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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

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African Journal of Plant Science

Review

Scope of medicinal flora as effective anti ulcer agents

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Gastric ulcer generally results from persistent erosions and damage of the stomach wall which become perforated and develops into peritonitis and massive haemorrhage as a result of inhibition of the synthesis of mucus, bicarbonate and prostaglandins. The commercially available antiulcer drugs usually have various side effects. Due to these side effects, there is a need to find new antiulcerogenic compound(s) with potentially less or no side effects and medicinal plants have always been the main source of new drugs for the treatment of gastric ulcer. Several hundred plant genera are used medicinally mainly in the form of herbal preparation in indigenous system of medicine in different countries for the treatment of ulcers. In this review, we have given a detailed description of ulcer and its causative factors along with medicinal plants with antiulcer potency.

Key words: *Helicobacter pylori*, Meckel's Diverticulum ulcer, gastroesophageal reflux disease, peptic ulcer disease.

INTRODUCTION

The human stomach contains enumerable muscles that carry out the process of digestion and switch the different forms of food into digestive fluids which are pepsin and hydrochloric acid. These fluids make the food digest in the stomach. In order to attain this several organs integrate with each other which includes the central nervous system (CNS) and hormonal systems. The ulcer in the stomach may be the result of the disparity in the digestive fluids (Hoogerwerf, 2001). The over production of pepsin or hydrochloric acid may harm the line up of the stomach and cause ulcers in the stomach. Every year 4 million people are diagnosed with this disease. An estimated 6000 people die every year because of the complications associated with stomach ulcer. 40, 000 people undergo surgery in order to get relief from the persistent symptoms of ulcer annually. An estimated 15,000 deaths occur as consequence of PUD. Indian pharmaceutical industry antacid and anti ulcer drugs share 6.2 billion rupees and occupy 4.3% in the market share (Jaikumar, 2010). Two main approaches for treating peptic ulcer include: reducing the production of gastric fluid and re-enforcing gastric mucosal protection (Valle, 2005).

Ulcers are defined as a breach in the mucosa of the alimentary tract, which extends through the muscularis mucosa into the submucosa or deeper (mayoclinic.com, 2011). Ulcus pepticum or PUD or peptic ulcer disease, is a chronic and most often solitary lesion that can be defined as mucosal erosions which is equal to or greater than 0.5 cm of an area of the gastrointestinal tract (mainly stomach and duodenum) which is usually acidic and extremely painful (Vakil, 2010; Chan, 2010).

Types of ulcers include, gastric ulcer (stomach), duodenal ulcer (duodenum), oesophageal ulcer (oesophagus) and meckel's diverticulum ulcer (meckel's diverticulum) (Podein, 2007).

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Signs and symptoms

Abdominal pain, naturally epigastric with severity relating to mealtimes is usually after 1 h of taking a meal. Duodenal ulcers are classically relieved by food, while gastric ulcers are exacerbated by it; bloating and abdominal fullness; water brash (rush of saliva after an episode of regurgitation to dilute the acid in oesophagus); nausea, copious vomiting; loss of apetite and weight loss; hematemesis (vomiting of blood); melena (tarry, foul-smelling feces due to oxidized iron from hemoglobin); heart burn, gastroesophageal reflux disease (GERD) and use of certain forms of medication can raise the suspicion of peptic ulcer; and sudden increase in the abdominal pain or sharpness in the quality of the pain; vomiting blood or material that looks like coffee grounds; blood in stool or black, tarry stools (pharmacology2000.com, 2011) .

A gastric ulcer will give epigastric pain during the meal as gastric acid is secreted, or after the meal, as the alkaline duodenal contents reflux into the stomach. Symptoms of duodenal ulcers will manifest mostly before the meal-when acid production stimulated by hunger is passed into the duodenum. This is not considered as reliable sign in clinical practice (Berroteran, 2002; Medina, 2010).

Helicobacter pylori

Helicobacter pylori was discovered in 1982 by two Australian Scientists, J. Robin Warren and Barry J. Marshall as a causative factor of ulcers. They showed that most stomach ulcers and gastritis were caused by colonization with this bacterium and not by stress or spicy food as had been assumed before. *H. pylori* is a spiral shaped Gram negative bacterium that lives in the acidic environment of the stomach. It is found in stomach along with acid secretion and can damage the tissue of the stomach and duodenum, causing inflammation and ulcers (Tiwari, 2005). *H. pylori* is believed to be transmitted from person to person through the oral cavity. The hypothesis that the mouth is a reservoir for *H. pylori* and a potential source of gastric infection is strengthened by several reports of *H. pylori* DNA in the saliva and dental plaque (Ahmed, 2006; Li, 1995; Olivier, 2006).

Other cause of ulcers is notably the gastric cancer. This especially occurs in ulcers of the greater curvature of the stomach in which most of them are a consequence of chronic *H. pylori* infection (Gloria, 2011; Saluja, 2002; Satoskar, 2007). It has been reported that *H. pylori* infection is a common problem in diabetic patients who have inadequate metabolic control where these micro organisms colonize the gastric antrum (Bikha, 2010). As diabetes and *H. pylori* are considered as major causes for dyspepsia, the incidence of *H. pylori* infection is higher in diabetic patients than in normal individuals (Roger and Walker,2005).

Acid and pepsin

Powerful digestive fluids are alleged to contribute to the formation of ulcers. The stomach can protect itself from these fluids in several ways. These are produced as lubricant like mucus that coats the stomach and shields stomach tissues. They produce bicarbonate that neutralizes digestive fluids and breaks them down into less harmful substances.

NSAIDs

The gastric mucosa protects itself from gastric acid with a layer of mucus, the secretion of which is stimulated by certain prostaglandins. NSAIDs block the function of cyclooxygenase1, which is essential for the production of prostaglandins. NSAIDs can make the stomach vulnerable to the harmful effects of acid and pepsin by interfering with the stomach‘s ability to produce mucus and bicarbonate.

Smoking

Tobacco smoking leads to atherosclerosis and vascular spasms, causing vascular insufficiency and promoting the development of ulcers through ischemia. Nicotine contained in cigarettes can increase parasympathetic nerve activity to the GIT by acting on the nicotinic receptors at synapses– increase stimulation to the enterochromaffin like cells and G cells increases the amount of histamine and gastrin secreted, and therefore increases acidity of gastric juice (Tiwari, 2005).

Caffeine

Beverages and foods that contain caffeine can stimulate acid secretion in the stomach. This aggravates an existing ulcer, but the stimulation of stomach acid cannot be attributed solely to caffeine.

Alcohol

Heavy consumption of alcohol causes liver cirrhosis. Ulcers are found in people with liver cirrhosis.

Stress

Emotional stress do not cause ulcers, but people who are experiencing this often report increased pain in existing

ulcers. Physical stress cause increase in risk of developing ulcers, especially in stomach. Examples of physical stress that can lead to ulcers are that suffered by people with injuries such as severe burns and people undergoing major surgery (Berroteran, 2002; Medina, 2010; Friedman,1998).

MECHANISM INVOLVED IN ULCERATION

Peptic ulcers are produced by an imbalance between the gastro duodenal mucosal defense mechanisms and damaging forces. Gastric acid and pepsin are requisite for all peptic ulcerations. Hyperacidity is not a prerequisite because only a minority of patients with duodenal ulcers has hyperacidity and is even less common in those with gastric ulcers (dyspepsy.com, 2011). Many bacteria have been found in the mucus, which was continuously secreted by mucus cells and removed on the luminal side. *H. pylori* is a bacterium, has flagella and moves through the stomach lumen and drills into the mucoid lining of the stomach. To avoid entry into the lumen, *H. pylori* senses the pH gradient within the mucus layer by chemotaxis and swims away from the acidic contents of the lumen towards the more neutral pH environment of the epithelial cell surface. *H. pylori* is also found on the inner surface of the stomach epithelial cells and occasionally inside epithelial cells. It produces cytotoxin associated gene A proteins (cag A) and vacuolating cytotoxins such as vacA, which activate the inflammatory cascade. *H. pylori* expresses sialic acid specific hemagluttinins and a lipid binding adhesins that mediate the binding to the mucosal surface. Gastrin is the main hormone involved in stimulating gastric acid secretion, and gastrin homeostasis is altered in *H. pylori* infection. The hyper acidity in duodenal ulcer may result from *H. pylori* induced hypergastrinemia. The elevation of gastrin may be a consequence of bacterial mediated decrease of antral D cells that secrete somastatin, thus using the inhibitory modulation of somatostatin on gastrin, or direct stimulation of gastrin cells by certain cytokines liberated during the inflammatory process. The organisms also elaborate phospholipases which damage surface epithelial cells and may release bioactive leukotrienes andeicosanoids. *H. pylori* produce large amounts of enzyme urease, molecules of which are localized inside and outside of the bacterium. Urease breakdown urea (which is normally secreted into the stomach) to carbondioxide and ammonia (ammonia is converted into the ammonium ion by taking hydrogen from water upon its breakdown into hydrogen and hydroxyl ions. Hydroxyl ions then react with carbondioxide, producing bicarbonate which neutralizes gastric acid. The survival of *H. pylori* in the acidic stomach is dependent on urease, and it would eventually die without the enzyme. The ammonia that is produced is toxic to the epithelial cells. Neutrophils attracted by *H. pylori* release myeloperoxidase which produces hypochlorous acid yield, in turn, monochloramine can destroy mammalian cells. In addition to *H. pylori* elaboration of enzymes, other antigens recruit inflammatory cells to the mucosa. The chronically inflamed mucosa is more susceptible to the acid injury. Finally, damaged mucosa is thought to permit leakage of tissue nutrients into the surface microenvironment thereby sustaining the bacillus. Mechanisms by which *H. pylori* could promote cancer are under investigation. One mechanism involves the enhanced production of free radicals near *H. pylori* and an increased rate of host cells mutation. The mechanism has been called “perigenetic pathway” and involves enhancement of the transformed host cell phenotype by means of alterations in cell proteins such as adhesion proteins. It has been proposed that *H. pylori* induces inflammation and locally high levels of TNF-α and/or interleukin 6. According to the proposed perigenetic mechanism, inflammation associated signaling molecules such as TNF-α can alter gastric epithelial cells adhesion and lead to the dispersion and migration of mutated epithelial cells without the need for additional mutations in tumor suppressor genes such as genes that code for cell adhesion proteins.

Complications associated with PUD

Gastrointestinal bleeding

Abrupt large bleeding which is life threatening can occur when the ulcer erodes one of the blood vessels.

Penetration

Herem the ulcer continues into the adjacent organs such as liver and pancreas.

Scarring and swelling

Scarring and swelling due to ulcers causes narrowing in the duodenum and gastric outlet obstruction, which causes severe vomiting.

Pyloric stenosis

Zollinger-Ellison syndrome

It is a rare syndrome which consists of a triad of non-beta islet cell tumors of the pancreas that contain and release gastrin, gastric acid hyper secretion and severe ulcer disease. Extra pancreatic gastrinomas are also common and may be found in the duodenal wall (Robbins and Cotran, 2006).

Diagnosis

An esophago gastro duodenoscopy (EGD) is a form of endoscopy, also known as gastroscopy, is carried out on patients in whom a peptic ulcer is suspected (Humphrey et al., 2008). The diagnosis of *H. pylori* can be made by: Urea breath test (non invasive and does not require EGD), direct culture from an EGD biopsy specimen, direct detection of urease activity in a biopsy specimen by rapid urease test, stool antigen test, histological examination and staining of an EGD biopsy.

If a peptic ulcer perforates, air will leak from inside the gastrointestinal tract which always contains some air into the peritoneal cavity which never contain air. This in turn lead to "free gas" within the peritoneal cavity. If the patient stands erect, while taking a chest X-ray, the gas will float to a point beneath the diaphragm. Thus in the peritoneal cavity, an erect chest X-ray or supine lateral abdominal X-ray will be obtained which is an open or perforated peptic ulcer disease.

Antiulcer drugs (Chaudri, 1991)

Drugs which neutralize gastric acid (antacids) are: Systemic antacids eg:- sodium bicarbonate; non systemic antacids; buffer type Eg: aluminium trioxide; non buffer type Eg:- MgO, magnesium hydroxide; Miscellaneous Eg: Alginates; drugs which reduce gastric acid secretion; H_2 receptor antagonists Eg: Cimetidine; Proton pump inhibitors Eg: omeprazole; anticholinergics Eg: propantheline; prostaglandin analogs Eg:- Misoprostol; mucosal protective drugs Eg: sucralfate; ulcer healing drugs Eg: carbenoxolone; anti *H. pylori* drugs Eg:- tetracycline, amoxicillin.

Side effects of antacids (Dharmani, 2006)

Osteomalacia, chronic renal failure, belching, flatulence, feeling of fullness, nausea, exacerbation of esophageal reflux.

Side effects of anti secretory agents

Rashes, diarrhoea, muscle pain, fatigue, bradycardia, blockade of cerebral H_2 receptors can cause drowsiness, mental confusion, delirium, hallucination. On long term use, it causes hepatotoxicity, gynecomastia, hyper prolactinemia.

Mechanism of proton pump inhibitors (PPI's)

PPI's decrease basal and stimulated gastric acid secretion during inhibition of acid secretion by parietal cells, the H^+/K^+ ATPase proton pump. These agents are most effective anti secretory agents. All medications in this class are week bases that must be activated by acid to inhibit the proton pump. Paradoxically, these prodrugs are acid labile compounds that can be degraded by stomach acid during oral administration and therefore available as enteric coated delayed release formulations. Once the drug reaches the higher pH of the duodenum, the enteric coating dissolves and the unprotanated prodrug readily penetrates the cell membranes, specifically that of parietal cells. As it traverses the parietal cell, and is exposed to intracellular acid, the prodrug becomes protonated and is no longer able to freely cross the cell membranes, thus the activated PPI becomes trapped in the parietal cell. Once formed, the active sulphonamide moiety covalently binds to H^+/K^+ ATPase and inhibits acid secretion. Food may delay the absorption of some agents, but because PPI's require accumulation and acid activation, and because they inhibit only proton pumps that are actively secreting acid, they are most effective when taken on an empty stomach, shortly before meals (Satoskar, 2007).

TREATMENT OF *H. PYLORI* INFECTION

Once *H. pylori* is detected in patients with peptic ulcer, it has to be eradicated and the ulcer is allowed to heal. The standard first line therapy is a one week triple therapy consisting of proton pump inhibitor such as omeprazole and antibiotics like clarithromycin amoxycillin.

An increasing number of infected individuals are found to wharf antibiotic-resistant bacteria. This results in initial treatment failure and requires additional rounds of antibiotic therapy or alternative strategies such as a quadruple therapy (Table 1), which adds a bismuth colloid. For the treatment of clarithromycin-resistant strains of *H. pylori* use of levofloxacin as part of therapy is optional (Tiwari, 2005).

Prevention

plenty of vegetables rich in beta carotene, fruit containing vitamin C, zinc rich foods such as whole grains and sea food (oysters) should be eaten. Eating more vegetables and fruit such as carrots, kale, red and green peppers, citrus fruits, apricots, kiwi fruit may promote healing of peptic ulcers and protect against further damage to the gut wall. The helpful nutrients in these foods are β-carotene, which the body converts to vitamin A and C. Foods rich in zinc such as whole grains and seafood, can also help in the healing process. Essential fatty acids (found in fish oils and seed oils) may help to protect against ulcers by increasing the production of prostaglandlins (a group of compounds, one function of which is protect the lining of the alimentary canal).

Table 1. Triple and quadruple regimen for treatment of *H. pylori* induced ulcers.

Regimen	Duration (days)	Efficacy
Amoxicillin + PPI	14	<70-80
Clarithromycin + PPI	14	>70-90
Clarithromycin + RBC	14	>70-90
Clarithromycin + Amoxicillin+ PPI	10-14	>80-90
Clarithromycin + Metranidazole+ PPI	10-14	>80-90
Clarithromycin + Tetracycline+ PPI	14	>80-90
Tetracycline + Metranidazole+ BSS+ PPI	7-10	>80-90
Tetracycline + Metranidazole +BSS+H_2RA	14 days	>80-90
Clarithromycin+Metranidazole+BSS+PPI	7-10 days	> 80-90

BSS: Bismuth subsalicylate; RBC: ranitidine bismuth citrate; PPI: proton pump inhibitor; H_2RA: histamine H_2 receptor antagonist.

Table 2. List of few medicinal plants scientifically proven for anti ulcer activity (Dharmani, 2006; Sandhya, 2010).

Botanical name	Plant part	Extract type	Ulcer model
Terminalia pallida	Leaves	Ethanol	Indomethacin, histamine, Alcohol
Allophylus serratus	Leaves	Ethanol	Aspirine, pylorus ligated, alcohol, cold resistant
Alpinia galangal	Rhizome	Ethanol	stress, pylorus ligated, ethanol,HCl
Anchusa strigosa	Root	Aqueous	Ethanol
Artemisia herba-alba	Leaves	Aqueous	Ethanol
Astronium urundeuva	Bark	Aqueous	Aspirin,stress,histamine
Atractyloids lancea	Rhizome	Acetone	Ethanol, HCl
Azadirachta indica	Leaves	Aqueous	Stress, ethanol
Baccharis triptera	Small branches	Aqueous	Pylorus ligated, stress, indomethacin
Bauhinia racemosa	Flower buds	Methanol	Aspirin
Bryophyllum pinnatum	Leaves	Methanol	Aspirin, indomethacin, serotonin, reserpine, stress, ethanol,
Caesalpinia ferrea	Stem	Crude	Acetic acid
Camellia sinensis	Leaves	Aqueous	Stress, ethanol, aspirin, indomethacin, reserpine, histamine, serotonin
Cassia nigrans	Leaves	Ethanol	Aspirin, pylorus ligated
Cistus incanus	Aerial part	Aqueous	HCl, ethanol, reserpine, serotonin
Curcuma longa	Rhizome	Ethanol	Pylorus ligated, cold-restraint, stress, indomethacin, reserpine, ethanol
Diodia sarmentosa	Whole plant	Ethanol	Aspirin, pylorus ligated
Entandrophragma utile	Bark	Aqueous	Ethanol,
Eremomastax speciosa	Leaves	Aqueous	Ethanol, HCl, pylorus ligated
Ficus exasperata	Leaves	Ethanol	Aspirin, pylorus-ligated
Laurus nobilis	Seeds	Ethanol	Ethanol
Maytenus aquifolium	Leaves	Aqueous	Indomethacin, cold-restraint stress
Microgramma squamulosa	Rhizome	Crude,ethanol, water	Stress, ethanol, HCl, acetic acid
Mikania cordata	Root	Methanol	Stress, ethanol, aspirin, phenyl butazone, pylorus ligated
Moringa pterygosperma	Flower buds	Methanol	Aspirin
Pistacia lentiscus	Resin from stem		Pylorus ligated, aspirin, reserpine restraint plus cold stress
Pluchea indica	Root	Methanol	Indomethacin, ethanol, aspirin
Punica granatum	Fruit peel	Aqueous	Ethanol
Pyrenacantha staudtii	Leaves	Aqueous	Aspirin, indomethacin, reserpine
Quercus ilex	Root bark	Aqueous	ethanol,
Saussurea lappa	Root	Acetone	Stress

Table 2. Cont.

Stachytarpheta cayennensis	Whole plant	Aqueous	Stress, ethanol, pylorus ligated
Stryphnodendron adstringens	Aerial parts	Total extract	Stress,ethanol, indomethacin
Styrax camporum	Stem	Ethyl acetate	Acetic acidl
Swertia chirata	Whole plant	Ethanol	Indomethacin, pylorus ligated, ethanol
Synclisia scabrida	Leaves	Ethanol	Aspirin, pylorus ligated
Tanacetum vulgare	Aerial parts	Chloroform	Ethanol
Trianthema pentandra	Whole plant	Methanol	Aspirin
Trichosanthes kirilowii	Fruit	Ethanol	Stress, histamine, serotonin, ethanol, HCl
Vernonia kotschyana	Root	Aqueous	Pylorus ligated, stress, indomethacin
Zingiber officinalis	Root	Methanol, acetone	HCl/ethanol
Amphipterygium adstringens	Stem bark	Methanol	Ethanol
Desmodium gangeticum	Root	Ethanol	Aspirin, alcohol, pylorus ligated, cold resistant
Ocimum sanctum	Leaves	Ethanol	Aspirin, alcohol, pylorus ligated, cold resistant,histamine
Hemidesmus indicus	Not mentioned	Ethanol	Aspirin, pylorus ligated
Asparagus racemosa	Fresh roots	Fresh juice	Aspirin, alcohol, pylorus ligated, cold resistant,histamine,cold resistant,cysteamine
Embelica officinalis	Fruits	Methanol	Aspirin, alcohol, pylorus ligated, cold resistant
Bacopa monniera	Not mentioned	Fresh juice	Aspirin, alcohol, pylorus ligated, cold resistant
Bidens pilosa	Not mentioned	Ethanol	Alcohol, pylorus ligated, indomethacin
Musa sapientum	Not mentioned	Powder	Pylorus ligated
Polyscias balfouriana	Leaves and root	n-butanol	Aspirin and physical stress induced

Intake of salt, soya sauce, spicy foods, caffeine in coffee, tea, cola drinks and alcohol should be cut down on. Large meals should be avoided, as they can encourage the production of excessive acid. Sufferers may also find that chilli peppers, black pepper, mustard and other strong spices such as those found in curries may aggravate their symptoms (Reader's Digest, 1996).

Medicinal plants as potent anti ulcer agents

Medicinal plants form the backbone of traditional systems of medicine in India. Phytochemicals from medicinal plants serve as lead compounds in drug discovery and design. Medicinal plants are rich source of novel drugs that forms the ingredients in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs. WHO pointed out that more than 80% of world's population depends on plants to meet their primary health care need. India is one of the 12 mega diversity countries in the world so it has a vital stake in conservation and sustainable utilization of its biodiversity resources. Plant extracts are some of the most attractive sources of new drugs and have been shown to produce promising results for the treatment of gastric ulcer. Nearly 240 medicinal plants and 21 plants based compounds were identified as anti ulcer agents so far (shodhganga.inflibnet.ac.in, 2011) (Table 2 and 3).

Conclusion

Medical treatments are effectual for some people, but not for everyone. They cause many superfluous side effects and make symptoms worse. As doctors continue to prescribe the same antibiotics, *H. pylori* resistance will continue to increase and the medications will become less effective. For minimizing the side effects, potent herbal drugs which can eradicate all traces of *H. pylori* is the better choice.

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Table 3. List of medicinal plants used by the folklore of Andhra Pradesh, India for ulcer treatment (Madhava et al., 2008).

Botanical name	Family	Parts used
Abutilon indicum	*Malvaceae*	Leaf
Acacia nilotica	*Mimosaceae*	fruit, seed, gum,resins
Albizia amara	*Mimosaceae*	Flower
Allium sativum	*Alliaceae*	Bulb
Ammannia baccifera	*Lythraceae*	Whole plant, leaf
Ampelocissus latifolia	*Vitaceae*	Leaf
Anacardium occidentale	*Anacardiaceae*	Rootbark
Antidesma ghaesembilla	*Stilaginaceae*	Leaf
Asphodelus tenuifolius	*Liliaceae*	Seed
Azadirachta indica	*Meliaceae*	Leaf
Balanites aegyptiaca	*Belanitaceae*	Leaf
Bambusa arundinacea	*Poaceae*	Root, leaf, fruit ,seed
Bauhinia variegate	*Caesalpiniaceae*	Stem bark
Boswellia ovalifoliolata	*Burseraceae*	Stem
Boswellia serrata	*Burseraceae*	Gum
Caesalpinia coriaria	*Caesalpiniaceae*	Fruit
Calophyllum inophyllum	*Clusiaceae*	Stembark
Calycopteris floribunda	*Combretaceae*	Leaf
Canavalia gladiate	*Fabaceae*	Pod
Canna indica	*Cannaceae*	Root
Carallia brachiata	*Rhizophoraceae*	Fruit
Cassia absus	*Caesalpiniaceae*	Leaf
Celosia argentea	*Amaranthaceae*	Whole plant
Clitoria ternatea	*Fabaceae*	Root
Combretum albidum	*Combretaceae*	Leaf
Coriandrum sativum	*Apiaceae*	Fruit
Cucurbita moschata	*cucurbitaceae*	Fruit seed, seed oil
Curcuma longa	*zingiberaceae*	Rhizome
Curcuma neilgherrensis	*zingiberaceae*	Rhizome
Cyclea peltata	*Menispermaceae*	Root
Dactyloctenium aegyptium	*Poaceae*	Fruit
Dalbergia latifolia	*Fabaceae*	Root
Dioscorea oppositifolia	*Dioscoreaceae*	Tuber
Dioscorea bulbifera	*Dioscoreaceae*	Tuber
Dioscorea hispida	*Dioscoreaceae*	Tuber
Dioscorea pentaphylla	*Dioscoreaceae*	Tuber
Dioscorea tomentosa	*Dioscoreaceae*	Tuber
Ficus benghalensis	*Moraceae*	Stem bark
Ficus benjamina	*Moraceae*	Leaf
Ficus religiosa	*Moraceae*	Stem bark
Ficus virens	*Moraceae*	Stem bark
Gardenia gummifera	*Rubiaceae*	Gum
Glinus oppositifolius	*Molluginaceae*	Whole plant
Gloriosa superba	*Cochlaceae*	Tuber
Hedera helix	*Araliaceae*	Leaf
Heliotropium indicum	*Boraginaceae*	Whole plant
Homonoia riparia	*Euphorbiaceae*	Root
Hydrolea zeylanica	*Hydroleaceae*	Leaf
Ipomoea eriocarpa	*Convolvulaceae*	Whole plant
Ixora coccinia	*Rubiaceae*	Flower

Table 3. Cont.

