CHARACTERIZATION AND EVALUATION OF TULSI (*Ocimum tenuiflorum* L.)

By

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(2018 - 21 - 005)

DEPARTMENT OF AGRONOMY

COLLEGE OF AGRICULTURE

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KERALA, INDIA

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THESIS

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DEPARTMENT OF AGRONOMY

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VELLANIKKARA, THRISSUR-680656

KERALA, INDIA

2021

DECLARATION

I, hereby declare that this thesis entitled **"Characterization and evaluation of tulsi (*Ocimum tenuiflorum* L.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"Characterization and evaluation of tulsi (*Ocimum tenuiflorum* L.)"** is a record of research work done independently by **Ms. Daly George** (2018-21-005) under my guidance and supervision and that it has not been previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

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Introduction

1. INTRODUCTION

Medicinal and aromatic plants (MAPs) have been ingredients of traditional medicine since time immemorial. Approximately 50,000 - 70,000 species of plants are used in traditional and modern medicine throughout the world, and many more species are being identified as raw drugs for plant based cosmetic and health products.

The genus *Ocimum* belonging to the Lamiaceae family (Order Lamiales), is an extremely versatile group consisting of more than 150 species and is widely distributed in the tropical, sub-tropical and warm temperate regions of the world (Runyoro *et al*., 2010). The *Ocimum* genus is characterized by great variability in morphology in terms of growth characteristics, reproductive behaviour and in their chemical compositions.

Ocimum tenuiflorum L. (synonym *Ocimum sanctum*) commonly known as holy basil or tulsi, is native to the Indian subcontinent. It is widespread as a cultivated plant throughout the Southeast Asian tropics (Warrier, 1995). Almost all parts such as leaves, stems, flowers, roots and seeds of tulsi have been used in numerous formulations of Ayurvedha, Sidha, Unani and Homeopathy. In Ayurveda, tulsi is known as "the queen of herbs" and is considered as an "elixir of life" that is without equal for both medicinal and spiritual properties (Singh *et al*., 2010).

Two major morphotypes of *Ocimum tenuiflorum* L. reported in India and adjoining regions are light green leaved 'Sri' or 'Rama tulsi' and purple coloured 'Shyama' or 'Krishna tulsi' (Kothari *et al*., 2005). Wide variability exists in tulsi accessions from diverse geographical regions varying in climate, habit and morphological traits. Diversity found in *Ocimum* spp is due to the combined influence of genotype and environment (Verma *et al*., 1989). Existence of intraspecific and interspecific hybridization between basil species can also be considered as a cause for morphotypic and chemotypic variation.

Ocimum tenuiflorum L. is a species prioritized by National and State Medicinal Plant Boards for commercial cultivation and the estimated annual trade is

2000-3000 MT (NMPB, 2020). With steadily rising interest in herbs, attention should be paid to the sustainable production and utilization of medicinal and aromatic plants. Shift from collection to cultivation will also ensure purity, authenticity and sustainable supply of raw materials. Biomass production, oil yield and oil composition of tulsi plants vary depending upon the growing condition, ontogenetical stage of the plant and method of harvest. Hence studies to identify the optimum light intensity, stage of harvest and method of harvest will be useful to ensure high yield and quality while recommending the crop for commercial cultivation.

Weeds, insects and plant parasitic nematodes cause significant damage to almost all crops. Genus *Ocimum*is well known for pesticidal properties due to diverse group of compounds in its essential oil, which may be utilized as bio-pesticides or organic amendments. Nematicidal phytochemicals (botanicals) are generally cheap and safe for the environment and humans. However, besides the pharmacological properties, very little is known about other prospects. *Ocimum* plants have been identified as potential allelopathic plants (Balicevic *et al*., 2015). Both weeds as well as cultivated crops are sensitive to allelochemicals, and allelopathy on weeds can be exploited as effective weed management strategy.

Tulsi (*Ocimum tenuiflorum* L.) is a valuable herb with diverse potential. However as a commercial crop it is less exploited in Kerala. Hence, the present research project was formulated with the following objectives:

- Evaluation of *Ocimum tenuiflorum* L. accessions
- Standardisation of shade requirement and method of harvesting
- Investigation of allelopathic effect on upland weeds and crops
- Assessment of the effect of tulsi on root-knot nematode (*Meloidogyne incognita*)

Review of Literature

2. REVIEW OF LITERATURE

Medicinal and aromatic plants (MAPs) are high value crops which have numerous applications in many industries such as food, beverage, flavour and fragrance, perfumery and cosmetics, pharmaceuticals and aromatherapy. In addition, it is used in traditional as well as modern medicine. MAPs have been used by humans since prehistoric period. Among early civilizations, India has been known as repository of medicinal plants. Indian forests are the principal source of a variety of medicinal and aromatic plants, which are largely collected as raw materials for the preparation of drugs and perfumery products. Increasing demand, habitat destruction and climate change are threatening the existence of many species harvested from the wild; hence expanding the cultivation of medicinal and aromatic plants is considered an important strategy (Rao *et al.,* 2004).

Among plants with medicinal properties, those belonging to the genus *Ocimum* collectively called ‘‘basil’’ are a very important group of aromatic herbs or shrubs. The word “Ocimum” was derived from the Greek word “ozo” which means smell (Paxton and Hereman, 1868). These plants are known to produce essential oil rich in a number of aromatic compounds and have immense use in traditional system of medicine, perfumery and pharmaceutical industry, hence tulsi is rightly known as the “queen of herbs” (Simpson and Ogorzaly**,** 1986).

2.1 Origin and distribution

Genus *Ocimum* was described in 1753 by Linneaus. According to him these plants are highly variable and possess wide genetic diversity at intra and inter-species levels. The genus *Ocimum* belongs to the subfamily Nepetoideae under Lamiaceae family (Order Lamiales) and is an extremely versatile group consisting of more than 150 species (Balyan and Pushpangadan, 1988), it is widely distributed in the tropical, sub-tropical and warm temperate regions of the world (Runyoro *et al*., 2010).

Based on molecular phylogenetic relations, tribe Ocimeae was originated in tropical Asia and later got introduced elsewhere. The taxonomy of *Ocimum* is complex due to inter-specific hybridization and polyploidy within the genus (Tucker, 1986). In India, nine species of *Ocimum* viz., *O. teniuflorum* L., *O. basilicum* L., *O. gratissimum* L., *O. kilimandscharicum* L, *O. micranthum* L., *O. campechianum* L., *O. americanum* L., *O. minimum* L., and *O. citriodorum* L., have been reported. Of these, three species namely *O. americanum* L., *O. minimum* L., and *O. citriodorum* L. are exotic (Willis, 1919). *Ocimum* species have shown vast variations in morphology, growth characteristics, reproductive behaviour and chemical compositions as influenced by environmental factors (Carovic-Stanko *et al*., 2010).

2.2 *Ocimum tenuiflorum* L.

Among the species of *Ocimum* found under cultivation, *Ocimum tenuiflorum* L. (synonym *Ocimum sanctum* L.), is an aromatic plant widely known as tulsi (Hindi), holy basil or sacred basil (English). This species is worshiped by the Hindus and commonly grown in courtyards and temples in India. *Ocimum tenuiflorum* L. with chromosome number 2n = 32 is native to India and can be seen in entire Indian sub-continent up to 1800 m in the Himalayas and the Andaman and Nicobar islands in the south (Kirtikar and Basu, 1984). It is widely distributed in Asia, Australia, and West Africa and also in some Arabian countries mostly in drier areas (Pistrick, 2001). North-Central India is considered as the geographical origin of this species, as per phylogeography and molecular data (Bast *et al*., 2014; Mishra *et al*., 2014).

2.3 Morphology

Ocimum tenuiflorum L. is an erect, tall (30-60 cm), aromatic, sub-shrub plant having hairy sub quadrangular branches. Leaves are simple, green or purple, ovate, elliptic-oblong, obtuse or acute, usually slightly toothed with entire or sub-serrate or dentate margins, pubescent on both sides, dotted with minute glands and slender hairy petioles. The aerial parts have glandular hairs on stalked and sessile glands which

secrete volatile oils. Inflorescence is purplish or white, hermaphrodite and zygomorphic flowers are arranged in elongate racemes as close whorls. Seeds are brownish-reddish-yellow coloured and globose-subglobose with shining seed coat that turns mucilaginous on wetting. *O. tenuiflorum* L. is easily distinguishable from the other species due to spreading pedicels and internally glabrous calyces.

Two main morphotypes of *Ocimum tenuiflorum* L. found in India and adjoining regions are light green-leaved (Sri or Rama tulsi) and purple/dark green-leaved (Shyama or Krishna tulsi) (Kothari *et al*., 2005), and their intermediate types are also found. Some authors have differentiated *O. tenuiflorum* L. in to three forms such as green, purple and mixed on the basis of external characters. Characterization based on morphology is an essential tool for phylogenetic studies even in the modern era of molecular systematics (Lee, 2006; Bruce *et al*., 2007). Ali and Ali (2012) indicated that morphologically indistinguishable types obtained from diverse ecological regions possess variable chemical constituents. In *Ocimum sanctum* colour of the leaves, new branches and inflorescence varied from green to purple-green (Saran *et al*., 2017). Dharmadasa *et al*. (2015) and Das *et al*. (2020) reported distinguishable features of the two morphotypes of tulsi (Table 1).

According to Ahmad and Khaliq (2002), inter and intra-specific hybridization between basil species leads to morphotypic and chemotypic variation. Biological diversity among *Ocimum tenuiflorum* L. due to the prevailing climatic conditions and natural selection is also important. Malav *et al*. (2015) studied morphological diversity with respect to phytogeographical distribution in *Ocimum tenuiflorum* L. They characterized 49 accessions, and high variation was observed for plant height, canopy, leaf characters, flower characters and inflorescence, though seed size showed little variation. Leaf number ranged from maximum 71 in accession IC583279 to minimum 18 in IC583306 and IC583312 accessions. Leaf size ranged from medium (3.36 cm) in KCB-25 to large (8.84 cm) in PM/12/6. Cluster analysis of 49 accessions of tulsi based on morphological quantitative traits identified two major clusters (cluster A and B) and cluster B was further separated into two sub groups. Similarly Malav *et al*. (2020) characterized 109 accessions of *O. tenuiflorum* based on

morphological characters and they grouped the accessions into seven major clusters. PCA analysis showed that first six principal components contributed to 72.33 per cent of total variation and was contributed mostly by leaf length, leaf width, petiole length, plant height, seed length, seed width, days to flower initiation, essential oil percentage, number of primary branches and fresh herbage yield.

Ahmad and Khaliq (2002) evaluated morpho-molecular variability in *O. tenuiflorum* accessions collected from different districts of North Himalayan regions of Pakistan. They found that significant variability occurred for morphological parameters such as leaf area, number of racemes per plant, number of flowers per raceme, plant height and number of days to maturity. Agarwal *et al*. (2013) compared sixteen different genotypes of *Ocimum* species to assess the variability in qualitative and quantitative characters. Morphological characterizations were carried out to estimate the biological diversity among different genotypes under same climatic condition and location. Analysis of data showed that location had significant effect on all the characters studied *viz.,* plant height, leaf length, leaf width, leaf area, branch number, length of inflorescence and days to flowering, and interaction between genotype and environment was also evident for all the characters.

Morphological variability found in *Ocimum* spp was due to the combined influence of genotype and environment (Verma *et al*., 1989). Morphological studies conducted have shown the relevance in taxonomy of the genus *Ocimum* (Singh and Sharma 1980; Singh *et al.,* 2012). Distinct variation in pigmentation, leaf shape and size reported in different basil species has been established through centuries of its cultivation (Ahmad and Khaliq 2002; Nazim *et al*., 2009).

Table 1. Morphological characters of the two *Ocimum tenuiflorum* L. morphotypes

Character	Morphotype 1 (purple)	Morphotype 2 (green)
Plant habit	Erect, much branched	Intermediate
Plant height	30-75 cm	40-60 cm
Stem	Purplish in colour in young stage and after maturity changes into reddish brown in colour	Greenish in colour in young stage and after maturity changes into brownish in colour
Leaf colour (dorsal surface)	Dark Purple	Purplish Green
Leaf colour (ventral surface)	Purplish Green	Green
Leaf margin	Serrate	Undulate
Leaf shape	Elliptic-Oblong	Subulate
Leaf apex	Acute	Cupsidate
Inflorescence	Peduncle dark purple	Peduncle green
Flower	Dark purplish	Light purplish
Calyx	Purple, size 0.31×0.38 cm and 1.28 cm in diameter	Green, size 0.3cm×0.4cm and 0.46 cm in diameter
Corolla	Purplish, size 0.34×0.46 cm and 0.48 cm in diameter	Whitish purple, size 0.39×0.44cm and 0.5 cm in diameter
Fruit	0.1 cm long, reddish brown	0.15 cm long, brownish
Seed	Reddish	Yellowish

2.4 Essential oil and chemical composition

The leaves of *Ocimum* plants upon steam distillation yield bright yellow coloured volatile oil with a pleasing clove oil odour. Essential oils extracted from *Ocimum* plants are complex mixtures of natural organic compounds and possess several biological properties. The therapeutic property of tulsi is ascribed to its essential oils content. The oil yield generally ranges from 0.2 to 1.0 per cent but can be as high as 1.7 per cent depending on the source and phenological stage. Almost all plant organs such as flowers, buds, stems, leaves, fruits, seeds and roots of aromatic plants contain essential oils and they accumulated in secretory cells, cavities, channels, and epidermic cells (Burt, 2004). Bunrathep *et al*. (2007) studied essential oil yield and constituents in different *Ocimum* species and according to them, *Ocimum tenuiflorum* yielded 0.7 per cent essential oil.

Basil is known to occur as several chemotypes that differ in their essential oil compositions (Simon *et al*., 1990). Polymorphism existed in the genus *Ocimum* and it formed a large number of subspecies, different varieties thereby producing essential oils with varying chemical composition and medicinal potential (Khalid *et al*., 2006). According to Awasthi and Dixit (2007), essential oil yield on fresh weight basis from two cultivars of *Ocimum sanctum* (Shyma and Rama) were 0.41 per cent and 0.43 per cent (w/v) respectively. *Ocimum* species exhibited clear variation in yield, constituents and composition of essential oil. Lawrence (1985) classified *Ocimum* oils into three large groups *viz*., European type, exotic or reunion type and African type based on their chemical composition and geographical origin. Afterwards, he also established four essential oil chemotypes (methyl chavicol, linalool, methyl eugenol and methyl cinnamate) and many subtypes (Lawrence, 1998).

Kitchlu *et al*. (2013) evaluated the chemotypic variability in 31 collections of *O. sanctum* collected from different ecological regions of India. Wide variability was observed in quantitative and qualitative attributes of essential oil, and essential oil content ranged from 0.16± 0.01 per cent to 0.55 ± 0.08 per cent and the other major constituent of the oil was β-Caryophyllene. Raina *et al*. (2013) characterized *O.*

tenuiflorum germplasm collected from various parts of India based on essential oil yield and composition. Yield of volatile oil exhibited high range of variation from 0.13 to 0.45 per cent on fresh weight basis. They identified IC 583284, IC 583278, IC 583279 and IC 583322 as promising accessions with high essential oil content. Saran *et al*. (2017) evaluated the essential oil content of eleven *O. sanctum* accessions and found that oil content in green herbage was maximum in DOS-1 (50 g/kg), followed by DOS-3 (44 g/kg).

2.5 Medicinal properties

Different parts of *Ocimum tenuiflorum* L. such as leaves, flowers, stems, root, seeds etc. are known to possess diverse medicinal potential and this plant is mentioned in the Charaka Samhita, an ancient ayurvedic text. This plant has been utilized for thousands of years in ayurvedic remedies for headache, common cold, stomach disorders, inflammation, heart disease, various forms of poisoning and malaria. In Ayurveda, tulsi was described as Dashemani Shwasaharni (antiasthmatic) and antikaphic (Kaphaghna) (Sirkar, 1989). The anticancer (Kathiresan *et al*., 1999), chemopreventive (Prashar *et al*., 1994), radioprotective, anticarcinogenic, antioxidant (Devi, 2001), antimicrobial, anti-inflammatory, analgesic, antipyretic, antistress and immunotherapeutic (Mukherjee *et al*., 2005) activities of tulsi have also been reported. Tulsi is renowned for treatment of bronchitis, bronchial asthma, malaria, diarrhea, dysentery, skin diseases, arthritis, painful eye diseases, chronic fever, and insect bite. It is also used for preventing stomach disorders (Prakash and Gupta, 2005). The aerial parts of the plants are known to be antispasmodic, stomachic and carminative in native medicine (Sajjadi, 2006).

Ethanolic extract of leaves of tulsi significantly increased the wound breaking strength, wound epithelializes fast and wound contraction was significantly increased along with increase in wet and dry granulation tissue weight and granulation tissue breaking strength (Udupa *et al*., 2006). Adhvaryu *et al*. (2008) reported the anticancer activity of *Ocimum sanctum* and found that ethanolic extract of tulsi caused reduction in tumor size and increased the life expectancy of mice that had Sarcoma-180 solid

tumors. Aqueous extract of *O. sanctum* L. significantly increases the activity of anti-oxidant enzymes such as superoxide dismutase and catalase level in extract-treated group compared to control (Gupta *et al*., 2006). Mondal *et al*. (2009) indicated the antidiabetic, adaptogenic, antimicrobial, anti-carcinogenic, hepato-protective, immunomodulatory, anti-inflammatory, radioprotective, neuro-protective, cardio-protective and mosquito repellent properties of *Ocimum tenuiflorum*.

2.6 Effect of growing condition and harvesting method on tulsi

The growth, development, yield and quality of medicinal and aromatic plants are significantly influenced by a number of extrinsic and intrinsic factors such as ecological and climatic parameters, geographical locations, season, developmental stage, method of harvest, processing of plant materials before extraction etc. Among the environmental factors which highly influence plant growth and development, both quantity and quality of light transmitted to canopy play a key role (Shahak *et al*., 2004). In depth knowledge on the optimum time and method of harvesting gives ample scope to improve the yield and quality of medicinal and aromatic plants (Smitha, 2019).

2.6.1 Biomass yield and growth parameters

Ocimum basilicum produced leaves with the highest leaf area under 35 per cent shade, followed by 50 per cent shade, and leaves with the smallest leaf area were found in full sunlight (Nelkin and Schuch, 2004). Chang *et al*. (2008) studied the effect of four levels of irradiance (*i.e*., no shade (control), 25, 50 and 75 %) on the growth of basil (*Ocimum basilicum* L.) and found that basil grew well under full sun, though it could tolerate light shade. High shading (75 %) produced shorter plants, lower plant dry weight, smaller leaf area, less shoots and higher specific leaf area, and it also reduced the rate of photosynthesis. *Ocimum selloi* plants grown under coloured shade nets were significantly taller than those grown under direct full sunlight, while total dry biomass and root shoot ratio of plants were higher in plants cultivated in full sunlight compared to those cultivated under shade (Costa *et al*., 2010).

Suvera *et al*. (2015) observed significantly higher fresh above and below ground and total herbage and oil yield of *Ocimum* spp under silvi-medicinal systems compared to sole cropping. Kumar *et al*. (2017) evaluated the performance of teak + *Ocimum* spp silvi-medicinal system. According to them all the *Ocimum* species studied recorded significantly higher plant height, number of branches per plant, number of leaves per plant and plant spread under sole cropping as compared to silvi-medicinal system. As per Stagnari *et al*. (2018), in basil, shading induced stem elongation, a greater leaf area expansion and a lower leaf thickness.

The yield of sweet basil was the highest in unshaded plants compared to different shading strategies (Castronuovo *et al*., 2019). Shaded plants of *Ocimum basilicum* L. had significantly greater plant biomass yield with higher leaf area index and leaf number than sun exposed plants. In addition, sweet basil grown under shade was taller than those grown under open due to increase in internode length (Milenkovic *et al*., 2019). Tulsi plants grown under red and silver shade net had 36.03 per cent and 31.31 per cent higher plant height than those grown under full sun light. Also shade grown plants had higher root length and density than those grown under full sun conditions (Guerra *et al*., 2020).

