications.

Chemical: Dextran Sulphate Sodium Salt (molecular weight: 36000-50000 Daltons) from MP Biomedicals, Solon, OH, USA

Plant Material: Purslane leaves were collected from the left over plant material in the phytotron which was used for the above chemical analysis. Plant material was also collected from the Agricultural Research Institute in Newe Yaár which together with that from the phytotron was oven dried for 14 days at a temperature of 40^0C. This was then blended in a kitchen blender into a homogenous fine powder. The powdered sample was then stored in a clean dried container, covered and stored in -20^0C.

2.4.1 Experimental animals

Female C57BL/6J mice weighing ~ 18 g, a strain known to be susceptible to oral administration of oral dextran sulphate sodium (DSS), were purchased from Harlan Animal Research Laboratories Ltd. (Jerusalem, Israel) at the age of 7-8 weeks. They were maintained on a standard laboratory diet (powder food, chow) (Harlan laboratories Ltd) and had free access to tap water in bottles *ad libitum*. They were kept in plastic cages (5 mice /cage) at the animal facility and were exposed to controlled conditions of humidity (5%), light (12/12 hr light /dark cycles) at a temperature of 23±2^0C. Animal care and experimental procedures were in accordance with the guidelines of the accredited animal ethics committee of the Hebrew University of Jerusalem.

Table 3: Basic nutrient composition of Standard diet (chow)

Nutrient	Quantity
Crude protein	18.8 %
Crude fibre	3.8 %
carbohydrates	50 %
Vitamin A	15.4 iu/g
Vitamin E(α-tocopherol)	100 mg/kg
Vitamin B1	16.5 mg/kg
Vitamin B6	18.5 mg/kg
β carotene	2.5 mg/kg
sodium	0.23 %
Iron	225 mg/kg
Palmitic acid	7.6 g/kg
Stearic acid	15 g/kg
Oleic acid	12.6 g/kg
Linoleic acid	31.3 g/kg
α-linolenic acid	2.8 g/kg
Total saturated fatty acids	9.6 g/kg
Total monounsaturated	12.8 g/kg
Total polyunsaturated	34.1 g/kg

Source: Adapted from Harlan Laboratories Ltd.

Experimental period: 18 days

2.4.2 Induction of colitis and administration of Purslane

Dextran sulphate sodium (DSS) was employed for the induction of colitis as previously described by (Okayasu et al., 1990). This was done by administering 3.5% (w/v) of the DSS in the drinking water of the mice for 8 days *ad libitum*. Twenty five (25) mice were divided randomly in five (5) groups. In each group, 5 mice were employed. The first group, designated as positive control (DSS-control group), received standard commercial powdered diet (25 g) and drinking water(20 ml) with 3.5 % DSS for 8days, 10 days after the start of the experiment. The second group designated as negative control received standard commercial feed (25 g) and drinking water (20 ml) *ad libitum* for the whole period of the experiment. The third group designated as high dose purslane, received 24 % (6 g) ground purslane of the total feed per day (25 g) starting from the first day of the experiment and 3.5% DSS for 8 days, just as in the DSS-control group. In this food mixture, **4800 mg** constituted of omega 3 fatty acid. The fourth group designated as medium dose also received 8% (2 g) purslane leaves mixed in the total standard feed per day and 3.5% DSS in drinking water for 8 days as described above in group 3. In this food mixture, **1600 mg** constituted of omega 3 fatty acids. The fifth group, designated as low dose received 0.8% (0.2g) of purslane leaves also mixed in standard feed per day and 3.5% DSS in drinking water for 8 days as shown above. In this concentration, **160 mg** constituted of omega- 3. To assess the extent of colitis, body weight, stool consistency and blood in the stool was monitored. Mice were fasted overnight, anaesthetized with isofluorane and then sacrificed 10 days after the induction of colitis (day 20 of the experiment). Colonic tissues were then removed and cleaned. Colonic length (cecum to rectum) was measured and divided into several sections for organ culture and histology.

Calculation: Quantity of omega-3 (in mg/g)

Average quantity of omega-3 in leave sample = 800 mg/g dry weight

Lets assume each mouse eats 5 g of food per day= 5 x 5= 25 g of food/cage/day

Percentage of leaves per 25 g of chow:

Group 1= 24%,

Group 2= 8%,

Group 3=0.8%

Therefore group 1 (24%) of 25 g = 6 g of leaves

If 1 g of leaves = 800 mg

Therefore 6 g of leaves will contain 4800 mg of n-3/ 25 g of chow

Group 2: 8 % of 25 g = 2 g of leaves

Therefore 2 g of leaves will contain 1600 mg of n-3 / 25 g of chow

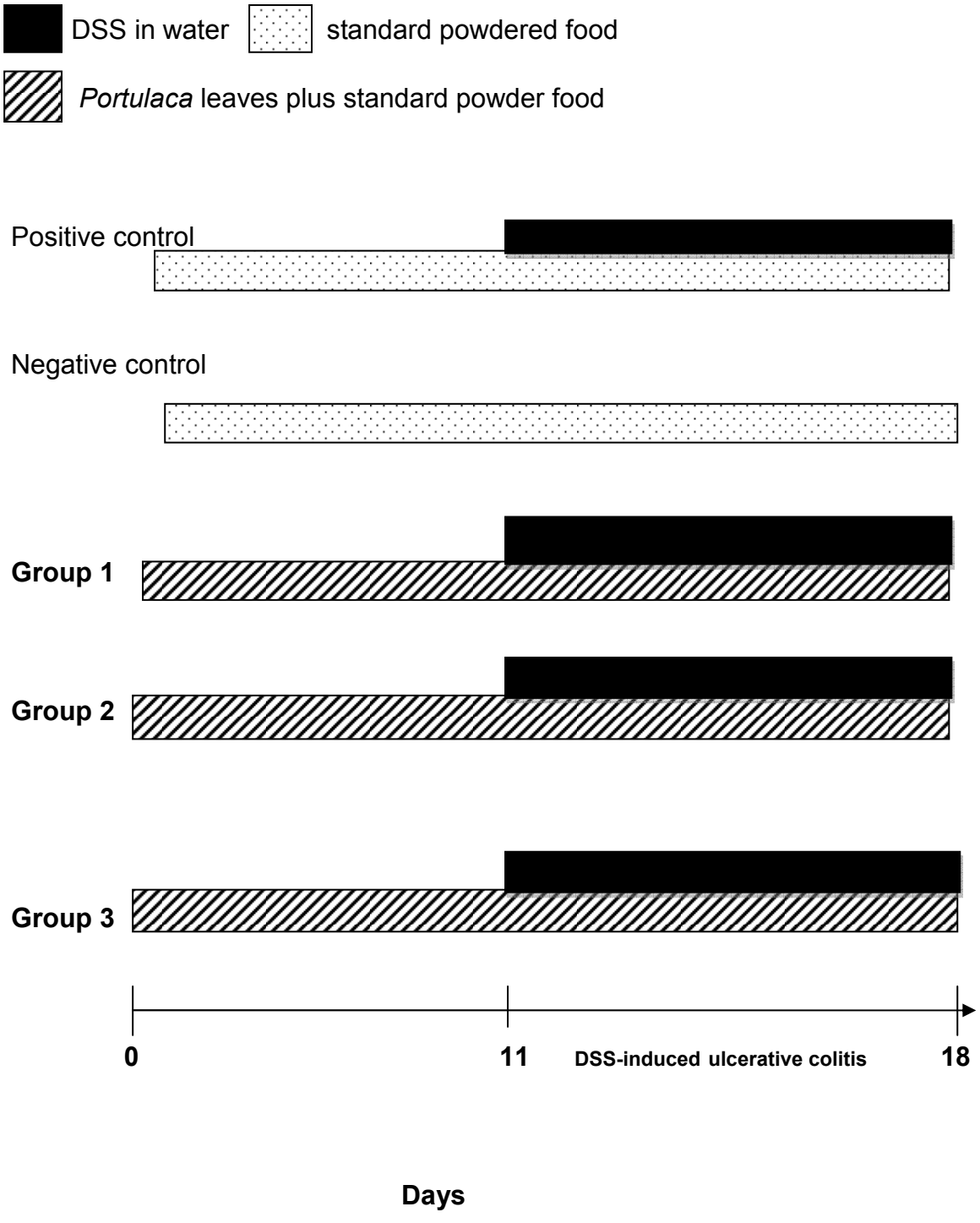
Group 3: 0.8% of 25 g = 0.2 g of leaves

Therefore 0.2 g of leaves will contain 160 mg of n-3/ 25 g of chow

Table 4: Experimental design for DSS- induced experimental colitis.

cage	Day 0-10	Day 11-18
Positive control	Standard powder food	3.5 % DSS in drinking water Standard powder food
Negative control	Standard powder food	Drinking water plus Standard powder food
Group 1	24 %(w/w) ground purslane leaves (**4800 mg** omega 3) in standard powder food per day	3.5 % DSS in drinking water plus plant omega 3 (4800 mg) in 24% (w/w) ground purslane leaves in standard powder food per day
Group 2	8 % (w/w) ground pursalne leaves (**1600 mg** omega 3) in standard powder food per day	3.5 % DSS in drinking water plus plant Omega 3 (1600 mg) in 8% (w/w) ground purslane leaves in standard powder food per day
Group 3	0.8 % (w/w) ground purslane leaves (**160 mg** omega 3) in standard powder food per day	3.5 % DSS in drinking water plus Plant Omega 3 (160 mg) in 0.8% (w/w) ground purslane leaves in standard powder food per day

Fig 3: Experiment *in vivo* (diagram)



2.4.3 Evaluation of colitis

Everyday, during the whole period of the experiment, the following parameters were evaluated for each mouse.

1. **Weight** (g)
2. **Fecal consistency**: normal normal/soft soft very soft diarrhea
3. **Hemoccult result**: +/- + ++ hemorrhage
4. **DAI score**: 0 1 2 3 4

Disease activity index: DAI was determined by scoring changes in weight, hem-occult results or gross bleeding and stool consistency in all animals. The scoring criteria and methodology were essentially described previously by Cooper et al., (1993) and is summarised in the table below. DAI/3

Table 5: DAI score evaluation

DAI score	Weight loss (%)	Faecal consistency	Blood in faeces
0	None	Normal	No blood
1	1-5	Normal/soft	Hemoccult +/-
2	5-10	Soft	Hemoccult +
3	10-20	Very soft	Hemoccult ++
4	> 20	Diarrhea	Hemorrhage or massive bleeding

Day of sacrifice: Ten days after the induction of colitis by DSS (day 20), mice were weighed and sacrificed. Their entire colon was resected from the colo-cecal junction to the anus and rinsed with sterile saline (0.9%) to remove faecal matter, weighed and their lengths (cm) measured. This was divided into 5 parts for the following measurements; RNA (saved in 200 µl of RNA tissue storage solution), Protein immunoblot, histology (saved in1 ml 4% buffered formaldehyde), Myeloperoxidase (MPO). However MPO and the histological measurements were determined.

2.4.4 Evaluation of blood in faeces (hemoccult test):

Fecal occult blood test (hemoccult test) was done in duplicate by collecting fresh fecal samples in the morning for all DSS administered groups on days (12, 14, 16 and 18 starting from the day of DSS administration). Small fecal specimen was then smeared onto both windows of the hemoccultsensa slides (Beckman Coulter Inc., USA) which contained guaiac paper using an applicator stick and then the cover flap closed. After 3-5 min, the back slide was opened and two drops of the developer was applied to the guaiac paper directly over each smear. The results were read within 60sec after developer was applied. A blue colouration showed positive for occult blood. This SENSA test is based on the oxidation of guaiac by hydrogen peroxide (the developer) to a blue-coloured compound. The heme portion of haemoglobin if present in the fecal specimen has peroxidase activity which catalyzes the oxidation of α-guaiaconic acid by hydrogen peroxide (developer) to form a highly conjugated blue quinine compound.

2.4.5 Histopathological analysis: The colon segment for this determination was kept in 4% buffered formaldehyde and submitted (transverse sections) for histological processing. Mucosal inflammation in the colon was evaluated in the haematoxylin and eosin (H&E) stained sections as described by Wallace et al., (1989).

2.4.6 Myeloperoxidase (MPO) determination

Between 20-50 mg of distal colonic tissue was taken and rinsed with ice-cold saline, blotted dry, weighed and homogenised in 0.6 g hexadecyltrimethyl-ammonium bromide (HTAB) (Fluka) in 120 ml phosphate buffer (pH 6.5) on ice using a Polytron homogenizer for 30 sec. The homogeniser was rinsed with ½ ml of HTAB and the procedure repeated twice. The homogenate was then frozen and thawed 3 times for 15mins each and then centrifuged at 11000xg for 45 minutes at 4^0C. The resulting supernatant was then freeze at -70^0C until determination of the enzyme activity.

MPO was assayed for determination spectrophotometrically as follows: 0.1 ml of the sample was mixed with 2.9 ml of 56 mM phosphate buffer at pH 6.5 containing 0.167 mg/ml *O*-Dianisidine dihydrochloride and 0.0005% hydrogen peroxide. These conditions were employed because they confer increased sensitivity to the assay (Bradley et al., 1982; Wallace et al., 1990). The change in absorbance at 460 nm was measured with a Shinuazdv UV-1601, Japan spectrophotometer. One unit of MPO activity is defined as that degrading one µmol of hydrogen peroxide/min at 25^0C.

Standard curve:

The standard curve consisted of 9 samples containing 5µl standard peroxidase diluted accordingly with *O*-Dianisidine dihydrochloride and H_2O_2. The standard curve was calculated by reading at time zero and after time 90 secs. Delta time was calculated (T90-T0) at 460 nm. Point below linear part of the graph (R^2 value was near 0.9).

Samples: MPO samples were measured in triplicate by spectrophotometer.

Table 6: Summary of sample amount per cuvette

	500µl cuvettes
Sample	13.3 µl
***O*-Dianisidine dihydrochloride (16.7 mg/100 ml PB)**	385 µl
H_2O_2 solution (0.0005%)	1.33 µl
	400 µl

Final sample delta was calculated from T=0 and T=90 values (T90-T0) and blank delta was subtracted from sample delta.

2.4.7 Blood fatty acid analysis

Blood collection: About 1ml of blood was collected into ethylenediaminetetraacetic acid (EDTA) vacuum tubes (BD vacutainer systems, Pre analytical solutions, Belliver Industries Estate, UK) on ice after an overnight fast before centrifugation(3000 x g for 10mins) at 4^0C to separate plasma and Red blood cells (RBC). Plasma was then aliquoted into microcentrifuge tubes and the Buffy layer of the white blood cells (WBC) removed by using a pasture pipette. The RBCs were then aliquoted into separate microcentrifuge tubes which were then washed thoroughly in normal saline (0.9% NaCl). Samples were frozen immediately and stored under nitrogen atmosphere in -80^0C until analysis.

Plasma fatty acid analysis

Fatty acids from plasma were isolated and methylated as described by Ji et al., (2009) with minor modification. Briefly, 125 µl of plasma was first mixed with 250 µl of ultrapure water and then with 1 ml methanol: dichloromethane (3:1 v/v). After addition of internal standard (50 nmol of heptadecanioc acid), 200 µl acetyl chloride was added drop by drop while vortexing in a fume hood using eye protection, laboratory coats and gloves. The samples were then incubated at 75^0C for 1hr. After removal and cooling to room temperature, the reaction solution was neutralised with 4 ml of 7% potassium carbonate (K_2CO_3) and the lipids were extracted into 2 ml of hexane, mixed vigorously and centrifuged for 10mins at 2500xg at room temperature. The hexane fraction was then washed with acetonitrile. The fatty acid methyl ester (FAME) mixture was then re suspended in 350 µl of hexane and analysed by GC as previously describe above.

2.5 Data analysis

For chemical analysis: Data were presented as mean± SEM in all tables and figures and analyzed by general analysis of variance (ANOVA), JMP and t-test (statistical version 7.0)(Liu et al.,2000)

For in vivo experiment: Data were expressed as mean± Standard error of means (SEM). The data obtained from the various groups were statistically analyzed using analysis of variance (ANOVA), JMP7.0. A value of $p<0.05$ was considered statistically significant (Zhang et al., 2009).

3.0 Results

3.1 Phenotypic evaluation of the three *Portulaca* microspecies.

3.1.1 Effects of temperature and day length on Plant Height

Plant height was measured within 8 weeks (once a week, on the same day for 8 weeks) starting from the day of transfer to the four different temperature rooms. By week eight, the greatest height in the long day regime was observed in the *P. sativa* (68±3.8 cm), followed by *P. papillato-stellulata* (37± 1.5 cm) and then *P .nitida* (30±3.8 cm) whiles in the short day regime, *P. sativa* (45±2.4 cm), *P .nitida* (37±3.6 cm), *P. papillato-stellulata* (29±4.3 cm) were the greatest heights by the end of week eight **(Fig. 4)**. The above heights were plants grown in the warmest temperature room (34/28^{0}C). Plants grown in this temperature condition (34/28^{0}C) had the greatest plant height and this decreased as the temperature also decreased. Plants in (16/10^{0}C) temperature condition actually showed retarded growth, caused colouration of plant chlorophyll and eventually plant death. About 70% of plants in this temperature room did not survive the cold conditions. Comparatively, plant height measurements indicated that in the long day regime (*P. sativa*) gain (1.5-fold) more height than *P .sativa* in the short day regime which was significant at 34/28^{0}C. Similarly, it was significantly 1.8-fold and 2.3-fold taller than *P. papillato-stellulata* and *P. nitida* respectively in the long day regime at the temperature of 34/28^{0}C. The lowest plant heights were seen for plants grown in (16/10^{0}C) temperature condition; *P. sativa* (9.7±0.6 cm), *P. nitida* (2.8±1.0 cm) and *P. papillato-stellulata* (4.6±0.6 cm) -long day and *P. sativa* (6.7±1.4cm), *P. nitida* (3.4±0.2 cm) for the short day regime. Decreasing the temperature condition for growth showed a significant reduction in plant height by (7-fold) for the Pos subspecies in both long and short day by the end of the eighth week.