Jasminum sambac	*Oleaceae*	Leaf
Jatropha curcas	*Euphorbiaceae*	Whole plant
Jatropha gossypifolia	*Euphorbiaceae*	Latex
Kalanchoe laciniata	*Crassulaceae*	Leaf
Lablab purpureus	*Fabaceae*	Leaf
Lactuca sativa	*Asteraceae*	Latex,stem
Lannea coromandelica	*Anacardiaceae*	Leaf
Lawsonia inermis	*Lythraceae*	Stem bark, leaf, flower
Luffa acutangula	*Cucurbitaceae*	Root
Macaranga peltata	*Euphorbiaceae*	Leaf
Madhuca longifolia	*Sapotaceae*	Root bark
Mangifera indica	*Anacardiaceae*	Root bark,stembark
Momordica charantia	*Cucurbitaceae*	Fruit
Morinda pubescens	*Rubiaceae*	Root, fruit
Myrtus communis	*Myrtaceae*	Fruit
Nelumbo nucifera	*Nelumbonaceae*	Whole plant
Nymphaea nouchali	*Nymphaceae*	Whole plant
Ochna obtusata	*Ochnaceae*	Leaf
Persea macrantha	*Lauraceae*	Leaf
Phoenix sylvestris	*Areceaceae*	Root
Phyla nodiflora	*Verbenaceae*	Whole plant
Pimpinella tirupatiensis	*Apiaceae*	Tuber
Plumbago zeylanica	*Plumbaginaceae*	Leaf
Polycarpaea corymbosa	*Caryophyllaceae*	Whole plant
Pongamia pinnata	*Fabaceae*	Root
Portulaca oleracea	*Portulacaceae*	Whole plant
Portulaca quadrifida	*Portulacaceae*	Whole pant
Pouzolzia wightii	*Urticaceae*	Leaf
Pouzolzia zeylanica	*Urticaceae*	Leaf
Pterocarpus santalinus	*Fabaceae*	Heartwood
Rosa centifolia	*Rosaceae*	Flower
Schleichera oleosa	*Sapindaceae*	Stem bark
Sesbania sesban	*Fabaceae*	Flower
Shorea tumbugggaia	*Dipterocarpaceae*	Resin
Sigesbeckia orientalis	*Asteraceae*	Whole plant
Solanum giganteum	*Solanaceae*	Leaf
Solanum melongena	*Solanaceae*	Root
Solidago virga aurea	*Asteraceae*	Whole plant
Sorghum vulgare	*Poaceae*	Fruit
Stachytarpheta jamaicensis	*Verbenaceae*	Whole plant
Syzygium alternifolium	*Myrtaceae*	Fruit
Talinum portulacifolium	*Portulacaceae*	Leaf
Tamarindus indica	*Caesalpiniaceae*	Leaf
Tectona grandis	*Verbenaceae*	Heartwood
Tephrosia calophylla	*Fabaceae*	Leaf, tuberous root
Tephrosia maxima	*Fabaceae*	Whole plant
Tephrosia purpurea	*Fabaceae*	Whole plant
Terminalia arjuna	*Combretaceae*	Fruit
Terminalia pallida	*Combretaceae*	Fruit
Trigonella foenum-graecum	*Fabaceae*	Seed
Triumfetta rhomboidea	*Tiliaceae*	Root
Tylophora fasciculata	*Asclepiadaceae*	Leaf

Table 3. Cont.

Viscum articulatum	*Viscaceae*	Whole plant
Wrightia tinctoria	*Apocyanaceae*	Latex
Xanthium indicum	*Asteraceae*	Leaf
Yucca gloriosa	*Agavaceae*	Whole plant

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Full Length Research Paper

Studies on floral phenology, fruit and seed maturation and harvest index based on fruit colour in *Pongamia pinnata* (L.) Pierre

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***Pongamia pinnata* is a leguminous tree having enormous potential of producing high seed oil that could be as a source of biofuel. The collection of pods with higher seed quality characters is much important for production of elite seedlings at nursery and hence studies related to seed maturation were initiated as matured seed is the basic character of quality seed. To evaluate the steps in obtaining quality seed from the tree, studies were initiated on floral phenology, fruit and seed maturation and on evaluation of fresh fruit colour variation on seed and seedling quality characters. The results revealed that in pungam, the flowers per inflorescence ranged from 28.4 to 44.0, and only 3-4 per cent of the flowers set as pods. But the seed set was obtained with 80 per cent of the pods. The studies on pod maturation revealed that seeds of pods attained physiological maturation at 26 weeks after anthesis and it was accompanied with the change in colour of pods from green to dark brown and the seed from greenish white to dark brown, where the seeds had potential for higher germination (98 per cent). Evaluation on harvest index based on fresh fruits colour (yellow, light brown and dark brown) revealed that the seeds of light brown fruits recorded the higher germination (96 per cent), and seedling quality characters suggesting the collection of light brown fruits for obtaining good quality seed for sowing. The colour of pods also coincided with the colour obtained with maturation of pod studied with tagged flowers.**

Keywords: *Pongamia pinnata*, floral phenology, pod maturation, fruit colours, seed and seedling quality characters.

INTRODUCTION

Pongamia pinnata (L.) Pierre (Leguminoseae, subfamily Papilionoideae) is a medium sized tree commonly called as Karanj or pungam (Sangwan et al., 2010) that generally attains a height of about 10-20 m and a trunk diameter of more than 50 cm (Troup, 1921). *P. pinnata* is widely distributed throughout tropical Asia and the Seychelles Islands, South Eastern Asia, Australia, India and naturally distributed along the sea coasts and river banks in India (Arote and Yeole, 2010). The species thrives in areas with an annual rainfall of 500- 2500 mm, and a temperature range of 1-38°C. It can resist drought and withstand water logging and slight frost (Beniwal, 2011). For the past one decade, oil from pungam seeds has been seen as a potential source for biodiesel. Pungam seeds contain 30-35% oil and its physical and chemical properties are almost similar to the diesel. However, pungam oil could not be used as such and this oil needs preheating due to high viscosity and conradson carbon residue (Shrivastava and Prasad, 2000). The pungam is being cultivated in large number of gardens

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and widely distributed in roadsides in India and has the potential for the biological industry (Scott et al., 2007).

It has been observed that reproductive biology is very important to determine the seed and fruit set, conservation, pollination and breeding systems that regulate the genetic structure of populations (Kukade and Tidke, 2013). Pungam has lot of variability in terms of flowering phenology, fruit color, and fruit maturation period between the ecological zones as well as within the ecological zone (Raut et al. 2011, Patil et al., 2011). Thus, the knowledge on variability and its association with pod and seed traits is prerequisite for genetic improvement of the species. Hence, it necessitates seed source testing prior to an intensive breeding work (Sniezko and Stewart, 1989; Zobel and Talbert, 1984).

A clear understanding of phenological behavior on time of anthesis, time and duration of stigma receptivity, fertilization, mode of pollination, seed development is necessary for breeding programmes to obtain better traits (Rout et al., 2009). Collection of quality seed is very important in this crop as this oilseeds crop loses viability at faster rate and are described by researchers as microbiotic. Seed maturation is the environmentally influenced genetic factor is the major factor that spokes on the quality of the seed. In this huge tree, application of duration for collection of matured seed is cumbersome and requires an alternate means for collection of quality seed. The size of fruits and seeds has been considered as an important variable in the reproductive biology of plants (Khan et al., 2002). But colour of fruit has long been considered as an easier technique for production of quality seeds in forestry (Srimathi et al., 2001). In the past, only very little efforts has been taken to study the phenology and seed maturation aspects in pungam. The present study aims to investigate the floral phenology, developmental variation in fruit and seed, maturation period and evaluating the harvest index based on fruit colour in *Pongamia pinnata*.

MATERIAL AND METHODS

Study area

Morphologically superior pungam trees of 20 years old were selected in a plantation at Coimbatore district, Tamil Nadu, India which is situated at11°1'6 N 76° 58'21 E and 320 MSL. The selected trees were used for studying flower phenology, pod development, seed and seedling quality characters. The laboratory experiments were conducted at the Department of Seed Science And Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Studies on floral phenology

Five different trees were selected from the plantation and ten inflorescences from each tree were tagged at the initiation of flowering and were observed up to pod maturation. During maturation the data on number of flowers per inflorescence, pod per inflorescence, and number of seeds per inflorescence were observed twice a day at morning and evening for the period of 30 days and observations were pooled as weekly intervals. In addition to that, pod setting percentage, seed setting percentage and pod to seed setting percentage were also calculated adopting the following formulae:

$$\text{Pod setting percentage} = \frac{\text{Number of pods formed}}{\text{Total number of flowers per inflorescence}} \times 100$$

$$\text{Seed setting percentage} = \frac{\text{Number of seeds formed}}{\text{Total number of flowers per inflorescence}} \times 100$$

$$\text{Pod to seed set percentage} = \frac{\text{Number of seeds in number of pods}}{\text{Total number of pods per inflorescence}} \times 100$$

Studies on fruit and seed maturation

At peak flowering phase more numbers of matured flower buds (will open on next day) were tagged and observed at weekly intervals for pod colours, pod length (cm), pod breadth (cm), pod weight (g), and seed colours upto 27 weeks . The seeds were also germinated to evaluate the germination potential of the seed.

Evaluation of harvest index based on fruit colour

At the time of maturation, from the whole plantation the fresh fruits (pods) were categorized based on the colour as yellow, light brown and dark brown. Each of the colour grades were observed for fruit length (cm) fruit breadth (cm), fruit moisture content (%) and seed moisture content (%). Fifty fresh fruits (pods) from each colour category were replicated seven times and measured for seed length (cm), seed breadth (cm) (AOAC, 1960). In addition the seeds were also evaluated for the seedling quality characters viz., 100 seed weight (g), germination (%) (ISTA, 1999), root length (cm), shoot length (cm), fresh and dry weight of 10 seedling-1 (g). Based on the values obtained on seed and seedling quality characters three types of vigour index values were computed for each of the colour categories of fruits by using the following formulae (Abdul – Baki and Anderson, 1973).

Vigour index^{1} = Germination (%) x Total seedling length (cm)
Vigour index^{2} = Germination (%) x Root length (cm)
Vigour index^{3} = Germination (%) x Dry matter production 10 seedling^{-1} (g)

Table 1. Flowering phenology and fruit set in *Pongamia pinnata.*

Individual tree	Number of flowers in inflorescence	pod per inflorescence	Number of seeds per inflorescence	Pod set percentage (Flower to pod)	Seed set percentage (Flower to seed)	Pod to seed percentage
T1	40.5	1.6	1.4	4.0	3.5	87.0
T2	29.0	0.6	0.5	2.0	1.7	88.9
T3	44.0	2.1	1.6	4.8	3.6	74.1
T4	28.4	0.5	0.4	1.8	1.3	73.3
T5	36.3	0.9	0.7	2.6	1.9	74.7
Mean	35.7	1.1	0.9	3.0	2.4	79.6
CD (P=0.05)	2.9	0.3	0.4	0.9	1.0	NS

NS-Non significant

Statistical analysis

Data were statistically scrutinized as per Panse and Sukhathme (1985) and F test was used to understand the level of significance (5%). Since all the experiments were laboratory basis, the experimental design adopted was completely randomized design. The percentage values were transformed to arcsine values before analysis.

RESULTS AND DISCUSSION

Flowering phenology

It was observed that *Pongamia pinnata* initiated flower buds from mid-April to mid of May. The data (Table 1) showed that on an average of 35.7 flowers were observed per inflorescence, which had the range varying from 28.4 to 44 flowers. The pod and seed set percentage obtained was only 3.0 and 2.4 per cent respectively. However, 80 per cent of the pods set seed. Formation of flowers, pod or seed setting characters are highly variable in pungam trees from locality to locality, the variation may be related with genetic effect of the tree (Nelsonnavamaniraj, 2005) and the environmental factors prevailing at the particular locality (Dhillon et al., 2009). Similar variation in seed setting and percentage was also reported in different crops by different researchers (Gassama-Dia et al., 2003, Ndoye et al., 2004, Natarajan and Srimathi, 2008, Bentos et al., 2008, Piechowski and Gottsberger, 2009, Adjaloo et al., 2012 and Kukade and Tidke, 2013).

Studies on fruit and seed maturation

Daily observations on flowers revealed that the flowers ended with the fruit bud formation from 19 to 25 days after anthesis (end of May). The pod colour was light green from first week and at fifteenth week the colour changed to dark green from 16th week to 20th week, while the colour was greenish brown in 21st and 22nd week, which changed to yellowish brown in 23rd and 24th week and finally changed to light brown in 25th and 26th week after anthesis (Figure 1). The seed colour also changed from yellowish white to light brown with advances in maturation. The seeds were able to germinate from 16th week after anthesis (Table 2) and the completion of the seed and pod development took almost 189 to 220 days after anthesis, i.e. 27 weeks after anthesis. In contrast, other study from north India stated that *Pongamia pinnata* needs 327-344 days after the anthesis (Dhillon et al., 2009) indicating that there will be great genetic variation in duration of seed and pod maturation, seed size, pod colours and other morphological features and these characters are strongly depends the species, those occurs in a particular ecological and climatic regions (Divakara et al., 2010, Patil et al., 2011).

Seeds of pungam changed their colour from greenish white to brown from first week of bud initiation to 26th week of maturity. The *Pongamia pinnata* fruit and seed quality characters revealed that the growth measurements increased with changes in fruit colours from light green to light brown and then it decreased on changes of fruit colours to dark brown when it was dropped from the tree due to formation of abscission layer . On 26th week the pod size recorded as pod length (5.2cm), pod breadth (3.1cm) and pod weight (6.4cm) were the maximum, concluding that 26 weeks after anthesis is the correct maturity stage

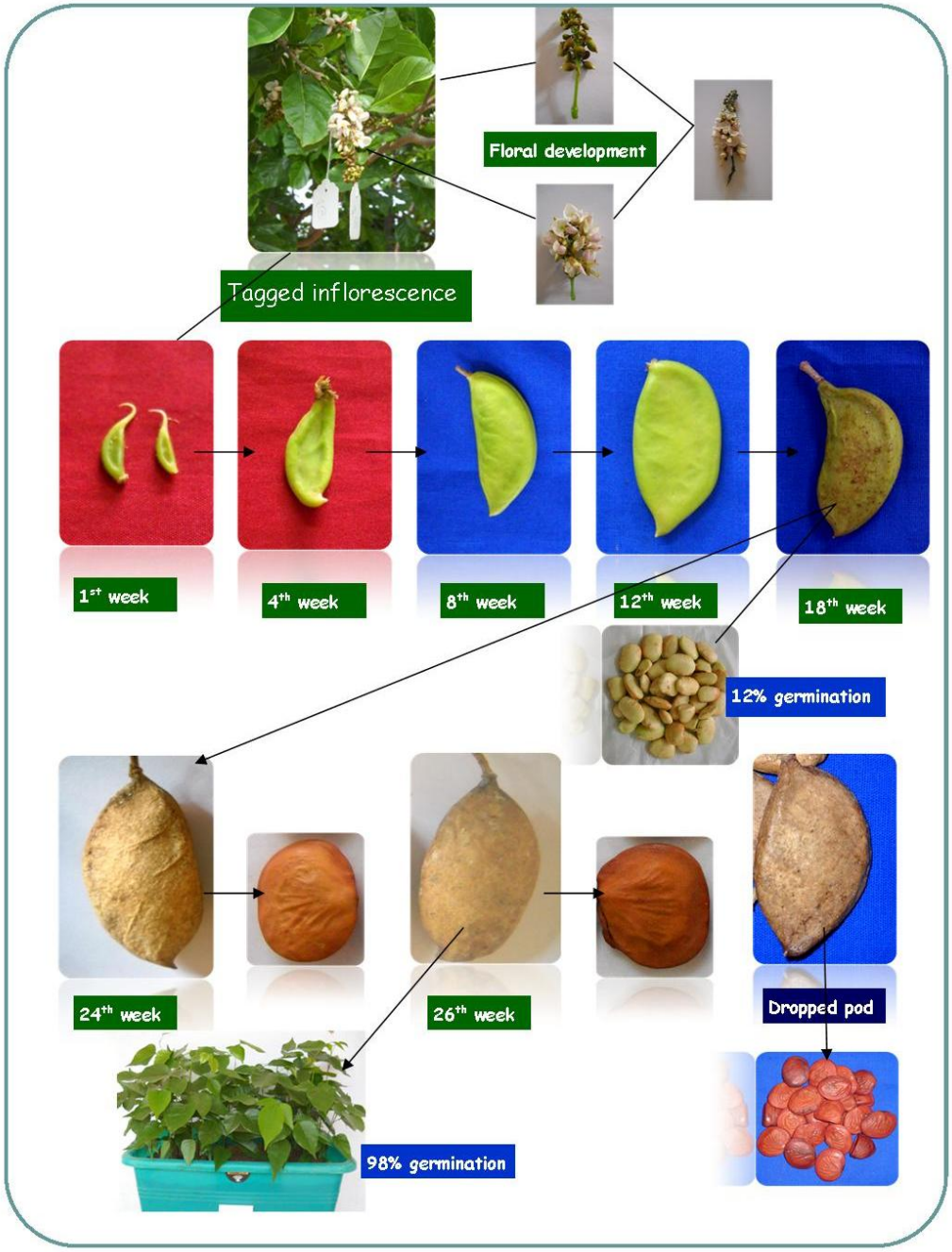

Figure 1. Pod and seed development in *Pongamia pinnata*.

to collect the fruits.

Varying periods of maturation for crops were also reported by Srimathi (1997) for fruit crops and Kathiravan (2004) for Jatropha. The pod maturity in this location (Coimbatore) was observed from mid-December to February. But Raut et al. (2011) reported that variation in fruit characteristics vary from place to place towards the point of maturation due to the variable environmental factors. Pollock and Roos (1972) reported that, after fertilization, as a result of cell division, there is a significant increase in seed size forming the embryonic cells that receive assimilates from the parent plant. During this period, seed moisture content remains constant and high. The significant decrease in seed moisture content occurs at the end of maturation when changes in cell membrane structure organization occurs as well as increases the enzyme synthesis in preparation for successful germination. Chlorophyll degradation and

Table 2. Studies on seed development and maturation in *Pongamia pinnata.*

Pod and seed development (weeks after anthesis)	Color of pod	Pod length (cm)	Pod breadth (cm)	Pod weight (g)	Color of seed	Germination (%)
1st week	Light green	1.0	0.2	0.6	Greenish white	-
2nd week	Light green	1.2	0.3	1.2	Greenish white	-
3rd week	Light green	1.3	0.4	1.3	Greenish white	-
4th week	Light green	1.4	0.5	1.5	Greenish white	-
5th week	Light green	1.6	0.6	1.7	Greenish white	-
6th week	Light green	1.8	0.9	2.1	Greenish white	-
7th week	Light green	1.9	0.9	2.7	Greenish white	-
8th week	Light green	2.0	1.0	3.0	Greenish white	-
9th week	Light green	2.2	1.2	3.2	Yellowish white	-
10th week	Light green	2.3	1.2	4.0	Yellowish white	-
11th week	Light green	2.4	1.3	4.2	Yellowish white	-
12th week	Light green	2.5	1.4	4.4	Yellowish white	-
13th week	Light green	2.6	1.6	4.5	Yellowish white	-
14th week	Light green	2.8	1.8	4.6	Yellowish white	-
15th week	Light green	2.9	1.9	4.7	Yellowish white	-
16th week	Dark green	3.1	2.1	4.9	Light brown	5 (12.9)
17th week	Dark green	3.3	2.2	5.0	Light brown	7 (15.3)
18th week	Dark green	3.5	2.2	5.1	Light brown	12 (19.6)
19th week	Dark green	3.6	2.4	5.2	Light brown	15 (22.5)
20th week	Dark green	3.7	2.5	5.4	Light brown	35 (36.2)
21st week	Greenish brown	3.9	2.6	5.5	Light brown	56 (48.5)

Table 2. Cont.

22nd week	Greenish brown	4.1	2.7	5.6	Light brown	60 (50.8)
23rd week	Yellow	4.3	2.9	5.7	Light brown	78 (62.1)
24th week	Yellow	4.4	2.9	5.9	Light brown	88 (69.8)
25th week	Light brown	4.7	3.0	6.2	Brown	92 (73.7)
26th week	Light brown (dry pod)	5.2	3.1	6.4	Brown	98 (84.2)
27 week	Dark brown (Pods dropped)	4.9	3.1	6.0	Dark brown	93 (74.3)
Mean	-	2.9	1.7	4.1	-	24 (21.1)
CD (P=0.05)	-	0.18	0.17	0.19	-	5.6 (5.2)

Figures in parentheses are arc sine transformed values

ethylene production is one of the reasons for the changing occurs in pod colours from initiation to maturity (Ward et al., 1995). Maturity has important implications in harvesting fruits at proper stage so as; the seeds have developed quality attributes (Singh et al., 2011).

Evaluation of harvest index based on fruit colour

Fresh fruit colours variations observed for fruit and seed characters, seed quality characters and for their performance in the present study were highly significant (Table 3). Standardization of fruit colours for each of the forestry species would be of immense help to seed collectors of forest trees as collection is a laborious process in these species owing to their inaccessibility for manual collection and the longer duration of harvesting period. Fruit colour is considered as an index of seed maturation particularly in forestry by several researchers (Khullar et al., 1991, Srimathi, 1997). Willan (1985) also revealed that fruit colours would serve as a tool for collection of good quality seeds in forestry as the persons involved in the collection process are mostly lacking in technical skill.

The analysis on colours of *Pongamia pinnata* fruit, on seed and seedling quality characters revealed that the growth measurements increased with changes in fruit colours. The fruit and seed moisture content decreased from yellow to dark brown as 27.4 to 17.6 per cent and as 21.2 to 8.9 per cent respectively. In seed characters, the seed length and breadth were highest with yellow fruits (2.5 and 1.9 cm respectively) and it reduced slightly with advances in fruit colours from light brown (2.4 and 1.7 cm respectively) to dark brown (2.2 and 1.6 cm). The highest seed weight of 100 seeds from green fruits recorded as 242.4g, however with light brown and dark brown pods it reduced as 232.1 and 192.4g respectively. The seed germination recorded by the fruits collected with various colours revealed that the seeds of yellow fruits were 80 per cent germination, while the seeds of light brown and dark brown (dropped pods) fruits recorded 96 and 91 per cent respectively suggesting the collection of light brown fruits for obtaining the seeds with maximum germination capacity. The vigour of seed evaluated through root length, shoot length, fresh weight, dry weight and vigour index were also higher in seeds obtained from light brown fruits and was followed by dark brown, and yellow fruits. The light brown colour of the fruit with higher seed and seedling quality characters were also coincided with the earlier results on seed maturation, where at 26 weeks after anthesis, the pod colour changes as light brown and that coincided with the seed germination recorded as the highest. Gurunathan et al. (2009), Kathiravan (2004), Kaushik et al. (2001) in *Jatropha curcas*, Srimathi et al. (2001) in Jamun found similar seed quality variations with fruit colours indicating that fuit colour would serve as an indication of seed maturation in pungam.

Conclusion

From the above results the study concluded that pungam trees were produced an average 35 flowers per inflorescence. Pod and seed set was very low as 3-4 percent though 80 percent of

Table 3. Influence of fresh fruit colour on *Pongamia pinnata* seed and seedling quality characters.

Fruit colours/character	Yellow	Light brown	Dark brown	SED	CD (P=0.05)
Fruit characters					
Fruit length (cm)	4.9	4.4	3.9	0.1	0.3
Fruit breadth (cm)	2.7	2.3	2.1	0.1	0.2
Fruit moisture content (%)	27.4	21.8	17.6	0.9	1.9
Seed moisture content (%)	21.2	15.9	8.9	0.5	1.0
Seed and seedling quality characters					
100 seed Weight (g)	242.4	232.1	192.4	2.2	4.6
Seed length (cm)	2.5	2.4	2.2	0.05	0.1
Seed breadth (cm)	1.9	1.7	1.6	0.04	0.1
Germination (%)	80 (63.6)	96 (79.4)	91 (72.9)	1.6 (1.9)	3.3 (4.1)
Shoot length (cm)	28.9	31.8	30.6	0.8	1.8
Root length (cm)	18.8	20.1	22.2	1.1	2.3
Fresh weight (10 seedlings^{-1})(g)	44.7	51.5	47.2	0.8	1.6
Dry weight (10 seedlings^{-1}) (g)	12.7	14.6	14.3	0.4	0.9
Vigour index^{1}	3827	5177	4808	132.5	278.3
Vigour index^{2}	1509	1977	2019	93.4	196.2
Vigour index^{3}	1016	1412	1301	40.3	84.6

Figures in parentheses are arc sine transformed values

pods setting seed. Pungam fruits (pods) attain physiological maturation 26 to 27 weeks after anthesis, and the seed collected at this stage recorded higher quality seed and seedling characters. Fruit (pod) colours of pungam vary as green, light brown and dark brown at the time of bulk collection for seeds. Among the three colours of fresh fruits the highest germination of 96 per cent was recorded for seeds of light brown fruits suggesting the collection of light brown fruits for obtaining the seeds with maximum germination capacity, which could also serve as harvest index for collection of quality seeds in pungam.

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In vitro antifungal effects of the essential oil of *Mentha piperita* L. and its comparison with synthetic menthol on *Aspergillus niger*

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The antifungal activity of *Mentha piperita* L. essential oil and its comparison with synthetic menthol on *Aspergillus niger* growth have been determined *in vitro*. The chemical compositions of essential oil of *M. piperita* provided from the aerial parts of plants grown in a village in Kerman Province in June 2012 were determined. The sample was cleaned and then dried in the shade. Essential oil was made by hydro-distillation method and analyzed by capillary gas chromatography (GC) using flame ionization (FID) and capillary gas chromatography coupled mass spectrometry (GC/MS). The main oil content from the plants of *M. piperita* was 3.26% (v/w). Twenty three (23) compounds were identified in the essential oil of *M. piperita, making* 96.25% of the total oil. The major components were menthol (38.33%), menthone (21.45%) and menthyl acetate (12.49%). For study of antifungal activity, the essential oil was tested against *A. niger* (strain PTCC = 5223) by disc diffusion method via average inhibition zone. The results showed that essential oil from *M. piperita* at 1 and 1/2 oil dilutions exhibited a strong antifungal activity than gentamycin (8 mg/ml) antibiotic on *A. niger* and exhibited a strong synthetic menthol at 10% dilution. The relative high amount of menthol and menthone in the *M. piperita* essential oil showed that they could display antifungal activity.