In *Ocimum* species herbage yield reached maximum when the plants were in between the stage of full flowering and the initiation of seed formation (Gupta, 1996). Kothari *et al*. (2004) reported that method of harvest affected biomass yield of *Ocimum tenuiflorum* L. grown in south India, and shoot biomass cut at 30 cm gave maximum biomass yield and harvesting of secondary branches produced minimum biomass yield. They also reported that irrespective of method of harvest, biomass yield was higher in first harvest and declined gradually in second, third and fourth harvests.

As per Singh *et al*. (2010), harvesting stage as well as cutting height significantly influenced the growth and fresh herb yield of *Ocimum basilicum* L. Plant height, plant spread and number of branches per plant increased significantly with increase in crop age, reaching a maximum at 100 days after transplanting (DAT) of the main crop. The crop harvested at 40 DAT at 15 cm above the ground produced the

highest plant spread (54.0 cm) and number of branches per plant (180.0) followed by the crop harvested at 7.5 cm height at same stage during second harvest. Total fresh herb yield (main crop + ratoon crop) was the highest when plants were harvested at 100 DAT at ground level, which was at par with the fresh herb yield of the treatment combinations of 40 DAT × 7.5 or 15 cm, 80 DAT at ground level and 100 DAT at 7.5 cm.

Harvesting method did not have a significant effect on yield or on the total fresh and dry mass of sweet basil (Maboko and Plooy, 2013). In contrast, according to Corrado *et al.* (2020), basil can be harvested more than once to increase productivity and yield was significantly affected by the cut factor. The fresh yield was higher at the first cut because the average leaf size and total leaf area per plant was significantly higher at the first cut, while the dry weight of the shoot biomass did not show any difference between the two harvests.

2.6.2 Photosynthetic pigments

Ocimum selloi plants grown under red and blue shading had significantly higher number and size of chloroplasts and contained larger pigment-rich chloroplasts (Costa *et al*., 2010). Chlorophyll content of index and leaf nitrogen of *Ocimum* spp were higher under silvi-medicinal system compared to sole cropping (Kumar *et al*., 2017).

Chlorophyll content in plants increased from youngest leaf to the leaf which could be described as “photosynthetically mature”, and after attaining this maximum value the chlorophyll content decreased (Sestak, 1963). The influence of plant age on chlorophyll content was also reported by Mauromicale *et al*. (2006). According to them, with increasing plant age, chlorophyll content decreased linearly. Drastic reduction in carotenoid content in plants grown under under shade was reported by Stagnari *et al*. (2015). Increased accumulation of chlorophyll in basil plants grown under shade was reported by Stagnari *et al*. (2018) and according to them, lower leaves showed 10 per cent higher pigment content than upper ones.

2.6.3 Essential oil yield and composition

Essential oil content and composition in *Ocimum* spp depended on soil and climatic conditions of the location, growing season and maturity stages (Laskar and Majumdar, 1988). In (*Ocimum gratissimum* L.), the oil yield, oil content and eugenol content were found maximum under open conditions compared to shade (Pillai, 1990). However Lee *et al*. (1994) indicated slight shading as beneficial for oil production in sweet basil (*Ocimum basilicum* L.). Chang *et al*. (2008) investigated the effect of solar irradiance levels on volatile oil content and composition in basil (*Ocimum basilicum* L.). They found that heavy shading (75 %) significantly reduced essential oil content, especially in older plants. The composition of essential oil was also influenced by the shading treatments; higher irradiance (no shade) significantly increased the relative contents of linalool and eugenol, whereas methyleugenol was increased by lower irradiance. Nonetheless, relative content of 1,8-cineole was not affected by shade treatments.

Costa *et al*. (2010) studied the effects of colored shade nets on the essential oil yield and composition of pepper basil (*Ocimum selloi*). Different light treatments had no effect on the yield of oil, although productivity (expressed in g of oil/plant) was higher in plants grown under full sunlight due to greater leaf biomass produced under such conditions. In this experiment the quality of light significantly influenced the compositions of the oils, and highest concentration of methyl chavicol was observed in plants grown under full sunlight (93.2 %) followed by plants grown under red and blue shading, respectively.

Ade-Ademilua *et al*. (2013) reported that essential oil (%) in African basil (*Ocimum gratissimum* L.) plants grown under shade was higher than that of plants growing in sunlight. According to Fernandes *et al*. (2013), light intensity did not affect the essential oil content in *Ocimum gratissimum* L., but the essential oil yield per plant increased linearly with light intensity due to increased leaf biomass production under these conditions. The major component in the essential oil was

eugenol (91 %), and it remained stable regardless of the light level. Oil yield of *Ocimum* species, intercropped under karanja and as sole crop, differed significantly and higher oil yield was achieved under silvi-medicinal systems (Karanja + *Ocimum* spp) compared to sole cropping. Among four *Ocimum* spp, *O. tenuiflorum* produced higher oil yield (Suvera *et al*., 2015). Milenkovic *et al*. (2019) revealed that accumulation of essential oil was higher in shaded plants compared to unshaded condition.

Plant ontogeny or growth stage had close relation with secondary metabolite accumulation in plants and thus had significant influence on oil yield and its composition (Dhar *et al*., 2006; Verma *et al*., 2010). In Indian basil, main crop harvested at 40 DAT at 15 or 7.5 cm above from the ground level and ratoon crop at 50 days after first harvest produced maximum oil yield (Singh *et al*., 2010). Padalia *et al*. (2013) reported that the essential oils of genus *Ocimum* were found to vary significantly during different phenophases. In all the landraces of *O. basilicum*, *O. americanum*, and *O. tenuiflorum*, the oil yield increased with plant growth stage up to full bloom stage (maximum oil content) followed by seed setting and half bloom stage, and the lowest oil yield was in vegetative stage.

In *Ocimum basilicum*, full flowering stage of the crop was the most profitable time of harvest with respect to oil yield and quality (Bahl *et al.,* 2000). Sims *et al*. (2013) reported that the date of seeding, transplanting, and harvest (plant maturity) significantly affected the essential oil content and composition in *O. tenuiflorum*, while the level of change depended upon the accession. In sweet basil, the yield of essential oil from different development stages varied between 1.2±1.6 per cent, andthe major components recognized in essential oils were linalool, eugenol and terpinen-4-ol. The highest linalool content was obtained from flowering stage and eugenol was rich in pre-flowering stage and the maximum amounts of terpinolen-4-ol were observed in post-flowering stage (Toncer *et al*., 2017).

2.7 Allelopathic potential of tulsi on weeds and crops

Allelopathy is defined as any direct or indirect inhibitory effect by one plant (including microorganisms) on another through the release of chemical compounds that escape into the environment (Rice, 1974). Allelopathic interactions between plants had significant positive and negative role in natural ecosystems (Rizvi *et al*., 1992), and both weeds as well as cultivated crops are sensitive to allelochemicals. Allelopathy on weeds can be exploited as effective weed management strategy; plant product based natural herbicides could serve as alternative to synthetic herbicides that were biodegradable and environment friendly. The *Ocimum* plants have been investigated as potential allelopathic plants (Balicevic *et al*., 2015).

Sharma and Singh (2003) evaluated allelopathic effect of tulsi (*Ocimum sanctum*) on the germination of some weed species such as radish (*Raphanus sativus* L.), redroot pigweed (*Amaranthus retroflexus* L.), hairy beggarticks (*Bidens pilosa* L.) and guineagrass (*Panicum maximum*). They observed complete inhibition of germination of all tested weed species with the application of 7.5 g basil leaf powder to 100 g of sand as compared to control. They also found that 10 per cent (w/v) basil leaf extract significantly inhibited germination of redroot pigweed (13 %) and hairy beggarticks (12 %) as compared to distilled water. Significantly lower germination of 58, 47 and 45 per cent in radish, redroot pigweed and hairy beggarticks respectively was found with basil stem+ root extract (at 2.5 % w/v).

Singh and Singh (2009) examined the effect of basil leachate on the emergence of ten weed species. Basil leachate reduced the germination of *Digitaria ciliaris*, *Sorghum halepense*, *Desmodium tortuosum*, *Amaranthus retroflexus*, *Senna obtusifolia* under the laboratory conditions and *Desmodium tortuosum*, *Amaranthus retroflexus*, *Senna obtusifolia*, *Bidens pilosa* and *Sida spinosa* under greenhouse conditions. Shoot leachates of *Ocimum sanctum* inhibited the germination of weed species *Parthenium hysterophorus*, where only 40 per cent and 30 per cent seeds germinated in 50 per cent and 100 per cent concentrations respectively (Knox *et al*., 2010). Inhibitory activity of *Ocimum tenuiflorum* extracts on the germination and

seedling growth of cress (*Lepidium sativum*), lettuce (*Lactuca sativa*), alfalfa (*Medicago sativa*), Italian ryegrass (*Lolium multiflorum*), barnyard grass (*Echinochloa crusgalli*) and timothy (*Phleum pratense*) were studied by Islam and Noguchi (2014). Concentrations of plant extracts greater than 30 mg dry weight equivalent extract/ml significantly reduced germination percent, germination index, germination energy, speed of emergence, seedling vigour index, and germination rate of all test plants except barnyard grass and germination percent in lettuce. Also, extracts at concentrations greater than 10 mg dry weight equivalent extract/ml significantly inhibited the shoot and root growth of all test species.

Dhima *et al*. (2009) found that aqueous extracts of above ground portion of sweet basil decreased seed germination and seedling growth of barnyard grass (*Echinochloa crusgalli* L.). Basil extracts in higher concentration completely inhibited germination and seedling growth of weed scentless mayweed (*Tripleurospermum inodorum* (L.), while root length was not inhibited (Balicevic *et al*., 2015). Spraying of up to 25 per cent *O. basilicum* leaf extracts inhibited growth of grass weed (*Phalaris minor*) and broad leaf weed (*Anagalis arvensis*) (El-Rokiek *et al*., 2018).

Fanaei *et al*. (2013) conducted an experiment to study the allelopathic effects of Sweet basil (*Ocimum basilicum*) extract and essence on growth of weeds like abutilon (*Abutilon theopharasti*), goosefoot (*Chenopodium album*) and centaury (*Centaurea depressa*). They found that dry weight of weeds were significantly decreased by increasing of extract concentration and high concentrations of sweet basil extract had much influence on the reduction of dry weight of centaury in comparison with abutilon and goosefoot. In addition, 100 per cent sweet basil essence caused the highest reduction of plantlet dry weight in abutilon, while application of sweet basil essence had no significant influence on the plantlet dry weight in goosefoot and centaury. Mekky *et al*. (2019) studied allelopathic potentiality of *Ocimum basilicum* extract against weeds and crops and found that *Ocimum* extracts inhibited germination and growth of *Amaranthus* weed seed more than both soybean and maize plants. The concentration required for 50 per cent germination of growth inhibition (ID_{50} values) were about 0.2 to 0.9 for *Amaranthus*, 10.0 to 30.0 and 17.0 to

23.0 for maize and soybean respectively. *Amaranthus* and *Portulaca* weeds were affected by post emergence application of extracts which gave 33-68 per cent reduction in fresh weight of *Amaranthus*/*Portulaca* mixture. They concluded from the study that use of 40.48 kg/ha of *Ocimum* were a promising strategy to control weeds in horticultural orchard fields during summer season.

The allelopathic effects of aromatic and medicinal plants depended on the plant and weed species and concentration or rate applied. Dried powder of *Ocimum sanctum* at 5 per cent had not much effect on growth of weed *Cassia uniflora*, while seed germination and growth rate of weed was very slow in 15 per cent and no seed germination was observed in 20 per cent *Ocimum* powder (Amrin and Abhijit, 2019). Sweet basil (*Ocimum basilicum*) aqueous extract had phytotoxic activity and could be used as selective natural herbicide to control broad leaved weeds associated with maize, wheat, clover, kidney bean, chickpea and onion. *Ocimum* aqueous extract had more effect on annual summer broad leaved weeds (*Portulaca oleraceae* L., *Amaranthus* spp, *Trianthema portulacastrum* L., *Chenopodium murale* L., *Ammi majus* L.) and perennial weeds (*Cyperus rotundus*, *Convolvulus arvensis*) while it did not affect annual grassy weeds and legume weeds (Mekky and Hassanein, 2021).

According to Chou and Patrick (1976), allelopathic effects of basil powder were probably due to the release of allelochemicals after decaying. As radicles are the first organs to emerge, they absorb allelochemicals released by the medicinal plants early (Gniazdowska and Bogatek, 2005). Dry powder of *Ocimum tenuiflorum* inhibited lettuce radicle growth by 74.0 per cent, and showed visual symptoms of radicle necrosis. In general, medicinal plants exhibited greater inhibitory effects on the lettuce radicles than the hypocotyls (Suwitchayanon *et al*., 2017).

Essential oil of *Ocimum americanum* exhibited allelopathic activity against two weed species *Mimosa pudica* and *Senna obtusifolia* and there was a positive relation between concentration and inhibitory effect. Seed germination of weeds was more affected by the essential oil, followed by radicle elongation and hypocotyl elongation, among which the weed *Mimosa pudica* found to be more sensitive

than *Senna obtusifolia* (Filho *et al*., 2009). Rosado *et al*. (2009) investigated allelopathic effect of aqueous extract and essential oil of *Ocimum basilicum* L. on lettuce, tomato and Melissa. Results showed that aqueous extracts of basil inhibited only germination velocity index of tomato seeds and reduced root length of lettuce and Melissa, while, essential oil of basil affected germination velocity index, germination percentage and seedling root length of lettuce, tomato and melissa seeds.

Extracts of *Ocimum sanctum* and *O. canum* plants had stimulatory effect on root weight of turnip (*Brassica rapa* L.), and maximum stimulation was observed at 80 per cent concentration. But there was gradual delay in the time of flowering with increasing concentration (Durrani and Prasad, 2009). Aqueous extracts derived from leaf, root and seeds of *Ocimum basilicum* plants inhibited seed germination of crops like wheat, gram lentil, mustard, barley, okra and pea, with maximum inhibitory effect found in leaf followed by root and seed extract. In addition, *Ocimum* extracts significantly affected the root and shoot elongation of all the test crops. Reduction of root length was 59 per cent, 40 per cent and 16 per cent by leaf, root and seed extracts respectively when compared to control. Overall, radicle elongation decreased by 64 per cent, 43 per cent and 40 per cent in leaves, root and seed extracts respectively, compared to control (Verma *et al*., 2012).

Purohit and Pandya (2013) studied allelopathic activity of *Ocimum sanctum* leaf extract on common legumes and weeds and found that it had differential effects on each legume at different concentration. Only chick pea seed germination was inhibited by the treatments and inhibition increased with increase in concentration. The radicle of all legumes showed the stimulatory effect under *Ocimum* 1 per cent leaf extract treatment, except green gram, chick pea, black gram, moth bean. They also observed that *Ocimum* at 1 per cent showed maximum inhibition to weeds such as *Amaranthus* (80 %) followed by *Chloris* (10 %) while it had no effect on *Acalypha* and *Dicanthium* seeds.

The allelopathic effects of sweet basil on seed germination and seedling growth of poaceous crops was reported by Dafaallah and Ahmed (2017). Similarly, Dafaallah and Ahmed (2019) investigated phytotoxic effects of the aqueous extract of aboveground parts of *Ocimum basilicum* L. on germination of sorghum, millet, maize and wheat. They found that aqueous extract of basil inhibited the seed germination of the tested cereal crops and there was direct positive relationship between concentration and inhibition. Among tested cereal crops, maize was most sensitive followed by the seeds of millet, wheat and sorghum, the LC_{50} values being 34.1, 46.3, 46.7, and 59.1, g/L, for maize, wheat, millet and sorghum respectively.

2.8 Nematicidal and insecticidal potential of tulsi

Plant parasitic nematodes cause significant damage to almost all crops. Among plant parasitic nematodes, root-knot nematode (*Meloidogyne incognita*) is the most frequently observed and key damaging genera (Mukhtar *et al*., 2017). Use of chemical nematicides is the common and most effective practice in nematode management but it causes severe environmental pollution (Wachira *et al*., 2009). Moreover, the use of synthetic pesticides has also been banned. Thus, alternative approaches involving the use of antagonistic plants for their pesticidal potential are now increasingly being implemented for the management of plant parasitic nematodes.

A wide variety of plant species are reported to have insecticidal and nematicidal properties and genus *Ocimum* is well known for pesticidal properties due to diverse group of compounds in its essential oil, which may be utilized as bio-pesticides or organic amendments. Popovic *et al*. (2006) reported that basil oil contained bioactive constituents that had insecticidal and repellent action.

Pandey *et al*. (2000) conducted bioassay tests to find out the nematicidal activity of *O. basilicum* oil against *Meloidogyne incognita* and reported that mortality increased with an increase in oil concentration and maximum mortality (100 %) was found at three concentrations (250, 500 and 1000 ppm). Nematicidal phytochemicals

(botanicals) were generally cheap and safe for the environment and humans (Chitwood, 2002). Bharadwaj and Sharma (2007) evaluated nematicidal potential of aqueous extracts of five plant species on root-knot nematode (*Meloidogyne incognita*). Leaf extracts of all the selected botanicals delayed the hatching of eggs of root-knot nematode and the best results were obtained with *O. sanctum*, with no hatching within 48 hours. African basil (*Ocimum gratissimum*) had nematicidal properties and it prevented egg-hatching, thereby reduced root knot nematode population in the soil (Olabiyi, 2008).

Claudius-cole *et al*. (2010) studied the effect of water extracts of *Ocimum gratissimum, Azadirachta indica, Vernonia amygdalina* and *Moringa oleifera* on the inhibition and mortality of root-knot nematode. They found that egg hatch inhibition ranged from 40 per cent-63.7 per cent in the extracts compared to the control with zero percent. Juvenile mortality in extracts was from 82 per cent to 93.8 per cent compared to the control with 25 per cent. They concluded that low to moderate concentrations of these indigenous botanicals were effective in reducing the pathogenicity of the root-knot nematode. Plant essences from *Ocimum basilicum* (sweet basil), *Mentha arvensis* (field mint), *Tagetes erecta* (Mexican marigold), and commercial product of neem seed (*Azadirachta indica*) could be used for nematicidal purposes (Douda *et al*., 2010).

According to Asimiea *et al.* (2015), nematicidal activity of botanicals depended on their application rates. According to them aqueous extract of *O. gratissimum* leaves at 20 ml/kg of soil was comparable to carbofuran in the management of *Meloidogyne incognita* in okra. They recommended *O. gratissimum* application as a cheap, easily available and environment-friendly method of controlling root knot nematode (*Meloidogyne incognita*). Aqueous extracts of turmeric, marwa tulsi, mint, aonla and jatropha were caused mortality in juveniles of root-knot nematode (Neeraj *et al*., 2017).

Kamaraj *et al*. (2008) reported that application of *Ocimum* extracts showed larvicidal activity against *Spodoptera litura*. Similarly, basil oil (*O. basilicum*) and its

major constituents trans-anethole, estragole, linalool were insecticidal to adult fruit flies (Chang *et al*., 2009). *Ocimum sanctum* leaf extract at 0.1 per cent concentration caused maximum mortality of grasshopper (*Acrida exaltata*) and minimum mortality (around zero) was observed at 0.005 per cent concentration (Sharma, 2010). Formulations based on essential oils of *Ocimum gratissimum* were safe alternative to control insect pests of stored products (Nguemtchouin *et al*., 2013).

Application of *Ocimum sanctum* extract could be included in integrated pest management (IPM) to control mustard aphids (Patel *et al*., 2016). Kayesth *et al*. (2018) exposed nymphs of red cotton bug to different concentrations of hexane extract of *Ocimum sanctum* and found that dose of 5 per cent or more caused high mortality, and those nymphs treated with extract concentrations of 1.25 per cent or more did not produce normal adults. *O. tenuiflorum* L. essential oil showed toxicity against rice weevil (*Sitophilus orzyae*) by inhibiting acetylcholinesterase and hence could be explored as a possible botanical insecticide (Bhavya *et al*., 2018). Bhavya *et al*. (2020) evaluated insecticidal activity of essential oil of *Ocimum tenuiflorum* and its constituent (eugenol) against *Callosobruchus maculatus*. In fumigant toxicity assay, essential oil and eugenol had LC_{50} value of 278.6 and 256.5 µL/L air, respectively after one hour exposure and the mode of action was inhibition of acetylcholinesterase activity. Pulses treated with *O. tenuiflorum* essential oil showed 70 per cent of *Callosobruchus maculatus* mortality after 24 h exposure.