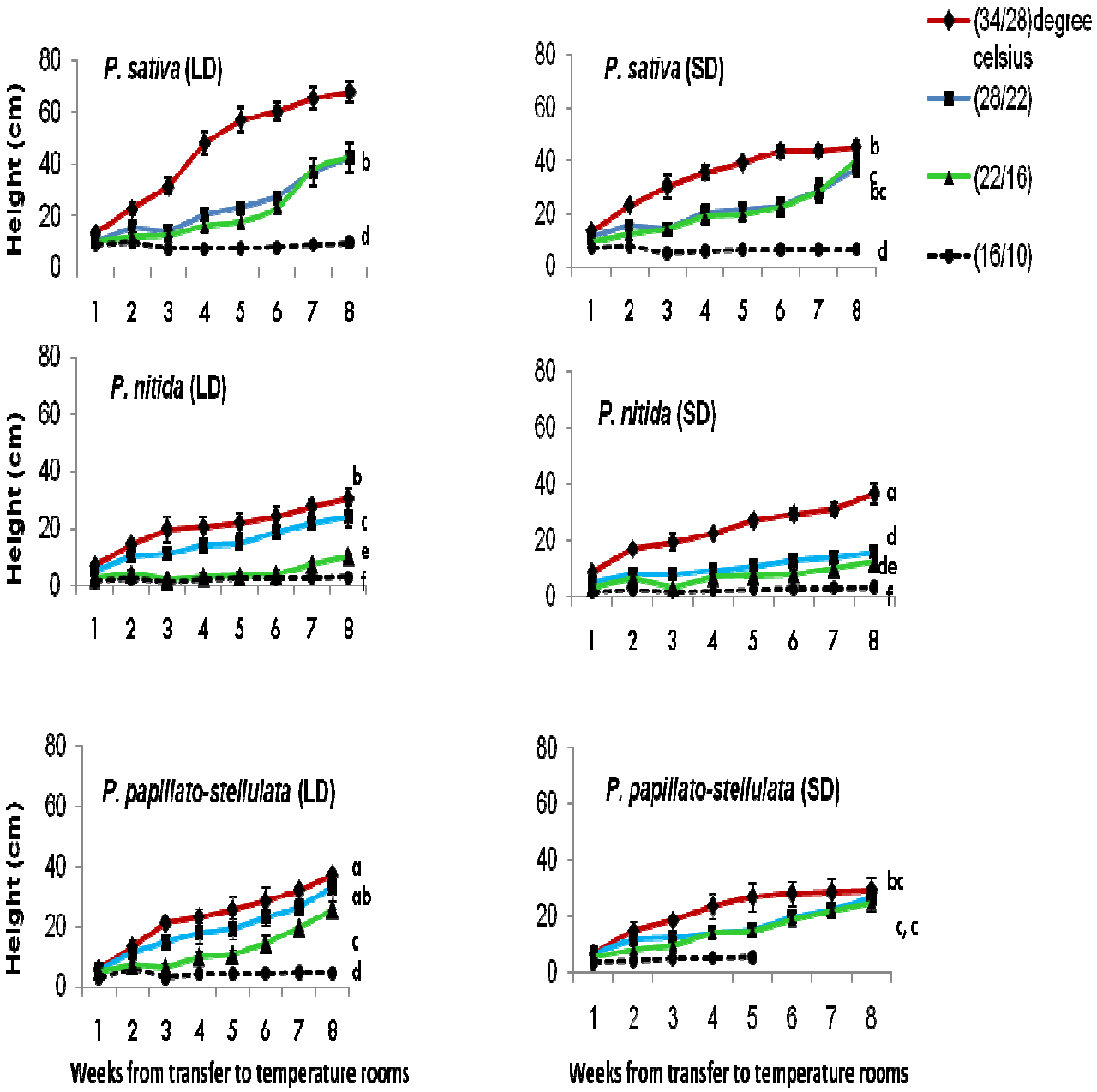


Fig. 4 Plant height (cm) among the three subspecies of purslane measured once a week, on the same day for eight weeks (from day of transfer to the different temperature rooms) in both long day (LD) and short day (SD) in response to four different temperature conditions (^{0}C day/night). Data represents means ± SEM of five replicates (n=5). Lines with different letters are significant different at $p<0.05$.

3.1.2 Effects of temperature and day length on Plant leaf length.

Similarly, the leaf length measurement recorded for 8 weeks showed lengthier and broader leaves (personal observation, data not shown) for the *P. sativa* (4.7±0.2 cm) microspecies followed by the *P. papillato-stellulata* (4.3±0.2 cm) and then *P. nitida* (3.9±0.4 cm) all in the long day regime. Similarly in the short day, *P. sativa* again with (5.6±0.06 cm) followed by *P. papillato-stellulata* (4.2±0.7 cm) then *P. nitida* (3.4±0.1cm) **(Fig. 5).** This observation was again seen among plants grown in the warmest temperature conditions (34-22^{0}C) whiles the shorter leaf length were seen for plants grown in the coldest temperature condition (16/10^{0}C). Relatively, leaf length measurement showed that *P.sativa* in the short day gained 1.2-fold more leaf length than *P. sativa* in the long day at 34/28^{0}C. Similarly in the long day regime, among the three microspecies, *P. sativa* gained 1.2 and 1.1-fold more leaf length than *P. nitida* and *P. papillato-stellulata* respectively. Looking within the short day, among the three microspecies again, *P. sativa* gained 1.6 and 1.3-fold more in length than *P. nitida* and *P. papillato-stellulata* respectively. Decreasing the temperature to 16/10^{0}C reduced the leaf length of *P.sativa* by 1.6 and 2-fold for both long day and short day respectively.

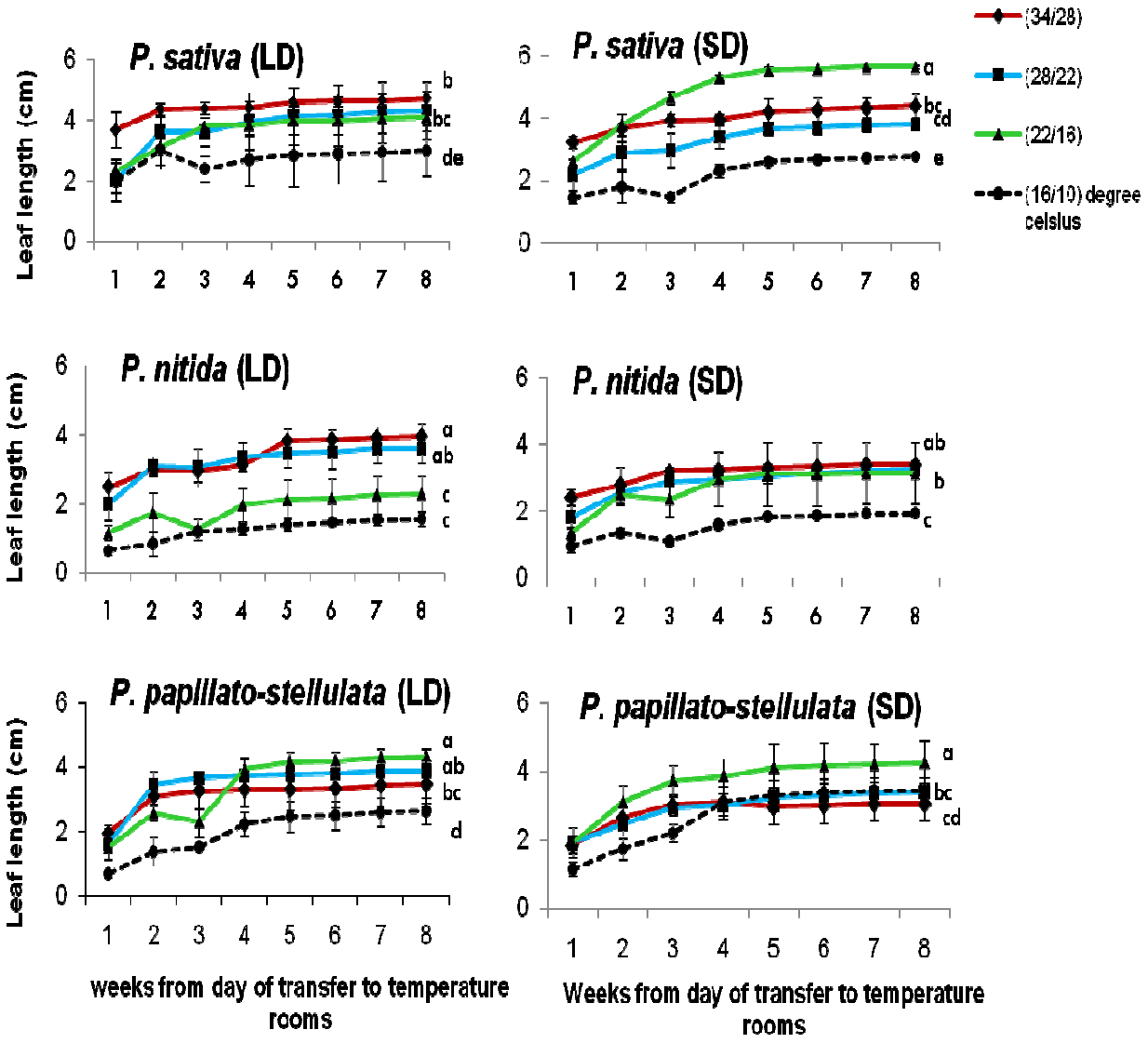


Fig. 5 Plant leaf lengths (cm) measurements among the three subspecies of purslane recorded once a week, on the same day for eight weeks (from day of transfer to temperature rooms) in both long day (LD) and short day (SD) in response to four different temperature conditions(^{0}C day/night). Data represents means ± SEM of five replicates (n=5). Lines with different letters are significantly different at $p<0.05$.

3.1.3 Effects of temperature and day length on Plant number of internodes.

Due to the highly branched nature and also the greater plant height of *P. sativa* as compared to *P. papillato-stellulata* and *P. nitida* as seen in both long and short day, day/night temperature regimes, the plant number of internodes was also greater in the *P. sativa* subspecies (10), followed by *P. papillato-stellulata* (8), then *P. nitida* (7) in the long day and *P. sativa* (8), *P. papillato-stellulata* (8), *P. nitida* (6) in the short day all grown in the warmest temperature condition (34/28^{0}C) **(Fig. 6).** In the coldest growth condition however, all plant microspecies in both long and short day had an average of 2 internodes each, (the lowest plant number of internodes). The number of plant internodes decreased by 1.3-fold in the *P. sativa* microspecies when it was grown in the short day temperature regime relative to the long day at the 34/28^{0}C. Consequently, within the long day regime, *P. sativa* gained 1.4 and 1.2-fold more internodes than *P. nitida* and *P. papillato-stellulata* respectively at the same temperature. Also in the short day, it was 1.3 and 1-fold more than *P. nitida* and *P. papillato-stellulata* respectively by the end of the eight week of measurement. It was also significantly decreased by 4.9 and 4-fold in the long and short day regime respectively as the temperature decreased to 16/10^{0}C.

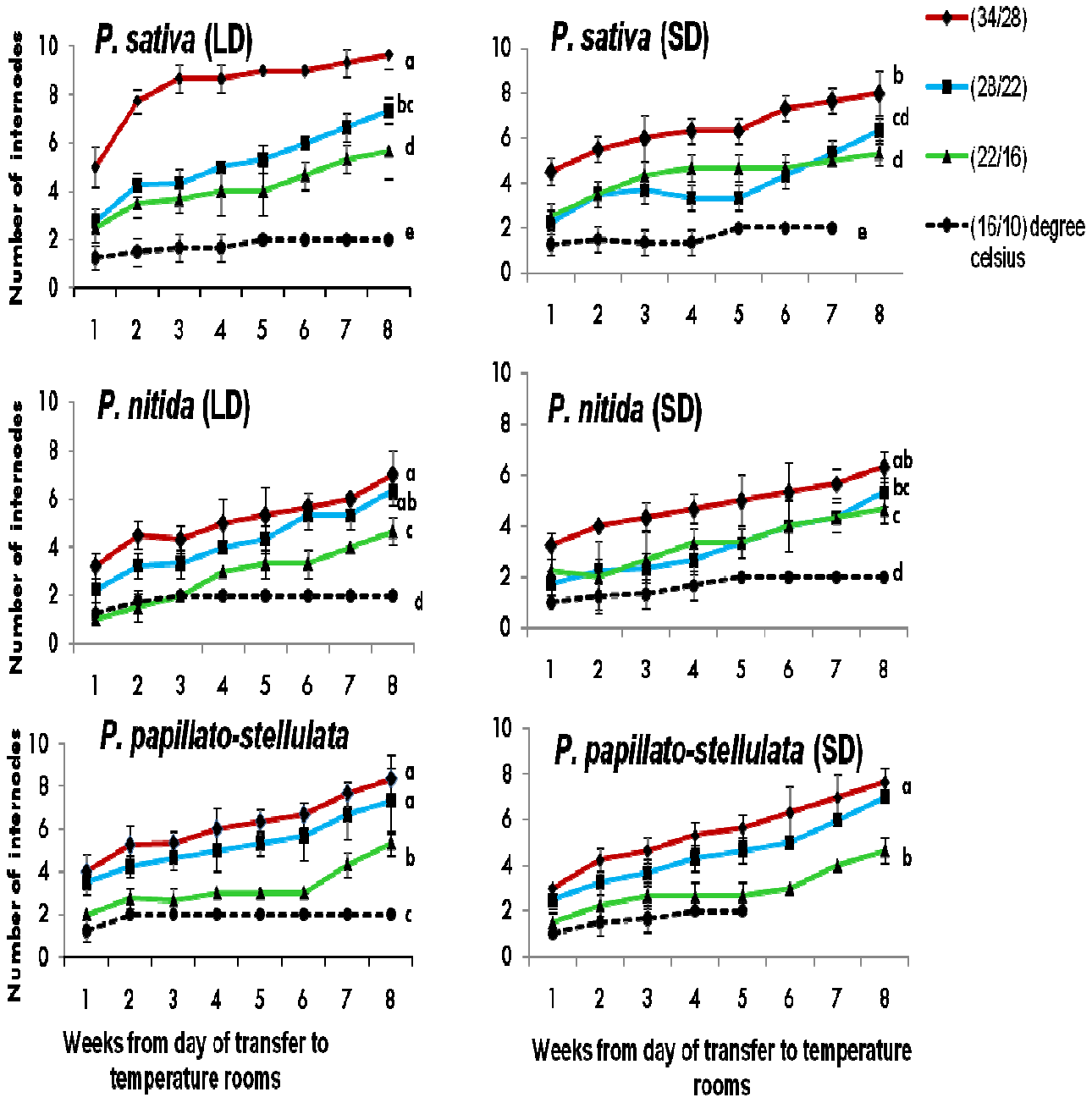


Fig.6 Plant number of internodes among the three subspecies of purslane measured once a week, on the same day for eight weeks (from day of transfer to temperature rooms) for both long day and short day in response to four different temperature conditions (^{0}C day/night). Data represents means ± SEM of five replicates (n=5). Lines with different letters are significantly different at $p<0.05$.

3.1.4 Effects of temperature and day length on average length of internodes

This measurement was an extrapolation from the measurements of plant height and the number of internodes. As shown in **(Fig. 7),** in the first week of measurement, the *P. sativa* microspecies grown in the temperature of (16/10^{0}C) recorded the highest length of 7.8±2.3 cm and 6.3±1.7 cm for long day and short day respectively. This later was measured at 4.9±0.3 cm and 3.4±0.7 cm, in long and short day respectively by the end of week eight. Also by the end of week eight, *P. sativa* (7.8±1.8 cm) recorded the highest and *P. nitida* (4.3±0.1 cm) the lowest length with *P. papillato-stellulata* (4.6±0.3 cm) an intermediate, all in the long day regime at 34/28^{0}C. In the short day also, *P. sativa* (7.7±0.9 cm), *P. nitida* (5.8±1.0 cm), *P. papillato-stellulata* (3.8±0.5 cm) were recorded also at the same temperature. There was no significant difference between the *P. sativa* microspecies in the long and short day by the end of week eight at 34/28^{0}C temperature condition. But within the long day regime, among the three microspecies, it was 1.8 and 1.7-fold more than *P. nitida* and *P. papillato-stellulata* respectively. Similarly, it was 1.3 and 2-fold more than *P. nitida* and *P. papillato-stellulata* respectively in the short day regime. By week eight, as the temperature decreased to (16/10^{0}C), *P. sativa* significantly decreased by 1.6 and 2.3-fold in the long and short day respectively.