Key words: *Mentha piperita* L., *Aspergillus niger*, menthol, antifungal activity.

INTRODUCTION

Pathogenic fungi cause diseases in humans or vegetable organisms. Aerosolized *Aspergillus* spores are found nearly everywhere, so we are routinely and almost constantly exposed to them. Such exposure is a normal part of human condition and generally poses no adverse health effects. Nevertheless, *Aspergillus* can and does cause disease in three major ways: through the production of mycotoxins, through induction of allergenic responses and through localized or systemic infections. With the latter two categories, the immune status of the host is pivotal. Allergies and asthma are thought to be caused by an active host immune response against the presence of fungal spores or hyphae. In contrast, with invasive aspergillosis, the immune system has collapsed and little or no defence can be mounted (Machida and Gomi, 2010). *Aspergillus niger* is a haploid filamentous fungi and is a very essential microorganism in the field of biology.

In addition to producing extracellular enzymes and citric acid, *A. niger* is used for waste management and biotransformations. The fungus is most commonly found in mesophilic environments such as decaying vegetation or

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soil and plants (Schuster et al., 2002). Some strains of *A. niger* have been reported to produce ochratoxins (Abarca et al., 1994) but other sources disagree, claiming this report is based upon misidentification of the fungal species. Recent evidence suggests some true *A. niger* strains do produce ochratoxin A (Schuster et al., 2002). It also produces the isoflavone orobol. *A. niger* causes black mold of onions. Infection of onion seedlings by *A. niger* can become systemic, manifesting only when conditions are conducive. *A. niger* causes a common postharvest disease of onions, in which the black conidia can be observed between the scales of the bulb. The fungus also causes disease in peanuts and in grapes. *A. niger* is less likely to cause human disease than some other *Aspergillus* species, but, if large amounts of spores are inhaled, a serious lung disease can occur.

Peppermint (*Mentha piperita* L.) belongs to *Labiateae* family and originated from Mediterranean Regions. It is widely cultivated in the world and is a hybrid mint, a cross between water mint and spearmint (Frampton, 2009). The plant, indigenous to Europe, is now widespread in cultivation throughout all regions of the world. It is found wild occasionally with its parent species. Peppermint was first described in 1753 by Carolus Linnaeus from speci mens that had been collected in England; he treated it as a species (Linnaeus, 1753), but it is now universally agreed to be a hybrid (Harley, 1975). It is an herbaceous rhizomatous perennial plant growing 30-90 cm (12-35 in) tall, with smooth stems, and square in cross section. The rhizomes are wide-spreading, fleshy, and bare fibrous roots. The leaves are from 4-9 cm (1.6-3.5 in) long and 1.5-4 cm (0.59-1.6 in) cm broad; are dark green with reddish veins, and with an acute apex and coarsely toothed margins. The leaves and stems are usually slightly hairy. The flowers are purple, 6-8 mm (0.24-0.31 in) long, with a four-lobed corolla about 5 mm (0.20 in) diameter; they are produced in whorls (verticillasters) around the stem, forming thick, blunt spikes. Flowering is from mid to late summer. The chromosome number is variable, with 2n counts of 66, 72, 84, and 120 recorded (Huxley, 1992).

Peppermint typically occurs in moist habitats, including stream sides and drainage ditches. Being a hybrid, it is usually sterile; it produces no seeds and reproduces only vegetation, spreading by its rhizomes. If placed, it can grow anywhere, with a few exceptions. Peppermint generally grows best in moist, shaded locations, and expands by underground stolons. Young shoots are taken from old stocks and dibbled into the ground about 1.5 feet apart. They grow quickly and cover the ground with runners if it is permanently moist. For the home gardener, it is often grown in containers to restrict rapid spreading. It grows best with a good supply of water, without being water-logged, and planted in areas with part-sun to shade. The leaves and flowering tops are used; they are collected as soon as the flowers begin to open and can be dried. The wild type of the plant is less suitable for this purpose, with cultivated plants having being selected for more and better oil content. They may be allowed to lie and wilt a little before distillation, or they may be taken directly to the still. Essential oils of peppermint are used in flavors, fragrances, and pharmaceuticals. Peppermint has a long tradition of medicinal use, with archaeological evidence placing its use at least as far back as ten thousand years ago. Peppermint has a high menthol content, and is often used in tea and for flavouring ice cream, confectionery, chewing gum and toothpaste. The oil also contains menthone and menthyl esters, particularly menthyl acetate. Dried peppermint typically has 0.3-0.4% of volatile oil containing menthol (7-48%), menthone (20-46%), menthyl acetate (3-10%), menthofuran (1-17%) and 1,8-cineol (3-6%). Peppermint oil also contains small amounts of many additional compounds including limonene, pulegone, eucalyptol, caryophyllene and pinene (Leung, 1980). It is the oldest and most popular flavour of mint-flavoured confectionery. Peppermint can also be found in some shampoos, soaps and skin care products. Menthol activates cold-sensitive TRPM8 receptors in the skin and mucosal tissues, and is the primary source of the cooling sensation that follows the topical application of peppermint oil (Eccles, 1994). Peppermint oil has a high concentration of natural pesticides, mainly polygone and menthone (Krieger, 2001). Mint essential oils are generally used externally for antipruritic, astringent, rubefacient, antiseptic, and antimicrobial purposes, and for treating neuralgia, myalgia, headaches, and migraines (Hendriks, 1998).

The well-known and widely used peppermint is a cultivated natural hybrid of *M. aquatica* L. (water mint) and *M. spicata* L. (spearmint). Although a native genus of the Mediterranean Regions, it is cultivated all over the world for its use in flavor, fragrance, medicinal, and pharmaceutical applications. Peppermint oil is one of the most widely produced and consumed essential oils (Foster, 1990). Menthol is an organic compound made synthetically or obtained from peppermint or other mint oils. It is a waxy, crystalline substance, clear or white in color, which is solid at room temperature and melts slightly above. The main form of menthol occurring in nature is (−)-menthol, which is assigned the (1R,2S,5R) configuration. Menthol has local anesthetic and counterirritant qualities, and it is widely used to relieve minor throat irritation. Menthol also acts as a weak kappa opioid receptor agonist. Natural menthol exists as one pure stereoisomer, which is nearly always the (*1R,2S,5R*) form. In the natural compound, the isopropyl group is in the *trans* orientation to both the methyl and hydroxyl groups. Menthol's ability to chemically trigger the cold-sensitive TRPM8 receptors in the skin is responsible for the well-known cooling sensation it provokes when inhaled, eaten, or applied to the skin (Eccles, 1994). In this sense, it is similar to capsaicin, the chemical responsible for the spiciness of hot peppers (which stimu-

lates heat sensors, also without causing an actual change in temperature). Menthol's analgesic properties are mediated through a selective activation of κ-opioid receptors (Galeottia et al., 2002). Menthol also blocks voltage-sensitive sodium channels, reducing neural activity that may stimulate muscles (Haeseler et al., 2002). Menthol also enhances the efficacy of ibuprofen in topical applications via vasodilation, which reduces skin barrier function (Braina et al., 2006). *M. arvensis* is the primary species of mint used to make natural menthol crystals and natural menthol flakes. This species is primarily grown in the Uttar Pradesh region in India. (−)-Menthol (also called *l*-menthol or (*1R,2S,5R*)-menthol) occurs naturally in peppermint oil (along with a little menthone, the ester menthyl acetate and other compounds), obtained from *Mentha x piperita*. Japanese menthol also contains a small percentage of the 1-epimer, (+)-neomenthol. Biosynthesis of menthol was investigated in *M. x piperita*, and all enzymes involved in its biosynthesis have been identified and characterized (Croteau et al., 2005).

M. piperita has been shown to possess strong antifungal activity, even when compared to synthetic fungicides. Peppermint oil showed antifungal activity against *A. niger*, *Alternaria alternata* and *Fusarium* sp. by agar well diffusion method (Aqil *et al*, 2000). The chemical responsible for this action was menthone (Soković et al., 2009). As with many widely used natural products, the demand for menthol greatly exceeds the supply from natural sources. In organic chemistry, menthol is used as a chiral auxiliary in asymmetric synthesis. For example, sulfinate esters made from sulfinyl chlorides and menthol can be used to make enantiomerically pure sulfoxides by reaction with organolithium reagents or Grignard reagents. Menthol reacts with chiral carboxylic acids to give diastereomic menthyl esters, which are useful for chiral resolution. This study evaluated and identified the chemical compounds of *M. piperita* mainly. Also, antifungal activity of *M. piperita* has been compared with synthetic menthol and gentamicin (8 mg/ml) antibiotic standard on culture of *A. niger* (strain PTCC=5223).

MATERIALS AND METHODS

Plant material collection and extration of essential oil

The aerial parts of peppermint (*M. piperita*) were obtained from this plant grown in a village in Kerman province (Iran) at full flowering stage in June 2012. The sample was cleaned in shade condition to prevent volatility of the plant material constituents and to keep the natural color of the sample fixed. Then they were air-dried and powdered using a milling machine and kept in a cool dry place until ready for extraction of the essential oil. Afterwards, essential oil was taken from 150 g of the powdered sample in hydrodistillation method with the help of Clevenger set for three hours. The sample oils were dried with anhydrous sodium sulfate and kept in sterile sample tubes in refrigerator. The oil yield from aerial parts of peppermint plant was calculated.

Analysis of essential oil

Gas chromatography

GC analysis was performed using a model HP-439 gas chromatograph equipped with column CP Sil, whose 5CB is 25 m length; internal diameter, 0.25 mm and film thickness, 0.39 µm. Oven temperature was from 60 to 220°C at a rate of 7°C slope per minute. Injector temperature was 280°C, detector (FID) temperature was 270°C and carrier gas was helium.

Gas chromatography/mass mass spectrometry

In order to analyze and identify the combinations forming the essential oil, the chromatograph gas set attached to a mass spectrometry, Model Hewlett Packard-5973 was used. The conditions of analysis and specifications of the GC/MC set were as follows: Capillary column HP 5MS, 60 m length; internal diameter, 0.25 mm; layer thickness, 0.25 µm; thermal program of oven (3 min), 60°C to 220°C with a 6°C slope per minute; and then 220°C in 3 min; the temperature of place of injection was 280°C; carrier gas was helium; the speed of gas was 1.0 milliliter per minute; the ratio of fission, 1 to 43; the rate of injection, 0.1 µl; temperature of the reservoir of ionization, 230°C; ionization mode EI, Ionization energy, 70eV. The series of normal Alkans C8-C17 was also injected to the set under the same condition with that of essential oil injection to calculate restrictive index (RI) of components of essential oil. The Restrictive Index of components of the sample was calculated by using a computerized program. Finally, the components of essential oil were identified by comparing the mass spectrums obtained with the existing standard mass spectrums at electronic library of Wiley 2000 existing in Absolution software of GC/Ms set. Calculation of standard restrictive index was done in accordance to C8-C17 Alkans and compared with the existing standard figurers in references (Adams, 2001).

Assessment of antifungal action

The solvent showing no antifungal activity from DMSO was selected as a diluting medium for the oil. Undiluted oil was taken as dilution 1, 1/2, 1/4, 1/8 and 1/16 dilutions of the oil were made DMSO. For antifungal activity (50 µl) of each dilution was used. The antifungal activity of the essential oil was evaluated by disc diffusion method using Mueller Hinton Agar (Baron and Finegold, 1995) and determination of inhibition zones at different oil dilutions against *Aspergillus niger* (PTCC=5223). The fungal strains under experiment were obtained from the Center for Fungi and Bacteria of Iranian Scientific and Industrial Researches Organization. The antifungal property of the oil was tested by agar well diffusion method using Sabouraud Dextrose Agar (SDA). Standard reference antibiotic was used in order to control the sensitivity of the tested fungi (gentamicin 8 mg/ml). The incubation condition used was 48 to 72 h at 24°C for fungi. All the experiments were carried out in triplicate and averages were calculated for the inhibition zone diameters.

RESULTS

The study of the analysis of peppermint essential oil under investigation showed that the output of essential oil is 3.26% (v/w). The combination of essential oil, restrictive index (RI), and quantitative percentage of the compounds were identified. Thirty three (33) compounds identified in the

Table 1. Combinations identified in the essential oil of *Mentha piperita* L.

Compound name	Restrictive index (RI)	Percentage (%)
α-Pinene	937	0.65
Sabinene	975	2.23
β-Pinene	982	1.22
Myrcene	992	0.76
3-Octanol	996	0.12
α-Terpinene	1024	0.54
P-Cymene	1028	0.17
Limonene	1034	5.33
1,8-Cineole	1039	3.27
(*E*)-β-Ocimene	1045	0.59
γ-Terpinene	1068	0.45
Terpinolene	1084	0.21
Iso-menthone	1092	2.87
Linalool	1126	0.36
Menthone	1148	21.45
Menthyl acetate	1156	12.49
Menthol	1171	38.33
α-Terpineol	1203	0.57
Pulegone	1238	1.34
Piperitone	1310	0.68
β-Caryophyllene	1415	1.23
Germacrene D	1482	0.58
γ-Cadinene	1521	0.81
Total		96.25

in the essential oil of this plant were 96.25%. The combinations of menthol (38.33%), menthone (21.45%) and menthyl acetate (12.49%) with 72.27% constitute the highest percentage of essential oil (Table 1). The indexes of restrictive have been calculated by injecting the mixture of normal hydrocarbons (C8-C17) to HP-5MS column

The results of studying the antifungal impacts of *M. piperita* essential oil show that the oil of this plant has an inhibitory effect in 1, 1/2, 1/4, 1/8 and 1/16 dilutions with average diameter growth of 26, 21, 16, 12 and 8 mm respectively. The results with standard antibiotic gentamicin (8 mg/ml) with a diameter of 18 mm had inhibitory effects. Synthetic menthol in 1 % dilution had moderrate inhibitory (14 mm) effect on *Aspergillus niger* growth, but at 10 % dilution it had a strong inhibitory (20 mm) effect on fungi growth.

The results show essential oil from *Mentha piperita* at 1 and 1/2 oil dilutions exhibited strong antifungal activity than gentamycin (8 mg/ml) antibiotic on *Aspergillus niger* and exhibited strong menthol at 10% dilution. The high percentages of antifungal activities of *Mentha* oil are related with menthol, the main organic compound (Table 2).

DISCUSSION

Essential oils are natural compounds, which have extensive applications in perfumery, food and pharmaceutical industries. Essential oils of *Mentha* species possess great antibacterial and antifungal potential and could be used as natural preservatives and fungicides. In this study, the chemical composition and antifungal effects of *M. piperita* are compared with that done by other researchers.

Comparing our results with other researchers, the essential oil of *M. piperita* has been studied in Iran and in the World. In a research, the chemical compositions and antibacterial and antifungal activity of essential oils from 4 medicinal plants consist of *M. piperita*, *M. spicata*, *Anethum graveolens* and *Foeniculum vulgare*. Antibacterial and antifungal activity of these oils and their components were assayed against a variety of human pathogenic bacteria. Main components in *M. piperita* oil were menthol, limonene, 1,8-cineole, sabinene, menthyl acetate and menthone, in *M. spicata* oil carvone, menthol, limonene and menthone. *M. piperita* showed strong antibacterial and antifungal activities (Kazemi et al., 2012).

Table 2. The zone diameter of inhibition of antibiotic, mentha oil and synthetic menthol on *aspergillus niger* (mm).

Antibiotic	Dilutions of menthane oil					Synthetic menthol	
Gentamicin (8 mg/ml)	1	1/2	1/4	1/8	1/16	1%	10%
18	26	21	16	12	8	14	20

The analysis of essential oil of *M. piperita* in Iran with GS/MS revealed that main compounds of oil include menthol (19.76%), menthan-3-one (19.31%), mentho-furan + isomenthone (9.12%), 1,8-cineole + beta phellan-dren (8.8%) and mentholacetate (5.63%). Inhibitory effect of essntial oil varied among different fungi. After 48 hours, results showed no significant difference between the growth of fungi at 800 and 1600 ppm as well as between water and alcohol controls; but differences between 200 and 400 ppm were significant.

The results of this study reveal that *M. piperita* oil exhibited a significant antifungal activity (Farshbaf Moghaddam et al., 2004). In a report, the antifungal activities of essential oils and herbal extracts have been demonstrated against a range of filamentous fungi. *In vitro* antifungal activity of a combination of some essential oils extracted from herbs (*Thymus vulgaris*, *Salvia officinalis*, *Eucalyptus globulus* and *Mentha piperita*) against some filamentous fungal strains (*Metrhizium sp.*, *Ophiostoma sp.*, *Trichoderma sp.* And *Penicillium expansum*) was determined. 1,8-cineol (21.37%), thymol (13.86%), camphor (7.92%), α-thujone (7.71%), menthon (6.8%) and menthol (6.2%) were the major constituents. This combination was found to have a wide spectrum of activity against all filamentous fungi examined in this study and may be proposed for control of fungal diseases (Mousavi and Raftos, 2012). In a research, the essential oils of *Origanum vulgare* subsp. *hirtum, Mentha spicata, Lavandula angustifolia*, and *Salvia fruticosa* exhibited antifungal properties against the human pathogens *Malassezia furfur, Trichophyton rubrum*, and *Trichosporon beigelii*. Among the main components of the four oils, carvacrol and thymol exhibited the highest levels of antifungal activity (Adam et al., 1998). Antimicrobial activity of four essential oils from lemon (*Citrus lemon*), mint (*Mentha piperita)*, juniper (*Juniperus communis*) and rosemary (*Rosmarinus officinalis*) was against *Aspergillus niger*, *Fusarium oxysporum*, *Monascus purpureus* and *Penicillium hirsutum* molds.

The results of this study confirm that essential oils from aromatic plants such as lemon, mint, juniper and rosemary possess antifungal activity. The most effective against all tested strains was the mint oil (Ferdes and Ungureanu, 2012). The essential oils of *Mentha arvensis* L. and *Zingiber officinale* R. were screened against *Staphylococcus aureus* Rosenbach, *Enterococcus faecium* Schleifer and Kilpper-Bälz, *Pseudomonas aeruginosa* Migula, *Escherichia coli* Castellani and Chalmers, *Proteus mirabilis* Hauser and yeast *Candida albicans* Berkhout. The oils showed a wide spectrum of antimicrobial activity (Mickienė et al., 2011). Essential oils of peppermint were investigated for their antimicrobial properties against 21 human and plant pathogenic microorganisms. The bioactivity of the oils of menthol and menthone was compared using the combination of *in vitro* techniques such as microdilution, agar diffusion, and bioautography. It was shown that all of the peppermint oils screened strongly inhibited plant pathogenic microor-ganisms, whereas human pathogens were only mode-rately inhibited.

Using the bioautography assay, menthol was found to be responsible for the antimicrobial activity of these oils (Iscan et al., 2002). The potential antifungal effects of *Thymus vulgaris* L., *Thymus tosevii* L., *Mentha spicata* L., and *Mentha piperita* L. (Labiatae) essential oils and their components against 17 micromycetal food poisoning, plant, animal and human pathogens are presented. In *M. piperita* oil menthol (37.4%), menthyl acetate (17.4%) and menthone (12.7%) were the main components, whereas those of *M. spicata* oil were carvone (69.5%) and menthone (21.9%). *Mentha* sp. showed strong antifungal activities. The commercial fungicide, bifonazole, used as a control, had much lower antifungal activity than the oils and components investigated.

It is concluded that essential oils of *Thymus* and *Mentha* species possess great antifungal potential and could be used as natural preservatives and fungicides (Soković et al., 2009). In a report, essential oils from peppermint (*Mentha* sp.), clove (*Syzgium aromaticum*) and eucalyptus (*Eucalyptus globus*) were evaluated for their antifungal activity against soil-borne fungi, including *Aspergillus niger, Alternaria alternata* and *Fusarium chlamydosporum* by agar well diffusion method. Maximum antifungal activity was detected in essential oil of clove oil followed by those of peppermint and eucalyptus. These observations indicate that these essential oils can be exploited as antifungal agents in the management of plant infectious diseases and post-harvest spoilage of crops (Aqil et al., 2000). In this study, the antifungal activity of essential oils of selected plant species, viz. *Piper nigrum* Linn., *Ricinus communis* Linn., *Cedrus deodara* (Roxb.) Loud., *Syzygium aromaticum* (Linn.) Merrill & Perry, *Eucalyptus globulus* Labill., *Citrus aurantium* Linn., C. *limon* (Linn.) Burm. f., *Olea europaea* Linn. and *Mentha piperita* Linn. were assayed for fungi toxicity against two genus, viz. *Aspergillus niger* and *Geotrichum candidum*. The highest and broadest activity was shown by the essential oils of *S. aromaticum*, *C.*

limon, C. aurantium and *M. piperita* as compared to standard drug, Ketoconazole. The 5 ppm concentration of essential oils of *S. aromaticum, C. limon* and *M. piperita* completely inhibited the mycelial growth of *A. niger* and *G. candidum* to the same extent as 5 ppm of Ketoconazole (Verma et al., 2011). In a study, menthol was found to be the active responsible for the antifungal effect (Edris, 2003). Essential oil samples of *Mentha x piperita* L. (peppermint) were analysed by GC/MS and assayed for their antibacterial, antifungal and antioxidant activities.

The oil samples from spring planted crops had a significantly higher menthol and lower terpinen-4-ol concentrations than those from autumn planted crops. The oil samples showed a different degree of inhibition against the twenty-five microorganisms tested. Peppermint oil exhibited a marked antifungal activity against *Aspergillus niger* (Marotti et al., 1994). *In vitro* antimicrobial activity of essential oils and the mechanisms of essential oils action on microorganisms are reported. This research gives an overview on the susceptibility of human and food-borne bacteria and fungi towards different essential oils and their constituents. Essential oils of spices and herbs (thyme, origanum, mint, cinnamon, salvia and clove) were found to possess the strongest antimicrobial properties among many tested (Kalemba and Kunicka, 2003). In a report, the effect of mint (*Mentha piperita*) essential oil (0·5, 1·0, 1·5 and 2·0%, v/w) on *Salmonella enteritidis* and *Listeria monocytogenes* in a culture medium and three model foods was studied. Mint essential oil antibacterial action depended mainly on its concentration, food pH, composition, storage temperature and the nature of the micro-organism (Tassou et al., 1995).

Conclusion

The peppermint oil recommended for large scale application is based on its strong antifungal as well as anti- *Aspergillus niger* efficacy. In this study, we find out that the antifungal effects of *Mentha piperita* essential oil under investigation compared to synthetic menthol on *Aspergillus niger* exhibited strong synthetic menthol. The high percentage of antifungal activities of *Mentha* oil is related to menthol, the main organic compound . This essential oil can be used for antifungal activity and as natural compound. The effectiveness of the oil concentration depends on the target pathogen and effects of natural compounds on fungus.

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Full Length Research Paper

Yield and quality of statice [*Limonium sinuatum* (L.) Mill.] as affected by cultivars and planting densities

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A study was conducted in a plastic greenhouse at Freesia Ethiopia PLC Sululta located in the central highlands of Ethiopia with the objective of determining the effect of cultivar, planting density and their interaction effect with reference to yield and quality of Statice. The experiment was laid out in a 3 x 4 factorial arrangement using a Randomized Complete Block Design (RCBD) with three replications. The treatments consisted of three cultivars ('Amazon Bird', 'Giant Blue Bird' and 'Yellow Sun Bird') and four different planting densities (4, 6, 8, and 10 plants per m^2). Data were collected on parameters pertaining to growth, yield and quality of Statice. The results of the study indicate a trend of rise in the number of marketable and unmarketable flowering stems per plot as the plant density increased from 4 to 10 plants per m^2. Conversely, flowers' vase life significantly ($P<0.01$) decreased when planted densely. In addition, the fresh weight of the marketable stems significantly ($P<0.01$) decreased as planting density increased. Among the cultivars, 'Yellow Sun Bird' was found to be more productive in terms of marketable stems followed by 'Giant Blue Bird' and 'Amazon'. In terms of stem length, 'Yellow Sun Bird' was found to be higher but was not statistically different from 'Giant Blue Bird'. With regards to vase life quality, the best result was obtained from 'Amazon Bird' cultivar. Overall, the study reveals that planting density and varietal differences had valid effects on the yield and quality of Statice. Cultivars 'Giant Blue Bird' and 'Yellow Sun Bird' had better performance in many of the parameters than the 'Amazon Bird' cultivar. The highest planting density (10 plants per m^2) demonstrated a positive influence on most of the evaluated parameters and therefore can be recommended for use by commercial growers in Ethiopia. However, further investigation is imperative on issues pertaining to the determination of nutrient supply for regulating the fresh weight of the product and other economic related topics.

Key words: Statice, *Limonium sinuatum*, planting density, Cultivar

INTRODUCTION

Among the many species of the genus *Limonium, Limonium sinuatum* is the most commonly cultivated and highly recognized cut flowers crop. The common name "Statice" is actually used in most references for this genus although "sea lavender" is alternatively used because of its lilac-colored flowers and the fact that it naturally inhabits mainly coastal areas (Steven, 2008). The cultivars of *L. sinuatum* is commonly known as the 'Sun Bird' series and are branded like 'Yellow Sun Bird' and 'Giant Blue Bird'.

Ethiopian flower exports to the global market have increased five-fold between 2006 and 2008. In 2008

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alone, Ethiopia earned 114 million dollars from the floriculture industry. The country's high altitude areas provide near ideal growing conditions for most high value cut flower crops, including Roses (*Rosa hybrida* L.*)*, Gypsophila or Baby's Breath *(Gypsophila paniculata* L.), Statice (*Limonium spp*), Carnations (*Dianthus caryophyllus* L.) and Chrysanthemum (*Chrysanthemum spp*) (EHPEA, 2009).

