EFFECT OF ORGANIC NUTRIENT SUPPLEMENT ON GROWTH, PRODUCTIVITY AND CAPSAICIN CONTENT OF *CAPSICUM FRUTESCENS*

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

M. MICHAEL PHAIRONG
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EFFECT OF ORGANIC NUTRIENT SUPPLEMENT ON GROWTH, PRODUCTIVITY AND CAPSAICIN CONTENT OF *CAPSICUM FRUTESCENS*

By

M MICHAEL PHAIRONG

Department of Botany

Supervisor

Prof. F. LALNUNMAWIA

Submitted

In partial fulfillment of the requirement of the degree of Doctor of Philosophy in Botany of Mizoram University, Aizawl

MIZORAM UNIVERSITY

(A Central University Established by an Act of Parliament of India)

F. Lalnunmawia
Professor

Department of Botany
School of Life Sciences
Tanhril- 796009
Aizawl, Mizoram
Phone: 91-9436153391
Email:fmawia@rediffmail.com

CERTIFICATE

This is to certify that the thesis entitled "***Effect of organic nutrient supplement on growth, productivity and Capsaicin content of Capsicum frutescens***" submitted by Mr. **M. Michael Phairong** for the degree of Doctor of Philosophy in Botany embodies the record of original investigation carried out by him under my guidance and supervision. He has been duly registered and the thesis presented is worthy of being considered for the award of the Ph.D degree. This thesis or any part thereof has not been submitted for any degree of any other University.

(Prof. F. LALNUNMAWIA)
Supervisor

DECLARATION

MIZORAM UNIVERSITY

APRIL , 2024

I **M Michael Phairong**, hereby declare that the subject matter of this thesis is the record of the work done by me, that the contents of this thesis did not form basis for the award of any previous degree to me or to anybody else, and the thesis has not been submitted by me for any research degree in any other University/ Institution.

This is being submitted to the Mizoram University for the degree of **Doctor of Philosophy** in the Department of Botany.

(Prof. F. LALNUNMAWIA)
Head & Supervisor
Department of Botany

(M MICHAEL PHAIRONG)
Candidate

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(M MICHAEL PHAIRONG)

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ABBREVIATIONS

ABTS	:	2, 2-azino-bis (3 ethylbenzothiazoline)-6-sulfonic acid
BSA	:	Bovine Serum Albumin
C	:	Carbon
^{o}C	:	Degree Celsius
%	:	per cent
Cm	:	centimetre
DPPH	:	*2, 2-diphenyl-1-picrylhydrazyl*
E	:	East
Ed	:	(eds.): Edition: editor(s) or edited
et al.	:	et alii: and others
etc.	:	etcetera or cetera: and the others
FYM	:	Farm Yard Manure
GAE	:	Gallic Acid equivalent
g/gms	:	gram(s)
ha	:	hectare
K	:	Potassium
m	:	metrum: metre
Mg	:	milligram
mm	:	millimetrum: millimetre
ml	:	millilitre
MZBEC	:	Mizoram Bird’s Eye chilli
N	:	North / Nitrogen
no.	:	numero: number

p., pp.	:	pigina: page, pages
P	:	Phosphorus
QE	:	Quercetin equivalent
Qt	:	quintal
SD	:	Standard Deviation
sp., spp	:	species (singular); species (plural)

CHAPTER I

INTRODUCTION

1.1 General

Capsicum species (Chilli) is a major spice used all over the world in diverse forms. India is the single major producer and exporter of chillies, meeting approximately half of the world's demand (Sajjan *et al*., 2020). It has been known since the beginning of civilization in the Western Hemisphere and has been a part of the human diet since about 7500 BC (Mac Neish, 1964). Chilli is an important vegetable and spice crop grown in the tropical and sub-tropical regions of the world. Chilli, one of the most important commercial crops in India, belongs to the Solanaceae family. The genus *Capsicum* includes 30 species, five of which are cultivated: *Capsicum annum* L., *C. frutescens* L., *C. chinense* Jacq, *C. pubescens* R. & P., and *C. baccatum* L. (Wang and Bosland, 2006). *Capsicum annum* is cultivated either for pungent fruited genotypes called Chilli or non-pungent fruited genotypes called sweet pepper. *C. annum* is one of the most common cultivated crops worldwide followed by *C. frutescens* (Ince *et al.*, 2010). The fruits are green when immature and turn orange-red or red at maturity. The plants are vigorous, upright, prolific bearer, fruits 3-4 lobed, medium thick flesh. It is a highly priced vegetable. It is an important remunerative crop of temperate regions, growing best at a temperature ranging between 20-30^{o}C.

1.2 Global Scenario of chilli

Chilli is produced in all the continents except Antarctica, and historically associated with the voyage of Columbus (Heiser, 1976). Columbus is given credit for introducing hot pepper to Europe, and subsequently to Africa and Asia. On his first voyage, he encountered a plant whose fruit mimicked the pungency of the black pepper; *Piper nigrum* L. Columbus called it 'Red pepper' because the pods were red. The plant was not the black pepper, but an unknown plant that was later classified as *Capsicum*. The crop spread rapidly across Europe into India, China, and Japan.

Throughout the world, *Capsicum* is grown in an area of 17,03,486 hectare with a production of 2,60,56,900 tonnes and productivity of 15.30 t/ha, while in India, it is grown in 5,761 hectares area with a production of about 53,198 tonnes and a productivity of 9.23 t/ha (Anonymous, 2014). Nutrition plays an important role in the growth and development of any crop including *Capsicum*, because it is known to exhibit positive response to the application of nitrogenous, phosphoric and potassium fertilizers particularly in light soils. Bio-fertilizers which release growth promoting substance including Auxin and vitamins improve germination of seeds and seedling growth of Chilli; and also help in improving biological activities of desirable micro-organisms in the soil and improve plant growth, yield and quality of produce. The micro-organisms such as Azotobacter are considered important not only for their nitrogen fixing efficiency, but also for their ability to produce antibacterial and antifungal compounds and growth regulators. Likewise, some phosphate solubilizing microbe like phosphoric are found to be effective in improving phosphorous use efficiency (Kumar and Srivastava, 2006).

After green revolution, production of vegetables has increased to a great extent due to use of chemical fertilizers, but their indiscriminate use has led the soil sickness, ecological hazards and depletion of non-renewable sources of energy. Moreover, in the developing countries like India, the escalating prices of fertilizers are hitting small and marginal farmers. To overcome the problems of ecological imbalance and increased cost of cultivation due to continuous use of chemical fertilizers, the latest trend of growing vegetable crops is by using organic sources of nutrients like bio-fertilizer together with inorganic fertilizers.

1.3 Classification and taxonomy of Chilli

Capsicum (chillies and other peppers) belong to family Solanaceae (tribe Solaneae, sub tribe Capsicinae), which also includes other economically important crops such as tomato, potato and tobacco (Dias *et al.,* 2013). They consist of annual or perennial herbs or shrubs and are native to South and Central America and the Galapagos (Walsh and Hoot, 2001). They are predominantly diploid (2n=24, infrequently 2n=26), except for a few (Moscone *et al.,* 2003). The genus *Capsicum* can be grouped into different categories based on the ability of members to successfully interbreed. These include *annum*, made up of the species *C. annum* (varieties *glabriusculum* and *annum*), *C. frutescens*, *C.* chinense, *C. chacoense* and *C.galapagoensis*; the baccatum group which consists of the species *C. baccatum* (varieties *baccatum, pendulum and praetermissum*) and finally C. *tovari,* and the pubescens group which is also made up of the species *C. cardenasii, C. eximium* and *C. pubescens* (Pickersgill, 1997). The genus has five major domesticated species of which *C. annum* is the most widely cultivated species worldwide (Andrews, 1984). Pepper, though a self-pollinated crop has been considered as a cross-pollinated crop as a result of its high rate of out crossing which ranges from 7 to 90% (Allard, 1960). Natural inter-specific crosses among *Capsicum* species are very high, resulting in intermediary forms which are complex to categorize (Allard, 1960). As a result, *C annum*, *C. chinense* and *C. frutescens* have been considered as one species (C *annum* L.) with four variety classes (Nsabiyera *et al*., 2013). These are the West Indies chilli (chinense group), bird chilli (frutescens group), hot chilli (annum group) and sweet pepper group.

1.4 Floral characters and morphology of Chilli

Capsicum is a highly heterogeneous plant which exhibits considerable morphological variation, especially in fruit shape, colour, and size (Walsh and Hoot, 2001). Pubescence of leaves and stems range from glabrous to very pubescent. Pepper produces bisexual flowers which are borne at the intersection between the stem and leaves at points where the stem splits into a fork. The inflorescences may vary from solitary to seven flowers at one node (Berke, 2000). The calyx may range from long, green sepals to truncate sepals to spine-like projections. The pedicel length varies among cultivars, ranging from 3 to 8 cm (Berke, 2000). In the species *C. annum* the petals are usually white with five to seven individual stamens which vary in colour from pale blue to purple anthers. Shaw and Khan (1928) observed greenish-white corolla in *C. frutescens* and added that corolla colour is one of the most consistent features of distinguishing *Capsicum* species. The pistil is made up of an ovary, which contains two to four carpels or lobules, and a stigma borne at the tip of a slender style (Berke, 2000). The length of the style and relative position of the stigma and the anthers vary among genotypes, and it is an important factor determining the level of natural cross pollinations of the flowers. The flower colour, shape, length and relative positions of the styles also vary with different species and cultivars. The fruits are, botanically, classified as berries with different varieties of shapes, colours and sizes that vary among cultivars. Seeds are cream coloured, except for *C. pubescens* which has black seeds (Berke, 2000).

Knowledge of the phenotype given by morphological descriptors is important in giving correct species identification (Dias *et al.,* 2013). Morphological markers are readily available and very easy to identify and in most cases do not require special skills. They offer simple 15 and straight-forward approaches to distinguishing different genotypes even at the farm level compared to molecular markers which in most cases require sophisticated laboratories. Morphological characterization is the only means by which plants can be differentiated based on their physical appearance. It is very essential in bringing to light traits of agronomic importance especially quantitative traits for crop improvement (Geleta *et al.,* 2005). Even though morphological characterization is important in variety identification, its application is influenced by prevailing environmental factors (Gepts, 1993; Geleta *et al.,* 2005) and, as such, make its use limited. It also falls short in its ability to detect differences between closely related individuals. Lack of polymorphism, environmental interference, and dependence on the state of crop growth and masking of recessive characters, limit the effectiveness of phenotypic characters though they can be effective in some cases (Costa *et al.,* 2009). The use of DNA-based molecular markers provides a high throughput method for assessing genetic heterogeneity among genotypes (Moreira *et al.,* 2013).

1.5 Ethnobotanical and economic Importance of Chilli

Pepper is a vital commercial crop, cultivated for vegetable, spice, and value-added processed products (Kumar and Rai, 2005). Besides vitamins A and C, the fruits contain mixtures of antioxidants notably carotenoids, ascorbic acid, flavonoids and polyphenols (Nadeem *et al.,* 2011). This makes it a very important constituent of many foods, adding flavour, colour and pungency and, hence, an important source of nutrition for humans. Peppers can be used whole, chopped or in various processed forms such as fresh, dried and ground into powder (with or without the seeds), or as an extract. In most advanced countries, the fresh fruits can be processed into paste and bottled for sale in supermarkets. Bell pepper, being a very rich source of vitamins A, C, B_6, folic acid and beta-carotene, provides excellent nutrition for humans (Nadeem *et al.*, 2011). Antioxidant compounds present in the different colours (green, yellow, orange, and red) in sweet bell peppers give them an anti-oxidative potential which helps protect the body from oxidative damage induced by free radicals when consumed (Simmone *et al*., 1997). This reduces the risk of cardiovascular diseases, asthma, sore throat, headache and diabetes. Red pepper on the other hand contains lycopene which is believed to possess anti-cancer properties (Simmone *et al.,* 1997). It is also used by the security agencies in the preparation of tear gas for crowd control. As a commercial crop, pepper was ranked as the second valuable vegetable crop. Notwithstanding the numerous advantages, the crop still remains a neglected crop that is of rare national priority in terms of agricultural development in many countries (FAO, 2010).

Chilli is a major spice cum vegetable crop used all over the world in diverse form. India is the single major producer and exporter of chillies, meeting approximately half of the world's intake demand (Sajjan *et al*., 2020). The compounds liable for the pungency of the chilli peppers fit to group of secondary metabolites known as Capsaicinoid. Two chief Capsaicinoid found in chillies are Capsaicin and dihydrocapsaicin. Capsaicin and dihydrocapsaicin characterize about 77-98% of total Capsaicinoids content in peppers. Capsaicin is a very steady alkaloid, which is stable under exposure to heat and cold, has no flavour, colour, and aroma and is insoluble in water, but effortlessly soluble in fat or some organic solvents (DeWitt and Bosland, 2009). Capsaicin is acknowledged for his pharmacological, neurological and dietetic effectiveness. It has also significant antibiotic activity and the ability to reduce the cholesterol level in blood (Gurnani *et al*., 2016). Capsaicin is also capable to kill some types of cancer cells (Anandakumar *et al*., 2013, Dawan *et al*. 2017, Prasad *et al*., 2006) and provide 861 November 8–9, 2017, Brno, Czech Republic 24 years relief in arthritis and respiratory ailments (Prasad *et al*., 2006), it is also antioxidant and has anticancer, anti-arthritic and analgesic properties (Dawan *et al*., 2017, Prasad *et al*., 2006).

Plant phenolic are secondary metabolites that constitute one of the most common and widespread groups of substances in plants. Phenolic biogenetically arise from the shikimate-phenyl-propanoids flavonoids pathways (Lattanzio *et al*., 2006*)*. Plants need phenolic compounds for pigmentation, growth, resistance to pathogens and for many other functions. Therefore, they represent adaptive characters that have been subjected to natural selection during evolution. Chilli pepper is also known for its richness in ascorbic acid, a very essential antioxidant for human nutrition and proper functioning of body (Igwemmar *et al*., 2013; Mohammed *et al*., 2013). Fresh green chilli pepper contains more vitamin C than citrus fruits and fresh red chilli pepper has more vitamin A than carrots (Chigoziri and Ekefan, 2013). Chilli pepper is also suitable for the diets of the obese and is useful in the control of cancer of the stomach and colon (Tayeb Rezvani *et al*., 2013; Dang *et al*., 2014).

1.6 Chilli production in India

India produced 36.57 tons of chilli from an area of 39.19 hectares in 2013. This accounted for about 27.24 per cent of the total area under spice cultivation and 25.65 per cent of total spice production in India. (Damodara, 2004). Andhra Pradesh stands first in the list of major chilli producing states in India and also has the maximum acreage under chilli cultivation in the country. It alone commands 57.80 per cent of the chilli production in India (Velayutham, 2015) with a production of around 7.7 lakh tones of chilli, followed by Karnataka (12.40 %), Orissa (4.82 %), West Bengal (4.80 %), Maharashtra (3.55 %), Gujarat (3.53 %) and Tamil Nadu (3.22 %). The major chilli producing states in India viz. Andhra Pradesh, Karnataka, Orissa, West Bengal, Maharashtra, Gujarat and Tamil Nadu contribute around 83 per cent of the total area under chilli cultivation in the country and 90 per cent of the total production.

India is the major consumer of chilli followed by China, Mexico, Thailand, USA, UK, Germany and Sweden. The major importers of Indian chilli are USA, Sri Lanka, Bangladesh, Nepal, Mexico, Canada, UK, Saudi Arabia, Singapore, Malaysia and Germany.

In 2020-21, total area of chilli in India was 7, 32,213 ha, with the production of 19, 88,304 lakh tons with vast majority of production occurring in Andhra Pradesh (42%) followed by Telangana (20.48%) and then by Madhya Pradesh (14.71%).

Total area and production of spices in Madhya Pradesh is 6, 99,994 ha and 32, 37,655 tons respectively. Chilli covers the area of 1, 13,366 ha (16.19%) and production of 2, 62,616 lakh tons (8.1%) (Spice board, India, 2020). Though Indian exports are showing satisfactory trends, nowadays India is facing a very tough competition in the international export market as price of the Indian chilli powder is considered too high and other competing countries are providing chilli at very competitive rates to the major importing countries. The exports can be further improved, provided India is able to meet the strict quality demands of the international market. Steps have to be taken by the government to encourage exporters.

1.7 Chilli Cultivation in North-East India

Chilli is widely cultivated in North -eastern states of India such as Assam, Nagaland, Manipur, Mizoram, etc. In North Eastern state the farmers practise Jhum cultivation (shifting cultivation) in paddy fields as sporadic with summer paddy and also in small homestead garden which can be kept for 2-3 years beyond which the fruit size reduces. In Jhum cultivation direct seeding is practiced in paddy fields and the peak harvest time is between August-September. The Chilli variety is indigenous to the North-East region of India and it is an important crop of the North-East India but scientifically it has not been explored to its fullest. According to Dhaliwal (2007), the Christian missionaries might have directly introduced chilli to the north-eastern India from South America. Majority of farmers in north eastern region of India still rely on the traditional systems of cultivation. Unregulated jhum (shifting) cultivation has not only been proved to be inefficient in terms of production but also a major threat to sustainable crop management. Scientific package of practices is not available for Chilli in this region where fertilizer uses is among the lowest in the country.

High rainfall causes leaching of nutrients and makes the crop vulnerable to weeds, insects and diseases resulting in poor yield. There is an urgent need to develop and popularize an effective production technology for this crop. Monsoon season with high humidity is ideal for the cultivation of the crop. In hilly areas, planting of chilli is done in February/March and the harvesting starts from May/June onwards. However, in kitchen gardens, the plants stay for 2–3 years and grow as high as 3 to 4m (Bhagowati and Changkija, 2009).

1.8 Chilli Cultivation in Mizoram

Under shifting cultivation in Mizoram, chilli is usually cultivated in the same field only for one year. However, most of the species of chilli can be grown for about three to five years under proper soil nutrient management under rain-fed condition. Although the agro-climatic conditions of north eastern India offer vast scope for the production of chilli, there exists a few bottlenecks in enhancing the production and promoting the export of this valuable commodity. There has been an increasing interest to use organic manures as a trail to compensate the decrease in soil fertility. The need to reduce costs of fertilizing crops has revived the use of organic fertilizers (Farhad *et al.*, 2009). The agricultural land in Mizoram is comprised of sloppy upland and lowland of valley (settled agriculture). In general, the upland and lowlands are under traditional system of cultivation without any improved input technologies. Among the North-Eastern states of India, Mizoram is known for presence of considerable diversity of Birds eye chilli with respect to fruit shape, size, colour, pungency, plant type, physiological characteristics, reactions to diseases and pests, adaptability and distribution. In Mizoram, Birds eye chill is locally known as *Hmarchate* or *Vaihmarchate* or *Mizoram Bird's eye chilli (*MZBEC) which belongs to species *Capsicum frutescens* and is widely grown in the state of Mizoram. Besides being used as spice and vegetable, they are also used as a very good source of ethno-medicines for a number of diseases by the traditional healers. It has also been used conventionally by different ethnic communities of the North-Eastern India in treating various human ailments (Bhagowati and Changkija, 2009). Besides being used as spice and vegetable, they are also used as a very good source of ethno-medicines for a number of diseases by the traditional healers. However, systematic and more extensive ethno medicinal investigations are not carried out to provide new insights into the other traditional uses of this important plant. In-depth research endeavours should also be directed towards phytochemical and pharmacological investigations of Mizo chilli that could lead to unearthing new bioactive compounds/activities. Mizo chilli is tiny, green and when matured the colour changes to bright red. The pungency varies from the place and environmental condition it receives. Due to the long history of cultivation, out crossing the nature and popularity of the crop large genetic diversity including local landraces have evolved. In hot chilli great range of variability for several attributes (Fruit shape, size, colour and bearing habit and semi - perennial and perennial and pungency) occurs throughout the North east region. In Mizoram itself three different varieties of Mizo chilli are cultivated in different parts. The production of chilli in the state can be traced up to the recorded history, B. c. Nuthara, in his book Mizoram society and politics mentions that when the village rand became free from the traditional clutches for the chiefs agriculture cash crops like cotton, ginger and chillies became a new source of wealth. The state is considered to be the germplasm bank for Mizo chilli.

It occupies an area of 100 ha. with a production of 200 tonnes and productivity of 2.0 tonnes/ha .The cultivation of Mizo chilli is scattered all over the state of Mizoram and some parts of Manipur. Three different varieties of Mizo chilli are being cultivated in all different districts of Mizoram. All these varieties are considered to be same with minor difference in quality. The local Government of Mizoram claimed that Mizoram is the world's native place of a Bird's Eye Chilli known locally as *Hmarchate.* It is evident that it is important to make a study on the application of organic fertilization and soil amendments with basic nutrient requirement to increase the productivity and life span of Mizo chilli. The present investigation will be taken to study which of the selected varieties of *Capsicum* would give better growth; productivity and life-span under the same treatment of organic fertilization comparison with control (no treatment). Moreover, the Capsaicin content of the selected *Capsicum* species will be determined using spectrophotometry method (Collins *et al.,* 1995). The study would be useful in finding improved technological package for cultivation and production of chilli.

1.9 Taxonomy and Morphology of selected varieties

Taxonomical classification of *Capsicum frutescens* (Carrizo Garcia *et al*., 2016) is given below

Kingdom - Plantae

Sub Kingdom - Trachobionta

Division - Magnoliophyta

Class – Magnoliopsida

Sub Class - Magnolidae

Order - Solanales

Family - Solanaceae

Genus - *Capsicum*

Species – *frutescens*

The plants of *Capsicum frutescens* are typically upright with one or sometimes two flowers per node, with green or greenish white corollas. Calyx teeth and a calyx constriction are lacking in *Capsicum frutescens*. Fruit are typically upright, soft, and deciduous. Mizo chilli/Hmarchate is characterized by a bushy type of plant growing up to 120 cm and the leaves are smooth, oval shaped up to 6.35 cm in size. Flowering in *Hmarchate* starts three months after planting and fruits continuously thereafter. The size of the *Hmarchate* ranges from 2.0 to 4.0cm in length and about 1.00cm in diameter. Three different colours of fruit at mature stage were orange, light red and red. Fruit are small sized and highly pungent and the colour of the mature fruit is dark red. The distinctive red colour of the *Hmarchate* is believed to be responsible for high availability of potash in the *Jhum* lands. According to the Spice Board of India, the Capsaicin content of Mizoram *Hmarchate* is 0.59 %. There are three popular varieties of differentiations in *Mizoram Bird's Eye chilli (*MZBEC) which are given below.

I. MZBEC A: The variety of *Hmarchate* MZBEC 'A' is the smallest, thin and most pungent among all the other variety. It is considered to be of the best quality and fetches the highest demand in the market. The chilli powder of this variety can be identified because of the slight difference of colour. Its colour is more shiny red in comparison of other two varieties.

II. MZBEC B: It is slightly thicker than MZBEC ‘A’ and marginal longer in size. The colour of dried red chilli changes to dark red and pungency is slightly less.

III. MZBEC C: The properties of MZBEC ‘C’ is almost similar to MZBEC ‘B’ but the size are a bit longer than other varieties of this segment.

Plate 1.Floral morphology and three varieties of Mizo chilli (*Capsicum frutescens*).

1.10 Application of Organic manure in Chilli cultivation

As agriculture technologies develops and becomes more intensive in its use of land and water resources in order to increase food production to meet the nutritional demand of vast growing population, its negative impacts on agricultural eco-systems was become more destructive (Millenium Ecosystem Assessment, 2005). Consequently, a great attention has been given to clean agriculture and application of eco-friendly practices. One of the most significant ways to achieve is using of organic and bio-fertilizers farming. Organic farming of vegetables is most appropriate as most of the vegetables are consumed in the fresh form and chemical residues have adverse effect on human health. Nitrogen is a major limiting nutrient for crop production. It can be applied through chemical or biological means. Over application can result in negative effects such as leaching, pollution of water resources, destruction of microorganisms and friendly insects. Due to the prohibitive cost of chemical fertilizers, majority of farmers who are mostly marginal and small, do not apply the recommended dose of fertilizers. The use of organic fertilizers provides soil with essential nutrients and adsorbs nutrients against leaching. Also improve soil texture, increase ion exchange capacity of soil, increase soil microbial populations and activity, improve moisture-holding capacity of the soil and enhanced soil fertility (Arancon *et al*., 2005). Lower availability of plant nutrients in plots applied with organic amendments is expected due to slower release rates of nutrients from organic materials particularly during initial years of conversion to organic production (Gopinath *et al*., 2009).

Farmers are using indigenous organic manures as sources of nutrients. These organics are bulky in nature but, contain reasonable amount of nutrients. Sweet pepper crop responds well to the application of both organic manures and inorganic fertilizers. The use of organic manures in INM helps in mitigating multiple nutrient deficiencies. The application of organics has also been shown to increase ascorbic acid content in chilli, which is one of the important quality parameters. Organic fertilizers are gaining importance because of their low cost, no residual toxicity and capacity to enrich soil fertility in addition to high returns under favourable conditions. There is greater demand in the international market for organically produced chilli. Application of inorganic fertilizers at the rate of 100:50:50kg N: P_2O_5: K_2O per ha produced highest fruit yield per vine (1.76kg/vine) and highest fruit yield per ha (117.33q/ha) in ridge gourd cv. DPL-RG-17 (Shinde *et al*., 2003). To determine the effects of foliar application of a novel organic liquid fertilizer on growth and yield in chilli (*Capsicum annum* L. var. Shama). The present investigation has revealed the consistent and significant results for growth parameters due to application of novel organic liquid fertilizer. Out of five different treatments, the 3% treatment resulted in maximum, plant height; number of branches per plant; leaf number; leaf area; fresh and dry weight of the plant; number of fruits per plant and total yield (Deore *et al*., 2010).Integrated nutrient management in chilli genotypes that the application of FYM @ 10 tones ha-1 along with RDF increased oleoresin content and yield by 16.97 per cent and 124.23kg ha-1, respectively over 100 per cent RDF alone. (Santoshkumar and Shashidhara, 2006). The nutrient requirement can be met through chemical fertilizers, organic manures and biofertilizer which constitute the main sources of plant nutrients. However, in order to trigger off production, indiscriminate use of chemicals has been observed in many parts of the country which has questioned the nutritional security of the crop and also its adverse impact on the environment. The long term sustainable production of the crop thus needs balanced supply of essential plant nutrients in available form, which involves systematic exploitation of potential soil resources, chemical fertilizers, biofertilizer and organic manures.

Organic manures supply the major nutrients, micronutrients, besides improving soil properties. In the light of the above facts, a field experiment was conducted involving organic manure (cow dung), poultry manure as source of nutrients to find out the best combination of manures for obtaining the higher yield of *Capsicum frutescens*.