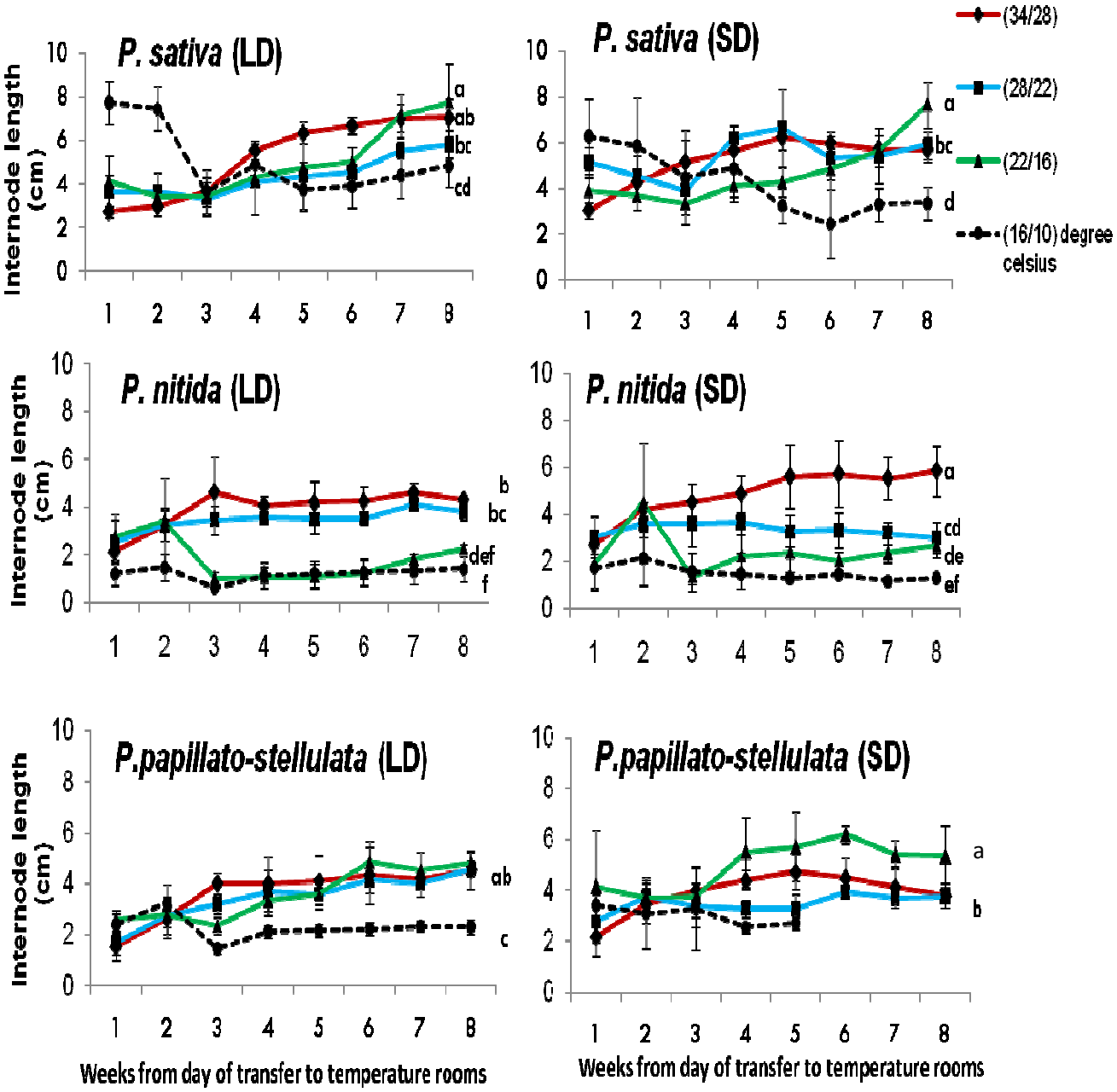


Fig. 7 Average length of internodes in the main stem among the three subspecies of purslane measured once a week, on the same day for eight weeks (from day of transfer to temperature rooms) in both long day (LD) and short day (SD) in response to four different temperature conditions (^{0}C day/night). Data shows mean ± SEM of five replicates (n=5).Lines with different letters are significantly different at $p<0.05$.

3.1.5 Effects of temperature and day length on Leaf (fresh and dry) weight.

The leaf and stem fresh weights in both long day (16 hrs) and short day (9 hrs) were significantly affected by the different temperature conditions. The commercial green-leaf *P. sativa* microspecies, (long day) were significantly higher in leaf FW among the three subspecies when the temperature was (34/28^{0}C). This wasn't the case with the plants grown in the temperature condition of (16/10^{0}C) in both day length regimes. However in the short day regime, *P. sativa* was only significantly different from *P. papillato-stellulata* at (34/28^{0}C) and *P. nitida* at (22/16^{0}C) as shown in **Fig. 8**. The dry weight measurements indicated that in the (34/28^{0}C) temperature condition, *P. sativa* was significantly more in biomass (2-fold) than *P. papillato-stellulata* and not *P. nitida* in the long day regime. In the short day, at the same temperature, *P. sativa* gained significantly 5.7-fold more biomass than *P. papillato-stellulata* and 2.4-fold more biomass than *P. nitida*. *P.nitida* was significantly similar to *P. sativa* in terms of plant dry weight when plants were grown in temperatures (28-22^{0}C) in both long and short day. Reducing the temperature reduced the fresh and dry weigh of the leaves. Compared to the (34/28^{0}C) temperature condition, all plant FW and DW under long and short day were significantly inhibited under the 16/10^{0}C temperature condition. The above observations were similar to stem fresh and dry weights for both long and short day (**Fig.9**).

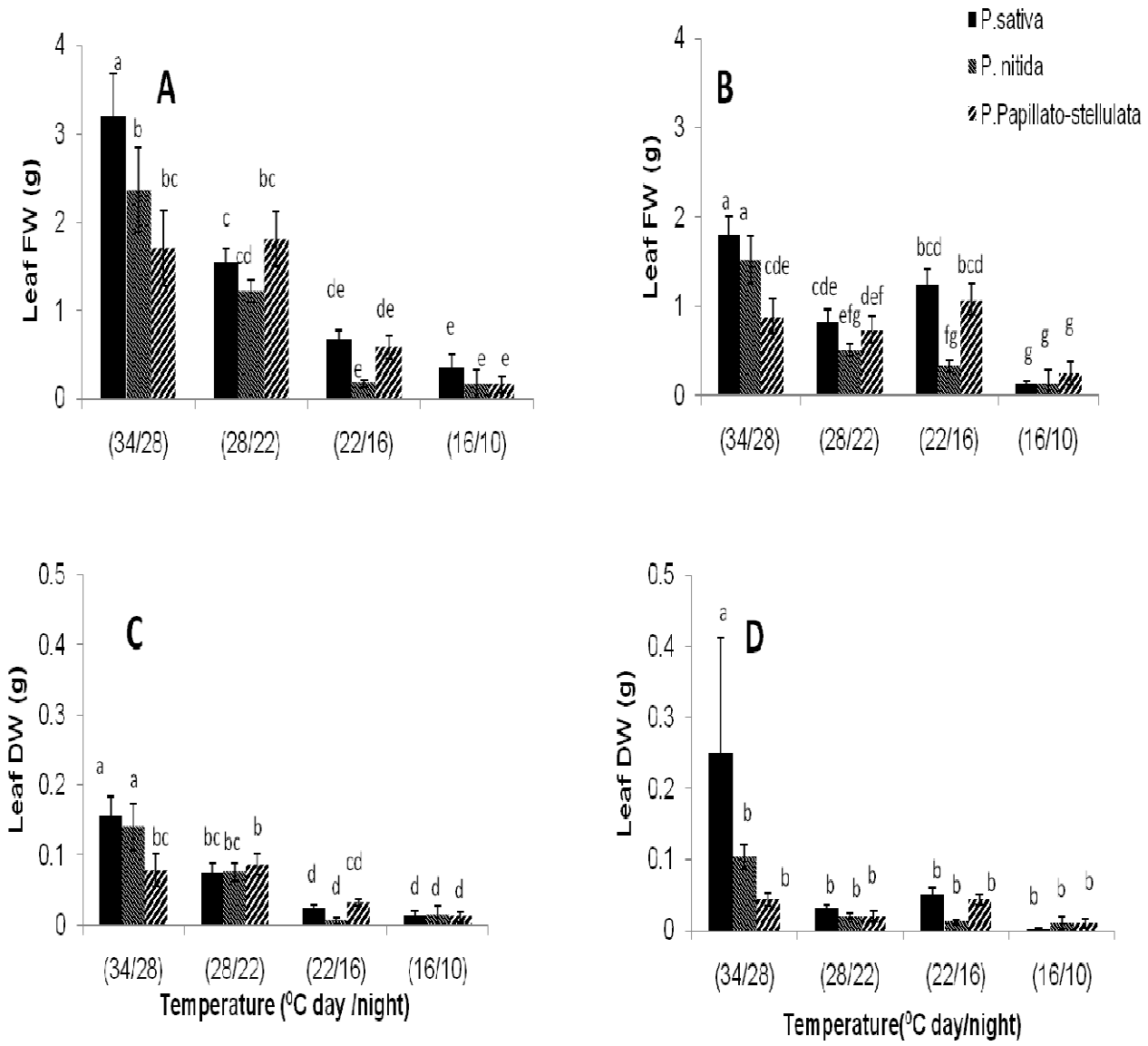


Fig 8. Leaf fresh weight (FW) and dry weight(DW) of three subspecies of purslane grown in four different temperature conditions (^{0}C day/night), **long day** (16 hrs) **(A and C**) and **short day** (9 hrs**) (B and D).** Data represents means ±SEM of five replicates each (n=5). Bars with different letters are significantly different at p<0.05.

3.1.6 Effects of temperature and day length on Stem (fresh and dry) weight .

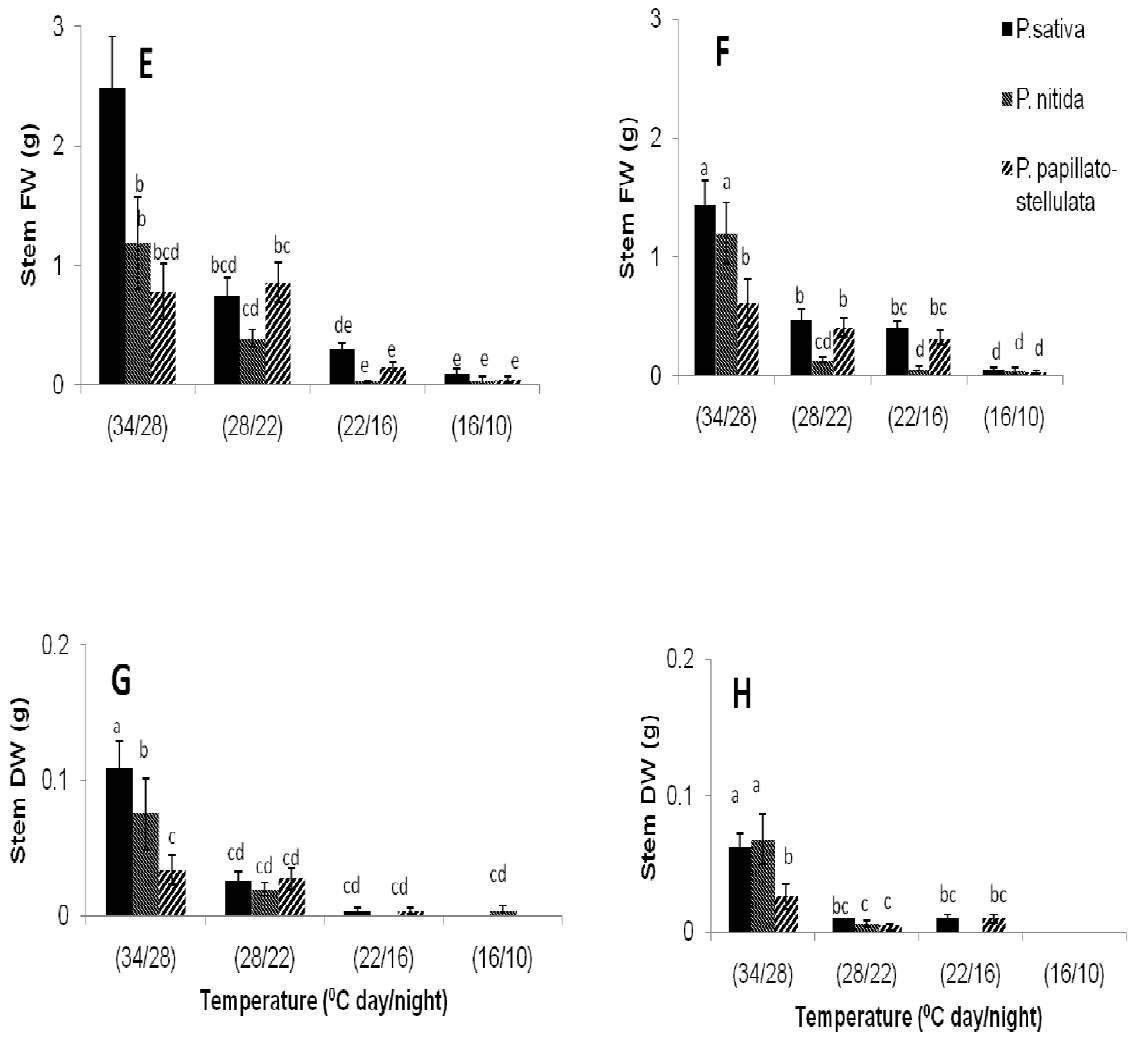


Fig 9. Stem fresh weight (FW) and dry weight (DW) of three subspecies of purslane grown in four different temperature conditions (^{0}C day/night), **long day** (16 hrs**) (E and G)** and **short day** (9 hrs) **(F and H**). Data shows means ± SEM of five replicates each (n=5). Bars with different letters are significantly different at $p<0.05$.

3.1.7 Effects of temperature and day length on Net plant (fresh and dry) weights.

The net plant fresh weight for *P. sativa* was significantly 4.4 and 1.6-fold more in biomass than *P. nitida* and *P. papillato-stellulata* respectively in the 34/28^{0}C temperature condition, long day (**Fig.10**). Similarly in the short day regime, it gained significantly 2.3 and 2.3-fold more biomass than *P. nitida* and *P. papillato-stellulata* respectively also in the same temperature condition. Decreasing the temperature reduced the fresh and dry weight of the leaves for all microspecies in both long and short day. In *P. papillato-stellulata*, short day, significant decrease was already evident in temperature condition of 34/28^{0}C. Dry weight measurements also indicated that *P. sativa* in the 34/28^{0}C gained 4.7 and 1.9-fold more biomass than *P. nitida* and *P. papillato-stellulata* respectively. Compared to the 34/28^{0}C temperature condition, all plants fresh and dry weights were inhibited in the 22/16^{0}C temperature condition . Due to the cold temperature condition in the 16/10^{0}C regime, plants did not survive to the time of collection of samples for this part of the experiment. So no records were made to this effect.

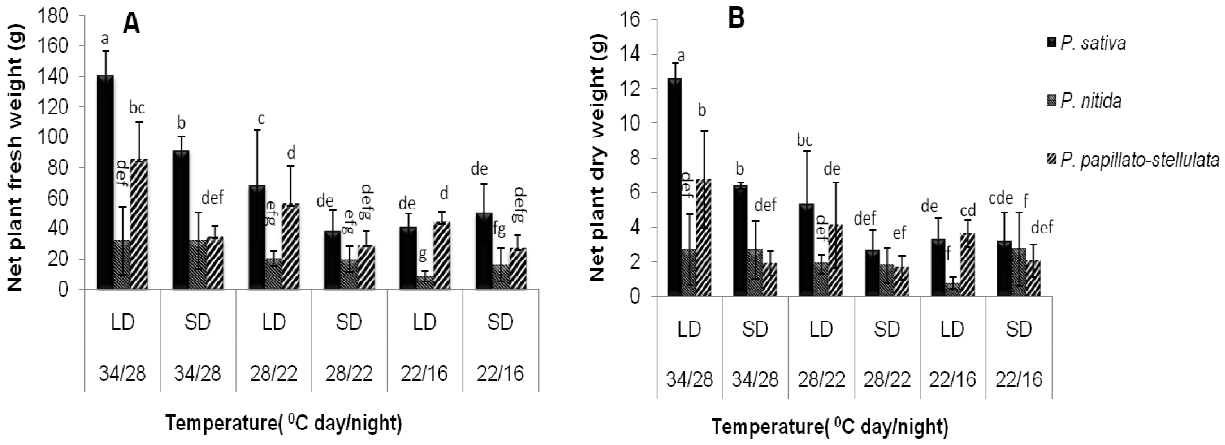


Fig.10 Comparison of net plant fresh weight (**A**) and net plant dry weight (**B**) in both long day (LD) and short day (SD) conditions in response to three different temperature conditions (^{0}C day/night). Data represents means ±SEM of five replicates (n=5). Bars with different letters are significantly different at $p<0.05$.