Among the cut flower crops grown in the country, Statice is currently in demand by new investors for a large scale production owing to its easy plant care requirement and good selling price in the auction market. Based on these facts, four well-known firms were involved in growing and exporting of this crop. However, among the operating farms, some have been forced to stop production because of the major quality and productivity issues related to fresh weight of the flowers, planting density, and cultivar selection. Despite the stated bottlenecks, a number of new farms have shown interest in growing of the crop. Among these, Freesia Ethiopia PLC is the one that decided to include the crop in its production scheme. However, unlike others, the company sought to ascertain some specific facts before launching production. Thus, finding out appropriate planting density and type of cultivars that brings better quality and yield in Ethiopian condition would be necessary to support growers to be competitive in the global market. Optimization of plant spacing in corresponding to adapted cultivar would be vital to ensure the best economic return (Khan et al., 2003). To this aim, a study was initiated to determine the effects of cultivar, planting density and their interaction effect on yield and quality of Statice.

A wide range of spacing has been used in Statice field production. Paparozzi and Hatterman (1988) used 30 cm between plants and 69 cm between rows which gives planting density of 4.8 plants/m^2 while Whipker and Hammer (1994) used 30 by 30 cm spacing between plants and rows, which is equivalent to 11.111 plants/m^2 or 111,111 plants per hectare. Wilfret et al. (1973) used two rows of plants with 30 cm between plants and 30 cm between rows. Plants were grown on raised beds 76 to 90 cm wide and 135 cm between bed centers, resulting in approximately 50,000 plants per ha.

The performance of Statice in terms of yield and quality has been reported to be substantially affected by the kind of variety selected (Dole and Wilkins, 2005; Burchi et al., 2006; Fascella and Zizzo, 2004), the growing environment and the management practice during the growing season (Starman et al., 1995).

MATERIALS AND METHODS

Study area

The study was conducted in a plastic greenhouse at Freesia Ethiopia PLC located at Sululta, Ethiopia which is 20 km away from Addis Ababa. Geographically, the area is situated at 9° 11' 0" latitude and 38° 39' 0" longitude at an average altitude of 2785 m.a.s.l. The inside temperature and relative humidity (RH) were controlled within the range of 15-20°C and 65-75%, respectively using a computerized system (Hogendoren systems) installed within the farm.

Materials

The seedlings of three *L. sinuatum* cultivars, namely 'Amazon Bird', 'Giant Blue Bird' and 'Yellow Sun Bird' were imported from The Netherlands and used for the study. Three blocks were then prepared with each having 12 experimental plots. Each plot had a size of 0.834 x 1.2 m = 1 m^2 with a distance of 80 cm between plots. The blocks were a meter apart from each other. After this, a standard wire mesh with 1.2 m width was laid along all the blocks which later helped as a support for the stems.

Experimental design and treatments

The experiment was laid out in a 3 x 4 factorial arrangement with Randomized Complete Block Design (RCBD). The treatments consisted of three cultivars ('Amazon Bird', 'Giant Blue Bird' and 'Yellow Sun Bird') and four different planting densities (4, 6, 8 and 10 plants/m^2). The experiment was replicated three times. Then, the 12 treatments combinations were assigned randomly to the experimental units within a block.

Fertilizer was applied as per the recommendation based on the result of soil analysis throughout the growing season and other management practices like weeding, raising the wire mesh, and removing of dry leaves were performed whenever necessary. Fertigation was conducted using a computerized sprinkler system in the vegetative stage and drip irrigation was used later during the flowering stage.

Measurements

Data were collected on parameters pertaining to growth, yield and quality of Statice. These parameters were studied from three to eight sample plants depending on the plant population size, except for yield parameters (Marketable and Unmarketable) wherein data were recorded on whole plot bases. Accordingly, the number of stems which have an upright stem, with sufficient stem length for export (above 45 cm), free of any mechanical and pest damage, with required fresh weight (30 to 80 g) were sorted and categorized as marketable; whereas, stems that deviated from the above stated quality parameters were considered as unmarketable stems. In addition, days to flowering was recorded as the number of days taken from the date of planting to the date on which 50% of plants in a plot started to open their flowers. The number of days taken for 50% of flowered plants to reach their harvestable stage was considered as days to first harvest. Accordingly, flowers were harvested when four spikes of a single stem had 75% of their florets opened. The first ten stems harvested were used for quality analysis and measurements were continued for six consecutive months.

Data analysis

The data collected for the different parameters were first checked for meeting the various ANOVA assumptions and the SAS version 9.2 Statistical software package was used for the analysis of variance and estimation of correlation among the response variables (Montgomery, 2005). Then data was subjected to analysis of variance using a factorial randomized complete block design (RCBD). Whenever treatment means were found to be significant,

Table 1. Effect of Planting Density and Cultivar on Days to 50% Flowering

Treatment	Days to 50% Flowering
Planting density	
4 plants per m^2	47.44
6 plants per m^2	48.55
8 plants per m^2	48.33
10 plants per m^2	48.66
LSD (5%)	ns
Cultivar	
'Amazon Bird'	53.50[a]
'Yellow Sun Bird'	45.25[b]
'Giant Blue Bird'	46.00[b]
LSD (5%)	1.1974
CV (%)	2.93

Means followed by different letters in the same column are significantly different at the 5 % level of probability; ns= non-significant difference

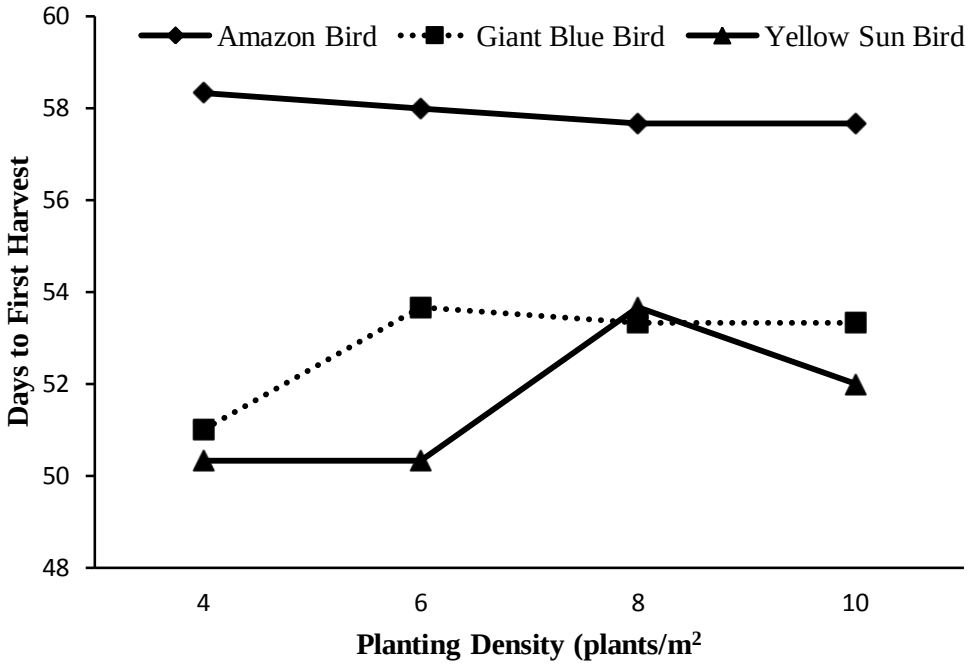

Figure 1. Influence of interaction between cultivar and planting density on days to first harvest

LSD at 5% was used to discriminate among the means.

RESULTS

Days to flowering

A highly significant (P<0.01) variation was noticed among cultivars with respect to days to 50% flowering. On the other hand, no significant results were obtained among the different planting densities and for the interaction effects between cultivars and planting densities. Accordingly, the mean comparison for cultivars revealed that 'Yellow Sun Bird' (45.25 days) and 'Giant Blue Bird' (46.0 days) flowered earlier than the 'Amazon Bird' (53.5 days) (Table 1).

Effect on days to first harvest

As depicted in Figure 1, the interaction effect between cul-

Table 2. Effect of Planting Density and Cultivar on the Number of Marketable Stems

Treatments	Number of marketable stems/m^2
Planting density	
4 plants per m^2	14.30[d]
6 plants per m^2	21.15[c]
8 plants per m^2	27.03[b]
10 plants per m^2	30.98[a]
LSD (5%)	1.0108
Cultivar	
'Amazon Bird'	19.79[c]
'Yellow Sun Bird'	26.23[a]
'Giant Blue Bird'	24.10[b]
LSD (5%)	0.8754
CV (%)	4.42

Means followed by different letters in the same column are significantly different at the 5 % level of probability. MS=Marketable Stems; ns= non-significant difference

tivars and planting densities on days to first harvest was found to be highly significant (P<0.01). Accordingly, 'Amazon Bird', in all planting densities, took a much longer time than the other cultivars, perhaps attributed to the fact that this cultivar took a longer time for its vegetative stage. On the contrary, the least number of days required for first harvest was noted for cultivar 'Yellow Sun Bird' in which planting at a density of 4 and 6 plants per m^2 resulted in a first harvest within a short time (50.33 days) probably because of the vigorous growth nature of the cultivar which was observed during the growing period. However, this was not significantly different from the 'Giant Blue Bird' cultivar which was planted at a density of 4 plants per m^2 (51.00 days).

Marketable stems per plot

A highly significant (P<0.0001) variation was observed among the cultivars and planting densities in relation to production of marketable stems per plot. However, there appeared to be no significant interaction effect among cultivars and planting densities. As indicated in Table 2, the highest number of marketable stems (30.98) was harvested at the maximum planting density (10 plants/m^2) while the least (14.30) was obtained at the lowest planting density (4 plants/m^2). As far as the variations among cultivars are concerned, 'Yellow Sun Bird' exhibited the highest number of marketable stems (26.23) when compared with the other two cultivars.

In order to determine the production dynamics, the trend for yield of marketable stems of the three cultivars under the different planting densities was observed for six months and the data were analyzed using the mean values for each month starting from the time of harvest (October). The result reveals that the number of marketable stems increased for the first four months and declined for the last two months in all the cultivars.

Among the cultivars, 'Yellow Sun Bird' gave the highest number of stems per plot in all the months under all planting densities. The three cultivars showed higher production from November to January. Such kinds of production dynamics could help growers to schedule their labor, agronomic practices, production period, and when to cut back the whole plant or replace it (Figure 2).

Unmarketable stems per plot

A highly significant variation was observed among the cultivars and planting densities in relation to production of unmarketable stems per plot. However, the interaction effect among cultivars and planting densities was found to be non significant. Consequently, the flower stems harvested from plots of the highest planting density (10 plants/m^2) resulted in the maximum number of unmarketable stems. It appears that increased plant density has significantly raised not only the number of marketable stems but also the number of unmarketable stems. The least number of unmarketable stems were recorded for cultivar Giant Blue Bird which was statistically different from other two cultivars (Table 3).

In view of the temporal distribution of unmarketable stems of the three cultivars with different planting density, the mean values of each month (October to March) were used to analyze and determine the production dynamics. The result reveals that the number of unmarketable stems was lower for all the cultivars with their respective planting densities for the first three months but started to rise from the fourth month and reached to its highest amount in March (Figure 3). In most cases 'Amazon Bird' had the highest reject rate due to short stem length.

Quality parameters

Fresh weight

The results pertaining to fresh weight revealed that there was a highly significant variation among cultivars and planting densities. On the contrary, the interaction effect between cultivars and planting densities was found to be non significant. Referring to the effect of planting densities on fresh weight of flower stems, the obtained results depict that as planting density increased from 4 to 10 plants per m^2, fresh weight significantly decreased from 72.4 to 54.0 g (Table 4). Pertaining to cultivars difference in terms of fresh weight, 'Yellow Sun Bird' showed the highest fresh weight followed by 'Giant Blue Bird' and 'Amazon Bird' with fresh weight of 75.9, 62.2, and 56.5 g, respectively.

The mean fresh weight of flower stems of each cultivar planted at different planting densities was taken for six consecutive months and the data were analyzed using the mean values for each month (October to March). The result reveals that the fresh weight of the harvested

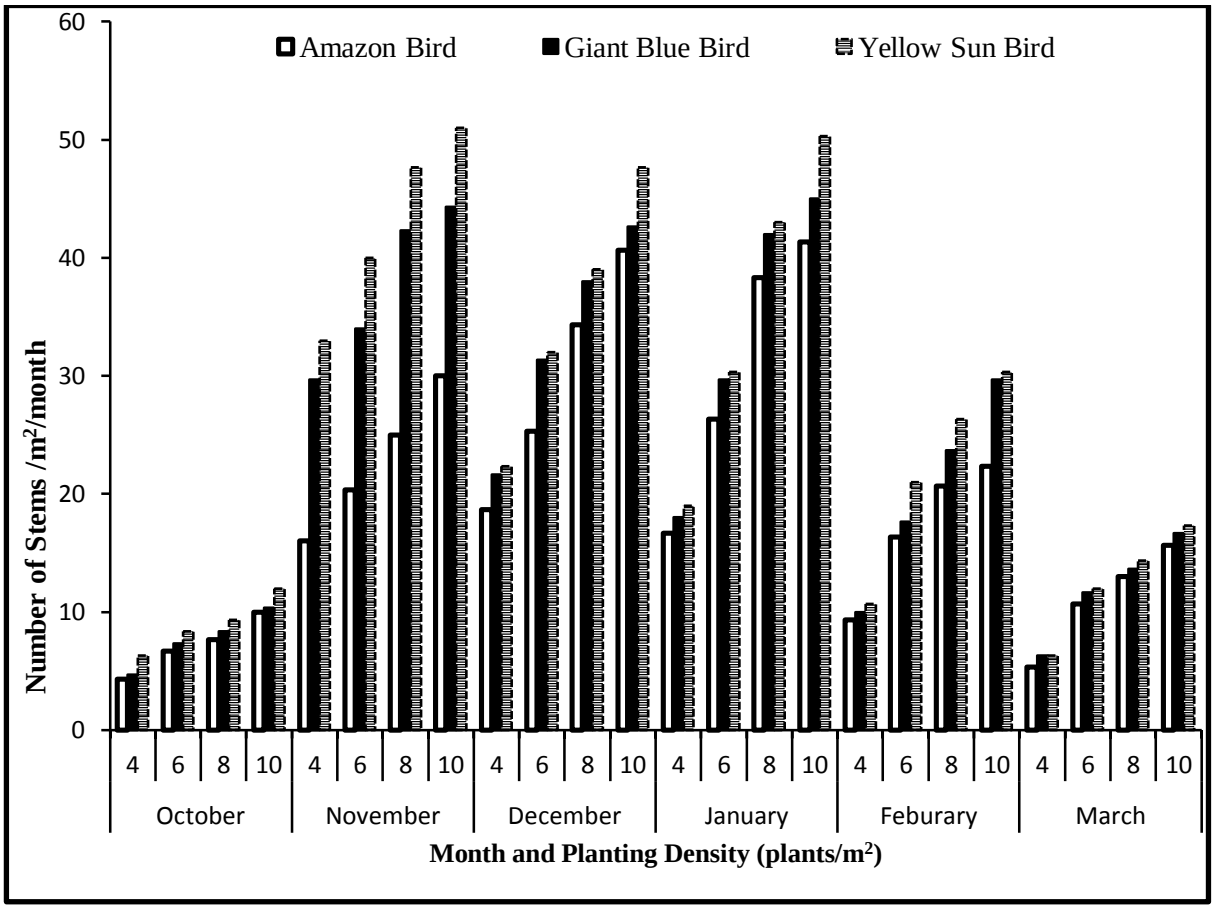

Figure 2. Trend of marketable stems of the three cultivars with different planting densities

Table 3. Effect of planting density and cultivar on the number of unmarketable stems/m^2.

Treatments	Number of unmarketable stems/m^2
Planting density	
4 plants per m^2	2.84^d
6 plants per m^2	4.53^c
8 plants per m^2	7.77^b
10 plants per m^2	11.16^a
LSD (5%)	0.5626
Cultivar	
'Amazon Bird'	7.08^a
'Yellow Sun Bird'	6.75^a
'Giant Blue Bird'	5.88^b
LSD (5%)	0.4872
CV (%)	8.75

Means followed by different letters in the same column are significantly different at the 5 % level of probability; ns= non-significant difference

stems increased for the first two months and started to decrease in the next four months in all the cultivars and planting densities (Figure 4). The stems started to become unmarketable starting from February when the weight of each stem was less than the required quality (< 30 g).

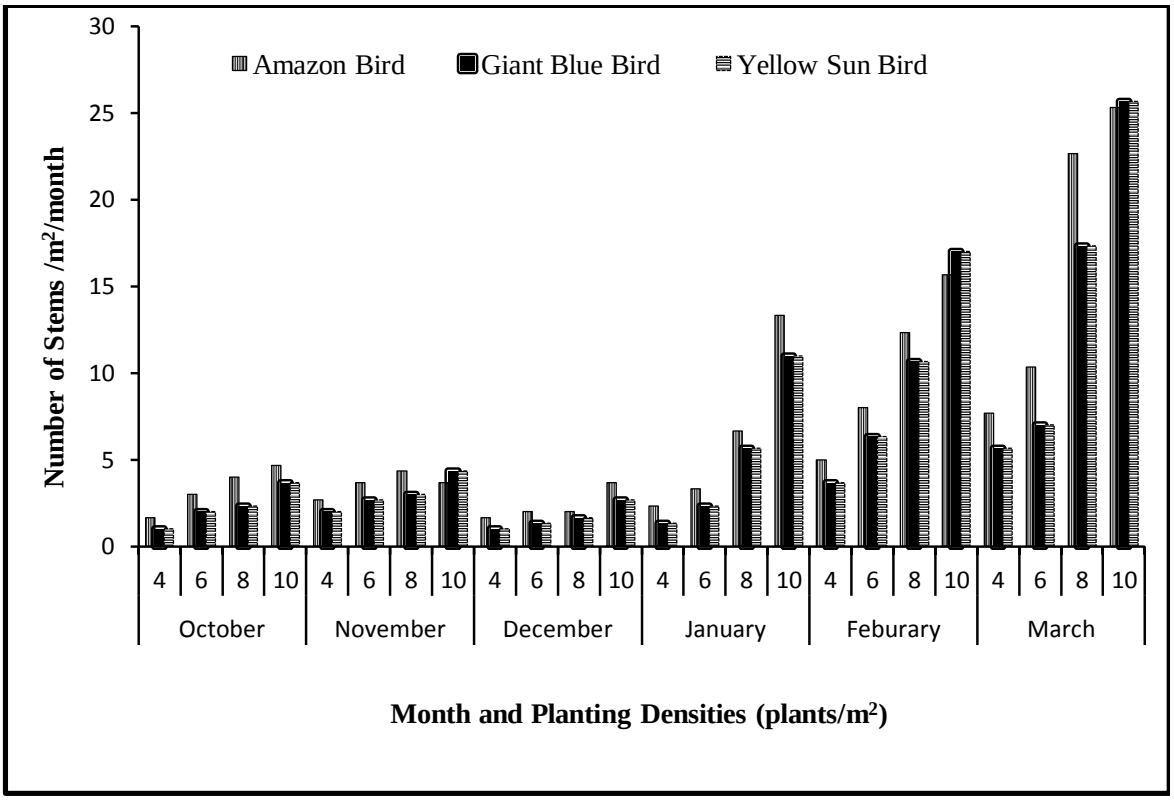

Figure 3. Trend of unmarketable stems of the three cultivars with different planting densities.

Table 4 . Effect of planting density and cultivar on fresh weight flower stems.

Treatment	Fresh weight of flower stems (g/m^2)
Planting density	
4 plants per m^2	72.38^a
6 plants per m^2	68.99^b
8 plants per m^2	64.06^c
10 plants per m^2	54.00^d
LSD (5%)	2.2381
Cultivar	
'Amazon Bird'	56.52^c
'Yellow Sun Bird'	75.87^a
'Giant Blue Bird'	62.17^b
LSD (5%)	1.9383
CV (%)	3.53

Means followed by different letters in the same column are significantly different at the 5 % level of probability; ns= non-significant difference

Number of branches per stem

Highly significant variation was observed among cultivars in terms of the number of branches produced. Accordingly, cultivar 'Giant Blue Bird' was revealed to have many more branches per stem when judged against the other two cultivars (Table 5).

Number of spikes

The tested cultivars varied significantly with respect to

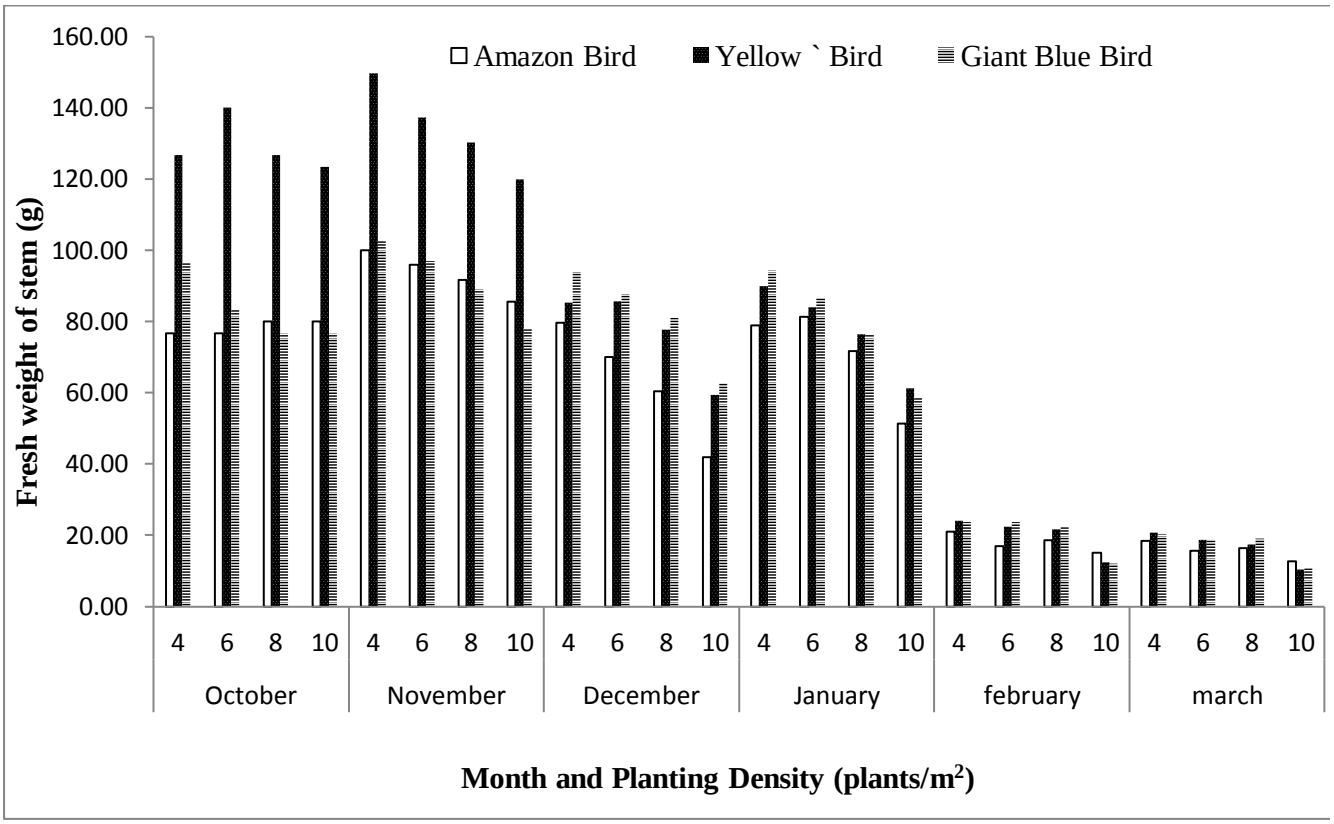

Figure 4. Trend of Fresh Weight of the three Cultivars with Different Planting Densities for six months

Table 5. Effect of planting density and cultivar on number of branches

Effects and levels	Number of branches per plant
Planting density	
4 plants per m^2	4.08
6 plants per m^2	4.19
8 plants per m^2	3.98
10 plants per m^2	3.93
LSD (5%)	ns
Cultivar	
'Amazon Bird'	3.70^c
'Yellow Sun Bird'	3.89^b
'Giant Blue Bird'	4.54^a
LSD (5%)	0.3404
CV (%)	9.94

Means followed by different letters in the same column are significantly different at the 5 % level of probability; ns= non-significant difference

the number of spikes produced. On the contrary, planting density and the interaction effect between the cultivars and the planting densities was found to be non signify-cant. The results in Table 6 shows that the number of spikes produced by cultivar 'Giant Blue Bird' were higher than the other cultivars.

Table 6. Effect of planting density and cultivar on number of spikes

Effects and Levels	Number of spikes
Planting density	
4 plants per m^2	8.30
6 plants per m^2	7.72
8 plants per m^2	7.55
10 plants per m^2	7.73
LSD (5%)	ns
Cultivar	
'Amazon Bird'	6.83^b
'Yellow Sun Bird'	6.90^b
'Giant Blue Bird'	9.75^a
LSD (5%)	0.5116
CV (%)	7.72

Means followed by different letters in the same column are significantly different at the 5 % level of probability; ns= non-significant difference

Table 7. Effect of planting density and cultivar on stem length

Effects and levels	Stem length (cm)
Planting density	
4 plants per m^2	71.57^c
6 plants per m^2	74.10^b
8 plants per m^2	75.14^b
10 plants per m^2	78.23^a
LSD (5%)	2.0621
Cultivar	
'Amazon Bird'	68.24^b
'Yellow Sun Bird'	78.54^a
'Giant Blue Bird'	77.50^a
LSD (5%)	1.7858
CV (%)	2.82

Means followed by different letters in the same column are significantly different at the 5 % level of probability; ns= non-significant

Stem length

The height of flower stems exhibited a highly significant variation among cultivars and planting densities. As a result, maximum stem length (78.23 cm) was obtained from the highest planting density (10 plants per m^2) while the lowest stem length was noticed from smallest planting density (4 plants per $m^{2)}$). Among the cultivars, highest stem length (78.54 cm) was recorded from 'Yellow Sun Bird' which however was at par with cultivar 'Giant Blue Bird' (Table 7).