Objectives

The specific objectives of the present study are given below:

1. To determine the soil Physico-chemical properties under different organic amendments.

2. To study the effect of soil organic amendments on growth, productivity and life span of the three varieties of *Capsicum frutescens* (Mizo chilli).

3. To determine the quantity of nutrients present in *Capsicum frutescens* (Mizo chilli).

4. To determine Capsaicin’s content of the three varieties of cultivated *Capsicum* frutescens species in relation to organic amendments.

5. To determine the quantity of phytochemicals present in *Capsicum frutescens* (Mizo chilli).

CHAPTER 2

LITERATURE REVIEW

The available literatures on the distribution, taxonomy, varieties, morphology of *Capsicum* species and its economic importance, cultivation, planting methods and nutrients management have been reviewed in this chapter.

2.1 Origin and distribution of chilli

The origin of *Capsicum* species is extended from Mexico in the North to Bolivia in the South of Latin America, where it has been part of human diet since about 7500BC (Purseglove *et al*., 1981). Spanish and Portuguese explorers spread pepper around the world. Pepper was introduced to Spain in 1493, England in 1548 and Central Europe in 1585. Then, from Europe it spread to Asia. Currently the crop is produced in various countries around the world including India, China, Pakistan, Indonesia, Sri Lanka, Thailand and Japan in Asia and Nigeria, Uganda and Ethiopia in Africa. India and Indonesia have been the largest producers. Chillies are the cheapest spices available in India and are eaten across all groups. India is the world leader in chilli production followed by China and Pakistan. In India, chilli is cultivated over an area of 0.81 million ha during 2010-11 with an annual production of 1.22 million tonnes green chillies (Anon., 2011).

Chilli occupies number one position in export of spices with 2,09,000 metric tonnes volume worth Rs.1097 crores. Organic fertilizers are essential for the proper development of plants, vegetables, flowers and fruits, as they offer rapid growth with superior quality to all species as they improve the physical properties of the soil as well (Nasef *et al*., 2004). Bhata and Shukla (1982) reported that organic fertilizer (farm yard manure) resulted in significant increase in soil carbon, nitrogen, pH, cation exchange capacity, and exchangeable Ca, Mg, and K which invariably enhance crop yield and productivity. Ludilov and Ludilova (1977) reported that the chilli responded well to application of 120 kg/ha each of nitrogen and phosphorus and potassium producing 54% higher yield than unfertilized control. Use of bio-fertilizers is one of the important components of integrated nutrient management, as they are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable agriculture. Several microorganisms and their association with crop plants are being exploited in the production of bio-fertilizers. They can be grouped in different ways based on their nature and function. *Capsicum* can grow on soils of all textures but moist, well-drained conditions and loose structure is best for rapid growth. Soil pH of 4.3 to 9.7 is better tolerated by this species. The species is intolerant of shade and frost, and fruits best in full sun and temperature above 7°C. These can be cultivated in areas that receive from 30 to 430cm of annual precipitation at elevations from near sea level to more than 2,000 meters.

2.2 Taxonomy and Morphology

Hot pepper (*Capsicum* species) belongs to the Family Solanaceae, Genus *Capsicum,* and species *frutescence* L., group of vegetables. Cultivated peppers are all members of the world *Capsicum* species. There are an estimated 1,600 different varieties of pepper throughout the world with five main domesticated species that includes *C. annum* L., *C. frutescens* L. *C. Chinenses., C. baccatum* L.*, and C. pubescens* R. (Bosland *et al*., 2000). *Capsicum* peppers are commercially classified by the concentration of Capsaicin ($C_{18}H_{27}NO_3$) which determines a variety's "hotness", *Capsicum* species are diploid, most having 24 chromosome number (2n=24). But recent studies indicated the chromosome number for non-pungent species is n=13. They vary in size, shape, colour, flavour and degree of hotness, from mild to very hot (Tong and Bosland, 2003). According to Salter (1985), their production and consumption have steadily increased worldwide during the 20th century due to their roles as both vegetable and spices. Just like their Solanaceous cousins, tomato, and potatoes, peppers have rapidly become important components of diverse cuisine around the world. This is reflected in the large acreages devoted to their production in such countries as India, Mexico, China, Korea, USA and Africa. In addition, interest in both sweet and pungent types of peppers is growing in many countries not traditionally associated with spicy cuisine; protected culture has developed in northern latitude countries such as Holland and Canada and also in Mediterranean countries such as Spain, and Israel, to supply the increased demand (Wien, 1997).

Capsicum species have a solitary (single) flower that starts at the axils of the first branching node with subsequent flowers forming at each additional node. Flower differentiation is not affected by day length, but the most important factor determining differentiation is air temperature, especially at night. The *Capsicum* flower is complete, bisexual, hypogenous and usually pentamerous (Bosland and Votava, 2000). Depending on the environmental conditions and variety, the period of receptivity of the stigma is 5-8 days, from several days before anthesis to fewer days afterwards, with maximum fertility on the day of anthesis (Aleemullah *et al*., 2000).

The most actively growing organ of a pepper plant after flowering is the fruit. The fruit is ordinarily seeded, but parthenocarpic forms exist. The seed set affects development and subsequent growth of the fruit. On average there is a direct linear relationship between the number of seeds per fruit and final fruit size, until saturation at perhaps over 200 seeds per fruit (Marcel *et al*., 1997). Typically cultivated fruit reaches the mature green stage in 35-50 days after the flower is pollinated. The fruits are characterized as non-climacteric in ripening (Bosland and Votava, 2000).

2.3 Economic Importance

Chillies are economically important crops all over the world and mostly used in spices and vegetables. Much of the recent attention focused on hot pepper can be attributed to their unique pungency that has made them an important spice in the cuisine of various countries. The proliferation of ethnic restaurants and food products from such as Mexico, India and Thailand has positively influenced the demand for peppers throughout the world. Both sweet and hot peppers are processed into many types of sauces, pickles, relishes and canned products. According to Bosland and Votava (2000), sweet pepper and hot pepper, like tomato and eggplant are rich in Vitamins A and C and a good source of B_2, potassium, phosphorus and calcium (Anonymous, 1998). It has been found that as hot peppers mature, the Pro- vitamin A (B Carotene) and ascorbic acid increase. This has led to extensive production of hot peppers in some countries for export markets. A substantial percentage of pepper acreage in the largest producing countries is dedicated to chilli powder. However, the higher prices received by farmers for fresh products have helped sustain the vegetative pepper industry, despite rising production costs competition and increased demand. This increasing demand for pepper to feed the growing human population and supply the ever expanding pepper industries at national and international level has created a need for the expansion of pepper cultivation in to areas where it has not ever been extensively grown (Beyene and David, 2007).

Hot pepper pungency is a desirable attribute in many foods. Pungency is produced by the Capsaicinoids, alkaloid compounds ($C_{18}H_{27}NO_3$) that are found only in the plant genus, *Capsicum*. The Capsaicinoids are produced in glands on the placenta of the fruit. While seeds are not the source of pungency, they occasionally absorb capsaicin because of their proximity to the placenta. No other plant part produces Capsaicinoids (Hoffman *et al*., 1983). Hot pepper pungency is expressed in Scoville Heat Units (Scoville, 1912). The Scoville Organoleptic Test was the first reliable measurement of the pungency of hot peppers. This test used a panel of limited human representatives, who tasted a *Capsicum* sample and then recorded the heat level. A sample was diluted until pungency could no longer be detected. The most common instrumental method to analyse pungency is high-performance liquid chromatography (HPLC). It provides accurate and efficient analysis of content and type of Capsaicinoids present in a *Capsicum* samples. High-performance liquid chromatography analysis has become the standard method for routine analysis by the processing industry. The method is rapid and can handle a large number of samples (Woodbury, 1980). The *Capsicum* species pungency level has genetic and environmental components. The Capsaicinoids content is affected by the genetic make-up of the cultivar, weather conditions, growing conditions, and fruit age. Plant breeders can selectively develop cultivars with varying degrees of pungency. Also, growers can somewhat control pungency by the amount of stress to which they subject their plants. Pungency is increased with increased environmental stress. More specifically, any stress to the hot pepper plant will increase the amount of Capsaicinoids level in the pods. A few hot days can increase the Capsaicinoids content significantly. In New Mexico, it has been observed that even after furrow irrigation, the heat level will increase in the pods. The plant has sensed the flooding of its root zone as a stress, and has increased the Capsaicinoids level in its pods. If the same cultivar was grown in both a hot semi-arid region and a cool coastal region, the fruit harvested from the hot semi-arid region would be higher in Capsaicinoids than that of the fruits harvested in the cool coastal climate (Lindsay and Bosland, 1995). *Capsicum* fruits are consumed as fresh, dried or processed, as table vegetables and as spices or condiments (Geleta, 1998), because, it increases the acceptance of the insipid basic nutrient foods. The nutritional value of hot pepper merits special attention, because it is a rich source of vitamin A, C and E. Both hot and sweet peppers contain more vitamin C than any other vegetable crops (Poulos, 1993). Oleoresins of paprika and capsicum are the two important extracts of pepper (Bosland and Votava, 2000). Medicinal use of *Capsicum* has a long history, dating back to the Mayas who used them to treat asthma, coughs, and sore throats. A survey of the Mayan pharmacopoeia revealed that tissue of *Capsicum* species is included in a number of herbal remedies for a variety of ailments of probable microbial origin (San Lin, 1994).

According to Bosland and Votava (2000), pepper is the most recommended tropical medication for arthritis. The pharmaceutical industry uses Capsaicin as a counter-irritant balm (cream), for external application of sore muscles (Thakur, 1993). Creams containing capsaicin have reduced pain associated with post-operative pain for mastectomy patients and for amputees suffering from phantom limb pain. Prolonged use of the cream has also been found to help reduce the itching of dialysis patients, the pain from shingles and cluster headaches. It is not only their nutritional quality and medicinal value that makes peppers an important food crops, but peppers also stimulate the flow of saliva and gastric juices that serve indigestion (Alicon., 1984). It has been said that peppers raise body temperature, relieve cramp, stimulate digestion, improve the complexion, reverse inebriation, and cure a hangover and increase passion. On the other hand among its many modern innovative uses it has been tried to use as a barnacle repellent. For example, anti-mugger aerosols with chillies pungency as the active ingredient have replaced mace and tear gas in more than a thousand police departments in the United States. The spray will cause attackers to gasp and twitch helplessly for 20 minutes (Bosland and Votava, 2000). Red and green pepper has increased a lot of medical acclaim due to their high ascorbic acid content, vitamin A and medicinally important Capsaicin. A lot of research is being carried out on different varieties of *Capsicum*, Talcott *et al* (2020), Brenes *et al* (2007), Villalon *et al* (2000). Because of high economic and medicinal importance of the family and its members, it has attracted the attention of morphologists, anatomists, embryologists, physiologists, geneticists, horticulturists and tissue culturists. In the report of Cichewicz and Thorpe, (1996) it has been mentioned that chilli peppers are used to treat microbial diseases.

According to the Baruah *et al.*, (2014) Chilli pepper was used to treat asthma gastrointestinal tract problems since ancient times. Deorani and sharma, (2007) have stated that Chilli pepper was used to treat headaches and night blindness. Salinity treatment increased the proline content in chilli plant. The accumulation of Nitrogen containing compatible solutes including proline is known to function in osmotic adjustment protection of cellular macro-molecular from damage by salt, storage of nitrogen and scavenging of free radicals. According to the Yuya ogiso *et al*., (2008), the antioxidant enzyme properties of hot water extracts of dried chilli pepper has been maintained through the postharvest ripening process at 10°C for eighteen months. In order to isolate the antioxidant from the ripe pepper, we will do fractionation of extract with hot-water and by size-exclusion gel chromatography. A certain fraction has been shown anti-oxidative activity via the 1, 1-diphenyl-2 picrylhydrazyl (DPPH) radical-scavenging activity assays. Structural analysis by reversed-phase high performance liquid chromatography (HPLC), LC-MS, and nuclear magnetic resonance (NMR) was revealed that the antioxidant was a known compound, p-coumaryl alcohol.

According to the Bhattacharya *et al*., (2010) it has been noted that the antioxidant enzyme quality of green chilli (*Capsicum annum*) was found improved through selection of breeding. Biochemical composition of screening of 10 cultivars has been conducted. The level of the antioxidant constituents vitamin C, carotene, phenol, and Capsaicin ranged between 0.160 and 2.987 mg/g wet weight, 0.167 and 0.913 mg/g wet weight, 0.09 and 0.214 mg/g wet weight, and 4.54 and 7.233 mg/g dry weight, respectively.

2.3 Cultivation practice

Capsicum like most other plants, prefer well drained, moisture holding loam soil (sandy loam) containing some organic matter (Lemma and Edward, 1994). A pH of 6.5-7.5 is suitable and the land should be level to 0.01- 0.03 % slope to allow adequate drainage and prevent root diseases. Adequate water supply is essential. Water stress can cause abscission of fruit and flowers, especially when it occurs during flowering (Matta and Cotter, 1994) and reduces yield through reduced pollination. The extreme case can result in increased risk of diseases. Poorer soil types and water stress are believed to produce lower yields (Haigh *et al.*, 1996). Numerous local landraces of *Capsicum frutescens* are cultivated in tropical and sub-tropical regions of the world. In India, *Hmarchate* (*C. frutescens*) is exclusively grown in the state of Mizoram and some parts of Manipur. The geographical boundaries of the production area of *Hmarchate* lies between 21° 58'North to 24° 35' North Latitude and 92° 15' East to 93° 29' East Longitude. Interestingly, *Hmarchate* can be found growing in the low-lying areas western part of Mizoram and also in the high elevated areas like the *Blue Mountain*, situated in the south-eastern part of the state (highest peak in Mizoram at 2,210 metres above sea level).

Capsicum fruits are consumed as fresh, dried or processed, as table vegetables and as spices or condiments (Geleta, 1998), because, it increases the acceptance of the insipid basic nutrient foods. The nutritional value of hot pepper merits special attention, because it is a rich source of vitamin A, C and E. Both hot and sweet peppers contain more vitamin C than any other vegetable crops (Poulos, 1993). Oleoresins of Paprika and *Capsicum* are the two important extracts of pepper (Bosland and Votava, 2000). Medicinal use of *Capsicum* has a long history, dating back to the Mayas who used them to treat asthma, coughs, and sore throats. A survey of the Mayan pharmacopoeia revealed that tissue of *capsicum* species is included in a number of herbal remedies for a variety of ailments of probable microbial origin (I-San Lin, 1994). According to Bosland and Votava (2000), pepper is the most recommended tropical medication for arthritis. The pharmaceutical industry uses Capsaicin as a counter-irritant balm (cream), for external application of sore muscles (Thakur, 1993). Creams containing Capsaicin have reduced pain associated with postoperative pain for mastectomy patients and for amputees suffering from phantom limb pain. Prolonged use of the cream has also been found to help reduce the itching of dialysis patients, the pain from shingles and cluster headaches. Mizo chilli is an important cash crop which supports the livelihood and generates income for the farmers. It is an integral part of *Mizo* dish where it is used for spicy cuisine in, pickle, chutneys and hot sauces to be served with noodles and has a very high demand in neighbouring states and countries like China, Thailand, Vietnam, Myanmar and Bangladesh and therefore, there is good export potential outside the state. Mizoram has enormous potential for large quantity production with proper market linkage and not less than 2000 tons is sold though unauthorized traders every year to Bangladesh and neighbouring states.

2.5 Factors Affecting Growth, Yield and Quality of Chilli

2.5.1 Planting methods

In Mizoram, Mizo Chilli is cultivated mostly under Jhuming system or Shifting Cultivation System, on hill slops. In this type of system, large tracts of hills are cleared by burning. Raised beds (Called bum) of about one meter width are made along the slope and again covered with farm wastes dried leaves etc. which are being burnt before sowing of seeds. The burning of field helps in reducing the weed growth, soft rot disease and increase the availability of certain plant nutrients, particularly ' Potash. This Jhum land is abandoned after 3-5 years and new piece of land is cleared in similar fashion.

Naveen *et al.,* (2009) analysed qualitative parameters of green chillies like ascorbic acid, Capsaicin, oleoresin and moisture content etc. and 100 % organic manure (composted coir pith 25% + vermicompost 25% + bio-digested slurry 25% + *Azospirillum*-PSB 25%) gave better results compare to all other treatments for quality parameters.

Deore *et al.,* (2010) revealed that, 3% novel organic liquid fertilizer application resulted in maximum plant height, number of branches per plant, leaf number, leaf area, fresh and dry weight of the plant, number of fruits per plant and total yield.

Application of green manure (sun hemp) + neem cake @ 2 t per ha + *Azospirillum* @ 2 kg per ha + burnt ash (crop residue) + phosphobacteria recorded higher growth parameters like plant height, plant spread, number of branches per plant of chilli over RDF alone (Bharathi *et al.,* 2011).

Patil *et al.,* (2014) studied the effect of organic nutrient and biological pest management practices on insect pests and disease dynamics in organic chilli production system. The result revealed that yield of dry chilli was highest with the application of organic manures. Similar results were noticed in tomato by Rajbir and Ram (2005).

2.5.2 Soil properties

Experiment conducted in Kenya also indicated that supplementing the inorganic fertilizers with well decomposed farmyard manure substantially increased both to improve soil fertility and potato tuber yield in a small holder farms (Muriithi and Irungu, 2004). The authors also assessed that considering cost of inorganic fertilizer and its negative effects on the environment, reduced usage at half the recommended rate combined with half rates of farmyard manure to be a feasible to the farmers, soil and environment. Nanthakumar and Veeragavathatham (2001) studied the effect of integrated nutrient management on the nutrient content of brinjal variety Palur-1. They found that nitrogen, phosphorous and potassium contents were highest in the treatments involving organic manure + inorganic fertilizers + bio fertilizer. Choudhary *et al.,* (2005) studied the effects of integrated use of organic manure, bio fertilizer and chemical fertilizers on nutrient status of soil and yield of brinjal. Soil available nitrogen, phosphorous and potassium increased significantly with the application of various organic and microbial sources of nutrients in combination with fertilizers over control. The organic carbon and available nitrogen status were increased significantly with conjunctive use of inorganic fertilizers, bio fertilizer and farm yard manure. Dass *et al.,*(2008) conducted an experiment with seven treatments including chemical fertilizers, vermicompost (VC), cow manure (CM), and microbial inoculants (*Azotobacter* and Phosphate Solubilizing Bacteria) for three consecutive years (2001 to 2003), at Koraput, India to determine the most effective integrated nutrient management for production, economics, and soil improvement in cabbage and bell pepper. They reported that bulk density of the surface soil after three years was reduced; its organic carbon and available nitrogen and phosphorous status improved due to treatment with cow dung manure and vermicompost. The data indicated that application of 5 t/ha of vermicompost can meet 50% of the fertilizer requirement of both crops while ensuring higher productivity, income, and residual soil fertility.

2.5.3 Varieties of Mizo chilli

Though Mizo chilli has been cultivated for many decades in typical tropical climate within Mizoram, the yield has remained very low due to limited improvement work on the crop**.** Each variety has its own significant effect on yield and yield components, and each variety has its own traits that are part and parcel as quality parameters of the crop (shape, size, colour, taste and pungency). The most important traits among others include, number of branches per plant (count), plant height, number of fruits per plant, days to maturity (count from days of transplanting), dry fruit yield per plant, fruit length and single fruit weight (Lemma *et al.*, 2008).

Owning to its specific climatic, topographic and cultivation characteristics the state is bestowed with some high quality crops verities that are peculiar and specifically associated with Mizoram. The Mizo Chilli or Mizoram Bird's Eye Chilli (MZBEC) grown in Mizoram is of a superior quality and has a very high demand in national and international market. In Mizoram, Mizo chilli is cultivated on hill slopes under shifting cultivation system or jhum cultivation system. In this type of system, large tracts of hills are cleared by burning and land occupancy of short periods is done by crops alternating with long fallow periods. One meter width raised beds also known as bum are made along the slopes and again covered with farm wastes, dried leaves etc. which are burnt before sowing of the seeds. This burning helps in checking the growth of weeds, soft rot diseases incidence and increase the availability of certain plant nutrients particularly potash. Mizo chilli/*Hmarchate* is not grown as single crop in Mizoram by the farmers. They are intercropped with paddy to generate some extra revenue because the yield is very low and there is high price fluctuation in the market. Seeds are sown between paddy crops in the month of April before the onset of monsoon by broadcasting and dibbling method.

Hmarchate are grown as rain fed in the state due to the occurrence of high rainfall spread over a period of six to eight months that helps the farmers to grow paddy and chilli without irrigation. Harvesting season for *Hmarchate* starts from October and ends till December. The yield of *Hmarchate* is normally low compared to other big size chilli varieties. *Hmarchate* is found throughout the year in almost all the roadside markets of Mizoram. Majority of the produce comes from traditional *Jhum* lands and farmers kitchen gardens. There are few commercial growers having extensive area under Bird's eye chilli. Mature red and green *Hmarchate* is sold in the form of bunches (carrying 100-200 berries) and loose in small cups. Both dry and green chillies are sold in the market. It is grown completely organically in the *Jhum* lands. Use of chemical fertilizers and pesticides is very rare in the state and because most of this chilli is grown on *Jhum* land which is already very fertile, farmers don't ever use any chemical fertilizers. This feature clearly differentiates it form other Bird's Eye Chilli grown elsewhere in the world. *Hmarchate* is completely free (Below Limit of Qualification) from any pesticide residues which is the unique characteristics of *Mizo chilli*. *Mizo chilli* which is exclusively grown in Mizoram is distinctly different from other chilli varieties grown in different parts of the country. *Hmarchate* is an important cash crop which supports the livelihood and generates income for the farmers. It is an integral part of *Mizo* dish where it is used for spicy cuisine in, pickle, chutneys and hot sauces to be served with noodles and has a very high demand in neighbouring states and countries like China, Thailand, Vietnam, Myanmar and Bangladesh and therefore, there is good export potential outside the state. Mizoram has enormous potential for large quantity production with proper market linkage and not less than 2000 tons is sold though un-authorized traders every year to Bangladesh and neighbouring states. However, no study has been made on the effect of soil nutrient amendments on growth and productivity of *Capsicum* in Mizoram and no literatures are available in this aspect.

Chilli is commonly cultivated under shifting cultivation in Mizoram in the same field only for one year. However, literatures have shown that most of the species of chilli can be grown for 3 years under proper soil nutrient management. Thus, it is important to make a study on the application of soil amendments with basic nutrient requirement to increase the productivity and life span of chilli, one of the most important crops in Mizoram. Nutrient supplementation by experimental increases in soil nutrient and moisture availability to plants to enhanced crop growth and increased economic yields remains a challenging issue, especially in hilly terrains. The pungent flavour of chilli is due to a group of closely related alkaloid called Capsaicinoid found only in the genus *Capsicum* (Hoffman *et al*., 1983). Among the Capsaicinoid, Capsaicin and dihydrocapsaicin together account for about 90% of pungency (Kawada *et al*., 1970). Capsaicin is also the active principle which accounts for the pharmaceutical properties of chillies. It has been used as a topical analgesic against arthritis pain and inflammation (Deal *et al*., 1991). Capsaicin binds to the same group of nociceptors which also leads to the sensation of pain from heat and acid (Caterina and Julius, 2001; Julius and Basbaum, 2001; Szallasi and Blumberg, 1999) and reduces pain and inflammation by depleting the neurotransmitters signalling pain. Capsaicin also shows anti-mutagenicity effect (Macho *et al.*, 2003) and a high antioxidant activity (Lee *et al*., 1995). Chilli pungency is measured in Scoville Heat Units (SHU) and Scoville organoleptic test was used initially for measuring SHU (Scoville, 1912). However, high-performance liquid chromatography (HPLC) method has replaced the organoleptic method since HPLC method is considered the most reliable and accurate method for determining both the amount of Capsaicin and pungency in a chilli sample (Collins *et al*., 1995). Thus, the present research proposal is made to improve soil fertility that would provide improved technological package for cultivation of chilli; further, the proposed study will give us information on the nutrient application on pungency in chilli plants.

2.5.4 Nutrient management

Animal manures, particularly cattle dung, were the main source of nutrients for the maintenance of soil fertility in settled agriculture until the advent of mineral fertilizers (Ofori and Santana, 1990). Farmyard manures are responsible to nutrient availability for crop in demand, improve soil physical properties (aggregation) and hence improve water retention capacity, infiltration rate and biological activity of soil (Aliyu, 2000.). The advantage of farmyard manure application, however, greatly depends, among others, on proper application methods, which increase the value, reduce cost, and effectiveness (Teklu *et al*., 2004).

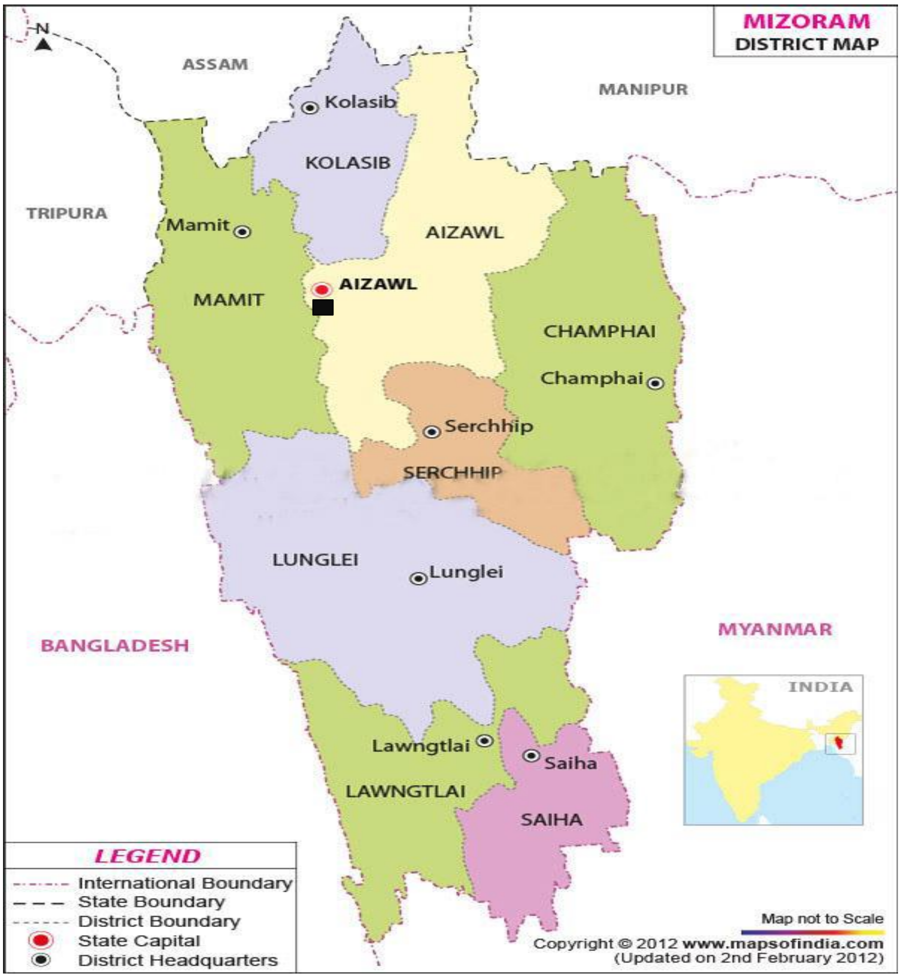
An integrated approach for the maintenance of soil productivity, with the complementary use of both mineral and organic fertilizers, offers a good opportunity to the small farmer to maintain yields at reasonable and sustainable levels (Ofori and Santana, 1990). Various research reports showed that as it improves quantity and quality of potato (Teklu *et al*., 2004). Experiment conducted in Kenya also indicated that supplementing the inorganic fertilizers with well decomposed farmyard manure substantially increased both to improve soil fertility and potato tuber yield in a small holder farms (Muriithi and Irungu, 2004). The authors also assessed that considering cost of inorganic fertilizer and its negative effects on the environment, reduced usage at half the recommended rate combined with half rates of farmyard manure to be a feasible to the farmers, soil and environment.