3.1.8 Effects of temperature and day length on Percentage plant moisture content

The student t-test analysis did not indicate any significant differences in moisture content among the three microspecies when the temperature conditions were 34/28^{0}C and 22/16^{0}C in the long day regime. Also, the same was observed for the plants grown in short day, temperature 22/16^{0}C (**Fig. 11**). However, in the long day regime at a temperature of 28/22^{0}C, *P. nitida* contained significantly (1.3-fold) moisture than *P. papillato-stellulata* but was not significantly different from *P. sativa*. The same was observed among *P. nitida* plants in the short day under temperatures 34/28^{0}C and 28/22^{0}C. It contained significantly (1.5-fold) more moisture than *P. papillato-stellulata* in both temperature conditions. Decreasing the temperature to 22/16^{0}C did not significantly affect the moisture content of all microspecies in the long day regime relative to 34/28^{0}C but did for *P. nitida* (1.6-fold less) in the short day regime. There were no plant samples available in 16/10^{0}C temperature condition for both short and long day since all plants died off before harvesting.

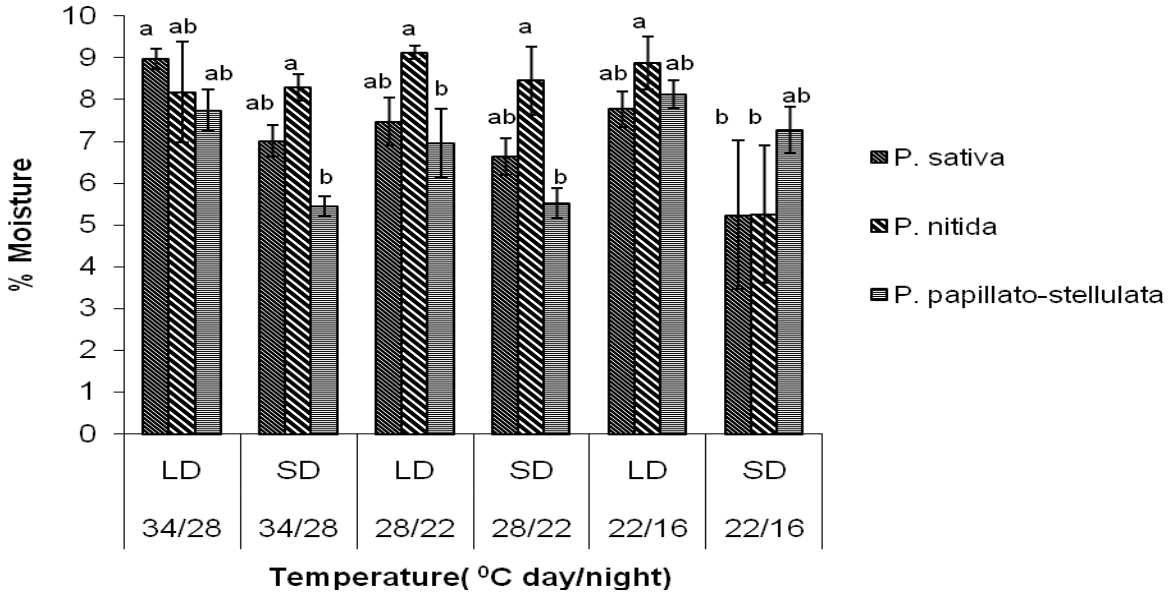


Fig.11 Comparison of plant moisture content (%) of the three microspecies of purslane in response to three different temperature conditions (^{0}C day/night), long day (**LD**) and short day (**SD**). Data represents means ± SEM of five replicates (n=5). Bars showing different letters are significantly different at $p<0.05$.

3.1.9 Effects of temperature and day length on Percentage leaf dry weight in net plant fresh weight.

The measurement was determined to show the amount of leaf dry weight (%) in the total plant fresh weight harvested. From the results below, *P. nitida* indicated significant differences with *P. sativa* but was relatively similar to *P. papillato-stellulata* in terms of leaf dry weight content in the long day regime for all temperatures with the exception of 22/16^{0}C. In this condition, *P. nitida* was significantly (2.2 and 1.5-fold) more than *P. sativa* and *P. papillato-stellulata* (**Fig.12**). The short day however showed no significant differences between *P. sativa* and *P. papillato-stellulata* for temperatures 34/28^{0}C and 28/22^{0}C but they were significantly different from *P. nitida*. This was not seen in 22/16^{0}C temperature condition as *P. nitida* and *P. papillato-stellulata* were comparatively similar but significantly different from *P. sativa*. Compared to the 34/28^{0}C temperature condition, *P. nitida* leaf dry weight gain was significantly enhanced under the 22/16^{0}C temperature condition, long day whiles under the short day, 28/22^{0}C.

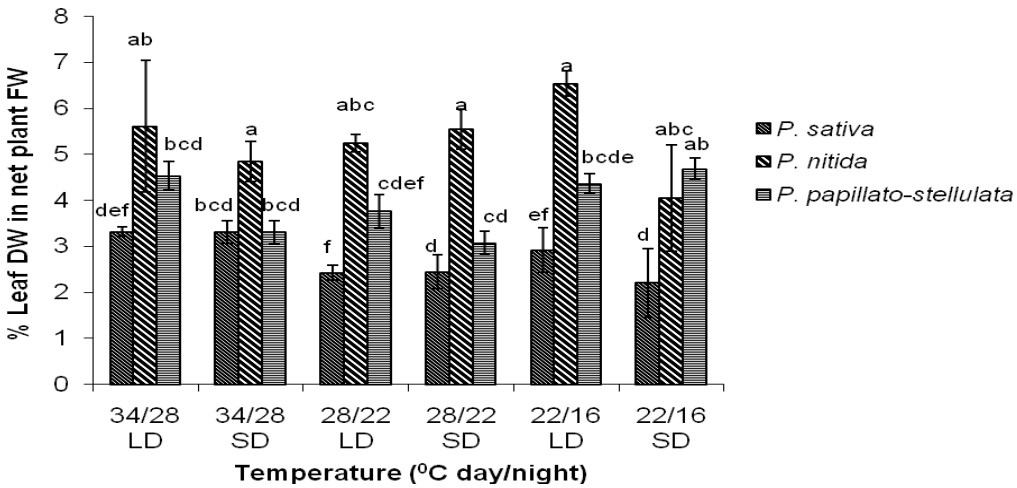


Fig. 12 Comparison of percentage leaf dry weight in net plant fresh weight of the three microspecies of purslane in response to three different temperature conditions (^{0}C day/night), long day (**LD)** and short day (**SD)**. Data represents means ± SEM of five replicates (n=5). Bars showing different letters are significantly different at $p<0.05$.

3.2 Effects of temperature and day length on Organic acids concentration.

3.2.1 Oxalic acid

Two organic acids were determined **(Fig. 13 and 14):** oxalic acid and ascorbic acid. In effect , oxalic acid was the one present in higher amounts in the leaves in both long day and short day across the four different temperature conditions. The highest and lowest quantity of oxalic acid in the long day regime was *P. sativa* (156.8 µg/g fresh weight) and *P.nitida* (135.6 µg/g fresh weight) respectively all in the 16/10^{0}C temperature condition whiles for the short day regime, the highest was recorded for *P. papillato-stellulata* (155.0 µg/g fresh weight) at 16/10^{0}C and the lowest for *P. sativa* (139.4 µg/g fresh weight) in the 34/28^{0}C temperature condition but this was similar to *P. nitida* in the 16/10^{0}C. In effect temperature had an influence on the oxalic acid concentration of the plant microspecies (*P. sativa* and *P. papillato-stellulata*) as this was seen to increasein the coldest temperature condition (16/10^{0}C) irrespective of long day or short day. *P. nitida* and *P. papillato-stellulata* were comparatively similar in oxalic acid concention but significantly different from *P. sativa* in the 34/28^{0}C temperature condition. As the temperature decreased to 16/10^{0}C, *P. sativa* increased and *P. nitida* decreased by 1.1-fold in the long day regime. *P. papillato-stellulata* was relatively similar in all the temperature conditions. In the short day too, *P. sativa* and *P. papillato-stellulata* increased by 1-fold each as the temperature reached 16/10^{0}C. Compared to the 16/10^{0}C temperature condition, *P. sativa* oxalic acid concentration was significantly inhibited in the 34/28^{0}C temperature condition. But this was not the same for *P. nitida* which showed a significant reduction in the long day regime for plants grown in the 16/10^{0}C temperature condition.

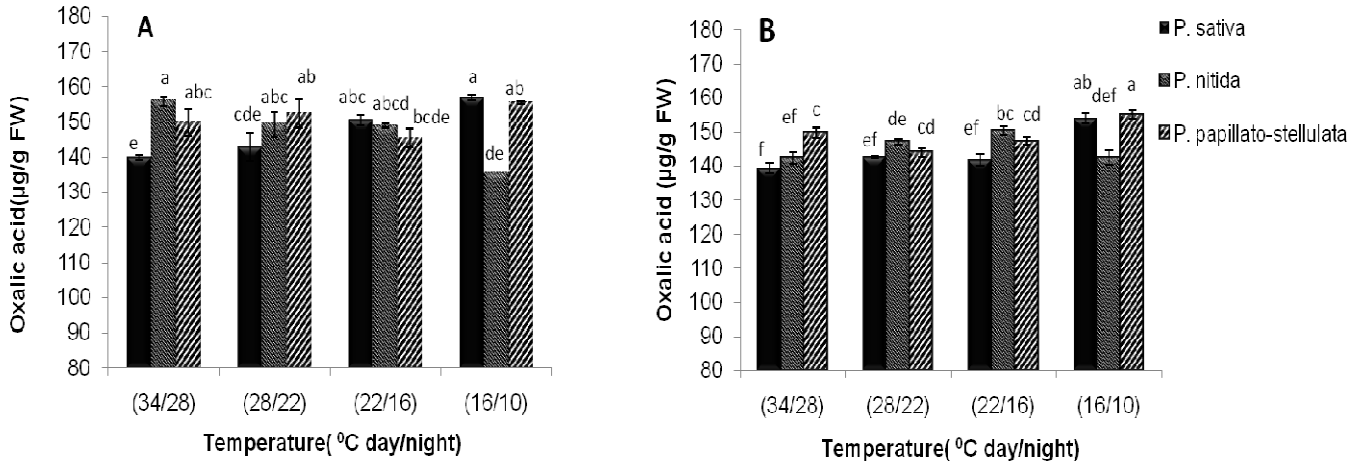


Fig13: Comparison of oxalic acid concentration (µg/g fresh weight) between the three purslane subspecies grown in four different temperature conditions (^{0}C day/night), long day **(A)** and short day (**B)**. Results represent means± SEM of five replicates. Bars with different letters are significantly different at $p<0.05$.

3.2.2 Ascorbic acid

The results below (**Fig.14**) show the ascorbic acid concentrations for the three purslane microspecies in long day and short day temperature regimes. As opposed the results on oxalic acid concentration, ascorbic acid was shown to decrease as the temperature decreased from the (34/28^{0}C) temperature condition to the (16/10^{0}C) temperature condition in both long and short day for all microspecies with the exception of *P. sativa* in the short day regime. Decreasing the temperature condition to 16/10^{0}C, significantly decreased the *P. sativa*, *P. nitida* and *P. papillato-stellulata* ascorbic acid concentrations by 1.7, 5 and 2.5-fold respectively in the long day regime. In the short day, *P. nitida* and *P. papillato-stellulata* were significantly reduced by 2.5 and 1.2-fold respectively in ascorbic acid concentration with *P. sativa* showing no change. Compared to the plants grown in 34-22^{0}C temperature condition, long day and short day, *P. nitida* and *P. papillato-stellulata* ascorbic acid accumulation was significantly inhibited at the 16/10^{0}C temperature condition.

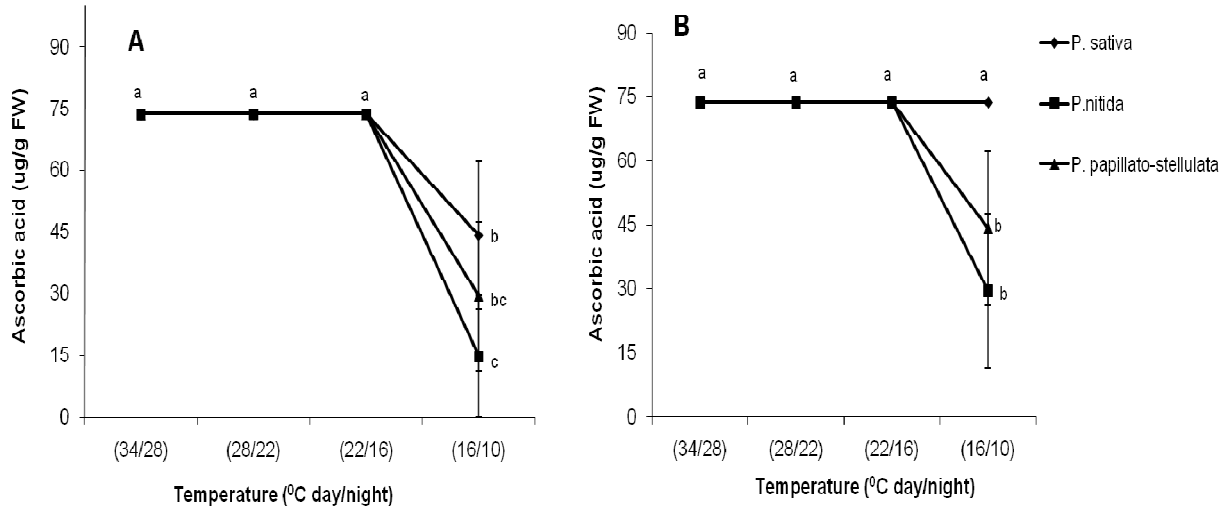


Fig.14 Comparison of ascorbic acid concentration (µg/g fresh weight) between the three purslane microspecies in response to four different temperature conditions (^{0}C day/night) for long day **(A)** and short day **(B)** regimes. Results represent means± SEM of five replicates. Lines with different letters are significantly different at $p<0.05$.

Table 7: Fatty Acid composition and concentration (%) of the three microspecies of purslane grown in four different temperature (^{0}C day/night), long day (16 hrs)

Temperature (^{0}C day/night)	34/28			28/22			22/16			16/10		
microspecies	*Pos*	*Pon*	*Pop*	*Pos*	*Pon*	*Pop*	*Pos*	*Pon*	*Pop*	*Pos*	*Pon*	*Pop*
Fatty acid	Fatty acid content (%)											
C 12:0	0.35±0.05^{ab}	0.28±0.12^{bc}	0.32±0.04^{abc}	0.21±0.09^{bc}	0.27±0.07^{bc}	0.58±0.26^{a}	0.09±0.06^{bc}	0.10±0.04^{bc}	0.11±0.07^{bc}	Nq	Nq	Nq
C 14:0	0.41±0.04^{a}	0.39±0.03^{ab}	0.30±0.01^{abc}	0.23±0.06^{cde}	0.27±0.01^{bc}	0.25± 0.03^{cd}	0.10±0.06^{ef}	0.09±0.06^{f}	0.12±0.08d^{ef}	Nq	Nq	Nq
C16:0	17.07±0.45^{a}	17.11±0.25^{a}	16.22±0.49^{a}	15.21±0.35^{a}	14.96±0.18^{a}	15.19±0.29^{a}	14.38±0.23^{a}	17.26±0.56^{a}	15.79±0.40^{a}	6.48±3.98^{b}	Nq	6.35±3.9^{ab}
C 16:1	0.08±0.04^{b}	0.07±0.04^{b}	Nq	Nq	0.11±0.03^{ab}	Nq	0.44±0.40^{a}	Nq	0.06±0.04^{b}	Nq	Nq	Nq
C 18:0	2.12±0.11^{a}	1.56±0.41^{abc}	1.65±0.05^{ab}	1.46±0.45^{abc}	1.53±0.07^{abc}	2.02±0.96^{ab}	1.21±0.76^{abc}	Nq	0.82±0.52^{abc}	1.37±1.06^{abc}	Nq	1.09±0.67^{abc}
C18:1nt9c	5.03±0.53^{ab}	5.08±0.33^{ab}	4.37±0.39^{ab}	2.85±0.73b^{cd}	4.66±0.18^{ab}	3.43±0.87^{bc}	1.48±0.61^{cd}	7.04±0.20^{a}	3.64±1.12b^{c}	3.36±2.77^{bc}	Nq	1.18±0.73^{cd}
C18:2n6t	Nq	Nq	0.06±0.04^{b}	0.08±0.03^{b}	0.08±0.04^{b}	0.02±0.03^{a}	Nq	Nq	Nq	Nq	Nq	Nq
C18:2n6c	13.02±1.25^{a}	9.82±0.85^{ab}	13.25±0.53^{a}	12.71±0.20^{a}	8.56±0.17^{ab}	12.71±0.25^{a}	14.23±0.27^{a}	10.27±0.19^{ab}	12.50±0.79^{a}	8.62±5.73^{ab}	Nq	5.75±3.52^{bc}
C18:3n6	0.38±0.02^{b}	0.39±0.01^{b}	0.38±0.01^{b}	0.30±0.07^{b}	0.38±0.00^{b}	0.38±0.01^{b}	0.22±0.09^{b}	0.20±0.08^{b}	0.23±0.09^{b}	Nq	Nq	0.14±0.09^{b}
C18:3n3	57.99±1.45^{a}	61.53±0.80^{a}	60.78±1.05^{a}	64.40±0.87^{a}	66.64±0.46^{a}	62.12±1.08^{a}	64.95±1.00^{a}	62.78±1.23^{a}	64.94±2.02^{a}	Nq	Nq	23.69±14.51^{b}
C 20:0	0.68±0.12^{a}	Nq	0.25±0.11^{b}	Nq	Nq	Nq	Nq	Nq	0.26±0.19^{b}	0.29±0.20^{b}	Nq	Nq