Vase life

A highly significant ($P<0.01$) difference was observed from the interaction between cultivars and planting densities with respect to the vase life of flower stems. The maximum number of days was recorded from cultivar 'Amazon Bird' planted at a density of 4 plants per m^2 (18.50). The shortest vase life was witnessed from 'Yellow Sun Bird' planted at 10 plants per m^2 (10.91) (Figure 5).

DISCUSSION

Determining the optimum plant density for high value crops like Statice is vital to effectively contend in international markets which always demands superior quality products. Plant density affects plant growth as well as the yield and quality of crops. Plant density effects may vary with genotype, agro-climate condition of the growing area, etc (Singh et al., 2011).

The findings of this study have shown obvious variations among the means of the evaluated parameters used to assess the yield and quality of cut Statice cultivars.

From the present experiment, the significant effect of cultivars on the days to 50% flowering was demonstrated. This result possibly occurred due to the inherent variability that exists in the respective cultivars. Zizzo et al. (2003) reported similar types of variations.

The combined influence of planting density and cultivars on days to first harvest might be attributed to the dissimilarity of the cultivars in terms of growth nature. Such possible explanation was also forwarded by other authors (Dole and Wilkins, 2005; Tabassum et al., 2002).

On the other hand, the number of marketable flowers revealed an increasing trend with increasing planting densities. This result illustrated the existence of a positive relationship between planting densities and marketable yield. Obviously, as the number of plants per unit area increased, more marketable stems per unit area could be obtained provided that each plant has the capability to produce more under high competition. These results are in agreement with those of Mili and Sable (2003). On the contrary, it appears that increased plant density had increased not only the number of marketable stems but also the number of unmarketable stems. This may be substantiated by the fact that as the plants were crowded due to space limitation, their stem apparently tended not to grow upright resulting in stem-interlocking and then twisting and bending. Moreover, competition for light in crowded conditions may lead to lower photosynthate production and hence results in weaker and lighter (< 30 g) stems. The result obtained in this study is in conformity with what has been reported by Hossain et al. (2003). These authors suggested that optimum plant density guarantees the plants to grow properly through efficient consumption of solar radiation and nutrients.

Regarding the temporal distribution of the flower stem gross product of the three cultivars under different planting densities, the result obtained reveals the existence of

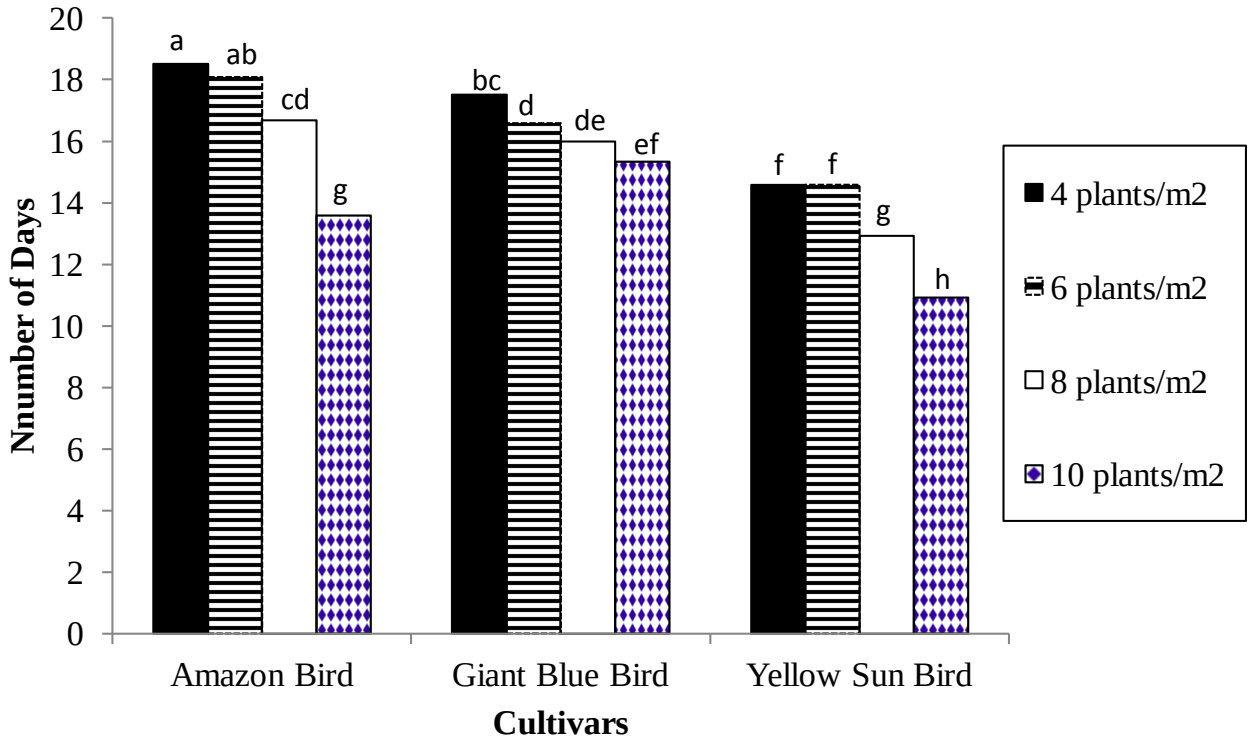

Figure 5. Influence of interaction effect of cultivar and planting density on vase life.

parallel relationship between marketable and unmarkedable yield. Proportionally, unmarketable yield for 'Amazon Bird' was found to be much higher than the other cultivars. Understanding of the yield dynamics throughout the length of the harvest period could help growers to schedule their labor, agronomic practices, production period, and when to cut back the whole plant or replace it.

The occurrence of variation in flower stem fresh weight due to differences in plant density and cultivar might be credited to the limited dry matter accumulation in the stems at higher planting densities owing the severe competition for light and nutrients and inherent variability of the genotypes. This result is in agreement with fact stated by Christine (1996).

The enlarged stem length due to varying of the plant density could be associated with the increased plant population per unit area which may cause poor light interception. Similar ideas were declared by Paparozzi and Hatterman (1988), Starman et al. (1995) and Christine (1996).

Similarly, in agreement with the present study, stem length was reported to vary with cultivar and spacing (Tabassum et al., 2002; Bakheit et al., 2012).

On the other hand, the observed higher vase life in the lower planting density might be due to the adequacy of the nutrients which ensured the presence of enough stored food in the harvested stems (Ghaffoor et al., 2000; Da Silva, 2003; Kazemi et al., 2011).

Conclusion

Foreign export is a key factor for building the economy of developing countries like Ethiopia. The floriculture industry in Ethiopia has been rapidly growing for the last five years. The country with all its resources has a big potential to expand its Agricultural export. Despite all these facts, research back up is known to be minimal.

In general, the investigation proved the existence of valid effects on the yield and quality of Statice as plant density and cultivars were varied. Cultivars 'Giant Blue Bird' and 'Yellow Sun Bird' exhibited the best performance in most of the considered quality and yield parameters and therefore can be recommended for the highlands area of Ethiopia. Moreover, from the tested planting densities, 10 plants per m^2 had shown better results as compared to the other densities in terms of marketability, premium fresh weight and stem length. For realistic recommendation, however, further research should be conducted to find ways on how to deal with issues such as vase life improvement and the like.

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African Journal of Plant Science

Full Length Research Paper

Chromosome numbers and karyotype in three species of the genus *Vernonia* Schreber in Southern Nigerian

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Detailed cytological studies were carried out on three species of the genus *Vernonia* namely *Vernonia amygdalina* (bitter leaf and non-bitter leaf), *Vernonia cinerea* and *Vernonia conferta* to ascertain their chromosome number. The taxa studied showed diploid number of chromosome for *V. cinerea* (2n = 18) and *V. conferta* (2n = 20) and tetraploid number for *V. amygdalina* (2n = 36). The karyotype show nine (9) pairs of submetacentric chromosomes in *V. cinerea* and 10 pairs of submetacentric chromosomes in *V. conferta.* The karyotype of *V. amygdalina* (bitter leaf) varied from that of *V. amygalina* (non-bitter) by being larger in size and with a pair of telocentric chromosome. The studies of the pollen fertility suggest that *V. amygdalina* is an amphidiploid.

Key words: Chromosome numbers, karyotype, polyploidy, *Vernonia*.

INTRODUCTION

Vernonia is a large tropical genus with about 1,000 species both in the old and new worlds (Jones, 1976, 1979). *Vernonia* belongs to the family compositae (Asteraceae). The family Asteraceae belongs to the order Asterales. The family compositae is the largest family of the flowering plants, comprising 950 genera and 23,000 species (Gills, 1988). The genus *Vernonia* is represented by about 500 species all over the world and 49 species in Flora of Ethiopia (Mesfin, 2004).

Adedeji and Jewoola (2008) noted that the family compositae possess simple leaves with alternate or opposite leaf arrangement. Among the species found in Nigeria, *Vernonia amygdalina* Del, *Vernonia cinerea* (Linn) Less and *Vernonia conferta* Benth form an interesting group to study because *V. amygdalina* is treated as a shrub while *V. cinerea is* a herbaceous weed and *V. conferta* is a small tree. Also the occurrence of bitter and non-bitter leaves of *V. amygdalina* is of interest. Chromosomes have been used to assign organisms to different taxa as members of the same species have similarity in their chromosome sets and related species have related chromosome sets (Gill and Singhal, 1998; Stace, 2000). It has been realized from the early years of this century that in general, the number of chromosome in each cell of the individuals of a single species is constant. Moreover, except for simple multiples of that number the more closely related species are the more likely to have the same chromosome number, and the more distantly related, the more likely they are to have different number. This relative conservativeness and inability of the environmental factors to alter it renders chromosome number an important and much used taxonomic character. It is consistently recorded in standard floras and the like (Stace, 1980).

Chromosome number may change in various ways and results in a new chromosome set which has effect on the general biology of the organism (Schubert, 2007). Polyploidy is the commonest of all changes in chromo-

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some number,especially in plants (Stace, 2000). This increase in chromosome number by complete set is of two types based on the origin of the additional chromosome set. Instudying published chromosome lists, it becomes evident that closely related species (within one genus) may differ in chromosome number, the most frequent variations based on the phenomenon of polyploidy (Swanson, 1968). Ikechukwu (2011) studied two species of Abrus in Nigeria and reported that *Anolis pulchellus* is a polyploidy of *A. pulchellus.* The structure of the chromosome together with the size and number has been found extremely useful at all levels of the taxonomic hierarchy. For members of the genus *Vernonia*, some chromosome counts and their taxonomic importance have been given and examples include *V. cinerea* (n = 9) (Olorode, 1974), *V. cinerea* (n = 9) (Jones, 1976) *V. cinerea* (2n = 18) (Andhra, 1981). According to Jones (1976), there are several kinds of polyploidy number relationship in flowering plants. *Vernonia* in the old world has a dibasic chromosome number of n = 9 or 10 with polyploids of n = 18, 20 or 30, whereas in the New world it has basic chromosome number of n = 17 with polyploids of 34, 51, 58 or 68. This paper is aimed at reviewing the number of chromosome of *V. amygdalina*, (bitter variety), *V. cinerea* and *V. conferta*. The chromosome number of the *V. amygdalina* (non-bitter variety) was determined. It will also investigate their polyploidy level and construction of a Karyotype for the taxa studied.

MATERIALS AND METHODS

Stem cuttings of the four different taxa were collected and grown in the field in small pots filled with wet soil. Auxiliary buds emerged and roots ranging from 10 to 40 mm in length were produced after two to three weeks, healthy roots were excised and transferred to collection bottles containing 0.002 m aqueous solution of 8 - hydroxyquinoline. This pretreatment was carried out to accumulate metaphase through spindle fibre inhibition (Darlington and Lacour, 1975). After 3 h in this solution, root tips were fixed in 3:1 ethanol acetic acid (V/V) for at least 24 h. The root tips were used immediately and some were stored in 70% alcohol in a refrigerator.

For microscopic observation a little portion of the root-tip, about 1 mm from the apex was excised and squashed in a drop of F.L.P. orcein stain (2 g of Orcein dissolved in 100 ml of solution of equal parts of formic acid, lactic acid, propanoic acid and water) under a cover slip, flattened out and examined under a microscope following the method of Okoli (1983). Photomicrographs of the chromosomes were taken from good temporary slides, using a leitz - habolux-12-microscope fitted with WILD - WPS camera. The flower heads of the different taxa studied were collected and pollen from anthers teased out on a slide, stained with cotton-blue lactophenol and viewed under the microscope to ascertain the percentage of fertility.

RESULTS

Mitotic studies of the four taxa studied show that *V. amygdalina* (bitter leaf) has mitotic chromosome number of 2n = 36, *V. amygdalina* (non-bitter leaf) 2n = 36, *V. cinerea* 2n = 18 and *V. conferta* 2n = 20 (Plates 1 to 4). The karyotype of *V. amygdalina* (bitter variety) consists of one (1) pair of telocentric chromosomes, one (1) pair of metacentric chromosomes and 16 pairs of submetacentric chromosomes (Plate 5A). *V. amygdalina* (non-bitter variety) consists of one (1) pair of telocentric chromosomes and 17 pairs of submetacentric chromosomes (plate 5B). *V. cinerea* consists of 9 pairs of large submetacentric chromosomes (Plate 5C). *V. conferta* consists of 10 pairs of submetacentric chromosomes (Plate D). Pollen fertility studies revealed that the fertility rate in *V. amygdalina* (bitter leaf) is 82.55% *V. amygdalina* (non-bitter leaf) is 74.57, 68.95% in *V. cinerea* and 66.00% in *V. conferta*

DISCUSSION

The results of the chromosome number of the *Vernonia* species studied show that *V. amygdalina* (bitter and non-bitter leaves) have 2n = 36, *V. cinerea* 2n = 18 and *V. conferta* 2n = 20. The chromosome count of *V. cinerea* corresponds with the work of Jones (1976) and Andhra (1981).

In studying published chromosome lists, it becomes evident that closely related species (within one genus) may differ in chromosome number, the most frequent variations based on the phenomenon of polyploidy (Swanson, 1968). Groups of organisms in which there is a range of chromosome numbers representing different degrees of polyploidy (ploidy levels) are known as polyploidy series. Interspecific variation in chromosome numbers has proved to be one of the richest sources of cytological data of value to taxonomists. At this level, there is usually a fairly obvious single base-number, from which the variations in chromosome number have been derived to produce aneuploids and polyploids (Stace, 1980).

The 36, 18, 20 chromosome counts made on these *Vernonia* species studied could be regarded as a polyploidy series. *V. amygdalina* 2n = 36 is tetraploid, *V. cinerea* 2n = 18 is a diploid and *V. conferta* 2n = 20 is also a diploid. According to Jones (1976), there are several kinds of polyploidy number relationship in flowering plants. *Vernonia* in the old world has a dibasic chromosome number of n = 9 or 10 with polyploids of n = 18, 20 or 30, whereas in the New world it has basic chromosome number of n = 17 with polyploids of 34, 51, 58 or 68. The ploidy level of the *Vernonia* species studied suggest basic chromosome number of n = 9 or 10 because *V. cinerea* is a diploid plant with chromosome number 2n = 18 while *V. conferta* which is also diploid has a chromosome number of 2n = 20. The dibasic chromosome number of the *Vernonia* species studied suggests that they all belong to the genus *Vernonia* in the old world. This conforms to the work of Jones (1976).

The karyotype of *Vernonia* species studied shows telo-centric, metacentric and often submetacentric chromo-

Plate 1. Mitotic chromosomes of *V. amygdalina* (bitter leaf), 2n = 36. 100x.

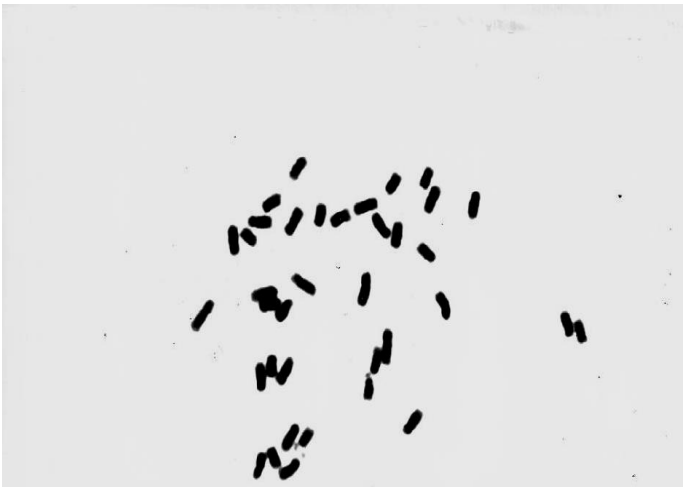

Plate 2. Mitotic chromosomes of *V. amygdalina* (non-bitter leaf) 20 = 36 100x.

Plate 3. Mitotic chromosomes of *V. cinerea* 2n = 18. 100x.

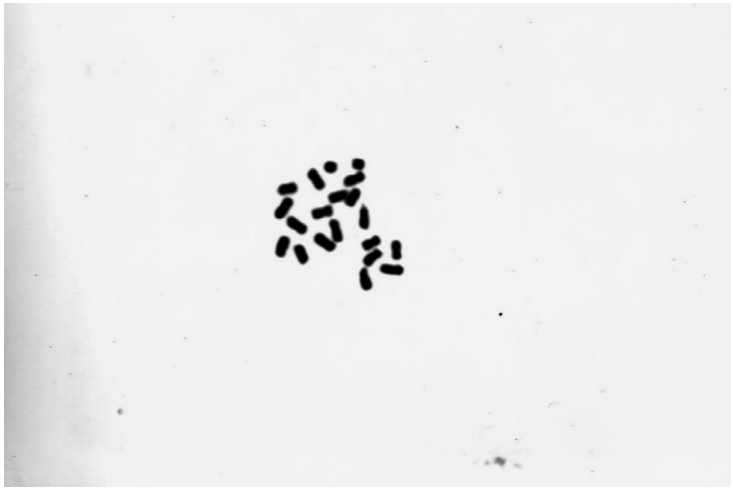

Plate 4. Mitotic chromosomes of *V. conferta* 2n = 20. 100x.

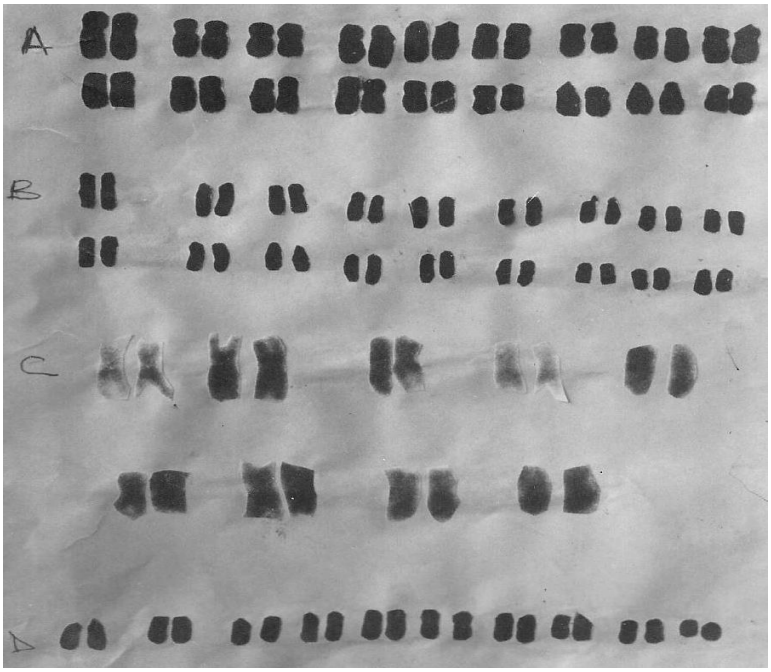

Plate 5. Karyotype of *Vernonia* species A: Tetraploid *V. amygdalina* (bitter leaf) 2n = 36. B: Tetraploid *V. amygdalina* (non-bitter leaf) 2n = 36. C: Diploid *V. cinerea* 2n = 18. D: Diploid *V. conferta* 2n = 20.

chromosome. The large nine pairs of submetacentric chromosome in *V. cinerea* suggest that the species is more primitive than the other *Vernonia* species studied. Polyploidy has been utilized in the past, as a positive marker of the direction of evolution which would indicate the primitive and the derived groups or at least derivations which are not possible using the negation principle. It has been widely held that diploids are more primitive forms from which polyploids arose and that this change is irreversible (Stace, 1980).

The study of the pollen fertility reveals that all the taxa of *V. amygdalina* are pollen-fertile. These suggest that both *V. amygdalina* (bitter leaved) and *V. amygdalina* non-bitter leaf which are polyploidy, are amphidiploids.

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Full Length Research Paper

Earliness and yield parameters of eggplant (*Solanum melongena* L.) grafts under different spacing and fertigation levels

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Field study was undertaken to standardize spacing and fertigation levels for eggplant (*Solanum melongena* L.) grafts. A strip plot design was adopted with four spacing levels (1 × 1, 2 × 1, 1.5 × 1.5 and 0.6 × 0.6 m) and three fertigation levels: 75, 100 and 125% recommended dose of fertiliser (RDF) replicated four times. After six months, the plants were pruned to obtain the ratoon crop which lasted four months. The days taken for 50% flowering and first harvest were affected by nutrition and neither spacing nor interaction. The 75 and 100% RDF were on par for early flowering (32.00 and 32.38 days in main crop; 16.88 and 17.00 days in ratoon crop); and early harvest (44.38 and 45.44 days in main crop; 34.38 and 35.56 days in ratoon crop). The treatment combination 1 × 1 m + 100% RDF recorded the highest marketable yield of 110.25 and 59.42 t/ha, in main and ratoon crops, respectively. Thus, cultivation of eggplant grafts under 1 × 1 m spacing along with 100% RDF (200:150:100 kg NPK/ha) through drip fertigation is recommended.

Key words: Fertigation, plant density, *Solanum melongena* L., vegetable grafting.

INTRODUCTION

Grafting of eggplant cultivars on perennial and wild *Solanaceous* species was proved to increase yield and availability period of the fruits (Gisbert et al., 2011; Lee, 1994). The use of *Solanum torvum* as rootstock was reported to confer resistance to *Verticillium* wilt, *Fusarium* wilt, bacterial wilt and root knot nematode (King et al., 2008; Sebahattin et al., 2005). Grafting is also high effective in ameliorating crop losses caused by adverse environmental conditions (Schwarz et al., 2010). The use of vegetable grafts will be most successful when complemented with sustainable farming system practices (Kubota, 2006). Among them, plant spacing is an important agronomic attribute since it is believed to have effects on light interception for photosynthesis which is the energy manufacturing medium using green parts of the plant. Also it affects rhizosphere exploitation by the plants (Ibeawuchi et al., 2008). Plant nutrition also plays an important role for enhancing yield of eggplant. Fertilizers applied under traditional methods are generally not utilized efficiently by the crop; while in drip fertigation nutrients are applied directly into the zone of maximum root activity and consequently fertilizer-use efficiency can be improved over conventional method of fertilizer application (Hebbar et al., 2004). However, according to

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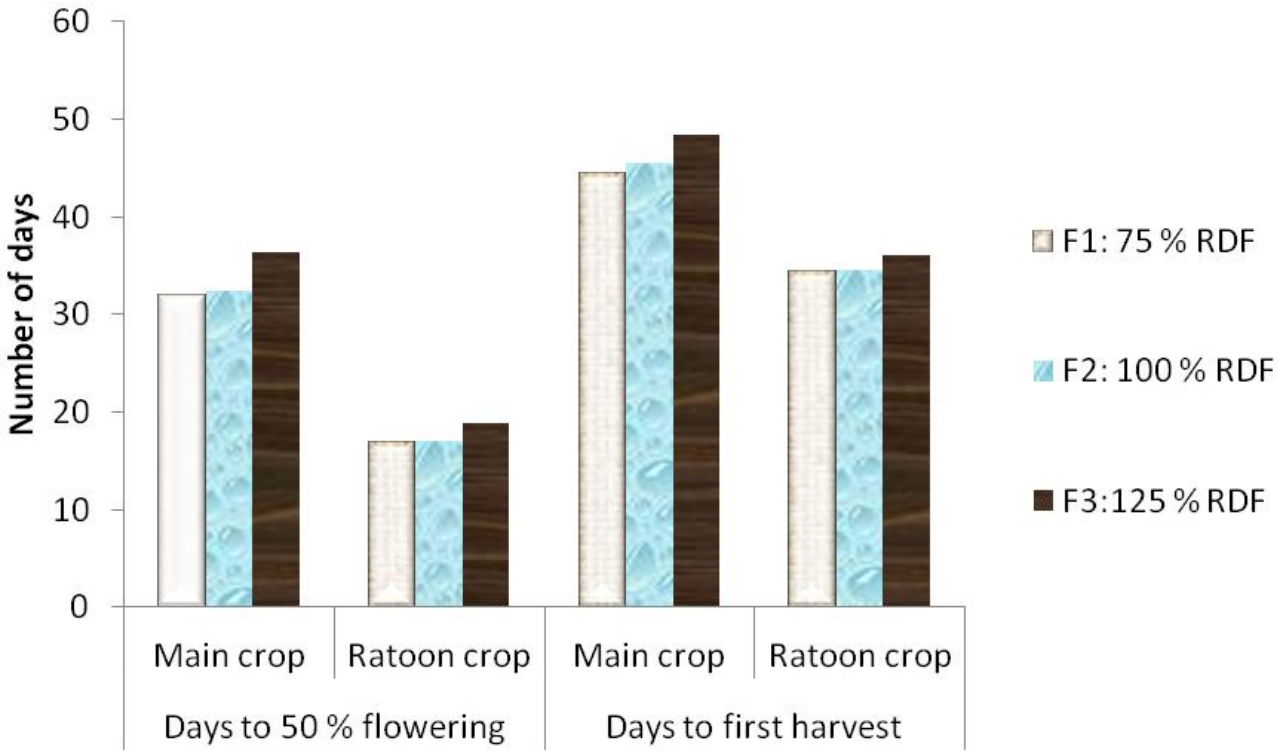

Figure 1. Effect of fertigation levels on days to 50% flowering and first harvest in eggplant grafts.