CHAPTER 3

MATERIALS AND METHODS

3.1 Description of the Study Area/ Experimental Site

The proposed site for conducting experiment is located at Horticulture Centre of Excellence, Thiak village, about 45 kms south of Aizawl, Mizoram. It is situated between 23.47^0 latitudes and 92.72^0 longitudes, and falls under sub-tropical climate and it enjoys a temperature ranging from 6^0C-30^0C during winter and 15^0-30^0C during summer. The altitude of this area is about 488 m above sea level (ASL) and received an annual rainfall of about 250-300cm.



■ Horticulture Centre of Excellence, Thiak village

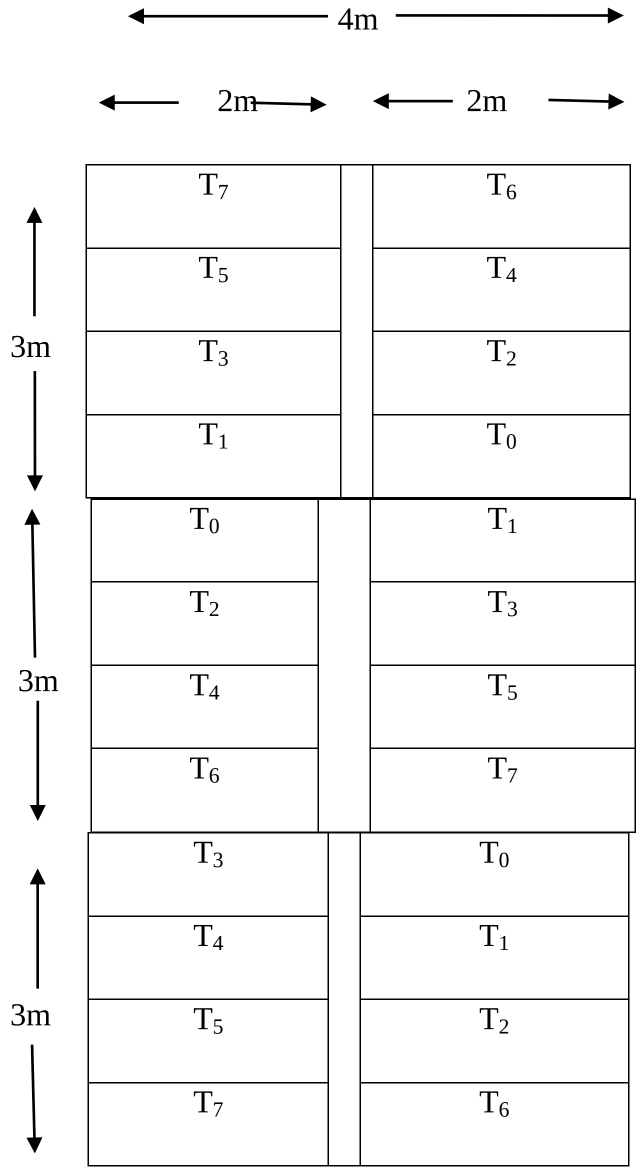
Figure 3.1. Geographical map of Mizoram showing expiremental site.

3.2 Experimental Design

The proposed experimental design is Randomized Block Design (RBD) with 3 replications with seven treatments *viz*. T_0, T_1, T_2, T_3, $T_{4,}$ $T_{5,}$ T_6, and T_7. T_0 a sub-plots will be used for the three varieties of *Capsicum frutescens* under control without Farm Yard Manure (FYM). On the other hand, sub plots $T_{1,}$ T_2 T_3, $T_{4,}$ $T_{5,}$ T_6 and T_7 will be for the three *Capsicum* varieties treated with different doses of Farm Yard Manure (FYM). FYM will be applied uniformly in the soil at a depth of 10 cm a week before planting and during pre-flowering of chilli plants. Each sub-plot will contain 6 chilli plants (Mizo chilli) at a spacing of 40-50 cm within and between the rows. Uniform sized seedlings would be transplanted manually. The experiment will be carried out under rain-fed condition. Soil moisture and temperature would be measured at 10 cm depth at monthly intervals. As to other agronomic practices, irrigation water was applied to the transplants on surface to facilitate plants establishments, and then up to the time of full plant establishments, water was applied using watering can once a day. Then based on the environmental conditions watering was done three times a week afterwards. Hand weeding was done frequently as per the emergence of the weeds.

3.3 Experimental Layout

Experimental Design: Randomized Block Design (RBD).



Treatments : T_0= MZBEC $_{A+B+C}$ Control (No Farm Yard Manure)

T_1= MZBEC $_{A+B+C}$ (Farm Yard Manure-1Kg/Plot)

T_2= MZBEC $_{A+B+C}$ (Farm Yard Manure-1.5Kg/Plot)

T_3= MZBEC $_{A+B+C}$ (Farm Yard Manure -2Kg/Plot)

T_4= MZBEC $_{A+B+C}$ (Farm Yard Manure-2.5Kg/Plot)

T_5= MZBEC $_{A+B+C}$ (Farm Yard Manure-3Kg/Plot)

T_6= MZBEC $_{A+B+C}$ (Farm Yard Manure-3.5Kg/Plot)

T_7= MZBEC $_{A+B+C}$ (Farm Yard Manure-4Kg/Plot)

Varieties of *Capsicum*	:	Three Varieties *viz*. *Mizoram Bird's Eye chilli* (MZBEC) A, B and C
Spacing	:	50x50 cm
Sub- Plot Size	:	2m x 1m
Plot size for 1 Replication	:	4mx4m (16m^2)
No. of Replications	:	3
Total Plot size	:	3 x 16m (48m^2)

3.4 Data analysis:

Data recorded on different aspects of crop, *viz*; growth, yield attributes and yield were tabulated and subjected to statistical analysis as per Gomez and Gomez, 1976.

The results were expressed in means of three replicates (Mean ± SE) and analysed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests for comparison of statistical significance (P<0.05). Pearson's correlation coefficients were calculated in order to measure the linear correlation between variables. All statistical calculations were performed using SPSS (V.16) statistical software.

Plate 2. Experimental site showing sub-plots with *Capsicum frutescens*

CHAPTER 4

DETERMINATION OF SOIL PHYSICO-CHEMICAL PROPERTIES UNDER DIFFERENT ORGANIC AMENDMENTS

4.1 Introduction

Plant establishment and survival is often determined by interactions with soil in a bottom-up manner; thus, soils directly impact many plant characteristics. Farm Yard Manure (FYM) is bulky organic manure which is rich in nutrient and supply nutrients required by the plants, however with low quantity. FYM also supplies macro and micro nutrients and maintains healthy positive nutrient balance besides being a source of organic matter; and further it emphasizes the need for integrated and balance nutrient management in potato Sharif *et al*., (2014). Nutrients need to be applied as accurately as possible to the zone of uptake, slightly before, or at the time that the crop needs them. Failure to ensure that each plant gets the right balance of nutrients can spoil crop quality and reduce yield.

The most significant property of soil is its pH level, its effects on all other parameters of soil. Therefore, pH is considered while analysing any kind of soil. If the pH is less than 6 then it is said to be an acidic soil, the pH range from 6-8.5 it's a normal soil and greater than 8.5 then it is said to be alkaline soil. Water content or moisture content is the quantity of water contained in a material, such as soil called soil moisture; Moisture is one of the most important properties of soil. Absorption of the nutrient by soil is largely depends on moisture content of the soil moisture of soil also shows its effect on the texture of soil. Recent economic literature on carbon sequestration was reviewed to gather insights on the role of agriculture in greenhouse gas emissions mitigation. Results from the most salient studies were presented in an attempt to highlight the general consensus on producer-level responses to C sequestration incentives and the likely mechanisms used to facilitate C sequestration activities on agricultural soils. Rates of carbon accumulation in EU soils are very difficult to estimate and the range of the estimated net yearly accumulation of carbon is from 1 to 100 million tonnes. The factors that influence soil organic matter accumulation include: climate, soil texture, hydrology, land use and vegetation. Carbon assimilation is a dynamic process necessary for nutrient availability and cycling. Different sources of organic matter have different assimilation and decomposition characteristics, and result in different soil organic matter fractions.

Nitrogen is the most critical element obtained by plants from the soil and is a bottleneck in plant growth (Gorde, 2013). About 80% of the atmosphere is nitrogen gas. Nitrogen gas diffuses into water where it can be "fixed" (converted) by blue-green algae to ammonia for algal use. Nitrogen can also enter lakes and streams as inorganic nitrogen and ammonia. Because nitrogen can enter aquatic systems in many forms, there is an abundant supply of available nitrogen in these systems. The Indian soils are generally deficient in organic matter thus unable to release N at the rate required to maintain adequate supply to the growing plant. Therefore, application of nitrogen in form of fertilizers and manures becomes indispensable to meet the needs of the crop (Trehan *et al.,* 2008). Total soil nitrogen includes inorganic and organic forms that may be available or unavailable for plant uptake. Measurements of total soil nitrogen ranked among the most expensive nitrogen diagnostic methods surveyed.

As previously mentioned a significant portion of the total soil nitrogen is present in forms associated with humid colloids, clay minerals, or microbial biomass and therefore is unavailable for plant acquisition. A measurement of total soil nitrogen includes unavailable forms; thus, the potential exists to significantly overestimate nitrogen available for plant uptake. Soils with variable nitrogen mineralization potentials exist with similar levels of total soil nitrogen. Total soil nitrogen fails to correlate with available nitrogen because the percentage of organic nitrogen mineralized tends to decrease as total soil nitrogen increases. For these reasons, total soil nitrogen is an inaccurate measure of nitrogen availability (Nelson & Sommer, 1996). Nitrogen is the fourth most abundant plant element, and it is an essential component of proteins, nucleic acids, hormones, and chlorophyll (Kozlowski, 1985). Although the plant nitrogen demand is large, the supply of nitrogen available for uptake is often small. Of the total global nitrogen, 99.96% is in the atmosphere as N_2 gas, and of the remaining 0.04%, only 6.5% (0.0026% of the global nitrogen) is in forms that plants can acquire from the soil and use for physiological functioning .Nitrogen is cycled among terrestrial plants, soil, and microorganisms. Nitrogen enters the terrestrial system through biological or industrial fixation, atmospheric deposition, or fertilization. Nitrogen is fixed in the soil lattices, oxidized, and assimilated by plants or microorganisms, or lost from the terrestrial system (Evangelou, 1998). Nitrogen assimilation by plants occurs directly through root uptake or indirectly through mycorrhizal association. Microbial assimilation of mineral nitrogen is referred to as immobilization. Upon death, senescence, exudation, or excretion, nitrogen enters the soil organic matter pool. As much as 90% of terrestrial nitrogen occurs as soil organic matter (Pulford, 1991).

Phosphorus is a most important element present in every living cell (Smita and Sangita, 2015). It is one of the most important micronutrient essential for plant growth. Phosphorus most often limits nutrients remains present in plant nuclei and act as energy storage. Phosphorus is important for early root and shoots development, providing energy for plant processes such as ion uptake and transports. Roots absorb phosphate ion only when they are dissolved in the soil water. P deficiencies can occur even in soils with abundant available P, if drought, low temperatures, or disease interfere with P diffusion to the root, through the soil solution. These deficiencies will result in stunt root development and inadequate function. Phosphorus is one the most essential elements for plant growth after nitrogen. However, the availability of this nutrient for plants is limited by different chemical reactions especially in arid and semi-arid soils. Phosphorus plays a significant role in several physiological and biochemical plant activities like photosynthesis, transformation of sugar to starch, and transporting of the genetic traits Mehrvarz *et al*., (2008).

Potassium plays an important role in different physiological processes of plants; it is one of the important elements for the development of the plant (Solanki and Chavda, 2012). It is involved in many plant metabolism reactions, ranging from lignin and cellulose used for the formation of cellular structural components, for regulation of photosynthesis and production of plant sugars that are used for various plant metabolic needs. Potassium has an important role in the control of the plant water status and internal ionic concentration of the plant tissues, with a special focus on the stomatal functioning. Potassium deficiencies reduce the yield, size, and quality of the potato crop. It also impairs the crop’s resistance to disease and its ability to tolerate stresses such as drought and frost. K^+ is an essential nutrient, and the most abundant cation in plants, whereas the closely related ion Na^+ is toxic to most of the plants at high mill molar concentration. K^+ is an important macronutrient and is necessary for phloem solute transport and for the maintenance of cation: anion balance in the cytosol as well as in the vacuole. K+-supply from soil can be rate-limiting for agricultural production. The closely related cation Na in contrast does not generally fulfil physiological functions (Wood & Turks, 1940). At deficient K supply in the root medium of various crops, a significant increase in K concentration and total K uptake was evidently due to the differences in ability of some genotypes to accumulate and take up K more efficiently than others. Differences were observed among sweet potato genotypes in K concentration, accumulation and K efficiency ratio in the field. Among various plant parts, petiole retained the highest K accumulation at maturity (Haby *et.al*., 1988).

4.2 Materials and methods

4.2.1 Determination of pH

The pH of the soil sample will be measure by the methods of soil to water ratio of 1:2. By this method 20g of soil is to be taken in a 100ml beaker to which 40ml of water is added. The suspension is stirred at regular intervals and kept overnight and the pH will be recorded with the help of pH meter.

4.2.2 Determination of soil moisture

Soil moisture loss on drying constant weight will be determined for 100g of fresh soil. The soil moisture content would be expressed as per cent fresh weight and calculates as follows:-

$$\text{Moisture (\%)} = \frac{FW - DW}{FW}$$

Where, M =Moisture

FW=Fresh Weight

DW=Dry Weight

4.2.3 Estimation of Total Carbon

The method given by Walkley and Black (1934) will be adopted to estimate total Carbon.

The method given by Walkley and Black (1934) was adopted to estimate Organic Carbon. Soil samples were grounded and completely passed through 0.2mm sieve and 1g of it was kept at the bottom of a dry 500ml conical flask. Then 10ml of 1N $K_2Cr_2O_7$ was pipetted in and swirled a little. The flask was kept on asbestos sheet. Then 20 ml of H_2SO_4 (containing 1.25% Ag_2SO_4) was run in an again two or three times. The flask was allowed to stand for 30 minutes; thereafter, 200ml of distilled water was added. Thereafter, 10ml of phosphoric acid or 0.5g Sodium fluoride and 1ml of diphenylamine indicator was added and titrated with ferrous Ammonium Sulphate solution till the colour flashes from the blue violet to green. Simultaneously, a blank was run without soil. The result was calculated by the following method:

$$\text{Organic Carbon (\%)} = \frac{10(B-T) \times 0.003 \times 100}{B \text{ Wt. of soil}}$$

Where,

B= Volume (in ml) of ferrous ammonium sulphate solution required for blank titration; & T= Volume of ferrous ammonium sulphate needed for soil sample.

4.2.4 Estimation of available Phosphorus

Olsen’s method (Olsen *et al.,* 1954) and Dickman and Bray’s (Dickman & Brays 1940), procedure will be used in the estimation of available phosphorus.

The methods developed by Olsen *et al.,* (1954) and Dickman & Brays (1940) were followed for the estimation of Phosphorus in the soil samples. 2.5g of the soil sample was taken in 100ml conical flask and a little of Dargo G 60 or equivalent grade of activated carbon (free of phosphorus) was added followed by 50ml of

Olsen's reagent. A blank is run without soil. Then the flasks were shaken for 30 minutes on a platform type shaker and the contents are filtered immediately through dry filter paper (Whatmann No. 1) into dry beakers or vials.

In the filtrate, phosphorus was estimated calorimetrically by Dickman and Bray's procedure (Dickman and Brays, 1940). 5ml of soil extract is pipette into a 25ml volumetric flask to which 5ml of the Dickman and Bray's reagent was poured in. The rock of the flask was washed down and the content was diluted to about 22ml. Therefore, 1ml of the diluted stannous chloride solution was added and the volume makes up to the mark level. The intensity of the blue colour was measured (using mμ filter) just after 10 minutes and the concentration of phosphorus was determined from the standard curve. With each sample a blank was maintained.

4.2.5 Estimation of exchangeable Potassium

The estimation of exchangeable K of water soluble forms will be determined with the help of Flame photometer (Ghosh *et al*., 1983).

Available Potassium (K) incorporates both exchangeable and water soluble forms of the nutrient present in the soil. The estimation of K of water soluble forms was carried out with the help of Flame photometer as suggested by Ghost *et al.,* (1983). 5gm of soil sample was shaken with 25ml of normal of Ammonium acetate (pH 7) for 5 minutes and filtered immediately through a dry filter paper (Whatmann No. 1). First few ml of the filtrate was rejected. Potassium concentration in the extract was determined in the flame photometer.

4.2.6 Estimation of Total Nitrogen Content (N)

The procedure for the analysis of Nitrogen content in the soil samples will be done by CHN analyzers.

The procedure for the analysis of Nitrogen content in the soil samples was divided into three steps, *viz*. digestion, distillation and titration.

Digestion: 1gm of soil sample was taken in each of Kjeldahl flask for digestion tube and 10ml conc. Sulphuric Acid was added in each flask. Also, 3gms of catalyst mixture (Kjeldahl catalyst) was added in each of digestion tube and the balance without a soil sample was maintained. Temperature was set at 420^{o}C and it was digested for approximately 1hr till the sample became green colour. Then, the digester was switched off and the flask was allowed to cool.

Distillation: Firstly, the conical flask was loaded (with 20ml of 40% Boric Acid) in the receiver side which will be pink colour as it contain 3 drops of Bromo cresol green and Methyl red solution of 5 drops. Then, the digested sample was loaded for distillation. Again, 40% of NaOH was added slowly in auto mode in the order of 10ml each time till the colour changes from bluish green to brown precipitation and the process time was set for 6 minutes for soil sample. After 6 minutes, the sample colour in a conical flask changed from pink to green to green colour which was the end point. The flask was then prepared for titration.

Titration: The distillates were titrated against 0.1N HCl. The titration was stopped when the colour changed from green to pake pink.

$$\% \text{ of Nitrogen} = \frac{14 \text{ x Titrant value x Normality of acid x } 100}{1000 \text{ x Sample wt.}}$$

4.3 Result and Discussion

The Physico-chemical properties of soil under different soil organic amendments in the experimental site have been presented below.

Table 4.1 Availability of different soil nutrients under different organic manure amendments

FYM	Soil pH (Mean±SD)	Carbon (%) (Mean±SD)	Nitrogen (Kg/ha) (Mean±SD)	Phosphorus (Kg/ha) (Mean±SD)	Potassium (Kg/ha) (Mean±SD)
T_0 (Control)	7.4±0.55	0.45±0.21	202.44±0.63	17.32±0.19	130.41±0.43
T_1	7.27±0.47	0.48±0.34	227.51±0.39	22.23±0.23	140.35±0.33
T_2	7.27±0.23	0.47±0.28	202.41±0.55	20.23±0.48	142.48±0.29
T_3	7.37±0.33	0.51±0.42	219.25±0.47	22.59±0.18	143.23±0.66
T_4	7.3±0.49	0.49±0.23	226.18±0.71	20.41±0.54	172.31±0.48
T_5	7.33±0.37	0.48±0.76	228.82±0.44	21.11±0.29	167.89±0.17
T_6	7.27±0.51	0.51±0.65	238.75±0.73	22.61±0.31	165.88±0.72
T_7	7.37±0.44	0.53±0.55	244.46±0.47	22.96±0.56	174.33±0.51

4.3.1 Soil pH

The highest pH (7.40) was recorded with treatment T_0 (Control) followed by treatment T_7 and T_3, whereas the lowest value was observed with treatment combination T_1, T_2 and T_6 (Table 4.1). The decrease in soil pH may be due to formation of bicarbonate and ammonium nitrate by the application of urea that react with H^+ ions and caused to reduce acidity. Slight decrease in pH may also be related with the application FYM due to formation of humic and carbonic acids. Release of organic acids during the mineralization of FYM helps to decrease soil pH. Similar findings were also reported by Roshan *et al*., (2014).

4.3.2 Available Organic carbon (%)

The result shows that soil Organic carbon (%) increases with increased in levels of FYM (Table 4.1). The maximum soil Organic Carbon (%) with 0.53 % was observed in treatment T_7 (Farm Yard Manure-4Kg/Plot), and the minimum soil Organic Carbon. (%) of 0.45 % was recorded in T_0 (Control). Similar findings have also been reported by Moharana *et al*., (2017), Rudrappa *et al.*, (2006) and Ghulam *et al*., (2016).

4.3.3 Available Nitrogen (kg/ha)

The maximum available N of 244.00 kg/ha was recorded at treatment T_7 (Farm Yard Manure-4Kg/Plot), which was followed by 238.75 kg/ha at treatment T_6. And the minimum available N was recorded at T_0 (Control) with 202.44 kg/ha (Table 4.1). The application of organic or inorganic fertilizers is widely known to ameliorate soil N status Ajebesone *et al.,* (2011); this explains why plots that received FYM had higher N. The increase in available N may be due to application of FYM, which is the major source of nitrogen and the soil physico-chemical characteristics are very much benefited by FYM. The increased in available N have also been reported by Shuh *et al.,* (2015) and Ghulam *et al.,* (2016).

4.3.4 Available Phosphorus (kg/ha)

The maximum available P of 22.96 kg/ha was recorded at treatment T_7 (Farm Yard Manure-4Kg/Plot), which was followed by 22.61 kg/ha at treatment T_6 (Farm Yard Manure-3.5Kg/Plot). And the minimum available P was recorded at T_0 (Control) with 17.32 kg/ha (Table 4.1). Phosphorus fertilizers and manure in the soil increase phosphorus uptake by plants, through favouring production of carbonic acid, the acid that increases solubility of phosphate compounds. Phosphorus is the second most important macro nutrient after nitrogen that plays significant role in physiological and biochemical reactions such as photosynthesis and transfer characteristics

Mehrvarz *et al.,* (2008). Das *et al.,* (1991) reported that application of FYM resulted in tremendous increase in available P status of soil which might be attributed to the build-up of available P owing to the formation of folic acid and other chelating agents which form soluble complexes with native P in soils. The increased in available P have also been reported by Shuh *et al.,* (2015) and Ghulam *et al.,* (2016).

4.3.5 Available Potassium (kg/ha)

The soil available potassium (kg/ha) increased significantly with increase in levels of FYM. The maximum available K of 174.33 kg/ha was recorded at treatment T_7 (Farm Yard Manure-4Kg/Plot).The minimum available K was recorded at T_0 (Control) with 130.41 kg/ha (Table 4.1). The increase in available K may be due to higher application of FYM which is advantageous as it improve soil physical properties, also due to availability of more nutrients as compared to their individual effects. FYM also supplies micro and macro nutrients and maintains healthy positive nutrient balance besides being a source of organic matter; and further it emphasizes the need for integrated and balance nutrient management in chilli. It is also suggested that continuous application of NP without K depletes soil K and may leads to problem in crop production (Sharif *et al.,* 2014). The results are conformity with the finding of Ghulam *et al.,* (2016).

The findings of the experiment concluded that the application of soil organic manure had significant effects on the properties of soil, which makes the application of organic manure (FYM) will be a beneficial approached towards an important source of plant nutrients and superior to all other treatments and profitable. Results indicated that there were great differences in the plot treated with fertilizers and plot with zero fertilizer; hence the combined use of FYM could be recommended as important nutrient management and sustainable for crop production.

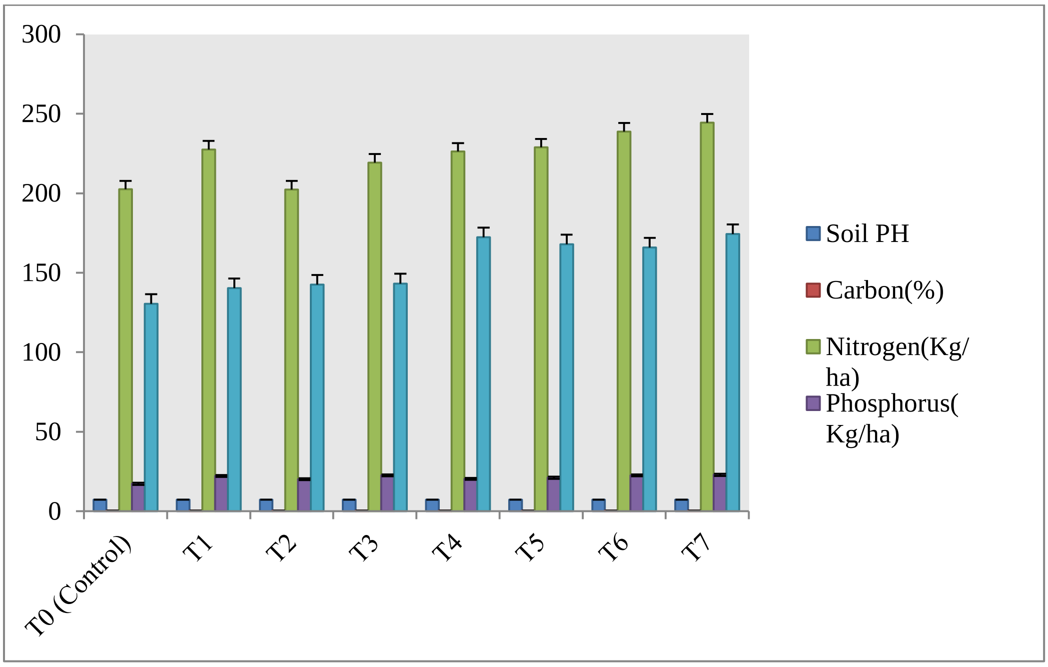
Table 4.2 Correlation between soil biochemical parameters (pH, C, N, P, and K)

	pH	CARBON	NITROGEN	PHOSPHORUS	POTASSIUM
pH		**0.021**	**-0.136**	**-0.305**	**-0.155**
		0.960	**0.748**	**0.462**	**0.713**
CARBON	**0.021**		**.814***	**.865****	**0.650**
	0.960		**0.014**	**0.006**	**0.081**
NITROGEN	**-0.136**	**.814***		**.779***	**.795***
	0.748	**0.014**		**0.023**	**0.018**
PHOSPHORUS	**-0.305**	**.865****	**.779***		**0.489**
	0.462	**0.006**	**0.023**		**0.219**
POTASSIUM	**-0.155**	**0.650**	**.795***	**0.489**	
	0.713	**0.081**	**0.018**	**0.219**	

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

Figure 4.1 Physico-chemical properties of soil under different organic amendments



CHAPTER 5

EFFECT OF ORGANIC MANURE (FYM) OF DIFFERENT TREATMENTS ON GROWTH, YIELD AND FRUIT QUALITY OF *CAPSICUM FRUTESCENS*

5.1 Introduction

Farm Yard Manure (FYM) refers to decomposed mixture of dung and urine of farm animals along with the litter (bedding material) and left over material from roughages or fodder fed to the cattle. Animal manures, particularly cattle dung, were the main source of nutrients for the maintenance of soil fertility in settled agriculture until the advent of mineral fertilizers (Ofori and Santana, 1990). Farmyard manures are responsible to nutrient availability for crop in demand, improve soil physical properties (aggregation) and hence improve water retention capacity, infiltration rate and biological activity of soil (Aliyu, 2000). The advantage of farmyard manure application, however, greatly depends, among others, on proper application methods, which increase the value, reduce cost, and effectiveness (Teklu *et al*., 2004). The sustainable production at higher levels is possible only by the proper use of inputs which will help to increase the organic matter content of soils, thus reducing the bulk density and decreasing compaction. Various organic manures like farm yard manure, vermicompost, green manure etc. are added to the soil from time to time further added to the store of organic matter (Palaniappan and Annadurai, 1999).