Long day condition Cont…

Temperature (^{0}C day/night)	34/28			28/22			22/16			16/10		
microspecies	Pos	Pon	Pop	Pos	Pon	Pop	Pos	Pon	Pop	Pos	Pon	Pop
Fatty acid												
C 21:0	Nq	0.47±0.12^{abc}	0.25±0.15^{bcd}	0.50±0.12^{abc}	0.53±0.13^{ab}	0.72±0.20^{a}	0.37±0.15ab^{cd}	0.35±0.14^{abcd}	Nq	Nq	Nq	0.22±0.14^{bcd}
C22:0	1.12±0.18^{ab}	1.19±0.19^{a}	0.64±0.06^{abcd}	0.48±0.12^{cd}	0.84±0.04^{abc}	0.50±0.05^{cd}	0.30±0.18^{cd}	0.81±0.21^{abc}	0.51±0.29^{cd}	0.17±0.11^{d}	Nq	0.60±0.39^{bcd}
C24:0	1.47±0.06^{ab}	1.31±0.03^{abc}	1.43±0.17^{abc}	1.52±0.08^{a}	1.01±0.05^{abcd}	1.33±0.04^{abc}	1.60±0.08^{a}	0.92±0.23^{abcd}	0.78±0.32^{cd}	0.36±0.31^{d}	Nq	0.81±0.51^{cd}
Total	*99.72±4.3*	*99.20±3.3*	*100±3.1*	*99.95±3.2*	*99.84±1.4*	*99.24±4.1*	*99.37 ±3.9*	*99.82±2.9*	*99.76±5.9*	*20.65±14.2*	*Nq*	*39.83±24.5*

Data are mean± SEM (n=5). Values within rows followed by different letters denote significant difference at p<0.05 Nq- Not quantified; Pos- *P.sativa*; Pon- *P.nitida*; Pop- *P.papillato-stellulata*.

Table 8: Fatty Acid composition and concentration (%) of the three microspecies of purslane grown in four different temperatures (^{0}C day/night), short day (9 hrs).

Temperature (^{0}C day/night)	34/28			28/22			22/16			16/10		
microspecies	*Pos*	*Pon*	*Pop*	*Pos*	*Pon*	*Pop*	*Pos*	*Pon*	*Pop*	*Pos*	*Pon*	*Pop*
Fatty acid	Fatty acid content (%)											
C12:0	0.66±0.42^{a}	0.29±0.08^{ab}	0.27±0.17^{ab}	0.09±0.05^{b}	Nq	0.10±0.07^{b}	0.30±0.07^{ab}	0.27±0.01^{ab}	0.28±0.08^{ab}	0.27±0.05^{ab}	Nq	0.32±0.16^{ab}
C14:0	0.23±0.10^{abc}	0.36±0.04^{a}	0.22±0.09^{abc}	0.10±0.06^{c}	0.17±0.07^{abc}	0.13±0.08^{bc}	0.30±0.06^{ab}	0.18±0.05^{abc}	0.23±0.06^{abc}	0.22±0.07^{abc}	0.12±0.08^{bc}	0.18±0.08^{abc}
C16:0	12.75±3.21^{ab}	17.17±0.50^{a}	15.15±3.79^{ab}	14.42±1.80^{ab}	15.97±0.62^{ab}	16.41±0.64^{ab}	16.34±0.63^{ab}	15.13±0.22^{ab}	11.71±2.93^{abc}	17.11±0.94^{a}	5.33±3.27^{c}	9.78±4.00^{bc}
C18:0	1.39±0.39^{bc}	1.71±0.06^{ac}	0.66±0.42^{c}	1.58±0.14^{abc}	1.44±0.07^{bc}	1.19±0.32^{c}	2.45±0.86^{ab}	1.74±0.20^{abc}	1.14±0.30^{c}	2.76±0.50^{a}	0.62±0.38^{c}	1.32±0.59^{bc}
C18:1nt9c	2.95±0.74^{bcde}	5.02±0.28^{abcd}	2.72±1.12^{bcde}	5.31±1.30^{abc}	5.44±0.48^{ab}	4.82±0.25^{abcd}	4.70±2.28^{abcd}	4.08±0.63^{abcde}	2.13±0.63^{cde}	6.25±1.50^{a}	1.19±0.75^{e}	2.07±0.65d^{e}
C18:2n6c	10.20±2.62^{cde}	8.40±0.15^{de}	10.26±2.59^{cde}	14.78±0.58^{abc}	9.48±0.32^{cde}	11.29±1.16^{bcd}	16.33±3.23^{ab}	12.03±1.40^{abcd}	10.30±2.58^{bcde}	18.02±1.66^{a}	5.01±3.07^{e}	6.73±2.77^{de}
C18:3n6	0.28±0.12^{b}	0.40±0.01^{b}	0.32±0.08^{b}	0.22±0.09^{b}	0.29±0.07^{b}	0.24±0.10^{b}	0.33±0.03^{b}	Nq	0.29±0.07^{b}	0.33±0.02^{b}	0.19±0.12^{b}	0.24±0.10^{b}
C18:3n3	48.01±12.05^{ab}	63.04±0.51^{a}	47.14±11.84^{ab}	52.68±6.87^{ab}	62.60±1.79^{a}	63.72±1.59^{a}	55.97±6.72^{a}	49.49±12.50^{ab}	52.03±13.01^{ab}	51.76±3.40^{ab}	24.64±15.14^{b}	37.89±15.47^{ab}
C21:0	0.22±0.14^{ab}	0.49 ±0.12^{a}	0.46±0.12^{ab}	0.33±0.12^{ab}	0.38±0.16^{ab}	Nq	0.46±0.10^{ab}	0.43±0.11^{ab}	0.51±0.13^{a}	0.45±0.08^{ab}	0.24±0.15^{ab}	0.34±0.14^{ab}
C22:0	0.52±0.14^{b}	0.97±0.05^{a}	0.54±0.15^{b}	0.81±0.30^{ab}	0.81±0.05^{ab}	0.52±0.18^{bc}	0.78±0.03^{ab}	0.78±0.11^{ab}	0.45±0.12^{bc}	0.74±0.08^{ab}	Nq	0.44±0.19^{bc}
C24:0	1.05±0.26^{ab}	1.27±0.02^{ab}	1.16±0.29^{ab}	1.54±0.13^{a}	1.26±0.01^{ab}	1.07±0.27^{ab}	1.61±0.32^{a}	1.16±0.06^{ab}	1.35±00.35^{a}	1.41±0.26^{a}	0.54±0.34^{b}	0.93±0.39^{ab}
Total	*78.26±20.2*	*99.12±2.2*	*78.90±20.7*	*91.86±11.4*	*97.84±3.6*	*99.49±4.7*	*99.57±14.3*	*85.29±15.3*	*80.42 ±20.3*	*99.32±8.6*	*37.88±23.3*	*60.24±24.5*

Data are mean± SEM (n=5). Values within rows followed by different letters denote significant difference at $p<0.05$ nq: Not quantified

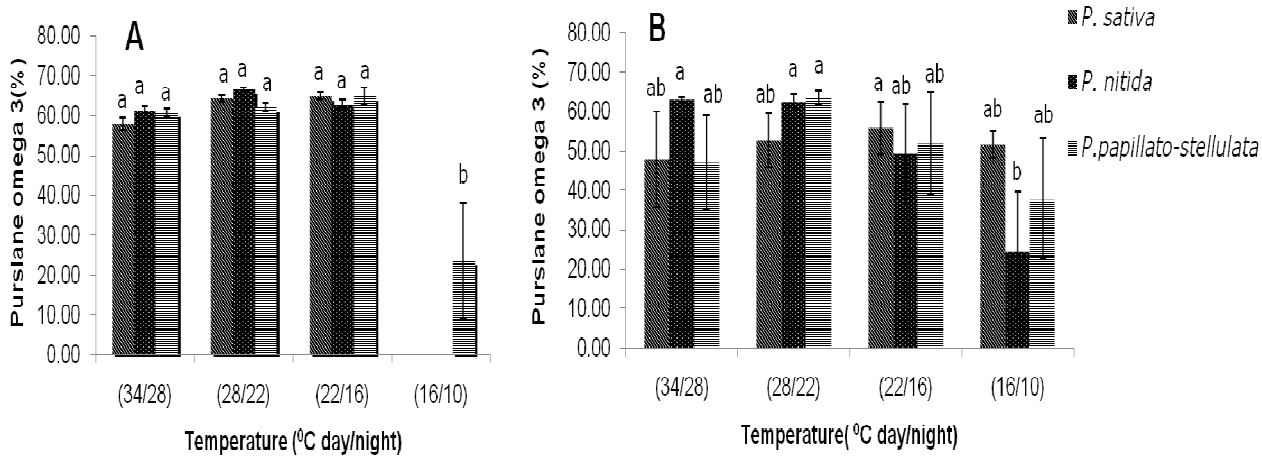


Fig.15 Percentage of α- Linolenic acid (C18:3n3) among the three microspecies of purslane grown in four different temperature (^{0}C day/night) conditions, long day **(A)** and short day (**B)**. Data represents mean ±SEM of five replications each. Bars with different letters are significantly different at $p<0.05$.

3.3 Effect of temperature and day length on fatty acids composition and concentration (in percentage).

The dry leaf fatty acid composition of the three purslane microspecies was similar and unaffected by temperature and day length conditions **(Tables 7 and 8)** In general, the major fatty acids present in the leaves were α-linolenic acid, ALA (18:3n3), Linoleic acid, LA (18:2n6) and Palmitic acid (16:0). Smaller amounts of Lauric acid (12:0), Myristic acid (14:0), Palmitoleic (16:1), Stearic (18:0), Oleic (18:1nt9c), Gamma linolenic acid, GLA (18:3n6), Behenic acid (22:0) and Lignoceric acid (24:0) were also detected. ALA was the most abundant one in all the microspecies making up about two-thirds of the total fatty acid in both long day and short day regimes. Hexadecatrienoic acid (16:3) as well as the longer chain fatty acids (EPA and DHA) were not detected in the leaves confirming previous reports that purslane is an 18:3 plant with little or no identification of the longer chain fatty acids. Regarding the major fatty acids found, the concentrations (%) increased from (16/10^{0}C) to the (34/28^{0}C) in both long and short day. As the case may be, the

lowest values of fatty acids were found in the coldest temperature room (16/10^{0}C) but the *P. sativa* microspecies (short day) in this temperature room showed comparatively higher percentages which were similar to those in the warmer rooms. The above regarding the major fatty acids were also observed in terms of amount of total fatty acids found but with the exception of *P. sativa* (Short day) **(Table 8).** In the long day temperature condition, the mean ±SEM (expressed as percentage dry weight) of the major fatty acids found ranged from; ALA (66.64 % in *P. nitida* to 23.69% in *P. papillato-stellulata*), LA (14.23 % in *P. sativa* to 5.75 % in *P. papillato-stellulata*) and palmitic (17.26 % in *P. nitida* 6.35% in *P. papillato-stellulata*)**(Table 7).**

In the short day regime, the major fatty acids ranged from ALA (63.72 % in *P. papillato-stellulata* to 24.64 % in *P. nitida*), LA (18.02 % in *P. sativa* to 5.01% in *P. nitida*) and palmitic (17.17% in *P. nitida* to 5.33% in *P. nitida*) **(Table 8).** From this it can be seen that *P. papillato-stellulata* had the lowest fatty acid concentrations (%) with regards the major ones in long day temperature regime whiles *P. nitida* had the lowest in the short day. From **Fig.15**, it can be seen that all three microspecies had a similar ALA percentage concentration across the three warmer rooms and not in the coldest room in the long day regime whiles in the short day temperature room, this increased with increasing temperature.

3.4 Assessment of the role of *Portulaca oleracea* leaves on acute inflammation in Dextran sulphate sodium (DSS)-induced experimental colitis.

3.4.1: Effect of Purslane intake on mice plasma fatty acid.

Treatment of mice with purslane as a preventive regime was initiated before the disease was established. We assessed the effect of added leaves in food rations on blood plasma fatty acid. Results showed the presence of omega 3 fatty acids in the plasma of the three groups which received the leave treatment and not the DSS control group or the normal group. A significant increase in plasma DHA was also observed in the group receiving the highest amount of the leaves (24% purslane) relative to the normal control group (**Fig. 16)**. Other fatty acids which were also present in considerable amounts can be shown in **Table 9**.

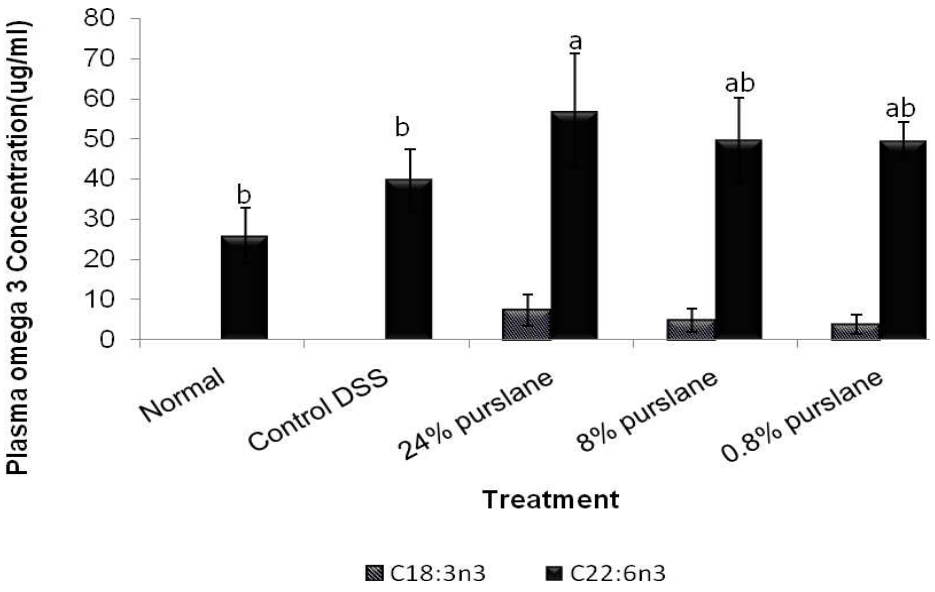


Fig. 16 Effects of *Portulaca* leaves (24 %, 8% and 0.8% concentration of leaves) on blood plasma omega 3 fatty acid, ALA (C18:3n3) and DHA (C22:6n3) (µg/ml) in dextran sulphate sodium (DSS) induced colitis in mice. Results are expressed as mean ± SEM of five observations**.** The DSS control group is not significantly different from the normal group. Purslane -fed groups was also compared to the normal group. Bars with the same letters are not significantly different at $p<0.05$.

Table 9: Fatty acid composition and concentration (µg) in blood plasma when mice were fed purslane leaves during dextran sulphate sodium induced colitis.

Fatty acid (µg/ml blood plasma)

Group/cage	C6:0	C14:0	C16:0	C18:0	C18:1nt9c	C18:3n6	C18:3n3	C20:4n6	C22:6n3
Normal	43.75±21.7	-	96.75±11.4	95.38±11.7	97.97±38.0	-	-	93.97±14.7	25.98±6.9
Control DSS	16.46±3.9	20.78±14.6	123.14±14.0	103.92±13.4	66.22±10.9	-	-	60.48±10.8	39.91±7.6
24% purslane	5.40±2.5	33.47±22.2	123.15±79.7	132.11±21.7	116.10±47.4	2.20±1.3	7.34±3.9	80.16±7.2	56.95±14.3
8% purslane	6.01±0.8	9.98±8.0	160.50±46.1	121.62±30.9	90.67±33.6	3.11±2.0	4.91±2.9	60.39±8.7	49.84±10.4
0.8% purslane	2.62±1.6	11.54±5.8	124.20±50.8	122.28±19.7	95.09±25.0	-	3.79±2.5	63.81±7.5	49.56±4.6

3.4.2. Effects of purslane leaves on body weight changes during DSS- induced colitis

We induced colitis in C57BL/6 mice by adding 3.5% DSS to drinking water for 8 days starting from day 11until the day of fasting when ordinary water was given for 12 hrs. DSS intake in drinking water did not differ significantly between the groups of mice receiving it as well as before its administration. Each group drank approximately 20 ml of water per day. Food intake among groups three and four who received DSS begun to decline 2-3 days after DSS administration (data not shown). All DSS groups showed different extent of diarrhea, more and grosser bleeding in the feaces as the disease progressed (data not shown) which suggested presence and development of inflammation. This was seen in more than 70% of the mice especially in day 14 (3 days after DSS induction). It was also observed that 1 mouse in the group which received 8% purslane and 1 in the group which received 24% purslane died a day before the sacrifice day. During the treatment with DSS, weight loss was noticed after day 14 and this remained so until they were sacrificed. Although all groups lost weight by the end of day 18, the DSS+ leaves treatment groups lost more weight much lower than the normal drinking water-treated mice though not significant. This was especially seen with group (8% purslane) **(Fig.17 and 18).** However a control group of mice fed purslane for 2 weeks did not show the above results seen with the DSS+ leaves fed groups (results not shown).

