



ADDIS ABABA UNIVERSITY
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE
CENTER FOR FOOD SCIENCE AND NUTRITION

**Effect of Organic and Inorganic Fertilizers on Biochemical Composition
and Antioxidant Capacity of Teff [*Eragrostis tef* (Zucc.)Trotter]**

Thesis submitted to the Center for Food Science and Nutrition Addis Ababa University in partial fulfillment of the requirement for the Degree of Master of Science in Food Science and Nutrition

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June, 2017

Addis Ababa, Ethiopia

DECLARATION

I, the undersigned, hereby declare that this thesis is my original work and all sources of materials used for the thesis have been acknowledged.

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LIST OF ABBREVIATION AND ACRONYMS

ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemistry
CSA	Central Statistical Agency
CRD	Complete Randomized Design
DAP	Diammonium phosphate
DPPH	2,2-diphenyl-1-picrylhydrazyl
E	East
Eqn	Equation
GAE	Gallic Acid Equivalents
GI	Glycemic Index
g	Gram
IFPRI	International Food Policy Research Institute
L	Liter
mL	Milliliter
N	Nitrogen
N	North
P	Phosphorous
RCBD	Randomized Complete Block Design
SEM	Scanning electron microscope
SNNPR	South Nations Nationalities and People's Region
SSPE	Statistical Product and Service Solutions
TSP	Triple Super Phosphate

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ABSTRACT

Teff is one of the major cereal that is used for making the Ethiopian staple food injera. Although studies have shown that teff has a good nutritional composition, whether this can be affected by various fertilizer applications remains unknown. The purpose of this study was to investigate the effect of organic and inorganic fertilizer treatments on nutrient composition, phytochemical composition, mineral content and anti-oxidant capacity of teff using DPPH (Diphenyl Picryl Picryl Hydrazyl) free radical scavenging activity. The kora teff variety was sown in an experimental field and was subjected to inorganic (recommended NP) and organic (recommended vermicompost and recommended compost) fertilizers. Standard methods were used to analyze the proximate composition, mineral content (Fe, Ca and Zn), phytochemicals (phytic acid and total phenolic content) and anti-oxidant capacity of teff with fertilizer treatments using DPPH. Teff grains with 50% recommended NP+50% recommended vermicompost had high protein content, calcium and phytic acid than others. Teff grain with recommended compost had the highest in iron content and scavenged the most free radical scavenging capacity at a lowest IC_{50} value accompanied by highest phenolic contents. Teff grains with recommended vermicompost were the highest in thousand kernel weight and bulk density than the others ($p<0.05$). Our study revealed that teff grown using organic fertilizers had better mineral content, total phenolic content and were effective in scavenging radicals at the lowest IC_{50} relative to chemical fertilizers. Application of recommended levels of vermicompost resulted in higher yield than other treatments including the conventional NP treatments. Yield and nutrient content are both improved when using vermicompost, suggesting that a transition from synthetic to organic fertilizers could be advantageous.

Key words: teff, fertilizer, organic, conventional, mineral, antioxidant

1. INTRODUCTION

1.1. Background

Teff is major cereal crop used for human consumption in Ethiopia. It is highly valued for its injera making quality. The composition of teff shows that it has good mineral content and generally higher amount of the essential amino acids. Besides, the crop can grow well in moisture stress and waterlogged conditions; can also grow from sea level up to 2800m, under various rainfalls, temperature and soil conditions (Ketema, 1997). Teff has also been described as nutritionally superior crop (Gebremariam *et al.*, 2014).

Nutritionally, teff has as much, or even more food value than the major grain crops like maize, rice, wheat, and sorghum. This is probably because teff is eaten as a whole grain. Teff grains contain 8-11 % proteins, 9.5-150 mg/100g iron, 17-178 mg/100g calcium and rich with potassium and phosphorous (Baye, 2014). Teff is a good source of limiting amino acids like lysine. This, along with its gluten-free nature, has increased the popularity of teff as a functional food. (Spaenij-Dekking *et al.*, 2005; Hopman, 2008). This global attention to teff has prompted the need to increase productivity and develop standards for teff. There are enough information exists about the nutrient composition of teff, but to what extent teff's nutrients composition is affected by agricultural practices remains unknown.

Fertilizer use is concentrated on cereals followed by pulses and oil seeds respectively (CSA 1995/96-2007/08). In 2007/08 the national level amount of fertilizer applied in cereals, pulses and oil seeds were 3,962, 160 and 136 thousands quintals, respectively (CSA, 2008/09). Teff is the crop with the largest share in fertilizer use among the cereals (40%), followed by wheat and maize with respective shares of 29% and 20% in the period 1994/95-2005/06 (CSA, 1995/96-2007/08). In terms of application rate per hectare of cultivated land, wheat accounted for the largest share (57 Kg/ha), followed by teff and maize respectively (CSA, 1995/96-2007/08). These statistics indicated that the national level intensity of fertilizer use is still lower than the recommended rate of 200 kg per ha (100 Kg of DAP and 100 Kg of Urea) (Alem *et al.*, 2010). Regardless of teff's wider adaptation, productivity of teff is low in the country with the national average grain yield of 1.379 tons/ ha (CSA, 2013). This is mainly

because of low soil fertility (Osman and Abera, 2015) and severe organic matter depletion (IFPRI, 2010) aggravated by low rate of chemical fertilizer application. The rate of chemical fertilizer application is low in the country due to unaffordable price for resource-poor smallholder farmers (Endale, 2011). The continued use of chemical fertilizers is not recommended as it causes health and environmental hazards such as ground and surface water pollution by nitrate leaching (Pimentel, 1996).

The prime purpose of fertilizers is to improve the quantity and quality of agricultural products (White, 2006); but fertilizers used by farmers in Ethiopia are only DAP and urea. It is clear that DAP and urea applications have notably increased yield of improved teff varieties up to 2.27 Kg/ ha (Tekalign *et al.*, 2000; Tulema *et al.*, 2005). However, the permanent use of these fertilizers could lead to facilitate mining of other essential plant nutrients which will limit crop yield and quality.

Alternatively, organic fertilizers can be used. Indeed, studies have shown that increasing rate of organic manure from 0 to 20 m³/fed markedly increased nutrient contents in lentil seeds. Plots receiving 20m³ organic manure/ fed gave higher figure in protein (14%), phosphorous (24%), potassium (29%) and iron (50%), and manganese (96%) in ppm than control (Zeidan, 2007).

1.2. Statement of the Problem

To meet the food requirements of a growing world population, intensification of crop production was a major challenge for agriculture in the past and will remain essential in the future.

Teff [*Eragrostis tef* (Zucc.) Trotter] is one of the most important food cereal crops in Ethiopia, occupying about 22.6% of the cultivated land from the total area of cereals (86.06%) which accounts 16% of the grain production (CSA, 2012). Teff yields until recently, have been decreasing in many parts of the highlands of Ethiopia (CSA, 2012), which could be the result of plant lodging, a decline in the soil fertility due to high soil erosion and others, and unbalanced chemical fertilizer applications.

Some of the major causes of low teff yield are inability of farmers to use the required quantities of mineral nutrition and unbalanced chemical fertilizer application (Nyalemegbe *et al.*, 2012). Besides, decline in soil organic matter and insufficient attention to crop nutrient studies greatly contributed to the loss of soil fertility resulting in severe nutrient reduction of soils (Kumwenda *et al.*, 1996). It is known that Ethiopia has the highest soil nutrient outflow rates of 10 Kg ha⁻¹ while inflows from application of fertilizers are very low (<10 Kg ha⁻¹) (Tesfahunegn *et al.*, 2014).

Knorr and Vogatmann, (1983) showed that crop variety, type of compost, and fertilizer level affected the yield from plants. The effect of excess nitrogen on nutritional quality of crops has received considerable attention. Excess N diminishes taste and flavor, lower resistance to disease (rust and downy mildew), lowers resistance to insect damage (mites and aphids), and reduce the biological value of plant protein (Schuphan,1972, 1974; Knorr and Vogtmann,1983; Linder,1985).

Excess nitrogen also can reduce carbohydrate synthesis; the resulting lower content of glucose can affect taste and flavor.

In Ethiopia, the need of adequate food supply will require raising teff yields, which will increase use of fertilizers. However, little is known about the impact of fertilizers on teff nutritional content.

1.3. Objective of the study

General objective:

The general objective of the study is to determine the effect of organic and inorganic fertilizers on proximate, mineral, phytochemical compositions and anti-oxidant capacity of teff.

Specific objectives:

The study has the following specific objectives

- To determine and compare the effect of organic and inorganic fertilizers on proximate composition (crude protein, ash, fiber, and fat) of teff.

- To determine and compare the effect of organic and inorganic fertilizers on mineral composition (Fe, Zn, and Ca) of teff.
- To determine and compare effect of organic and inorganic fertilizers on phytochemicals content (phytate and total phenolic content) of teff.
- To evaluate the effect of organic and inorganic fertilizers on total anti-oxidant activity (free radical scavenging activities) of teff.
- To investigate the effect of organic and inorganic fertilizers on grain characteristics and functional properties of teff flour.

2. LITERATURE REVIEW

2.1. Description of Teff

Ethiopia's major staple crops include varieties of cereals, pulses, oilseeds, and coffee. Grains are the most important field crops and chief element in the diet of most Ethiopians. Among which the principal grain is Teff. Teff is an important food source in Ethiopian diet which is used to make injera (pancake). The word 'teff' is thought to have been derived from Ethiopian Amharic word *teffe*, which means lost due to small size of grain and how easily it can be lost if dropped. It is the smallest grain in the world, measuring only about 1/32 of inch in diameter. It takes 150 rains of teff to weigh as much as one grain of wheat. Because the grains of teff are so small, the bulk of grain consists of bran and germ. This makes teff nutrient-dense. (<http://chetday.com/teff.html>).

Teff (*Eragrostis tef* (Zucc.) Trotter) belongs to the family Poaceae, subfamily Eragrostoideae, tribe Eragrosteae and genus *Eragrostis*. The genus contains about 300 species (Demissie, 2001). Within the genus *Eragrostis* 43% of the species seem to have originated in Africa. In South America 18%, in Asia 12%, in Australia 10%, in Central America 9%, in North America 6% and in Europe 2% (Costanza, 1974). 54 *Eragrostis Species* in Ethiopia, 14 (or 26%) are endemic (Cufodontis, 1974). The fact that the genetic diversity for teff exists nowhere in the world except in Ethiopia. (Vavilov, 1951) has identified Ethiopia as the center of origin and diversity of Teff.

2.2. Overview of teff production in Ethiopia

Teff can grow under wide and diverse agro-ecologies. Even though there are areas where the crop is grown during the short rainy season (Belg), teff is mainly cultivated during the main rainy season (Meher). The length of the growing period (LGP) ranges from 60 to 180 days (depending on the variety and altitude) with an optimum of 90 to 130 days (Deckers *et al.*, 2001).

In Ethiopia, teff is mainly produced in Amhara and Oromia, with smaller quantities in the Tigray and SNNP regions. There are 19 major teff producing zones in the country. The Central and South Tigray zones are the major teff producing zones in Tigray. Within the Amhara Region, East Gojjam, West Gojjam, North Gonder, South Gonder, North Wollo,

South Wollo, North Shoa and Awi Zones are the major producers of tef. In Oromia region the major tef producing zones include the East Shoa, West Shoa, South West Shoa, North Shoa, East Wallega, Horo Guduroo Wallega, Jimma, Illubabor and Arsi (CSA, 2012).

2.3. Nutritional composition and health benefit of teff

2.3.1. Carbohydrates

Carbohydrates are the major source of energy for human nutrition and play an important role in metabolism and homeostasis. Complex carbohydrates make up 80 percent of the teff grain. It has a starch content of approximately 73 percent, making teff a starchy cereal. The amylose content of 13 teff varieties tested ranged from 20 to 26 percent, comparable to other grains, such as sorghum (Bultosa, 2007).