Materials and Methods

3. MATERIALS AND METHODS

The research work entitled "Characterization and evaluation of tulsi (*Ocimum tenuiflorum* L.)" was conducted at College of Agriculture, Vellanikkara, Kerala Agricultural University, from March 2019 to May 2021. The objectives of the study included the evaluation of *Ocimum tenuiflorum* L. accessions, standardization of shade requirement and method of harvesting, investigation on allelopathic effect on upland weeds and crops, and also the assessment of the effect of tulsi on root-knot nematode (*Meloidogyne incognita*). The research work consisted of four experiments and the details of the materials used and methodology adopted for each experiment are given below.

3.1 Experiment 1. Evaluation of *O. tenuiflorum* L. accessions

A total of 25 local accessions were collected from different places of 14 districts of Kerala. Along with local accessions, 10 NBPGR accessions of tulsi were also procured from the Department of Floriculture and Landscaping, College of Agriculture, Vellanikkara. The evaluation of morpho-physological and phytochemical characters of these 35 accessions (25 local + 10 NBPGR accessions) were conducted in the field.

3.1.1 Geographical specification of the experimental area

The field evaluation of *Ocimum tenuiflorum* L. accessions was conducted during *Kharif* 2019 at the farm of Department of Floriculture and Landscaping, College of Agriculture, Vellanikkara, Thrissur, Kerala. The field is situated at 13° 32'N latitude and 76° 26'E longitude, at an altitude of 22.50 m above mean sea level. The average annual rainfall during 2019 was 3128.3 mm. The mean weekly weather data which prevailed during the study period are presented in Appendix I and Fig. 2. The soil of the experimental site was acidic in reaction (4.65), medium in available nitrogen (297 kg/ha) and high in available phosphorus (31 kg/ha) and potassium (299 kg/ha).

3.1.2 Experimental details

The evaluation of morpho-physological and phytochemical characters of 35 accessions of *Ocimum tenuiflorum* L. was conducted in the field during *Kharif* 2019 (June to September). The experiment was arranged in randomized block design (RBD) with 35 treatments and three replications. The spacing adopted was 40 cm x 40 cm. The details of treatments are given in Table 2 and the layout plan of the experimental field is given in Fig.1.

Table 2. List of *Ocimum tenuiflorum* accessions used for the study

Sl. No.	Details	Source
1	IC 583278	Uttar Pradesh - Mathura
2	IC 583288	Uttar Pradesh - Aligarh
3	IC 583296	Uttar Pradesh - Muzaffarnagar
4	IC 583303	Uttar Pradesh - Bijnor
5	IC 583304	Uttar Pradesh - Bijnor
6	IC 583305	Uttarakhand - Udham Singh Nagar
7	IC 583314	Uttar Pradesh - Badaun
8	IC 583317	Uttar Pradesh - Saharanpur
9	IC 583318	Uttar Pradesh - Saharanpur
10	IC 583322	Uttar Pradesh - Hathras
11	KAU OC 11	Kerala - Thrissur - Vellanikkara
12	KAU OC 12	Kerala - Thrissur - Cherpu
13	KAU OC 13	Kerala - Thrissur - Chirakkekodu
14	KAU OC 14	Kerala - Kannur - Kuttiyattur
15	KAU OC 15	Kerala - Palakkad - Nagalassery
16	KAU OC 16	Kerala- Thrissur - Mukkattukara

17	KAU OC 17	Kerala - Thrissur - Mukkattukara
18	KAU OC 18	Kerala - Kasaragod - Pilicode
19	KAU OC 19	Kerala - Kannur -Parassinikkadavu
20	KAU OC 20	Kerala - Wayanad - Pulpally
21	KAU OC 21	Kerala - Kozhikode - Malaparambu
22	KAU OC 22	Kerala - Alapuzha - Kayamkulam
23	KAU OC 23	Kerala - Pathanamthitta - Adoor
24	KAU OC 24	Kerala - Pathanamthitta - Kozhencherry
25	KAU OC 25	Kerala - Thiruvananthapuram - Vellayani
26	KAU OC 26	Kerala - Malappuram - Wandoor
27	KAU OC 27	Kerala - Palakkad - Nenmara
28	KAU OC 28	Kerala - Ernakulam - Moothakunnam
29	KAU OC 29	Kerala - Idukki - Thodupuzha
30	KAU OC 30	Kerala - Kottayam - Ettumanur
31	KAU OC 31	Kerala - Kollam - Ayathil
32	KAU OC 32	Kerala - Thiruvananthapuram - Vellanad
33	KAU OC 33	Kerala - Idukki - Moonnilavu
34	KAU OC 34	Kerala - Wayanad - Ambalavayal
35	KAU OC 35	Kerala - Kozhikode - Koodaranhi

3.1.3 Field operation

Nursery practices

Seeds of *Ocimum* accessions collected from different areas were sown separately in protrays filled with mixture of coir pith compost, sand and soil. Twenty days old seedlings were transferred into small polythene bags and these were irrigated from time to time to provide sufficient moisture.

Land preparation, sowing and fertilizer application

The experimental area was thoroughly ploughed, made into fine tilth and raised beds of 3.2 x 1 x 0.5 m size were prepared. The beds were covered with 30 micron silver black polythene sheet. The experimental plot was provided with inline drip irrigation with a dripping capacity of 4 l/h. Farm yard manure (FYM) was incorporated into the beds at 10 t/ha and Pseudomonas was applied to each bed at the rate of 300 g/bed. Two month old healthy seedlings were transplanted in the prepared beds at a spacing of 40 cm x 40 cm. The crop was uniformly fertilized with N: P_2O_5: K_2O @ 120: 60: 60 kg/ha. Half the dose of N and K and full dose of P were given as basal, and remaining N and K were applied in two equal split doses at 30 and 60 DAT. Layout of experimental field is given in Fig. 1.

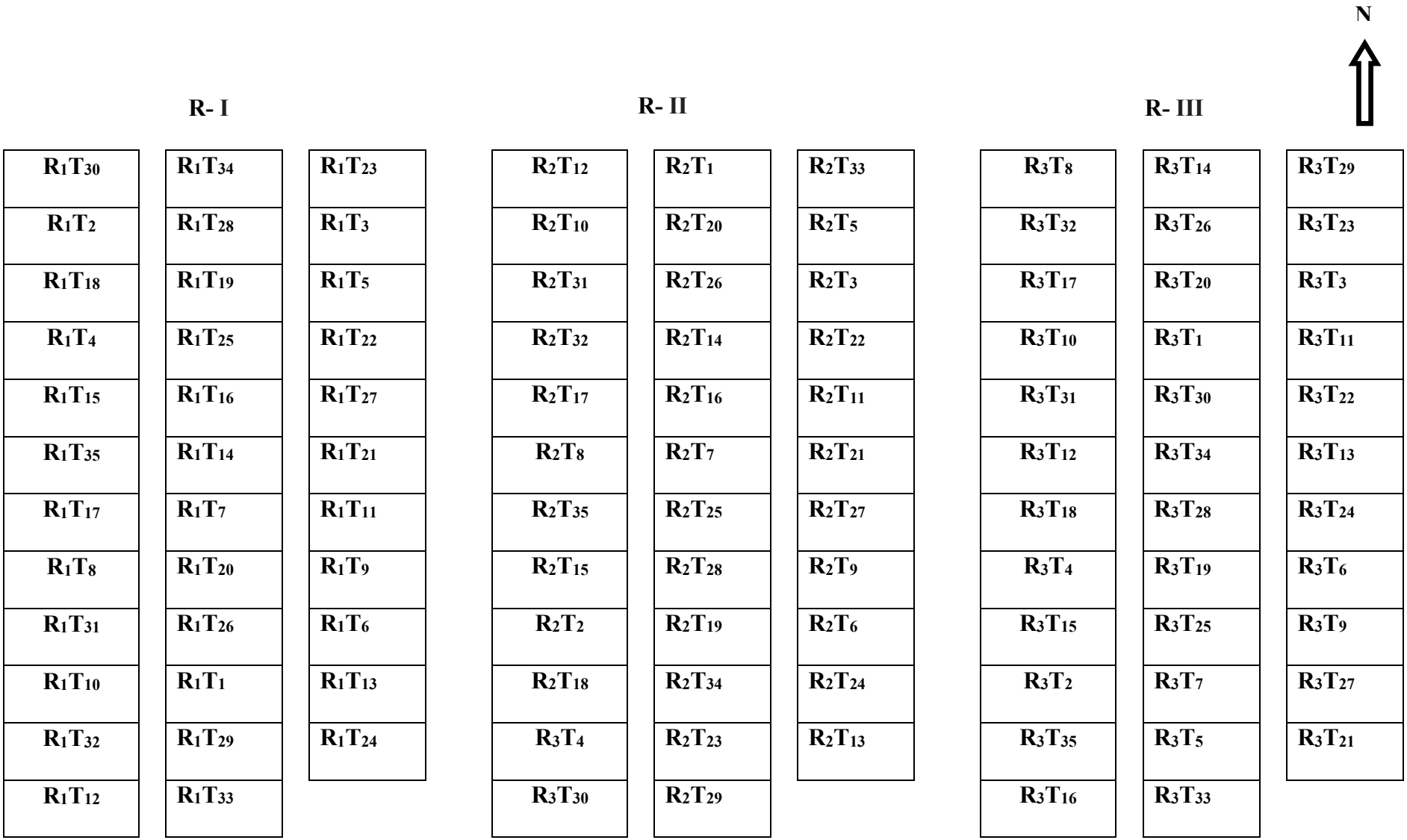
The experimental area was kept weed free by regular hand weeding and no serious incidence of pests or diseases were noticed during the crop period. *Ocimum* accessions were harvested at 90 DAT.

3.1.4 Observations

3.1.4.1 Morphological characters

Plant height

Five plants were selected and height of the plant was measured from the ground level to the growing tip at 30, 60 days after transplanting (DAT) and at harvest. The average was expressed in cm.



T_1 –IC 583278, T_2 –IC 583288, T_3–IC 583296, T_4–IC 583303, T_5–IC 583304, T_6–IC 583305, T_7–IC 583314, T_8–IC 583317, T_9–IC 583318, T_{10}–IC 583322
T_{11}–KAU OC 11, T_{12}–KAU OC 12, T_{13}–KAU OC 13, T_{14}–KAU OC 14, T_{15}–KAU OC 15, T_{16}–KAU OC 16, T_{17}–KAU OC 17, T_{18}–KAU OC 18, T_{19}–KAU OC 19,
T_{20}–KAU OC 20, T_{21}–KAU OC 21, T_{22}–KAU OC 22, T_{23}–KAU OC 23, T_{24}–KAU OC 24, T_{25}–KAU OC 25, T_{26}–KAU OC 26, T_{27}–KAU OC 27,
T_{28}–KAU OC 28, T_{29}–KAU OC 29, T_{30}–KAU OC 30, T_{31}–KAU OC 31, T_{32}–KAU OC 32, T_{33}–KAU OC 33, T_{34}–KAU OC 34, T_{35}–KAU OC 35

Fig.1 Layout of experiment 'Evaluation of *O. tenuiflorum* L. accessions'

Primary branches, secondary branches and number of leaves per plant

Primary branches, secondary branches and total number of leaves per plant was counted at 30, 60 DAT and at harvest in each replication. The average was taken and denoted as number per plant.

Length of inflorescence

The length of five randomly selected inflorescences in each plot was measured and means length was expressed in centimeters.

Stem colour

Stem color of all the accessions were recorded using RHS colour chart.

Leaf colour and inflorescence colour

Colour of both upper and lower surfaces of leaf, and inflorescence colour of each accession was recorded using RHS colour chart.

3.1.4.2 Yield Attributes

Total biomass yield

Plants were uprooted from each replication at 30, 60 DAT and at harvest, and their fresh weight was recorded. The average weights were taken and expressed in grams per plant.

3.1.4.3 Physiological and biochemical parameters

Leaf area index

Leaf area index at 30, 60 DAT and at harvest was calculated as the ratio of total leaf area per plant to ground area occupied by the plant.

Tulsi seeds in protrays

Seedlings of tulsi in polythene bags

Plate 1. Nursery of experiment 1

Plate 2. General field view of experiment 1

Days to first flowering

The number of days taken by the plants to produce first flower in each treatment was recorded during the experiment.

Days to 50 per cent flowering

The days taken for fifty per cent of the plants to produce flowers in each treatment were recorded.

Crop growth rate (CGR)

Crop growth rate explains the dry matter accumulated per unit land area per unit time and it was estimated at 30, 60 DAT and at harvest using the following formula (Watson, 1952) and expressed as gram of dry matter produced per day ($g/m^2/day$).

$$CGR\ (g/m^2/day) = \frac{W_2 - W_1}{A\ (t_2 - t_1)}$$

Where W_1 and W_2 are dry weights of plants at time t_1 and t_2, respectively
A is the ground area

Relative growth rate (RGR)

Relative growth rate shows the rate of growth per unit dry matter per unit time and it was calculated at 30, 60 DAT and at harvest. RGR was determined using the following formula (Blackman, 1919) and expressed as gram of dry matter produced by a gram of existing dry matter in a day (g/g/day).

$$CGR\ (g/g/day) = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

Where W_1 and W_2 are dry weights of plant at time t_1 and t_2, respectively

Chlorophyll and carotenoids

Chlorophyll and carotenoid content (mg/g of fresh weight) in leaves at 30, 60 DAT and at harvest were estimated by using dimethyl sulphoxide (DMSO) extraction technique (Hiscox and Israelstam, 1979). The intensity of colour was read using spectrophotometer at 663, 645, 480 and 510 nm and chlorophyll and carotenoid contents were calculated using the following formulae.

$$\text{Total Chlorophyll} = (8.02 \times \text{OD at } 663) + (20.2 \times \text{OD at } 645) \times \frac{V}{1000 \times W}$$

$$\text{Carotenoids} = (7.69 \times \text{OD at } 480) - (1.49 \times \text{OD at } 510) \times \frac{V}{1000 \times W}$$

Where; V= volume of extract (ml)
W= fresh weight of sample (g)

Essential oil content

Essential oil concentration (%) in fresh herbage at 30, 60 DAT and at harvest were estimated by hydro distillation method using Clevenger's apparatus (ASTA, 1968). Fresh sample of about 30 g was harvested and hydro distilled in a Clevenger's apparatus and the temperature was maintained at 90° C till boiling and then kept at 70° C for 3 hours for distillation. Distillate was cooled to room temperature and oil is allowed to settle to obtain a clear layer. The volume was measured and oil content (%) was calculated as follows.

$$\text{Essential oil (\%)} = \frac{\text{Volume of oil (ml)} \times 100}{\text{Weight of sample (g)}}$$

3.1.5 Statistical analysis

The data were analysed statistically using analysis of variance (ANOVA) with statistically package 'WASP-2.0' (Statistical package, ICAR - Central Coastal Agricultural Research Institute, Goa).

3.1.5.1 Diversity analysis

D^2 Statistics

The genetic association among accessions based on quantitative and qualitative characters of tulsi was calculated using non-hierarchical Euclidian cluster analysis. The Euclidean distances were calculated by the Ward's method and dendrogram were constructed (Sokal and Rohlf, 2003).

3.1.5.2 Principle component analysis

Principle component is complementary to D^2. Assembly of data, wilk's test and transformation of correlated variables (Xi) into uncorrelated ones (Yj) as the sequential same steps followed in this analysis as in D^2 statistics. Differences start with handling of Y_j values.

Development of A-matrix

Worked out sum of squares and sum of products of uncorrelated (Y_j) variables and arranged them in matrix form as A-matrix.

Derivation of canonical vector I (Z_1)

Vectors are derived by repeated iteration or approximation and getting the better approximation of canonical vector than the preceding ones. To obtain the first vector, iteration commences with a trial (unit) vector (1,1,1,1) multiplying with A-matrix

Vector i

This vector is essentially the row total of A-matrix is multiplied by unit values as column matrix. By dividing this vector with the highest element of the vector, the latter approximates are reduced to Vector I (better approximated than unit vector).

***Vector* ii**

The second iteration / approximation were carried out by multiplying vector I with A-matrix. This is vector II (better approximated than vector i). Now divide the elements of this vector by the highest element of the vector to get the reduced vector ii.

Vector iii and iv

Following the same procedure of literation we developed vectors iii and iv or even further till we obtain stable values of the elements in succeeding vectors. The stable values of the elements in succeeding vectors form the element so fun standardized vector I. To standardize this vector, get the square root of the sum of squares of all the elements, which serves as the common divider. By dividing each element of the unstandardized vector I by the common divider, we get the standardised vector I as Z_1.

Deriving canonical root I (λ1)

The highest element of last interaction which was used as divider to get the best approximated vector serves as λ1. The λ1 corresponds with the proportion of total variation accounted for by the vector I (Z_1)-the first or primary axis of differentiation.

Development of B-matrix

This is a reduced matrix obtained by subtracting the product: $\lambda_1 Xi^{th}$ element X_j^{th} element of the A matrix. B-matrix+ $(ij)^{th}$ element of A-matrix-($\lambda_1 Xi^{th} X_j^{th}$ elements of vector I).

Derivation of canonical vector 2 (Z2) and canonical root 2(λ2)

Using the same process of literation and approximation starting with trial (unit) vector (1, -1, 1, 1,) as done for vector I, we easily derive vector 2 (Z_2) and λ_2.

Development of other matrices

Reduced matrices of C, D, *etc*. are also developed just like B-matrix from A-matrix. However, this may not require if $\lambda 1+\lambda 2$ account for more than 80 per cent variation.

Means of canonical variates

The two vectors Z_1 and Z_2 supply the best two linear functions and the two roots, λ_1 and λ_2 the two proportions of total variation. Now, using the elements of Z_1 and Z_2 and corresponding Y-values for each genotype, we can develop the means of canonical variates as:

Z_1 for variety I = Σ Yik. Z_1
(1,k) Z_2 for variety I = Σ Yik.
Z_2 (1,k)

Thus, we develop Z_1 means for each variety and Z_2 for each variety using Y_1 values. Check that sum of squares of Z_1 and sum of squares of Z_2 correspond to λ_1 and λ_2 respectively. Any difference in this relationship would indicate mistakes in computation.

λ 1 –λ 2 chart

If $\lambda 1+\lambda 2$ is more than 80 per cent of total variation, a two dimensional pictorial representation (scatter diagram) of all the varieties is possible. Now using Z_1 and Z_2 means as the two coordinate axes, fairly accurate configuration of varieties, in terms of spatial distance can be presented in a graph.

3.1.5.3 Correlation analysis

Correlation analysis was done by using the formula given by Weber and Moorthy (1952) as follows

$$\text{Phenotypic correlation } (vp_{12}) = \frac{\text{COV.P}_{12}}{\sqrt{VVVVVV}.PP_1 \times \sqrt{VVVVVV}.PP_2}$$

Cov. P_{12}= Phenotypic covariance of character x_1 and x_2

Var. P_1= Phenotypic covariance of character x_1

Var. P_2= Phenotypic covariance of character x_2

3.1.5.4 Path coefficient analysis

Path coefficient analysis developed by Wright (1921) and elaborated by Dewey and Lu (1959) was worked out to find the direct and indirect effect of quantitative characters on biomass yield. The path coefficient is estimated by setting up simultaneous equation and solving by elimination method or metric inversion method.

$$Po_1 + Po_2r_{12} + \text{----------} + Pop\ r_{1p} = r_{o1}$$
$$Po_1 + r_{12} + Po_2 + \text{----------} + Pop\ r_{2p} = r_{o2}$$
$$Po_1 + r_{1p} + P_{o2}r_{2p} + \text{---------} + Pop = r_{op}$$

P_{o1}, P_{o2}, ---- P_{op} = Direct path coefficients of variable 1, 2...P on depended variables

r_{12}, r_{13}------- r_{1p} ---- rp (p-1) = correlation coefficients between independent variables

ro_1, ro_2-------rop = correlations between dependent and independent variables.