In organic farming nutrient are supplied through different sources of nutrients *viz*., FYM, vermicompost, poultry manure, and sheep manure etc. vermicompost is the casting from the earth worms produced by different species of *Eisenia fetida*, *Eudrilus eugeniae*, and *Perionyx excavates* etc. extensively used in vegetable production. Farm yard manure is a decomposed organic matter obtained by the action of microbial population in a warm and moist aerobic environment using cow dung, cow urine and other waste materials available from backyard cattle (Ramprasad *et al.*, 2009). Usage of liquid organic manures such as jeevamrutha, microbial consortia and decomposer results in increased growth and yield of crops and improve the soil physicochemical and biological properties. They contain micro and macro nutrients, many vitamins, essential amino acids, beneficial microorganisms and growth promoting substances *viz*., IAA, GA (Devakumar *et al*., 2008 and Tharmaraj *et al*., 2011). Jeevamrutha is eco-friendly organic preparation made from cow products. The products from cow have the ability to bring the flow of cosmic energy which in turn can revitalize the growth process. Chilli plants response well to inorganic fertilizers in terms of early vegetative growth but it decreased at later stage. Farmers nowadays keep on applying inorganic fertilizer for their crop as it can provide rapid nutrition but it will increase cost of production. Inorganic fertilizer is made up from synthetic materials, when excess of application, the soil will become toxic. Thus, this study intends to provide an alternative method by using several of organic treatment for enhancing growth, quality and NPK content of Chilli.

Spices are aromatic vegetable substances used for seasoning of foods. Spices are known to have prophylactic and therapeutic value. Chilli is one important spice used all over the world in one form or the other. Chilli both in ripe and green fruit stage is an indispensable spice in Indian cuisine. Chillies are known to stimulate the flow of saliva and simultaneously increase amylase activity thus increasing digestion of Carbohydrates. Pungency of chillies is felt deep in the throat rather than in the front edge of root of the tongue. Chillies are well known for their flavour and pungency and form important source of vitamin C. In chillies which are used in food preparations, quality is of much importance and is based on the following characters: a good pungency level, a bright red colour, a good flavour, and medium -sized fruit with a moderately thin pericarp, a smooth glossy surface, and few seeds in the fruit with firm stalk. Medium sized fruits are preferred to long pods, owing to the fact that in storage they remain intact better than longer pods, which tend to break at the distal ends. A fairly thin pericarp is preferred as drying is easily accomplished. Chillies with a bright red colour command high prices than those which are dull red or orange or yellow in colour and deep red fruits tend to retain their colour in storage longer than those which are of lighter shade. In case of pungent chillies which are used for the preparation of capsicum oleoresin, the appearance is of much less importance but a high Capsaicin is essential. Soil type, manuring practice, management of pest and diseases, harvesting, drying and handling methods significantly influence the quality characters. Chilli crop raised in red soils have bright red colour, than the crop raised in black cotton soils. Fruits maturing during post rainy season have bright red colour while fruits maturing in rainy season have dull colour. The pungency in chilli is due to capsaicin and other vanillyl amides. The red colour of fruits is due to the Carotenoid pigment of which Capsanthin, the most important. Only 25-30% of dry chilli is obtained from fresh ripe chilli fruits. The term quality implies the degree of excellence of a product or its suitability for a particular use. Quality of produce encompasses appearance, texture, nutritive values, chemical constituents, mechanical properties, functional properties and defects. Shelf life affects food quality, which in turn influences the consumer's buying decisions. Product quality is very important to growers because it determines marketable yield and can affect price.

Long-term food production needs sustainable agricultural practices including organic production. The entire production process conforms to the rules of organic production. The main features of organic crop production are the removal of chemical crop protectants, the use of organic inputs instead of chemical fertilisers and the certification of the organic production system. Hence the first step in organic farming is producing high quality organic fruits with the same quality standards as conventional farming (Groot and Raaijmakers, 2018). Quality organic fruits production is crucial since preventive measures to keep plant healthy, i.e. using pesticides and herbicides, are not allowed and competition with weeds is fierce (Sripathy *et al* ., 2012). There are numerous studies on longevity of the productive life of chilli (Verma *et al*., 2018). Nevertheless, organic fruit production systems are different than conventional ones and require specific measures for production. The purpose of the study was to determine the productive lifespan of organically produced chilli and compare with the application of different treatments. From this study, the best of organic treatment can be determined for optimum growth, quality and NPK content of Chilli. Farmers may use those treatments as an alternative ways in order to avoid excessive application of chemical fertilizer to the soil. Besides, farmers will have a proper management toward nutrients required for growth and completion of life cycle of the Chilli crop. Organic fertilizers may help farmer to increase soil structure, provide food source for soil micro-organisms, provides cation exchange capacity, increases water holding capacity, decrease toxicity at low pH and act as reservoir of plant nutrients (Zaccheo *et al*., 2002, Evanylo *et al*., 2008 and Mitchell *et al*., 2006)

5.2 Materials and Methods

5.2.1 Plant height (cm)

Plant heights were recorded in at 30, 60 and 90 Days after transplantation (DAT) by randomly selecting three plants from each plot and heights were measured by linear scale from the ground level up to the flag leaf. The mean values were computed and expressed in centimetre (cm).

5.2.2 Days to first harvest

The number of days from transplanting to the date of first harvest was recorded from six sample plants selected from central rows of each sub-plots under different organic manure amendments.

5.2.3 Number of fruit per plant

The numbers of fruits harvested from plants in each treatment were counted at 90 DAT and 120 DAT from different pickings and average was worked out and expressed as number of fruits per plant.

5.2.4 Fresh weight of fruit per plant (g)

Fresh weight of five randomly selected fruits from each treatments were immediately measured after harvest with the help of an electronic balance and the average fruit weight for each treatment was worked out and represented in terms of gram (g).

5.2.5 Fruit dry weight content (g)

The average weight were considered for the ten freshly harvested plants per replicate and then dried in an oven at 60°C to a constant weight.

5.2.6 Fruit length (cm)

Length of ten marketable fruits from each plot for each varieties were measured at red and dried stage using venire calliper and mean values were taken.

5.2.7 Fruit diameter (cm)

Fruit wall was measured at 90 DAT from ten marketable fruits of sample plants from each plot at red ripe and dried stage using venire calliper and mean values were recorded.

5.2.8 Life span of *Capsicum* under different treatments

The productive life of chilli under different treatments would be recorded. An attempt would be made to correlate the productive lifespan of the crop with and without organic fertilization.

5.3 Result and Discussion

The growth and yield of chillies under different organic treatments in the present study is presented below.

5.3.1 Plant height (cm)

Plant height measurement was made from the soil surface to the top most growth points of above ground plant part. The measurement was taken as the length from three plants of central rows of each plot. At 30 DAT, maximum plant height was found in T_7 of MZBEC-C variety of Mizo chilli (23.22 ± 0.43) and minimum was found in T_0 of MZBEC -A variety (16.17 ± 0.85)(Table 5.1). At 60 DAT maximum average plant height per plant was found in T_7 of MZBEC-C (40.20±0.47 cm) and minimum was recorded in T_0 of MZBEC-A (29.80±0.86 cm.)(Table 5.2) and maximum average plant height per plant at 90 DAT was recorded in T_7 of MZBEC-A (46.28±0.74cm) and minimum was recorded in T_0 of MZBEC-A variety (34.28±0.45 cm)(Table 5.3). The increment in plant height with increasing dose Farm Yard Manure (FYM) application might be better root growth; cell multiplication, elongation and cell expansion in the plant body by higher dose of FYM which ultimately increased the plant height similar results have been reported by Amirthalingam (1988). Moreover increase in plant height could mainly be due to better availability of soil nutrients in the growing areas, especially Nitrogen and Phosphorus which have enhancing effect on the vegetative growth of plants by increasing cell division and elongation and the varietal variability to absorb the nutrients from the soil (Vos and Frinking, 1997; El-Tohamy *et al*., 2006). The result of this study confirms the finding of Gonzalez *et al.,* (2001) who reported that organic manure supplied most of the essential nutrients at growth stage resulting in increase of growth variables including plant height.

Table 5.1 Effect of different doses of organic manure on Plant height at 30 days after transplantation

Capsicum varieties	T_0 (mean±SD) (cm)	T_1 (mean±SD) (cm)	T_2 (mean±SD) (cm)	T_3 (mean±SD) (cm)	T_4 (mean±SD) (cm)	T_5 (mean±SD) (cm)	T_6 (mean±SD) (cm)	T_7 (mean±SD) (cm)
MZBEC-A	16.17 ±0.85[a]	16.48 ±0.54[a]	17.19 ±0.39[a]	17.23 ±0.43[a]	18.09 ±0.05[a]	18.61 ±0.36[a]	19.12 ±0.30[a]	19.34 ±0.35[a]
MZBEC-B	17.23 ±0.41[b]	17.45 ±0.56[b]	18.22 ±0.88[b]	18.58 ±0.51[b]	19.18 ±0.50[b]	20.04 ±0.75[b]	20.53 ±0.65[b]	21.15 ±0.72[b]
MZBEC-C	19.11 ±0.77[c]	19.22 ±0.82[c]	19.70 ±0.86[c]	20.11 ±0.56[c]	20.38 ±0.51[c]	21.22 ±0.34[c]	22.30 ±0.38[c]	23.22 ±0.43[c]

Means followed by different letters within same column are significantly different according to Duncan's multiple range comparisons (DMRTs (P<0.05). Means followed by same letter are not significantly

Table 5.2 Effect of different doses of organic manure on Plant height at 60 Days after transplantation

Capsicum varieties	T_0 (mean ±SD) (cm)	T_1 (mean±SD) (cm)	T_2 (mean±SD) (cm)	T_3 (mean±SD) (cm)	T_4 (mean±SD) (cm)	T_5 (mean±SD) (cm)	T_6 (mean±SD) (cm)	T_7 (mean±SD) (cm)
MZBEC-A	29.80 ±0.86[a]	30.72 ±0.71[a]	32.11 ±0.59[a]	34.12 ±0.53[a]	35.16 ±0.57[a]	37.12 ±0.49[a]	38.11 ±0.42[a]	39.15 ±0.47[a]
MZBEC-B	28.39 ±0.47[b]	29.04 ±0.45[b]	30.10 ±0.44[a]	32.12 ±0.53[b]	35.11 ±0.44[a]	37.20 ±0.41[a]	38.19 ±0.48[a]	40.11 ±0.41[b]
MZBEC-C	29.17 ±0.52[c]	31.23 ±0.44[c]	34.27 ±0.50[a]	36.10 ±0.49[c]	38.12 ±0.55[b]	39.11 ±0.45[b]	39.20 ±0.45[b]	40.20 ±0.47[b]

Means followed by different letters within same column are significantly different according to Duncan's multiple range comparisons (DMRTs (P<0.05). Means followed by same letter are not significantly different. Each value was represented as

Table 5.3 Effect of different doses of organic manure on Plant height at 90 Days after transplantation

Capsicum varieties	T_0 (mean ±SD) (cm)	T_1 (mean± SD) (cm)	T_2 (mean± SD) (cm)	T_3 (mean ±SD) (cm)	T_4 (mean ±SD) (cm)	T_5 (mean ±SD) (cm)	T_6 (mean ±SD) (cm)	T_7 (mean± SD) (cm)
MZBEC-A	34.28 ±0.45^a	34.65 ±0.30^a	36.10 ±0.50^a	39.12 ±0.38^a	41.23 ±0.82^a	44.49 ±0.54^a	43.29 ±0.48^a	47.46 ±0.55^a
MZBEC-B	34.19 ±0.46^b	35.42 ±0.42^b	35.71 ±0.39^b	38.41 ±0.44^b	42.17 ±0.47^b	44.46 ±0.62^a	41.87 ±0.83^b	45.44 ±0.56^b
MZBEC-C	35.63 ±0.43^c	36.33 ±0.54^c	37.28 ±0.48^c	39.53 ±0.63^c	40.28 ±0.44^c	42.58 ±0.61^b	44.89 ±0.26^c	46.38 ±0.74^c

Means followed by different letters within same column are significantly different according to Duncan's multiple range comparisons (DMRTs (P<0.05). Means followed by same letter are not significantly different. Each value was represented as

Figure 5.1 Effect of soil organic amendments on Plant height at 30, 60 and 90 days after transplantation

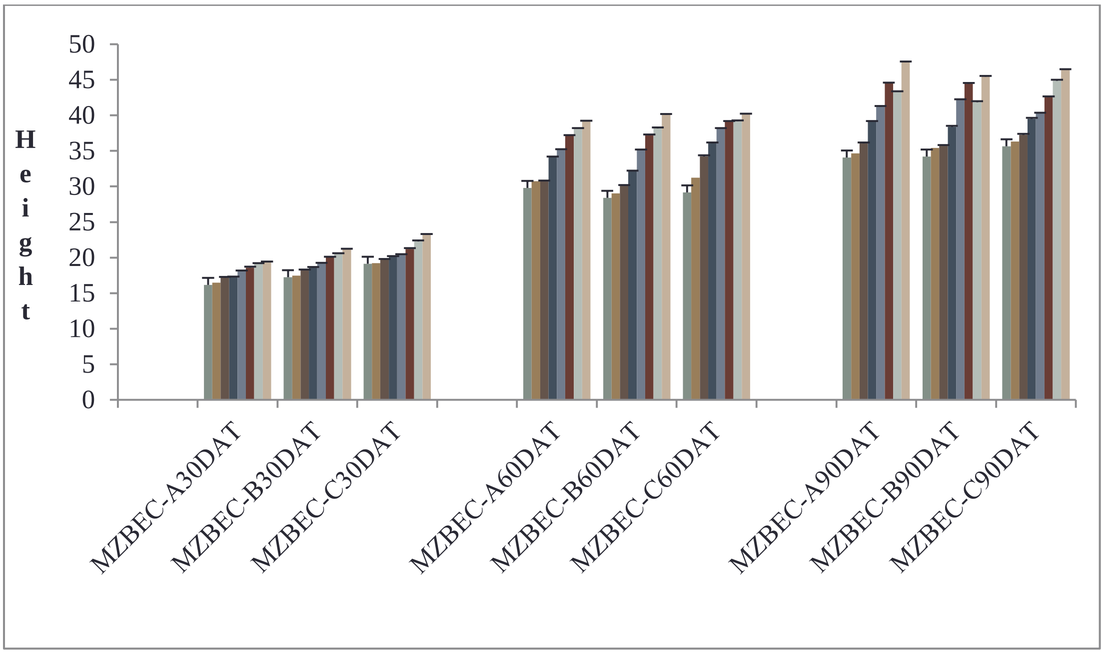


PHOTO PLATES

Plate A: Plant heights at 30 days after transplantation under different doses of soil organic amendments

Plate B: Plant heights at 60 days after transplantation under different doses of soil organic amendments

Plate C: Plant heights at 90 days after transplantation under different doses of soil organic amendments

5.3.2 Days to first harvest

The interaction effect of organic manure by varieties indicated very highly significant variation (*p<0.001*) on hot pepper on days to first harvest. Accordingly, the shortest number of days to first harvest was recorded from variety MZBEC-A (84.25±0.45 Days) (Table 5.4). The longest day to attain days to first harvest was recorded from variety MZBEC-C (94.05±0.11 Days), though it is statistically similar with MZBEC-B, which is in line with the works of MARC (2005) that reported, cultivars like Melka Zala are later than others to mature. The variations in days to first harvest (maturity) could be due to the differences in the growing environment climatic conditions and or due to the genetic make-up of the varieties. For best growth and fruit maturity and quality, it should be grown in an area with a temperature of (21-29 ^{0}C day) and (15-20 ^{0}C night) and soil pH of 6.5-7.5.

Table 5.4 Days to first harvest as affected by different doses of organic manure amendments

***Capsicum* varieties**	**T_0 (mean± SD) (Days)**	**T_1 (mean± SD) (Days)**	**T_2 (mean± SD) (Days)**	**T_3 (mean± SD) (Days)**	**T_4 (mean± SD) (Days)**	**T_5 (mean± SD) (Days)**	**T_6 (mean± SD) (Days)**	**T_7 (mean± SD) (Days)**
MZBEC -A	**94.05 ±0.11^a**	**94.15 ±0.04^a**	**94.10 ±0.01^a**	**85.68 ±0.01^a**	**85.45 ±0.21^a**	**85.03 ±0.05^a**	**84.17 ±0.12^a**	**84.25 ±0.61^a**
MZBEC -B	**94.15 ±0.23^C**	**94.31 ±0.42^b**	**94.17 ±0.16^b**	**85.77 ±0.45^b**	**84.33 ±0.17^b**	**85.05 ±0.62^b**	**84.22 ±0.11^a**	**84.34 ±0.36^b**
MZBEC -C	**94.17 ±0.71^c**	**94.23 ±0.51^c**	**94.22 ±0.73^b**	**85.51 ±0.55^c**	**85.47 ±0.83^c**	**85.09 ±0.53^c**	**84.27 ±0.39^b**	**84.25 ±0.45^c**

Means followed by different letters within same column are significantly different according to Duncan's multiple range comparisons (DMRTs (P<0.05). Means followed by same letter are not significantly different. Each value was

5.3.3 Number of fruits per plant

Results of analysis of variance indicated a very highly significant interaction ($p<0.001$) among the varieties and treatments in terms of number of fruits per plant at 90 DAT (Table 5.5) Variety MZBEC-A (T_7) had the highest number of fruits, while the least number of fruits per plant was recorded from variety MZBEC-C (T_0) and MZBEC-B (T_0) at experimental field respectively. The variations in fruit yield might be due to the influence of the growing environment's temperature, associated traits like canopy diameter that could limit the number of branches. Because, as a number of primaries, secondary and tertiary branches increased, there could be a possibility of increasing the number of fruit producing buds which are the locations for fruit production. Moreover, the variations in fruit development among varieties at both locations could also be due to the temperature stress of the growing environment and the capability of each varieties to with stand the stress specially on the reproductive development, which is more sensitive to high temperature stress (day and night temperature) than vegetative development. On the other hand, number of fruit can be affected by fruit abortion and predation have all been proposed as factors explaining low fruit set in plants. This also is in agreement with Schemske (1980) who reported that, Pollination can be the first factor limiting fruit production. In general the interaction of treatments by varieties had relatively better effect on the number of fruits per plant as it has been observed at the experimental sites. The relative earliness in flowering and maturity could also have enabled the varieties to produce more pods per plant, which contributed for higher productivity of the varieties per unit area.

Table 5.5 Number of fruits per plant under different doses of organic manure treatment at 90 days after transplantation

Capsicum varieties	T_0 (mean±SD)	T_1 (mean ±SD)	T_2 (mean ±SD)	T_3 (mean ±SD)	T_4 (mean±SD)	T_5 (mean ±SD)	T_6 (mean±SD)	T_7 (mean ±SD)
MZBEC-A	**22 ±2.51^a**	**29 ±3.51^a**	**33 ±2.51^a**	**35 ±2.51^a**	**44 ±3.60^a**	**43 ±3.51^a**	**48 ±3.60^a**	**45 ±5.13^a**
MZBEC-B	**23 ±2.01^b**	**29 ±2.51^b**	**32 ±3.05^a**	**38 ±5.03^a**	**41 ±4.58^b**	**37 ±2.64^b**	**40 ±3.51^b**	**34 ±6.02^a**
MZBEC-C	**19 ±3.12^b**	**27 ±1.52^b**	**35 ±3.70^b**	**34 ±5.69^a**	**33 ±5.61^b**	**39 ±4.04^b**	**42 ±3.00^b**	**37 ±6.65^a**

Means followed by different letters within same column are significantly different according to Duncan's multiple range comparisons (DMRTs ($P<0.05$). Means followed by same letter are not significantly different. Each value was represented

Table 5.6 Number of fruit per plant under different doses of organic manure treatment at 120 days after transplantation

Capsicum varieties	T_0 (mean ±SD)	T_1 (mean ±SD)	T_2 (mean± SD)	T_3 (mean± SD)	T_4 (mean ±SD)	T_5 (mean ±SD)	T_6 (mean ±SD)	T_7 (mean ±SD)
MZBEC-A	6 ± 1.0^{a}	11 ± 0.57^{a}	17 ± 1.52^{a}	16 ± 2.08^{a}	19 ± 2.00^{a}	23 ± 1.52^{a}	17 ± 2.00^{a}	23 ± 5.68^{a}
MZBEC-B	4 ± 1.5^{a}	8 ± 2.00^{b}	10 ± 2.08^{b}	9 ± 1.52^{b}	12 ± 1.00^{b}	17 ± 2.00^{b}	15 ± 1.52^{a}	19 ± 1.52^{a}
MZBEC-C	5 ± 1.26^{a}	7 ± 1.74^{b}	8 ± 2.01^{b}	7 ± 2.00^{b}	10 ± 1.52^{b}	13 ± 1.52^{b}	11 ± 1.52^{b}	16 ± 2.51^{a}

Means followed by different letters within same column are significantly different according to Duncan's multiple range comparisons (DMRTs (P<0.05). Means followed by same letter are not significantly different. Each value was represented as

Figure 5.2 Effect 0f soil organic manure on numbers of fruit per plant under different doses of organic manure at 90 and 120 days after transplantation

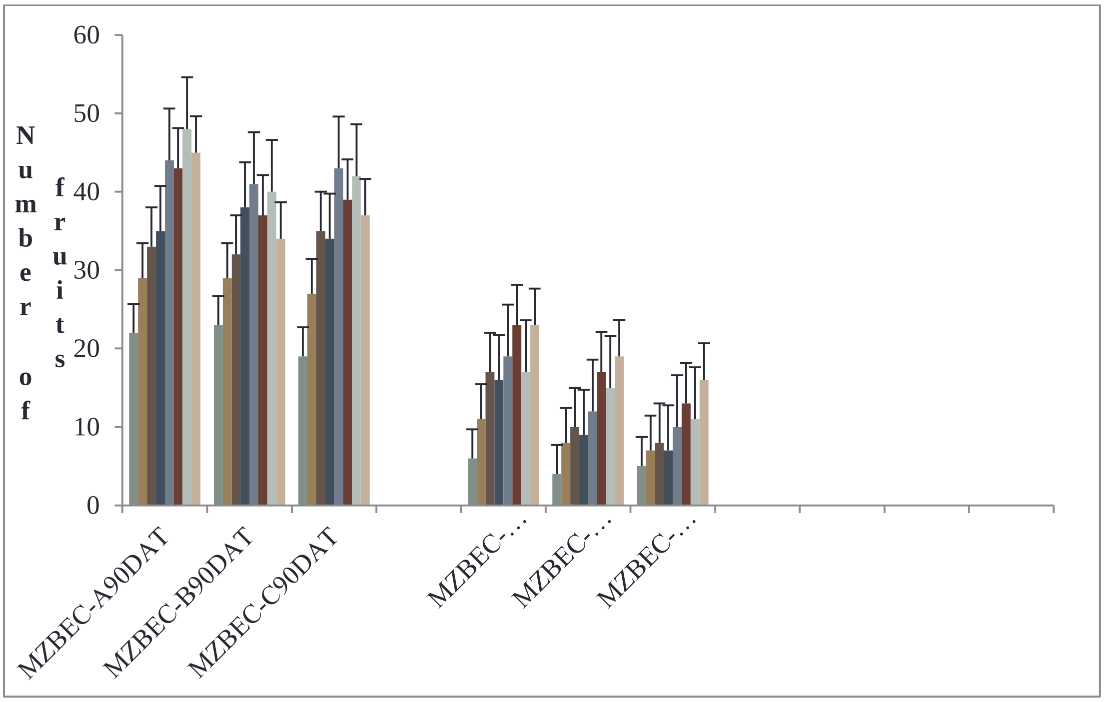


Plate A: Numbers of fruits per plant at 90 DAT under different doses of soil organic amendments

5.3.4 Fresh weight of fruit

The data on fresh weight of individual fruits as given in table 5.7 shows that variety MZBEC-C (T_7) has the maximum fresh weight among the varieties and MZBEC-A (T_0) shows the minimum fresh weight among the varieties. This may be due to the phenotypic differences among the varieties and amount of organic manure content in the soil in each plot of the experiment.