Colla et al. (2010) and Arao et al. (2008), available information on spacing and nutrition for grafted vegetables is not sufficient for commercial cultivation.

Therefore, the aim of this article is to provide the results of field experiment conducted to find out the effect of spacing and fertigation levels on earliness and yield parameters for eggplant (*Solanum melongena* L.) grafted onto *S. torvum*.

MATERIALS AND METHODS

The experiment was carried out at the University Orchard, Department of Vegetable Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. The study location is situated at 11° North latitude and 77° East longitude and at an elevation of 426.6 m above mean sea level. *S. torvum* was used as rootstock and the scion was eggplant (*S. melongena* L.) F_1 hybrid, COBH 2. A strip plot design was adopted with four levels of spacing (S1: 1 × 1 m, S2: 2 × 1 m, S3: 1.5 × 1.5 m and S4: 0.6 × 0.6 m) and three levels of fertigation (F1: 75% RDF, F2: 100% RDF and F3: 125% RDF) replicated four times. The recommended dose of fertilizer (RDF) adopted was 200:150: 100 kg of N: P: K / ha. Drip lines were laid to cover entire area of the field and planting was done in June 2011 by using forty days old, vigourous and healthy grafts. The 75% of P was given as basal application along with 25 ton ha of farm yard manure while 25% of P was given along with N and K through fertigation in equal splits from third week after planting. Fertilizers applied through fertigation were in the form of NPK 19:19:19, mono ammonium phosphate (12:61:0), potassium nitrate (13:0:45) and urea (46:0:0). Recommended cultural practices were followed. After six months, the plants were pruned to obtain ratoon crop which was maintained for four months. The data were recorded for days to 50% flowering, days to first harvest, fruit weight, number of marketable fruits per plant, marketable yield per plant and marketable yield per hectare.

Analysis of variance was performed for all the recorded data by using *M Stat-C* Software package and the level of significance was set at $p < 0.05$. LSD test was conducted for pair-wise comparisons of means.

RESULTS

Effect of spacing and fertigation levels on days to 50% flowering and first harvest in eggplant grafts

The days taken to 50% flowering were significantly affected by fertigation levels and neither spacing nor interaction. The 75 and 100% RDF were on par for early flowering (32.00 and 32.38 days in main crop; 16.88 and 17.00 days in ratoon crop); and early harvest (44.38 and 45.44 days in main crop; 34.38 and 35.56 days in ratoon crop). Plants receiving 125% RDF recorded the delayed 50% flowering and first harvest in both main and ratoon crops (Figure 1).

Effect of spacing and fertigation levels on yield parameters in eggplant grafts

Spacing, fertigation levels and their interaction significantly

Table 1. Effect of spacing and fertigation on yield parameters in eggplant grafts.

Treatment	Main crop			Ratoon crop		
	Fruit weight (g)	Number of marketable fruits/plant	Marketable yield/plant (kg/plant)	Fruit weight (g)	Number of marketable fruits/plant	Marketable yield/ plant (kg/plant)
Factor A: Spacing (S)						
S1: 1 ×1 m	61.12[c]*	173.24[b]	10.36[c]	60.21[c]	95.85[c]	5.56[c]
S2: 2 × 1 m	64.90[b]	233.31[a]	14.82[b]	62.06[b]	119.88[b]	7.23[b]
S3: 1.5 × 1.5 m	67.44[a]	243.30[a]	16.07[a]	63.71[a]	126.32[a]	7.84[a]
S4: 0.6 × 0.6 m	54.99[d]	48.96[c]	2.71[d]	54.07[d]	33.20[d]	1.58[d]
CD (*P* = 0.05)	0.49	16.68	1.12	1.03	5.00	0.23
Factor B: Fertigation (F)						
F1: 75% RDF	53.71[c]	163.76[b]	8.87[b]	52.45[c]	89.16[c]	4.58[b]
F2: 100% RDF	62.85[b]	191.43[a]	12.23[a]	61.63[b]	98.79[a]	6.12[a]
F3: 125% RDF	69.79[a]	168.94[b]	11.87[a]	65.96[a]	93.49[b]	6.05[a]
CD (*P* = 0.05)	0.81	10.05	0.69	1.59	3.92	0.30
Interaction (S × F)						
S1F1	52.11[i]	162.92[e]	8.42[e]	51.21[g]	88.26[h]	4.29[e]
S1F2	62.17[e]	190.50[d]	12.30[d]	61.22[d]	105.83[f]	6.26[d]
S1F3	69.09[c]	166.32[e]	12.38[d]	67.22[ab]	93.45[g]	6.14[d]
S2F1	56.14[g]	217.23[c]	12.09[d]	54.79[f]	115.68[e]	6.11[d]
S2F2	65.74[d]	258.61[a]	17.49[b]	64.84[c]	124.20[bc]	7.83[b]
S2F3	72.82[b]	224.10[bc]	17.398[b]	66.55[b]	119.75[d]	7.74[b]
S3F1	59.06[f]	229.25[b]	13.40[c]	57.16[e]	121.34[cd]	6.71[c]
S3F2	68.90[c]	267.72[a]	18.92[a]	66.75[b]	130.23[a]	8.47[a]
S3F3	74.37[a]	232.93[b]	18.35[a]	68.19[a]	127.41[ab]	8.34[a]
S4F1	47.54[j]	45.64[f]	2.17[g]	46.64[h]	31.34[i]	1.23[g]
S4F2	54.61[h]	48.88[f]	2.67[f]	53.71[f]	34.90[i]	1.66[f]
S4F3	62.83[e]	52.39[f]	3.29[f]	61.86[d]	33.35[i]	1.85[f]
CD (*P* = 0.05)	0.83	10.00	0.60	1.15	3.81	0.36
Grand mean	62.11	174.71	10.99	60.01	93.81	5.55
CV (%)	0.90	3.85	3.92	1.29	2.73	4.39

*The mean followed by the same letter(s) are not significantly different at p = 0.05.

affected yield parameters namely, fruit weight, number of marketable fruits per plant, marketable fruit yield per plant and per hectare in both main and ratoon crops (Table 1 and Figure 2).

The 1.5 × 1.5 m spaced plants recorded the highest fruit weight (67.44 and 63.71 g) and number of 243.30 and 126.32 fruits/plant in main and ratoon crops, respectively (Table 1). The highest marketable yield (98.47 and 52.84 t/ha) was recorded under 1 × 1 m spacing in main and ratoon crops, respectively (Figure 2). The highest fruit weight (69.79 and 65.96 g) was recorded under highest nutrition level (125% RDF) while the highest number of 191.43 and 98.79 marketable fruits/plant and marketable yield (87.24 and 44.79 t/ha) were observed under 100% RDF in main and ratoon crops, respectively (Table 1 and Figure 2).

DISCUSSION

The spacing levels did not affect significantly the days to 50% flowering and first harvest; the reason could be that, at the initial stage up to the first harvest, there was no competition for space among different spacing levels. Similar results were also recorded by Birbal et al. (1995) in okra and Saglan and Yazgan (1995) in tomato. The delayed flowering and first harvest recorded by the plants fertilised with 125% RDF might have been due to the influence of higher level of nitrogen in delaying initiation of flowering caused by prolonged vegetative phase (Rajangam, 1991). Similar results were found by He and Chen (1996) in tomato and Suthar et al. (2005) in eggplant. In both main and ratoon crops, the 1.5 × 1.5 m spaced plants had the highest fruit weight and number of

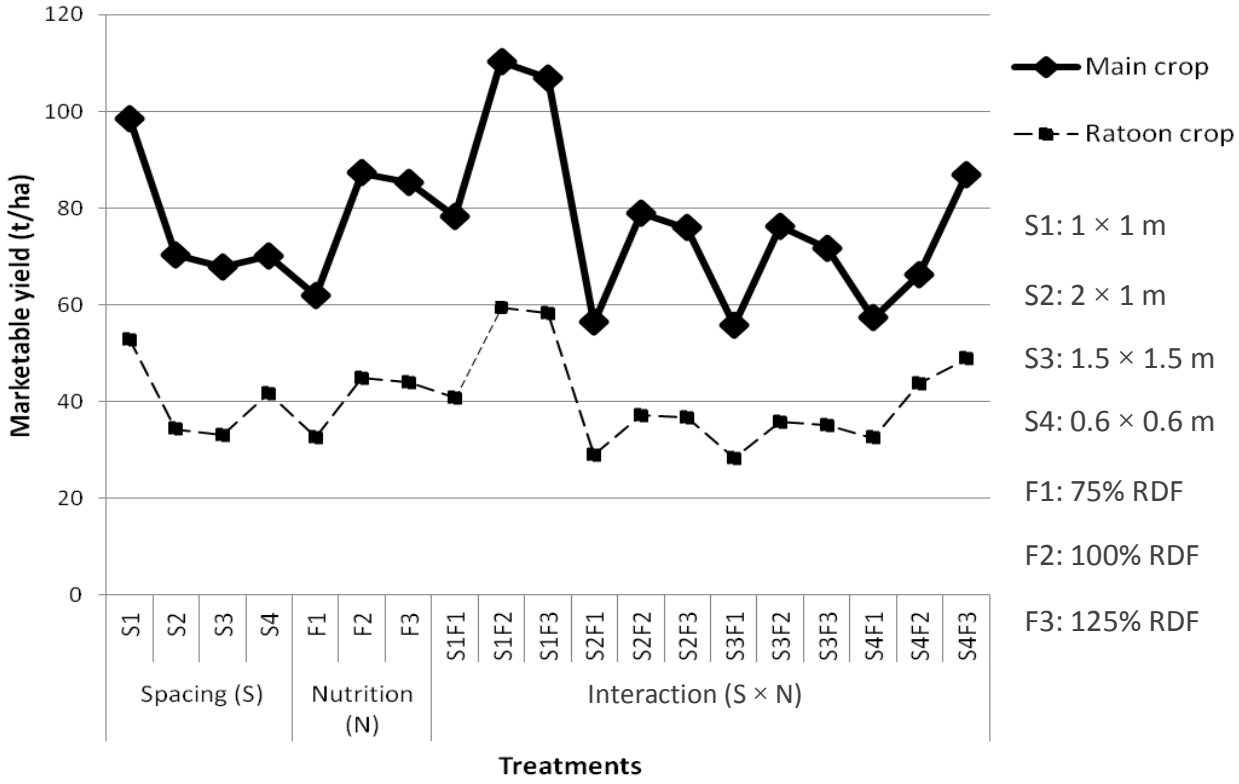

Figure 2. Marketable yield of eggplant grafts as affected by spacing and fertigation.

fruits per plant. Since these widest spaced plants had lowest competition for soil nutrients and light, it was most likely that they would produce more and bigger sized fruits (Sanchez et al., 1993). On other side, the overlapping of plants at the closest spacing might have resulted in competition for light and soil nutrients leading to low fruit performances when compared to the wider spaced plants. Similar findings were also reported by Nanthakumar and Veeraraghavathatham (2000) and Anburani et al. (2003) in eggplant.

The closer spacing (1 × 1 m) recorded the highest marketable yield in both main and ratoon crops. This could be attributed to the highest number of plants per hectare. Similarly, Reddy et al. (1990) observed in eggplant that the highest yield per hectare was obtained at the closest spacing. Mishriky and Alphonse (1994) reported in bell pepper cv. California wonder that the number of fruits and yield per plant were decreased; however, total yield per hectare was increased with closer spacing. In addition, Singh and Saimbhi (1998) opined that the magnitude of yield is influenced by plant population and its distribution pattern, which are important for getting maximum economic yield from a given field area. Comparable results were also obtained by Saglan and Yazgan (1995) in tomato.

The highest nutrition level (125% RDF) resulted in highest fruit weight; this might be due to the fact that nitrogen up to certain level increases shoot and leaf growth, which would have helped in the synthesis of greater amount of carbohydrates and more efficient protein synthesis to the developing fruits and that may have resulted in increased number of cells as well as elongation of individual cells. This in turn might have enhanced the size of fruits. Similar findings were also quoted by Reddy et al. (1990) in eggplant and Gare (2000) in chilli. Furthermore, phosphorus as an important constituent of nucleoproteins is involved in high energy transfer compounds such as adenosine diphosphate and adenosine triphosphate and plays a key role in energy transfer in the metabolic processes and thus, it would have contributed to the process. The potassium up to certain level would have also encouraged better utilization of assimilates through efficient transport to the developing fruits which acts as active sinks in eggplant (Clarkson and Hanson, 1980). In fact, the essential elements, particularly the primary nutrient elements N, P and K are supplied to plants to increase crop production (Nandekar and Sawarkar, 1990). Prabhu et al. (2006) also reported in eggplant that the fruit size and weight increased with increasing levels of N and P. The superiority of 100 over 125% RDF for fruit weight and number of marketable fruits per plant could be attributed to the fact that the excess nitrogen fertilizer application is associated with vigorous vegetative growth and extended duration for flower bud appearance, leading to the reduction in potential number of fruits per plant.

Batal et al. (1994) and Everaarts (1994) opined that

excessive nitrogen fertilization may increase crop susceptibility to pests, diseases and physiological disorders, and will not always ensure that marketing yield is increased. Similar results were found by Birbal et al. (1995) in okra, He and Chen (1996) in tomato and Suthar et al. (2005) in eggplant.

Conclusion

In eggplant grafts, the 1 × 1 m spaced plants and applied with 100% RDF recorded lowest number of days to 50% flowering (31.50 and 16.50 days) and days to first harvest (45.50 and 33.25 days) as well as the highest marketable fruit yield (110.25 and 52.42 t/ha) for main and ratoon crops, respectively. Therefore, cultivation of eggplant grafts under 1 × 1 m spacing along with 100% RDF (200:150:100 kg NPK/ha) through drip fertigation is recommended, due to its superiority for earliness and high marketable yield over other treatment combinations.

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Full Length Research Paper

Study on malting barley genotypes under diverse Agroecologies of north western Ethiopia

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The study was conducted with the objectives to determine the magnitude effect of genotype, environment, and their interactions on economically important traits and identify stable malt barley (*Hordeum distichon* L.) genotypes. Combined analysis of variance indicated that the main effects due to environment, genotype and GxE interaction were highly significant for grain yield and economically important malting quality triats indicated that development of both specific and wide adaptable varieties are essential. The GxE interaction of grain yield was further partitioned using AMMI and it showed the first two IPCA axes explained most of the sum of squares. According to stability analysis measures genotype G1 was the most stable whereas G13 showed specific adaptation in low potential environments. Protein content and seed size variability measures revealed G9 and G11 in protein content and G1 and G11 in seed size, respectively as the least varying genotypes across environments.

Key words: GxE interaction, kernel protein content, malting barley, stability analysis.

INTRODUCTION

Barley (*Hordeum distichon* L.) is an important grain crop in Ethiopia. It has diverse ecologies being grown from 1800 to 3400 m altitude in different seasons and production systems. In the highest altitudes, barley is grown as a sole crop. The total area covered by the crop is about one million hectares with a total annual production of 1.3 million tons (CSA, 2005). Northwestern high and mid altitudes belong to the major barley producing areas however all of the produce is used for food purpose. On the other hand, breweries have been setup in the country, which require lots of malt annually. Majority of their requirement is obtained from import. Since malting barley is a new crop to the northwestern Ethiopia, information is unavailable about cultivar performance across diverse environments. The performance of malting barley grain yield and quality characteristics depends greatly on environmental conditions, which results in differential expression of grain quality from environment to environment (Verme and Nagaragam, 1996). The genotype by environment interaction is another important aspect in affecting performance of genotypes across diverse environments. The GxE interaction arises when there is differential response of genotypes in environmental changes. It reduces the correlation between the genotype and phenotype, hindering the genetic potential of the cultivar (Kang and Gorman, 1989). Selection of stable genotype is described as one of the strategies to reduce GxE interaction effect. High yield stability refers to a genotype's ability to perform consistently, whether at high or low yield levels, across a wide range of environments. According to Finlay and Wilkinson (1963), yield response and stability are heritable and can be measured. Lin et al. (1986) have described nine parametric stability statistics. Among them, are conventional coefficient of variation (CV_i), variance of genotypes across environments

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(Si^2), Finlay and Wilkinson (1963) regression coefficient (b_i), Eberhart and Russell (1966) deviation from regression (S_{di}^2), Shukla's (1972) stability variance (σi^2) and Wricke's (1962) ecovalence (W_i^2) are parametric stability measures of genotypes.

The objectives of this paper were to: i) assess phenotypic performance of malting barley genotypes across northwestern Ethiopia, ii) determine the magnitude of effect of genotype, environment, and their interaction on yield and quality traits, and iii) to identify stable genotype in grain yield and consistence performance in protein content and seed size across environments.

MATERIALS AND METHODS

Field experiment

The field experiment was carried out with 20 advanced malting barley genotypes; EH1609-F5.B3-10 (G1), 37622 (G2), 118173 (G3), Bekoji sel-8 (G4), 118007 (G5), EH1510-F6.10H.3 (G6), 108932 (G7), 118173 (G8), 118146 (G9), EH1746-F6.B2-109 (G10), EH1606-F5.B2-7 (G11), EH1601-F5.B2-2 (G12), EH1603-F5.B1-4 (G13), EH1612-F5.B3.13 (G14), HB1533-sels (G15), and Miscal-2 (G16), Miscal-1 (G17), HB-242-sels (G18), along with standard checks; HB-52 (G19) and HB-120 (G20). The experiment was conducted under rainfed conditions in four locations that represent different barley growing agro-ecologies during three growing seasons (2004, 2005, 2007) where E1 = Adet-2004, E2 = Adet-2005, E3 = Adet-2007, E4 = Debretabor-2004, E5 = Debretabor-2005, E6 = Debretabor-2007, E7 = Dabat-2004, E8 = Dabat-2005, E9 = Adet-2007, E10 = Laygaint-2004, E11 = Laygaint-2005 and E12 = Laygaint-2007. At each location, the genotypes were planted in a randomised block design in three replicates. Sowing was done by hand in plots of 3 m^2 with six rows measuring 2.5 and 0.2 m within row spacing. Fertiliser application was 41 kg N ha^{-1} and 46 kg P_2O_5 ha^{-1} at planting. The four middle rows with an area of 2 m^2 were harvested.

Data collected

Harvesting and data collection were done from the four central rows. Yield data was recorded on clean, dried samples and plot yields were adjusted to 12.5% moisture level and converted to kilogram per hectare. Thousand kernels were counted by using electronic seed counter and weighted (g). Every sample was measured in standard hectoliter and then weighted to know their hectoliter weight. Germination energy in percent was determined from 100 seeds germinated in a petridish after 120 h. Two hundred seeds were soaked in a flask with 0.3M H_2O_2 (hydrogen peroxide) and counting after 24 h and converted to percentage to determine germination capacity. Seed size test was carried out using 2.2, 2.5, 2.8 mm size sieves and proportion of the seed trapped by each sieves were weighted and converted to percentage. Eight gram samples from each plot were grounded and 5.0 g flour was placed in moisture dishes and was oven dried for 1 h at 100°C. Percent moisture was determined from the mass of water lost on drying to the original milled sample and kernel protein content was also determined using Kjeldahl method.

Statistical analysis

Analyses of variance were performed on all traits. Thereafter, combined analyses of variance were performed using fixed linear model where both genotypes and environments were fixed. Mean separation was carried out using least significant (LSD) at 5% level of significant. The G x E interaction was further partitioned using additive main effects and multiplicative interaction (AMMI) statistical model. The AMMI analysis of variance summarizes most of the magnitude of genotype x environment interaction into one or few interaction principal component analysis (Zobel et al., 1988; Guach, 1988). The larger the IPCA scores, either negative or positive, the more specifically adapted a genotype is to a certain environments; the smaller the IPCA scores, the more stable the genotype is over all environments studied. AMMI analysis was performed for grain yield. The method of Eberhart and Russell (1966) was used to calculate the regression coefficient (b_i), deviation from regression (Sd_i^2) and coefficient of determination (R_i^2). It was calculated by regressing mean grain yield of individual genotypes on environmental index. Shukula stability variance (σi^2) and Ecovalence (Wi^2) suggested by Wricke (1962) were also computed, where stability values with minimum values are considered stable.

Variability of genotypes for protein content and seed size across environments were also measured by coefficient of variation (CV_i) (Francis and Kannenberg, 1978) and genotypic variance across environmental (Si^2).

RESULTS

Phenotyping

Grain yield

Mean grain yield of locations averaged over genotypes was between 3376.0 and 831.0 $kgha^{-1}$ at E5 and E11, respectively (Table 2). The mean grain yield over all the genotypes and locations was 2178.7 $kgha^{-1}$. Moreover, performances of genotypes were not consistent across locations. G1, G12 and G13 won all the genotypes at all most all environments except E4, E5 and E6. At E4, G12 with grain yield of 4427.0 $kgha^{-1}$ and at E5, G5 with 4614.7 $kgha^{-1}$ were the top performing genotypes. G17 produced the least when genotypes are ranked according to their grain yield performance. When environments were compared, the highest mean grain yield (3376.0 $kgha^{-1}$) was obtained at E5, while E11 (831.0 $Kgha^{-1}$) and E12 (1791.50 $Kgha^{-1}$) were relatively low yielding environments. The low grain yield at these environments that represent Dabat could be because of the water logging of the trial site. Moreover, relatively small amount of annual rainfall coupled with high disease pressure resulted in poor stand and low grain yield (Table 1).

Malting quality traits

Highest mean thousand kernel weight was recorded at Adet (40.85 g) and lowest mean at Dabat (37.42 g). Genotypes G13 (46.1 g), G11(45.9 g), G3 (43.37) and G1(42.49 g) provided the highest thousand kernel weight but genotype G2, G16 and G17 had low mean thousand kernel weight over locations with corresponding values of

Table 1. Total annual rainfall, soil type and altitude of the locations.

Location	Altitude (m.a.s.l)	Total annual rainfall (mm)	Soil type
Adet	2240	1331.8	Nitosol
Debretabor	2630	1378.6	Luvisol
Dabat	2620	963.4	Cambisol
Laygaint	2500	950.4	NA

Sources: NMSA, BBO, Tsige (2002) and Yihenew (2004), NA: not Available.

33.1, 35.1 and 34.4 g. G15 also produced highest hectoliter weight 60.56 $kghl^{-1}$ and G2 with 56.66 $kghl^{-1}$ gave the lowest. The standards set for thousand kernel weight and hectoliter weight by National Standard Authority ranged from 35 to 45 g and 60 to 65 $Kghl^{-1}$, respectively. The mean thousand kernel weight of most genotypes, but G2 and G17 fulfill the requirement; however, hectoliter weight of genotypes averaged over all locations except G7, G10, G14 and G15 did not meet the standards. Kernel protein content of genotypes averaged from all locations was between 9.25% for G19 and G20 and 11.0% for G2 (Table 3). Protein content for G2, G4 and G5 was high with 11.0, 10.5 and 10.5%, respectively. All genotypes gave acceptable mean kernel protein and met the standards set by the National Standard Authority for malting barley that should be between 9 and 11.5. Kernel protein content that exceeds recommended levels is undesirable for malting because it increase steep times and cause uneven water uptake during steeping, uneven germination during malting, increased malt loss due to abnormal growth, excessive enzymatic activity, low extract yield, excessive nitrogenous compounds in the wort during brewing, and chill haze formation in beer (Burger et al., 1979). Table 4 reveals that Adet (11.49) and Debretabor (10.45) were the locations where high protein content was recorded while the mean kernel protein contents obtained from Dabat and Laygaint was 8.35 and 8.82, respectively and the standard set by Ethiopian National Standard Authority was not achieved at these locations. This might be due to low fertility and low temperature.

G1, G11 and G19 had the large mean percentage of kernels trapped by 2.8, 2.5 and 2.8 + 2.5 mm size sieves (Table 3). From the tested genotypes, only G1 and G11 fulfills the standard greater than 80% of the kernel that passed through 2.5 + 2.8 mm size sieve. Genotype that had high percent of screen loss was G2. These genotypes had small kernel size much below the standard as observed in the test using 2.2 and <2.2 mm size sieve. Other genotypes provided kernel sizes in between these two ranges. Highest percentage of acceptable kernel size (92.5%) was recorded at Laygaint. Adet was the locations where higher kernel sizes were recorded next to Laygaint (Table 4). From the result it can be deduced that genotypes grown at these location meet the standard. It can be seen from Table 3 that G4 obtained germination energy of 99.75% followed by G1 (99.42%). The least germination energy was obtained from G9 and G3 (97%). All genotypes showed germination energy that meets the standard (> 95% in 120 h). The highest germination energy (99.55%) was observed from seeds harvested at Dabat because of early harvesting (Table 4). All genotypes had germination capacity above 97% that is the minimum requirement set by National Standard Authority. G4 was the top in germination capacity with 100% seeds germinated.

Genotype by environment interaction components

The combined analysis of variance revealed a highly significant ($p<0.01$) variation for the genotype, environment and genotype by environment interaction effects for grain yield, thousand grain weight and hectoliter weight (Table 5). This indicated genotype, environment and their interaction are important in governing the expression of these traits. It agrees with the finding that yield and quality traits are influenced by genotype (G), environment factors (E) and their interaction (Uhlen et al., 1998). The presence of variation between environments point out the presence of dissimilarity and provide a baseline information to categorize potential, low potential and intermediate yielding agro ecologies. The genotype effect depicted clear variation among tested genotypes thus wide and specific adaptation of genotypes is crucial for vertical increase of production. The significant GxE interaction indicated the differential genotypic performance across environments. It reduces the association between phenotypic and genotypic values, and thus, genotypes that perform well in one environment may perform poorly in another (Fox et al., 1996).

Generally, larger interaction component cause difficulties in selection of widely adapted, high yielding genotypes under diverse environments. The presence of significant G x E interaction and environment effects on yield traits were reported by Finlay and Wilkinson (1963) and Tesfaye et al. (1998) in barley.