Using a scanning electron microscope (SEM), the size of teff starch was found to be 2-6 μm (Bultosa, Hall, and Taylor 2002; Wolter *et al.*, 2013). This makes teff starch granules smaller than those of wheat (A type 20-35 μm), sorghum (20 μm) and maize (20 μm) (Delcour *et al.*, 2010). Given their larger surface area, smaller starch granules are more susceptible to enzymatic attack (Tester *et al.*, 2004). Nonetheless, in comparison to wheat which has larger starch granules, the in vitro starch digestibility of teff was found to be significantly lower (Wolter *et al.*, 2013). In line with this, the predicted glycemic index of teff (74) was significantly lower than that of white wheat (100) but comparable to that of sorghum (72) and oats (71) (Wolter *et al.*, 2013). This somewhat lower GI for teff than expected may be explained by its amylose content, lower starch damage, and the possible formation of amylose-lipid complexes that can hinder enzymatic access and thus starch digestibility (Singh *et al.*, 2010; Wolter *et al.*, 2013). In addition, the high (68-80 °C) gelatinization temperature of teff (Bultosa 2007; Wolter *et al.*, 2013) can hinder gelatinization and thus decrease susceptibility to enzymatic attack by α -amylase (Fardet *et al.*, 2006).

2.2.2. Protein

According to (Wolter *et al.*, 2013) the average crude protein content of teff is in the range of 8 to 11 percent, similar to other more common cereals such as wheat. Teff's fractional protein composition suggests that glutelins (45 percent) and albumins (37 percent) are the major

protein storages, while prolamins are a minor constituent (~ 12 percent) (Bekele *et al.*, 1995; Tatham *et al.*, 1996).

Teff's amino acid composition is well-balanced (Wolter *et al.*, 2013). A relatively high concentration of lysine, a major limiting amino acid in cereals, is found in teff. Similarly, compared to other cereals, higher contents of isoleucine, leucine, valine, tyrosine, threonine, methionine, phenylalanine, arginine, alanine, and histidine are found in teff.

Another important feature of teff is that it has no gluten (Hopman *et al.*, 2008). Spaenij-Dekking *et al.* (2005) investigated the presence or absence of gluten in pepsin and trypsin digests of 14 teff varieties. The digests were analyzed for the presence of T-cell-stimulatory epitopes. In contrast to known gluten containing cereals; no T-cell stimulatory epitopes were detected in the protein digests of all the teff varieties assayed, thus confirming the absence of gluten in teff. This makes teff a valuable ingredient for functional foods destined for celiac patients who are gluten intolerant.

2.2.3. Minerals

2.2.3.1. Calcium, Iron, Zinc and Copper

Calcium is essential for developing and maintaining healthy bones and teeth, assists in blood clotting, muscle contraction, nerve transmission and oxygen transport. Optimal intakes reduce the risk of osteoporosis Iron is necessary for red blood cells formation and required for oxygen transport throughout the body. Zinc is essential part of more than 200 enzymes included in the digestion, metabolism, and reproduction and wound healing. It plays critical role in immune response and is an important antioxidant (Mohammed, 2004).

Notwithstanding the differences described above, teff has a higher iron, calcium and copper content than other common cereals (Mengesha, 1966). The zinc content of teff is also higher than that of sorghum and wheat.

The difference in mineral content between and within teff varieties is wide ranging. Red teff has a higher iron and calcium content than mixed or white teff (Abebe *et al.*, 2007). On the other hand, white teff has a higher copper content than red and mixed teff. However, the very

high mineral (i.e. iron) content of teff has been contested and in many instances attributed to soil contamination (Ketema 1997; Abebe *et al.*, 2007).

Table 1.Mineral content of teff grain, mg/100g

Minerals	mg/ 100g
Iron	7.63
Zinc	3.63
Calcium	180
Copper	0.810
Magnesium	184
Manganese	9.240
Sodium	12

Sources: USDA, 2014.

2.3. Phytochemicals in teff

2.3.1. Phytate (phytic acid)

Phytate, myo-inositol hexaphosphate is a naturally occurring phosphorus compound which significantly influences the functional and nutritional properties of foods. Although the presence of these compounds has been known for over a century, their biological role is not completely understood. It is primarily present as a salt of the mono and divalent cations of potassium, magnesium and calcium and accumulates in the seeds during the ripening period (Gemedede and Ratta, 2014). The high quantities of this anti-nutrient in the seed reduce their food value hence causing low utilization unless processed prior to use (Udayasekhara Rao and Deosthale, 1988). Phytate is an effective chelator of positively charged cation when consumed in feeds it will bind the nutritionally important mineral cations that it encounters in the intestinal tract, such as calcium, iron, and zinc and to proteins as well, forming complexes; the net result is reduced protein and mineral bio-availability.

Degradation of phytate can occur both during food processing and in the gastrointestinal tract. This degradation is of nutritional importance because it has been demonstrated that such controlled degradation improves the uptake of essential dietary minerals, i.e., Fe and Zn (Sandberg, 2002). Major efforts are therefore made to reduce the amount of phytate in foods by means of phytate degrading enzymes, phytases, present naturally in the plant foods or present in yeasts or other micro-organisms used in food processing. These enzymes can successively remove, one after the other, the six phosphorus groups attached to the inositol ring until it no longer binds iron and other minerals (Sandberg and Andlid, 2002; Hurrell *et al.*, 2003). Fermentation is one of the processes that decrease the levels of antinutrients in food grains. It has been indicated that fermentation of processed pearl millet grains caused significant reduction in antinutritional factors (Hassan *et al.*, 2006). Thus, Phytic acid has been completely degraded in weaning cereals by adding commercial exogenous phytases or by activating the native phytases by a combination of soaking, germinating, and fermentation processes (Hurrell *et al.*, 2003).

Phytates have been shown to prevent kidney stones by serving as crystallisation inhibitor of calcium salts in biological fluids (Curhan *et al.*, 2004). They also have glucose lowering (Lee *et al.*, 2005, 2006) and anti-cancer properties (Singh *et al.*, 2003). Given these positive effects of phytates, it remains unknown as to whether there is an optimal concentration of phytate where the beneficial effects can be appreciated with little or no compromise to mineral bioavailability.

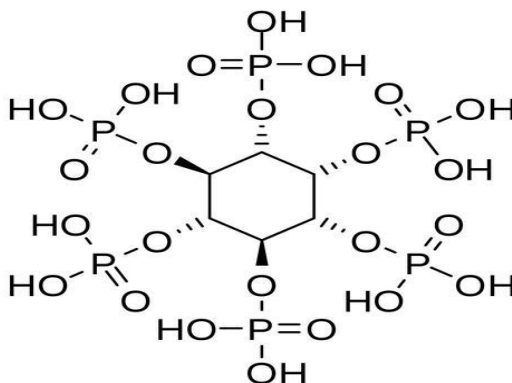


Figure 1. Chemical structure of phytic acid (Reddy *et al.*, 1982)

Teff contains high amounts of phytate with a wide range of variability, probably due to differences in varieties and growing conditions (Baye, 2014). Teff's phytate content is comparable to values reported for wholegrain cereals (Schlemmer *et al.*, 2009). Such high values in phytate are likely to impair the absorption of iron and zinc (Hurrell and Egli, 2010). The mechanism by which phytate inhibits mineral absorption is based on the formation of insoluble phytate-mineral or peptide-mineral phytate complexes in the gastrointestinal tract (Weaver and Kannan, 2002). Furthermore, phytates form complexes with endogenously secreted minerals such as zinc (Sandström, 1997; Manary *et al.*, 2002) and calcium (Morris and Ellis 1985), making these minerals unavailable for reabsorption into the body.

2.3.2. Phenolics compound in teff

Phenolic compounds (phenolics) in cereal grains encompass a diverse group of secondary plant metabolites and primarily located in the grain outer layers. The general definition of phenolic compound containing a benzene ring or derivatives of benzoic or cinnamic acid. They contain hydroxyl or methoxyl groups substituted at various position on the aromatic ring (Hahn *et al.*, 1984). Phenolic compounds can be conveniently divided into three broad groups, phenolic acid, flavonoids, and polymeric flavanols including condensed tannins (Harborne, 1980).

Baye (2013) reported the total polyphenol content of teff, wheat, barley and sorghum whole grains using the modified Folin-Ciocalteu's method. Red sorghum has the highest content of total polyphenols per 100 g gallic acid equivalents (GAE) of flour (1,607 mg), followed by barley (310 mg), wheat (143 mg), teff (140 mg), and white sorghum (81 mg). Polyphenols can hamper iron absorption from plant-based foods (Hurrell and Egli, 2010). Consequently, reducing polyphenol contents in predominantly plant-based diets has been encouraged (Matuschek and Svanberg, 2002). The galloyl content of teff was similar to that of wheat, white sorghum, and barley, whereas the catechol content was comparable to that of barley, but higher than that of wheat and white sorghum (Baye, 2013).

The polyphenol profile of teff was reported by McDonough *et al.* (2000). Ferulic acid is the major constituent of phenolic acid in teff. Vanillic, cinnamic, and coumaric are also important constituents of the phenolic acids. These major constituents of phenolic acids in teff do not

have galloyl and catechol functional groups and thus are less likely to hamper iron absorption. This suggests that it may be possible to benefit from the anti-oxidative properties of the polyphenols in teff while not compromising on iron bioavailability.

2.4. Antioxidants in Cereals

Historically, Africa's indigenous cereal grains have been major food source for human and other animals and as constituents of nutritional and technological importance, cereals nutritional constituents have been studied extensively. Cereals grains varieties contain high levels of antioxidant compounds (Harborne, 1980). Most of the literatures plant phytochemicals focus on mainly on those fruits, vegetables, wines and teas however; many antioxidant compounds in fruits and vegetables (i.e., phenolic acid flavonoids) are also reported in cereals.

All plant- derived foods contains phytochemicals such as polyphenols which affect their organoleptic and nutritional properties. Awareness of their importance in human nutrition has been aroused because of their potential beneficial effects on human health (Andreso *et al.*, 2009). Phytochemicals represent a heterogeneous group of substances including glucosinolates, the wide group of polyphenols and carotenoids. Benzoic and cinnamic acids derivatives are the two main groups of phenolic acids presents in cereals. Polyphenols in cereals have been receiving considerable attentions largely because of their adverse influence on colour, flavor, antioxidants and nutritional quality.

Wide range of factors can influence the mix of antioxidants that a plant manufactures, as well as the levels the plant producers at any given of point. These factors include soil type and chemistry, available nitrogen and level of other plant nutrients, moisture levels, temperature, pest pressure (Brandt and Mølgaard, 2002; Daniel *et al.*, 1999; Romero *et al.*, 2004; Wang *et al.*, 2000; Wang *et al.*, 2002). In general, Factors that impose stress on plant tends to trigger a plant's innate defense mechanisms and these mechanisms are driven by and/or entail the synthesis of antioxidants.

The level of IC₅₀ values of free radical and reducing power of various teff varieties varied from 0.6 to 0.88 mg/mL and 2.25 to 2.5 mg/mL respectively. The highest free radical scavenging activities (0.6 mg/ml) and reducing power (2.25 mg/ml) was observed in red tef

while the lowest scavenging activities and reductive potential were shown in white tef (0.86 mg/ml) and (2.5g/ml), respectively(Boka, *et al.*, 2013).

2.5. Plant fertilizer

The term fertilizers refer to chemically synthesized plant nutrient compounds which are usually applied to the soil to supplement its natural fertility. Fertilizer could also be defined as any organic or inorganic materials of natural or synthetic origin which are added to a soil or foliage to supply certain elements essential for plant growth (Asiegbu, 2006). They are the most effective means of increasing crop production and of improving the quality of food and fodder. Fertilizers may contain one or more of the essential nutrients.

Those that contain only one of the major elements are described as single, simple or straight fertilizers. Those that contain two or more of the major elements are classified as mixed or compound fertilizers. Nitrogen, phosphorus and Potassium are the main plant nutrients and these three provide the basis for the major groups of fertilizers (Loks *et al.*, 2014).

In Ethiopia, DAP (diammonium phosphate) and urea are the only chemical fertilizers used for crops production with initial understanding that nitrogen and phosphorus are the major limiting nutrients of Ethiopian soils(Fufa and Hassan, 2006).There are many studies to improve agricultural productivity using urea and DAP in Ethiopia. Plant growth and crop production require an adequate supply and balanced amounts of all nutrients but the use of urea and DAP are totally neglected the use of micronutrients such as iron, manganese, boron zinc and copper (Mengel and Kirkby, 1987).