3.1.5.5 Binary logistic regression model

Binary logistic regression model (Logic model) is a uni/multivariate technique that is used to estimate the probability that a character is present by predicting a binary dependent outcome from a set of explanatory variables and it is used for model binary response data. When response is binary, it takes the value zero and one which indicates success/failure or resistant/susceptible depending upon the type of study conducted. In this model the dependent variable was categorical.

A logistic model is used to predict the effect of change in the independent variable on the probability of belonging to a group when the dependent variables are dichotomous (Mafini and Omoruyi, 2013).

$$Pi = E(Y=1/X_i) = \frac{1}{1+ee^{-(\alpha+\beta iXi)}}$$

Where, P_i is the probability

X_i is the vector of independent variables

B_i are the coefficients to be estimated

$$P_i = \frac{1}{1+ee^{-ZZZZ}} = \frac{ee^{ZZZZ}}{1+ee^{ZZZZ}}$$

Where,

$$Z_i = \alpha + \beta_i X_i$$

$$1-P_i = \frac{1}{1+ee^{-ZZZZ}}$$

3.2 Experiment 2, Effect of shade and harvesting method on performance of tulsi

3.2.1 Geographical specification of the experimental area

Location and climate

The field experiment was conducted during *Kharif* 2019 and 2020 at the Agronomy farm, Department of Agronomy, College of Agriculture, Vellanikkara, Thrissur, Kerala. The field is situated at 13° 32'N latitude and 76° 26'E longitude, at an altitude of 40 m above mean sea level. The average annual rainfall was 3128.3 mm during 2019 and 2697.3 mm during 2020. The mean weekly weather data which prevailed during the study period are presented in Appendix I and Fig. 2 and 3.

Soil

The texture of the experimental site is sandy clay loam and is acidic in reaction (4.68), low in available nitrogen (142 kg/ha), and high in available phosphorus (27 kg/ha) and medium in available potassium (214 kg/ha).

3.2.2 Experimental details

The experiments were conducted in *Kharif* 2019 and repeated in *Kharif* 2020 to standardize the shade requirement and method of harvesting of *Ocimum tenuiflorum* L. The experiments were laid out in split plot design (RBD) with two main plots and four sub plot treatments and five replications. The plot size and spacing adopted were 4.8 m x 2.0 m and 40 cm x 40 cm respectively. The layout of the field experiment is shown in Fig.4.

Treatments

Main plot: Growing condition

M_1. Open

M_2. 50 per cent shade

Sub plot: Harvesting method

S_1. Harvesting at 20 cm height from ground level at 75 and 135 DAT

S_2. Harvesting at 30 cm height from ground level at 75 and 135 DAT

S_3. Harvesting at 20 cm height from ground level at 90 and 150 DAT

S_4. Harvesting at 30 cm height from ground level at 90 and 150 DAT

3.2.3 Field operations

Land preparation, sowing and fertilizer application

The experimental area was thoroughly ploughed with a disc plough and made in to fine tilth by working with cultivator. The individual plots were laid out in open and 50 per cent shade as per the layout plan. Artificial shading was provided by using green colour shade net with 50 per cent permeability to sunlight.

The seeds of tulsi accession KAU OC 35 were collected from AICRP on MAP&B and seedlings were raised in small polythene bags. Two month old healthy, uniform sized seedling were selected and transplanted in the main field at 40 cm × 40 cm spacing. The crop was uniformly fertilized with farm yard manure (FYM) @ 10 t/ha and N: P_2O_5: K_2O @ 120: 60: 60 kg/ha. After leveling, farm yard manure (FYM) @ 10 t/ha and half the dose of N and K and full dose of P were given as basal, and remaining N and K were applied in two equal split doses at 45 DAT and after first harvest.

Weed management and plant protection

The experimental plots were kept weed-free by hand weeding at 30, 60 DAT and at first harvest. No serious incidence of pests and diseases were noticed during the crop period.

Harvesting

Harvesting was done manually with secateurs at different times and heights as per the treatments.

3.2.4 Observations

3.2.4.1 Growth and yield attributes

Plant height

Five plants from each plot were selected at random and height of the plants was measured from the ground level to the growing tip at first and second harvest. The average was expressed in cm.

Total biomass

After the first and second cuts, total fresh biomass yield per plot was computed and converted into t/ha.

3.2.4.2 Chemical and biochemical parameters

Chlorophyll and carotenoid content

Chlorophyll and carotenoid content (mg/g of fresh weight) in leaves at harvests were estimated by using dimethyl sulphoxide (DMSO) extraction technique (Hiscox and Israelstam, 1979).

$$\text{Total Chlorophyll} = (8.02 \times \text{OD at } 663) - (20.2 \times \text{OD at } 645) \times \frac{V}{1000xW}$$

$$\text{Carotenoids} = (7.69 \times \text{OD at } 480) - (1.49 \times \text{OD at } 510) \times \frac{V}{1000xW}$$

Where; V= volume of extract (ml)

W= fresh weight of sample (g)

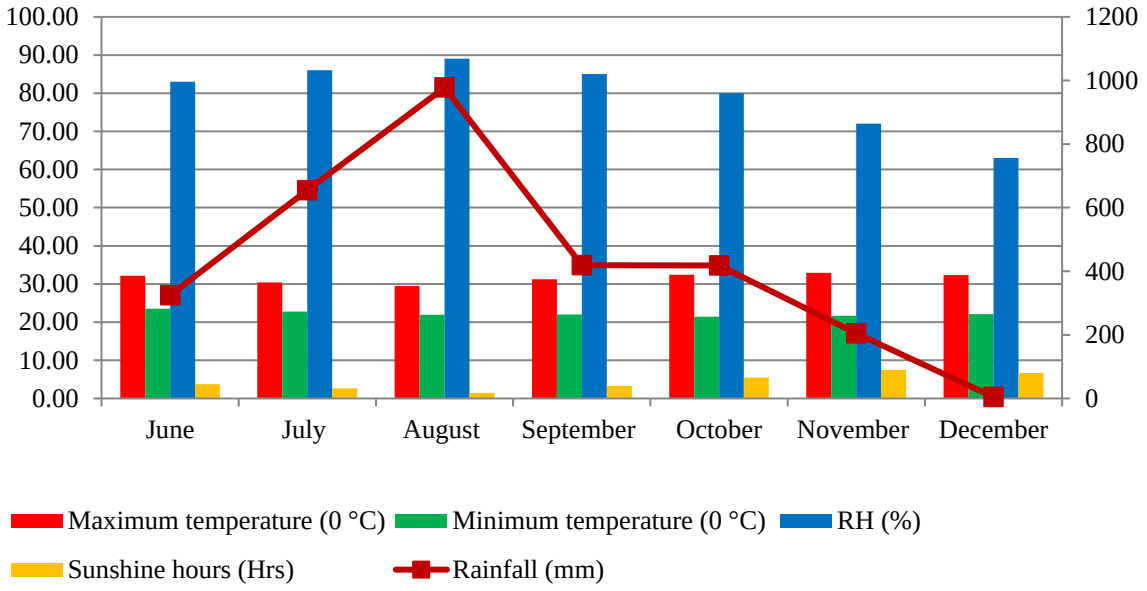


Figure 2. Weather data during the period of study- (June- December, 2019)

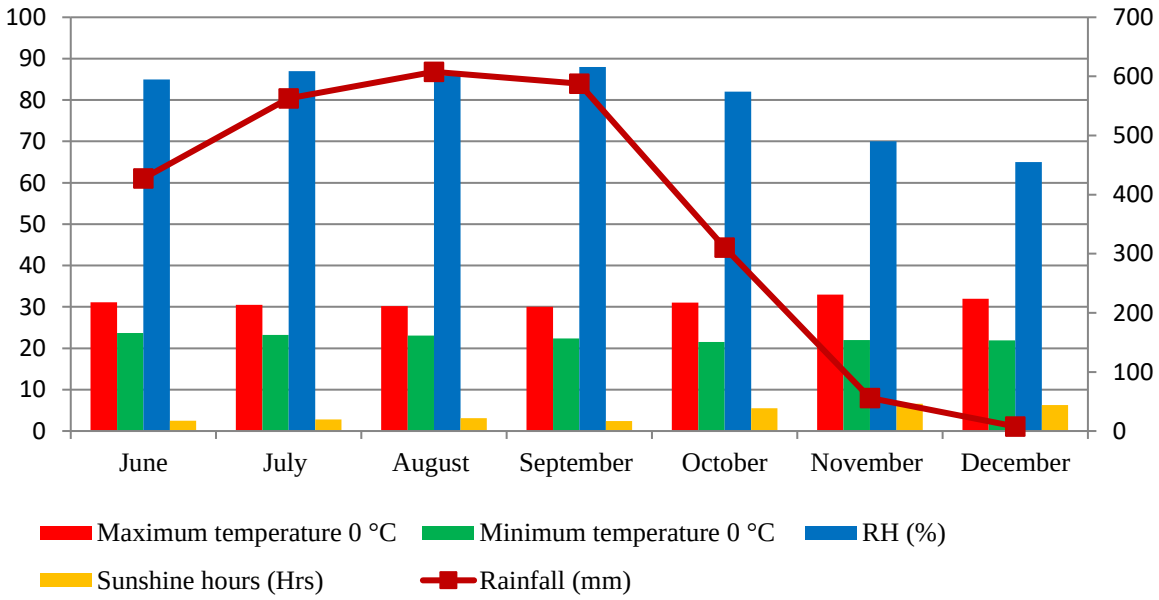
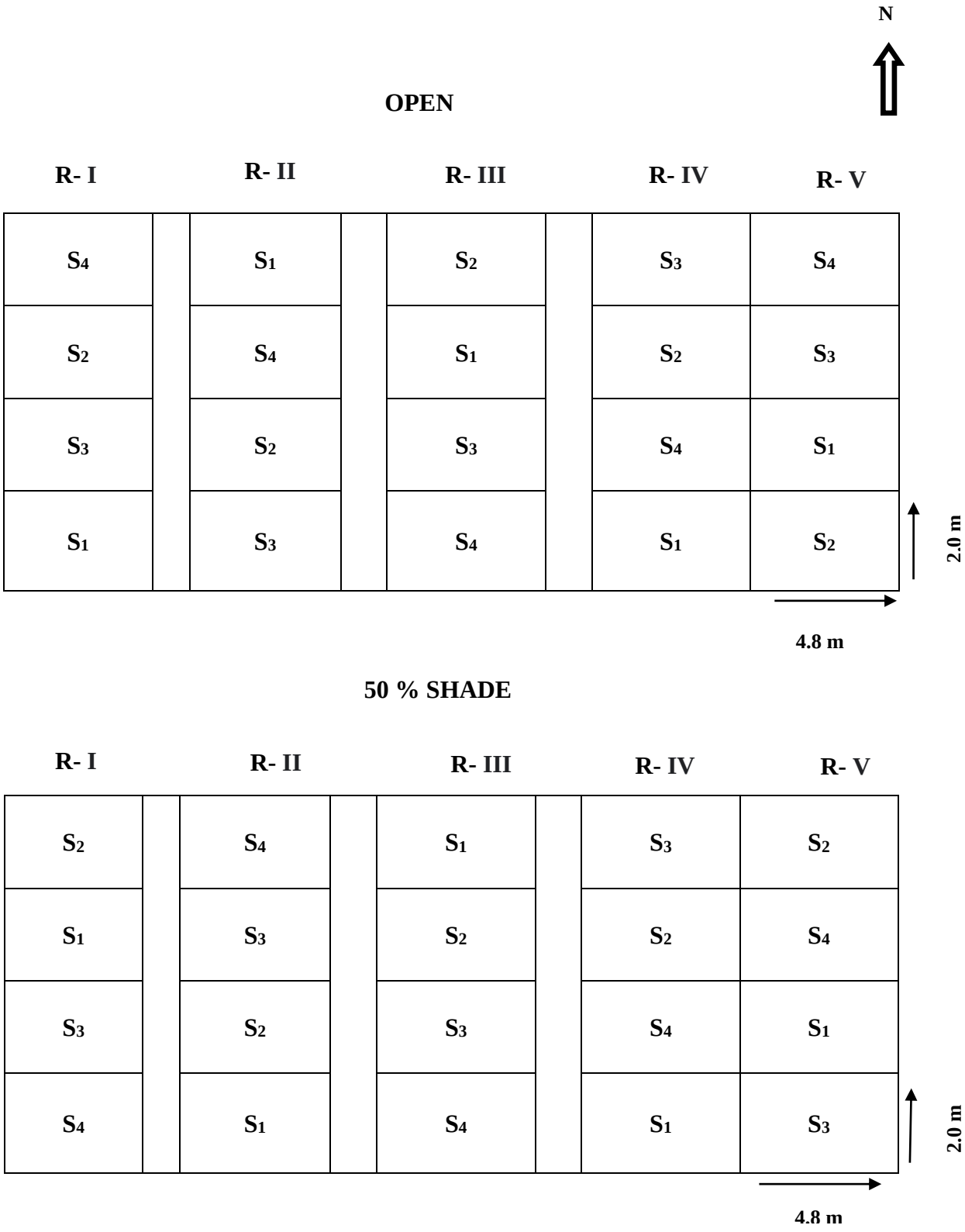


Figure 3. Weather data during the period of study - (June- December, 2020)



Treatments

S_1. Harvest at 20 cm height at 75 and 135 DAT
S_2. Harvest at 30 cm height at 75 and 135 DAT
S_3. Harvest at 20 cm height at 90 and 150 DAT
S_4. Harvest at 30 cm height at 90 and 150 DAT

Fig.4 Layout of the experiment 'Effect of shade and harvesting method on the performance of tulsi'

Plate 3. General view of experiment -2 under open condition

Plate 4. General view of experiment -2 under 50 per cent shade

Essential oil yield

Essential oil concentration (%) in fresh herbage was estimated by hydro distillation method using Clevenger's apparatus (ASTA, 1968). Fresh sample of about 30 g was harvested and hydro distilled in a Clevenger's apparatus and the temperature was maintained at 90° C till boiling and then kept at 70° C for 3 hours for distillation. The distillate was cooled to room temperature and oil was allowed to settle to obtain a clear layer. The volume was measured and oil content (%) was calculated as

$$\text{Essential oil (\%)} = \frac{\text{Volume of oil (ml)}}{\text{Weight of sample (g)}} \times 100$$

The essential oil yield was computed by multiplying the oil concentration (%) with respective biomass yield and expressed in kg/ha (Dhar *et al*., 1996)

Eugenol content

Eugenol content of tulsi at first and second harvests was estimated by Gas Chromatograph-Mass Spectrometry (GC-MS) analysis. GC-MS of the essential oil samples was recorded in an instrument model QP2010S manufactured by Shimadzu, Tokyo, Japan. The column used was ELITE-5MS of 30 m × 0.25 mm × 0.25 µm dimensions with injector temperature of 250 °C and detector temperature of 300 °C. Total volatile oil was calculated as the sum of all GC peak areas of individual compounds in the chromatogram, and the spectra was compared using two spectral libraries NIST 11 and WILLEY 8 and eugenol content was expressed as relative percent area (%).

3.2.4.3 Rhizosphere observations

Soil microorganisms

Total population of bacteria, fungi, actinomycetes, nitrogen fixers, phosphorus solubilizers, fluorescent Pseudomonads and *Trichoderma* were analyzed at sowing, 45 DAT and at harvests. Soil samples were collected from the root zone of the crop and total population of microflora was enumerated by serial dilution and plate count

technique (Wollum, 1982). The particulars of media used for the enumeration are presented in Table 3.

Table 3. Media used for enumeration of soil microorganisms

Sl. No.	Microbes	Medium	Reference
1	Bacteria	Nutrient agar	Agarwal and Hasija (1986)
2	Actinomycetes	Kenknight's agar	
3	Fungi	Martin's Rose Bengal agar	
4	*Trichoderma* sp.	Potato dextrose agar	
5	Nitrogen fixers	Jenson's N free agar	Jensen (1955)
6	Phosphorus solubilizers	Pikovskya's agar	Pikovskya (1948)
7	Fluorescent pseudomonas	King's medium B agar	Gould *et al*. (1985)

Population of plant parasitic nematodes

A composite sample of 250 cc soil was collected from the root zone of tulsi at sowing, 45 DAT and at harvest and plant parasitic nematodes were extracted from the soil samples by Cobb' s sieving and decanting technique (Cobb, 1918) followed by modified Baermann's method (Schindler, 1961). The nematode suspension obtained was made up to a constant volume (50 ml) by adding water. An aliquot of 1 ml was pipetted out in to a counting dish and the total number of plant parasitic nematodes were counted under stereoscopic microscope - Motic - SMZ -168 (50 X). Total population of plant parasitic nematodes in 250 cc soil was determined by multiplying the average population with dilution factor.

3.2.4.4 Observations on weeds

Weed count

Weed count at 30, 60 DAT and at harvest were computed by placing a 50 cm × 50 cm (0.25 m^2) quadrant in each plot at random. The count was expressed in nos./m^2.

Weed dry weight

From the quadrant weeds were uprooted, cleaned air dried and oven dried at 80 ± 5°C and then expressed in g/m^2.

3.2.4.5 Soil analysis

Soil pH, EC, organic carbon and available N, P and K were analyzed before and after the experiment. Soil samples were collected from a depth of 15 cm, air dried, powdered and composite samples were prepared by quartering. Soils were sieved with 0.5 mm sieve for organic carbon and 2 mm sieve for N, P, K estimation. The methods used for physico-chemical analysis of soil are given in Table 4.

Table 4. Methods adopted for physico-chemical analysis of soil

Sl. No.	Particulars	Method used
1	pH	pH meter (Jackson, 1958)
2	EC (dS/m)	Conductivity meter (Jackson, 1958)
3	Organic carbon (%)	Walkley and Black rapid titration method (Walkley and Black, 1934)
4	Available N (kg/ha)	Alkaline permanganate method (Subbiah and Asija, 1956)
5	Available P (kg/ha)	Bray-1 extraction ascorbic acid reduction method (Watanabe and Olsen, 1965)
6	Available K (kg ha)	Ammonium acetate method (Jackson, 1958)

3.3 Experiment 3, Evaluation of allelopathic potential of tulsi on upland weeds and test crops

3.3.1 Experimental details

The experiment was carried out at College of Agriculture, Kerala Agricultural University, Vellanikkara, using rectangular plastic trays of dimensions; L 25 cm × B 20 cm × H 5 cm. The experiment was conducted in completely randomized design (CRD) with 13 treatments and 3 replications.

Part A: Soil was collected from an open area and sieved to remove pebbles and stones. The plastic trays were filled with uniform quantity of soil (1.5 kg) and treatments were imposed to investigate the effect of tulsi on weed germination and growth.

Part B: To test effect of tulsi extracts and powders on crops, twelve seeds each of test crops *i.e*., rice and cowpea, were dibbled in trays filled with soil (1.5 kg).

Treatments

T_1. Aqueous extract of shoot@ 5%
T_2. Aqueous extract of shoot @ 10%
T_3. Aqueous extract of root @ 5%
T_4. Aqueous extract of root @ 10%
T_5. Hot water extract of shoot@ 5%
T_6. Hot water extract of shoot @ 10%
T_7. Hot water extract of root @ 5%
T_8. Hot water extract of root @ 10%
T_9. Powder of shoot@ 10g/kg soil
T_{10}. Powder of shoot@ 20g/kg soil
T_{11}. Powder of root @ 10g/kg soil
T_{12}. Powder of root @ 20g/kg soil
T_{13}. Control

The treatments were applied uniformly to the plastic trays immediately after filleing trays with upland soil or after sowing seeds of test crops. Only a single application of treatments was given. Trays were examined daily for germination and kept for one month for taking observations on growth parameters.