Additive main effects and multiplicative interaction analysis for grain yield

Additive main effects and multiplicative interaction

Table 2. Mean grain yield (Kgha^{-1}) of twenty malting barley genotypes evaluated at four testing sites in the 2004, 2005 and 2007 main rain cropping season.

Genotype	Environment												Mean ($\bar{x}$)
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	
G1	4225.0	3548.3	3763.7	4164.0	3552.0	2889.7	1774.0	1805.7	1949.0	2869.7	885.5	2120.3	2795.5
G2	2592.0	1930.0	2138.0	1356.3	1881.3	2511.0	904.8	767.7	1223.5	1743.7	772.7	1309.3	1594.2
G3	3520.0	3296.0	3285.0	3370.0	3878.7	2830.3	1364.7	712.1	1413.0	1997.7	1663.7	1318.3	2387.3
G4	2460.0	2465.7	2340.0	3345.3	2395.3	2598.7	1377.0	997.3	1295.7	1964.7	1029.3	806.7	1923.0
G5	2785.7	2415.0	2477.0	3964.7	4614.7	2905.0	1562.3	735.8	805.2	1905.7	841.2	1857.7	2239.1
G6	3414.0	2829.3	2998.3	4061.0	3701.7	3942.0	406.3	834.3	1970.7	2052.3	175.3	1619.7	2333.7
G7	3395.7	2617.0	2883.3	2362.0	2190.3	2063.0	1235.3	1564.0	920.2	1488.7	622.3	522.0	1821.9
G8	3639.7	3279.0	3336.0	3436.7	3693.7	2625.3	863.5	1159.7	1794.7	2142.0	920.8	1257.0	2345.6
G9	3580.3	3213.7	3273.7	3085.7	3616.7	2801.0	716.2	771.8	1073.2	2236.0	1271.5	1218.0	2238.1
G10	3633.7	2808.7	3098.3	3851.3	3812.3	3308.3	1116.2	607.7	894.4	2360.7	1091.9	497.3	2256.8
G11	3732.7	3190.0	3338.3	3589.0	3887.0	3386.0	769.2	1165.2	1078.0	1895.0	987.8	1181.3	2349.9
G12	4103.7	3806.0	3832.0	4427.3	3113.3	3460.3	822.3	1505.3	1529.7	1685.7	660.8	1127.3	2506.2
G13	3933.0	3962.7	3824.7	3918.7	4038.7	2645.3	1289.7	1678.0	1611.0	2196.0	1068.8	1785.3	2662.6
G14	3527.7	3221.3	3251.0	3563.0	3629.0	3135.3	1429.0	2154.3	1340.5	1905.0	840.0	1117.7	2426.0
G15	3583.3	3248.7	3293.0	3820.3	3654.3	2948.0	1344.0	1241.7	1823.7	1965.3	600.2	247.7	2314.2
G16	2590.3	1925.0	2134.7	2861.7	3426.7	2348.0	1177.7	905.2	1388.5	2480.0	1055.6	687.5	1915.0
G17	2066.0	1417.0	1618.7	1545.0	1865.0	1446.7	1481.3	529.9	963.1	2799.7	212.5	539.6	1373.7
G18	2593.0	2457.0	2402.0	4377.7	3803.0	3251.7	1022.8	1386.0	1376.2	1886.7	607.8	1231.0	2199.6
G19	3620.7	2983.3	3179.0	2777.3	3112.0	1683.3	1420.3	1198.7	1408.7	1392.3	435.7	623.2	1986.11
G20	2608.7	3031.0	2696.7	1838.0	3657.0	2515.7	465.6	1350.7	995.1	1727.7	879.0	1088.9	1904.45
Mean ($\bar{x}$)	3280.0	2882.0	2958.0	3286.0	3376.0	2765.0	1127.0	1153.0	1343.0	2035.0	831.0	1108.0	2178.7
SE ±	220.0	236.1	164.8	220.9	266.7	137.6	58.09	58.9	42.13	146.1	101.4	44.30	65.2
LSD (5%)	629.72	675.8	471.9	632.5	763.5	394.0	166.3	168.7	120.6	418.4	290.4	126.8	181.2
C.V (%)	11.61	14.18	10.0	12.0	14.0	9.0	9.0	9.0	5.0	12.0	21.0	7.0	18.0

Table 3. Mean grain protein content and other quality traits of twenty malting barley entries, tested at Adet, Debretabour, Laygaint and Dabat (2005).

Genotype	Germination		Protein (%)	HLW (kgha^{-1})	TGW (g)	Sieving test (%)				
	GE (%)	GC (%)				2.8 mm	2.5 mm	2.8+2.5 mm	2.2 mm	<2.2 mm
G1	99.42	99.67	9.90	59.66	42.49	56.05	30.83	86.93	9.08	3.98
G2	97.58	98.75	10.85	56.77	33.10	1.30	10.43	11.73	33.81	54.43
G3	97.67	97.83	9.78	58.03	43.37	33.91	44.28	78.18	16.41	5.33
G4	99.75	100.00	10.55	59.66	37.46	7.21	48.38	55.59	31.58	12.72
G5	99.17	99.92	10.47	58.93	42.37	39.63	37.23	76.86	14.50	8.48

Table 3. Cont.

G6	97.75	99.50	9.65	59.02	39.16	18.62	39.13	57.75	24.64	17.47
G7	98.83	99.25	9.51	60.01	37.70	32.89	41.93	75.18	17.79	7.18
G8	97.25	98.67	9.82	57.31	42.98	28.23	44.38	72.60	20.85	6.42
G9	96.42	97.50	9.98	57.84	42.89	28.84	41.97	70.81	21.49	7.57
G10	98.67	99.42	9.25	60.03	37.24	3.52	36.57	40.08	36.93	22.91
G11	98.67	99.58	9.80	59.53	45.90	37.48	46.48	83.95	12.46	3.53
G12	98.42	99.67	9.49	58.85	38.76	18.83	46.18	65.02	22.89	11.99
G13	99.33	99.42	10.32	59.73	46.06	28.00	47.98	75.98	14.82	9.14
G14	97.58	98.25	9.81	60.37	39.87	37.54	40.56	78.11	13.50	8.32
G15	99.00	99.92	9.73	60.56	41.37	18.49	45.13	63.62	23.30	12.98
G16	98.00	98.08	9.41	57.20	35.11	20.08	37.46	57.54	25.62	16.76
G17	97.50	97.83	9.51	57.95	34.37	12.42	29.80	42.19	29.48	28.70
G18	98.42	98.50	9.35	58.39	41.59	32.26	38.28	70.54	20.77	8.60
G19	98.00	98.50	9.07	58.65	38.80	35.29	43.45	78.78	14.74	6.37
G20	98.00	99.25	9.28	59.33	37.68	8.89	51.58	60.47	28.97	10.47
Mean ($\bar{x}$)	98.3	98.9	9.8	58.89	39.91	24.9	40.1	65.1	21.7	13.2
SE ±	1.48	1.27	0.36	0.75	0.56	8.03	5.92	9.06	5.31	5.04
LSD (5%)	2.48	2.13	0.60	2.09	1.55	13.44	9.89	15.15	8.87	8.42
C.V (%)	2.13	1.82	5.2	7.6	8.4	45.36	20.82	19.67	34.56	54.18

TSW = Thousand seed weight, PC = protein content, GE = germination energy, GC = germination capacity.

Table 4. Mean values of malting quality traits of malting barley genotypes grown at four representative locations in the 2004 main cropping season.

No.	Location	TSW (g)	PC (%)	GE (%)	GC (%)	Kernel size test using sieve sizes of (mm)				
						2.8	2.5	2.5+2.8	2.2	<2.2
1	Adet	40.85	11.49	96.51	97.55	28.05	41.39	69.45	20.08	10.34
2	Debretabor	41.14	10.45	98.83	99.70	33.86	42.73	76.65	16.19	7.11
3	Dabat	37.42	8.35	99.55	99.9	23.65	43.18	66.82	20.92	12.32
4	Laygaint	39.16	8.82	98.35	98.75	14.34	33.11	47.47	29.55	22.91
Mean ($\bar{x}$)		9.8	98.9	39.91	98.3	24.9	40.1	65.1	21.7	13.2
SE ±		0.36	1.27	0.56	1.48	8.03	5.92	9.06	5.31	5.04
LSD (5%)		0.60	2.13	1.55	2.48	13.44	9.89	15.15	8.87	8.42
C.V (%)		5.2	1.82	8.4	2.13	45.36	20.82	19.67	34.56	54.18

TSW = Thousand seed weight, PC = protein content, GE = germination energy, GC = germination capacity.

Table 5. Combined analysis of variance for twenty genotypes for yield and quality traits grown in 2004, 2005 and 2007 main rain cropping season.

Yield and quality traits	Mean squares (MS)		
	Environments (E)	Genotypes (G)	G x E
GY	200582000**	4365559.1**	60865505.0**
TKW	322.97**	469.51**	154.50**
HLW	403.39**	43.48**	51.6**
KPC	42.31**	0.88**	0.17 ns
GE	34.42**	2.53 ns	4.43 ns
GC	23.01**	2.45ns	3.30ns
SS	1356.32**	784.94**	128.76 ns
ST	446.30**	320.68**	69.66 ns
SY	3108.22**	1275.69**	164.17 ns
SL	634.47**	236.21**	56.39 ns

ns = non significant, * = significant (P<0.05) and ** = highly significant (P<0.01).

Table 6. Additive main effects and multiplicative interactions (AMMI) analysis of variance for grain yield of 20 genotypes of malting barley across 12 environments.

Source	Degree of freedom	Mean square	Variance explained (%)
Total	719	-	-
Treatment	230	3868773.39	92.39
Blocks	24	60865505.0	3.91
Environment	11	60865505.0**	75.24
Genotypes	19	4365559.1**	9.32
Interactions	209	657185.2 **	15.44
IPCA 1	29	1695438.4**	35.80
IPCA 2	27	1123189.2**	22.08
IPCA 3	25	637724.8**	11.61
IPCA 4	23	554398.6**	9.28
IPCA 5	21	439107.1**	6.71
Error	456	78097.8	3.70

** P< 0.01, ** Highly significant at the 0.01 probability level, *Significant at the 0.05 probability level, IPCA- Principal component axis for interaction.

(AMMI) analysis proved significant (P<0.05) main effects and interaction effects for grain yield (Table 8). It showed that 75.24% of the total sum of squares was attributable to environmental effects, 9.32% of genotypic effects and 15.44% to GxE effects (Table 7). A large sum of squares for environments indicated that the environments were diverse; with large differences among environmental means causing most of the variation in grain yield. The magnitude of the G x E sum of squares was highly significant (p<0.01) indicating that there were large differences in genotypic response across locations. Results from AMMI analysis also showed that the first principal component axis (IPCA1) of the interaction captures 35.80% of the interaction sum of squares at 29 of the degrees of freedom (Table 6). Similarly, Purchase et al. (2001) and Romagosa et al. (1996) reported 41 and 72% of the G x E interaction explained by the first IPCA in wheat and barley. The second interaction principal component axis explained a further 22.08% of the GxE sum of squares and only 11.6% by the third IPCA axis. The mean squares for the IPCA1, IPCA2 and IPCA3 were significant at P = 0.01 and cumulatively contributed to 69.5% of the total GxE. F-test at P = 0.01 revealed that the first four principal component axes of the interaction were significant for the model. However, the prediction assessment indicated that AMMI 2 with only two interaction principal component axes was the best predictive model (Zobel et al., 1988). Further interaction principal component axes captured mostly noise and therefore did not help to predict validation observations. Thus, the interaction of the twenty genotypes with twelve environments was best predicted by the first two principal components of genotypes and environments and genotypes and environments with similar signs of their

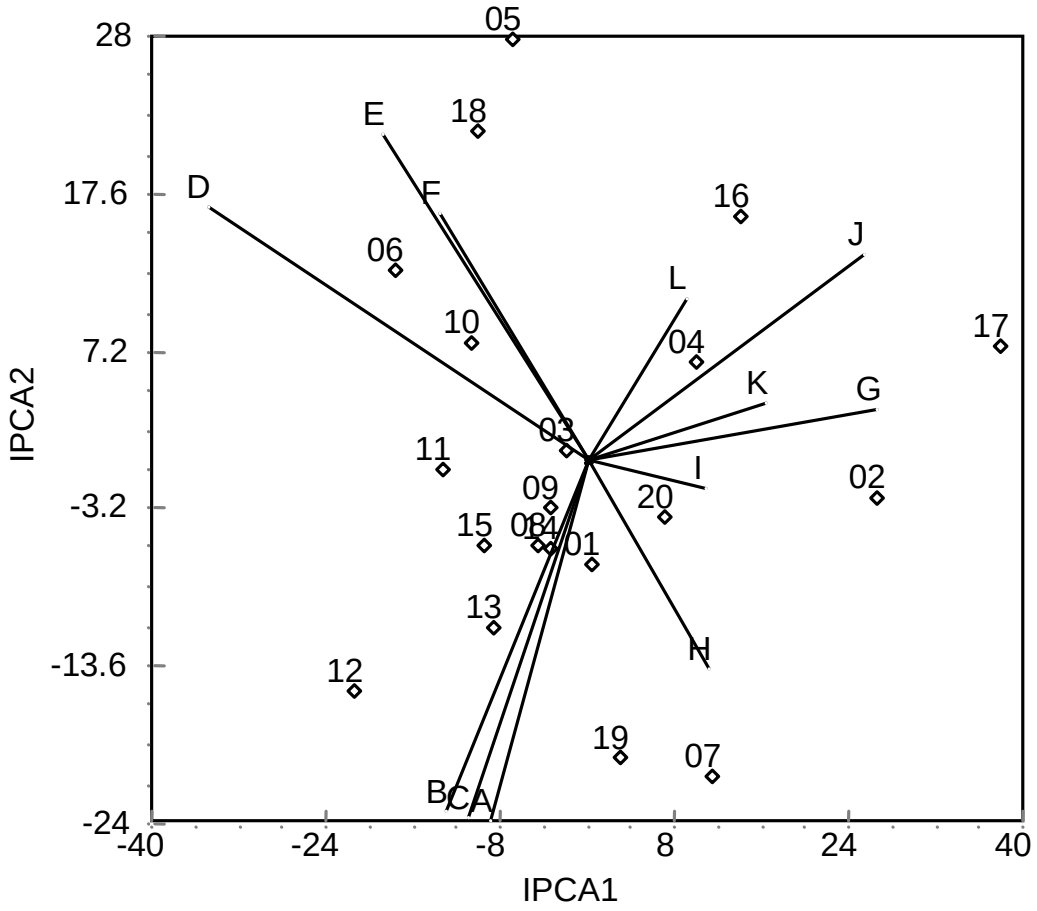

Figure 1. Biplot of principal component analysis axis (PCA) 1 against principal component analysis axis (PCA) 2 of twenty genotypes grown at twelve environments. 01 = G1, 02 = G2, 03 = G3, 04 = G4, 05 = G5, 06 = G6, 07 = G7, 08 = G8, 09 = G9, 10 = G10, 11 = G11, 12 = G12, 13 = G13, 14 = G15, 16 = G16, 17 = G17, 18 = G18, 19 = G19, 20 = G20. A = Adet-2004, B = Adet-2005, C = Adet-2007, D = Debretabor-2004, E = Debretabor 2005, F = Debretabor-2007, G = Dabat-2004, H = Dabat-2005, I = Adet-2007, J = Laygaint-2004, K = Laygaint-2005, L = Laygaint-2007.

IPCA scores interact positively for that trait.

AMMI 2 biplot as shown in Figure 1 has four sections: genotype G12, G19 and G13 were good for locations Adet: genotype G6, G18 and G10 were good for location Debretabor G2 and was good for Laygaint; and for Dabat genotypes G16 was good. Genotypes G1, G3, G9 and G4 located near the plot origin have low GxE interaction than the vertex genotypes and thus stable. Genotypes G5, G17 and G7 located far from the vertex were unstable over locations.

Stability analysis

Stability in performance of genotypes across environments using Eberhart and Russell's regression coefficient, deviation from regression, Wricke ecovalence and Shukula stability variance were performed for grain yield. According to Eberhart and Russell's model, genotypes performance across environments is generally expressed in terms of three parameters, mean yield, the regression coefficient (β_i) and the deviations from regression (s^2d_i). A stable genotype should have a high mean yield, unit regression coefficient and deviation from regression nearly equals to zero (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966). Accordingly, genotype G1 was the most stable because it had high grain yield, its regression coefficient was almost equal to unity and it had none significantly the least deviation from regression. In contrast, G6, G10 and G11 had Sd_i^2 values significantly different from zero and β_i greater than one that is, and below average stability, so were regarded as sensitive to environmental changes. Genotypes G2 and G17 had regression coefficients less than unity (βi <1.0) that is, above average stability and significantly different deviation from regression. These genotypes are therefore, insensitive to environmental changes and have specifically adapted to low potential environments.

Table 7. Mean grain yield and genotypic stability parameters for 20 malting barley genotypes grown over 12 environments.

Genotype	Mean	W_i^2 %	b_i	S^2d_i	σ_i^2
G1	2795.53	2.56	1.02	65798.92	359720.73
G2	1594.16	9.43	0.51	108814.66	503106.53
G3	2387.33	2.34	1.03	55164.67	324273.23
G4	1922.96	3.63	0.74	40716.72	276113.39
G5	2239.09	8.51	1.06	334503.28	1255401.92
G6	2333.73	7.72	1.27	223435.63	885176.43
G7	1821.91	5.15	0.81	142847.81	616550.35
G8	2345.64	1.21	1.09	-5105.23	123373.56
G9	2238.10	2.16	1.11	35010.17	257091.55
G10	2256.75	4.21	1.28	54990.73	323693.41
G11	2349.88	2.36	1.24	-5447.04	122234.18
G12	2506.15	7.14	1.33	155931.46	660162.52
G13	2662.63	2.84	1.14	57028.42	330485.73
G14	2426.04	2.00	1.02	40127.77	274150.21
G15	2314.17	3.49	1.22	56997.44	330382.46
G16	1915.03	4.55	0.78	102283.13	481334.76
G17	1373.73	14.50	0.46	289886.81	1106680.35
G18	2199.57	5.94	1.05	218517.97	868784.21
G19	1986.11	4.64	0.99	161276.34	677978.77
G20	1904.45	5.61	0.87	186810.96	763094.18

GY = Grain yield, bi = slopes of regressions, S2di = deviations from regression, Wi = Wricke ecovalence, = Sukula stability variance.

The result of Wricke's ecovalence (W_i) and Shukula's stability variance (σi^2) showed that G8, G14 and G19 were comparatively stable as their contribution to the GxE interaction sum of squares was least and with minimum stability variance (σi^2). On the other hand, G17 and G2 were unstable in grain yield performance because these genotypes had relatively higher ecovalence (Wi) and stability variance (σi^2) (Table 7). Kernel protein content and seed size variability of genotypes across environments using Francis and Kannenberg (1978) conventional coefficient of variation (CV_i) and variance of genotypes across environments (Si^2) was performed as indicated in Table 8. The model identified G15, G19 and G12 in kernel protein content and G4, G1 and G3 in seed size respectively showing relatively consistent performance across locations. Kernel protein content and seed size of these genotypes might have a relatively small variation on varied locations while grain protein content of G3, G9 and G11 and seed size of G1, G11 and G19 show considerable variation across environments.

DISCUSSION

Despite its potential and market demand, production of barley for malt is not expanded in northwestern Ethiopia. These could be attributed to the lack of information on the effect of genotype, predictable and unpredictable environmental variations and their interaction on yield and quality attributes of the crop. Thus, twenty genotypes were tested at four locations under rain-fed conditions in 2004, 2005 and 2007 main cropping seasons to assess phenotypic performance, determine the effect of genotype, environment, and their interaction and to identify stable ones in yield, consistent performance in protein content and seed size. Data were collected on grain yield and quality traits and analysis of variance (ANOVA), additive main effects and multiplicative interactions analysis (AMMI) and stability analysis were undertaken. The combined analysis of variance over locations has shown highly significant difference among genotypes, environments and genotype by environment interaction on grain yield. The effect of environment on this trait was greater than genotypes and genotype by environment interaction. Even though genotypes' performance ranking was not consistent across environments, most genotypes performed well in grain yield at Debretabor and Adet locations. Kernel protein content, thousand kernel weight and hectoliter weight of genotypes were promising at Debretabor and Adet.

Additive main effects and multiplicative interactions (AMMI) model analysis was used to partition the GxE interaction of grain yield. The first two principal component

Table 8. Estimates of variability in performance for protein content and seed size for twenty malting barley genotypes at four environments.

No.	Genotype	Protein (%)	Protein content variability		seed size	Seed size Variability	
			Si^2	CV_i	(2.8+2.5 mm)	Si^2	CV_i
1	G1	9.90	1.43	14.6	86.93	21.53	13.0
2	G2	10.85	1.42	12.9	11.73	21.33	40.7
3	G3	9.78	1.36	11.5	78.18	21.65	17.8
4	G4	10.55	1.36	16.5	55.59	21.47	15.9
5	G5	10.47	1.33	12.3	76.86	21.23	30.1
6	G6	9.65	1.30	17.5	57.75	21.38	38.4
7	G7	9.51	1.30	15.4	75.18	21.85	18.6
8	G8	9.82	1.30	14.1	72.60	21.82	31.0
9	G9	9.98	1.29	11.5	70.81	21.89	26.9
10	G10	9.25	1.28	16.2	40.08	21.83	48.8
11	G11	9.80	1.28	11.5	83.95	22.67	9.5
12	G12	9.49	1.27	13.6	65.02	22.61	32.1
13	G13	10.32	1.25	16.7	75.98	22.95	34.6
14	G14	9.81	1.20	12.9	78.11	23.06	35.3
15	G15	9.73	1.17	17.5	63.62	23.03	37.2
16	G16	9.41	1.15	20.2	57.54	23.46	24.3
17	G17	9.51	1.11	18.2	42.19	23.58	25.1
18	G18	9.35	1.10	16.2	70.54	23.58	22.7
19	G19	9.07	1.09	16.2	78.78	23.54	9.4
20	G20	9.28	1.08	16.2	60.47	23.44	20.9

GP = Kernel protein content, Si^2 = variance of genotypes acrose environments; CV_i = Francis and Kannenberg (1978) conventional coefficient of variation.

axes for interaction alone captured most of the interaction sum of squares. The sign and magnitude of IPCA scores revealed the relative contribution of each genotype and environment for the genotype and environment interactions and the biplot graph of AMMI scattered genotypes and environments based on their interaction (Zobel et al., 1988; Guach, 1988). It helped to summarize the pattern and magnitude of GxE interaction and main effects that reveal clear insight into the adaptation of genotypes to environments and helped enhanced understanding of GxE interactions. Stability analysis using Eberharts and Russell's coefficient of regression (β_i) (1966), deviation from regression (s^2d_i), Wricke's (1962) ecovalence (W_i) and Shukula's (1972) stability variance (σi^2) on genotypes performance across environments have shown similar results in identifying the stable genotypes. Relatively, G1 had better grain yield stability. G13 and G12 showed specificity in environments; however, a potential high yielder averaged over locations. G3, G11 had better consistent performance across environments in protein content and G1, G11 in seed size. Therefore, G1 could be used for production and further malting barley improvement programs in the sub-region.

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African Journal of Plant Science

Full Length Research Paper

Evaluation of allelopathic potential of an aromatic exotic tree, *Melaleuca leucadendron* L.

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An attempt was made to evaluate the allelopathic potential of an exotic tree species, *Melaleuca leucadendron* L. using mung bean (*Vigna radiata* L.) seeds as responsive bioassay material. This was recorded in terms of the plant extract and plant leachate-induced changes of seed germination behaviour, the levels of DNA and RNA as well as amylase activity in the seed kernels. The results of the present investigation clearly revealed that pretreatment of mung bean seeds with various concentrations [1:1 and 1:2 (w/v)] of *M. leucadendron* bark extract, leaf extract and leaf leachates for 24 h duration, significantly reduced percentage seed germination and increased the T_{50} hours. Levels of DNA and RNA were also significantly reduced with concomitant increase of amylase activity in mung bean seed samples pretreated with the bark extract, leaf extract and leaf leachates of *M. leucadendron*. Tender bark extract and leaf extract showed more inhibitory action on mung bean seed than leaf leachates. Putative allelochemical-induced inhibitory effect, that is, reduction of seed germinability along with stimulation of amylase activity in seeds, being the important allelopathic indices, it can be concluded that *M. leucadendron* can potentially render allelopathic action on the experimental bioassay material.

Key words: Allelopathic potential, *Melaleuca leucadendron*, mung bean, seed germination, DNA and RNA levels, amylase activity.

INTRODUCTION

The term allelopathy was derived from the Greek words ‘allelon’ meaning ‘of each other’ and ‘pathos’ meaning ‘to suffer’ refers to the chemical influence of one species by another. Although the term allelopathy is most commonly used to describe the chemical interaction between two plants, it has also been used to describe microbe-microbe, plant-microbe, plant-insect or plant-herbivore chemical communication. Allelopathy is defined as “any process involving secondary metabolites produced by plants, microorganisms, viruses and fungi that influence the growth and development of agricultural and biological systems including positive and negative effects” (Torres et al., 1996). In plants, allelochemicals may be present in the leaves, barks, roots, flowers and fruits. Many exotic and invasive plants are threat to ecological diversity throughout the world and the invasive plants are uncommon in their native range but become very abundant in their new habitats (Louda et al., 1990; Callaway, 2002; Dogra et al., 2011). In fact, in some parts of India, the *Melaleuca* species has made a monospecific stand by displacement of the growth of local herbs growing under its canopy.

There are some common indices for assessing allelopathic action of plants or plant parts. These include, among others, germination behaviour and other physio-biochemical

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Table 1. Effect of seed treatment with bark extracts, leaf extracts and leaf leachates of *M. leucadendron* on percentage of germination and time (h), to 50% germination (T_{50}) of mung bean seeds.