2.5.1. Role of compost in plant nutrition

Compost is a homogenous and friable mixture primarily composed of stabilized (no longer decaying) organic matter and it is the product of mostly aerobically processed organic (waste) materials, with a broad origin (CCME, 1995). Prior to the utilization of mineral fertilizers, compost often was the only source of nutrients, and in biological and ecological farming this situation still exists. It is also applied to soils to maintain or increase the organic matter content of the soil, resulting in improved moisture and temperature characteristics and an increased biological activity (Chuck, 2005). Even in areas that have become reliant upon the

use of synthetic fertilizers, organic material often employed to improve soil structure, reduce evapotranspiration rates, supply small amounts of nutrients, and to improve the efficiency of nutrient cycling (Lal and Reddy, 1999; Schroth *et al.*, 2001).

Compost application results in range of environmental benefits, including improved soil health, water saving, synthetic fertilizer reduction and improved crop productivity. The beneficial effects of compost on soils have led to an increase in usage together with or instead of chemical fertilizers (Davies *et al.*, 1972; Beyer *et al.*, 2002; Schroth *et al.*, 2003). Plants cannot utilize organic nutrients from compost directly for their growth, but organic matter from compost is vital component from a soil fertility perspective as it improves soil aggregation, structural stability and water infiltration and provides nutrients after mineralization (Aggelides and Londra, 2000; Schroth *et al.*, 2003; Tajada and Gonzalez, 2003). Compost contains large amounts of nitrogen and nutrients in organic and inorganic forms (Paradelo *et al.*, 2007; Chamini *et al.*, 2008; Curtis and Claaseen, 2009; Wang *et al.*, 2010).

Compost has more advantages than other organic amendments because of reduced volume and slower decomposition rate and absence of pathogens (Bernal *et al.*, 2009). The effect of compost on plant growth and crop production not only depends on nutrients from compost, but also due to increased soil water content because compost application can reduce evaporation and erosion (Pinamonti, 1998; Arthur *et al.*, 2010).

However, nutrients added with the compost may be lost via leaching (Shepherd and Bennett, 1998; Mamo *et al.*, 1999; Basso and Ritchie, 2005). The composting process is affected by starting materials (feedstock), decomposing organisms and environmental factors (Annabi *et al.*, 2007; Farrell and Jones, 2009).

All composts work as a 'slow-release fertilizer' whereas chemical fertilizers release their nutrients rather quickly in soil and soon get depleted. Nitrogen and phosphorus particularly are not all available to plant roots in the first year because N & P in organic matter are resistant to decay. Nitrogen is about one half effective as compared to chemical fertilizer, but phosphorus & potassium are as effective as chemical fertilizers. With continued application of compost the organic nitrogen tends to be released at constant rate from the accumulated 'humus' and the net overall efficiency of nitrogen over a period of years is considerably

greater than 50% of that of chemical fertilizers. Availability of phosphorus is sometimes much greater (Bonbatkar and Vasanthrao, 1996; Reganold *et al.*, 1990). Moreover, significant amount of nitrogen is lost from soil due to oxidation in sunlight (Suhane, 2007) calculated that upon application of 100 kg urea (N) in farm soil, 40-50 kg gets oxidised and escapes as 'ammonia' (NH₃) into the air, about 20-25 Kg leaches underground polluting the groundwater, while only 20-25 Kg is available to plants.

The following procedures are the steps used to prepare pit composting in Debrezeit Agricultural Research Centre (DARC).

- ✓ Dig the compost pit in semi-shaded and water logged area.
- ✓ Place dry plant materials as the first layer. This should be about 20-25cm thick. Sprinkle enough water to make the composting materials moist but not wet.
- ✓ The next layer will be composed of green materials, either wilted grasses or weeds. Twigs and branches can also be added unless they are chopped in to smaller pieces. This layer should also be 20-25cm thick.
- ✓ Top this mixture of animal manure, soil and ash. This layer should be 10-115cm thick.
- ✓ Repeat the steps 1-3 until the pile reaches a height 1m. Make the pile thicker in the middle (than the sides) to create adome shaped pile. This makes turning the pile easier.
- ✓ Place the sticks vertically into pile to allow the air to circulate in to various layers.
- ✓ Cover the pit with broad leaves like banana leaves and taro leaves, etc.
- ✓ Turn the pile every two weeks. The compost will be ready after 3-4 months.

2.5.2. Role of Vermicompost in plant nutrition

Vermicompost is a nutritive 'organic fertilizer' rich in NKP (nitrogen 2-3%, potassium 1.85-2.25% and phosphorus 1.55-2.25%), micronutrients, beneficial soil microbes like 'nitrogen-fixing bacteria' and 'mycorrhizal fungi' and are scientifically proving as 'miracle growth promoters & protectors' (Sinha *et al.*, 2009). Kale and Bano, (1986) reports as high as 7.37% nitrogen (N) and 19.58% phosphorus as P₂O₅ in worms vermicast. Suhane, (2007) showed that exchangeable potassium was over 95% higher in vermicompost. There are also good amount of calcium, magnesium, zinc and manganese. Additionally, vermicompost contain enzymes like amylase, lipase, cellulase and chitinase, which continue to break down organic

matter in the soil (to release the nutrients and make it available to the plant roots) even after they have been excreted (Chaoui *et al.*, 2003; Lunt and Jacobson, 1994; Tiwari *et al.*, 1989).

Annual application of adequate amount of vermicompost also leads to significant increase in soil enzyme activities such as 'urease', 'phosphomonoesterase', 'phosphodiesterase' and 'arylsulphatase'. The soil treated with vermicompost has significantly more electrical conductivity (EC) and near neutral pH (Tiwari *et al.*, 1989). Vermicompost has very 'high porosity', 'aeration', 'drainage' and 'water holding capacity'. They have a vast surface area, providing strong absorbability and retention of nutrients. They appear to retain more nutrients for longer period of time. Study showed that soil amended with vermicompost had significantly greater 'soil bulk density' and hence porous & lighter and never compacted. Increase in porosity has been attributed to increased number of pores in the 30-50 μm and 50-500 size ranges and decrease in number of pores greater than 500 μm (Neilson, 1951).

Conventional composting and vermicomposting are quite distinct processes particularly with respect to optimum temperatures for each process and the type of decomposer microbial communities that predominate during active processing. While 'thermophilic bacteria' predominate in conventional composting, 'mesophilic bacteria & fungi' predominate in vermicomposting. Although the conventional composting process is completed in about 8 weeks, but additional 4 weeks is required for 'curing'. Curing involves the further aerobic decomposition of some compounds, organic acids and large particles that remain after composting. Less oxygen and water is required during curing. Compost that has had insufficient curing may damage crops.

Vermicomposting takes nearly half the time of conventional composting and vermicompost do not require any curing and can be used straightway after production (Dominguez *et al.*, 1995). Vermicomposts have much 'finer structure' than ordinary compost and contain nutrients in forms that are readily available for plant uptake. Vermicomposts have outstanding chemical and biological properties with 'plant growth regulators' (lacking in other composts) and significantly larger and 'diverse microbial populations' than the conventional thermophilic composts (Edwards and Burrows, 1998; Edwards *et al.*, 2004; Umberto and Galli, 1995).

Atiyeh *et al.*, (2000) found that the conventional compost was higher in ‘ammonium’, while the vermicompost tended to be higher in ‘nitrates’, which is the more available form of nitrogen. They also found that vermicompost has higher N availability than the conventional compost on a weight basis and the supply of several other plant nutrients e.g. phosphorus, potassium, sulfur and magnesium, were significantly increased by adding vermicompost as compared to conventional compost to soil (Atiyeh *et al.*., 2000).

Vermicompost retains nutrients for long time and while the conventional compost fails to deliver the required amount of macro and micronutrients including the vital NKP (nitrogen, potassium & phosphorus) to plants in shorter time, the vermicompost does (Bonkowski and Schaefer, 1997; Hammermeister *et al.*,2004; Subler, Edwards and Metzger,1998). This was verified by (Bhatia, 2000; Sinha and Bharambe 2007; Valani, 2009).

The nutritional quality of vermicompost is determined primarily by the type of the substrate (raw materials) and species of earthworms used for composting, along with microbial inoculants, liming, aeration, humidity, pH and temperature. Similar was findings of (Singh, 2009). Vermicompost processed by earthworms showed higher values of important plant nutrients as compared to those available in composts made from the same feed stock ‘food & garden wastes ’by aerobic & anaerobic methods.

Table 2: Important nutrients contents in vermicompost and conventional (mg/g).

Nutrients	Vermicompost	Aerobic compost	Anaerobic compost
1) Nitrogen (N)	9.500	6.000	5.700
2) Phosphorus (P)	0.137	0.039	0.050
3) Potassium (K)	0.176	0.152	0.177
4) Iron (Fe)	19.730	15.450	17.240
5) Magnesium (Mg)	4.900	1.680	2.908
6) Manganese (Mn)	) 0.016	0.005	0.006
7) Calcium (Ca)	0.276	0.173	0.119

Source: Singh (2009); Master’s Degree Project Report, Griffith University, Australia.

The following steps are followed for vermicompost preparation in Debrezeit Agricultural Research Centre (DARC).

- ✓ Vermicomposting unit should be in a cool, moist and shady site
- ✓ Cow dung and chopped dried leafy materials are mixed in the proportion of 3: 1 and are kept for partial decomposition for 15 – 20 days.
- ✓ A layer of 15-20cm of chopped dried leaves/grasses should be kept as bedding material at the bottom of the bed.
- ✓ Beds of partially decomposed material of size 6x2x2 feet should be made.
- ✓ Each bed should contain 1.5-2.0 quintals of raw material and the number of beds can be increased as per raw material availability and requirement.
- ✓ Red earthworm (1500-2000) should be released on the upper layer of bed.
- ✓ Water should be sprinkled with can immediately after the release of worms.
- ✓ Beds should be kept moist by sprinkling of water (daily) and by covering with gunny bags/polythene.
- ✓ Bed should be turned once after 30 days for maintaining aeration and for proper decomposition.
- ✓ Compost gets ready in 45-50 days
- ✓ After two days compost can be separated and sieved for use.

2.5.3. Role of nitrogen fertilizer in plant nutrition

Nitrogen is the principal raw material required for the growth of a plant. Since it is a necessary component of all proteins, N is involved in all plant growth processes. Generally, N is involved in cell multiplication, giving rise to the increase in size and length of leaves and stems, especially the stalks of grains and grasses; increases in chlorophyll contents, giving the leaves their dark green colour; plays a part in the manufacture of proteins in the plant and is part of many compounds in the plant, including certain types of basic acids and hormones(Ortiz-Monasterio *et al.*, 1997).

Chlorophyll is a green pigment found in plants, and it is necessary for photosynthesis and enables plant development, nutrient storage in different organs and nutrient cycling through transferring energy from sunlight by photosynthesis. Nitrogen is one of the basic components of chlorophyll inside. In addition, it is also effective in the enzymatic carbon metabolism and photosynthetic electron carriers system. Therefore, nitrogen supply to the plant will influence cell size and leaf area, chlorophyll formation and photosynthetic activity. In turn, this influences the amount of protein (Hansen *et al.*, 2005).

Photosynthesis capacity is affected by nutrient elements, especially nitrogen. Net photosynthesis rate of C₃ and C₄ plants varies depending on the amount of nitrogen. Nitrogen accumulated in the leaves delays aging of the leaf. In cereals, leaves remain green for long, especially in the greens for a long time in period of ear emergence increases photosynthetic activity (Kara and Mujdeci, 2010). Leaves are the organ contributing to the formation of yield in plants most. Approximately 70 - 90% of the final grain yield is derived from photosynthates (products of photosynthesis) produced by the plant during the grain filling. The flag leaf and head usually contribute most, but certainly not all, of the photosynthate to the grain (Yildirim *et al.*, 2009).