3.3.2 Method of extraction

Aqueous extract

The shoots and roots of tulsi for the experiment were collected from the Agronomy farm, COA, Vellanikkara. Two hundred and fifty grams each of samples were washed with tap water to remove soil and debris and then crushed and added to 500 ml of distilled water. The mixture was shaken for 1 hr continuously in an electric shaker. It was kept at room temperature for 48 hours and filtered through No.2 Whatman filter paper to obtain the concentration of 50 per cent w/v and the stock solution was prepared. These extracts were diluted with distilled water to obtain desired concentrations *viz*., 5 per cent and 10 per cent (El-Rokiek and El-Nagdi, 2011).

Hot water extract

To prepare hot water extract, 500 ml of hot water (70 °C) was added to two hundred and fifty gram of crushed tulsi samples and kept for 12 hours and filtered through No. 2 Whatman filter paper to obtain the stock solution (50 % w/v). From the stock solution, further dilutions *viz*., 5 per cent, and 10 per cent concentration were made (Asimiea *et al*., 2015).

Powder

Fresh plant samples of tulsi (shoots or roots) were air dried under shade for two weeks. The dried shoots and roots of tulsi were powdered separately into fine particles using an electric grinder.

3.3.3 Observations

3.3.3.1 Observations on weeds

Germination count at weekly intervals

Germination was determined at weekly interval by counting the number of germinated seeds.

Density of weeds at one month after application

Weeds grown in the tray were uprooted, counted and categorized into grasses and broad leaf weeds at one month after application. Then the weed density was expressed in nos./m^2.

Dry weight of weeds at one month after application

Weeds were uprooted from plastic trays at one month after application, cleaned, air dried and oven dried at 80 ± 5°C. The weed dry weight was recorded in grams and expressed as g/m^2.

3.3.3.2 Observations on crops

Speed of germination

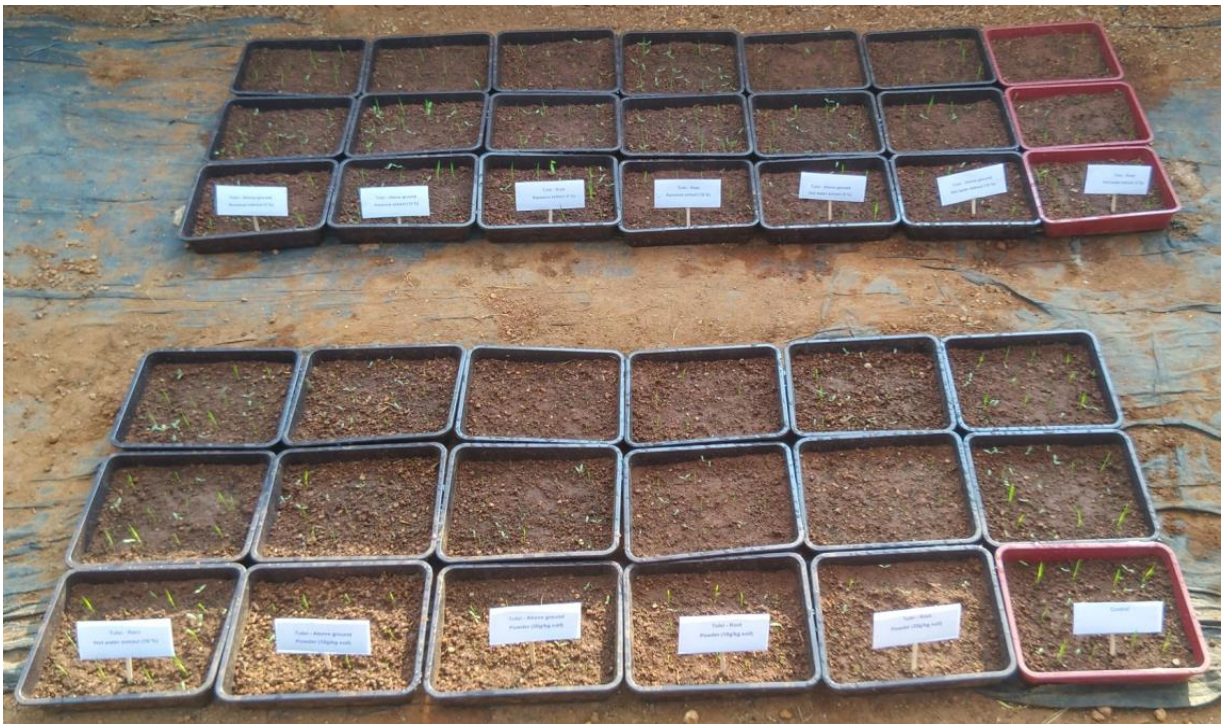
Speed of germination was calculated as per formula of Allan *et al*. (1962) and expressed as numbers per day.

Speed of germination: $S = N_1 / T_1 + N_2 / T_2 + N_3 / T_3 + \cdots\cdots + N_k / T_k$;

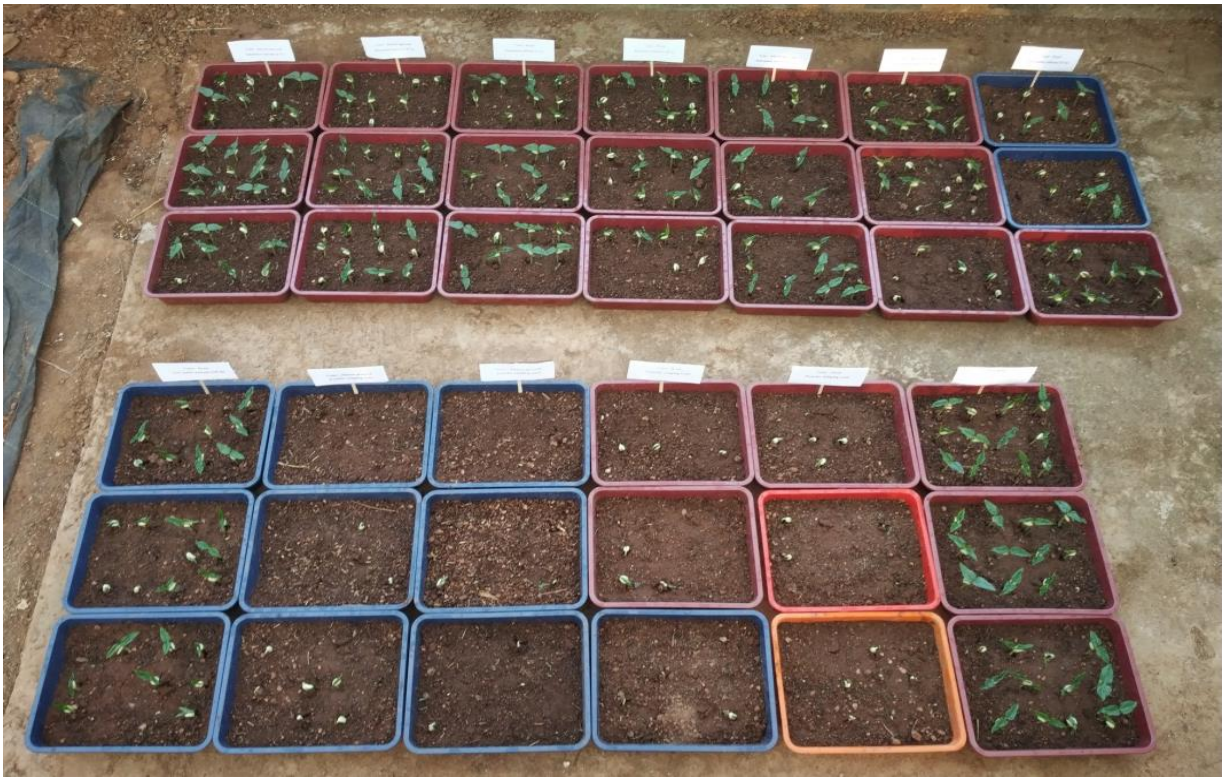
Where,

N_1, N_2, N_3, ……, N_k are the number of seeds germinated at T_1, T_2, T_3,……, T_k days after sowing.

Plate 5. Experimental setup: Allelopathic effect of tulsi on upland weeds



Test crop - Rice



Test crop - Cowpea

Plate 6. Experimental setup: allelopathic effect of tulsi on test crops

Mean germination time

Mean germination time was calculated by the following formula and expressed in days (Basra *et al*., 2005).

Mean germination time: $MGT = \sum(n \times d) / N$

Where n = number of seeds germinated on each day, d = number of days from the beginning of the test, and N = total number of seeds germinated at the termination of the experiment.

Germination percentage

The number of germinated seeds was counted daily and germination percentage was calculated using the following formula for each treatment.

$$\text{Germination percentage (GP)} = \frac{\text{Seeds germinated at the end of trial}}{\text{Number of seeds sown}} \times 100$$

Shoot length

The length of shoot was measured in centimetre from the point where the root and shoot joined the top of the shoot.

Seedling vigor index

The average shoot and root lengths of five randomly selected seedlings and per cent germination from each tray were recorded and seedling vigor index (SVI) was calculated as per formula of van Staden *et al*. (2006).

Seedling vigor index (SVI) = (Shoot length + Root length) x Per cent germination

Root length

Root length was measured in centimeters from the point where the root and shoot joined to the end of the root.

Fresh weight

Plants were uprooted after one month; their fresh weight was recorded and expressed in grams.

Dry weight

Plants used for fresh weight determination were first shade dried and then dried in hot air oven till they attained constant weight. The dry weights were recorded and expressed in grams.

3.3.3.3 Biochemical estimation of extracts

Biochemical parameters like pH, EC, total phenols, tannins, alkaloids and flavanoids of different extracts and powders used for the study were estimated and data are presented in Table 5.

pH and EC of the extracts and powders were measured with digital pH meter and conductivity meter. Total phenols, tannins, alkaloids and flavanoids were determined using the method of Harborne (1973).

Total phenols

Total phenolic content was determined by using Folin-Ciocalteu (FC) reagent with Catechol as standard. One ml of the test sample was diluted with 2 ml with distilled water and then 0.5 ml of Folin-Ciocalteu (FC) reagent was added. After 3 min, 2 ml of 20 per cent sodium carbonate was added and the contents were mixed thoroughly. The mixture was kept for boiling in water bath for 1 minute. The colour

was developed and absorbance measured at 650 nm in spectrophotometer and calculated as follows.

$$\text{Total phenols (mg/ml)} = \frac{\text{Reading of sample} \times \text{concentration of standard}}{\text{Reading of standard}}$$

Total tannins

Total tannin content was estimated by using Folin-Denis method with tannic acid (0.5 mg/ml) as standard. Five ml of test sample was mixed with 5 ml of Folin-Denis reagent and 10 ml of Na_2CO_3. It was mixed well and the total volume was made up to 100 ml with distilled water. The absorbance was recorded at 700 nm in spectrophotometer.

$$\text{Total tannin (mg/ml)} = \frac{\text{Reading of sample} \times \text{concentration of standard}}{\text{Reading of standard}}$$

Total alkaloid content

Samples (5g for powder and 5 ml for extracts) were added into a 250 ml beaker and 200 ml of 10 per cent acetic acid in ethanol was added, covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue which was the alkaloid, was dried and weighed.

Total flavanoids

Total flavanoid content was determined by aluminum chloride colorimetric method with catechin (flavon-3-ol catechin) as standard. An aliquot (0.5 ml) of extract was added to 10 ml flask containing 2 ml distilled water. To this 0.15 ml of 5 per cent

$NaNO_2$ and 0.15 ml of 10 per cent $AlCl_3$ were added. After 6 min, 2 ml of 4 per cent NaOH and 0.2 ml of distilled water was added, mixed well and kept for 15 minutes for colour development. Absorbance was read at 510 nm with a spectrophotometer and calculations were made using the following formula.

$$\text{Total flavonoids (mg/ml)} = \frac{\text{Reading of sample} \times \text{concentration of standard}}{\text{Reading of standard} \times \text{volume of test}}$$

Table 5. Biochemical properties of tulsi extracts and powders

Extracts and powder	pH	EC (dSm^{-1})	Phenols (%)	Tannins (%)	Alkaloids (%)	Flavanoids (%)
Aqueous extract - Shoot	6.16	0.220	0.005	0.0005	0.167	0.042
Aqueous extract -Root	5.85	0.015	0.004	0.0005	0.407	0.026
Hot water extract - Shoot	5.97	0.240	0.005	0.0005	0.127	0.034
Hot water extract -Root	5.76	0.018	0.004	0.0006	0.267	0.023
Powder - Shoot	5.67	0.230	0.005	0.0005	0.557	0.038
Powder -Root	5.56	0.210	0.004	0.0007	0.813	0.024

3.3.4 Statistical analysis

The data were analyzed statistically using analysis of variance (ANOVA) with statistical package 'WASP 2' (Statistical package, ICAR - Central Coastal Agricultural Research Institute, Goa).

3.4 Experiment 4, Evaluation of nematicidal potential of tulsi against root-knot Nematode (*Meloidogyne incognita*)

3.3.2 Experimental site

The experiment was conducted in the laboratory and glass house of the Division of Entomology-cum-Nematology, Banana Research Station, Kannara, Thrissur, which is geographically situated at 10° N 76° E.

3.4.2 Multiplication and maintenance of root knot nematode culture

Pure mother cultures of root knot nematode (*Meloidogyne incognita*) were procured from National Research Centre for Banana (NRCB), Tiruchirappalli, Tamil Nadu. Rooted cuttings of *Plectranthus ambonicus* (Panikoorkka) was used as host plant for multiplication of root-knot nematode cultures. Cuttings of panikoorka were planted in pots filled with sterilized soil and that were kept inside the glasshouse. The potted plants were inoculated with infected juveniles of *M. incognita* and irrigated regularly for the multiplication of nematodes on the host plant. Repotting and sub culturing were carried out from the mother culture to ensure sufficient population of nematodes (Plate 7).

3.4.3 Extraction of second stage juveniles of *M. incognita*

The second stage juveniles of *M. incognita* were extracted using Modified Baermann Funnel Method (Schindler, 1961). From the pure culture pots, heavily infested plants were uprooted and roots were washed gently with water to remove adhering soil particles. Egg masses in the galled roots were picked up using forceps and kept over tissue paper supported on a wire mesh. Then the wire mesh was placed over a petri dish filled with water enough to touch the egg masses on tissue paper and was kept in the laboratory at room temperature. In order to obtain required number of second stage juveniles, sufficient numbers of petri dishes were kept for extraction. The active juveniles settled at the bottom of petri dishes. They were collected in a beaker and this nematode suspension was used for further study.

3.4.4 Preparation of tulsi extracts

Aqueous extract

Fifty gram fresh leaves of tulsi were mixed thoroughly with 100 ml distilled water in an electric blender for 5 minutes. The mixture kept for 72 hours and filtered through No. 2 Whatman filter paper to obtain the concentration of 50 per cent w/v and the stock solution was prepared (Chedekal, 2013). From the stock, further dilutions *viz*., 10 per cent, 20 per cent and 30 per cent concentration were made.

Hot water extract

Fifty grams fresh leaves of tulsi were crushed in a mortar and pestle. 100 ml of hot water (70°C) was added to the paste and kept for one hour and filtered through No. 2 Whatman filter paper to obtain the concentration of 50 per cent w/v. The extract obtained was considered as the stock solution and it was stored in a sterile plastic container at 4°C and from this further dilutions *viz*., 10 per cent, 20 per cent and 30 per cent concentration were made (Asimiea *et al*., 2015).

3.4.5 Experimental details

The experiment was set up in completely randomized design (CRD) with seven treatments and five replications. The treatments were imposed on second-stage juveniles (J_2s) of *M. incognita* extracted from pure culture maintained in the glasshouse on *Plectranthus ambonicus* (Panikoorkka)

Treatments

T_1 - Aqueous extract of tulsi @ 10%

T_2 - Aqueous extract of tulsi @ 20%

T_3 - Aqueous extract of tulsi @ 30%

T_4 - Hot water extract of tulsi @ 10%

T_5 - Hot water extract of tulsi @ 20%

T_6 - Hot water extract of tulsi @ 30%

T_7 - Control

Root-knot nematode pure culture pots

Root-knot nematode sub culture pots

Plate 7. Multiplication and maintenance of root-knot nematode using panikoorka as host plant

Nine millilitres of each treatment (aqueous and hot water extracts) was added to 1 ml of nematode suspension containing 100 J_2 of *M. incognita* in petri dishes. Double distilled water was used as the control. All the Petri dishes were kept at ambient temperature and the numbers of alive and dead nematodes were counted after 12, 24, 48 and 72 h exposure.

3.4.6 Observations

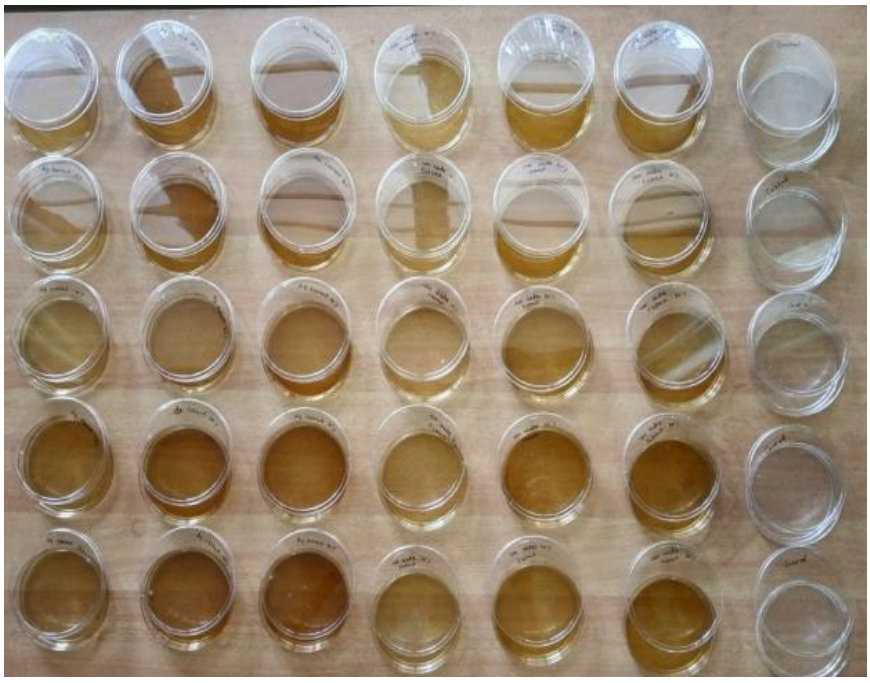
The mortality of second stage juveniles (J_2s) was recorded after 12, 24, 48 and 72 hours of incubation using stereoscopic microscope of make Motic-SMZ -168 (50 X). The juveniles were considered as dead when they attained the shape of straight line and were not responding to touch with a fine needle (Siddiqui and Shaukat, 2004). The mortality of J_2s for each treatment was calculated as the ratio of dead J_2s/number of total J_2s and expressed as percentage.

3.4.7 Statistical analysis

The data were analyzed statistically using analysis of variance (ANOVA) with statistical package 'WASP 2' (Statistical package, ICAR - Central Coastal Agricultural Research Institute, Goa).

Egg mass of root-knot nematode

Second stage juveniles of root-knot nematode



Exposure of J_2s to tulsi extracts in petri dishes

Plate 8. Root-knot nematode and exposure of J_2s to tulsi extracts

Results

4. RESULTS

The research work entitled "Characterization and evaluation of tulsi (*Ocimum tenuiflorum* L.)" was conducted at College of Agriculture, Vellanikkara, Kerala Agricultural University. Study consisted of four experiments and the results obtained from each experiment are presented below.

4.1 Experiment 1: Evaluation of *O. tenuiflorum* L. accessions

4.1.1 Morphological characters

4.1.1.1 Plant height at 30, 60 DAT and at harvest

Plant height of tulsi accessions differed significantly at 30, 60 DAT and at harvest (Table 6). Among the accessions, NBPGR collections had more plant height than local collections.

At 30 DAT, significantly taller plants were observed in accessions IC 583314 (61.04 cm) and IC 583288 (60.84 cm), followed by IC 583318 (55.25 cm) and IC 583322 (54.53 cm). KAU OC 14 recorded the lowest plant height (33.90 cm) and it was significantly lower than all other accessions.