Treatments	Concentration (W/V)	Germination (%)	T_{50} Hours
Bark extract	1 : 1	32 ± 1.05	37.54 ± 0.80
	1 : 2	36 ± 1.48	33.38 ± 0.90
Leaf extract	1 : 1	29 ± 1.30	40.18 ± 1.34
	1 : 2	31 ± 1.38	37.72 ± 0.65
Leaf leachate	1 : 1	44 ± 1.30	27.22 ± 2.01
	1 : 2	52 ± 1.64	23.12 ± 1.03
Control	0	94 ± 1.58	12.72 ± 0.91
LSD (P = 0.05)		7.82	3.45

responses of test species (Dogra et al., 2011). The present investigation is an attempt to evaluate the allelopathic potential of *Melaleuca leucadendra* L. (Myrtaceae), an exotic tree growing abundantly in the coastal belt of Digha in West Bengal state of India. For *prima facie* screening of allelopathic action of this plant, some selected physiological and biochemical parameters were analyzed using mung bean seed as a potential and responsive bioassay material.

MATERIALS AND METHODS

Fresh, mature and healthy leaves and tender bark (500 g each) of *M. leucadendron* L., collected from the coastal belt of Digha in West Bengal state of India, were thoroughly homogenized separately by mortar and pestles using 300 ml distilled water. The homogenate was strained using fine cloth and then centrifuged at 5000 *g* for 15 min. The supernatant was then made up to 500 ml using distilled water and these were considered 1:1 (W/V) proportion stock solution of leaf extract and tender bark extract, respectively. From these stock solutions another concentration grade of leaf and bark extract in the proportion of 1:2 (W/V) was prepared using distilled water and these were taken as the two concentration graded solutions of the leaf extract and the bark extract.

Another lot of dry leaves (500 g) sample of the *M. leucadendron* was kept immersed in 300 ml distilled water in a 100 ml beaker for 48 h and the leachate was decanted in a separate beaker. The total volume of the leachate was made up to 500 ml using distilled water and this was taken as the 1:1 (W/V) proportion of leaf leachate. From this stock solution another concentration grade in the proportion of 1:2 (W/V) was prepared using distilled water and this was taken as the two concentration graded solutions of leaf leachate. These three concentration grades of each leaf extract, bark extract and leaf leachate were used for possible allelopathic analysis.

Fully viable 200 g mung bean (*Vigna radiata* L.) seeds were surface sterilized with 0.1% $HgCl_2$ solution for 90 s. The seed lots were then separately presoaked in the three concentration grades of the bark extract, leaf extracts and leaf leachate for 24 h.

From the treated seed samples germination behavior (percentage and T_{50} of seed germination), DNA and RNA levels and activity of amylase enzyme in seeds were recorded.

To analyze germination behavior from continuous treatment sets, seven groups of 100 fresh seeds (700 fresh seeds) were transferred to separate Petri dishes containing filter paper moistened with 10 ml each of leaf extract, bark extract or leaf leachates and distilled water for control. Germination data were recorded after 120 h of seed soaking following the International Rules of Seed Testing (ISTA, 1976), DNA and RNA levels were analyzed as per the method described by Cherry (1967) modified by Choudhuri and Chatterjee (1970). Extraction and estimation of the enzyme amylase was done as per the method described by Khan and Faust (1967). For the assay of this enzyme, the blank was taken as zero time control. The activity of this enzyme was expressed as $[(\Delta A \times Tv)/(t \times v)]$, where ΔA is the absorbance of the sample after incubation minus the absorbance of the zero time control, Tv is the total volume of the filtrate, t is the time (minutes) of incubation with the substrate and v is the volume of the filtrate taken for incubation (Fick and Qualset, 1975).

Statistical analysis of the data was done in terms of least significant difference (LSD) which was calculated at 95% confidence limits and as per the method of Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

Effect of treatment on percentage and T_{50} value of seed germination

It is evident from the result (Table 1) that the putative allelochemicals of bark extract, leaf extract and leaf leachates of *M. leucadendron* strongly inhibited the percentage germination of mung bean seeds. Highest inhibitory allelopathic effect was recorded in the case of leaf extract followed by bark extract and lowest in treatment with leaf leachate. The data also reveals that more concentrated extracts were more injurious. Concomitantly, T_{50} value is increased due to the treatment with bark extract, leaf extract and leaf leachate of *M. leucadendron* on mung bean seeds.

Analysis of germination behaviour is considered to be a reliable index for evaluation of allelopathic action (Datta and Chakraborty, 1982; Pati, 2007; Pati and Bhattacharjee, 2008). It is reported that reduced germinability is an important effect of allelopathic action of plants and such action is chiefly exerted by a number of inhibitors of diverse chemical nature (Ghosh and Dutta, 1989). Thus, inhibition of percentage germination and increase of T_{50} value of mung bean seeds with bark extract, leaf extract and leaf

Table 2. Effect of seed pretreatment with bark extracts, leaf extracts and leaf leachates of *M. leucadendron* on DNA and RNA levels and amylase activity in kernels of mung bean seeds.

Treatment	Concentration (W/V)	DNA level (µg/g fresh weight)	RNA level (µg/g fresh weight)	Amylase activity (unit/h/g fresh weight)
Bark extract	1 : 1	16.04 ± 0.72	86.26 ± 1.00	26.44 ± 0.78
	1 : 2	18.64 ± 0.79	91.36 ± 0.48	24.78 ± 0.56
Leaf extract	1 : 1	15.96 ± 0.89	82.64 ± 0.47	30.56 ± 0.52
	1 : 2	16.22 ± 0.33	87.06 ± 0.69	27.16 ± 0.56
Leaf leachate	1 : 1	22.16 ± 0.51	118.34 ± 0.73	18.30 ± 0.56
	1 : 2	24.84 ± 0.73	129.50 ± 1.34	15.86 ± 0.68
Control	0	31.25 ± 0.62	153.84 ± 1.38	11.60 ± 0.50
LSD(P=0.05)		2.00	2.29	2.93

leachate of *M. leucadendron* are clear indicative of the allelopathic action of the test material.

Effect of treatment on changes of DNA and RNA levels and amylase activity in seed kernels

The results of Table 2 show that DNA and RNA levels of the kernel of mung bean seeds decreased significantly with leaf extract, bark extract and leaf leachate of *M. leucadendron* where leaf extract exhibited more significant effect. On the other hand, amylase activity increased in seed kernels irrespective of the treatments with two concentration grades of leaf extract, bark extract and leaf leachate of the selected plant.

Reduction of DNA and RNA levels and increase of amylase activity are suggestive of allelopathic potentiality of the test plant parts. Various inhibitors present in plants having allelopathic property reduce the overall metabolism of plants and anabolic activities of individual plant parts are also reported to be strongly impaired (Datta and Chakraborti, 1982). Reduced plant growth and slowed rate of plant establishment are also convincing evidence of allelopathic action (Ghosh and Dutta, 1989; Pati, 2007; Pati and Bhattacharjee, 2011).

The unreported and preliminary findings of the present investigation point out that the leaves and tender bark of *M. leucadendron* possess some allelochemicals, which efficiently render inhibitory action on the bioassay material. The observation further reemphasized the fact that the experimental exotic plant having growth suppressing property should be treated as a potential threat to plant biodiversity in a natural ecosystem in Indian soil.

Thus, a conclusion can be drawn from this study that the exotic plant *M. leucadendron* is a potential species having allelopathic property. Further work is in progress in our laboratory to identify, by advanced chromatographic technique, the specific allelochemicals which are causal factors for exhibiting allelopathic action.

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Full Length Research Paper

Variability, heritability and genetic advance in upland cotton (*Gossypium hirsutum* L.)

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The analysis of variance study indicated the presence of significant difference among all the traits in *Gossypium hirsutum* accessions. The highest phenotypic coefficient of variation (GCV) and genotypic coefficient of variation (GCV) were recorded by seed index, plant height, lint index and boll weight. Genotypic co-efficient of variation had a similar trend as PCV. High heritability along with high genetic advance was observed in traits viz., number of sympodia per plant, single plant yields, seed index and micronaire value. The combinations of high heritability with high genetic advance will provide a clear base on the reliability of that particular character in selection of variable entries. Based on *per se* performance, the accessions MCU5, TCH1715, TCH1716 and G cot 16 were identified as potential donors for single plant yield (g), number of bolls per plant, 2.5% span length (mm) and bundle strength (g/tex). So these accessions may be utilized for crossing programme to improve a particular character in crop improvement.

Key words: Genetic variability, heritability, genetic advance, upland cotton.

INTRODUCTION

Cotton is an important fibre crop of global importance which is grown in tropical and subtropical regions of more than 60 countries of the world. Despite threat from synthetic fibre or manmade fibre, cotton retains its reputation as “queen of the fibre plants””. For multiple uses of lint and by-products, cotton is also referred to as “white gold”. In any crop improvement programme, knowledge on nature of gene action and inheritance of traits is essential so as to choose a suitable breeding methodology in crop improvement (Vineela et al., 2013). Development of an effective breeding programme depends on the existence of genetic variability for various economic characters in the gene pool. Selection is effective only when there is enough magnitude of variability in the breeding population. An understanding of precise magnitude of variability present in a population is important in formulating the most appropriate breeding technique for improvement of various characters. The present investigation was carried out with 54 *Gossypium hirsutum* lines of diverse origin to estimate their *per se* performance, variability, heritability and genetic advance on the genetic architecture of 16 yield, yield components and fibre quality traits.

MATERIALS AND METHODS

The study was conducted in the Department of Cotton, Tamil Nadu Agricultural University, Coimbatore during winter 2010. Fifty four *G. hirsutum* cotton genotypes were planted in randomized block design with two replications. Uniform spacing of 90 x 45 cm and all the recommended field operations were carried out. In each replication five competitive plants were randomly selected and observations were recorded for 16 characters viz., days to 50% flowering, plant height (cm), internode length (cm), number of sympodia/plant, number of ovules/plant, number of bolls/plant, boll weight (g), number of seeds/plant, seed setting percentage, seed cotton yield/plant, lint index, seed index, ginning outturn (%), 2.5% span length (mm), bundle strength (g/tex) and fibre fineness.

Analysis of variance was carried out statistically utilizing the mean values (Panse and Sukhtame, 1995). The phenotypic and genotypic coefficient of variation was estimated using the formula suggested by Burton (1952) and expressed in percentage. The phenotypic and genotypic variances were calculated by utilizing the

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Table 1. Mean and range performance for different characters among G. *hirsutum* accessions.

Characters	Mean	Minimum	Maximum
Days to 50% flowering	56.6	50.0	65.0
Plant height (cm)	108.8	78.0	147.0
Inter node length(cm)	5.4	4.0	6.5
No of Sympodia per plant	19.6	14.0	27.0
No of bolls per plant	23.6	19.5	38.5
Boll weight (g)	4.3	12.4	5.5
No of seeds per boll	27.4	23.0	35.5
No of ovules per flower	30.7	24.0	38.0
Seed setting percentage	89.6	71.5	100.0
Single plant yield (g / plant)	57.6	31.7	91.6
Lint index	5.7	4.0	7.9
Seed index	9.6	6.4	16.3
Ginning outturn (%)	37.3	30.8	46.1
2.5 % span length (mm)	28.4	24.3	34.0
Bundle strength (g / tex)	20.4	16.2	23.7
Micronaire value	4.3	3.5	5.2

respective mean sum of square from variance table (Lush, 1940). Heritability, expected genetic advance and genetic gain in the broad sense was calculated according to the formula suggested by Johanson et al. (1955).

RESULTS AND DISCUSSION

The *per se* performance of yield and fibre quality characters were recorded on 54 germplasm accessions and the range of variations observed in respect of all the 16 traits studied are presented in the Table 1. Based on *per se* performance, the accessions such as MCU 5, TCH1715, TCH1716 and G cot 16 in *G. hirsutum* were identified as potential donors which recorded highest mean values for single plant yield, number of bolls per plant, 2.5% span length (mm) and bundle strength (g/tex). These accessions may be exploited for further improvement of the above traits by breeding programmes. Regarding the identification of donor for specific trait, the highest performer of that particular trait can be considered. Among the accessions, TCH 1715 (5.5 g of boll weight), RB 488 (46.1% of GOT) were expressed the highest *per se* for that particular traits. The culture TCH 1710 (34 mm, 23.1g/tex) recorded the highest 2.5% span length. So these accessions may be utilized for crossing programme to improve that particular character in crop improvement. Anjali was found to be compact type which can be utilized in development of genotype suitable for high density planting.

The analysis of variance showed highly significant differences among genotypes for all the characters studied (Table 2) and infers existence of considerable genetic diversity among genotypes. Phenotypic variance, genotypic variance, phenotypic coefficient of variation, genotypic coefficient of variation, heritability in broad sense and genetic advance as percentage of mean which were estimated for 16 characters are shown in Table 3. The knowledge of nature and magnitude of variability available in the genotypes for different characters is an important prerequisite for making simultaneous selection over more number of characters to bring remarkable improvement in cotton. The analysis of variance study indicated the presence of significant difference among all the traits in the accessions. The heritable (genotypic) variation is usually masked by non-heritable variation creating

Table 2. Analysis of variance for the different characters among the germplasm accessions of *G. hirsutum*.

Source of variation	Degrees of freedom	Days to 50% flowering	Plant height (cm)	nter node length (cm)	Number of sympodia / plant	Number of bolls / plant	Boll weight (g)	Number of sees / boll	Number of ovule / flower	Seed setting percentage	Single plant yield (g)	Lint index	Seed index	Ginning out turn (%)	2.5 % span length (mm)	Bundle strength(g /tex)	Micronaire value
Genotypes	53.0	10.4**	466.6**	0.7**	14.1**	18.3**	0.6*	10.8**	17.1**	90.4**	441.9**	1.3**	4.6**	21.1**	10.2**	6.1**	0.3**
Error	53.0	2.7	218.6	0.4	2.8	5.0	0.3	5.50	6.5	40.1	2.1	0.5	1.2	10.9	0.2	0.3	0.1

*,** Significant at 5 and 1% levels, respectively.

difficulty in exercising selection. Hence it becomes necessary to partition overall variability into heritable and non-heritable components to enable the breeders to plan for proper breeding programme. The plant height recorded the highest value for phenotypic variance (342.6) and the single plant yield recorded highest genotypic variance (219.9). The micronaire value exhibited the lowest phenol-typic variance (0.2) and in case of genotypic variance, the traits *viz*., inter node length, boll weight and micronaire value had recorded the lowest value (0.1). The co-efficient of phenotypic and genotypic variance were calculated for all the characters under study. The PCV ranged from 4.5 (days to 50% flowering) to 25.9% (single plant yield). The highest PCV was followed by seed index (17.7%), plant height (17.0%), lint index (17.0%) and boll weight (15.4%). Genotypic co-efficient of variation had a similar trend as PCV. The range varied from 3.5 (Days to fifty percent flowering) to 25.8 (Single plant yield). The maximum genotypic coefficient of variation (GCV) was observed for single plant yield (25.8) followed by seed index (13.5), number of sympodia per plant (12.1), lint index (11.2) and number of bolls per plant (10.9), plant height (10.2). The lowest PCV (4.5%) and GCV (3.5) values was observed in days to 50% flowering. In the present study, there was a close correspond-dence between phenotypic and genotypic variance for days to 50% flowering, inter node length, boll weight, single plant yield, lint index, micronaire value and 2.5% span length indicating less environmental influence. But plant height, number of sympodia per plant, number of bolls per plant, number of seeds per boll, seed setting percentage and ginning outturn showed higher variation indicating the influence of environment on these characters. Since the variations are influenced by the magnitude of the units of measurements of different traits, a measure of coefficient of variation which is independent of the unit of measurement is more useful in comparing between populations. In *G. hirsutum* accessions PCV was higher than the GCV for all characters. From this, we can understand that the environment plays a major role on expression of all these traits leading to increase in the PCV more than GCV. The highest PCV and GCV estimates were recorded for single plant yield indicating the presence of significant genetic variability in this character. Selection pressure can be applied on this character to isolate promising genotypes. Similar observations in cotton was reported by Dheva and Potdukhe (2002) and Preetha and Raveendran (2007).

Moderate PCV and GCV estimates were noticed for the characters such as plant height, number of sympodia per plant, number of bolls per plant, lint index and seed index. Girase and Mehetne (2002) and Harshal (2010) also reported the moderate PCV and GCV in various traits with the suggestion that these characters can be improved though rigorous selection. The characters such as days to 50% flowering, 2.5% span length and micronaire value exhibited low PCV and GCV which indicated that the breeds should go for source of high variability for these characters to make improvement. Similar suggestion were given by Kowsalya and Raveendran (1996), Do Thi Ha An et al. ((2006) in their conclusion. In a population, the observed variability is a

Table 3. Components of variance for yield and fibre quality characters of *G. hirsutum* accessions.

Characters	Phenotypic variance	Genotypic variance	PCV (%)	GCV (%)	h^2 (%)	A	GA as percentage of mean
Days to 50% flowering	6.6	3.9	4.5	3.5	58.5	4.0	7.0
Plant height (cm)	342.6	124.0	17.0	10.2	36.2	17.7	16.3
Inter node length(cm)	0.5	0.1	13.5	6.6	23.5	0.5	8.4
No of sympodia per plant	8.5	5.7	14.8	12.1	66.9	5.1	26.2
No of bolls per plant	11.7	6.7	14.4	10.9	57.2	5.2	21.8
Days to 50% flowering	6.6	3.9	4.5	3.5	58.5	4.0	7.0
Plant height (cm)	342.6	124.0	17.0	10.2	36.2	17.7	16.3
Boll weight (g)	0.4	0.1	15.4	8.4	29.8	0.5	12.1
No of seeds per boll	8.2	2.6	10.4	6.0	32.5	2.5	9.0
No of ovules per flower	11.8	5.3	11.2	7.5	44.5	4.0	13.1
Seed setting percentage	65.3	25.1	9.0	5.6	38.5	8.2	9.2
Single plant yield (g / plant)	222.0	219.9	25.9	25.8	89.1	39.0	27.3
Lint index	0.9	0.4	17.0	11.2	43.9	1.1	19.6
Seed index	2.9	1.7	17.7	13.5	58.0	2.6	27.1
Ginning outturn (%)	16.0	5.1	10.7	6.0	31.6	3.3	9.0
2.5 % span length (mm)	5.2	5.0	8.0	7.9	96.2	5.8	20.4

combined measure of genetic and environment causes, where as the genetic variability is the only estimate heritable from generation to generation. However, a measure of heritability alone does not give an idea about the expected gain in the next generation but it has to be considered in conjunction with genetic advance. The traits which expressed high heritability and high genetic advance as percentage of mean could be used as a powerful tool in selection process. According to Panes and Sukhatme (1995) such characters were found to be governed by additive genes and had minimum environment influence. The heritabi-lity estimates ranged from 23.5 (internode length) to 89.1% (single plant yield). The high heritability estimates of 89.1% were recorded by single plant yield followed by 2.5% span length (96.2 %) and bundle strength (91.8%) where as the low heritability was observed in inter node length (23.5%).

The high heritability was registered in the traits *viz.,* number of sympodia per plant, single plant yield, seed index, 2.5% span length and bundle strength. The inter node length exhibited low heritability in this investigation. For efficient selection, we cannot completely depend on heritability alone. The combinations of high heritability with high genetic advance will provide a clear base on the reliability of that particular character in selection of variable entries. The genotypic advance as percent of mean for 16 traits ranged from 7.0 to 27.3%. The higher genetic advance as percent of mean was recorded by single plant yield (27.3%) followed by seed index (27.1 %), number of sympodia per plant (26.2 %), number of bolls per plant (21.8 %). The lowest value of 7.0% was observed in the trait days to 50% flowering. High heritability along with high genetic advance was observed in traits *viz.,* number of sympodia per plant, single plant yields, seed index and micronaire value in *G. hirsutum* (Do Thi Ha An et al., 2008). These traits are highly reliable during selection. High heritability combined with moderate genetic advance was found in the 2.5% span length. It was in accordance with of Muhammad et al. (2004). Among the study materials some of the accessions were identified as potential donors for the improvement of different characters (Table 4). The accessions with high mean performance are generally preferred for all the traits except days to 50% flowering, since earliness is the preferred attribute and early flowering was taken into consideration. From the results of the present study, it can be concluded that direct selection can be done for most of the yield attributing traits since it exhibited high genetic variability and high range of variation. A high PCV over GCV for the characters studied indicated that environment influences the expression of these characters under study. High genetic advance, genetic gain and heritability were recorded for number of sympodia per plant, single plant yield, seed index, micronaire value and 2.5% span length indicated that selection can be resorted for the improvement of these characters in the future crop improvement programmes.

Table 4. Potential donors for yield and fibre quality traits.

S/N	Characters	Potential accessions
1	Days to 50% flowering	MCU-7, SVPR-3, TCH-1716, SVPR-2
2	Plant Height	SCS 102, T CH 1715, Okra narrow
3	Inter node length	MCU 5, RAC9740, SCS 102, F-1946
4	No. of Sympodia	PSCL VII, Okra narrow, RHC 1694, SCS 102
5	No. of ovules	NDLH 1588, TCH 1608
6	No. of Bolls	Okra Narrow, G cot-16, F-1946
7	Boll weight(g)	TCH 1715, MCU 13, LRA 5166
8	No. of seeds per boll	NDLH 1588, SVPR 4
9	Seed setting %	MCU 12, MCU 13, KC 3, TCH 1710
10	Single plant yield	MCU 5, MCU 12, Surabhi, SVPR 2, SVPR 4, KC 2, TCH 1715, TCH 1716, TCH 1732, TCH 1734, TCH 1744, G. COT-16, CCH 2117, Pusa 953, ARB 2001, RHC 1694, LH 1961, RB 488, GISV201, MHIS-5, MHIS-7, Sara 2,Giza 1461, Sumangala
11	Lint index	GISV 201, RAC 9544
12	Seed index	SVPR 4, L-752, TCH 1715
13	Ginning Out Turn	RB 488, TCH 1705
14	2.5 % span length	TCH 1608, CNH 152, TCH 1705, RAC 9740, TCH 1732, MCU 5, MCU 13, TCH 1710, TCH 1715, TCH 1716
15	Bundle strength	TCH 1710, TCH 1715, MCU 5, TCH 1732, TCH 1734, TCH 1744
16	Micronaire value	MHIS-7, MCU 7, CCH 2117, NDLH 1588

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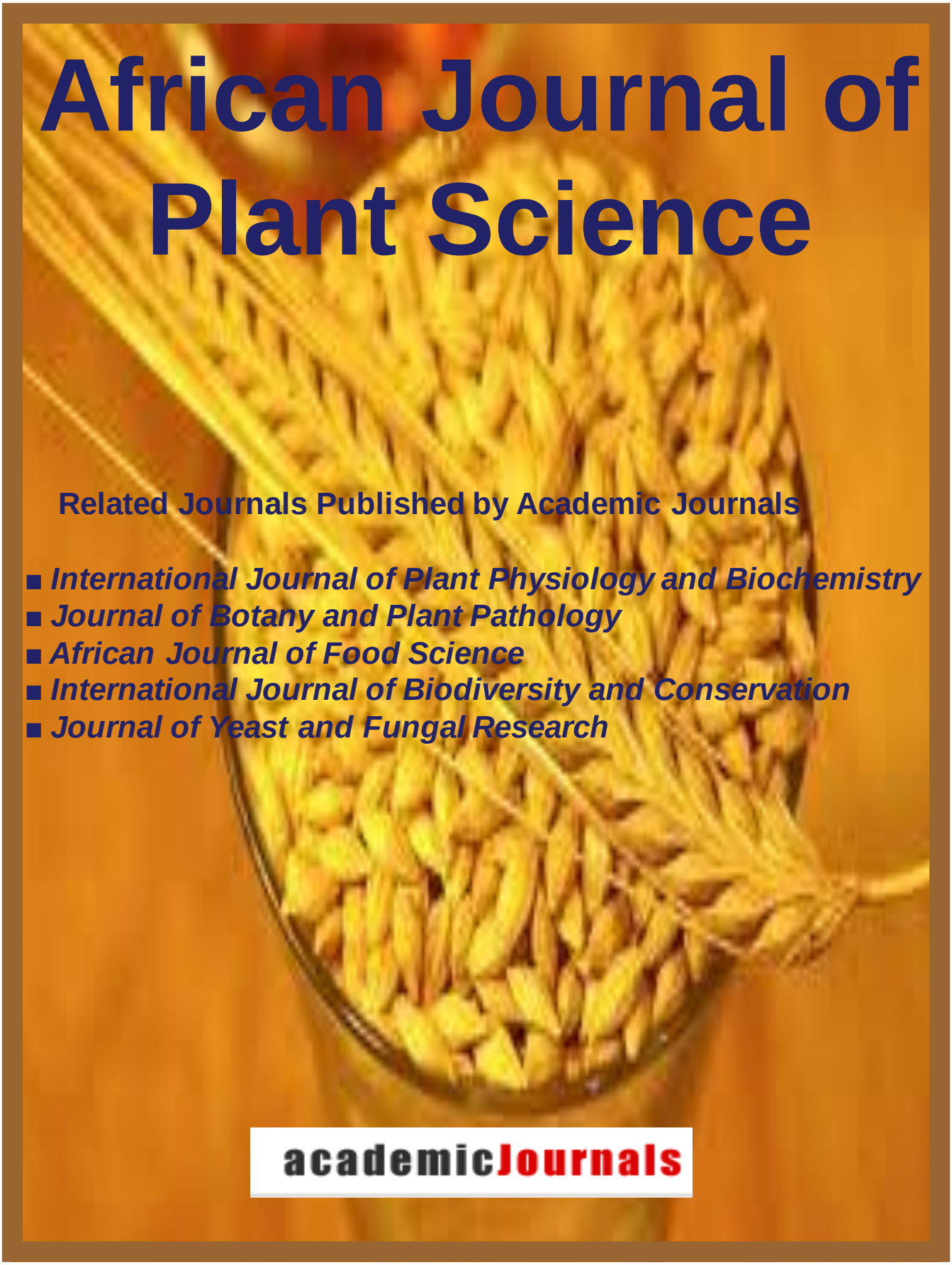

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