Deficiency in the supply of nitrogen has a profound effect on crop growth and development and can lead to a loss of grain yield in extreme cases (Miller and Donahue, 1995). The most easily observed symptom of nitrogen deficiency is the yellowing (chlorosis) of leaves due to a drop in chlorophyll content. Lack of chlorophyll inhibits the capacity of the plant to assimilate CO₂ and synthesize carbohydrates, leading to poor and premature flowering and fructification, with shortening of the growth cycle (IAEA, 2000). This symptom is usually noticed first in the more mature leaves, and last in the upper actively growing leaves, because the N is translocate from older to new leaves to sustain growth. Thus, the older leaves will wither and result in poor plant growth and yield reduction. Generally, growth is slowed; stunted and firing of the leaf tips and margins is evident (Evans, 1996). Nitrogen deficient plants respond quickly to the addition of N fertilizers if applied in a timely manner and properly. However, adverse effects on annual plants caused by early-stage lack of N cannot usually be corrected by late application of N (IAEA, 2000).

Nitrogen affects crop performance through its ability to determine photosynthetic capacity. Application of nitrogen at onset of stem elongation greatly stimulated leaf area growth, which resulted in significantly greater assimilation capacity, both before and after flowering. Increases in grain yield achieved through improved grain indices have, however, often outstripped improvements in the uptake and partitioning of N to grain (Gooding and Davies, 1997). Nitrogen stimulates tillering probably due to its effect on cytokinin synthesis (Botella *et al.*, 1993).

The presence of N fertilizers in excess promotes development of the aerial organs with relatively poor root growth. Synthesis of proteins and formation of new tissues are stimulated, and thus carbohydrates of high molecular weight are synthesized in insufficient amounts, resulting in abundant dark green (high chlorophyll) tissues of soft consistency. This increases the risk of lodging, and reduces the plant's resistance to harsh climatic conditions and to foliar diseases and insect predation. A non-limited supply of N also extends the growth cycle (extends the life span of a leaf), delaying maturity, and often reduces the quality of the harvestable products due to loss of chlorophyll during grain filling stage (Yang *et al.*, 2000).

Nitrogen fertilization boosts the grain yield of crops to a certain point through its influence on yield components, phenology and leaf traits. Nitrogen availability influences the efficiency of assimilated mobilization to the sink during leaf senescence, and thus affects leaf viability and activity. Dry plant material contains about 1 to 4% N and N is an indispensable element constituent of numerous organic compounds of general importance; amino acids, proteins, nucleic acids (Mengel and Kirkby, 2012). It is involved in all major processes of plant development and yield formation. Besides, a good supply of nitrogen to the plant stimulates root growth and development as well as uptake of other nutrients (FAO, 2000; Weil and Brady, 2002).

2.5.4. Role of phosphorous fertilizer in plant nutrition

Phosphorus is the second most important element for crop production next to N to achieve maximum yield. The P concentration in plants ranges from 0.05 to 0.50% dry weight. This element plays a role in an array of processes, including energy generation, nucleic acid

synthesis, photosynthesis, glycolysis, respiration, membrane synthesis and stability, enzyme activation/inactivation, redox reactions, signaling, carbohydrate metabolism, and nitrogen (N) fixation. However, it is deficient in most soils of the world. Phosphorus is unavailable because it rapidly forms insoluble complexes with cations, particularly aluminum and iron under acid conditions. The acid-weathered soils of the tropics and subtropics are particularly prone to P deficiency (Sanchez *et al.*, 1980). Application of P-containing fertilizers is usually the recommended treatment for enhancing soil P availability and stimulating crop yields.

Deficiency of P in Ethiopian soil is an indication of the complex reactions of P with soil minerals that go through intensive weathering, leaching, erosion and depletion, thus comprising diverse mineralogy and organic nature. Abebe, (1996) dealt with these phenomena depending on climatic differences and consecutive crop management practices caused considerable variability in available soil P. High affinity of P with soil minerals leads to fixation of the element which limits its availability. According to Fairhurst *et al.* (1999), deficiency of soil P might be due to low P status of parental material, weathering and soil reaction, loss by erosion and surface runoff and human mismanagement that lead to import and export of nutrient P imbalance.

It is highly extracted by crops accumulated to grains of cereals or seeds of legumes and hence removed from the soil at harvest. Moreover, P from plant residues and manure is generally insufficient to meet crop production requirements. Hence, sustainable crop production is impossible without application of P fertilizer (Sanchez *et al.*, 1996). Plants deficient in P show stunted growth and in contrast to shortage of N, are often dark green in colour. As well, maturity is often delayed compared to plants containing abundant phosphate (Salisbury and Ross, 1992).

3. MATERIALS AND METHODS

3.1. Location of the study area

The study was conducted in Tedecha wereda of Oromia Regional State on a farmer's field from August – November during the main cropping season of 2016/17. The place is located at 09° 8' 54' N latitude and 39° 38' 56' E longitude at an altitude of 1907 meters above sea level. The study area receives an annual average rainfall of about 776mm, the average minimum and maximum temperatures reach about 10 and 25.5⁰C, respectively.

The dominant soil of the study area is vertisols with high water retention capacity by its nature. The area is intensively used for crop production. Many crop types are also widely grown in the area: maize (*Zea mays* L), wheat, barley, lentils, and indigenous Ethiopian crop teff (*Eragrostis teff*) (Yemenu and Chemed, 2010)

3.2. Treatments and sample collection

The experiment was conducted in the main cropping season under rain fed condition on vertisol of farmer's field. The field experiment comprised 5 treatments including without fertilizer treatments (0NP).

T1- Control (0NP)

T2-3 tones of vermicompost

T3-5 tones of compost

T4-50% recommended NP + 50% Vermicompost

T5- Recommended NP (60KgN/ha + 10KgP/ha)

The proximate analysis, phytochemicals, extraction, digestion, sample readings for mineral test (Fe, Ca, and Zn), grain characteristics and functional properties were carried out at Addis Ababa University, College of Natural and Computational Sciences, in Center for Food Science and Nutrition, Ethiopia. Crude fiber was determined at Ethiopian Public Health Institute (EPHI).

Table 3.Type and rate of fertilizer treatments applied to teff

Fertilizer Treatments	Fertilizer to be applied per plot	Image for fertilizer treatments
Control(no fertilizer)	0NP	
Recommended NP (60Kg/ha+10KgP/ha)	156gm urea+ 60gm TSP	
Recommended rate of vermicompost (3 tones/ha)	4.3Kg	
Recommended rate of compost (5 tones/ha)	7.2Kg	
50%recommendedNP+50%vermicopost	78gm urea+30gm TSP+2.15kg vermicompost	

Field experiment was laid out in a randomized complete block design (RCBD) with three replications. The plot size was 3m x 4m (12m²) each containing 20 rows of 4m length. The total area of the experimental field was 546m² with spacing between blocks and row planting 1 m and 20cm respectively. It was sowed at seed rate of 18g per plot.

Sources of fertilizers: Triple supper phosphate (TSP), vermicompost, compost and urea fertilizers were applied to soil at the time of sowing. Urea was applied in two splits ½ at sowing plus ½ at 50% tillering stage (35 days after planting).

Teff variety:-The teff variety named *kora*, which was developed in 2015 and released by Debrezeit Agricultural Research Centre was used for the experiment.

All teff samples were treated with five fertilizer treatments (T1-control, T2-VC, T3-COM, T4-NPVC and T5-NP respectively) were brought from Debreziet Agricultural research institute, Ethiopia.

3.2. Experimental design

This study was designed to carry out an investigation on the effect of organic and inorganic fertilizers on proximate, mineral, phytochemical composition and anti-oxidant capacity of teff [(*Eragrostis tef*(Zucc.)Trotter]. The design used for study is CRD (completely randomized design).

The model for the study was:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where:

Y_{ij} = j th observation of the i th treatment

μ = population mean

T_i = treatment effect of the i th treatment

ε_{ij} = random error

3.3. Sample Preparation

All samples were taken at one lot, cleaned and stored in closed bins and used for entire study. All teff seeds with five fertilizer treatments were cleaned manually to remove husks, dust, stalks, and other extraneous materials then the teff seeds were cleaned using a sieve with size of 0.0425mm. Then some amount of teff were washed using distilled water which was available in the center for mineral analysis for case of iron determination and grinded with grinding mill of model (High Speed Universal Disintegrator FW100) which is available by the center.

3.4. Proximate composition determination

Proximate analyses of all prepared samples were conducted according to (AOAC, 2000) and total carbohydrate was determined by difference. All proximate analyses were conducted in triplicates.

3.4.1 Determination of moisture content

Moisture content of the samples were determined according to Association of Official Analytical Chemistry (AOAC, 2000) using the official method 925.09 by oven drying method. A crucible was cleaned and dried in an oven (CHINCAN, GHG-9055A, china) at 105°C for 1 hour and placed in desiccators to cool. The weight of the crucible (W_1) was determined. 5gm

samples (in triplicate) was weighed in the dry crucible (W2) dried at 105°C for 3 hours and after cooling in desiccators to room temperature it is again weighed (W3). The moisture content was determined using Equation.

$$\text{Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} * 100 \dots\dots\dots (\text{Eqn 1})$$

Where:

W1= the weight of the crucible

W2= the weight of the crucible plus weight of the sample before dried

W3= the weight of the crucible plus weight of the sample after dried

3.4.2. Determination of ash content

The ash content was determined by (AOAC, 2000) using the official method 923.03. Porcelain dishes were placed in a muffle furnace (Carbolite, Aston Lane, Hope, Sheffield s30 2RR, England) for 30min at 550°C. The dishes were cooled in desiccators (with granular silica gel) for about 30 minutes at room temperature and weighed to the nearest milligram (W1). About 2.5g of fresh sample (in triplicate) were placed in dish and weighed (W2). Dishes were placed on a hot plate furnace (Carbolite, Aston Lane, Hope, Sheffield s30 2RR, England) under a fume-hood and the temperature was slowly increased until smoking ceases and the samples become thoroughly charred. The dishes with sample were placed inside the muffle furnace at 550°C for 5 hours and cooled in desiccators for 1 hour. The ash in each dish was clean and white in appearance. When cooled to room temperature, each dish with ash was reweighed to the nearest milligram (W3).

$$\text{Total ash (\%)} = \frac{W_3 - W_1}{W_2 - W_1} * 100 \dots\dots\dots (\text{Eqn 2})$$

3.4.3 Determination of crude fat

Crude fat was determined according to (AOAC, 2000) using the official method 4.5.01. About 2g of flour was extracted with 50 ml petroleum ether or diethyl ether for a minimum period of 4 hours in the soxhlet extractor (SZC-D Fat determinator China). The solvent was then

evaporated and the extracted fat was dried in the oven and cooled in a desiccator. The crude fat was determined according to the following equation.

$$\text{Crude fat, percent by weight} = \frac{W_2 - W_1}{W} * 100 \dots\dots\dots (\text{Eqn 3})$$

Where:

W1= weight of the extraction flask (g)

W2 = weight of the extraction flask plus the dried crude fat (g)

W = weight of sample (g)

Where: (W2-W1) is sample mass in g on dry base and (W3-W1) mass of ash in g.

3.4.4. Determination of crude protein

Protein content was determined according to (AOAC, 2000) using the official method 979.09 by the Kjeldahl method.

Digestion: Fresh samples of 0.5g was taken in a tecator tube and 6ml of acid mixture (5 parts of concentrated ortho-phosphoric acid and 100 parts of concentrated sulfuric acid) was added and mixed, and 3.5 mL of 30% hydrogen peroxide was added step by step. As soon as the violet reaction had ceased, the tubes were shaken and placed back to the rack. Three gram of catalyst mixture (ground 0.5 g of selenium metal with 100 g of potassium sulfate) was added into each tube, and allowed to stand for about 10 minutes before digestion. When the temperature of the digester attained 370°C, the tubes were lowered into the digester. The digestion was allowed to continue until a clear solution was obtained, about 4 hours. The tubes in the rack were cooled in a fume hood; 25 mL of de-ionized water was added, and shaken to avoid precipitation of sulfate in the solution.

Distillation: A 250mL conical flask containing 25 mL of boric acid, 25 mL of distilled water and an indicator solution was placed under the condenser of the distiller with its tips immersed into the solution. The digested solution was transferred into the sample compartment of the distiller. Sodium hydroxide solution (35%) was added (40 mL) into the digested and diluted solution. The distillation process was continued for 9 minute until a total volume reached

between 200 mL and 250 mL. The tip of the distiller was rinsed with a few milliliter of water before the receiver was removed.