At 60 DAT also accessions IC 583288 (91.41 cm) and IC 583314 (90.33 cm) had greater plant height and they were on par with IC 583305 (88.45 cm), IC 583318 (87.24 cm), IC 583322 (86.81 cm), IC 583303 (86.43 cm), IC 583278 (86.02 cm), IC 583296 (85.41 cm) and IC 583317 (84.46 cm).

At harvest, accession IC 583288 recorded significantly greater plant height (117.60 cm) and it was on par with IC 583305(109.09 cm), IC 583318 (109.02 cm) and IC 583303 (108.13 cm). At 60 DAT and at harvest KAU OC 14 had lower plant height (67.08 and 88.18 cm respectively).

Table 6. Plant height of tulsi accessions at 30, 60 DAT and at harvest

Accessions	Plant height (cm)		
	30 DAT	60 DAT	Harvest
IC 583278	48.22	86.02	106.67
IC 583288	60.84	91.41	117.60
IC 583296	49.20	85.41	106.77
IC 583303	46.30	86.43	108.13
IC 583304	48.28	80.64	105.79
IC 583305	49.59	88.45	109.09
IC 583314	61.04	90.33	106.67
IC 583317	49.63	84.46	106.17
IC 583318	55.25	87.24	109.02
IC 583322	54.53	86.81	105.93
KAU OC 11	45.30	81.49	99.58
KAU OC 12	45.94	79.27	99.67
KAU OC 13	47.81	74.28	99.89
KAU OC 14	33.90	67.08	88.18
KAU OC 15	46.66	74.11	96.85
KAU OC 16	42.60	76.39	95.45
KAU OC 17	45.17	75.40	92.67
KAU OC 18	45.39	78.30	101.55
KAU OC 19	44.20	76.73	97.03
KAU OC 20	47.54	72.32	91.17
KAU OC 21	45.22	76.47	97.22
KAU OC 22	47.00	75.02	100.17
KAU OC 23	49.03	77.02	101.73
KAU OC 24	46.39	73.83	94.00
KAU OC 25	46.56	75.25	101.50
KAU OC 26	45.85	72.49	91.88
KAU OC 27	44.43	75.54	100.13
KAU OC 28	44.29	74.71	96.44
KAU OC 29	46.49	77.81	101.60
KAU OC 30	44.76	76.21	102.32
KAU OC 31	45.16	72.14	94.54
KAU OC 32	46.94	73.94	92.56
KAU OC 33	43.42	74.22	94.31
KAU OC 34	44.64	74.60	92.67
KAU OC 35	44.70	74.93	95.72
SE m (±)	0.820	1.010	1.092
CD (0.05)	5.20	7.59	10.14

4.1.1.2 Number of primary branches per plant at 30, 60 DAT and at harvest

Data pertaining to the number of primary branches per plant at 30, 60 DAT and at harvest of different tulsi accessions are presented in Table 7. Tulsi accessions showed variation with respect to number of primary branches at all growth stages. At 30 DAT, accession IC 583288 produced higher number of primary branches per plant (9.11) and it was on par with KAU OC 23, KAU OC 16, IC 583303, IC 583305, KAU OC 31, KAU OC 21, IC 583278, KAU OC 28, IC 583304, IC 583317, KAU OC 29, KAU OC 20, KAU OC 12 and KAU OC 17. Number of primary branches was found lower in KAU OC 19 (4.11).

At 60 DAT also accession IC 583288 recorded higher number of primary branches (13.00) and it was on par with KAU OC 16 (11.11). At this stage of crop growth, lower value for primary branches was recorded by KAU OC 19 and KAU OC 22 (7.33).

Similarly at harvest, IC 583288 had higher number of primary branches (13.44) and it was on par with KAU OC 30 (12.45), IC 583317 (12.33), IC 583296 (12.33), KAU OC 27 (12.11), KAU OC 23 (12.00), IC 583318 (11.78) and KAU OC 28 (11.78). The number of primary branches per plant was lower in KAU OC 24 (9.00).

4.1.1.3 Number of secondary branches per plant at 30, 60 DAT and at harvest

The number of secondary branches per plant at all growth stages differed significantly among the tulsi accessions (Table 8). IC 583288 recorded higher number of secondary branches per plant (23.44, 60.33 and 67.78) at 30, 60 DAT and at harvest. At 30 DAT, it was on par with accessions KAU OC 35, KAU OC 12, KAU OC 34, KAU OC 27, KAU OC 17, KAU OC 14, KAU OC 20, KAU OC 24, KAU OC 27, KAU OC 30, KAU OC 23, KAU OC 25, IC 583296, KAU OC 16 and IC 583317.

At 60 DAT, IC 583288 was on par with KAU OC 34, KAU OC 30, KAU OC 20, KAU OC 17 and KAU OC 29.

At harvest, IC 583288 was on par with KAU OC 17, KAU OC 30, KAU OC 29, KAU OC 34, KAU OC 20, KAU OC 35, KAU OC 32, KAU OC 11 and KAU OC 28. At 30 DAT, KAU OC 26 had the lower number of secondary branches (17.11), at 60 DAT and at harvest IC 583278 recorded lower values (38.11 and 45.11 respectively).

4.1.1.4 Number of leaves per plant at 30, 60 DAT and at harvest

The data on number of leaves per plant of different accessions are depicted in Table 9. The number of leaves ranged from 172.66 to 239.33 at 30 DAT, 247.89 to 308.11 at 60 DAT, 238.11 to 294.11 at harvest.

At 30 DAT, 60 DAT and at harvest, tulsi accession KAU OC 12 recorded significantly higher number of leaves *i.e*., 239.33, 308.11 and 294.11 respectively. At 30 DAT, KAU OC 13 had lower number of leaves (172.66). At 60 DAT and at harvest, accession KAU OC 22 recorded lower leaf number (247.89 and 238.11).

4.1.1.5 Length of inflorescence

The data pertaining to the length of inflorescence of different tulsi accessions are given in Table 10. Length of inflorescence varied from 10.73 cm to15.56 cm. Among different accessions of tulsi, KAU OC 16 (15.56 cm), IC 583303 (15.46 cm), KAU OC 24 (15.38 cm), KAU OC 20 (15.34 cm), KAU OC 15 (15.27 cm), KAU OC 27 (15.27 cm), KAU OC 21 (15.04 cm), KAU OC 22 (14.98 cm) and KAU OC 30 (14.95 cm) recorded greater inflorescence length. Accession IC 583278 recorded lower inflorescence length of 10.73 cm.

Table 7. Number of primary branches per plant at 30, 60 DAT and at harvest

Accessions	Number of primary branches per plant		
	30 DAT	60 DAT	Harvest
IC 583278	7.45	9.11	9.78
IC 583288	9.11	13.00	13.44
IC 583296	8.00	10.33	12.33
IC 583303	8.22	9.67	10.11
IC 583304	7.44	8.78	9.78
IC 583305	8.11	8.22	10.56
IC 583314	5.78	8.44	9.44
IC 583317	7.33	11.00	12.33
IC 583318	6.78	9.89	11.78
IC 583322	6.89	10.33	11.00
KAU OC 11	6.00	9.22	10.44
KAU OC 12	7.22	8.78	9.89
KAU OC 13	5.11	7.78	9.11
KAU OC 14	4.44	8.00	9.11
KAU OC 15	7.00	8.44	10.00
KAU OC 16	8.33	11.11	11.11
KAU OC 17	7.11	10.22	11.67
KAU OC 18	4.44	7.45	9.11
KAU OC 19	4.11	7.33	9.56
KAU OC 20	7.33	9.89	11.67
KAU OC 21	7.45	9.78	10.44
KAU OC 22	4.45	7.33	9.78
KAU OC 23	9.00	10.22	12.00
KAU OC 24	4.78	7.78	9.00
KAU OC 25	5.00	8.11	9.89
KAU OC 26	4.89	8.33	9.66
KAU OC 27	7.78	10.33	12.11
KAU OC 28	7.44	10.89	11.78
KAU OC 29	7.33	9.78	10.33
KAU OC 30	7.55	10.89	12.45
KAU OC 31	8.11	10.00	11.55
KAU OC 32	4.89	8.33	9.66
KAU OC 33	5.67	9.22	10.67
KAU OC 34	6.67	9.56	10.78
KAU OC 35	4.56	9.11	11.56
SE m (±)	0.246	0.22	0.216
CD (0.05)	2.00	1.98	1.70

Table 8. Number of secondary branches per plant at 30, 60 DAT and at harvest

Accessions	Number of secondary branches per plant		
	30 DAT	60 DAT	Harvest
IC 583278	17.33	38.11	45.11
IC 583288	23.44	60.33	67.78
IC 583296	20.78	38.55	45.89
IC 583303	18.22	40.67	48.44
IC 583304	19.11	46.89	53.56
IC 583305	18.67	45.11	56.89
IC 583314	21.22	38.78	46.11
IC 583317	20.56	47.22	52.66
IC 583318	20.11	49.89	57.44
IC 583322	18.89	50.44	53.22
KAU OC 11	18.78	51.78	61.00
KAU OC 12	22.22	52.67	58.11
KAU OC 13	17.89	50.33	59.34
KAU OC 14	21.78	52.00	57.89
KAU OC 15	18.45	38.33	48.11
KAU OC 16	20.67	51.89	59.67
KAU OC 17	22.11	55.11	66.67
KAU OC 18	20.22	50.67	56.89
KAU OC 19	20.11	51.78	56.55
KAU OC 20	21.44	55.67	62.55
KAU OC 21	19.78	52.55	59.33
KAU OC 22	18.45	51.33	56.00
KAU OC 23	21.11	50.78	56.67
KAU OC 24	21.33	50.00	55.33
KAU OC 25	21.00	47.56	54.00
KAU OC 26	17.11	42.00	53.89
KAU OC 27	22.11	52.67	55.44
KAU OC 28	20.22	52.33	60.89
KAU OC 29	20.11	53.89	64.11
KAU OC 30	21.22	56.00	64.67
KAU OC 31	19.44	51.11	59.78
KAU OC 32	20.11	52.00	61.44
KAU OC 33	20.00	51.89	57.33
KAU OC 34	22.22	57.00	63.56
KAU OC 35	23.22	52.45	62.11
SE m (±)	0.268	0.941	0.963
CD (0.05)	3.1	6.84	7.08

Table 9. Number of leaves per plant at 30, 60 DAT and at harvest

Accessions	Number of leaves per plant		
	30 DAT	60 DAT	Harvest
IC 583278	198.11	269.89	261.11
IC 583288	207.34	271.78	263.89
IC 583296	212.00	279.67	253.44
IC 583303	208.78	277.22	258.11
IC 583304	193.88	264.67	252.22
IC 583305	209.78	281.33	269.67
IC 583314	208.78	282.67	272.22
IC 583317	182.00	252.22	244.55
IC 583318	192.44	270.55	263.00
IC 583322	205.33	276.23	254.89
KAU OC 11	230.78	288.75	277.89
KAU OC 12	239.33	308.11	294.11
KAU OC 13	172.66	250.89	239.11
KAU OC 14	180.55	266.44	261.33
KAU OC 15	225.33	290.56	278.33
KAU OC 16	205.55	279.00	260.89
KAU OC 17	231.78	282.45	266.33
KAU OC 18	203.00	268.00	251.56
KAU OC 19	209.56	274.34	261.66
KAU OC 20	186.77	253.22	243.56
KAU OC 21	228.11	277.78	263.66
KAU OC 22	187.56	247.89	238.11
KAU OC 23	200.45	261.67	255.33
KAU OC 24	208.00	278.67	261.67
KAU OC 25	224.33	276.66	267.11
KAU OC 26	176.89	261.67	248.89
KAU OC 27	222.34	276.67	259.55
KAU OC 28	229.44	282.00	270.00
KAU OC 29	219.89	278.45	267.22
KAU OC 30	221.78	283.89	266.00
KAU OC 31	236.22	291.77	272.22
KAU OC 32	199.89	257.77	251.11
KAU OC 33	227.78	295.45	278.33
KAU OC 34	233.33	280.55	275.56
KAU OC 35	210.88	290.66	276.77
SE m (±)	3.045	2.267	2.064
CD (0.05)	28.91	27.22	26.93

Table 10. Length and colour of inflorescence of tulsi accessions

Accessions	Length of inflorescence (cm)	Colour of inflorescence
IC 583278	10.73	136 Green group, Dark yellowish green B
IC 583288	11.06	138 Green group, Moderate yellow green C
IC 583296	10.98	136 Green group, Dark yellowish green B
IC 583303	15.46	148 Yellow green group, Moderate yellow green B
IC 583304	11.23	148 Yellow green group, Moderate yellow green B
IC 583305	11.91	148 Yellow green group, Moderate yellow green B
IC 583314	11.66	79, Purple group, Dark purple B
IC 583317	11.25	148 Yellow green group, Moderate yellow green B
IC 583318	11.37	144 Green group, Strong yellow green A
IC 583322	12.11	77 Purple group, Strong purple B
KAU OC 11	13.09	79 Purple group, Dark purple A
KAU OC 12	12.52	79 Purple group, Dark purple A
KAU OC 13	10.91	79 Purple group, Dark purple B
KAU OC 14	11.19	148 Yellow green group, Moderate yellow green B
KAU OC 15	15.27	79 Purple group, Dark purple A
KAU OC 16	15.56	77 Purple group, Strong purple B
KAU OC 17	12.79	136 Green group, Dark yellowish green B
KAU OC 18	10.92	N77 Purple group, Greyish reddish purple C
KAU OC 19	13.45	136 Green group, Dark yellowish green B
KAU OC 20	15.34	136 Green group, Dark yellowish green A
KAU OC 21	15.04	136 Green group, Dark yellowish green B
KAU OC 22	14.98	182 Purple violet group, Light purple C
KAU OC 23	11.56	N82 Purple violet group, Strong purple A
KAU OC 24	15.38	79 Purple group, Dark purple A
KAU OC 25	13.15	77 Purple group, Strong purple B
KAU OC 26	11.29	79, Purple group, Dark purple B
KAU OC 27	15.27	79, Purple group, Dark purple B
KAU OC 28	11.61	137 Green group, Moderate yellow green C
KAU OC 29	12.80	N77 Purple group, Greyish reddish purple C
KAU OC 30	14.95	137 Green group, Moderate olive green B
KAU OC 31	13.06	137 Green group, Moderate olive green A
KAU OC 32	13.15	N 79 Purple group, Moderate reddish purple D
KAU OC 33	12.53	N 79 Purple group, Moderate reddish purple D
KAU OC 34	12.72	N 79 Purple group, Moderate reddish purple D
KAU OC 35	12.76	N 79 Purple group, Moderate reddish purple D
SE m (±)	0.276	
CD (0.05)	1.49	

4.1.1.6 Colour of inflorescence

The accessions exhibited variation in inflorescence colour (Table 10). As per RHS colour chart, inflorescence colour showed different shades of green and purple colours. Among green group itself, the colour ranged from 136 A to 148 B, in purple group colour ranged from 77 B to 182 C. Among 35 accessions, there were 16 accessions with green colour inflorescence (IC 583278, IC 583288, IC 583296, IC 583303, IC 583304, IC 583305, IC 583317, IC 583318, KAU OC 14, KAU OC 17, KAU OC 19, KAU OC 20, KAU OC 21, KAU OC 28, KAU OC 30 and KAU OC 31) and 19 with purple group inflorescence (IC 583314, KAU OC 11, KAU OC 12, KAU OC 13, KAU OC 15, KAU OC 16, KAU OC 18, KAU OC 22, KAU OC 23, KAU OC 24, KAU OC 25, KAU OC 26, KAU OC 27, KAU OC 29, KAU OC 32, KAU OC 33, KAU OC 34 and KAU OC 35).

4.1.1.7 Stem colour

Variations were also observed in the tulsi accessions for stem colour (Table 11). There were 19 accessions with purple stem colour and 16 accessions with green colour stem. Purple group ranged from N77 C to 79 D and green group ranged from 137 D to 143 B.

Green stem colour was observed in accessions IC 583278, IC 583296, IC 583303, IC 583304, IC 583305, IC 583317, IC 583318, IC 583288, KAU OC 14, KAU OC 17, KAU OC 19, KAU OC 20, KAU OC 21, KAU OC 28, KAU OC 30 and KAU OC 31.

Among the different accessions, IC 583314, IC 583322, KAU OC 11, KAU OC 12, KAU OC 13, KAU OC 15, KAU OC 16, KAU OC 18, KAU OC 22, KAU OC 23, KAU OC 24, KAU OC 25, KAU OC 26, KAU OC 27, KAU OC 29, KAU OC 32, KAU OC 33, KAU OC 34 and KAU OC 35 showed purple stem.

Table 11. Stem colour of tulsi accessions

Accessions	Stem colour
IC 583278	138 Green group, Moderate yellow green B
IC 583288	143 Green group, Strong yellow green B
IC 583296	138 Green group, Moderate yellow green B
IC 583303	138 Green group, Moderate yellow green B
IC 583304	138 Green group, Moderate yellow green B
IC 583305	138 Green group, Moderate yellow green B
IC 583314	79 Purple group, Dark purple A
IC 583317	137 Green group, Moderate yellowish green D
IC 583318	137 Green group, Moderate yellowish green D
IC 583322	79 Purple group, Moderate purple D
KAU OC 11	79 Purple group, Dark purple A
KAU OC 12	79 Purple group, Dark purple A
KAU OC 13	79 Purple group, Dark purple B
KAU OC 14	138 Green group, Moderate yellow green B
KAU OC 15	79 Purple group, Dark purple A
KAU OC 16	79 Purple group, Moderate purple D
KAU OC 17	137 Green group, Moderate yellowish green D
KAU OC 18	79 Purple group, Moderate purple D
KAU OC 19	137 Green group, Moderate yellowish green D
KAU OC 20	137 Green group, Moderate yellowish green D
KAU OC 21	137 Green group, Moderate yellowish green D
KAU OC 22	79 Purple group, Dark purple B
KAU OC 23	79 Purple group, Dark purple C
KAU OC 24	N77 Purple group, Greyish reddish purple C
KAU OC 25	79 Purple group, Dark purple B
KAU OC 26	79 Purple group, Dark purple A
KAU OC 27	79 Purple group, Dark purple A
KAU OC 28	138 Green group, Moderate yellow green C
KAU OC 29	79 Purple group, Dark purple C
KAU OC 30	138 Green group, Moderate yellow green B
KAU OC 31	138 Green group, Moderate yellow green B
KAU OC 32	79 Purple group, Dark purple A
KAU OC 33	79 Purple group, Dark purple A
KAU OC 34	79 Purple group, Dark purple A
KAU OC 35	79 Purple group, Dark purple A