Table 5.7 Fresh weight of fruit per plant under different organic manure treatments (g)

***Capsicum* varieties**	**T_0 (mean± SD) (g)**	**T_1 (mean ±SD) (g)**	**T_2 (mean ±SD) (g)**	**T_3 (mean ±SD) (g)**	**T_4 (mean ±SD) (g)**	**T_5 (mean ±SD) (g)**	**T_6 (mean ±SD) (g)**	**T_7 (mean ±SD) (g)**
MZBEC-A	**5.41 ±0.79[a]**	**7.12 ±0.73[a]**	**9.06 ±0.36[a]**	**11.23 ±0.12[a]**	**15.17± 0.65[a]**	**15.11 ±0.30[a]**	**18.05± 0.12[a]**	**17.4 ±0.70[a]**
MZBEC-B	**9.19 ±0.34[b]**	**11.32 ±0.83[b]**	**16.37± 0.20[b]**	**17.07 ±0.45[b]**	**19.46± 0.80[b]**	**21.34 ±0.41[b]**	**20.09± 0.04[b]**	**23.05 ±0.08[b]**
MZBEC-C	**10.13± 0.23[c]**	**9.28±0 .45[c]**	**16.17± 0.20[b]**	**22.23 ±0.36[c]**	**25.83± 0.14[c]**	**27.07 ±0.41[c]**	**26.46± 0.88[c]**	**27.22 ±0.58[c]**

Means followed by different letters within same column are significantly different according to Duncan's multiple range comparisons (DMRTs ($P<0.05$). Means followed by same letter are not significantly different. Each value was

5.3.5 Fruit dry weight (g)

A significant variation was recorded in consideration of dry weight of plant in different combination of organic and inorganic fertilizer application and the result was presented in (Table 5.8). In case of fruits dry weight, the highest dry weight MZBEC-C (4.70±0.20gm) was found in T_7 treatment where the lowest dry weight (2.18±0.08gm) was obtained from MZBEC- A in T_0 treatment (control). The plant dry weight of sweet pepper in the experiment shows the following gradation in the decreasing order: $T_7 > T_6 > T_5 > T_4 > T_3 > T_2 > T_1 > T_0$. The present results showed in general an increase in vegetative growth of the inorganic and organic treatments compared to the control treatment; which could be due to the higher availability of nutrients in integrated treatment especially nitrogen (Palomaki *et al*., 2002).

The analysis of variance on interaction effect of treatments with varieties showed a very highly significant difference ($p<0.001$) on fruit dry weight per plant. The increase in pod dry weight in this study is in conformity with the work of Hedge (1997) and Guerpinar and Mordogan (2002) who reported that pod dry matter content of peppers was directly related to the amount of nutrient taken from the soil, which was proportional to the nutrients present in the soil or the amount of organic fertilizers applied to the soil. The variations in fruit dry weight among varieties may be due to the genetic make-up of .the varieties, and or due to the agro-ecological variations in which the varieties were evaluated.

Table 5.8 Dry weight of fruit per plant under different organic manure treatments (g)

Capsicum varieties	T_0 (mean ±SD) (g)	T_1 (mean ±SD) (g)	T_2 (mean± SD) (g)	T_3 (mean± SD) (g)	T_4 (mean ±SD) (g)	T_5 (mean ±SD) (g)	T_6 (mean ±SD) (g)	T_7 (mean ±SD) (g)
MZBEC - A	**2.18 ±0.8^a**	**2.22 ±0.12^a**	**2.1 ±0.5^a**	**2.25 ±0.23^a**	**2.19 ±0.22^a**	**2.28 ±0.07^a**	**2.23 ±0.15^a**	**2.27 ±0.14^a**
MZBEC- B	**2.21 ±0.37^a**	**2.32 ±0.11^b**	**2.38 ±0.05^b**	**2.29 ±0.12^a**	**2.41 ±0.08^b**	**2.88 ±0.24^b**	**3.08 ±0.19^b**	**3.07 ±0.17^b**
MZBEC- C	**3.11 ±0.17^b**	**3.17 ±0.10^c**	**3.23 ±0.09^c**	**3.33 ±0.11^b**	**3.27 ±0.09^c**	**3.77 ±0.05^c**	**4.11 ±0.18^c**	**4.70 ±0.20^c**

Means followed by different letters within same column are significantly different according to Duncan's multiple range comparisons (DMRTs (P<0.05). Means followed by same letter are not significantly different. Each value was represented

5.3.6 Fruit length (cm)

A highly significant ($p<0.01$) interaction was observed between varieties and treatments in terms of their fruit length (Table 5.9). Consequently, the longest fruits were recorded from variety MZBEC-C (3.76±0.01cm) in T_7, followed by MZBEC-B (2.85±0.07cm) in T_6, and MZBEC-A (2.04±0.02cm). The result agrees with that of MARC (2005) which reported that the long fruit length of (5cm) and the short fruit length with (2.4cm) at similar variety trial. The variations were most probably being attributed to their inherited traits or the growing environment. This current result was supported by the findings of Haileslassie *et al*., (2015) and Seleshi *et al.*, (2014). Moreover, this finding was supported by the work of Tibebu and Bizuayehu (2014).

Table 5.9 Fruit length of different varieties of Mizo chilli under different organic amendments at 90 DAT

Capsicum varieties	T_0 (mean± SD) (cm)	T_1 (mean± SD) (cm)	T_2 (mean± SD) (cm)	T_3 (mean± SD) (cm)	T_4 (mean± SD) (cm)	T_5 (mean ±SD) (cm)	T_6 (mean ±SD) (cm)	T_7 (mean ±SD) (cm)
MZBEC-A	1.3 ±0.83^{a}	1.8 ±0.30^{a}	1.11 ±0.06^{a}	1.9 ±0.10^{a}	2.1 ±0.04^{a}	2.0 ±0.03 a	2.2 ±0.03^{a}	2.4 ±0.02^{a}
MZBEC-B	2.1 ±0.11^{b}	2.2 ±0.30^{b}	2.5 ±0.03^{b}	2.7 ±0.03^{b}	2.5 ±0.03^{b}	2.8 ±0.02 b	2.6 ±0.03^{b}	2.8 ±0.07^{b}
MZBEC-C	2.7 ±0.03^{c}	2.9 ±0.96^{c}	3.2 ±0.06^{c}	3.4 ±0.03^{c}	3.3 0.02^{c}	3.6 ±0.02 c	3.5 ±0.05^{c}	3.7 ±0.01^{c}

Means followed by different letters within same column are significantly different according to Duncan's multiple range comparisons (DMRTs (P<0.05). Means followed by same letter are not significantly different. Each value was represented as means ± SD (n=3)

5.3.7 Fruit diameter (cm)

A very highly significant *($p<0.001$)* interaction effect of organic soil amendments by variety was recorded on fruit diameter (Table 5.10). The widest fruit diameter was obtained from variety MZBEC-B (1.3±0.43), followed MZBEC-C (0.9±0.36), while the least fruit width was observed from MZBEC-A (0.6±0.74). The variations in fruit diameter could be due to the difference in varieties inherited characteristics and or due to environmental conditions of the growing areas. This result is in line with MARC (2005) which showed that variety Mareko Fana had a fruit diameter of 2 cm. The pod width difference among varieties could be due to different dry matter partitioning ability of plants and the soil fertility status of the growing locations. Larger and wider hot pepper pods are considered to be the best in quality and have better demand for fresh as well as dry pod use in Ethiopian markets (Beyene and David, 2007). Therefore, subjectively this quality attribute, along with pod length and pericarp thickness could be of better preference to consumers over thinner and shorter pods.

Table 5.10 Fruit diameter of Mizo chilli varieties at 90 days after transplantation (cm)

***Capsicum* varieties (MZBEC)**	**T_7 (mean±SD) (cm)**
MZBEC-A	**0.6 ± 0.74^{a}**
MZBEC-B	**1.3 ± 0.43^{b}**
MZBEC-C	**0.9 ± 0.36^{c}**

Means followed by different letters within same column are significantly different according to Duncan's multiple range comparisons (DMRTs (P<0.05). Means followed by same letter are not significantly different. Each value was represented as means ± SD (n=3)

5.3.8 Life span of *Capsicum* under different treatments

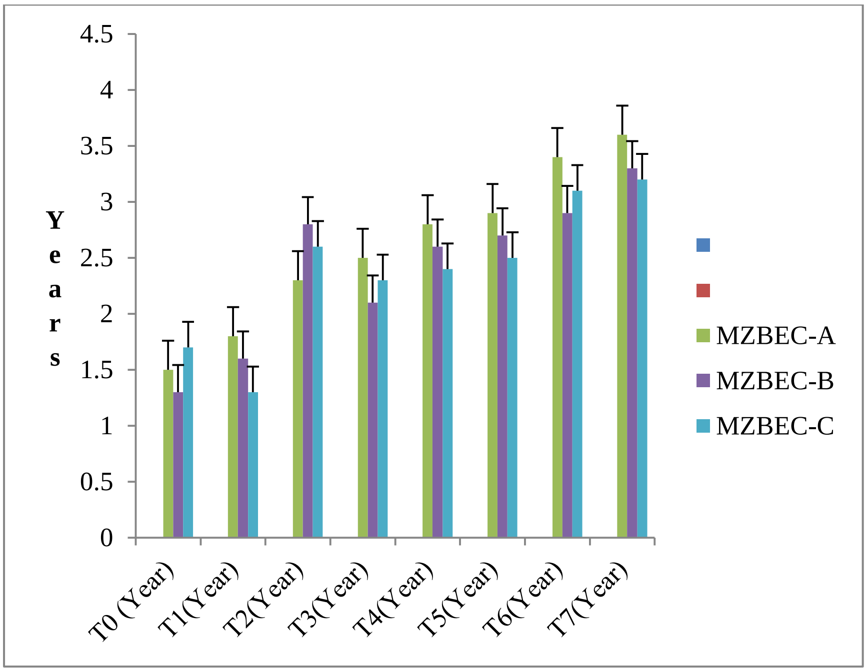
Life span of different varieties of Mizo chilli genotypes varied significantly (Figure 5.3). MZBEC-A had longest shelf life (3.6 years) followed by MZBEC-B (3.3 years) but were statistically at par ($p>0.05$) (Table 5.11). Shortest shelf life was recorded for MZBEC-C but it was statistically similar ($p>0.05$) with MZBEC-B. (Shil *et al.*, 2018) had reported difference in productive life span among different Chilli genotypes irrespective of storage condition. Accordingly, they can be categorized to different types of market. For example, MZBEC-A could be best for distant market whereas MZBEC-C could be the worst. The longevity in productive lifespan of any fruit lot depends on various factors. One of which is the genetic structure (Wiebach *et al.*, 2019). Some species deteriorate faster than others. Chillies are also considered to be a faster deteriorating species (Passam and Lambropoulos, 1997). The rapid decline of productive life in some cultivars compared to others requires special attention for some cultivar fruits. Incidence ageing is common in hot and humid environments particularly for chilli that are grown in such regions.

Table 5.11 Productive life span of different varieties of *Capsicum frutescens* under different organic amendments

Capsicum varieties (MZBEC)	T_0 (mean ±SD) (Year)	T_1 (mean ±SD) (Year)	T2 (mean ±SD) (Year)	T_3 (mean± SD) (Year)	T_4 (mean ±SD) (Year)	T_5 (mean ±SD) (Year)	T_6 (mean ±SD) (Year)	T_7 (mean ±SD) (Year)
MZBEC-A	1.5 ±0.83[a]	1.8 ±0.30[a]	2.3 ±0.06[a]	2.5 ±0.10[a]	2.8 ±0.04[a]	2.9 ±0.03[a]	3.4 ±0.03[a]	3.6 ±0.02[a]
MZBEC-B	1.3 ±0.11[b]	1.6 ±0.30[b]	2.8 ±0.03[b]	2.1 ±0.03[b]	2.6 ±0.03[b]	2.7 ±0.02[b]	2.9 ±0.03[b]	3.3 ±0.07[b]
MZBEC-C	1.7 ±0.03[c]	1.3 ±0.96[c]	2.6 ±0.06[c]	2.3 ±0.03[c]	2.4 ±0.02[c]	2.5 ±0.02[c]	3.1 ±0.05[c]	3.2 ±0.01[c]

Means followed by different letters within same column are significantly different according to Duncan's multiple range comparisons (DMRTs (P<0.05). Means followed by same letter are not significantly different. Each value was represented as means ± SD (n=3)

Figure 5.3 Effect of soil organic manure on productive life span of different varieties of Mizo chilli under different organic amendments



5.4 Conclusion

The beneficial effect of organic manure associated with different doses of treatments was found to have high efficiency in improving chilli peppers' nutrient content and crop productivity. The application of organic manure substantially increased the growth performance of chilli peppers at all conditions, whereas the treatment rate of (3 to 4 kg/plots) exhibited a higher yield increase. Therefore, applying the 3 to 4 kg of dry organic manure is considered the optimum for obtaining a maximum chilli pepper fruit yield. The excessive use of organic manure may result in the accumulation of more vegetative biomass than the increase in chilli pepper yield value. Thus, the rational use of organic amendments for chilli pepper cultivation is highly recommended depending on soil quality and environmental conditions. Further studies are required to understand the long-term effect of organic manure application on chilli pepper production under variable temperature conditions. Furthermore, it is important to develop a proper simulation model to elucidate the long-term effect of an organic amendment on growth and yield of chilli peppers.

The term quality implies the degree of excellence of a product or its suitability for a particular use. Quality of produce encompasses appearance, texture, nutritive values, chemical constituents, mechanical properties, functional properties and defects. Shelf life affects food quality, which in turn influences the consumer's buying decisions. Product quality is very important to growers because it determines marketable yield and can affect price. In present investigation application of organic manure improved the quality parameters and productive life of Chilli under different doses of organic manure. The productive life of Chilli was significantly higher with organic manure. These observations might be due to thick rind and heavier fruits with low moisture content and lower crude fibre content which might have enhanced the shelf life of fruits.

CHAPTER 6

DETERMINATION OF TOTAL NUTRITIONAL AND CAPSAICIN CONTENT IN DIFFERENT VARIETIES OF *CAPSICUM FRUTESCENS*

6.1 Introduction

Chilli is considered as an excellent source of bioactive compound. Moreover, an average amount of nutrients are found in chilli extract. Beside, chilli content high amount of vitamin C as compared to other spices and vegetables. Qualitative phytochemical screening and total nutritional content of the three Mizo chilli varieties *viz*; A, B and C was determined. The principle includes preliminary phytochemical screening and total estimation of ash, proteins, carbohydrates and ascorbic acid content. The output of these analyses indicates that sufficient amount of nutrients are present in these indigenous varieties of *Capsicum frutescens* and nutritional content varies from varieties to variety. *Capsicum sp.* is a major spice crop commonly cultivated in different parts of the world. Besides chilli content important nutrients for human consumption because of its nutritional and vitamins content. It is one of the most common valuable crops of India which belongs to family Solanaceae and cultivated throughout the country. Moreover the extracts of Chilli are used in pharmaceuticals, cosmetic products, paints and chilli sprays.

Plants are the reservoirs of biological active compounds to combat various pathogens (Khan and Nasreen. 2010). Phytonutrients provides crucial linked between health and nutrition. Chilli is a rich source of ascorbic acid and an average amount of carbohydrates and proteins are present. It is found in the literature that 100 grams of the edible portion of chilli provides 24 k Cal of energy, 1.3 grams of protein 4.3 grams of carbohydrate and 0.3 grams of fat (Anon, 2001). Vitamin C cannot be endogenously synthesized by human body, so it is very essential for our dietary component (Li and Schellhorn, 2007). Vitamin C is instrumental in neutralizing free radicals, which are harmful to the body, assimilation of iron, healing of wounds, helps to build collagen which aids the skin, and also defence against bacteria and virus infection (Medina-Juarez *et al.*, 2012). Vitamin C has been considered greatly as an important ingredient for boasting immune system during this Covid-19 pandemic. It is considered as most effective biochemical for immune response against Covid-19 virus recently with the outbreak of Covid-19 pandemic. Vitamin C or Ascorbic acid is the biologically active form of dehydroascorbic acid and it also prevents some important illness such as cancer, anaemia, diabetics, and cardiovascular diseases. It is fundamentally provided by fruits and vegetables (Vanderslice *et al.,* 1990). Higher amount of ascorbic acid is found in chilli than other fruits and vegetables (Ghasemnezhad *et al.,* 2011).In addition, ascorbic acid content of the fresh peppers increases during the ripening and ascorbic acid content decreases during the post-harvesting period. Also, this value might be changed with the cultivar, production practices, maturity at harvest and storage conditions (Lee and Kader, 2000).

The extremely hot or burning sensation of chilli is due to the presence of Capsaicinoids found only in *Capsicum* (Hoffman *et al*., 1983). The Capsaicinoids present in the *Capsicum* fruit are predominantly capsaicin and dihydrocapsaicin making up 80 to 90%.The ratio of capsaicin to dihydrocapsaicin ranges between 1:1and 2:1 (Govindarajan and Sathyanarayana, 1991). The capsaicin content of fruits of *Capsicum frutescens* has been found to be very high in comparison to the fruits of the other chilli species (Sanatombi and Sharma, 2008). The pharmaceutical applications of Capsaicinoids are attributed to their analgesic, antiarthritic, anticancer, and antioxidant properties (Prasad *et al*., 2005). In fact, Capsaicin has been at the centre of intense research for elucidating the basis of its pharmacological properties and exploiting the therapeutic potential (reviewed by Prasad *et al*., 2005).

Capsaicin has become a promising molecule for the development of a new generation of analgesic-anti-inflammatory agents targeting the nociceptive primary afferent neurons (Szolcsanyi, 2003). It has also been reported to inhibit the growth of prostate cancer cells (Mori *et al*., 2006). The anti-oxidative capacity of chillies are higher than ginger, garlic, mint and onion (Shobana and Naidu, 2000), which may play an important role in the process of chemo prevention (Yu *et al*., 2002) .The genus *Capsicum* (Solanaceae) consists of five domesticated species: *Capsicum annum* L., *Capsicum baccatum* L., *Capsicum chinense* Jacq., *Capsicum frutescens* L. and *Capsicum pubescens* Ruiz & Pav as well as around 25 wild species (IBPGR, 1983). *Capsicum annum* is one of the major vegetable and spice crops cultivated worldwide. Dietary spices are important ingredients commonly prescribed in Indian systems of medicine including Ayurveda, Siddha and Unani systems (Pruthi, 1976; Kochhar, 1996) and during the past years have received renewed attention for treating chronic and acute diseases. One of such dietary spice is the fruit of *Capsicum frutescens* a source of the highly pungent Capsaicinoids and of antioxidants, which may play a role in preventing or reducing chronic and age-related diseases. The 'Mizo chilli', which is native to Mizoram in the north-eastern part of India, has received the attention due to its high pungency and unique aroma. It is known by various names in Mizoram such as Mizoram Birds 'eye chilli (MZBEC) or 'Hmarchate' or 'Vaihmarchate'. (Dutta SK *et al*., 2018). It has also been used conventionally by different ethnic communities of the north-eastern India in treating various human ailments (Bhagowati and Changkija, 2009). Besides being used as spice and vegetable, they are also used as a very good source of ethno-medicines for a number of diseases by the traditional healers.

However, systematic and more extensive ethno medicinal investigations are not carried out to provide new insights into the other traditional uses of this important plant. In-depth research endeavours should also be directed towards phytochemical and pharmacological investigations of Mizo chilli that could lead to unearthing new bioactive compounds/activities. The objective of this study was to determine Capsaicin content of these high yielding chilli varieties to better guide farmers, consumers and pharmaceutical industries.

In North eastern parts of India, Jhum cultivation or shifting cultivation is the main agriculture practice where most of the farmers still rely on. The Chilli variety is indigenous to the Northeast region of India and it is an important crop of the North-East India but scientifically it has not been explored to its fullest. According to Dhaliwal (2007), chilli was introduced directly by the Christian missionaries to north eastern India from South America.

Capsicum frutescens which is exclusively grown in Mizoram is distinctly different from other varieties grown in different parts of the country. It is cultivated on hill slopes under shifting cultivation system or *Jhuming* system which are grown completely organically in the *Jhum* lands. In Mizoram, Bird's eye chill is locally known as *Hmarchate* or Mizo chilli or *Mizoram Bird's eye chilli (*MZBEC) which belongs to species *Capsicum frutescens* and is widely grown in the state of Mizoram.

In Mizoram itself three different varieties of Mizoram Bird's eye chilli (MZBEC) *viz*; A, B and C are cultivated in different parts of the state. The government of Mizoram claimed that Mizoram is the native place of Bird's eye chilli locally called Hmarchate or Mizo chilli. The ethnic communities of the north-eastern India also used in treating various ailments (Bhagowati and Changkija, 2009). However, systematic and more extensive ethno medicinal investigations are not carried out to provide new insights into the other traditional uses of this important plant. Moreover, no scientific study has been made on nutrient content and phytochemical screening of *Capsicum frutescens* in Mizoram and no literatures are available in this aspect.

6.2 Materials and Methods

6.2.1 Collection of Sample Material and Extraction

Fresh varieties of Mizo chilli were air-dried and used as the samples in these experiments. The samples were collected from Horticulture Centre of Excellence, Thiak village, about 45 kilometres South of Aizawl, Mizoram. Air-dried ripe fruits of Mizo chilli were powdered and extracted by Soxhlet extraction method.

Photo plate A. Collection of Chilli samples

6.2.2 Preliminary phytochemical screening

Phytochemical screening of the chilli extract was done using the standard protocol described below.

6.2.2.1 Test for Alkaloids (Mayer's reagent test)

2 ml of the extract was dissolved in 2 ml of 2N Hydrochloric acid and two to three drops of Mayer's reagent was added. The creamy white precipitate formation indicated the presence of alkaloids (Harborne, 1998; Doughari, 2012).

6.2.2.2 Test for Terpenoids (Salkowski test)

In 5 ml of each extract 2 ml of chloroform was added, 3 ml of sulphuric acid was added with a careful overlaying. Reddish brown precipitate formation at the interface indicated the presence of Terpenoids. (Harborne, 1998)

6.2.2.3 Test for Saponins (Foam test)

The extract of 3 ml was diluted with distilled water upto 10 ml and was shaken vigorously for two minutes. White froth formation indicated the presence of Saponins.

6.2.2.4 Test for Flavonoids (H_2SO_4 test)

A few drops of concentrated sulphuric acid were treated with 5 ml of the extract. Orange-yellow colour appearance indicated the presence of flavonoids (Harborne, 1998).

6.2.2.5 Test for Tannins (Ferric chloride test)

0.5g or 500mg of the plant extract was boiled in 20ml of distilled water and filtered. To the filtrate a few drops of 0.1% ferric chloride was added. Blue-black or brownish green colour formation indicted the presence of tannins. (Doughari, 2012).

6.2.3 Determination of total nutritional content

6.2.3.1 Estimation of Total Ash content:

Dry ashing method was used for the estimation of total ash content in the sample. In this method, 1g of the oven dried sample was ignited in a muffle furnace with a temperature of 550°C for three hours. The process was repeated until a constant weight of the dried crucibles was observed.

$$\text{Ash (\%)} = \frac{weight\ of\ Ash}{weight\ of\ sample} \times 100$$

6.2.3.2 Determination of Ascorbic acid is done by 2, 4-dinitrophenylhydrazine method.

5 grams of Chilli pepper fruits were washed with tap water and cut into small pieces and homogenized with the help of mortar and pestle by adding 5 ml of 4% oxalic acid. The homogenates were centrifuged at 5,000 rpm for 10 minutes then the supernatants were filtered with 540 Whatmann filter paper. The obtained residues were made up to 25 ml with 4% oxalic acid. The ascorbic acid content was estimated by using 2, 4 dinitrophenylhydrazine (DNPH) reagents in conjunction with spectrophotometer at 540 nm (Sadasivam and Manickam, 1992). Pure Ascorbic acid reagent is used as standard reagent for preparing the standard curve against which the unknown concentration of Vitamin-C was expressed in the milligrams per 100 milligrams of the sample.

6.2.3.3 Determination of Total Protein content:

Protein content of the fruit was estimated by Lowry method (1951). Reagent A**:** 2gm NaOH +500ml. double distilled water + 1gm Sodium carbonate. Reagent B**:** 1gm potassium sodium tartrate + 50ml double distilled water +0.25gm CuSO4 Reagent C**:** 1ml of Reagent B + 50ml of reagent A Reagent D: 1ml of foline + 1ml double distilled water Phosphate Buffer: Solution A: 1.36gm potassium dihydrogen orthophosphate + 100ml double distilled water Solution B**:** 1.74 g dipotassium orthophosphate + 100ml double distilled water Solution C**:** 39ml of solution of A + 61ml of solution B. Normal NaOH; 4gm NaOH+100ml double distilled water 0.5 gm fruit weight and crushed in 5 ml of Phosphate buffer and centrifuge at 3000 rpm for 5 minutes. 0.5 ml of the Supernatant was collected in the test tube and 0.5 ml of 1N NaOH, 2.5 ml reagent C and 0.5ml reagent D was added to it after adding the reagent D blue colour was appeared and absorbance was read at 660 nanometres. Bovine Serum Albumin (BSA) is used as standard reagent for preparing the standard curve (Figure 2) against which the unknown concentration of proteins was expressed in the milligrams per 100 milligrams of the sample.

6.2.3.4 Quantification of Total Carbohydrates content:

The total carbohydrates content was determined by Anthrone method (Dubois *et al.,* 1951). Anthrone reagent: Dissolved 2grams of Anthrone in 1 litre of concentrated H_2SO_4. The freshly prepared reagent is used for the assay. Glucose stock solution: 200µg glucose per mL distilled water. It was pipette out into a series of test tubes different volumes of glucose solution from the supplied stock solution (200µg /ml) and the volume is made upto 1 mL with distilled water. Then 4 mL of the Anthrone reagent (supplied) were added to each tube and mixed well by vortexing. After the tubes were cooled, the tubes were covered with aluminium on top and incubate at 90°C for 17 minutes. Then the tubes were cool to room temperature and the optical density was measured at 620nm against the blank. D-glucose is used as a reagent for preparing a standard curve against which the unknown concentration of total carbohydrates was expressed in the milligrams per 100 milligrams of the sample.

6.3 Estimation of Capsaicin content

6.3.1 Preparation of Chilli Powder extracts

The extract was prepared employing Soxhlet extraction method. About 5gm of dried chilli powder material was uniformly packed into a thimble and extracted with THF. The process of extraction continues for 24 hours or till the solvent in siphon tube of an extractor become colourless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40ºC till all the solvent got evaporated. Dried extract was kept in refrigerator at 40^{o}C for their future use in Capsaicin analysis.