Titration: The distillate was titrated using 0.1N hydrochloric acid until reddish color appeared.

The crude protein was determined using the following formula.

$$\text{Nitrogen (\%)} = \frac{\text{VHCL for sample} - \text{VHCL for blank} * \text{NHCL}}{W_o} * 14 * 100 \dots\dots\dots (\text{Eqn 4})$$

$$\text{Protein(\%)} = 6.25 * \% \text{ Nitrogen}$$

Where: V is the volume of HCL in liter consumed to the end point of titration, N is the normality of HCL (0.1N was used), Wo is sample weight on dry matter basis and 14.00 is the molecular weight nitrogen. The % of nitrogen is converted to % of protein by using appropriate conversion factor 6.25.

3.4.5. Determination crude fiber

Crude fiber was determined by the method of (AOAC, 2000), as the combustible and insoluble organic residue was obtained after the samples were subjected to acid digestion and then alkaline distillation. Clean crucible was dried one gram celite in oven maintained at 105⁰C for one hour and placed in desiccators to cool. One gram of each sample was measured in the dried crucible using analytical balance (W₁). Two hundred ml of 1.25% H₂SO₄ (R1) solution was added to each beaker and allowed to boil for 37 minutes. The acid later drained using vacuum pump; sample was cooled for five minutes and then washed three times using distilled water. The same step (as H₂SO₄) was followed using 1.25% NaOH solutions NaOH solution (R2) except that column was used instead of beaker. Crucibles containing residue was dried at 130⁰C for two hours by drying oven cooled in desiccators and weighted (W₂). The crucibles were transferred to muffle furnace and kept for three hours at 525⁰C. Crucible containing ash was later cooled in desiccators and weighted (W₃). The crude fiber content was measured using the following formula.

$$\text{Crude Fiber in } \frac{\text{g}}{100\text{g}} = \frac{W_2 - W_3}{W_1} * 100 \dots\dots\dots (\text{Eqn 5})$$

Where,

W2=mass of the crucible

W3=mass of the crucible and the sand

W1= weight of sample

3.4.6. Determination of Carbohydrates

The available carbohydrate content of teff flour samples were determined by difference that is by subtracting the sum percentage of crude protein, crude fat, crude ash and crude fiber from 100.

% Available carbohydrates = $100 - [\% \text{Moisture} + \% \text{Fat} + \% \text{Protein} + \% \text{Ash} + \% \text{Dietary fiber}]$

3.4.7. Gross energy determination in kilo calories

The gross energy of each teff flour samples were estimated (in Kcal/g) by multiplying the percentage of crude protein, crude fat and total carbohydrate with recommended factors.

Total energy (Kcal/100g) = $(4 * \text{crude protein} + 9 * \text{crude fat} + 4 * \text{carbohydrate})$

3.5. Mineral determination

Ash was obtained from dry ashing of food sample by the procedure described above in the total ash analysis. The ash was wetted completely with 7ml of 6N HCl, and dried on a low temperature hot plate. Fifteen (15ml) of 3N HCl was added to the dried ash and heated on the hot plate until the solution boiled. The ash solution was cooled to room temperature in a hood and filtered into a 50ml graduated flask using a filter paper (Whatman 45,125mm). Fifteen (15ml) of 3N HCl was added into each crucible dishes and heated until the solution boiled, was then cooled and filtered into the flask. The crucible dishes were again washed three times with de-ionized water; the washing was filtered into a flask. Since calcium is to be determined 2.5 ml of 10 % Lanthanum chloride solution were added to the flask. Then, the solution was cooled and diluted to 50ml with de-ionized water. A blank solution was prepared in 50ml volumetric flask with the same procedure for minerals reading. Sample reading was conducted using atomic absorption spectrophotometer.

Standard solutions: Six series of working standard metal solutions (0, 2.0, 4.0, 6.0, 8.0 and 10.0 ppm for Ca, and 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 ppm for Zn and 0, 1.0, 2.0, 3.0, 4.0, and 5.0 ppm for Fe of the minerals were prepared by appropriate dilution of the metal stock solution (nitrate of the metal) with deionized water in 10ml volumetric flask. Calibration curve (concentration versus absorbance) for each element (appendix III-V) using the prepared standard solution was prepared.

The absorbance of the samples was measured using flame atomic absorption spectrophotometer by aspirating de-ionized water. Sample blank solution was run with the sample solution. The concentrations of the samples were calculated from the absorbance values of each samples using Beer-Lambert Law plot.

$$\text{Metal content} \left(\frac{\text{mg}}{100\text{g}} \right) = \frac{A-B}{W} * V * 100 * D \dots\dots\dots (\text{Eqn 6})$$

Where, W= Weight (g) of sample, V= Volume of extract in liters, A= Concentration (mg/l) of sample solution, B= Concentration (mg/l) of blank solution and D= Dilution factor.

3.6. Determination of phytochemicals of teff

3.6.1 Phytic acid

Phytic acid analysis was determined by using Latta and Eskin (1980) as modified by Vaintraub and Lapteva (1988). About 5 mg of dry sample was extracting with 100 ml 2.4% HCl for 1h at an ambient temperature and centrifuged (3000 rpm/30min). The supernatant was used for phytate estimation. About 1 ml of Wade reagent was added to 3ml of the sample solution and centrifuged. The absorbance at 500 nm was read using UV-VIS spectrophotometer (UV-7804C spectrometer, China).

A series standard solution was prepared containing 0, 5, 10, 20, and 40 µg/ml of phytic acid (analytical grade sodium phytate) in 0.02N HCl. A 3ml of standard was added into 15ml of centrifuge tubes with 3ml of water which were used as a blank. A 1ml of the Wade reagent was added to each test tube and the solution was mixed on a Vortex mixer for 5 second. The mixtures were centrifuged for 10 min and the absorbance of the solutions (both the sample and the standard) were measured at 500nm by using de-ionized waters a blank. A standard curve

was made from absorbance versus concentration and the slope and intercept were used for calculation.

The Phytic acid in mg/100g will be determined using Equation.

Phytic acid in $\mu\text{g/g} = \frac{[(\text{Ab}-\text{As})-\text{intercept}]*10}{\text{Slope} \times \text{W} \times 3}$ (Eqn 7)

Slope X W X 3

Where As=sample absorbance

Ab=blank absorbance

W=weight of sample

3.6.2. Total Phenolic Determination

Phenolic Compounds concentration in teff methanolic extracts were estimated based on procedures described by (Ferreira *et al.*, 2007). One milliliter of sample (2000 μg) was mixed with 1ml of Folinand Ciocaitu's phenol reagent. After 3 min, 1ml with of saturated sodium carbonate(35%) solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 765nm. Gallic acid was used to construct the standard curve (7.5-50 $\mu\text{g/ml}$). The results were mean values $\pm$ standard error of mean and expressed as mg of gallic acid equivalents/g of extract (GAE). Total content of phenolic in teff extract in gallic acid equivalents (GAE) was calculated by the following formula:

$$C = \frac{c \times V}{m} \dots\dots\dots \text{(Eqn 8)}$$

Where C is the total content of phenolic compounds, mg/g fresh material, in GAE; the concentration of gallic acid established from the calibration curve ($y=10.259x+0.0106$; $R^2=0.9991$) V the volume of extract in liter; m is the weight of extract, g.

3.7. Antioxidant capacity determination

3.7.1. Sample Extraction

Samples were extracted based on the procedures as outlined by Barros and Babtista *et al.* (2007) and Ferreira *et al.* (2007). The teff grains with five fertilizer treatments processed in to flour, weighted in to ten grams. The teff powder was extracted by stirring with 100ml of methanol at 25⁰C at 150rpm for 24hrs using temperature shaker incubator (ZHWY-103B) and then filtered using WhatmanNo.1filter paper. The residue was then extracted with two

additional 100ml portions of methanol as described above. The combined methanolic extract were evaporated at 40°C to dryness using rota evaporator (StuartR3300) and re-dissolved in methanol at concentration of 50mg/ml and stored at 4°C for further uses.

3.7.2. Determination of Free Radical Scavenging Activity

The DPPH molecule is a stable free radical which has a deep-violet color, characterized by absorption at 515–520 nm.

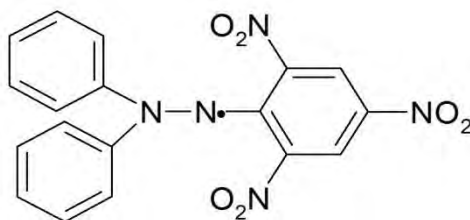


Figure 2. Chemical structure of 2,2-diphenyl-1-picrylhydrazyl (Kiers *et al.*, 1976).

The DPPH radical-scavenging assay is based on the reduction of DPPH when mixed with an antioxidant such as polyphenol, which leads to loss of its violet color and a reduction in its absorption at 520 nm (Molyneux, 2004). The effect of methanolic extracts on DPPH radical was estimated according to (Kirby and Schmidt, 1997). A 0.004% solution was mixed with 1ml of various concentrations (0.25-7.5mg/mL) of extracts in methanol. Finally, the samples were incubated for 30min in the dark at room temperature. Scavenging capacity was read spectrophotometrically by monitoring the decrease in the absorbance at 517nm. This absorption maximum was first verified by scanning freshly prepared DPPH from 200-800nm using the scan mode of spectrophotometer. Ascorbic Acid was used as standard and mixture without extract was used as the control. Inhibition of free radical DPPH in percent (I%) was then calculated:

$$\text{Radical Scavenging Activity} = \frac{A_0 - A_1}{A_0} \times 100\% \dots\dots\dots (\text{Eqn 9})$$

Where,

A₀ = Absorbance of control

A₁ = Absorbance of test sample

The extract concentration providing 50% of radicals scavenging activities (IC₅₀) was calculated RSA percentage against extract concentration (Barros & Baptita *et al.*, 2007; Barros & Ferreira *et al.*, 2007).

3.8. Grain characteristics and functional properties of teff

❖ Thousand kernel weight (TKW)

Weight of randomly selected one thousand grains was recorded in grams using electronic balance with a sensitivity of 0.0001 g and the results are expressed as the mean of triplicates observation.

❖ Bulk density of the flour

Bulk density was determined based on the methods used by Wang and Kinsella (1976). A sample of 5 g was put in to a 50 ml graduated measuring cylinder. The cylinder was tapped on a laboratory bench continuously until a constant volume was obtained. Then the volume of sample was recorded. The bulk density was calculated as weight of the ground flour (g) divided by its volume (cm³).

$$\text{Bulk Density} \left(\frac{\text{g}}{\text{cm}^3} \right) = \frac{\text{Weight of Sample (g)}}{\text{Volume of Sample after tapping (cm}^3\text{)}} \dots\dots\dots (\text{Eqn 10})$$

❖ Water absorption index and water solubility index

Water absorption index and Water solubility index were determined based on the methods used by (Solusulski, 1962). Dried the crucibles and centrifuge tubes in the oven 105⁰C for 20 minutes and cooled in desiccators and weight. Then weight 1 (g) each sample in to the tube and 10ml of distilled water and stir gently with a s stirring rod for 30 minutes. Then centrifuged the tube containing the paste at 4000rpm for 15 minutes. After 15 minutes; decant the supernatants in to crucibles and dried in the oven at 105⁰C until the supernatant is dried off. Finally, weight the residue in the tubes and the crucible after dried with the supernatant. Water absorption index and Water solubility index were expressed as the weight of water bound by 100g dried flour.

$$\text{Water absorbtion index} \left(\frac{\text{g}}{100\text{g}} \right) = \frac{W_{tr} - W_t}{W} * 100 \dots\dots\dots (\text{Eqn 11})$$

Where,

W_{tr} = Weight of tube + residue after centrifuge

W_t = weight of empty tube

W = weight of sample

Water solubility index $\left(\frac{g}{100g}\right) = \frac{W_2 - W_1}{W} * 100$ (Eqn 12)

Where,

W_1 = weight of empty crucible

W_2 = weight of crucible after drying

W = Weight of sample

3.9. Yield and yield components

- ❖ **Plant height:** Plant height was measured at physiological maturity from the ground level to the tip of panicle from ten randomly pre-tagged mother plants in each plot.
- ❖ **Panicle length:** It is the length of the panicle from the node where the first panicle branches emerge to the tip of the panicle which was determined from an average of ten randomly pre-tagged mother plants per plot.
- ❖ **Number of tillers:** The numbers of effective tillers was determined by counting the tillers from an area of 0.25 m × 0.25 m by throwing a quadrat into the middle portion of each plot.
- ❖ **Biomass yield:** At maturity, the whole plant parts, including leaves, stems and kernels from the net plot area was harvested and after sun drying for five days the biomass was measured.
- ❖ **Grain yield:** Grain yield was measured by harvesting the crop from the net plot area of 2 × 3 m² excluding border effects.