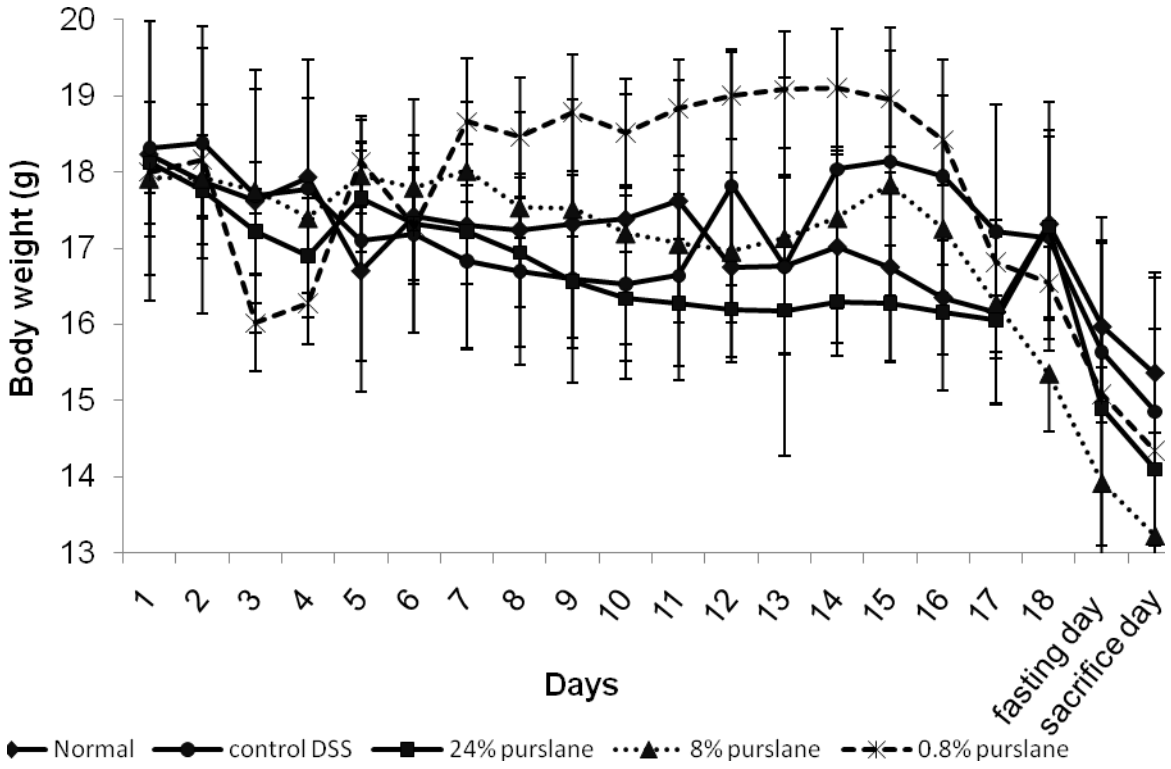


Fig. 17 Time course of changes in mice body weight during the DSS-induced colitis fed 24%, 8% and 0.8% purslane leaves. C57BL/6 mice were administered 3.5% DSS by drinking water from day 11 to 18. Weight changes are shown as an average of 5 observations in each group (n=5).

3.4.3: Mice body weight measurement on sacrifice day

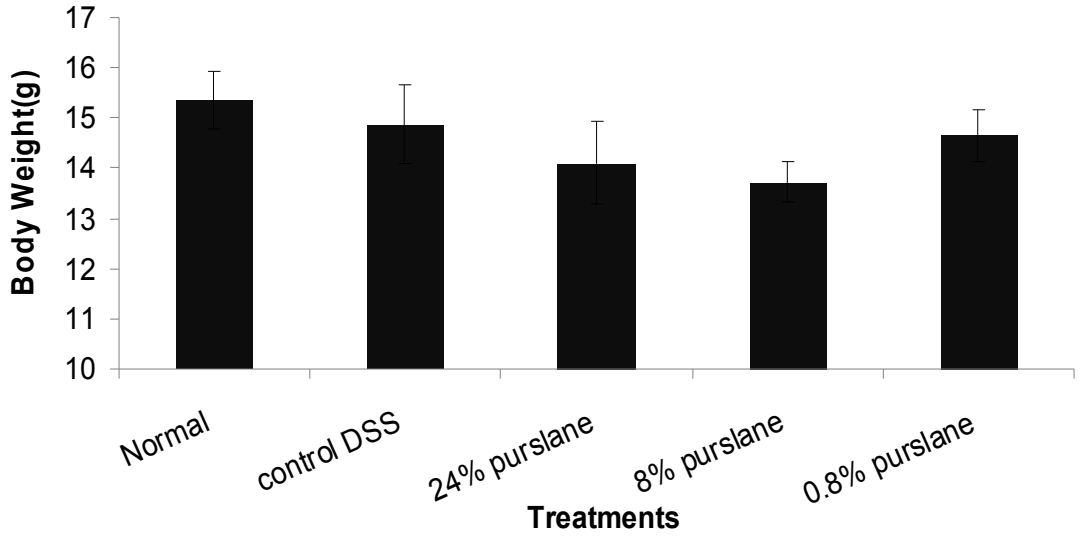


Fig.18 Effects of the different concentration of purslane leaves (24%, 8% and 0.8%) on the body weight of mice on the sacrifice day induced with dextran sodium sulphate for 8 days. Weights represent mean ±SEM of five mice in each group. The DSS control group is not significantly different from the normal group and also with the rest of the treatments at $p<0.05$.

3.4.4 Effect of purslane leaves on DSS- induced macroscopic changes in colon.

Colon length was also measured to determine the severity of colitis. We found that DSS could lead to significant reduction of colon length during the treatment as described in **Fig.19** Treatment with the different concentrations of *Portulaca* however did not significantly attenuate DSS-induced shortening of the colonic length.

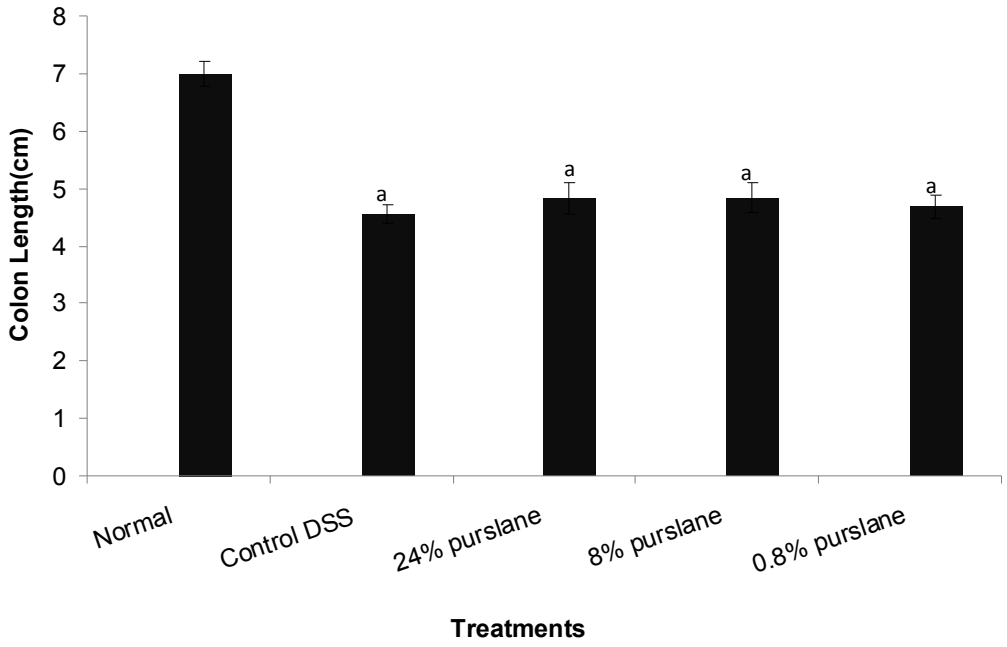


Fig. 19 Mice colon length changes during DSS treatment. The effect of different concentration of purslane leaves on colon length in dextran sulphate sodium induced colitis in mice. Colon length removed on the sacrifice day is depicted as mean± SEM in each group. Bars with the same letters are not significantly different at $p<0.05$. **(a)** $p< 0.05$ was compared to control DSS group.

3.4.5: Effects of purslane leaves on DSS-induced disease severity (DAI).

Administration of DSS significantly increased disease severity in terms of disease activity index, with its peak at day 18. Treatment with the three different concentrations of purslane did not have any significant reduction in DSS-induced disease activity. On the contrary, the disease activity rose up higher than that seen with DSS control group by the end of day 18.This was especially seen for the group with the lowest concentration of Portulaca (0.8% purslane) **(Fig. 20).**

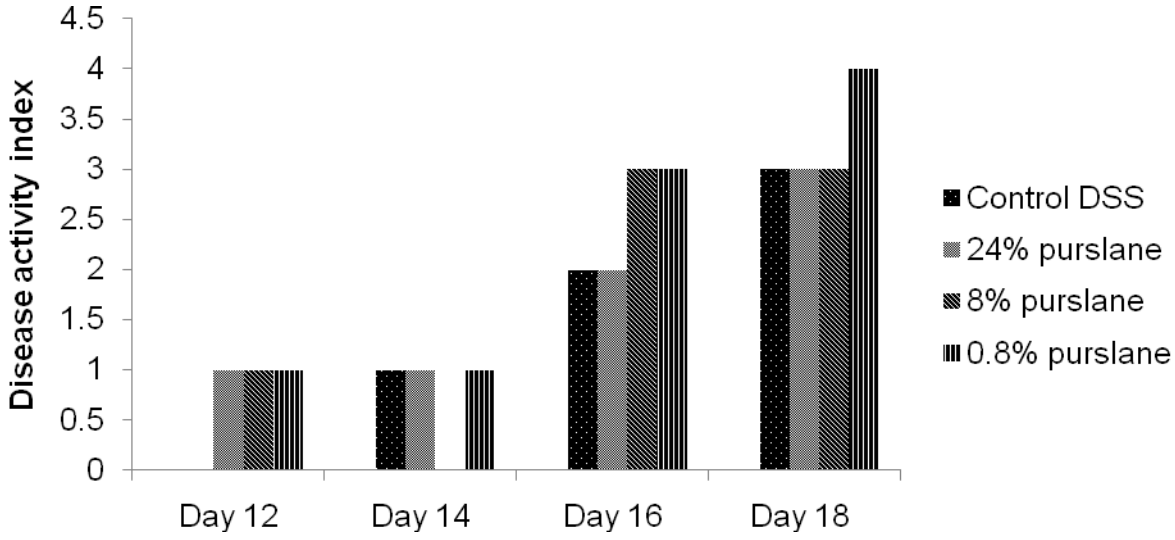


Fig. 20 Determination of the effect of different concentrations of *Portulaca* leaves on severity of disease measured in terms of disease activity index (DAI) in dextran sulphate sodium (DSS) induced colitis in mice.

3.4.6 Effect of Purslane leaves on DSS-induced histopathological changes in colon.

Administration of DSS resulted in significant histopathological changes in the colon as in terms of marked destruction of surface epithelium, cell disruption, and severe inflammatory lesions extensively through out the mucosa. On the contrary, normal control mouse colon sections showed intact epithelium, well defined crypt length with no edema, neutrophil infiltration in mucosa and sub mucosa, and no ulcers and erosions. Treatment with the different leave concentrations of purslane did not show any significant attenuation of DSS-induced histopathological changes in the colon but the groups which received 24% and 8% leaves did show some level of protectiveness with mild cellular infiltration **(Fig. 21).**

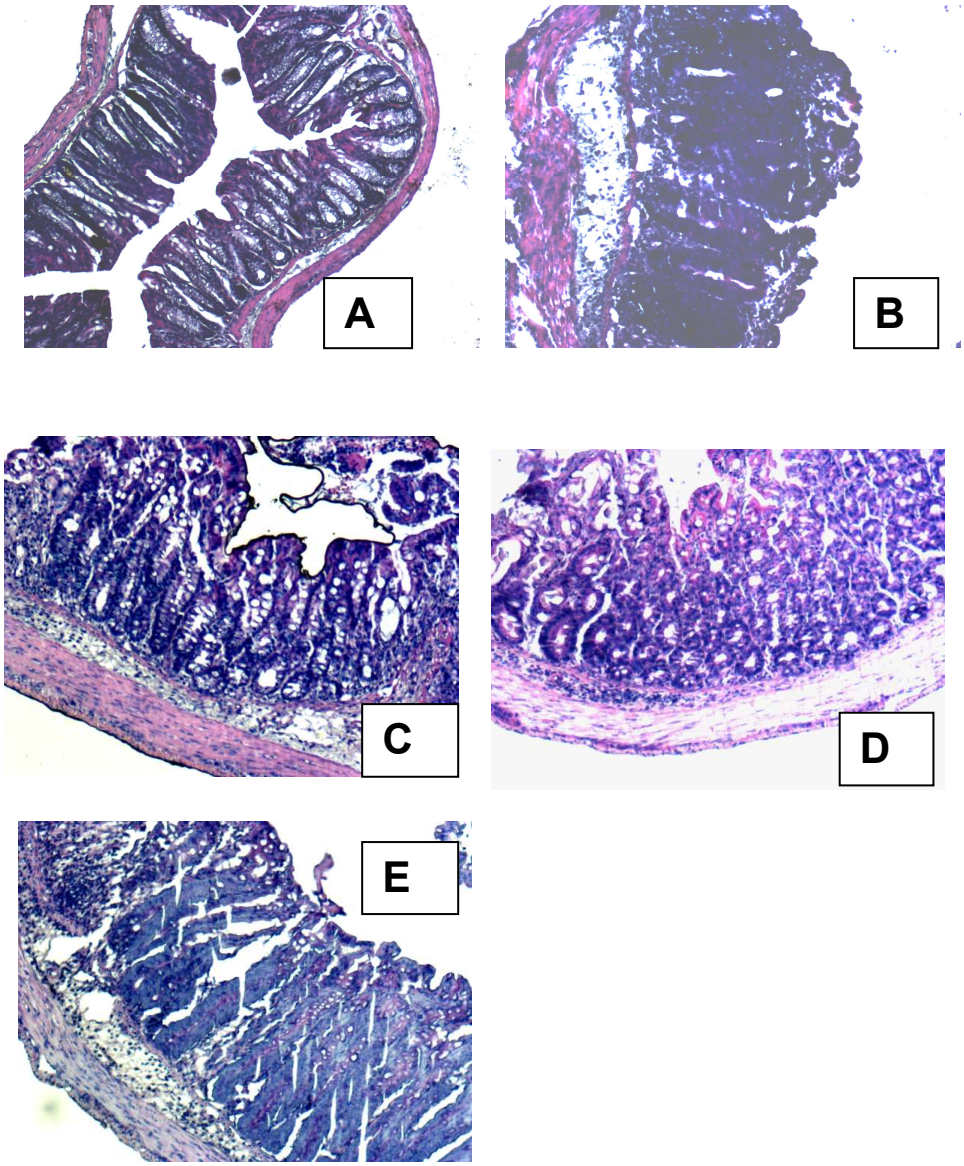


Fig. 21 Effect of purslane leaves on histological injury in DSS-induced colitis in colon. Hematoxylin and eosin staining (magnification x 100) of colonic mucosal tissue section from mice receiving water (**A,** normal control), dextran sulphate sodium **(B,** control DSS), *Portulaca* (**C,** 24% leaves), (**D**, 8% leaves), **(E**, 0.8% leaves). In normal control group (**A)**, the haematoxylin and eosin stained section of mice colon showed intact epithelial surface. In the control DSS group (**B)**, the colonic mucosa showed extensive necrotic destruction of epithelium with loss of crypts, sub mucosal oedema and also intense inflammatory infiltration in the layers. Treatment with all leaves concentrations of *Portulaca* did not exert a significant protective effect against DSS –induced damage.

3.4.7 Effects of purslane leaves on myeloperoxidase (MPO) activity.

We measured colonic MPO activity as an indicator of the extent of neutrophil infiltration into mucosa. Administration of DSS significantly increased MPO activity ($p<0.05$) in colonic tissue compared with the normal control group **(Fig. 22).** Treatment with *Portulaca* (24%, 8%, and 0.8%) leave concentrations again did not show a significant attenuation of the DSS-induced rise in colonic MPO activity in colonic tissue. As can be seen from the graph, the group which received 8% leave concentration did have a minimal reduction but was not statistically significant from the DSS control group.