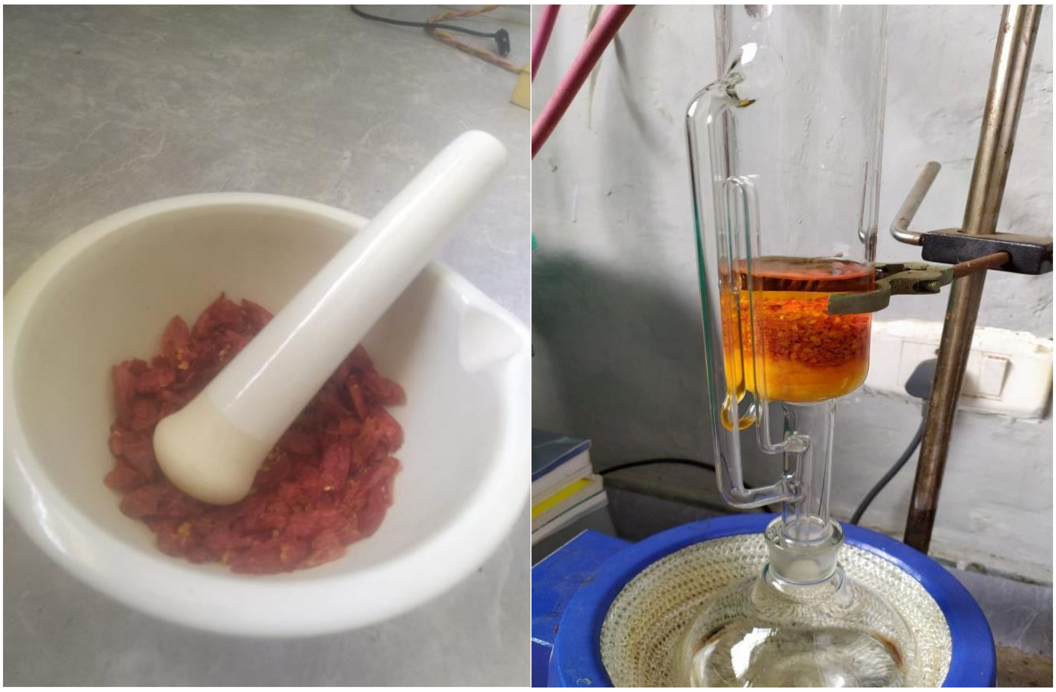


Photo plate B. Extraction of plant specimen using Soxhlet apparatus for analysis.

6.3.2 Extraction and determination of Capsaicin content

Capsaicin content in the samples was estimated by spectrophotometric measurement of the blue coloured component formed as a result of reduction of phosphomolybdic acid to lower acids of molybdenum following Ademoyegun *et al*., (2011). One gram (1g) of each dry sample was extracted with 10 ml of dry acetone using pestle and mortar. The extract was centrifuged at 10,000 rpm for 10 min and 1ml of supernatant was pipetted into a test tube and evaporated to dryness in a hot water bath (60°C). The residue was then dissolved in 0.4 ml of NaOH solution and 3 ml of 3% phosphomolybdic acid. The contents were shaken and allowed to stand for 1 h. The solution was filtered to remove any floating debris and centrifuged at 5,000 rpm for 15 min. Absorbance was measured for the clear blue solution, thus obtained, at 650 nm using reagent blank (5 ml of 0.4% NaOH+ 3ml of 3% phosphomolybdic acid). Capsaicin content is calculated from the standard curve and expressed as percentage per one gram of the sample on dry basis.

6.3 Results and Discussion:

6.3.1 Preliminary phytochemical screening

From the result (Table 6.1) all the varieties of Mizo chilli showed the presence of alkaloids, Terpenoids, Saponins, Flavonoids and Tannins. It indicates that most of the bioactive compounds are present in these varieties of chilli. So the presence of phytochemical constituents plays a significant role in maintaining the vital health condition of the human body. The phytochemical can have complementary and overlapping mechanisms of oxidative agents, stimulating the immune system, regulator of gene expression in cell proliferation, and apoptosis (Praveena *et al.*, 2014). The alkaloids show strong antioxidant activity. It has a wide range of pharmacological activities including antimalarial, antiasthma, anticancer, and antibacterial properties (Coe *et al.*, 2012).

Table 6.1 Preliminary phytochemical screening of different varieties of Mizo chilli

Mizo Chilli varieties	Alkaloids	Terpenoids	Saponins	Flavonoids	Tannins
MZBEC-A	++	++	+	++	+
MZBEC-B	++	+	+	+	+
MZBEC-C	+	++	+	++	+

6.3.2 Estimation of total Nutrition content: The nutritional estimation has demonstrated the potential benefits of Mizo chilli varieties. The proximal values were calculated against the standard curve and depicted in the Table 6.2.

Table 6.2, indicates that high amount of vitamin C is present in the fruits of Mizo chilli that varies from 0.79 mg/100mg of the sample to 2.18 mg/100mg of dry weight of the sample. From the result, MZBEC-A variety has the highest Vitamin-C content (2.18mg/100mg) followed by MZBEC-C (1.68mg/100mg) and MZBEC-B has the lowest Vitamin-C content (0.79mg/100mg). With conformity with the present finding Teodoro *et al*., (2013) also reported the vitamin C contents in the range of 54.1mg/100g to 129.8 mg/100g in Habanero pepper accessions (*Capsicum chinense*). Similarly, Orobiyi *et al*., (2015) reported 84.64 -192.64 mg/100g ascorbic acid content in the fresh fruits of chilli pepper. From the result, the Mizo chilli varieties has high amount of Vitamin- C as compared to other chilli varieties. Proteins content was found far highest in MZBEC-A (4.22mg/100mg) as compared to MZBEC-B (0.9mg/100mg) and MZBEC-C (1.1mg/100mg). But carbohydrates content was found highest in MZBEC-B (1.18mg/100mg) followed by MZBEC-C (1.18mg/100mg) and lowest in MZBEC-(1.15mg/100mg). The Total Ash content of the sample was found to be highest in MZBEC-A variety (6.02%) followed by MZBEC-B (5.74%) and lowest in MZBEC-C (4.4%) in one gram of each sample. The total estimation of ash content indicates the presence of inorganic minerals by burning the organic substances in the samples. It is important because the amount of minerals can determine physiochemical properties of the chilli. From the experimental result, it collaborate with those of which state that plant morphological variation and fruit shape, as well as fruit colour, strongly depend on the cultivated chilli varieties (Bosland and Votava, 2000). These results also confirmed that nutrients vary from one stage to another because of climatic conditions, variety of cultivars and differences in analytical procedures (Bhutia and Meetei, 2018).The significant differences among the varieties were probably due to the different factors like soil texture, climate, growing conditions, cultivars, postharvest handlings and storage conditions (Martinez *et al.,* 2005).

Figure 6.1 (a) Standard curve for Vitamin-C using Ascorbic acid (b) Total Vitamin-C content (mg/100mg of sample) in Mizo chilli varieties.

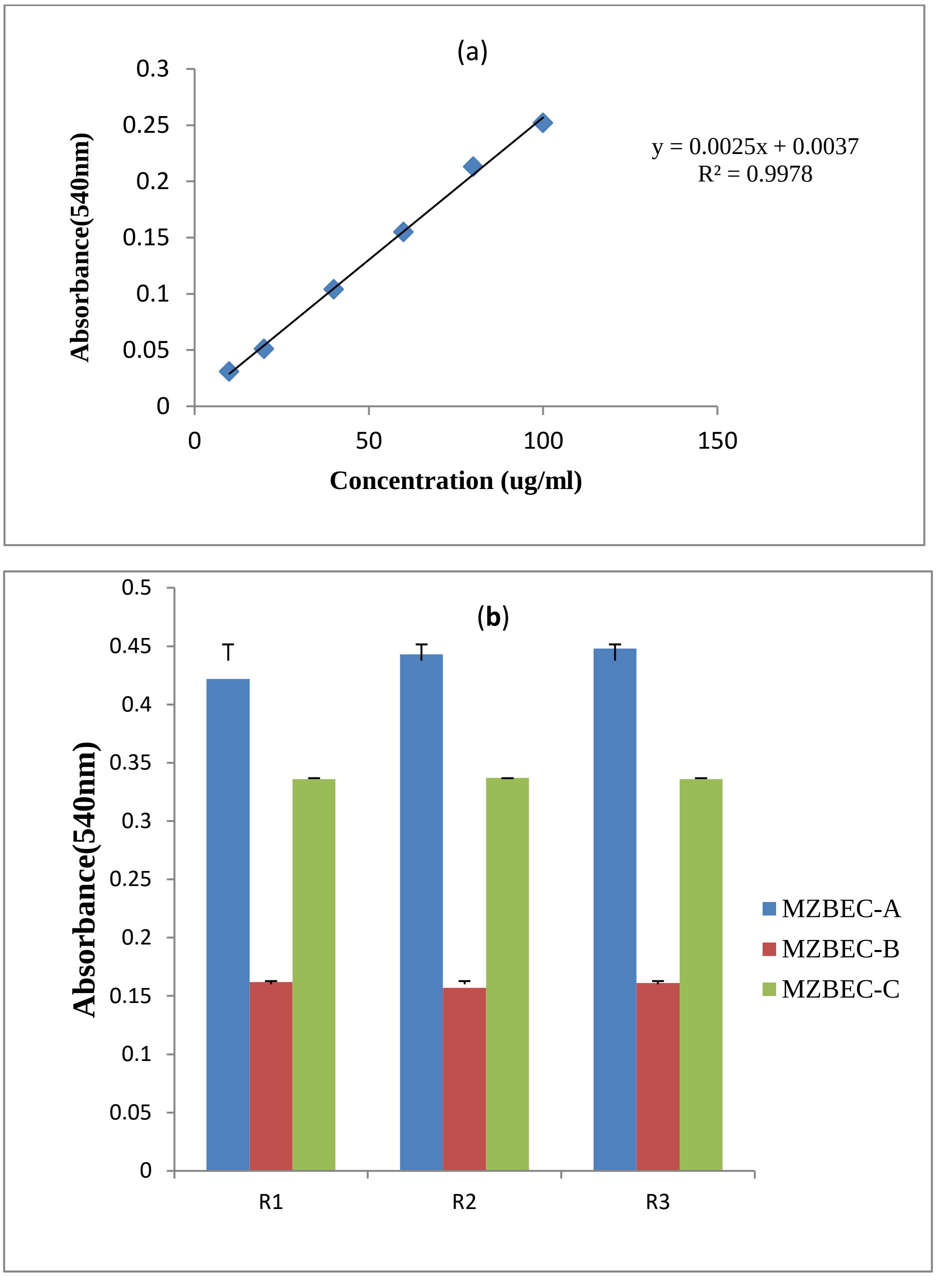
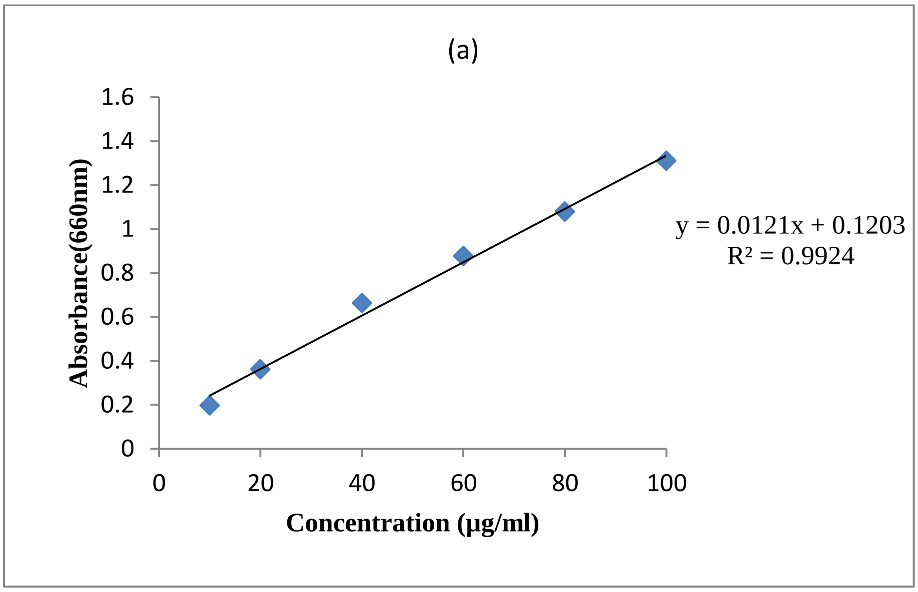


Figure 6.2. (a) Standard curve for proteins using Bovine Serum Albumin (BSA) (b) Total proteins content (mg/100mg of sample) in Mizo chilli.



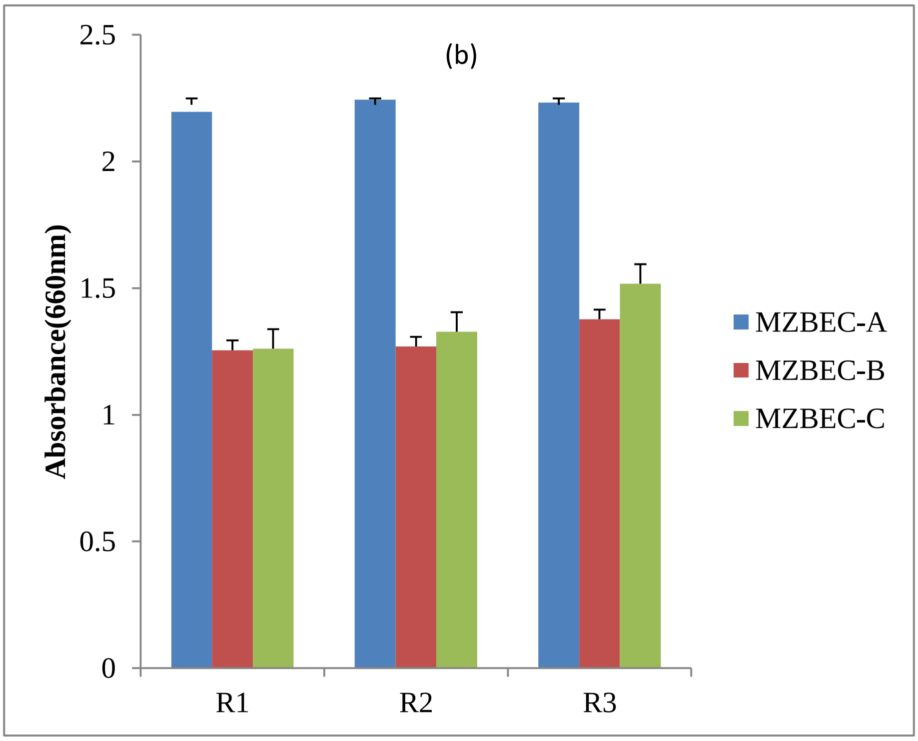
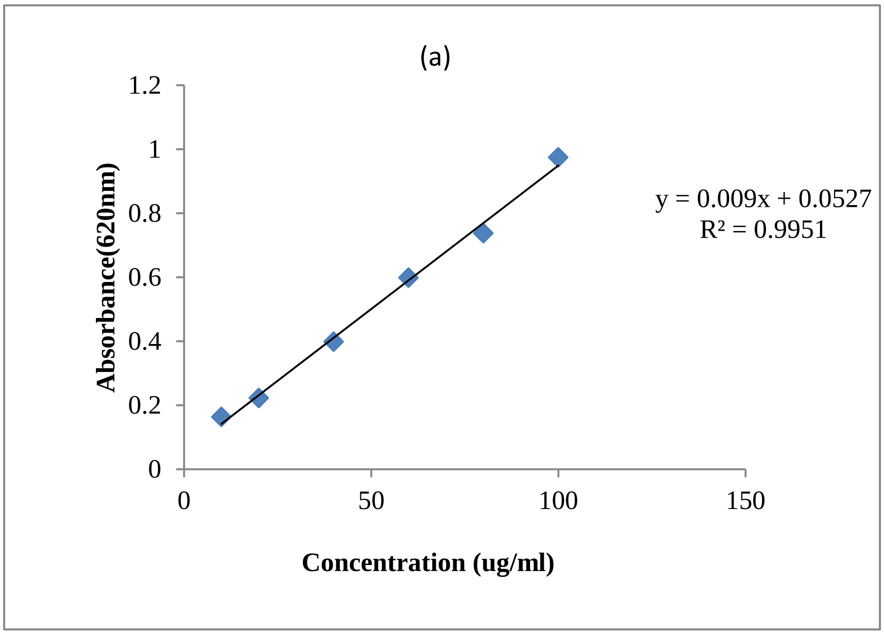


Figure 6.3 (a) Standard curve for Carbohydrates using D-Glucose (b) Total carbohydrates content (mg/100mg of sample) in Mizo chilli.



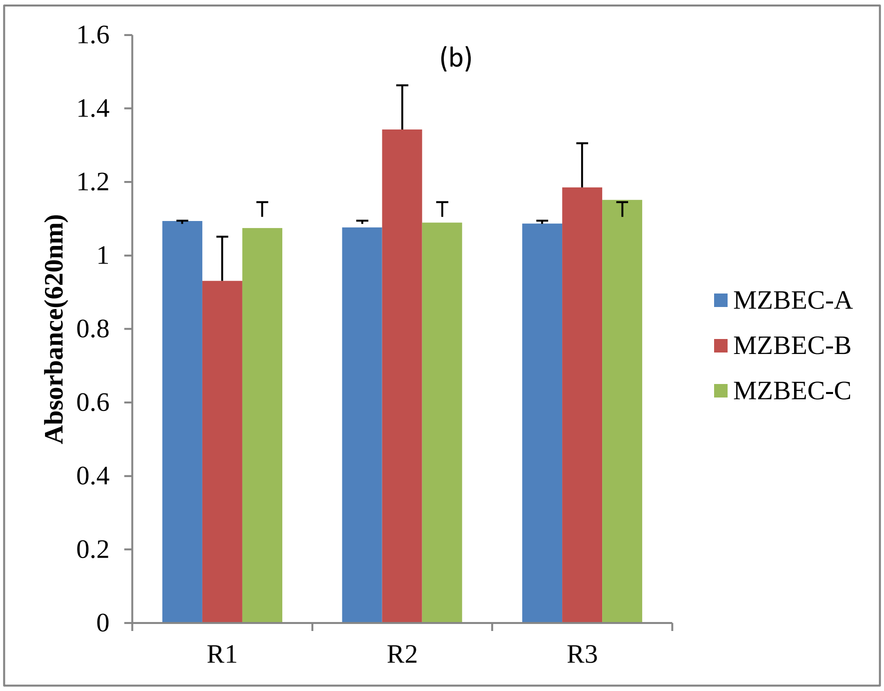


Figure 6.4 Total Ash content (%) in the three varieties Mizo chilli

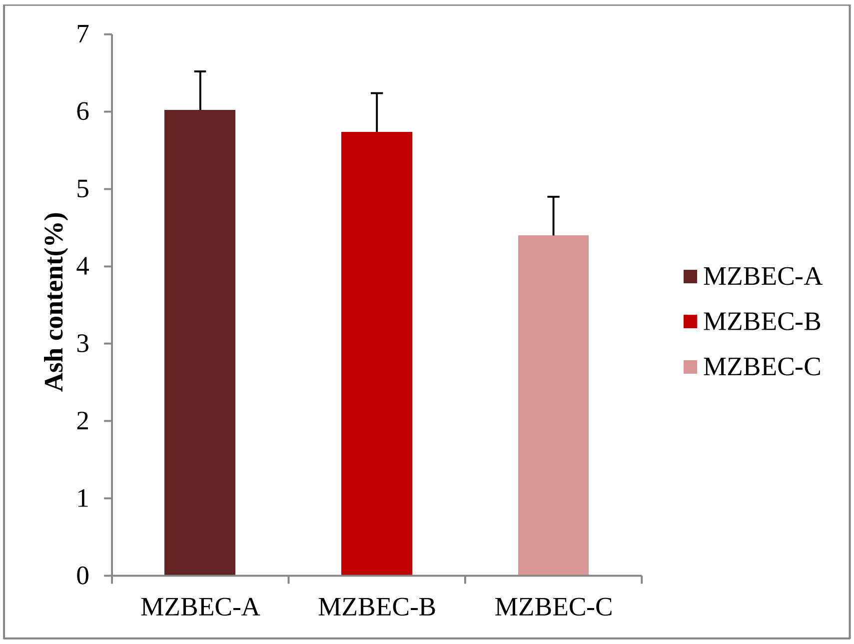


Table 6.2 Total nutrition contents (Ascorbic acid, Proteins, Carbohydrates, Ash content) in different varieties of Mizo chilli

Mizo Chilli varieties	Ascorbic acid (mg/100mg±SD)	Proteins (mg/100mg±SD)	Carbohydrates (mg/100mg±SD)	Ash content (%±SD)
MZBEC-A	2.18±0.013[a]	1.75±0.025[a]	1.15±0.009[a]	6.02±0.022[a]
MZBEC-B	0.79±0.002[b]	0.9±0.066[b]	1.22±0.21[b]	5.74±0.068[b]
MZBEC-C	1.68±6.103[c]	1.1±0.133[c]	1.18±0.040[a]	4.4±0.208[c]

Means followed by different letters within same column are significantly different according to Duncan's multiple range comparisons (DMRTs (P<0.05). Means followed by same letter are not significantly different. Each value was represented as

6.3.3 Capsaicin content

The standardization curve equation gotten is: Y = 0.0083X + 0.01431 (Figure 6.3). It was used to calculate the content of the Capsaicin (expressed in % of dry weight) contained in each sample of chilli pepper analysed. The content varied from a variety to another. The highest Capsaicin content was found in MZBEC-A (2.11±0.47 % of dry weight) in T_7 in followed by MZBEC-C (1.92±0.43 %) in T_7 and MZBEC-C (1.81%) while the lowest was found in MZBEC-B (0.81±0.34 % of dry weight) in T_0 (Table 6.3.3.1).The results show that all the three varieties of Mizoram bird's eye chilli (MZBEC) content rich amount of Capsaicin.

Figure 6.5 Standard curves for Capsaicin

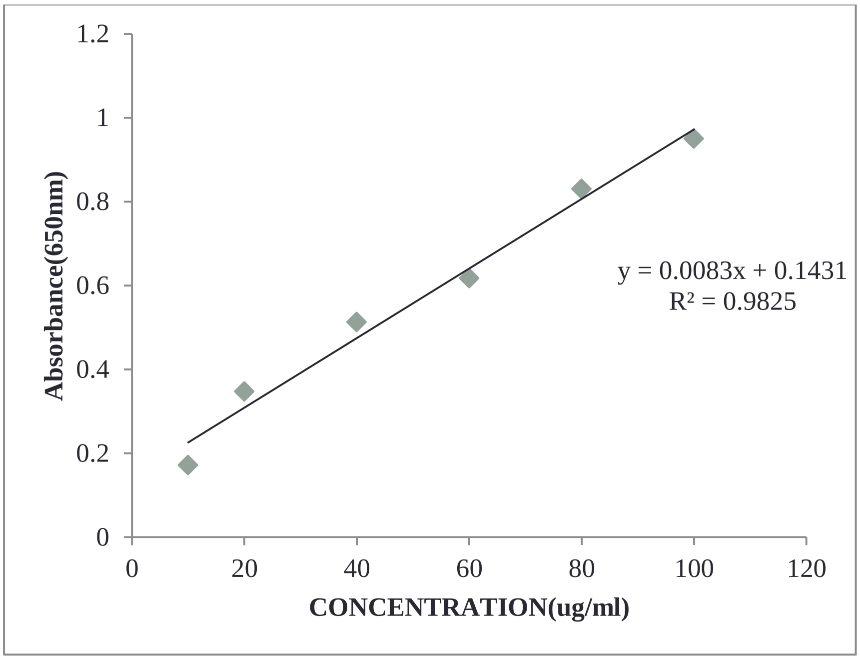
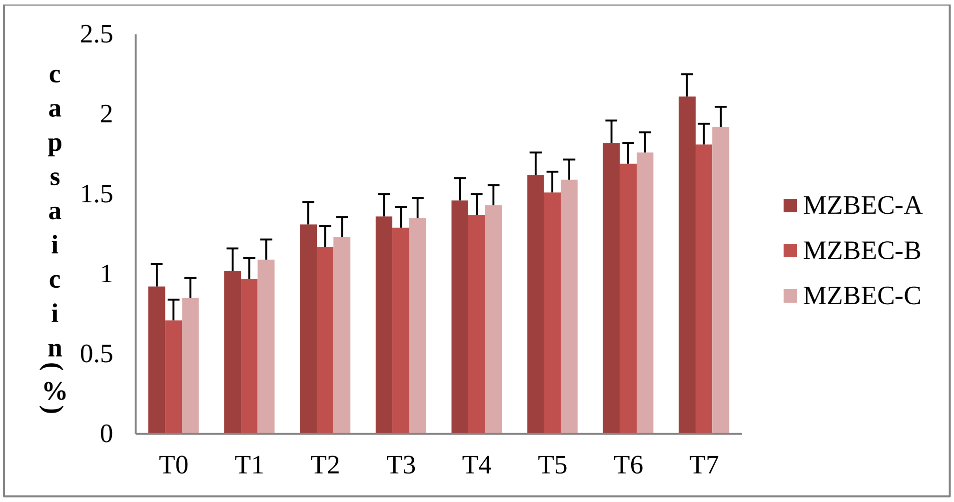


Table 6.3 Amount of Capsaicin present in each varieties of chilli against different doses of organic amendments

Chilli Varieties	T_0 (mean ±SD) (%)	T_1 (mean ±SD) (%)	T_2 (mean ±SD) (%)	T_3 (mean ±SD) (%)	T_4 (mean ±SD) (%)	T_5 (mean ±SD) (%)	T_6 (mean ±SD) (%)	T_7 (mean ±SD) (%)
MZBEC -A	**0.92 ±O.45**^{a}	**1.02 ±0.51**^{a}	**1.31 ±0.44**^{a}	**1.36 ±0.27**^{a}	**1.46 ±0.36**^{a}	**1.62 ±0.55**^{a}	**1.82 ±0.33**^{a}	**2.11 ±0.47**^{a}
MZBEC -B	**0.71 ±O.32**^{b}	**0.97 ±0.23**^{b}	**1.17 ±0.39**^{b}	**1.29 ±0.43**^{b}	**1.37 ±0.41**^{b}	**1.51 ±0.39**^{b}	**1.69 ±0.51**^{b}	**1.81 ±0.34**^{b}
MZBEC -C	**0.85 ±0.38**^{c}	**1.09 ±0.46**^{b}	**1.23 ±0.31**^{c}	**1.35 ±0.17**^{c}	**1.43 ±0.25**^{a}	**1.59 ±0.26**^{c}	**1.76 ±0.42**^{c}	**1.92 ±0.43**^{c}

Means followed by different letters within same column are significantly different according to Duncan's multiple range comparisons (DMRTs (P<0.05). Means followed by same letter are not significantly different. Each value was represented as

Figure 6.6 Amount of Capsaicin (%) present in each variety against the treatments



6.4 Conclusion

The fruits of Mizo chilli varieties were found to be rich in Vitamin-C, Proteins, Carbohydrates and Ash content and average amount of phytochemicals. There is a great diversity within the *Capsicu*m species consumed in India. From the result, it indicates that Mizo chilli varieties can be considered to be a good source of nutrients and bioactive compounds. The variability presented in Mizo chilli's varieties germplasm for nutrition and bioactive substances can be exploited for breeding of new varieties with improved nutritional qualities. Considering the significance of Vitamin-C in human health and diseases, and its application in pharmaceutical product and commercial implication in food industry and current Covid-19 pandemic, Mizo chilli's offers great potential for future exploitation.