Figure 3. Tillers of teff plant



Figure 4. Panicles of teff plant

3.10. Statistical analysis:

Comparisons between sample treatments were made using one way ANOVA statistically using duncan's multiple range post-hoc test. Mean difference with $p < 0.05$ were significantly different. SPSS version 21.0 for Windows, (SPSS Inc, Illinois, USA) was used for statistical analyses.

4. RESULTS AND DISCUSSION

4.2. Proximate composition

The proximate composition of teff flour samples with four fertilizer treatments including the control which is without fertilizer were summarized in the Table.

Table 4. Proximate composition of teff (/100 g DM)

Teff Treatment	Moisture (g)	Protein (g)	Ash (g)	Fat (g)	Fiber (g)	Total CHO (g)	Energy (Kcal)
CON	10.73±0.12 ^c	9.6±0.18 ^e	1.80±0.00 ^c	2.80±0.00 ^c	2.78±0.11 ^{bc}	71.48±0.22 ^a	352.78±0.42 ^a
VC	12.07±0.12 ^a	11.64±0.10 ^c	2.29±0.01 ^b	2.84±0.00 ^a	2.65±0.9 ^c	68.66±0.42 ^b	346.86±1.60 ^b
COM	10.8±0.20 ^c	10.4±0.21 ^d	2.70±0.01 ^a	2.80±0.01 ^c	3.05±0.6 ^a	71.94±0.05 ^a	351.44±0.40 ^a
50%NP+50%VC	11.4±0.20 ^b	12.93±0.18 ^a	1.81±0.45 ^c	2.82±0.01 ^b	2.83±0.12 ^b	68.21±0.90 ^b	349.98±3.05 ^a
NP	11.2±0.20 ^b	12.41±0.13 ^b	1.81±0.00 ^c	2.82±0.01 ^b	3.19±0.10 ^a	68.73±0.42 ^b	349.92±0.94 ^a

Results were expressed as Mean ±standard deviation, n=3. Means in the same column with the same letter are not significantly different at $p < 0.05$ as assessed by Post Hoc multiple comparison tests. CON=teff without fertilizer treatment (0NP),VC=teff with recommended vermicompost(3 tones/ha), COM=teff with recommended compost(5 tones/ha), 50%NP +50%VC= 50% recommended NP+50% recommended vermicompost, NP=recommended NP(60NKg/ha and10PKg/ha).

Moisture

The moisture content of teff flours obtained from the various fertilizer treatments ranged between 10-12% of the flour weight. Slightly higher moisture was observed for VC, while the lowest value was for the CON and COM flour. The increase in transpiration especially towards the end of drying stage could also be due to enhanced water retention in soils as a result of reduced evaporation (Johnson *et al.*, 2009) and higher soil organic matter. The moisture content of all teff flour samples obtained was comparable with Bultosa, (2007).

Crude protein

Protein content was highly variable among fertilizer treatments and ranged from 10-13 g/100g DW. The highest values were obtained for the 50%NP+50%VC (12.93 g/100g), which is not surprising given that protein content is analyzed based on N conversion, and since this type of fertilizer provides N, it may be interpreted as increased protein. This is in line with the findings for rice reported by Farnaz *et al.* (2013). The lowest protein content was for the CON (10.4 g/100g). The protein content of teff flour samples with VC and COM was 12.07 and 10.8%, respectively.

Conventional compost is reported to be higher in ‘ammonium’, while the vermicompost tends to be higher in ‘nitrates’, which is a more available form of nitrogen in which the plant can absorb in the soil Atiyeh *et al.*, (2000). These findings are in line with previous studies Zahedifard *et al.* (2014); Montemurro, (2009); Barczak, (2008) and suggest that N absorption and utilization may be influenced by the type of fertilizer applied. As proteins play a critical role in human nutrition and health, this finding suggests that nutrition-sensitive agriculture programs should also aim in looking at the relationship between agronomic practices and protein content and quality.

Crude fat

The fat content for the various teff fertilizer treatments was ranged between 2.80-2.84 g/100g, which was in line with values reported by Bultosa, (2007). Although statistically significant differences were observed among the various treatments, a 0.04 g/100g difference in fat content would not be considered as biologically relevant. Unless fertilizer treatments lead to

significant changes in the physical composition of the grains, for instance lead to higher proportion of germ (higher in lipids), a plausible mechanism by which fertilizer application will lead to changes in fat contents is limited. Accumulations of fat are relatively uniform because of the effect of organic fertilizers to maintain a constant ratio between the endosperm and embryo growth by providing optimal conditions physiological processes (Simion *et al.*, 2010).

Crude ash

Ash gives an indication of inorganic elements that are present in food as minerals. The ash content of teff flour from COM and VC was significantly higher than the other treatments. This suggests that organic fertilizer could possibly provide better content of inorganic elements than the conventional chemical fertilizer Sinha *et al.* (2009). Compared with chemical fertilizer, application of organic fertilizers could also improve the supplement soil nutrients such as organic matter and micronutrients. Soil organic matter exerts a significant and direct impact on the availability of inorganic elements such as N, P, K, Cu, Zn, Fe and Mn are essential nutrients for crop growth, and their plant availability affects the uptake from soil to crop, and crop yield and quality (Song *et al.*, 2016).

Crude fiber

The maximum fiber content was obtained for teff flour with the COM (3.09 g/100g) and NP (3.19) fertilizer applications, while the lowest value was obtained for VC (2.65 g/100g). The study showed that the fiber content of all teff flour samples was ranging from 2.65 to 3.19g/100g which was comparable with Boltosa, (2007).

Total carbohydrate and energy

The total carbohydrate content teff flour obtained from the CON (no fertilizer) and the COM had significantly higher carbohydrate contents than all the other applications. Although these differences are statistically significant, they were not high enough to induce a substantial difference in the energy content of the flours.

4.2. Effect of fertilizers on minerals contents

Total Iron, Zinc and Calcium

The total iron content of teff flours from both washed and unwashed grains as well as the zinc and calcium concentrations is reported in table 5.

There was a significant reduction in iron content in the washed than in the unwashed, suggesting that part of the iron is from extraneous source due to contamination. Indeed various studies have indicated that teff is extremely contaminated with soil during the threshing process. However, the iron values obtained in the washed grains were lower than those reported earlier by various scholars (Baye, 2014). Considering that the possible effect of the contamination is removed with washing, one can see that the fertilizer applications had a different effect in the plants iron absorption. In line with the trends observed for the ash content, the highest iron content was obtained for the COM and VC applications. This suggests that perhaps the organic fertilizers COM and VC provide additional nutrients to soil or enhance the absorption of existing minerals in the soil. This will however need a more systemic evaluation, possibly using a tagged marker.

Table 5. Iron both unwashed and washed, zinc and calcium content (mg/ 100g) DM of teff

Teff Treatments	Fe Unwashed	Fe Washed	Zn	Ca
CON	3.47±0.25 ^d	2.82±0.25 ^d	2.51±0.08 ^b	163.43±0.94 ^b
VC	9.53±0.94 ^b	3.74±0.02 ^b	2.27±0.07 ^{ab}	145.89±0.42 ^c
COM	9.37±0.02 ^b	4.06±0.06 ^a	2.58±0.06 ^a	131.82±0.26 ^d
50%NP +50%VC	13.90±0.14 ^a	2.76±0.01 ^e	2.60±0.04 ^a	172.89±1.73 ^a
NP	8.82±0.08 ^c	3.36±0.02 ^c	2.37±0.28 ^{ab}	164.17±0.50 ^b

Results were expressed as Mean ±standard deviation, n=3. Means in the same column with the same letter are not significantly different at $p < 0.05$ as assessed by Post Hoc multiple comparison tests. CON=teff without fertilizer treatment (0NP), VC=teff with recommended vermicompost (3 tones/ha), COM= teff with recommended compost (5 tones/ha), 50%NP +50%VC= 50% recommended NP+50% recommended vermicompost, NP=recommended NP (60NKg/ha and10PKg/ha).

The study showed that the maximum zinc content was obtained for teff flour with COM (2.58 mg/100g) and the 50% VC + 50%NP (2.6 mg/100g), respectively. However, the differences in zinc concentrations among treatments were not statistically significant. This finding is in contrast with the report by Maleki-Farahani *et al.* (2011), who reported that barely with 50% recommended NP +50% chemical fertilizer had a maximum zinc content, but this may be explained by different plant physiology between barley and teff, as well as difference in the zinc content of the soil.

High levels of iron and zinc concentrations in vermicompost resulted in more Iron and zinc in vermicompost and vermicompost +chemical fertilizer fertilizing systems (both contained vermicompost). Soil organic matter directly affects Zinc and iron availability (Li *et al.*, 2007). So, higher levels of Fe and Zn concentration can be expected in treatments containing vermicompost. Probably, Fe concentration increases with microbial activities and release of siderophores which are compounds with high-affinity iron-chelating compounds secreted by microorganisms such as bacteria and fungi (Rengel *et al.*, 1999).

The highest calcium content was obtained for teff flour from 50%NP+50%VC. This was significantly higher than average of values obtained from NP and VC alone, suggesting that there is a possible synergistic effect that led to higher calcium content. Such synergistic effects have been previously reported Ali, (2007). In addition, in the materials and methods sections as tried to indicate about the experimental field soil type which was vertisols characterized by high cation exchange capacity(CEC).

Cation exchange capacity (CEC) is the total capacity of a soil to hold exchangeable cations. Calcium is the most dominant cation accounting for 52 to 85% of the total exchange complex. There for, it wasn't surprising that teff without any fertilizers applications (CON) was obtained 165.43mg/100g next to 50%NP+50%VC. Teff with NP fertilizers was the highest calcium content compare to the organic fertilizers. This was due to the TSP could provide another 15% Calcium to the soil. The unexpected lower calcium amount of teff with organic fertilizers could be water stress, too much or too little, can affect calcium relations within the plant, causing deficiency in the location where calcium was needed at the time of stress but, it needs further investigation for future work.

Altogether the finding from the mineral analyses suggests that fertilizer applications can influence the mineral content of teff. This is particularly true for iron and calcium concentrations. Among the various fertilizer applications, the VC and the COM lead to a higher mineral composition than the applications with no or synthetic fertilizer. Besides the mineral content of the food, its bioavailability is of importance, and thus the concentrations of phytochemicals and how they are influenced by the different fertilizer applications is of equal importance.

4.3. Phytochemicals

Data on phytic acid and total phenolic content of teff flour with four fertilizer treatments including without fertilizer (control) are presented in Table.

Phytic acid

Teff flours from fertilizer applications had significantly higher phytic acid concentrations than the control without any fertilizer. This is not surprising as phytate is a phosphorous storage of the plant; a higher phytic acid is expected with fertilization. The phytate content ranged from 256-281 mg/100g, with the highest values obtained for 50% NP+50%VC.

Table 6. Phytic acid content (mg/100g) and Total phenolic content (mgGAE/g) DM of teff

Teff treatments	Phytic acid (mg/100g)	Total phenolic content (mgGAE/g)
CON	256.39±0.37 ^e	7.15±0.10 ^d
VC	276.88±1.38 ^b	9.13±0.13 ^b
COM	268.48±1.24 ^c	10.07±0.15 ^a
50%NP +50%VC	281.53±1.77 ^a	7.10±0.21 ^d
NP	263.92±1.29 ^d	7.5 ±0.05 ^c

Results were expressed as Mean± standard deviation, n=3. Means in the same column with the same letter are not significantly different at $p < 0.05$ as assessed by Post Hoc multiple comparison tests. CON=teff without fertilizer treatment (0NP), VC=teff with recommended vermicompost (3 tones/ha), COM= teff with recommended compost (5 tones/ha), 50%NP +50%VC= 50% recommended NP+50% recommended vermicompost, NP =recommended NP (60NKg/ha and10PKg/ha).