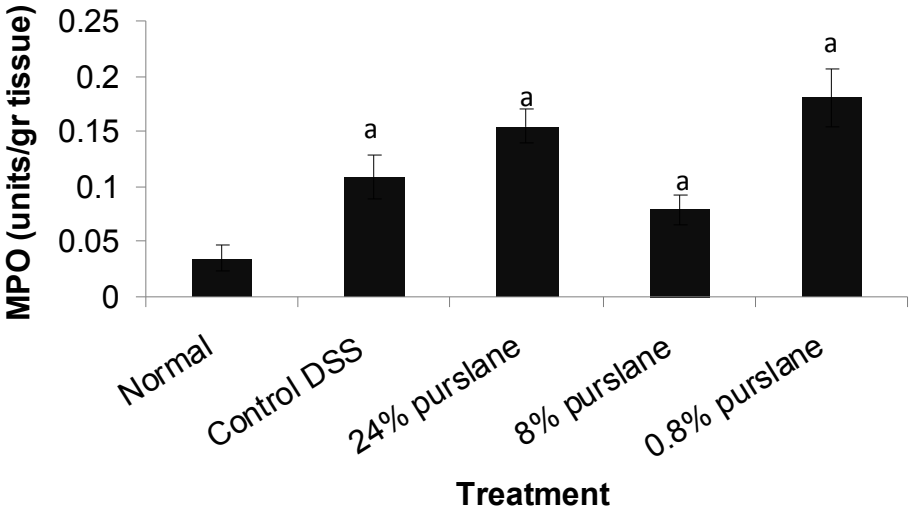


Fig. 22 Effects of different concentration of *Portulaca* leaves on colonic myeloperoxidase (MPO) levels in dextran sulphate sodium (DSS) induced colitis in mice. Results are expressed as mean ± SEM of five observations. Bars with the same letters are not significantly different at $p<0.05$. **(a)** $p < 0.05$ was compared to the control DSS group.

4.0 Discussion

In this study, we initially investigated the effects of temperature and day length on puslane microspecies looking at parameters such as organic acid and fatty acid accumulation as well its phenotypic evaluations. By this we then evaluated the ameliorative effect of these plants on an animal model of human colitis bearing in mind their high ALA content.

Temperature and day length had an effect on the plant height, leaf length, the number of plant internodes as well as the average length of internodes among the three microspecies examined. It also had a significant effect on the accumulation of organic acids in the leaves of *Portulaca* and also on the leaf concentration of essential fatty acids without altering its composition. Several studies have shown the effect of different growth conditions (light intensity, day length) as well as the effect of nutrient and stage of harvest on the accumulation of oxalic acid and α-linolenic acid in the plants (Palaniswamy et al., 2000; Palaniswamy et al., 2001; Palaniswamy et al., 2002; Palaniswamy et al., 2004; Fontana et al., 2006). Whereas others have shown purslane to be an excellent source of omega -3 fatty acids (Liu et al., 2000; Cros et al., 2007), our study indicates that temperature can affect the nutritional value of the harvested crops.

The complex of *Portulaca* consists of many microspecies as newly described by Danin et al.,(2008) some of which are new to science and whose taxonomic classification has not yet been eluded. So therefore, the microspecies of this complex differ in ploidy and in their seed coat characteristics (Danin et al., 1979; Danin and Reyes-Betancort, 2006).

In this study, we saw the effect of temperature and day length on three microspecies of the *Portulaca* complex in order to evaluate their agronomic potential (crop production) as well as the nutritional value of their leaves harvested at 8-true-leaf stage.

Our results for plant height measurements show that on the average, plants gained more height when cultivated in $34/28^0$C temperature condition in the long day regime and comparatively less height when cultivated in $16/10^0$C, short day (**Fig**. **4**). Since

the commercial green-leaf *P. sativa* subspecies grows in a more erect and decumbent form (Radhakrishnan et al., 2001), it grew taller and gained significantly 1.8 and 2.3-fold more height than *P. papillato-stellulata* and *P. nitida* in the long day regime at the 34/28^{0}C temperature condition. Comparing *P. sativa* in the two day length regimes at the same temperature, also showed an increase in height by 1.5-fold in the long day as compared to the short day buttressing the fact that purslane grew better and taller in long day conditions (Fontana et al., 2006). The average heights observed in *P. nitida* (30±3.8 and 37±3.6) and *P. papillato-stellulata* (37±1.5 and 29±4.3) in both long and short day respectively at 34/28^{0}C were in agreement with previous reports (Palaniswamy et al., 2000; 2004).

Purslane as an annual green herb has obovate to spathulate leave forms which are about 1-5cm long (Oliveira et al., 2009). From the results shown in **Fig.5**, the trends of leaf length measurements were similar to that of plant height. The results showed the commercial green –leaf *P. sativa* recording the greatest leaf length in both long (1.2, 1.1-fold) and short day (1.6, 1.3-fold) comparable to *P. nitida* and *P. papillato-stellulata* respectively at the 34/28^{0}C temperature condition. Relative to the 34/28^{0}C temperature condition, the *P. sativa* microspecies grown in 16/10^{0}C temperature condition caused a significant reduction in leaf length by 1.6 and 2-fold in the long and short day respectively (**Fig. 5**).This again goes to show that purslane is a plant which thrives and grows better at high temperatures in long day.

Temperature and day length also influenced the number of internodes the three plant microspecies had. Again, *P. sativa* gained significantly more internodes than *P. nitida* but this was comparatively similar to *P. papillato-stellulata* in the long day regime (**Fig. 6).** Statistically, *P. sativa* decreased significantly by 1.3-fold when grown in the short day regime relative to long day at the 34/28^{0}C temperature condition. This was not seen with *P. papillato-stellulata* and *P. nitida* as there was no significant difference among the two day lengths. Growing *P. sativa* in the temperature condition of 16/10^{0}C significantly decreased it by 4.9 and 4-fold in the long and short day respectively. This was expected and similar to the rest of the microspecies since at this temperature condition, plants failed to grow and mature.

Since the average length of the internodes measured were based on plant height and the number of internodes (height/number of internodes), the *P. sativa* microspecies grown in the coldest (16/10^{0}C) temperature condition in both day length regimes had the greatest length at the very first week of measurements obviously due to its relatively greater height and fewer internodes than *P. papillato-stellulata* and *P. nitida*. This however reduced by 1.6 and 1.9-fold in long and short day respectively as the plants reached week eight (**Fig.7**). However *P. sativa* plants grown in the warmest temperature conditions (34/28^{0}C) showed a significant increase in the length of internodes (1.6 and 2.3-fold) in the long and short day respectively by week eight relative to 16/10^{0}C temperature condition. Consequently *P. nitida* relatively had lengthier internodes in the short day than *P. papillato-stellulata* because of its fewer internodes and greater plant height measured at week eight for the 34/28^{0}C temperature condition. This was not the case in the long day since *P. papillato-stellulata* was relatively taller then *P. nitida*. *P. sativa* in the long and short day was significantly greater in length than *P. nitida* and *P. papillato-stellulata* at (34/28^{0}C) and it was also significantly reduced at the temperature (16/10^{0}C) by week eight. From this it can be said that the average length of plant internodes was largely influenced by the plant height and not the number of internodes.

The leaf and stem fresh and dry weights of the three purslane microspecies varied greatly with the different day/night temperatures as well as in the day length. The results show an increasing trend in both leaf and stem fresh weights as the temperature increased in both long and short day. This is in accordance with research done by Singh, 1973 who reported that purslane flushes much better in the summer (high temperatures) than the winter (cold temperatures). *P. sativa* was shown to be the one with highest leaf fresh weight at the 34/28^{0}C temperature condition as well as having more leaf fresh weight than *P. nitida* but comparable to *P. papillato-stellulata* in the 28-16^{0}C temperatures in both long and short day. However the dry weight measurements indicated no significant differences between *P. nitida* and *P. sativa* in the long day regime along all the temperature rooms. A similarly observation was noticed in the short day regime and this was not different from the stem measurements too (**Fig. 8 and 9**). This goes to show potential

agronomic traits of the local species (*P. nitida*) when cultivated. Again the highest net plant fresh weight was also seen to favour *P. sativa* (140.8g) at the warmest temperature condition (34/28^{0}C) due to its thick and broad stem characteristics whiles being comparable to *P. papillato-stellulata* and significantly different from *P. nitida* as the temperature decreases in the long day regime (**Fig. 10**). In the short day however, *P. nitida* and *P. papillato-stellulata* show similar trend. In general, plants (*P. sativa*, *P. nitida*, *P. papillato-stellulata*) net weight decreased as the temperature decreased and also had relatively more biomass in the short day conditions than the long day regime with the exception of *P. nitida* which did not show any significant change in fresh weight as the temperature decreased and also with day length. This goes to show the high adaptation of the commercial green-leaf *P. sativa* and *P. papillato-stellulata* which have similar growth patterns (Szalai et al., submitted) to the warm summer (long day) and high temperature conditions of tropical countries and less adaptation in the temperate regions as opposed *P. nitida* but a similar adaptation of *P. papillato-stellulata* and *P. nitida* in the short day regime still concluding that purslane grows much better in the long day, high temperature conditions as opposed the short day, cold temperature conditions. The dry weighs measurements too showed similar results as above.

All plants of the genus are known to be persistent and succulent due to their perceived high moisture content (Ezekwe et al., 1999). Various reports have shown that leaves contain moisture that range between 78.5 to 90.3% (Ezekwe et al., 1999) and 93.0-97.3% (Oliveira et al., 2009). But the values of moisture obtained in this experiment were lower than the ones mentioned above (**see Fig.11**). The *P. nitida* subspecies reported the highest percentage moisture content at 28/22^{0}C in both long and short day. This was significantly different from *P.papillato-stellulata* but comparative to *P. sativa* when plants were cultivated in the 28/22^{0}C temperature condition in the long and (34-28^{0}C) temperature condition in the short day. This also goes to support its similarity statistically, with *P. sativa* in terms of leaf and stem fresh weight at the same temperature condition in both long day and short day (**Fig. 8 and 9).** By means of comparison, *P. nitida* tends to show similar traits with the commercial green-leaf *P. sativa* in terms of moisture and dry weight content which is perceived to be associated with a high amount of total number of leaves, thin stems

and fewer nodes it produces as compared to *P. sativa* which has fewer but broader leaves. This is also evident with the percentage leaf dry weight as a measure of the total (net) fresh weight of the plants (**Fig. 12**). In conclusion, *P. nitida* shows similar agronomical potentials with *P. sativa* (leaf and stem fresh and dry weights, high moisture content, % leaf dry weight as a measure of plant fresh weight) when grown in the warmer temperature conditions (Fontana et al.,2006) in both long and short day regimes. However, the plants show relatively better growth in the long day conditions than the short day as shown in the figures above.

Oxalic acid is a strong dicarboxylic acid used extensively in industrial and household applications (Guo et al., 2005). It also doubles as a metal chelator inhibiting the bioavailability of iron and calcium in our diets when consumed in extensively high quantities together with food containing good amounts of such nutrients (Caliskan, 2000; Palaniswamy et al., 2002). This has developed so much concern in human diet because of the negative health effects which comes with it. Its presence and distribution in various organisms including plants, fungi have been enormously reported and in plants, higher concentrations are found in the leaves. It is also well established that plants are capable of metabolising oxalate by sensing changes in concentrations under certain conditions; (one may be to balance the normally occurring anions in plants). This has also been shown not to affect the normal plant growth or cause toxicity to the plants (Palaniswamy et al., 2004; Guo et al., 2005). A report by Caliskan, (2000), shows that oxalate contents in plants may vary according to their age of harvest and also the season or climate (long day or short day). In this experiment, plants were harvested at the stage when they had 8-true leaves during actively growing stage. The results indicate that oxalic acid accumulation, increased at $16/10^0$C temperature conditions in both long (1.1 and 1-fold respectively) and short day (1.1 and 1-fold respectively) relative to $34/28^0$C for the *P. sativa* and *P. papillato-stellulata* microspecies and not the *P. nitida* (**Fig. 13**). Earlier reports by Palaniswamy et al., (2004) have shown a positive correlation with increased light (long day) and the formation of oxalic acid in rhubarb and buck wheat but this was not the case with purslane when comparing the two day lengths. *P. nitida* rather decreased its oxalic acid concentration as the temperature decreased and this was more evident in the $16/10^0$C temperature condition. In this study, the oxalic acid

concentrations were high and this could be due to the fact that plants were harvested at an early developmental stage during active growth and during which many researchers have reported elevated amounts of oxalic acid in leave sample (Palaniswamy et al., 2004). This goes to suggest that purslane is better off in terms of lower oxalic acids when harvested at a more matured age (16 true-leaf stage) as recommended by previous researches and also when grown in high temperature conditions especially for the commercial green-leaf *P. sativa* and *P. papillato-stellulata* and not *P. nitida* which should be grown in the coldest temperature condition (16/10^{0}C) to minimise oxalic acid concentration irrespective of the day length.

Ascorbic acid as an antioxidant against oxidative damage has been suggested by several other researches as a precursor for the formation of oxalic acids in plants (which works in tolerating heavy metal toxicity in plants) and its conversion has been observed in a number of species (Loewus, 1999; Keates et al., 2000; Weir et al., 2006). However some other studies think otherwise (Palaniswamy et al., 2004). With the results shown in **Fig.14**, it can be seen that ascorbic acid concentrations increased significantly at temperatures (34-22^{0}C) for all the three microspecies in both long and short day which is in agreement with earlier reports by Guo et al., (2005) and Weir et al., (2006). This however decreased significantly in plants (*P. nitida* especially) grown in the 16/10^{0}C temperature condition in both long and short day which is in line with results obtained from the oxalic acid measurements. The commercial green-leaf *P. sativa* in the short day regime also did not show any change in concentration at 16/10^{0}C relative to 34/28^{0}C (**Fig.13).** These observations may well be due to technical lapses during the experiment. The above gathered information makes *P. nitida* a better plant over *P. sativa* and *P. papillato-stellulata* in terms of lowering its oxalic acid content when grown in the 16/10^{0}C temperature condition and not 34-22^{0}C irrespective of the day length.

Another parameter looked at is the composition and concentration of various fatty acids as a function of temperature and day length. Whereas various studies have shown that purslane is an excellent source of omega-3 fatty acids (Liu et al., 2000; Cros et al., 2007), it is evident from our study that fatty acid concentrations(%) in purslane leaves vary with the temperature condition but shows no significant

differences with day length among the *P. nitida* and *P. papillato-stellulata* microspecies but with *P. sativa* in the short day regime (**Tables 7 and 8**). Our results indicate that by far the highest total fatty acid content obtained was with plants grown in the (34-22^{0}C) temperature condition with the lowest values being obtained in 16/10^{0}C (long and short day). The values obtained in the warmer temperature conditions (long day, short day) irrespective of the microspecies were significantly higher than that recorded by Oliveira et al., (2009) for common purslane plants. These differences may have been the results of differences in plant microspecies as well as the stage of harvest of the plants. Interestingly, there were also no significant differences in fatty acids contents among the three microspecies in warmer temperature conditions (34-22^{0}C). This is very interesting because FW and DW measurements indicated comparably higher values for *P. sativa* relative to *P. nitida* and *P. papillato-stellulata* (**Fig. 8 and 10)** equating possible higher fatty acid content for *P. sativa* as compared to the rest but this is not so as shown by the results. However, fatty acids contents were lower in the coldest room (16/10^{0}C) among *P. nitida* and *P. papillato-stellulata* in the short day and all three in the long day as shown by the results. The values of *P. sativa* (99.32%) in the short day (16/10^{0}C) were comparable to that in the warmer temperature. This observation with *P. sativa* in the short day at the 16/10^{0}C temperature condition is quite consistent with a remark made by Ezekwe et al., (1999) who suggested that the level of antioxidants in purslane leaves may have a contribution to the mechanism of accumulation of essential fatty acids considering the fact that *P. sativa* showed a significantly high ascorbic acid concentration in the short day regime at the 16/10^{0}C temperature condition (**Fig. 14**). Regarding the major fatty acids identified, the most abundant was ALA, and followed by palmitic acid then linoleic acid and this was evident over the four temperature conditions and also the day length. Values for ALA were comparable to that reported by Liu et al., (2000) but higher than that from Oliveira et al., (2009) in leave samples (**Fig. 15**). Linoleic acid was however within the range of 5-18%, similar to values of Oliveira et al., (2009) whiles palmitic acid was lower. These differences as mentioned before may well be the result of differences in plant microspecies, sample material or sample procedures. Diets rich in PUEFA especially omega- 3 fatty acids are of great concern to modern day nutrition considering the health benefits associated with their consumption. Hence,

the identification of omega-3 in purslane has come as an addition to restoring the balance of nutrients in human diets. We could not however identify the longer chain omega-3 fatty acids, EPA and DHA in any of the plant samples grown in the various temperature conditions which is in agreement with other studies (Liu et al., 2000; Palaniswamy et al., 2001) since these fatty acids are exclusively confined to fish, fish oil and other lower plants (mosses algae and ferns).