The results show that all the three varieties of Mizoram bird's eye chilli (MZBEC) content rich amount of capsaicin. The Capsaicin contents vary from a chilli pepper variety to another and don't depend on any morphological similarity (Orobiyi *et al.*, 2015). Organic and inorganic fertilizers increasing the hormonal activities of plants like ethylene which helps in repining of fruits and capsaicin formation. The same result has been shown by (Balasubramani, 1988).

The inverse relationship between size and pungency confirms report by Derek and Wibberley (1981) that peppers are hotter if they are smaller. These varieties can be exploited by pharmaceutical industries in the manufacturing of some remedies against cancer, diabetes, and cardiovascular illnesses. It is therefore necessary to follow up on the analyses within the whole existing diversity in order to find the landraces that are naturally rich in Capsaicin. Moreover other programs must be put in place in order to find within the whole existing diversity or to create some very productive and rich landraces of chilli pepper in capsaicin and that will be very valuable in pharmacology.

CHAPTER 7

QUANTITATIVE ANALYSIS OF TOTAL PHYTOCHEMICALS CONTENT AND ANTIOXIDANT ACTIVITIES IN *CAPSICUM FRUTESCENS*

7.1 Introduction

Plants with several bioactive compounds are increasingly gaining attention because of their effectiveness in improving human health and nutrition (Hale *et al.,* 2008).The correct identification of these plant species with medicinal importance is the first and basic step in any improvement programme. This allows reliable and effective selection of suitable parental genotypes (with authentic purity and quality) in plant breeding programmes that are developed for various nutritional and pharmacological purposes. They are vastly valued not only because of their economic importance but also for their rich nutritional value. There is evidence that the consumption of fruits and vegetables is associated with decreased risk of cancer, heart disease, and degenerative diseases associated with ageing (Charles, 2013). One such vegetable in which a variety of antioxidants can be found is the chilli. *Capsicum* is widely used due to its strong pungency, aroma, colour, and nutritional value. Indian, Chinese, and North American traditional medicines have also used chillies for the treatment of arthritis, rheumatism, stomach aches, skin rashes, dog/snake bites, and flesh wounds (Meghvansi *et al.,* 2010).

Plant phenolic are secondary metabolites that constitute one of the most common and widespread groups of substances in plants. Phenolic biogenetically arise from the shikimate-phenyl-propanoids flavonoids pathways (Lattanzio *et al*., 2006*)*. Plants need phenolic compounds for pigmentation, growth, resistance to pathogens and for many other functions. Therefore, they represent adaptive characters that have been subjected to natural selection during evolution. Fresh *Capsicum annuum* has been recognized to have antioxidant activity due to the presence of metabolites with well-known antioxidant capacities such as ascorbic acid, vitamin E, provitamin A, carotenoids, xanthophyll, and phenolic compounds, which are present in connection with sugars (Materska and Perucka, 2005). The fruit of pepper contains a range of bioactive phytochemicals including flavonoids, carotenoids, phenolic, and other antioxidant compounds (Alvarez *et al.,* 2011). Besides the nutritional benefits of pepper and their use as food additives, the hot *Capsicum spp*. (due to their Capsaicin content) have a significant role in pharmacy and are currently used for different therapeutic purposes. Several classes of phytochemicals (phenolic compounds, particularly flavonoids and phenolic acid) and antioxidants are sufficiently available in high amounts in vegetables and fruits; thus they form an important part of human diet. They are known to protect the body cells by fighting off free radicals in the body by disallowing oxidation process. Series of reactions initiated by free radicals caused damage to membrane and disruption of metabolic pathways thereby increasing mutations in DNA and alteration of platelet function among others (Palevitch and Craker, 1996). Since numerous studies have suggested that eating foods rich in phytochemicals and antioxidants has been linked with lessened risk of certain forms of cancer, stroke, and cardiovascular diseases, much attention is recently given to natural foods which are rich in these bioactive compounds.

Several studies have documented the effectiveness of the antioxidative components of various pepper species (Khan *et al.*, 2014). For example, Loizzo *et al*., (2015) reported the inhibitory effect of *C. annuum* var. *acuminatum* on the enzyme acetyl cholinesterase. The inhibition of this enzyme is one of the therapeutic methods for the symptomatic management of Alzheimer's disease. Similarly, Trehan *et al*., (2008) also reported high antioxidant properties of the fruit extracts of *C. frutescens* from green to red stages based on the oxygen radical absorbance capacity (ORAC) and DPPH tests. In another study, methanolic extracts from *C. annuum* L. were reported to inhibit 4-hydroxy-2nonenal-induced and H_2O_2-induced DNA damage; this study was done on human leucocytes and a potential toxicity was reported against HT-29 cells (Park *et al.*, 2012).

Mizo Chilli also referred as Hmarchate or Vaimarcha or Mizoram Bird's eye chilli belongs to species *Capsicum frutescens* widely grown in the state of Mizoram. It is grown for its pungency, spicy taste besides the appealing colour it adds to the food. This Chilli is small but packs quite a lot of heat. The hot flavour of chillies is due to the presence of a group of seven closely related compounds called Capsaicinoids, but capsaicin (8-methyl-N-vanillyl- 5-nonenamide) and dihydrocapsaicin are responsible for approx. 90% of the pungency. Mizo chilli is one of the most popular and widely grown vegetable and spice crops in Mizoram. It is one of the important cash crops supporting the livelihood and improving the economic life of farmers/ tribes because it is mostly marketed in dried form and therefore, it is non-bulky and has long shelf life which makes it easy to transport. The state is considered to be the germplasm bank for Mizo chilli Mizoram is a major producer of Mizo Chilli where production of other verities of chilli is almost non-existent. In Mizoram, Mizo Chilli is cultivated mostly under Jhuming system or Shifting Cultivation System on hill slops. In Mizoram itself three different varieties of Mizo chilli are cultivated in different parts as Mizoram bird's eye chilli (MZBEC) A, B and C. However, systematic and more pharmaceutical investigations are not still carried out to provide new insights into the traditional uses of this important crop plant. The objective of this study was to determine the phytochemical content and antioxidant scavenging capacity of the three varieties of Mizo chilli.

7.2 Materials and Methods

7.2.1 Collection of Plant Materials

Three varieties of fresh red Mizoram bird's eye chilli (*Capsicum frutescens*) as MZBEC- A, B and C were used as the materials in this study. The samples were collected from Horticulture Centre of Excellence, Thiak village, about 45 kilometres south of Aizawl, Mizoram. The samples were put into polyethylene bags and immediately transported to the laboratory and then stored at –18◦C until extraction for analysis. Air-dried ripe fruits of Mizo chilli were powdered and extracted by Soxhlet extraction method.

7.2.2 Total phenol estimation:

Total phenolic content in each plant extract were assayed spectrophotometrically using Folin-Ciocalteu reagent with slight modification (Andrew, 2003).Phenols were reacted with phosphomolybdic acid in Folin-Ciocalteu reagent in alkaline medium producing blue colour**.**1 ml of the sample was centrifuged and 0.5 ml Folin-Ciocalteu reagent was added and incubate for 5-8 minutes. 1.5 ml of sodium carbonate with 7 ml of distilled water was added and incubates for 1 h at room temperature. The absorbance was measured at 750 nm alongside methanol as blank. All samples were analysed in triplicate. The total phenol in each variety was expressed as milligram per 100 mg of extract's total phenol content in Gallic acid equivalent (mg GAE/100mg) using the standard curve: y=0.0057x + 0.1076, R_2=0.9941, where R is the determined coefficient, x is the concentration, and y is absorbance.

7.2.3 Total Flavonoid content:

Total flavonoid content in each variety was determined by using colorimetric aluminium chloride assay (Idris *et al.*, 2017). Briefly, distilled water (2ml) and 0.15ml of 5% Sodium nitrite ($NaNO_3$) were mixed and added to an aliquot of each extract and were allowed to stand for five minutes. Thereafter, 0.15ml of 10% aluminium chloride was added to the solution. After five minutes, 1ml of 4% NaOH was added. The solution was vortexed and incubated at 40°C for 15 minutes. Varying the concentrations of the standard solution (quercetin) was prepared following the same procedure as standard curve. Absorbance of the solution was measured at 510 nm and total flavonoid contents in each variety were expressed as mg/100mg quercetin equivalents (mg QE/100mg) of extract: y= 0.0028X + 0.0016, R_2=0.9984. All samples were analysed in triplicate

7.2.4 Determination of Antioxidant Activity: The antioxidant activities of the three varieties of Mizoram Bird's eye chilli (MZBEC) were determined using DPPH and ABTS assays.

7.2.4.1 *DPPH (2, 2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity Assay:* - DPPH radical scavenging activity for each variety of chilli was determined by a modified method previously described by Blois (1958). Briefly 1mm of the solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml methanolic extract of chilli varieties at different concentration. The mixture was shaken vigorously and allowed to stand in room temperature for 30 minutes. Then the absorbance of the mixture was measured spectrophotometrically at 517nm against the blank and control. The DPPH radical scavenging activity was calculated according to the following equation:

DPPH scavenging activity (%) = $\frac{Ao-A1}{Ao}\ x\ 100$

Where A_o =Absorbance of control

A_1=Absorbance of the sample

The relationship between percentage inhibition and equivalent sample concentration was plotted to determine the half inhibitory concentration (IC_{50}) value of each sample.

7.2.4.2 ABTS (2, 2-azino-bis (3 ethylbenzothiazoline)-6-sulfonic acid) *Radical Scavenging Activity Assay:* A modified method of Ohekhena (2018) was adopted for the determination of ABTS activity in each chilli extract. Equal proportions (1:1) of ABTS (7mM) mixed with Potassium persulphate ($K_2S_2O_8$) (2.45mM) were left in the dark for 18 hours for the formation of a green coloured. ABTS solution was further diluted with methanol to an absorbance of 0.700 ±0.005 at 745nm which serve as the working solution. Then, 1ml of chilli extract or standard Butylated Hydroxytoluene (BHT) at varying concentration was mixed with the resulting ABTS solution and was allowed to stand in the dark for 7 minutes. Thereafter, the absorbance at 745nm was read against methanol as blank. The percentage inhibition of samples and standards was calculated using the following equation (Iqbal *et al.*, 2015):

$$\text{ABTS (\%)} = \frac{Absorbance\ of\ Control - Absorbance\ of\ Sample}{Absorbance\ of\ Control} \times 100$$

The sample concentrations providing 50% (IC_{50}) of antioxidant activity were calculated from graph by plotting percentage inhibition of ABTS by the sample against the corresponding sample's concentration.

7.3 Result and Discussion

7.3.1 Phytochemical Analysis:

The phytochemical content of the three varieties of Mizo Bird's eye chilli were analysed and the values of phenols and flavonoids were determined on dry weight basis and the mean values of their phytochemical contents of both aqueous and methanol extract are presented in table 7.1.

Phytochemical analysis showed that phenols and flavonoid were present in the extract of the three varieties of *Capsicum frutescens* (Mizo chilli) studied. The varieties showed higher phytochemical constituents in aqueous extract compared to those of methanolic extract.

From the result shown in Table 7.1, MZBEC-B showed higher phenolic content (21.16±0.088mg GAE/g) in aqueous extract and (16.32±0.267mgGAE/g) in methanolic extract than other varieties. However, a large variability was seen across the varieties. In methanolic extract the total phenolic content of variety MZBEC-B (21.16±0.088) and MZBEC C (21.09±0.139) are almost similar. And in methanolic extract, MZBEC-B variety (16.32±0.267) still has the highest total phenolic content but followed by MZBEC-A (13.45±0.127) where MZBEC-A has lowest phenolic content in aqueous extract. The difference in the values of the phenolic contents from the two solvents used is an indication that they have different extractive capabilities for phenols from the studied chilli sampled. Similar observation was made by Akeem *et al.,* (2016) where higher phytochemical content was recorded in the ethanolic extracts of some Nigerian spices including pepper when compared to their corresponding aqueous extracts. Zhuang *et al.,* (2012) also reported variability in phenolic contents of nine *Capsicum* varieties. Our result showed a lower phenolic content than those reported by other studied on chilli. The difference may be attributed to different cultivars used as well as the growing conditions of the varieties (Alvarez *et al.,* 2011) (Oboh and Rocha, 2007).

Figure 7.1 Standard curves for Phenolic content (Gallic acid)

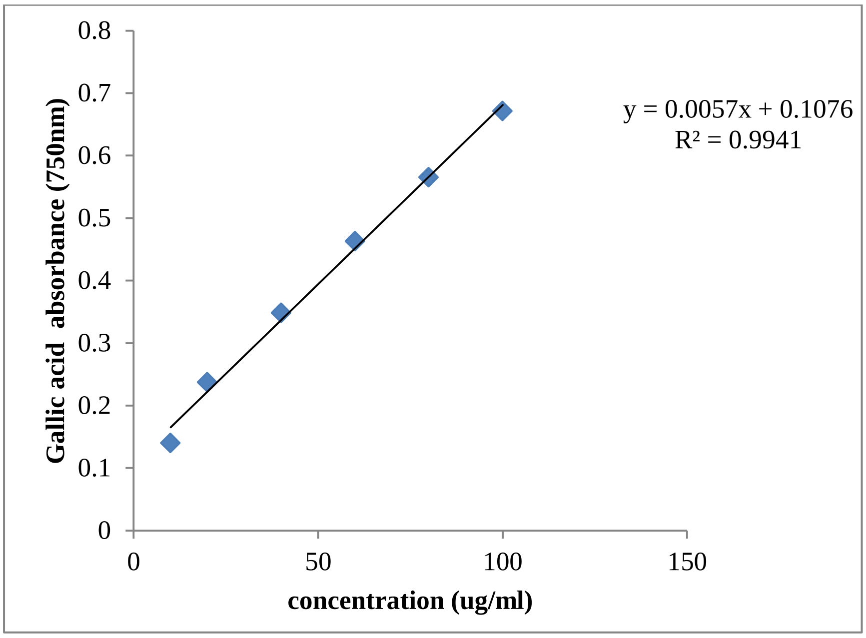
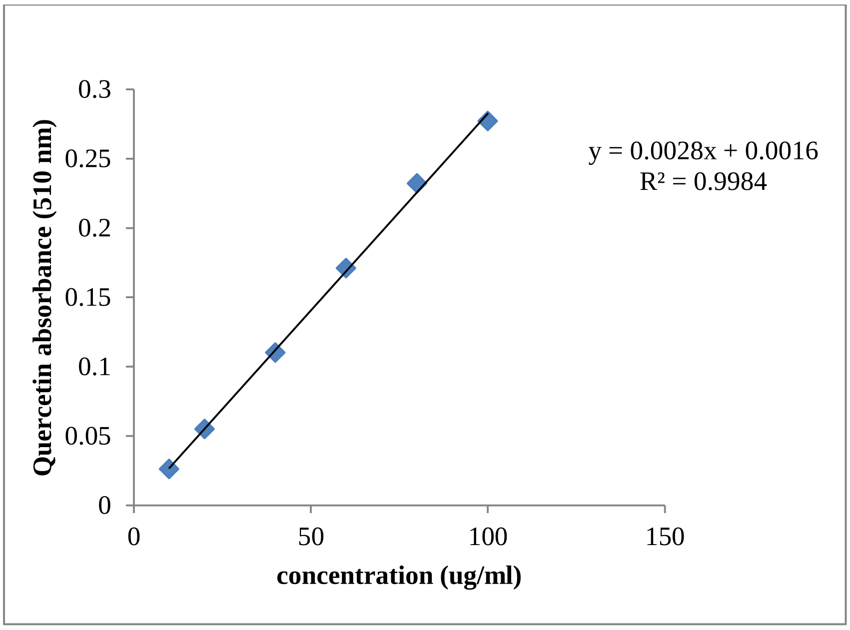


Figure 7.2 Standard curves for Flavonoids (Quercetin)



As shown in table 7.1, the total flavonoid contents of both aqueous and methanolic extracts differed among the varieties of Mizo chilli. The highest total flavonoids contents of aqueous extract was again obtained from MZBEC-B (8.95±0.01 mgQE/g of Sample) as in total phenolic content followed by MZBEC-A(6.95±0.008 mgQE/g of Sample) and MZBEC-C (5.8±0.013 mgQE/g of Sample). In methanolic extracts, the total flavonoids contents was again obtained highest in MZBEC-B (7.15±0.014±0.01mgQE/g of Sample) but followed by MZBEC-C (5.22±0.004 mgQE/g of Sample) and MZBEC-A (5.18±0.002 mgQE/g of Sample). Flavonoids account for 60% of total phenolic content, making them the largest naturally occurring phenolic compound. Their free radical scavenging capacity is attributed to their biological activity that includes antioxidant, anticancer and anti-inflammatory (Ohekhena *et al.*, 2018; Singh, 2016; Olajuyigbe and Afolayan, 2011; Khanam *et al.*, 2012). Aqueous extracts of the three varieties of Mizo chilli revealed high content of flavonoids as compared to methanolic extracts with considerable variation in the flavonoid content of the three varieties. These results agree with Scalzo *et al.,* 2005 who emphasized the role of plant genotype i.e. species and varieties within a species in the determination of bioactive contents as very close species varieties in a species are likely to show similar values than varieties distantly related.

Table 7.1 Phenols and Flavonoids content in three varieties *Capsicum frutescens* (Mizo chilli)

Chilli varieties	Phenols (mgGAE/g of Sample)		Flavonoids (mgQE/g of Sample)	
	Aqueous Extract	Methanol Extract	Aqueous Extract	Methanol Extract
MZBEC-A	13.9±0.058	13.45±0.127	6.95±0.008	5.18±0.002
MZBEC-B	21.16±0.088	16.32±0.267	8.95±0.01	7.15±0.014
MZBEC-C	21.09±0.139	11.87±0.002	5.8±0.013	5.22±0.004

Data are mean±SD values of triplicates determination

7.3.2 Antioxidant Activity

The evaluation of antioxidant activity gives valuable information about the functional quality of plant and can be used to better characterize plant species. Because of the complexity in the nature of antioxidative compounds, the overall antioxidant capacities of vegetable crops cannot be determined based on a single assay; two or more assays have been suggested by several researchers to better evaluate the antioxidant capacities of plants. In this study, we evaluated the antioxidant capacity of the varieties of *Capsicum* species using DPPH and ABTS assays.

7.3.2.1 *DPPH (2, 2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity Assay:*

DPPH scavenging activity of the three Mizo chilli varieties in comparisons to standard antioxidant Butylated Hydroxytoluene (BHT) are shown in Figure 7.3. The IC_{50} values (concentration of extracts/standard required to scavenge 50% of the radicals) are presented in Table 7.2 and this value was found to be inversely proportional to its scavenging activity. DPPH scavenging activity based on IC_{50} values were in the order BHT>MZBEC-B>MZBEC-A>MZBEC-C.

Figure 7.3 Comparisons between the percentages of DPPH radical scavenging activity with positive control BHT

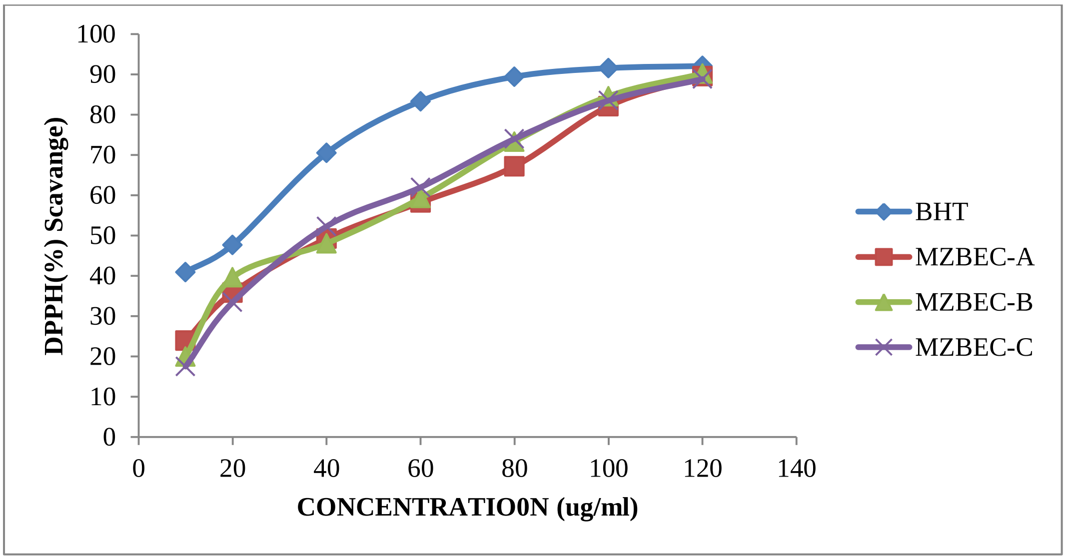


Table 7.2 IC_{50} values of different varieties of *Capsicum frutescens* (Mizo chilli)

Sample	IC_{50} value
MZBEC-A	**47.62±0.016**
MZBEC-B	**46.18±0.007**
MZBEC-C	**47.41±0.002**

7.3.2.2 ABTS (2, 2-azino-bis (3 ethylbenzothiazoline)-6-sulfonic acid) *Radical Scavenging Activity Assay*

The three varieties of Mizo chilli show significant amount of ABTS scavenging activity as shown in Figure 7.4 and Table 7.3. The variety MZBEC-B has somewhat high radical scavenging activity than the other variety. Overall, the ABTS antioxidant activities based on the IC_{50} values were in the order MZBEC-B>MZBEC-C>MZBEC-A.

From the study, the result showed that Mizo chilli varieties exhibited strong antioxidant activities. Determination of half inhibitory concentration (IC_{50} value) was used to measure the antioxidant efficiency of the varieties as lower IC_{50} values indicate strong capacity in scavenging the free radicals (Idris *et al.,* 2017). The antioxidant capacities of plants have also been linked to their phytochemical contents (Unuofin *et al.,* 2017).The presence of high amount of phenols and flavonoids content in the chilli contributes to their antioxidant activities. It is evident from the results that considerable variation existed in term of antioxidant capacities among the varieties of Mizo chilli. The differences observed in the antioxidant capacities among the varieties could be due to the impact of genotype, complexity and diversity of antioxidant compounds present in them (Dubey *et al.*, 2015).

Figure 7.4 Comparisons between the percentages of ABTS radical scavenging activity with positive control BHT

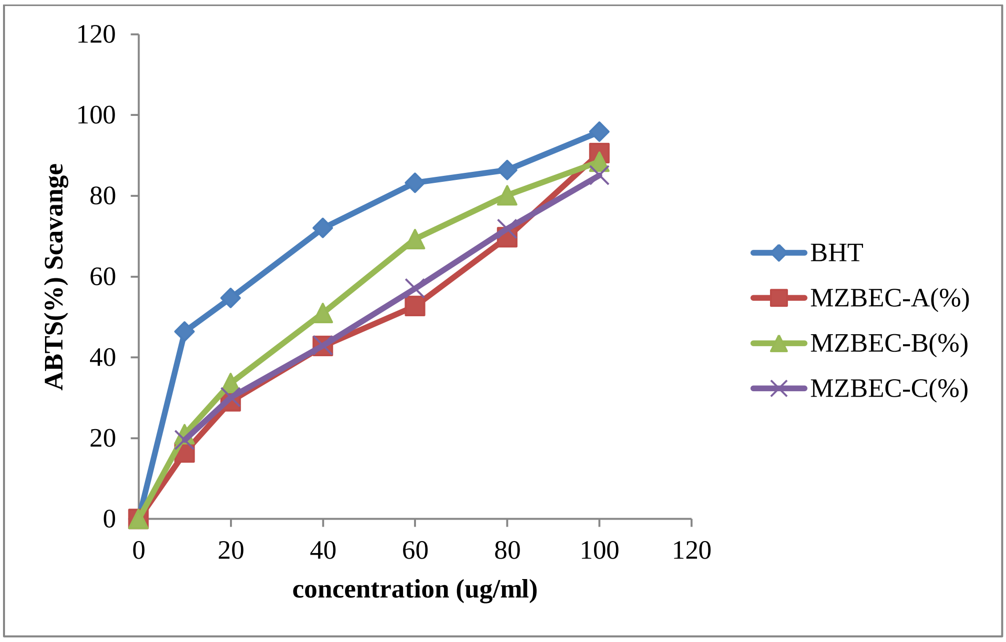


Table 7.3 IC_{50} values of different varieties of *Capsicum frutescens* (Mizo chilli)

Sample	IC_{50} value
MZBEC-A	**52.21±0.003**
MZBEC-B	**42.04±0.004**
MZBEC-C	**50.14±0.008**

Table 7.4 Evaluation of antioxidant activities (DPPH and ABTS), total phenol and total flavonoid contents of *Capsicum frutescens* varieties

***Capsicum* varieties**	**DPPH IC_{50}±SE(μg/ml)**	**ABTS IC_{50}±SE(μg/ml)**	**TPC (mg GAE/g±SE)**	**TFC (mg QE/g±SE)**
MZBEC-A	**47.62±0.016[a]**	**52.21±0.003[a]**	**13.45±0.127[a]**	**5.18±0.002[a]**
MZBEC-B	**46.18±0.007[a]**	**42.04±0.004[b]**	**16.32±0.267[b]**	**7.15±0.014[b]**
MZBEC-C	**47.41±0.002[a]**	**50.14±0.008[a]**	**11.87±0.002[b]**	**5.22±0.004[a]**

Means followed by different letters within same column are significantly different according to Duncan's multiple range comparisons (DMRTs) (P<0.05). Means followed by same letter are not significantly different. Each value was represented

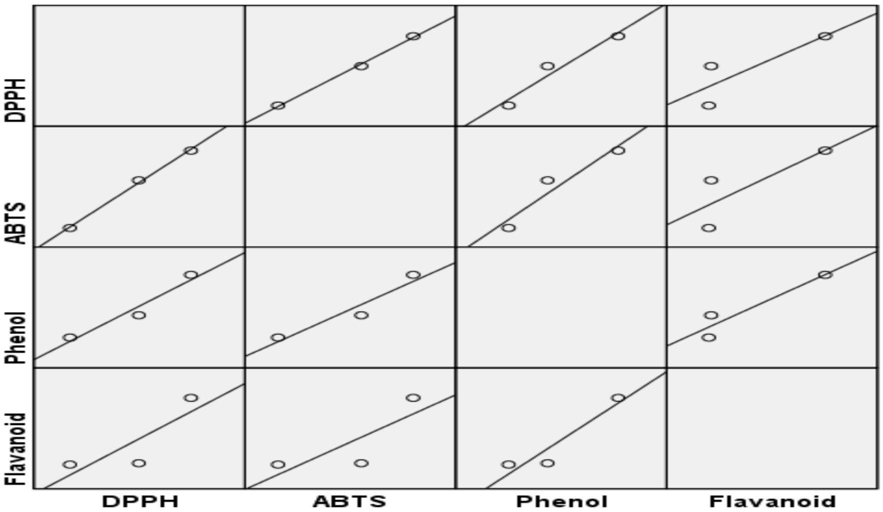
Table 7.5 Pearson's Correlation coefficients between antioxidant activity, total phenol and flavonoid content

Antioxidant assays	Total phenol		Total flavonoid	
	R value	P- Value	R value	P value
DPPH radical scavenging activity	0.970**	0.156	.833*	0.075
ABTS radical scavenging activity	0.956**	0.190	0.803*	0.407

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

Figure 7.5 Linear regression graph showing Pearson's correlation co-efficient of DPPH, ABTS, Phenol and Flavonoids



7.4 Conclusion

The varieties of Mizo chilli (*Capsicum frutescens*) were found to be rich in phytochemicals like phenols and flavonoids and have strong antioxidant properties. The antioxidant potential was found to be in good correlation with total phenolic content of the varieties. The variety MZBEC-B show somewhat higher phenolic content and result in higher free radical scavenging activity with less variation in the result. The total antioxidant activity differences reported between our investigation and the above-mentioned results could possibly be due to the diversity and complexity of antioxidants compounds present in chillies. In this sense, many studies showed that differences in the total antioxidant activity in peppers may be explained in terms of growing conditions, maturity, and the cultivar (Álvarez-Parrilla *et al.*, 2011).