The phytate content of our samples ranged from 256 to 281mg/100g, which was lower than reported by Baye *et al.*, (2014). This can be partly explained by the difference in methods

(HPLC vs UV-vis) used to determine phytic acid content. Differences in growing conditions can also be related to this difference.

Total phenolic content

The maximum and minimum phenolic content was found for teff flour with recommended compost and without fertilizer (control) each with 10.07 and 7.15 mg GAE/g, respectively. Teff flour obtained from VC and COM had higher polyphenol contents ($P < 0.05$). The phenolic content of teff flour obtained from recommended applications of VC and COM was 9.13 and 10.07 mg GAE/g, respectively. This could be due to the difference in phenolic compound among the organic fertilizer.

It has been postulated that relative differences in release of nutrients from various fertilizers could lead to different carbon/nitrogen ratios (i.e., low C and high N levels) initially accelerate microbial growth and decomposition and consequently differences in the production of secondary metabolites (such as phenolics) in plants (Bryant, 1983).

As known, the balance of carbon to nitrogen is necessary to produce phenolics in plants (Bryant *et al.*, 1983). Due to the plants cannot allocate the resources to growth simultaneously; added organic fertilizers with abundant carbon may push the teff plant to allocate more resources to synthesize phenolics (Riipi *et al.*, 2002).

According to Boka *et al.*, (2013) the total phenolic compound content in red teff was highest (11.47 mg GAE/g). Brown teff extract was ranked second (9.87 mg GAE/g) possessing higher antioxidant properties than white teff (8.248 mg GAE/g). Applying compost to teff crops could give comparable amount of total phenolic compounds with brown teff. So, applying organic fertilizers can replace the total phenolic compounds obtained from brown and red teff.

4.4. Antioxidant capacity

4.4.1. Free radical scavenging activity of teff

The IC₅₀ of teff flour with recommended rate vermicompost, recommended compost and without fertilizer is 1.5, 1.0 and 2.0mg/mL respectively as compared to positive control: ascorbic acid (IC₅₀=0.018).Teff with recommended rate compost was the most potent of all that could scavenge most free radical as shown in figure 3.In contrast, teff without fertilizer had the highest IC₅₀, (i.e. the least potent).

In this study the IC₅₀ teff with NP and 50% NP+50% VC were 1.75 and 1.25mg/mL respectively. From the finding both teff with the organic fertilizer had the most potent that could scavenge free radical as compared to teff with the inorganic (chemical) fertilizer one. This was because in adverse environmental condition such as slow availability of nutrients to plants under organic fertilization, plants become highly tolerant providing higher antioxidant enzyme (Ahmed *et al.*, 2010).The result obtained were in agreement with (Kesarwani *et al.*, 2014) evaluated that the free radical scavenging activities of two rice cultivar grown under organic farming had higher compared to conventional farming.

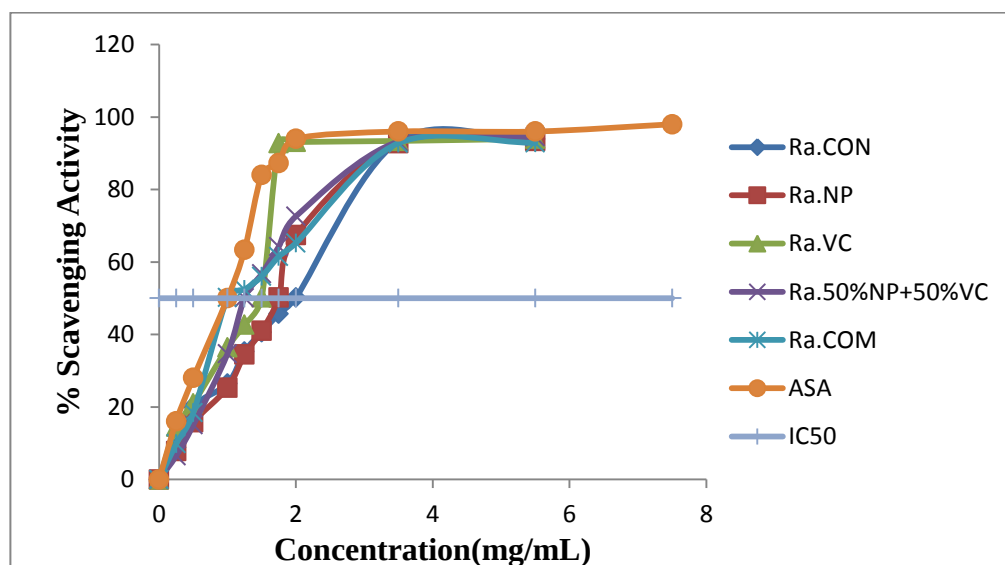


Figure 5. DPPH scavenging activity of raw teff extracts

4.5. Grain characteristics and Functional properties of teff

Thousand Kernel Weight (TKW)

The thousand kernel weight teff grains with four fertilizer treatments including without fertilizer treatments (control) was significantly different ($p < 0.05$). The highest thousand kernel weight was observed on teff flours with recommended rate of vermicompost which is 0.32g. The thousand kernel weight teff grains with recommended rate of compost, recommended rate NP, 50% recommended NP+50% recommended vermicompost and Control was 0.28, 0.26, 0.30 and 0.28 g, respectively. The thousand kernel weight among the teff grains with four fertilizer treatments was in range (0.19-0.42 g) which was reviewed for teff grain by (Assefa *et al.*, 2001).

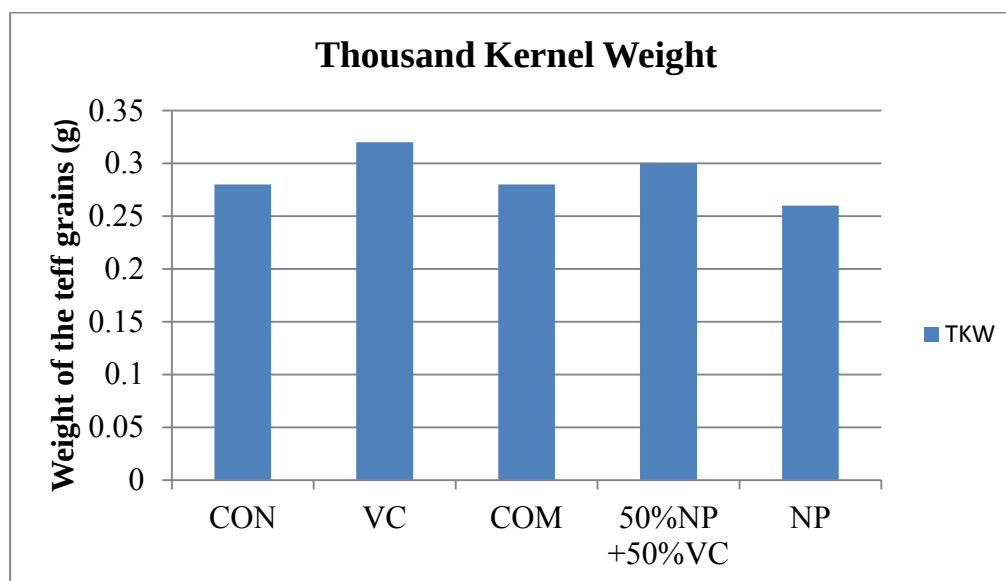


Figure 6. Thousand Kernel Weight of teff grains

Bulk density

The bulk density of teff flours with four fertilizer treatments including teff grains without fertilizer treatments (control) was significantly different ($p > 0.05$). The highest bulk density was observed on teff grains with recommended rate of vermicompost which is 0.9380g/cm³. According to Padmasshree *et al.* (1987) higher bulk density is desirable for greater ease of dispersibility of flours. In contrast, lower bulk density would be an advantage formulation of complementary foods. The bulk density of teff flours with recommended rates

of COM, NP and 50% NP+50% VC and without fertilizer (Control) was 0.8931, 0.8671, 0.8153 and 0.8671 g/cm³ respectively. The bulk densities of all teff flour samples were higher than reported by Galante, (2013) for teff.

Data on Thousand kernel weight (TKW), bulk density of the flour Water absorption index and Water solubility index are presented in Table 7.

Table 7. Bulk density of the flour, Water absorption index (WAI) and Water solubility index (WSI) of teff flour

Teff treatments	BD (g/cm ³)	WAI (g/100g)	WSI (g/100g)
CON	0.8671±0.01 ^b	203.16±0.68 ^e	4.73±0.04 ^e
VC	0.9380±0.03 ^a	211.00±0.55 ^b	5.76±0.02 ^b
COM	0.8931±0.01 ^b	205.06±0.48 ^c	5.32±0.01 ^d
50%NP +50%VC	0.8153±0.01 ^c	213.82±0.08 ^a	7.22±0.05 ^a
NP	0.8288±0.01 ^c	208.82±0.65 ^d	5.15±0.04 ^c

Results were expressed as Mean ±standard deviation, n=3. Means in the same column with the same letter are not significantly different at $p < 0.05$ as assessed by Post Hoc multiple comparison tests. CON=teff without fertilizer treatment (0NP), VC=teff with recommended vermicompost (3 tones/ha), COM= teff with recommended compost (5 tones/ha), 50%NP +50%VC= 50% recommended NP+50% recommended vermicompost, NP=recommended NP(60NKg/ha and10PKg/ha).

Water absorption index and Water solubility index

The minimum water absorption index and water solubility index obtained for teff flour without fertilizer (control) was 203 and 4.73g/100g respectively. The maximum water absorption index and water solubility index obtained for teff flour with 50% NP+50% VC was 213 and 7.22g/100g, respectively.

The increase in the water absorption index has always been associated with increase in the amylose leaching and solubility, and loss of starch crystalline structure. The flour with high water absorption may have more hydrophilic constituents such as polysaccharides. Protein has both hydrophilic and hydrophobic nature and therefore they can interact with water in foods.

The good WAI of teff flour (NP+VC) may prove useful in products where good viscosity is required such soups and gravies.

The observed variation in different flours may be due to different protein concentration, their degree of interaction with water and conformational characteristics (Butt and Batool, 2010).

4.6. Effect of integrated organic and inorganic fertilizers on yield and yield components of teff

The fertilizer treatments had a significant effect in teff yield ($p < 0.05$). The maximum grain yield was obtained for teff flour from VC fertilizer application, while the lowest was for the treatment without fertilizer (control). Inorganic fertilizers rapidly release nutrients during the early growth stages and that of organic fertilizers do release gradually up to the later developmental stages as they are slow nutrient releasers (Lorry *et al.*, 2008). Furthermore, organic fertilizers potentially increase moisture retention capacity of the soil, which enables the crop to access water even during the dry period. Edwards, (2007) indicated that crops treated with organic fertilizers resist wilting for about two weeks longer than chemically treated plots when encountering drought. Data on yield and yield components are presented in the table below.

Table 8. Effect of integrated organic and inorganic fertilizer on yield and yield components of teff

Treatments	Grain yield (Kg/ha)	Biomass yield (Kg/ha)	No. of tillers	Panicle length (cm)	Plant height (cm)
CON	1228.67±35.92 ^d	4949.97±577.35 ^d	3.57±0.21 ^{ab}	43.37±1.56 ^a	112.07±1.10 ^b
VC	2341.00±8.54 ^a	6486.97±1576.53 ^c	3.9±0.10 ^a	40.00±0.72 ^b	112.80±2.77 ^b
COM	1400.67±174.67 ^{cd}	6181.63±358.80 ^{cd}	2.8±0.20 ^c	39.93±2.33 ^b	115.23±2.58 ^{ab}
50%NP +50%VC	1619.0±340.55 ^{bc}	8783.3±500.00 ^b	3.37±0.35 ^b	42.8±0.35 ^a	112.90±2.15 ^b
NP	1803.17±96.90 ^b	11280.63±4.61 ^a	3.80±0.20 ^a	42.8±0.80 ^a	116.632±1.66 ^a

Results were expressed as Mean ±standard deviation, n=3. Means in the same column with the same letter are not significantly different at $p < 0.05$ as assessed by Post Hoc multiple comparison test. CON=teff without fertilizer treatments (0NP), VC=teff with recommended vermicompost (3tones/ha), COM=teff with recommended compost (5tones/ha), 50%NP+50%VC= 50% recommended NP +50% recommended vermicompost, NP= recommended NP (60kg/haand10kg/ha).