Table 12. Leaf colour (upper and lower surface) of tulsi accessions

Accessions	Leaf colour (upper surface)	Leaf colour (lower surface)
IC 583278	137 Green group, Moderate yellow green C	138 Green group, Moderate yellow green B
IC 583288	137 Green group, Moderate olive green B	137 Green group, Moderate olive green B
IC 583296	138 Green group, Moderate yellowish green A	138 Green group, Moderate yellow green B
IC 583303	137 Green group, Moderate olive green B	138 Green group, Moderate yellow green B
IC 583304	137 Green group, Moderate olive green B	138 Green group, Moderate yellow green B
IC 583305	137 Green group, Moderate yellow green C	138 Green group, Moderate yellow green B
IC 583314	79 Purple group, Dark purple A	137 Green group, Moderate olive green B
IC 583317	138 Green group, Moderate yellowish green A	137 Green group, Moderate yellow green C
IC 583318	137 Green group, Moderate olive green B	137 Green group, Moderate yellow green C
IC 583322	137 Green group, Moderate olive green B	137 Green group, Moderate yellow green C
KAU OC 11	79 Purple group, Dark purple C	137 Green group, Moderate yellow green C
KAU OC 12	79 Purple group, Dark purple C	137 Green group, Moderate yellow green C
KAU OC 13	79 Purple group, Dark purple C	137 Green group, Moderate yellow green D
KAU OC 14	137 Green group, Moderate olive green B	137 Green group, Moderate yellow green C
KAU OC 15	79 Purple group, Dark purple B	137 Green group, Moderate yellow green C
KAU OC 16	79 Purple group, Dark purple B	137 Green group, Moderate yellow green C
KAU OC 17	137 Green group, Moderate olive green B	137 Green group, Moderate yellow green D
KAU OC 18	137 Green group, Moderate yellow green C	138 Green group, Moderate yellow green B
KAU OC 19	137 Green group, Moderate olive green B	137 Green group, Moderate yellow green C
KAU OC 20	137 Green group, Moderate yellow green C	137 Green group, Moderate yellow green C
KAU OC 21	137 Green group, Moderate yellow green C	137 Green group, Moderate yellow green C
KAU OC 22	137 Green group, Moderate yellow green C	137 Green group, Moderate yellow green B
KAU OC 23	79 Purple group, Dark purple B	138 Green group, Moderate yellow green B
KAU OC 24	79 Purple group, Dark purple B	137 Green group, Moderate yellow green C
KAU OC 25	79 Purple group, Dark purple A	137 Green group, Moderate yellow green C
KAU OC 26	79 Purple group, Dark purple A	137 Green group, Moderate yellow green C
KAU OC 27	79 Purple group, Dark purple A	137 Green group, Moderate yellow green D
KAU OC 28	137 Green group, Moderate yellow green C	138 Green group, Moderate yellow green B
KAU OC 29	137 Green group, Moderate olive green B	137 Green group, Moderate yellow green C
KAU OC 30	137 Green group, Moderate yellow green C	137 Green group, Moderate yellow green C
KAU OC 31	137 Green group, Moderate olive green B	137 Green group, Moderate yellow green C
KAU OC 32	79 Purple group, Dark purple A	138 Green group, Moderate yellow green B
KAU OC 33	137 Green group, Moderate olive green B	138 Green group, Moderate yellow green B
KAU OC 34	79 Purple group, Dark purple A	138 Green group, Moderate yellow green B
KAU OC 35	79 Purple group, Dark purple A	137 Green group, Moderate yellow green C

Table 13. General colouration of leaf, stem and inflorescence of tulsi accessions

Sl. No.	Accessions	Leaf colour	Stem colour	Inflorescence colour
1	IC 583278			
2	IC 583288			
3	IC 583296			
4	IC 583303			
5	IC 583304			
6	IC 583305			
7	IC 583314			
8	IC 583317			
9	IC 583318			
10	IC 583322			
11	KAU OC 11			
12	KAU OC 12			
13	KAU OC 13			
14	KAU OC 14			
15	KAU OC 15			
16	KAU OC 16			
17	KAU OC 17			
18	KAU OC 18			
19	KAU OC 19			
20	KAU OC 20			
21	KAU OC 21			
22	KAU OC 22			
23	KAU OC 23			
24	KAU OC 24			
25	KAU OC 25			
26	KAU OC 26			
27	KAU OC 27			
28	KAU OC 28			
29	KAU OC 29			
30	KAU OC 30			
31	KAU OC 31			
32	KAU OC 32			
33	KAU OC 33			
34	KAU OC 34			
35	KAU OC 35			

4.1.1.8 Leaf colour (upper and lower surface)

The colour of upper and lower leaf surface of different accessions of tulsi is given in Table 12. Upper leaf surface colour showed wide variation from green group to purple group, while lower leaf surface colour was shades of green in all the 35 accessions studied. Upper leaf surface colour of 21 accessions of tulsi was of green group and 14 accessions were in purple group.

Accessions, IC 583278, IC 583288, IC 583296, IC 583303, IC 583304, IC 583305, IC 583317, IC 583318, IC 583322, KAU OC 14, KAU OC 17, KAU OC 18, KAU OC 19, KAU OC 20, KAU OC 21, KAU OC 22, KAU OC 28, KAU OC 29, KAU OC 30, KAU OC 31 and KAU OC 33 had purple colour on the upper side of leaf.

Accessions, IC 583314, KAU OC 11, KAU OC 12, KAU OC 13, KAU OC 15, KAU OC 16, KAU OC 23, KAU OC 24, KAU OC 25, KAU OC 26, KAU OC 27, KAU OC 32, KAU OC 34, and KAU OC 35 had green colour on the upper side of leaf.

In general, the tulsi accessions had either green or purple colour on stem, leaf and inflorescence as depicted in Table 13.

4.1.2 Yield attributes

4.1.2.1 Total biomass yield per plant at 30, 60 DAT and at harvest

Total biomass yield per plant at 30 DAT, 60 DAT and at harvest was significantly differed among the accessions (Table 14). At all stages of crop growth, accession IC 583288 recorded significantly higher biomass yield (205.3 g, 393.3 g and 443.7 g respectively) and accession KAU OC 14 had significantly lower biomass yield (108.0 g, 272.0 g and 319.7 g respectively).

At 30 DAT, biomass yield of IC 583288 was on par with KAU OC 34 (203.3 g), KAU OC 23 (200.7 g), IC 583314 (197.3 g), KAU OC 17 (197.0 g), IC 583296

(194.7 g), IC 583305 (192.3 g), KAU OC 35 (185.0 g), KAU OC 31 (184.0 g), KAU OC 27 (182.7 g), KAU OC 28 (182.0 g) and IC 583318 (181.0 g).

At 60 DAT, biomass yield of IC 583288 was on par with IC 583314 (383.3 g), KAU OC 34 (381.7 g), KAU OC 23 (381.0 g), KAU OC 30 (379.7 g), KAU OC 35 (376.0 g), IC 583318 (375.7 g), KAU OC 28 (374.0 g), IC 583296 (373.7 g), KAU OC 16 (365.7 g), IC 583305 (358.0 g) and KAU OC 32 (354.7 g).

At harvest stage, biomass yield of IC 583288 was on par with KAU OC 30 (433.7 g), IC 583318 (431.7 g), IC 583296 (427.0 g), IC 583314 (426.7 g), KAU OC 34 (424.3 g), KAU OC 23 (421.0 g), KAU OC 28 (420.0 g), KAU OC 35 (419.3 g), KAU OC 31 (414.0 g) and KAU OC 16 (412.7 g).

4.1.3 Physiological parameters

4.1.3.1 Leaf area index at 30, 60 DAT and at harvest

Data on leaf area index of different tulsi accessions are furnished in Table 15. At 30 DAT, significantly higher value for leaf area index was observed in IC 583288 (0.84) and it was on par with IC 583314 (0.81), IC 583296 (0.79) and IC 583305 (0.76). Lower value of LAI at 30 DAT was observed in KAU OC 14 (0.36) and it was on par with KAU OC 20 (0.36), KAU OC 26 (0.38) and KAU OC 13 (0.38).

At 60 DAT also IC 583288 recorded higher leaf area index (1.32) and it was on par with IC 583314 (1.25), followed by IC 583296 (1.2) and IC 583305 (1.19). Lower value for LAI was observed in KAU OC 14 (0.56) and it was statistically on par with KAU OC 13 (0.56), KAU OC 20 (0.59), KAU OC 11 (0.61), KAU OC 26 (0.62) and KAU OC 30 (0.64).

Similarly at harvest, IC 583288 recorded significantly higher LAI (1.19) and it was on par with IC 583318 (1.15) and IC 583314 (1.12), followed by IC 583305 (1.10), IC 583296 (1.08), IC 583317 (1.07). Accession KAU OC 13 recorded lower value for LAI (0.55) at harvest.

Table 14. Total biomass yield per plant at 30, 60 DAT and at harvest

Accessions	Biomass yield per plant (g)		
	30 DAT	60 DAT	Harvest
IC 583278	118.7	304.0	366.0
IC 583288	205.3	393.3	443.7
IC 583296	194.7	373.7	427.0
IC 583303	119.3	317.7	384.0
IC 583304	130.7	324.0	406.7
IC 583305	192.3	358.0	405.3
IC 583314	197.3	383.3	426.7
IC 583317	128.3	332.3	403.7
IC 583318	181.0	375.7	431.7
IC 583322	160.7	340.3	410.7
KAU OC 11	145.7	281.0	331.3
KAU OC 12	167.3	319.0	401.0
KAU OC 13	118.7	288.7	340.7
KAU OC 14	108.0	272.0	319.7
KAU OC 15	158.7	313.3	386.3
KAU OC 16	159.7	365.7	412.7
KAU OC 17	197.0	343.3	402.7
KAU OC 18	135.7	292.3	380.0
KAU OC 19	141.0	296.0	404.3
KAU OC 20	110.0	311.7	358.3
KAU OC 21	152.0	312.3	405.3
KAU OC 22	153.3	290.7	377.7
KAU OC 23	200.7	381.0	421.0
KAU OC 24	137.7	290.7	390.3
KAU OC 25	125.7	291.7	398.3
KAU OC 26	114.0	296.7	351.3
KAU OC 27	182.7	317.0	393.7
KAU OC 28	182.0	374.0	420.0
KAU OC 29	173.7	312.7	400.3
KAU OC 30	171.0	379.7	433.7
KAU OC 31	184.0	333.3	414.0
KAU OC 32	160.3	354.7	407.7
KAU OC 33	174.7	300.0	407.3
KAU OC 34	203.3	381.7	424.3
KAU OC 35	185.0	376.0	419.3
SE m (±)	5.10	6.121	4.954
CD (0.05)	25.33	42.27	32.77

Table 15. Leaf area index at 30, 60 DAT and at harvest

Accessions	Leaf area index		
	30 DAT	60 DAT	Harvest
IC 583278	0.62	1.14	0.96
IC 583288	0.84	1.32	1.19
IC 583296	0.79	1.20	1.08
IC 583303	0.69	1.11	0.95
IC 583304	0.69	1.11	1.00
IC 583305	0.76	1.19	1.10
IC 583314	0.81	1.25	1.12
IC 583317	0.64	1.04	1.07
IC 583318	0.73	1.16	1.15
IC 583322	0.72	1.15	1.06
KAU OC 11	0.45	0.61	0.59
KAU OC 12	0.63	0.84	0.80
KAU OC 13	0.38	0.56	0.55
KAU OC 14	0.36	0.56	0.55
KAU OC 15	0.61	0.75	0.72
KAU OC 16	0.54	0.70	0.69
KAU OC 17	0.62	0.78	0.72
KAU OC 18	0.51	0.65	0.61
KAU OC 19	0.50	0.73	0.68
KAU OC 20	0.36	0.59	0.56
KAU OC 21	0.66	0.79	0.75
KAU OC 22	0.50	0.69	0.66
KAU OC 23	0.58	0.72	0.70
KAU OC 24	0.48	0.72	0.68
KAU OC 25	0.62	0.78	0.73
KAU OC 26	0.38	0.62	0.59
KAU OC 27	0.64	0.79	0.75
KAU OC 28	0.67	0.81	0.78
KAU OC 29	0.66	0.81	0.77
KAU OC 30	0.61	0.64	0.74
KAU OC 31	0.65	0.82	0.76
KAU OC 32	0.46	0.64	0.65
KAU OC 33	0.61	0.81	0.78
KAU OC 34	0.66	0.83	0.80
KAU OC 35	0.69	0.88	0.84
SE m (±)	0.021	0.037	0.032
CD (0.05)	0.08	0.09	0.08

4.1.3.2 Days to first flowering

Number of days from planting to first flowering varied among accessions (Table 16) and it ranged from 21.67 to 31.67 days. The accession KAU OC 16 was earlier with respect to first flowering (21.67days), and was on par with KAU OC 15, KAU OC 12, KAU OC 30, KAU OC 29, KAU OC 34, KAU OC 24, KAU OC 20, KAU OC 19, KAU OC 28, KAU OC 13, KAU OC 21 and KAU OC 11. Days to first flowering was more in IC 583305 (31.67 days) and it was on par with all other accessions other than KAU OC 21, KAU OC 13, KAU OC 28, KAU OC 19, KAU OC 20, KAU OC 24, KAU OC 34, KAU OC 29, KAU OC 30, KAU OC 12, KAU OC 15 and KAU OC 16.

4.1.3.3 Days to 50 per cent flowering

Number of days taken to 50 per cent flowering was significantly different among different accessions (Table 16). Days to 50 per cent flowering ranged from 26.67 to 39.0 days. Accession KAU OC 16 took lower number of days for 50 per cent flowering (26.67 days) and it was on par with all the accessions except KAU OC 33, IC 583278, KAU OC 26, KAU OC 32, IC 583305, IC 583296, IC 583314, IC 583317, KAU OC 31, IC 583304, IC 583288, KAU OC 25 and IC 583322. Accession IC 583322 took 39 days to reach 50 per cent flowering stage.

4.1.3.4 Crop growth rate (CGR) at 30, 60 DAT and at harvest

Data on crop growth rate (CGR) of different tulsi accessions at 30, 60 DAT and at harvest are given in Table 17. At 30 DAT, CGR was found higher in IC 583288 (1.24 g/m^2/day) and it was on par with KAU OC 34, KAU OC 23, IC 583314, KAU OC 17, IC 583296, KAU OC 31, IC 583305, KAU OC 35, KAU OC 27 and KAU OC 28. Lower CGR of 0.65 was observed in accession KAU OC 14.

At 60 DAT, KAU OC 30 recorded higher CGR of 1.31 g/m^2/day and it was on par with all accessions except KAU OC 14, KAU OC 21, KAU OC 18, KAU OC 19,

KAU OC 15, KAU OC 24, KAU OC 12, KAU OC 17, KAU OC 31, KAU OC 29, KAU OC 22, KAU OC 27, KAU OC 11 and KAU OC 33.

At harvest stage, CGR was higher in KAU OC 33 (0.67 g/m^2/day), which was on par with KAU OC 19 (0.66 g/m^2/day), KAU OC 25 (0.66 g/m^2/day) and KAU OC 24 (0.61 g/m^2/day). At this stage of crop growth, lower CGR was found in KAU OC 20 (0.29 g/m^2/day).

4.1.3.5 Relative growth rate (RGR) at 30, 60 DAT and at harvest

Data on relative growth rate (RGR) of different tulsi accessions at 30, 60 DAT and at harvest are presented in Table 18. At 30 DAT, IC 583288 recorded higher RGR (0.118 g/g/day), which was on par with all accessions except KAU OC 11, KAU OC 19, KAU OC 24, KAU OC 18, IC 583304, IC 583317, KAU OC 25, KAU OC 13, IC 583278, IC 583303, KAU OC 26, KAU OC 20 and KAU OC 14.

At 60 DAT, higher RGR was found in KAU OC 20 (0.035 g/g/day) and was on par with IC 583303 (0.033 g/g/day), IC 583317 (0.032 g/g/day), KAU OC 26 (0.032 g/g/day) and IC 583278 (0.031 g/g/day). Lower RGR of 0.018 g/g/day was observed in KAU OC 33.

At harvest stage, higher RGR was observed in KAU OC 19 (0.011 g/g/day), KAU OC 25 (0.010 g/g/day), KAU OC 33 (0.010 g/g/day) and KAU OC 24 (0.010 g/g/day). Lower RGR was found in KAU OC 23 (0.003 g/g/day).

Table 16. Days to first flowering and 50 per cent flowering for tulsi accessions

Accessions	Days to first flowering	Days to 50 per cent flowering
IC 583278	29.00	33.67
IC 583288	31.00	37.00
IC 583296	29.67	35.00
IC 583303	27.67	32.33
IC 583304	29.33	36.67
IC 583305	31.67	34.67
IC 583314	28.33	35.67
IC 583317	28.33	36.00
IC 583318	28.00	31.67
IC 583322	30.00	39.00
KAU OC 11	26.67	30.00
KAU OC 12	23.33	27.33
KAU OC 13	25.67	28.67
KAU OC 14	28.33	30.33
KAU OC 15	22.33	27.67
KAU OC 16	21.67	26.67
KAU OC 17	29.00	32.33
KAU OC 18	27.67	31.67
KAU OC 19	25.00	30.33
KAU OC 20	24.67	30.67
KAU OC 21	26.00	30.33
KAU OC 22	27.33	31.33
KAU OC 23	28.00	32.33
KAU OC 24	24.67	30.00
KAU OC 25	30.00	37.33
KAU OC 26	27.00	33.67
KAU OC 27	28.00	32.00
KAU OC 28	25.67	30.67
KAU OC 29	24.33	30.00
KAU OC 30	23.67	29.67
KAU OC 31	30.00	36.00
KAU OC 32	28.67	34.00
KAU OC 33	29.33	33.00
KAU OC 34	24.67	30.00
KAU OC 35	27.67	31.67
SE m (±)	0.419	0.509
CD (0.05)	5.31	6.02

Table 17. Crop growth rate of tulsi accessions at 30, 60 DAT and at harvest

Accessions	CGR (g/m^2/day)		
	30 DAT	60 DAT	Harvest
IC 583278	0.71	1.16	0.38
IC 583288	1.24	1.18	0.31
IC 583296	1.17	1.13	0.33
IC 583303	0.71	1.24	0.39
IC 583304	0.79	1.20	0.51
IC 583305	1.16	1.04	0.30
IC 583314	1.19	1.17	0.39
IC 583317	0.76	1.27	0.44
IC 583318	1.09	1.22	0.35
IC 583322	0.96	1.13	0.44
KAU OC 11	0.88	0.85	0.31
KAU OC 12	1.02	0.95	0.50
KAU OC 13	0.72	1.07	0.48
KAU OC 14	0.65	1.03	0.29
KAU OC 15	0.96	0.97	0.45
KAU OC 16	0.97	1.28	0.40
KAU OC 17	1.19	0.92	0.36
KAU OC 18	0.82	0.98	0.54
KAU OC 19	0.86	0.97	0.66
KAU OC 20	0.66	1.26	0.29
KAU OC 21	0.92	1.00	0.57
KAU OC 22	0.93	0.87	0.54
KAU OC 23	1.21	1.13	0.40
KAU OC 24	0.84	0.96	0.61
KAU OC 25	0.76	1.04	0.66
KAU OC 26	0.68	1.14	0.34
KAU OC 27	1.10	0.85	0.47
KAU OC 28	1.09	1.20	0.45
KAU OC 29	1.05	0.87	0.54
KAU OC 30	1.03	1.31	0.33
KAU OC 31	1.17	0.88	0.50
KAU OC 32	0.97	1.22	0.32
KAU OC 33	1.05	0.79	0.67
KAU OC 34	1.22	1.13	0.39
KAU OC 35	1.12	1.20	0.40
SE m (±)	0.030	0.025	0.019
CD (0.05)	0.15	0.27	0.09

Table 18. Relative growth rate of tulsi accessions at 30, 60 DAT and at harvest

Accessions	RGR (g/g/day)		
	30 DAT	60 DAT	Harvest
IC 583278	0.100	0.031	0.006
IC 583288	0.118	0.027	0.004
IC 583296	0.116	0.022	0.004
IC 583303	0.100	0.033	0.007
IC 583304	0.102	0.030	0.008
IC 583305	0.116	0.021	0.004
IC 583314	0.117	0.022	0.004
IC 583317	0.102	0.032	0.006
IC 583318	0.114	0.024	0.005
IC 583322	0.109	0.025	0.006
KAU OC 11	0.106	0.022	0.005
KAU OC 12	0.111	0.021	0.008
KAU OC 13	0.100	0.027	0.006
KAU OC 14	0.096	0.026	0.005
KAU OC 15	0.109	0.023	0.007
KAU OC 16	0.109	0.028	0.004
KAU OC 17	0.116	0.018	0.005
KAU OC 18	0.104	0.026	0.009
KAU OC 19	0.105	0.025	0.011
KAU OC 20	0.097	0.035	0.004
KAU OC 21	0.108	0.024	0.009
KAU OC 22	0.108	0.021	0.009
KAU OC 23	0.117	0.021	0.003
KAU OC 24	0.104	0.025	0.010
KAU OC 25	0.101	0.028	0.010
KAU OC 26	0.098	0.032	0.006
KAU OC 27	0.114	0.018	0.007
KAU OC 28	0.114	0.024	0.004
KAU OC 29	0.112	0.020	0.008
KAU OC 30	0.111	0.030	0.005
KAU OC 31	0.114	0.020	0.007
KAU OC 32	0.109	0.027	0.005
KAU OC 33	0.112	0.018	0.010
KAU OC 34	0.117	0.021	0.004
KAU OC 35	0.114	0.027	0.004
SE m (±)	0.001	0.0007	0.0004
CD (0.05)	0.011	0.004	0.001

4.1.4 Diversity analysis based on qualitative characters

Euclidean cluster analysis grouped the 35 accessions of tulsi into three clusters at standard Euclidean distance of 4.00 (Figure 5) using qualitative traits. The accessions having similar qualitative characters were placed in the same cluster of Euclidean dendrogram and accessions included in each cluster are given in Table 19. Among the clusters, cluster I was largest consisting of 16 accessions including 8 NBPGR collections and 8 local collections. These accessions shared common features such as green colour on upper leaf surface, stem and inflorescence. Cluster II consisted of 14 accessions including one NBPGR collection (IC 583314) and 13 local collections and they were similar with respect to purple coloured upper leaf surface, stem and inflorescence. Cluster III consist of 5 accessions including one NBPGR collection (IC 583322) and 4 local accessions based on green coloured upper leaf surface, stem and purple coloured inflorescence.