In conclusion, purslane microspecies grown in different temperature conditions and day length showed significant differences in crop production (height, leaf length, number of internodes, average length of internodes, leaf and stem fresh and dry weights, net plant fresh and dry weights, percentage moisture content and percentage leaf DW in plant fresh weight). The commercial green-leaf *P. sativa* and *P. papillato-stellulata* grew taller, had broader leaves and had higher fresh plant biomass relative to *P. nitida* when grown in the warmer temperature conditions in both day lengths. However dry weight measurements and moisture contents showed higher values for *P. nitida* in the warmer temperature rooms which suggest potential agronomic traits for this microspecies. Significant trends were found for ascorbic acid indicating a favourable accumulation in the warmer temperature conditions (34-22^{0}C) and a lower accumulation in (16/10^{0}C). Day length did not have any effect on the oxalic acid concentration but temperature did. *P. sativa* and *P. papillato-stellulata* but not *P. nitida* were seen to be high in the 16/10^{0}C in both day length regimes for oxalic acid. ALA and LA also significantly decreased as the temperature decreased irrespective of the microspecies and the day length. Thus decreasing the temperature (16/10^{0}C) without much influence of day length reduces the nutritional quality and crop production of purslane. The optimal temperature condition might be between (34-22^{0}C).

Ameliorative effect of plant omega3 fatty acid on DSS-induced animal model of colitis.

With this part of the experiment, three groups of five C57BL/6J mice were fed different concentrations of purslane followed by the administration of 3.5 % DSS in drinking water for 8 days to induce colitis and compared to respective negative and positive control groups of five mice each.

The mechanism of ulcerative colitis (UC) as a human inflammatory condition of the colon is still poorly understood, but evidence suggest the possible increase in proinflammatory cytokines as well as arachidonic acid -derived eicosaniods and other inflammatory agents as contributing to the pathogenesis of UC (Barbosa et al., 2003; Calder, 2006; Zhang et al., 2009). Therefore, extensive studies have been conducted on the beneficial role omega 3 fatty acids (EPA and DHA especially) may play in the management of this disease condition since these fatty acids have been established by many animal and clinical research to have anti inflammatory effects used to ameliorate UC symptoms [Belluzzi et al., 2000; Simopoulos, (2002**a**, 2006); Calder,(2006,2008)] since current drugs used (5-aminosalicylic acid, glucocorticiods) are not devoid of severe undesirable side effects. The results for many of these studies on UC however show weak evidence (Calder, 2006). Relatively fewer studies have been conducted on the role of plant-derived omega -3 fatty acids (ALA) on inflammatory conditions. Its conversion however to the longer chain fatty acids is possible in humans and animals but modest and inefficient if factors such as the presence of high omega 6 fatty acids (which compete for the same desaturase enzyme), the high ratio of LA to ALA, and also the presence of other trans fatty acids and saturated fatty acids (Caughey et al., 1999; Crawford et al., 2000; Simopoulos, 2002**b,** Maroon et al., 2006) are present in the diet. Even with the experiments which show positive and conclusive results, it is even still unclear whether these effects seen are due to ALA itself or its subsequent conversion to the longer chain derivatives since naturally, the latter fatty acids are more potent and biologically more active then ALA (Simopoulos, 2002**b**; Calder, 2008).

In the present study mice fed with different concentrations of purslane (ALA) as described above showed different concentrations of the longer chain DHA and not

EPA in their plasma and this was significant from the normal control group for the 24%-fed group **(Fig.16**). The presence of DHA and not EPA in the plasma is in contrast to other investigations which suggest that feeding plant-derived omega 3 fatty acids resulted in an increase in EPA and not DHA (Caughey et al., 1996; De Groote et al., 2008). Also, the presence of other saturated fatty acids (C16:0, C18:0) was high in purslane fed group as compared to control group (**Table 9**) which may have resulted to its inefficient conversion.

The DSS model of human colitis is a simple method with high degree of uniformity and reproducibility of colonic lesions manifested by conditions such as bloody diarrhea, weight loss, shortening of the colon (Clapper et al., 2007; Yan et al., 2009). It can be seen from the results that the four groups of mice who received DSS for eight days including the normal group lost weight relative to the start of the experiment (**Fig. 17**). This case of weight loss may probably be due to changes in feeding regimes as well as aging of the mice. This was however more evident in the purslane –fed groups (23% weight lose on the average) (**Fig. 18**) who were also seen to suffer severe diarrhea, weakness of the fore and hind limbs (which progressed to an inability of the animals to stand and move) and as well as chronic bleeding in the stool (data not shown). This may have been due to the low ingestion of food following DSS induction and also the ingestion of other compounds (e.g organic acids, coumarins, alkaloids, anthraquinones) in purslane (Obied et al., 2003). Coumarins as reported for oxalic acids are found in high concentrations in many plant species. As a rodenticide, mice and other rodents consuming it largely metabolize it to 3, 4-coumarin-epoxide, a toxic compound that can cause internal hemorrhage and eventually death. It has also been shown to be an appetite suppressing compound. These therefore suggest the low body weight and chronic bleeding seen in the purslane –fed groups and also the death of one mouse in both the 24% and 8% fed groups. Current research on the role of omega 3 fatty acids on bleeding also shows that omega 3 fatty acids decreases platelet aggregation, it is anti-thrombotic, reduces blood viscosity and influences on clotting factors (Abeywardena et al., 2001; Irish et al., 2009). Hence its association with the ulceration caused by DSS may have resulted in the profuse bleeding seen among the purslane- fed groups.

Experimentally, colitis is characterised macroscopically by mucosal edema, bleeding, erosion ulceration and tissue necrosis whiles histopathologicaly, the disease is associated with mucosal damage, cell disruption, ulceration and inflammation (Wallace et al., 1989). Again in this study, administration of DSS resulted in the development of colitis as assessed above plus disease activity index. However none of the three treatments with purslane (24%, 8% and 0.8%) was able to significantly attenuate the DSS induced macroscopic and histopathological alteration in the colon (**Fig.19 and 21**) along with reduction in disease activity (**Fig.20**). However mild protectiveness was seen among 24% and 8% purslane –fed groups as shown histologically but knowledge about this is unclear. The observed 'non performance' of purslane in ameliorating the disease condition may also be due to the fact that low doses of the plant ALA were used (800 mg/g/d in 6 g or 24% leaves, 2 g or 8% leaves and 0.2 g or 0.8% leaves) considering the fact that ALA is a less potent omega 3 fatty acid as compared to EPA. Simopoulos, (1999) indicated that 3.7 g ALA will appear to have biological effects similar to those of 0.3 g long chain omega-3 PUEFA with the conversion factor of 11 g ALA to 1g long chain omega-3 PUEFA. Calder, 2006 and Calder et al., 2009 also did indicate that >10 g/d ALA is required for anti-inflammatory effects to be seen and that the conversion of ALA to the longer chain derivatives was hindered by the presence of omega-6 and other saturated fatty acids (Simopoulos, 1999; Calder (2006, 2008)) which has been shown to be quite high in the plant (**Table 9**). Also the presence of other compounds as already mentioned which may be toxic according to research cannot be ruled out (Fontana et al., 2006).

MPO is an enzyme released from neutrophils and has been as an index of inflammation (Bradley et al., 1982; Wallace et al., 1989). Administering DSS was associated with significant rise in the colonic MPO activity indicating acute inflammation. Again feeding with various concentrations of purslane did not show any significant attenuation of colonic MPO activity associated with ulceration possibly due to the above mentioned reasons (**Fig. 22**).

In conclusion, even though purslane is a rich source of omega- 3 fatty acids as documented by research (Palaniswamy et al., 2001; Liu et al., 2000; Simopoulos, 2002**c**) and having a good omega -3 / omega 6 ratio, its ameliorative effects in this

experiment of DSS-induced experimental colitis is not encouraging possibly due to the fact that the n-3 rich fraction was not isolated and purified from the rest of the other 'unimportant' compounds of the plant and whose association with the induced inflammation worsens it instead of ameliorating it or may be the quantities used were not enough. In addition, purslane administration did not cause significant concentrations of EPA levels in serum indicating that conversion of omega 3 fatty acids (α-linolenic acid) is not transformed readily by the desaturase enzyme in the body. It is possible that EPA and not DHA are responsible for beneficial effects exerted by omega 3 fatty acids in acute IBD.

Overall conclusion

Research on the Portulaca complex show that the various microspecies yet examined is different in terms of their seed coat characteristics and also in their morphology. In this experiment, it was evident again that these microspecies were different as their agronomic traits (plant height, leaf length, fresh and dry weights) were seen differ with the effect of the four temperature regimes. However no much difference was established among the microspecies in terms of fatty acids and ascorbic acid with respect to temperature. Nevertheless, plants grown in the warmer temperature conditions showed higher fatty acid and ascorbic acid concentrations. Also the oxalic acid measurements showed that plants gained higher concentrations when grown in the cold temperatures especially for the commercial green-leaf *P. sativa* and *P. papillato-stellulata.* Attenuating colitis in this experiment with purslane was not successful probably due to the ingestion of other compounds in purslane in higher amounts which may be detrimental to the ulceration already induced by DSS. Isolation and purification of the omega-3 component of the plant may be helpful in using this plant as a preventive therapy for UC.

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Appendix 1

Three *Portulaca* microspecies (*P.sativa, P.nitida, P.papillato-stellulata*) grown in four different temperature conditions and day length regimes (Phytotron experiment, Hebrew University of Jerusalem, 2008)

אבסטרקט

רגלת הגינה (*Portulaca oleracea*) ידועה כגידול ירק למאכל וכמקור עשיר לחומרי טבע כמו חומצת לינולאית, חומצה אלפא לינולנית, אנטיאוקסידנטים וויטמינים החיוניים לגדילה, התפתחות ומניעת מחלות. זאת בנוסף ליתרונה החקלאי הנובע מקצב צמיחתה המהיר והיכולת לגדלה במשך תקופה ארוכה בשנה.

מחקרים רבים הראו את האפקט של הזנת הצמח על היבול והערך התזונתי שלו, אך מעט מאוד נלמד על הטמפרטורה ואורך היום המתאימים הנדרשים לשיפור פוטנציאל היבול ואופטימיזציה של הערך התזונתי, דהיינו רמה גבוהה של חומצות שומן בלתי רוויות וחומצה אסקורבית ופחות חומצה אוקסאלית שהיא המזיקה לבריאות.

לפיכך, מטרת המחקר הנוכחי הייתה לבחון את השפעת הטמפרטורה ומשטר אורך היום על היבול הצמחי וריכוז חומצות השומן והחומצות האורגניות בעלי רגלת הגינה. לשם כך נבחנו שלושה מיקרו-מינים של רגלת הגינה: *P .sativa*, *P .nitida*, *P. papillato-stellulata* . הצמחים גודלו בתנאים מבוקרים בארבעה משטרי טמפרטורה (16/10 ,22/16 ,28/22 ,34/28 מ"צ לילה/יום) כ"א ביום ארוך (LD) או יום קצר (SD) במשך 8 שבועות.

במשך הניסוי נמדדו וחושבו המדדים הבאים: גובה, אורך העלה הגדול ביותר, מספר הפרקים בענף הארוך ביותר, משקל טרי ויבש, תכולת החומר היבש ואחוז העלים היבשים מכלל משקל הצמח. בנוסף, בשלב של 8 עלים אמיתיים נדגמו ענפים לצורך אנאליזה של תכולת חומצות השומן והחומצות האורגניות.

נמצא שמשקל הצמח, אורך העלה, מספר הפרקים, אורך הפרק הממוצע בענף והיבול הטרי והיבש בכל מיקרו ירדו המינים שנבחנו עם ירידת הטמפרטורה בטווח שבין 16-34 מ"צ בשני משטרי אורך היום.

מיקרו-המין *P. sativa* , שהוא הזן המסחרי, הניב את הערכים הגבוהים ביותר של כל המדדים הנ"ל, חוץ מיבול עלים יבשים ותכולת החומר היבש בעלים. במדדים אלה *P. nitida* היה דומה ל- *P.sativa* בעיקר בטווח הטמפרטורות 22-34 מ"צ. בכל הצמחים חוץ מ- *P. nitida* היתה עלייה מובהקת ברמת החומצה האוקסאלית ככל שירדה הטמפרטורה עד 16/10 מ"צ לילה/יום. כמו כן, מגמת ירידה בתכולת החומצה האסקורבית וחומצות השומן העיקריות שנמדדו (ח. אלפא לינולנית, ח. לינולאית, וח. פלמיטית) עם ירידת הטמפרטורה במיוחד ב- *P. nitida*.

עם זאת, לא היו הבדלים מובהקים בתכולת חומצות השומן העיקריות בין מיקרו המינים בטמפרטורה 34/22 לילה/יום מ"צ השני משטרי אורך היום. לפי התוצאות, אם כך, יש מגמת ירידה בצימוח והערכים התזונתיים בכל מיקרו-המינים שנבחנו עם ירידת הטמפרטורה, עד כדי מוות בתנאי 16/10 מ"צ יום/לילה. בנוסף, התוצאות מצביעות על פוטנציאל חקלאי למיקרו המין המקומי- *P. nitida* .

מטרת מחקר נוספת הייתה לחקור את השפעתה המשפרת של רגלת הגינה על מודל של מחלת מעי-קוליטיס-בעכברי מעבדה. מחלת הקוליטיס הושרתה בנקבות מזן *C57BL/6* באמצעות חשיפתן ל-3.5% *DSS* (דקסטרן-סולפט סודיום) במי השתייה למשך שמונה ימים. המחקר כלל חמש קבוצות של עכברות : 3 קבוצות מהן קיבלו במזון 24% של עלים טחונים של רגלת הגינה אשר הכילו 4800 מ"ג *ALA* (סה"כ 6 גרם), 8% של עלים טחונים אשר הכילו 1600 מ"ג *ALA* (סה"כ 2 גרם), ו 0.8% של עלים טחונים אשר הכילו 160 מ"ג *ALA* (סה"כ 0.2 גרם). המזון האבקתי המעורב עם העלים ניתן בהזנה חופשית*AD LIBITUM* במשך 10 ימים לפני מתן *DSS*, ועד לסוף הניסוי. *DSS* גם נכלל בקבוצת ביקורת אחת אשר ניזונה בדיאטה רגילה. בסוף הניסוי העכברות הוקרבו, והנזק במעי נמדד היסטולוגית וביוכימית. נקבעו רמות של חומצות שומן בפלסמה, אורך המעי, אינדקס פעילות המחלה(*DAI*)) ו *MPO*. מתן של רגלת הגינה גרם לעליה מובהקת בכמות ה-*DHA* בפלסמה בקבוצה שקבלה 24% אך לא 8% או 0.8% כמנוגד לקבוצת הביקורת. *EPA* לא נמצאה באף אחת מהקבוצות. מתן של *DSS* גרם להתפתחות משמעותית של כיב במעי ,לעליה בפעילות המחלה ועליה ברמת *MPO*. מתן אבקה של עלי רגלת גינה מיובשים ברכוזים שונים במזון לא גרם לירידה בחומרת דלקת המעי שהושרתה ע"י *DSS* וגם לא ב-*MPO* ו-*DAI*. בבדיקות היסטולוגיות נראתה הגנה מתונה בקבוצות שקיבלו במזון 24% ו 8% רגלת הגינה. לא ברור מדוע הגנה דומה לא נצפתה בבדיקות הביוכימיות. חוסר היכולת של עלי רגלת הגינה למתן את ההתכייבות שנגרמה ע"י *DSS* יכולים להיות מוסברים ע"י כך שהחיות עיכלו חומרים אורגניים אחרים (חומצות אורגניות, קומרינים, אלקלואידים, אנתראקינונים) בעלי השפעות טוקסיות ברכוזים גבוהים, רכוזים נמוכים של *ALA* שהשתמשו בהם או אי הפיכה של *ALA* ל-*EPA*. המסקנה היא שעלי רגלת הגינה יכולים להיות גורם חדשני במזון למניעת קוליטיס שהושרה בחיות מעבדה רק אם רכיבי אומגה 3 מבודדים ומנוקים מחומרים אורניים נלווים. דרוש מחקר נוסף בצמח זה על מנת לאמוד את ההשפעות התרפויטיות שלו על מחלת קוליטיס המעי.

עבודה זו נעשתה בהדרכתם של:

ד"ר נתיב דודאי, מנהל המחקר החקלאי, מרכז מחקר נווה יער

פרופ' בטי שוורץ, המכון לביוכימיה מדעי המזון והתזונה

השפעת הטמפרטורה ואורך היום על התכולה וההרכב של חומצות שומן וחומצות אורגניות בעלים של רגלת הגינה (*Portulaca oleracea*)

השפעת הזנת עכברים בעלים האלה על דלקת מעיים מושרת בעכברים.

עבודת גמר

מוגשת לפקולטה לחקלאות, מזון וסביבה

ע"ש רוברט ה. סמית' האוניברסיטה העברית בירושלים

לשם קבלת תואר "מוסמך" במדעי התזונה

מאת

חנה אודורו

טבת תש"ע רחובות דצמבר 2009