The biomass yield of teff with NP was the highest ($P<0.05$). The significant increase of biomass yield could be resulted by increasing the growth parameters (plant height, panicle length and tillers) since it was measured the whole teff plant parts. The biomass yield of teff grains with recommended rate of compost was 6181.63Kg/ha which was not significantly different ($p<0.05$) than treatments with VC alone or without fertilizer (control).

The highest number of tillers was founded on both teff grains with recommended rate vermicompost and recommended NP with 3.90 and 3.80 tillers, respectively. Giday *et al.* (2014) also reported positive and significant increase in number of productive tillers with increasing rates of N fertilizer on teff. Since N plays an important role vegetative growth of the plant. There was no significant difference($p>0.05$) on number of tillers between the teff grains without fertilizer (control), teff grains with recommended vermicompost and 50% recommended NP+50% recommended NP, respectively. The finding showed that the lowest number of tillers was recorded for teff grains with recommended rate of compost which was 2.8 tillers.

The panicle length for teff crop with recommended NP, 50% NP+50% VC and without fertilizer (control) were 42.8, 42.8 and 43.37 cm, respectively. However, there was no significant difference among them ($P>0.05$).The finding was in agreement with (Assefa *et al.*,2016).The minimum length was observed for teff grains with recommended rate of compost and vermicompost, with panicle length 39.93 and 40.00cm, respectively.

The highest plant height (118.23cm) was observed for teff grown with recommended rate of NP. The increase in plant height with applying recommended NP could have resulted due to sufficient supply of nutrient, which encourages plant growth: nitrogen plays critical role in the structure of chlorophyll, while phosphorous is the main element involved in energy transfer for cellular metabolism in addition to its structural role (Wiedenhoeft, 2006). The result was in agreement with the findings of Aregu *et al.*(2001), but the application of either recommended rate of VC or VC+NP did not significantly affect plant height ($p>0.05$).

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

The present study evaluates the effect of fertilizer treatment on teff grain biochemical and phytochemical composition. In addition, possible effects on functional property were evaluated. The treatments were then compared taking into account yield figures.

Teff grains with recommended 50% NP+50% VC had high protein, calcium, and phytic acid contents, while teff grown with compost application had the highest iron and polyphenol content. The compost grown teff scavenged most free radical at the lowest IC₅₀ value. Fertilizer application had also an effect on the functional property of the teff. For instance, teff flour obtained under vermicompost treatment had the highest thousand kernel weight and bulk density.

This study revealed that teff grown under organic fertilizers has higher iron and phytochemical content. Its antioxidative property, as measured by DPPH radical scavenging assay, was also the highest.

Vermicompost fertilizer application can consider as best alternative to replace chemical fertilizer considering both nutritional, mineral aspect as the same time in the grain yield.

5.2. Recommendation

The current study with its own limitation has investigated the effect organic and inorganic of fertilizer treatments on proximate, minerals, phytochemicals and antioxidant activity of teff. The following recommendations are made based the study conducted.

- Teff grains with recommended rate of compost fertilizer should get attention and emphasis by the consumers due to its good antioxidant capacity, iron and zinc content relative to what is obtained with a chemical fertilizer
- Vermicompost should be advised for farmers to improve yield, while maintaining a good nutritional profile.

Recommendation for future further work

- The effect of fertilizer on nutrient and phytochemicals compositions and antioxidant activities and should be evaluated for other varieties of teff as well as other cereals.
- Based on these findings, we recommend that awareness creation efforts towards the use of organic fertilizers can have both agronomic, economic, and health benefits.
- Further investigation should be carry out on the effect of fertilizers on sensory quality of injera made from teff.
- Studies should be conducted on the effect of organic and inorganic fertilizers starch characteristics of teff.
- Agricultural research centers should develop an easy way preparation technique for organic fertilizers since it is tedious for farmers to prepare in the conventional methods.

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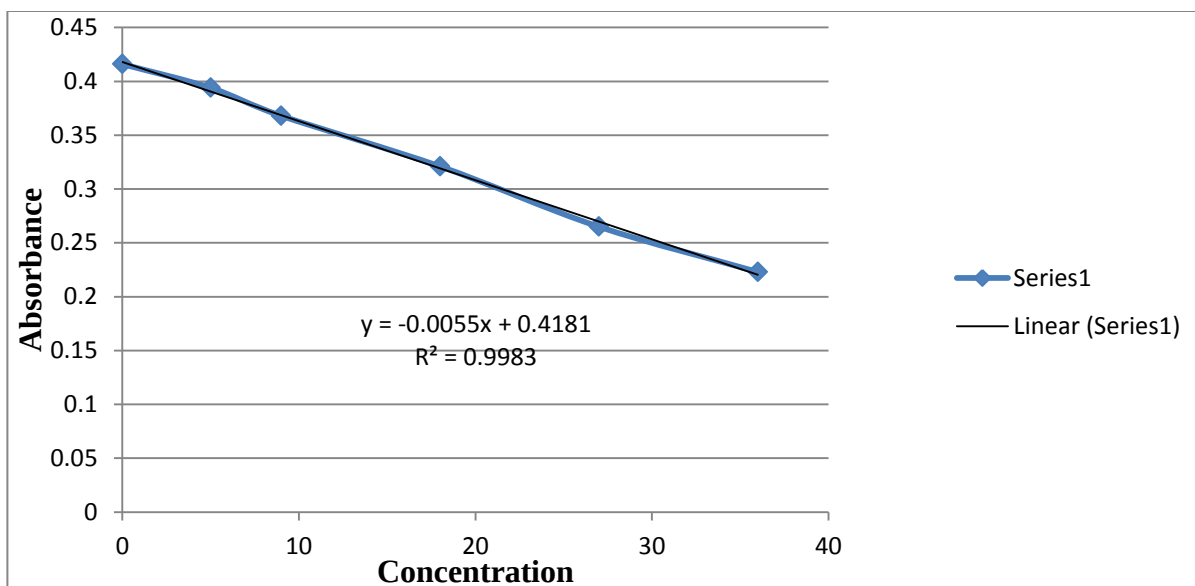
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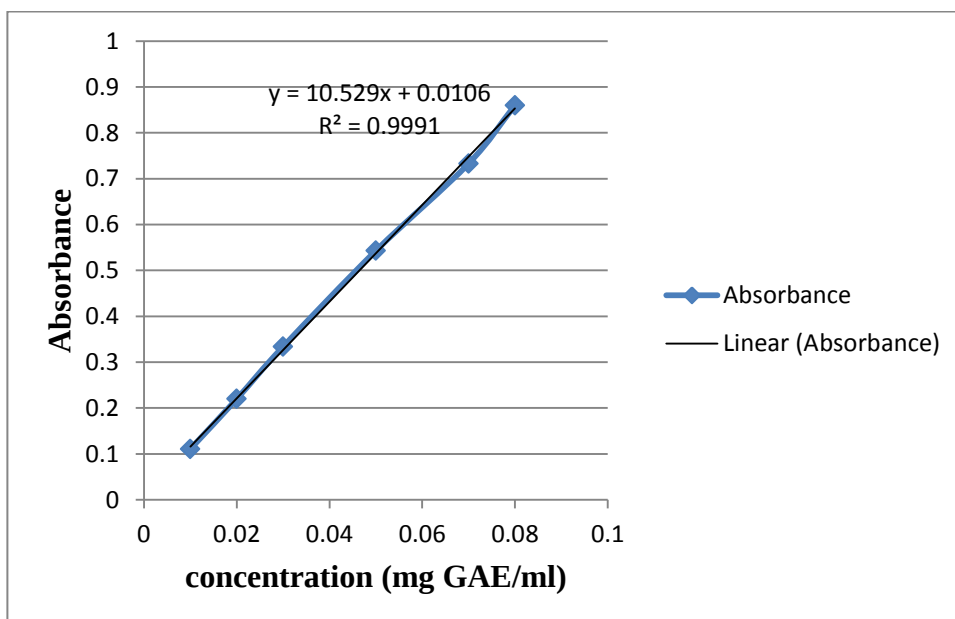
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APPENDEX

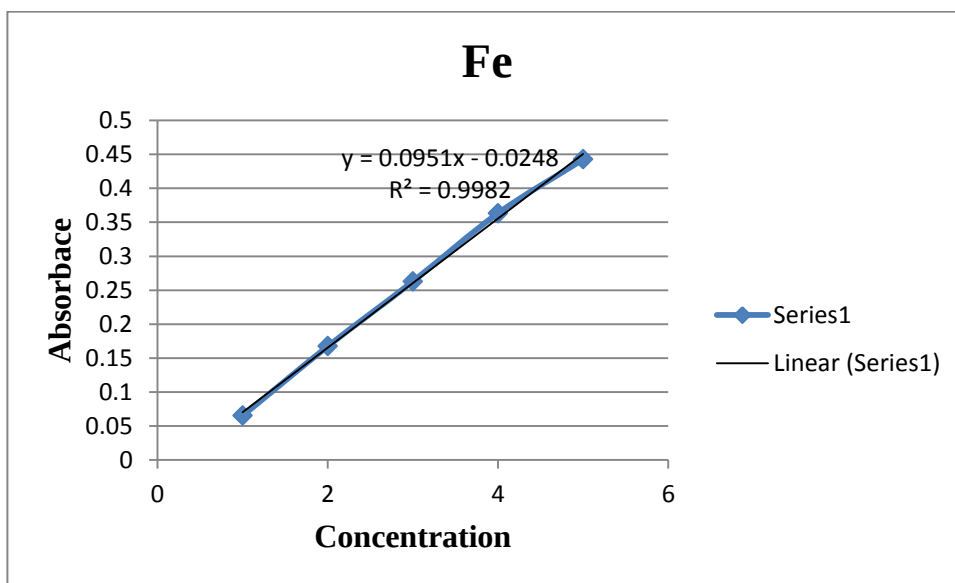
Appendix I: a standard curve for phytate determination ($R^2=0.9983$)



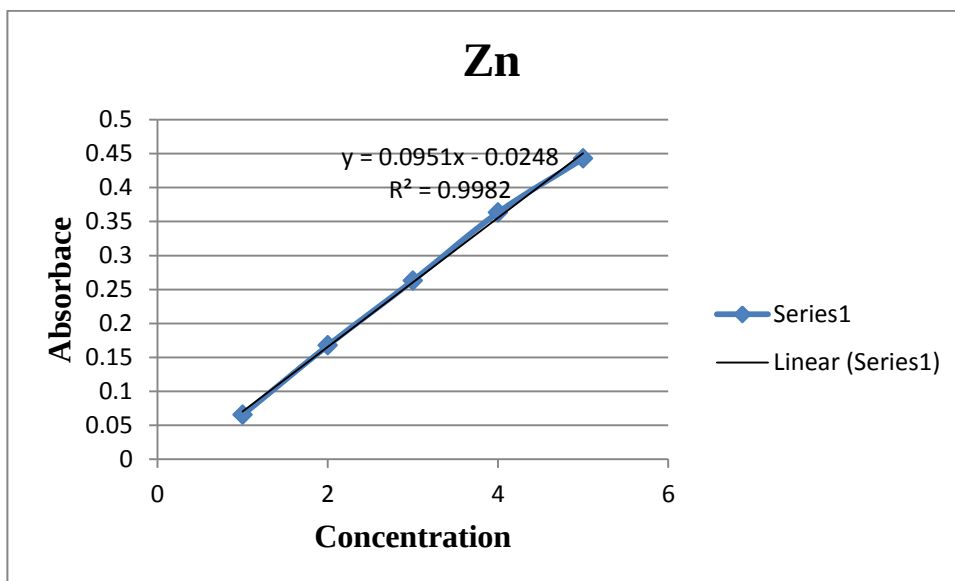
Appendix II: a standard curve for total phenolic determination ($R^2=0.9991$)



Appendix III: Iron standard calibration curve



Appendix IV: Zinc standard calibration curve



Appendix V: Calcium standard calibration curve

Appendix V: Calcium standard calibration curve