According to the literature, several research groups have demonstrated a relationship between the phenolic content and the antioxidant capacity in extracts from plants and vegetables, whereas other researchers found no such correlation. Research reported by Velioglu *et al.,* (1998) demonstrated a strong correlation between the total phenolic content and the antioxidant activity in some fruits, vegetables and grains. In contrast, Kähkönen *et al.,* (1999) did not find a correlation between the antioxidant capacity and the phenolic content in some plant extracts. Additionally, no correlation between the total phenolic content and the radical scavenging activity was found in pepper fruits (*C. annuum* var. acuminatum) at two maturity stages (small green and green). In addition, the synergism between the antioxidants in the mixture makes the antioxidant action not only dependent on the concentration but also on the structure and the interaction between the antioxidants (Conforti *et al.*, 2006).

According to the results obtained, the pepper cultivars studied have levels of phenolic constituents that contribute to a high antioxidant activity and may be considered as a good source of natural antioxidants. MZBEC-B had the highest antioxidant capacity, which correlated with the highest levels of total phenols and flavonoids. However, other compounds (e.g. ascorbic acid) present in the peppers could contribute to the antioxidant activity and therefore should be considered in order to understand know the individual contribution of each group of phytochemicals to the total antioxidant activity. The information presented in this study can help promote the consumption of peppers in fresh form. We can conclude that Mizo chilli content sufficient amount of bioactive compound and result in good antioxidant properties. Therefore, it is necessary to carry out further studies on these underutilized chilli varieties which will be very valuable in pharmaceutical and health benefits.

From the above discussion, it is clear that Capsaicin and other phytochemicals present in chilli play a vital role in different medicinal benefits, including anticancer, anti-diabetic, anti-obesity, antibacterial, antifungal, anti-clotting, and anti-inflammatory activities. The phytochemicals involved along with their corresponding molecular mechanisms have been characterized to exhibit their modes of action. Such revelations were confirmed using animal models and human cancer cells. The handful of information that is currently available with meticulous research on biomedical perspectives of chilli pepper by skilful harmonization with technology, IT, computerized drug designing, molecular docking and other sophisticated tools can be used to develop drugs to combat cancers along with other important ailments.

Thus, chillies can serve as reservoir of potent drug formulations, which could precisely upgrade and enrich our pharmaceutical industries for one or more medical and pathological conditions and towards human wellbeing. The evaluation of levels of genetic variation which aids in proper delineation of plant species is fundamental in agriculture as it helps to effectively conserve, manage, and develop improved cultivars of plants that are endowed with bioactive compounds for various pharmacological uses. Determination of the level of variation of plant bioactive compounds has been successfully used as an additional tool for classification in some plant species. In this study, evaluation of phytochemical content and antioxidant activities proved to be useful in the continuous quest to better characterize and classify the varieties and the species of *Capsicum frutescens* in the state of Mizoram.

More importantly, the high phytochemical contents and antioxidant activities displayed in the various extracts of the varieties of *Capsicum* species indicate their pharmacological and nutritional value. Consumption of this beneficial crop as a source of natural antioxidant is recommended.

CHAPTER 8

SUMMARY AND CONCLUSIONS

The present experiment aims at studying the effect of different sources of manuring on growth yield and quality of *Capsicum* under low cost polyhouse conditions. An experiment was conducted to assess the effect of the organic manure (FYM) on the three varieties of *Capsicum frutescens* (Mizo chilli) on growth, yield and quality related characters during 2017-2019. The experimental site is located at Horticulture Excellence Centre, Thiak village, about 45 kms south of Aizawl, Mizoram.

The experimental design is Randomized Block Design (RBD) with 3 replications with seven treatments viz. T_0, T_1, T_2, T_3, $T_{4,}$ $T_{5,}$ T_6, and T_7. T_0 asub-plots will be used for the three varieties of *Capsicum frutescens* under control without Farm Yard Manure (FYM). On the other hand, sub plots $T_{1,}$ T_2 T_3, $T_{4,}$ $T_{5,}$ T_6 and T_7 will be for the three *Capsicum* varieties treated with different doses of Farm Yard Manure (FYM). FYM was applied uniformly in the soil at a depth of 10 cm a week before planting and during pre-flowering of chilli plants. Each sub-plot contained 6 chilli plants (Mizo chilli) at a spacing of 40-50 cm within and between the rows.

The result of the study revealed that almost all of the parameters considered were significantly affected by the treatments or their interaction effects. Days to first harvest were affected significantly by the organic amendments and the variety itself. Accordingly, the shortest day to first harvest was obtained from variety MZBEC-A (84.25 days), while the longest days to first harvest was recorded from variety MZBEC-C (84.35 days) at the experimental field.

Fruit length and fruit dry weight of chillies exhibited significant difference for interaction effects of the organic manure treatments and varieties. The highest records for parameters (fruit length and fruit dry weight) where, the highest fruit length was recorded from MZBEC-C (3.76±1.0cm) in T_7, followed by MZBEC-B (2.85cm) in T_6, and MZBEC-A (2.04cm), while the highest fruit dry weight was obtained from MZBEC-C (8.46g) from T_7. Yield related traits were also affected significantly by the interaction effect of varieties with different doses of organic manure amendments. Such higher yield was attributed to the agro-ecological conditions (temperature, soil type, soil pH) and or due to the heritable traits of these varieties. Moreover, the selection criteria of their marketable yield includes, long fruit size, thick fruit wall and dark-red pod colour as a components of good quality which was highly demanded in the market.

Besides, the fruits of Mizo chilli varieties were found to be rich in Vitamin-C, Total Proteins, Total Carbohydrates and Total Ash and Phytochemicals contents. The result of the present study indicates that Mizo chilli is a good source of nutrients and bioactive compounds. The results also showed that all the three varieties of Mizoram bird's eye chilli (MZBEC) are rich in Capsaicin. Further, it is necessary to examine the whole existing diversity of *Capsicum* species in order to find the landraces that are naturally rich in Capsaicin.

The beneficial effect of organic manure associated with different doses was found to have high efficiency in improving the nutrient content of chilli (*Capsicum frutescens*) and crop productivity. The application of organic manure from T_4 (Farm Yard Manure-2.5Kg/Plot) substantially increased the growth parameters of chilli peppers at all conditions. Therefore, applying organic manure from T_4 rate to T_7 rate of organic manure is considered the optimum for obtaining a maximum chilli pepper fruit yield. The excessive use of organic manure may result in the accumulation of more vegetative biomass than the increase in chilli yield value. The comparative higher level of nutrients and vitamin C upon treatments with integration may be due to action of specific soil nutrients which may be made more readily available into the soil for plant absorption as a result of organic manure integration effect which in turn may activate specific enzymes for the synthesis of these compounds. It is therefore, certain that specific nutrients in soil play a vital role in determining these quality parameters.

In general, the overall result of the study indicated that the variety trial at different doses of organic manure amendments substantially improve plant growth, the dry pod yield and quality of chilli which will benefits to the large scale producers in general and small scale producers in particular in the state of Mizoram. However, as the study was the first of its kind in the study area, it is recommended to further evaluate the varieties of Mizo chilli at different organic manure amendments to establish sound production system. From this experiment, it is evident that low soil nutrient under is the major limiting factor for growth and yield of Mizo chilli in the hilly state of Mizoram. In general, most of the treatment applied was found effective in increasing the plant growth at all stages significantly as compared to control.

Mizoram has a potential in organic farming since the extent of chemical consumption in farming is far less than the national average. In fact, the use of fertilizer and pesticides in agriculture and horticulture fields in Mizoram is almost non-existing. While the national average consumption of fertiliser is over 95 kg per hectare, it is only around 13 kg per hectare in Mizoram. On July 12, 2004, Government of Mizoram has also passed the Mizoram Organic Farming Act, 2004 to promote organic farming in the state. In case of Mizo Chilli, Mizoram has a huge marketable surplus and the production can increase with promotion. Moreover, the varieties of Mizo chilli (*Capsicum frutescens*) were found to be rich in phytochemicals like phenols and flavonoids and have strong antioxidant properties. The antioxidant potential was found to be in good correlation with total phenolic content of the varieties. The variety MZBEC-B show somewhat higher phenolic content and result in higher free radical scavenging activity with less variation in the result. The total antioxidant activity differences reported between our investigation and the above mentioned results could possibly be due to the diversity and complexity of antioxidants compounds present in chillies.

The present study indicated significant and positive correlation of soil organic manure treatments and yield parameters of chillies. Significant positive correlations were found between treatments and growth parameters, yield parameters, quality parameters and the Capsaicin content. These include positive correlation between organic manure treatments and growth parameters(r=0.978** in MZBEC-A, r=0.944** in MZBEC-B and r=0.989** in MZBEC-C), between organic treatment and yield parameters (r=0.861** in MZBEC-A, r=0.955** in MZBEC-B and r=0.926** in MZBEC-C), between organic manure treatments and quality of fruits (r=0.976** in MZBEC-A, r= 0.952** in MZBEC-B and r=0.924** in MZBEC-C). Similarly, a positive correlation was observed between organic manure treatments and capsaicin content (r=0.985** in MZBEC-A, r=0.990** in MZBEC-B and r=0.994** in MZBEC-C). This is in agreement with the observation of Aleemullah *et al*. (2000), who reported that yield and quality of Chilli mainly depend on the environment and or their inherent characteristics.

According to the results obtained in the present study, the pepper cultivars studied contain. Phenolic compounds that contribute to a high antioxidant activity and are a good source of natural antioxidants. MZBEC-B had the highest antioxidant capacity, which correlated with the highest levels of total phenols and flavonoids. However, other compounds (*e.g*. ascorbic acid) present in the peppers could contribute to the antioxidant activity and therefore should be considered in order to understand know the individual contribution of each group of phytochemicals to the total antioxidant activity. We can conclude that Mizo chilli contains sufficient amount of bioactive compound and exhibited good antioxidant properties. Therefore, it is necessary to carry out further studies of these chilli varieties which could be valuable in pharmaceutical industries. From the above result, it may be inferred that Capsaicin and other phytochemicals present in chilli could have medicinal properties as anti-cancer, anti-diabetic, anti-obesity, antibacterial, antifungal, anti-clotting, and anti-inflammatory.

Thus, the rational use of organic amendments for chilli cultivation is recommended depending on soil quality and environmental conditions. Further studies are required to understand the long-term effect of organic manure application on chilli pepper production under variable organic manure amendments. Furthermore, it is important to develop a proper simulation model to elucidate the long-term effect of organic amendments on growth and yield of chilli (*Capsicum frutescens*).

The long term sustainable production of the crop thus needs balanced supply of essential plant nutrients in available form, which involves systematic exploitation of potential soil resources, chemical fertilizers, biofertilizer and organic manures. The use of organic manures was suggested a long time in *Capsicum* and the efficiency of FYM, vermicompost, green manures, compost etc. are established, as its application triggers the microbial activity and results in increased mineralisation of soil nutrients. There is also a positive response of crops to biofertilizer application. It is evident that *Capsicum* responds well to application of plant nutrients in terms of growth, yield and quality. Further study needs to be carried out to determine the exact effect of individual nutrient sources, and in combination with organic manures.

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BRIEF BIODATA OF THE CANDIDATE

Name: M Michael Phairong

Mother's Name: Lalmuanpuii

Date of birth: 13th January 1991

Gender: Male

Nationality: Indian

Category: Tribal

Address: T-163, ITI Veng, Aizawl, Mizoram. Pin.796001

Contact: 9774952595

Email: michaelmyke20@gmail.com

Research Interest: Plant Ecology and Biodiversity, Agronomy, Phytochemistry, Ethnobotany

EDUCATION DETAILS:

Degree	**Year**	**Board/University**	**Subject**	**Division**	**Percentage**
HSLC	2006	Board of Secondary Education Manipur	General	Second	49.67
HSSLC	2008	Mizoram Board of School Education	Science	Second	53.03
B.Sc.	2012	Mizoram University	Botany	First	62.75
M.Sc.	2014	Mizoram University	Botany	First	64.02

PUBLICATIONS

M Michael Phairong, J Lalbiaknunga and F Lalnunmawia. Mizo chilli (Capsicum frutescens): A potential source of capsaicin with broad-spectrum ethno pharmacological applications. *Journal of Pharmacognosy and Phytochemistry,* 9(5): 670-672, 2020.

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CONFERENCES/ SEMINAR/WORKSHOP ATTENDED

1. National Seminar on Biodiversity, Conservation and Utilization of Natural Resources with reference to North-East India (BCUNRNEI)' organised by Department of Botany, Mizoram University, Aizawl, 30th – 31st March, 2017.

2. National Workshop on 'Statistical and Computing Methods for Life-Science Data Analysis' jointly organised by Biological Anthropology Unit, Indian Statistical Institute, Kolkata and Department of Botany, Mizoram University, Aizawl, 05th -10th March, 2018.

3. National Conference on recent advances in Plant Biology with special references to North-East India organised by Department of Botany, Mizoram University, Aizawl, 20th – 21 April, 2023.

4. International Conference on Advancements in Science, Technology and Management (ICASTM) 2024, jointly organised by Department of Computer Science, St. Xavier's College of Management & Technology, Patna, Bihar, India & Global Conference Hub, Coimbatore, Tamilnadu, India on 25/04/2024 & 26/04/2024.

PARTICULARS OF THE CANDIDATE

NAME OF THE CANDIDATE	**: M MICHAEL PHAIRONG**
DEGREE	**: DOCTOR OF PHILOSOPHY**
DEPARTMENT	**: BOTANY**
TITLE OF THESIS	**: EFFECT OF ORGANIC NUTRIENT SUPPLEMENT ON GROWTH, PRODUCTIVITY AND CAPSAICIN CONTENT OF *CAPSICUM FRUTESCENS***
DATE OF ADMISSION	**: 13/08/2015**

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HEAD

DEPARTMENT OF BOTANY

ABSTRACT

EFFECT OF ORGANIC NUTRIENT SUPPLEMENT ON GROWTH, PRODUCTIVITY AND CAPSAICIN CONTENT OF *CAPSICUM FRUTESCENS*

AN ABSTRACT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

M. MICHAEL PHAIRONG

MZU REGISTRATION NO: 1013 of 2008-09

Ph.D. REGISTRATION NO. MZU/Ph.D./844 of 22.04.2016

DEPARTMENT OF BOTANY

SCHOOL OF LIFE SCIENCES

APRIL, 2024

EFFECT OF ORGANIC NUTRIENT SUPPLEMENT ON GROWTH, PRODUCTIVITY AND CAPSAICIN CONTENT OF *CAPSICUM FRUTESCENS*

By

M MICHAEL PHAIRONG

Department of Botany

Supervisor

Prof. F. LALNUNMAWIA

Submitted

In partial fulfillment of the requirement of the degree of Doctor of Philosophy in Botany of Mizoram University, Aizawl

ABSTRACT

A field experiment was conducted to investigate the performance of different varieties of *Capsicum frutescens* for growth, productivity and capsaicin content under different doses of organic amendments thereby, to recommend best adapting and high yielding variety (varieties) for the farmers in the study area. The study was conducted from October, 2016, to March, 2019, at Horticulture Centre of Excellence, Thiak, Aizawl district, Mizoram. The proposed experimental design is Randomized Block Design (RBD) with 3 replications with seven treatments viz. T_0, T_1, T_2, T_3, $T_{4,}$ $T_{5,}$ T_6, and T_7. T_0 a sub-plots will be used for the three varieties of Capsicum frutescens under control without Farm Yard Manure (FYM). On the other hand, sub plots $T_{1,}$ T_2 T_3, $T_{4,}$ $T_{5,}$ T_6 and T_7 will be for the three Capsicum varieties treated with different doses of Farm Yard Manure (FYM). FYM will be applied uniformly in the soil at a depth of 10 cm a week before planting and during pre-flowering of chilli plants. Each sub-plot will contain 6 chilli plants (Mizo chilli) at a spacing of 40-50 cm within and between the rows. Uniform sized seedlings would be transplanted manually.

The result of the study showed significant interactions between organic manure treatments and the three varieties of *Capsicum frutescens* on plant height, days to first harvest, number of fruits per plant, fruit dry weight (g), fruit length and fruit diameter. As a result, the earliest variety to attain shortest days to first harvest was recorded from variety MZBEC-A (84.25 Days), while the highest number of fruits per plant was again from MZBEC-A., whereas, the widest fruit diameter was recorded from MZBEC-B (1.3cm) .The high yielding capacities were attributed to their early flowering and maturity, days to first harvest dry weight content of the varieties as well as their reaction to disease.

The crop responds to various biotic and abiotic factors, of which the growth environment and requisite nutrient supplementation are of foremost importance. The nutrient requirement can be met through chemical fertilizers, organic manures and biofertilizer which constitute the main sources of plant nutrients. However, in order

to trigger off production, indiscriminate use of chemicals has been observed in many parts of the country which has questioned the nutritional security of the crop and also its adverse impact on the environment. The long term sustainable production of the crop thus needs balanced supply of essential plant nutrients in available form, which involves systematic exploitation of potential soil resources, chemical fertilizers, biofertilizer and organic manures. The use of organic manures was suggested a long time in *Capsicum* and the efficiency of FYM, vermicompost, green manures, compost etc. are established, as its application triggers the microbial activity and results in increased mineralization of soil nutrients. It is evident that *Capsicum* responds to incorporated plant nutrients in terms of growth, yield and quality. But very few inquisitions have been carried out to determine the exact effect of individual plant nutrient sources and their combination with organic manure. It was therefore felt necessary to investigate the effect of different sources of manuring on growth yield and quality of *Capsicum frutescens*.

The major focus of the present study was also to estimate the quantity of Capsaicin present in different verities of *Capsicum frutescens*. For these three varieties of Mizo chilli or Mizoram Bird's Eye chilli (MZBEC) samples has been used. The grounded chilli samples were subjected to Soxhlet extraction with Tetrahydrofuran as a solvent. Measurements of the concentration of Capsaicin in the extracts were evaluated through their absorbencies measured on λ=280nm by UV spectrophotometer. The amount of Capsaicin in sample extracts was calculated using the following equation ($y = 0.0083x + 0.1431$). Among the three varieties of chilli, MZBEC-A (2.11%) has the highest percentage of capsaicin content followed by MZBEC-C (1.92%) and MZBEC-B (1.82%).

Qualitative phytochemical screening and total nutritional content of the three Mizo chilli varieties viz; A, B and C was determined. The principle includes preliminary phytochemical screening and total estimation of ash, proteins, carbohydrates and ascorbic acid content.

Maximum protein content was recorded from MZBEC-A (4.22mg/100mg) in T_7 and minimum protein content was recorded from MZBEC-B (0.9mg/100mg) in T_0. The protein content was maximum due to higher concentration of FYM, Application of FYM helped to the slow release of nutrients from organic manures. Further, micro-organisms might have helped in faster decomposition of organic manures there by increasing the availability of nutrients, specially protein synthesis further it was suggested that increase in fruit weight might have accelerated the mobility of photosynthates from source to the sink which was influenced by the growth hormones which released from vermicompost, the organic source Sivakumar *et al*., 1999 in *Capsicum***.**

Beside, *Capsicum frutescens* content high amount of vitamin C as compared to other spices and vegetables. High amount of vitamin C is present in the fruits of Mizo chilli that varies from 0.79 mg/100mg of the sample to 2.18 mg/100mg of dry weight of the sample. From the result, MZBEC-A variety has the highest Vitamin-C content (2.18mg/100mg) followed by MZBEC-C (1.68mg/100mg) and MZBEC-B has the lowest Vitamin-C content (0.79mg/100mg). With conformity with the present finding Teodoro et al. (2013) also reported the vitamin C contents in the range of 54.1mg/100g to 129.8 mg/100g in Habanero pepper accessions (*Capsicum chinense*). But carbohydrates content was found highest in MZBEC-B (1.18mg/100mg) followed by MZBEC-C (1.18mg/100mg) and lowest in MZBEC-(1.15mg/100mg). The Total Ash content of the sample was found to be highest in MZBEC-A variety (6.02%) followed by MZBEC-B (5.74%) and lowest in MZBEC-C (4.4%) in one gram of each sample. The total estimation of ash content indicates the presence of inorganic minerals by burning the organic substances in the samples. It is important because the amount of minerals can determine physiochemical properties of the chilli.

The output of these analyses indicates that sufficient amount of nutrients are present in these indigenous varieties of *Capsicum frutescens* and nutritional content varies from varieties to variety.

Fresh Dried fruits were quantitatively analysed for their total phenolic and flavonoid content spectrophotometrically while the antioxidant activities were determined by 2,2-diphenyl-1-picrylhydrazyl(DPPH) and 2,2-azino-bis(3 ethylbenzothiazoline)-6-

sulfonic acid(ABTS). The study demonstrated that the three varieties of Mizoram Bird's eye chilli (MZBEC) are good source of phytochemicals and antioxidant components and thus have a large potential for neutraceutical and pharmaceutical development. The phytochemical content of the three varieties of Mizo Bird's eye chilli were analysed and the values of phenols and flavonoids were determined on dry weight basis and the mean values of their phytochemical contents of both aqueous and methanol extract. Phytochemical analysis showed that phenols and flavonoid were present in the extract of the three varieties of *Capsicum frutescens* (Mizo chilli) studied. The varieties showed higher phytochemical constituents in aqueous extract compared to those of methanolic extract. MZBEC-B showed higher phenolic content (21.16±0.088mg GAE/g) in aqueous extract and (16.32±0.267mgGAE/g) in methanolic extract than other varieties. However, a large variability was seen across the varieties. In methanolic extract the total phenolic content of variety MZBEC-B (21.16±0.088) and MZBEC C (21.09±0.139) are almost similar. And in methanolic extract, MZBEC-B variety (16.32±0.267) still has the highest total phenolic content but followed by MZBEC-A (13.45±0.127) where MZBEC-A has lowest phenolic content in aqueous extract. The difference in the values of the phenolic contents from the two solvents used is an indication that they have different extractive capabilities for phenols from the studied chilli sampled. Similar observation was made by Akeem *et al.*, (2016) where higher phytochemical content was recorded in the ethanolic extracts of some Nigerian spices including pepper when compared to their corresponding aqueous extracts.

The total flavonoid contents of both aqueous and methanolic extracts differed among the varieties of Mizo chilli. The highest total flavonoids contents of aqueous extract was again obtained from MZBEC-B (8.95±0.01 mgQE/g of Sample) as in total phenolic content followed by MZBEC- A(6.95±0.008 mgQE/g of Sample) and MZBEC-C (5.8±0.013 mgQE/g of Sample). In methanolic extracts, the total flavonoids contents was again obtained highest in MZBEC-B (7.15±0.014±0.01mgQE/g of Sample) but followed by MZBEC-C (5.22±0.004 mgQE/g of Sample) and MZBEC-A (5.18±0.002 mgQE/g of Sample). Flavonoids account for 60% of total phenolic content, making them the largest naturally occurring phenolic compound.

DPPH scavenging activity of the three Mizo chilli varieties in comparisons to standard antioxidant Butylated Hydroxytoluene (BHT) are determined. DPPH scavenging activity of the three Mizo chilli varieties in comparisons to standard antioxidant Butylated Hydroxytoluene (BHT) are determined. The IC_{50} values (concentration of extracts/standard required to scavenge 50% of the radicals) are presented and this value was found to be inversely proportional to its scavenging activity. DPPH scavenging activity based on IC_{50} values were in the order BHT>MZBEC-B>MZBEC-A>MZBEC-C.

The three varieties of Mizo chilli show significant amount of ABTS scavenging activity. The variety MZBEC-B has somewhat high radical scavenging activity than the other variety. Overall, the ABTS antioxidant activities based on the IC_{50} values were in the order MZBEC-B>MZBEC-C>MZBEC-A.

From the study, the result showed that Mizo chilli varieties exhibited strong antioxidant activities. Determination of half inhibitory concentration (IC_{50} value) was used to measure the antioxidant efficiency of the varieties as lower IC_{50} values indicate strong capacity in scavenging the free radicals.

From the experiment, it is clear that Capsaicin and other phytochemicals present in chilli play a vital role in different medicinal benefits, including anticancer, anti-diabetic, anti-obesity, antibacterial, antifungal, anti-clotting, and anti-inflammatory activities. The phytochemicals involved along with their corresponding molecular mechanisms have been characterized to exhibit their modes of action. Such revelations were confirmed using animal models and human cancer cells. The handful of information that is currently available with meticulous research on biomedical perspectives of chilli pepper by skilful harmonization with technology, IT, computerized drug designing, molecular docking and other sophisticated tools can be used to develop drugs to combat cancers along with other important ailments. Thus, chillies can serve as reservoir of potent drug formulations, which could precisely upgrade and enrich our pharmaceutical industries for one or more medical

and pathological conditions and towards human wellbeing. The information presented in this study can help promote the consumption of peppers in fresh form. We can conclude that Mizo chilli content sufficient amount of bioactive compound and result in good antioxidant properties. Therefore, it is necessary to put further studies on these underutilized chilli varieties which will be very valuable in pharmaceutical and health benefits.

Keywords: *Capsicum frutescens*, Farm Yard Manure (FYM), Mizoram Bird's Eye chilli, Phytochemicals.