Inter and intra cluster distances among the accessions are presented in Table 20. The inter cluster distance between the clusters was 1.5. The cluster I and cluster II showed higher intra cluster distance (3.00) and the minimum (0.67) was seen in the cluster III.

4.1.5 Diversity analysis based on quantitative characters

The Euclidean cluster analysis grouped the 35 accessions of tulsi into 6 clusters at standard Euclidean distance of 10.00 (Figure 6). Accessions included in each cluster are given in Table 21. In Euclidean dendrogram, cluster I and cluster III consisted of 9 accessions each and cluster V comprised of 8 accessions. Cluster VI included 5 accessions and Cluster IV included 3 accessions. Cluster II contained only one accession named IC 583288 and this cluster recorded higher mean values for all the traits except length of inflorescence.

Inter and intra cluster distances among clusters are presented in Table 22. The higher inter cluster distance (16410.14) was observed between cluster V and VI.

Minimum inter cluster distance (654.35) was found between cluster II and VI. The intra cluster distance was higher in cluster IV (376.84) and thus more variations were found within cluster IV.

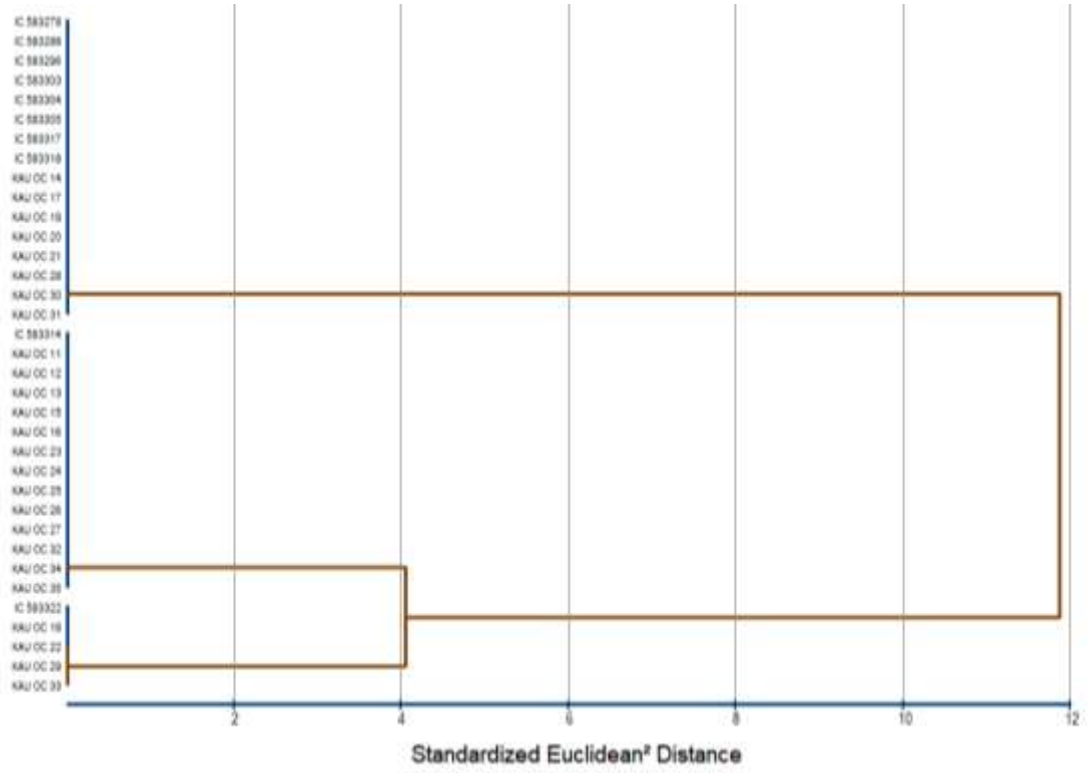


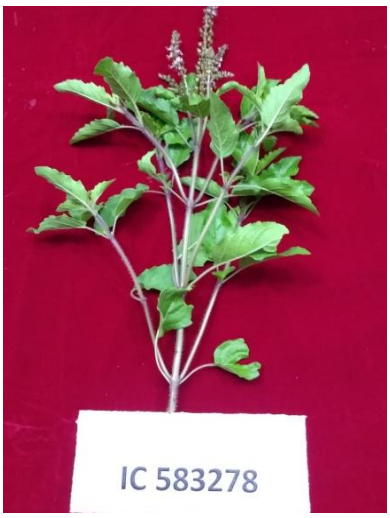
Figure 5. Dendrogram based on qualitative characters of tulsi

Table 19. Clustering based on qualitative characters intulsi

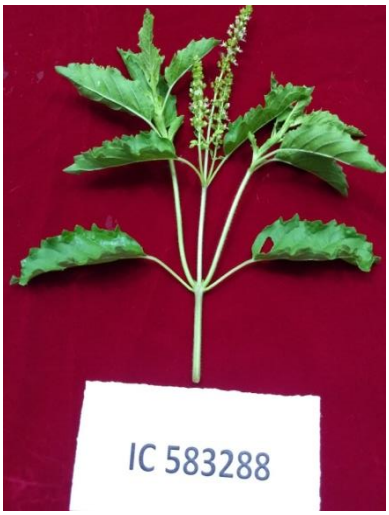
Cluster No.	No. of accessions	Accessions	Similar characters shared
Cluster I	16	IC 583278, IC 583288, IC 583296, IC 583303, IC 583304, IC 583305, IC 583317, IC 583318, KAU OC 14, KAU OC 17, KAU OC 19, KAU OC 20, KAU OC 21, KAU OC 28, KAU OC 30, KAU OC 31	Upper leaf surface, stem and inflorescence green coloured
Cluster II	14	IC 583314, KAU OC 11, KAU OC 12, KAU OC 13, KAU OC 15, KAU OC 16, KAU OC 23, KAU OC 24, KAU OC 25, KAU OC 26, KAU OC 27, KAU OC 32, KAU OC 34, KAU OC 35	Leaf, stem and inflorescence purple coloured
Cluster III	5	IC 583322, KAU OC 18, KAU OC 22, KAU OC 29, KAU OC 33	Upper leaf surface and stem green coloured. Inflorescence purple coloured

Table 20: Inter and intra cluster distance among tulsi accessions for qualitative characters

	Cluster 1	Cluster 2	Cluster 3
Cluster 1	3.00		
Cluster 2	1.50	3.00	
Cluster 3	1.50	1.50	0.67



IC 583278



IC 583288

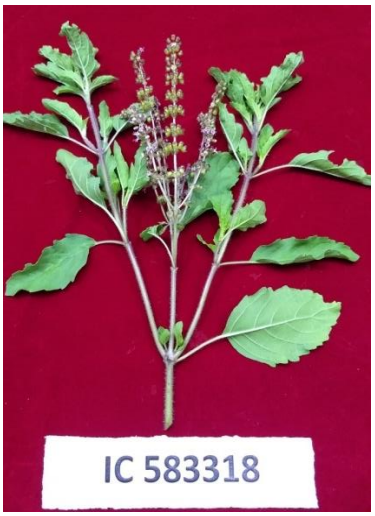
IC 583296

IC 583303

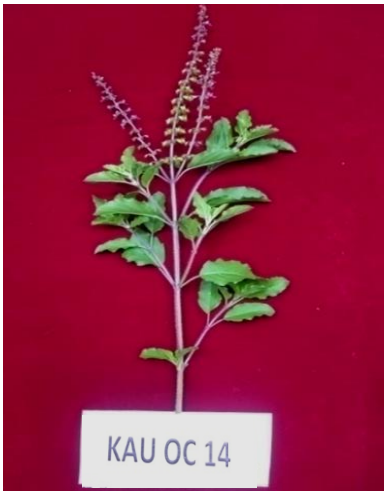
IC 583304

IC 583305

IC 583317



IC 583318



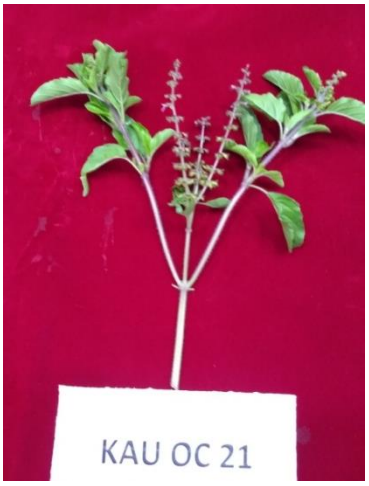
KAU OC 14

Plate 9. Green type tulsi accessions

KAU OC 17

KAU OC 19

KAU OC 20



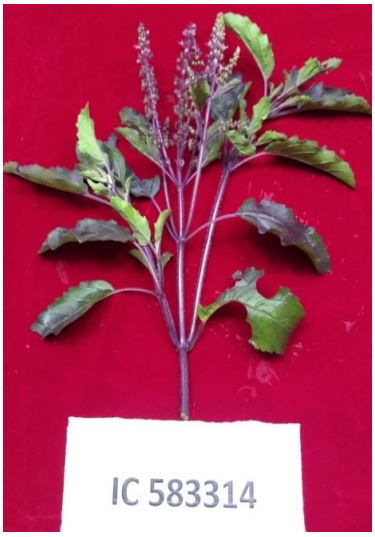
KAU OC 21

KAU OC 28

KAU OC 30

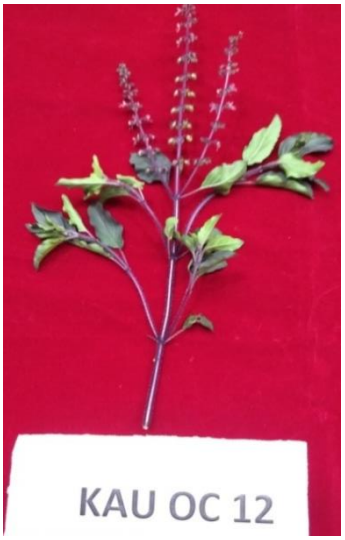
KAU OC 31

Plate 9. Green type tulsi accessions (Contd.)



IC 583314

KAU OC 11



KAU OC 12

KAU OC 13

KAU OC 15

KAU OC 16

KAU OC 23

KAU OC 24

KAU OC 25

Plate 10. Purple type tulsi accessions

KAU OC 26

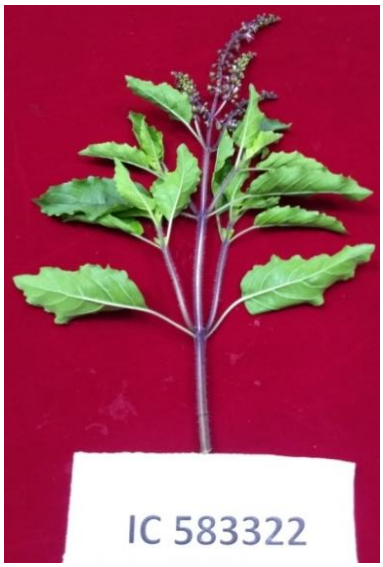
KAU OC 27

KAU OC 32

KAU OC 34

KAU OC 35

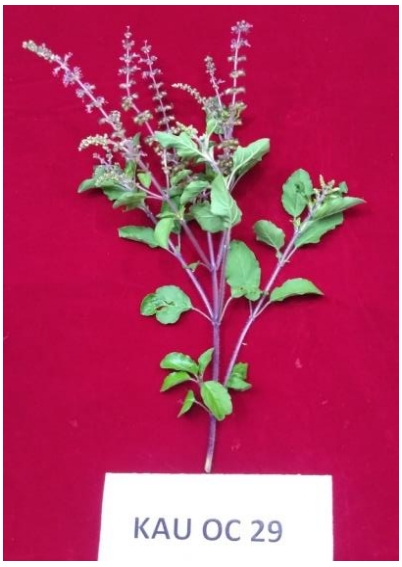
Plate 10. Purple type tulsi accessions (Contd.)



IC 583322

KAU OC 18

KAU OC 22



KAU OC 29

KAU OC 33

Plate 11. Intermediate type tulsi accessions

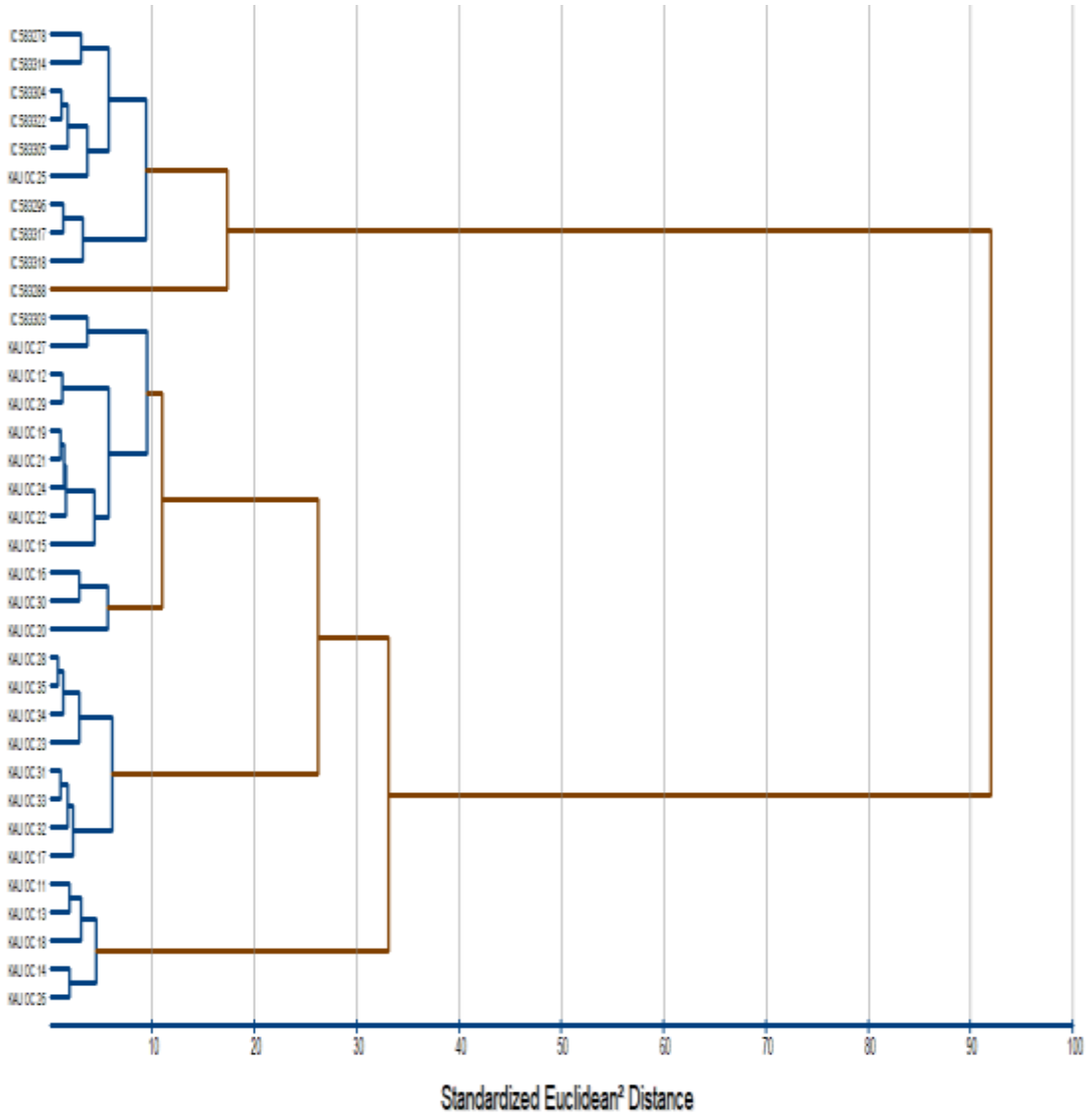


Figure 6. Dendrogram based on quantitative characters of tulsi accessions

Table 21. Clustering based on quantitative characters of tulsi accessions

Cluster No.	No. of accessions	Accessions
ClusterI	9	IC 583278, IC 583314, IC 583304, IC 583322, IC 583305, KAU OC 25, IC 583296, IC 583317, IC 583318
ClusterII	1	IC 583288
ClusterIII	9	IC 583303, , KAU OC 27, KAU OC 12, KAU OC 29, KAU OC 19, KAU OC 21, KAU OC 24, KAU OC 22, KAU OC 15
ClusterIV	3	KAU OC 16, KAU OC 30, KAU OC 20
ClusterV	8	KAU OC 28, KAU OC 35, KAU OC 34, KAU OC 23, KAU OC 31, KAU OC 33, KAU OC 32, KAU OC 17
ClusterVI	5	KAU OC 11, KAU OC 13, KAU OC 18, KAU OC 14, KAU OC 26

Table 22: Inter and intra cluster distance among tulsi accessions for quantitative characters

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Cluster 1	250.96					
Cluster 2	718.27	248.64				
Cluster 3	1002.72	2484.71	294.25			
Cluster 4	4340.10	7506.52	1730.41	376.84		
Cluster 5	8246.06	12568.33	4289.92	867.99	0.00	
Cluster 6	1786.67	654.35	4451.84	10437.90	16410.14	0.00

4.1.6 Principal component analysis (PCA) for quantitative variables

Principal component analysis was carried out for genetic diversity among all tulsi accessions for 8 quantitative variables. The values of the eigen vectors and their contribution to variation are given in Table 23. In the scree plot analysis of PCA (Figure 7), the first three principal components having eigen values greater than 1.0, contributed to 79.7 per cent of variability. The first principal component (PC1) with an eigen value of 3.583 accounted for 44.8 per cent of total variance, and the important contributing traits were leaf area index (0.896), days to 50 per cent flowering (0.828), plant height (0.799), days to first flowering (0.767) and biomass yield (0.549), while, length of infloresence (-0.543) and secondary branches (-0.281) had negative effect on PC1. The second and third principle components contributed to about 21.5 per cent and 13.4 per cent respectively of the total variation.

Table 23. Eigen value and eigen vectors of the eight principal components of quantitative characters

Variable	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**
Eigen values	3.583	1.723	1.072	0.633	0.426	0.339	0.122	0.102
Per cent variance	44.8	21.5	13.4	7.9	5.3	4.2	1.5	1.3
Cumulative variance (%)	44.8	66.3	79.7	87.6	93	97.2	98.7	100
Plant height (cm)	0.799	0.039	-0.334	-0.238	-0.327	0.252	-0.137	-0.06
Primary branches per plant	0.44	0.747	0.172	0.064	-0.277	-0.37	-0.02	-0.005
Secondary branches per plant	-0.281	0.624	0.638	-0.131	-0.045	0.316	0.031	0.061
Leaf area index	0.896	0.128	-0.3	-0.112	0.023	0.041	0.241	0.136
Days to 1st flowering	0.767	-0.324	0.412	0.279	-0.059	0.07	0.124	-0.188
Days to 50 per cent flowering	0.828	-0.246	0.237	0.371	0.076	0.034	-0.152	0.175
Length of inflorescence (cm)	-0.543	0.388	-0.418	0.571	-0.152	0.169	0.041	0
Biomass yield per plant (g)	0.549	0.664	-0.182	0.026	0.456	0.045	-0.065	-0.101

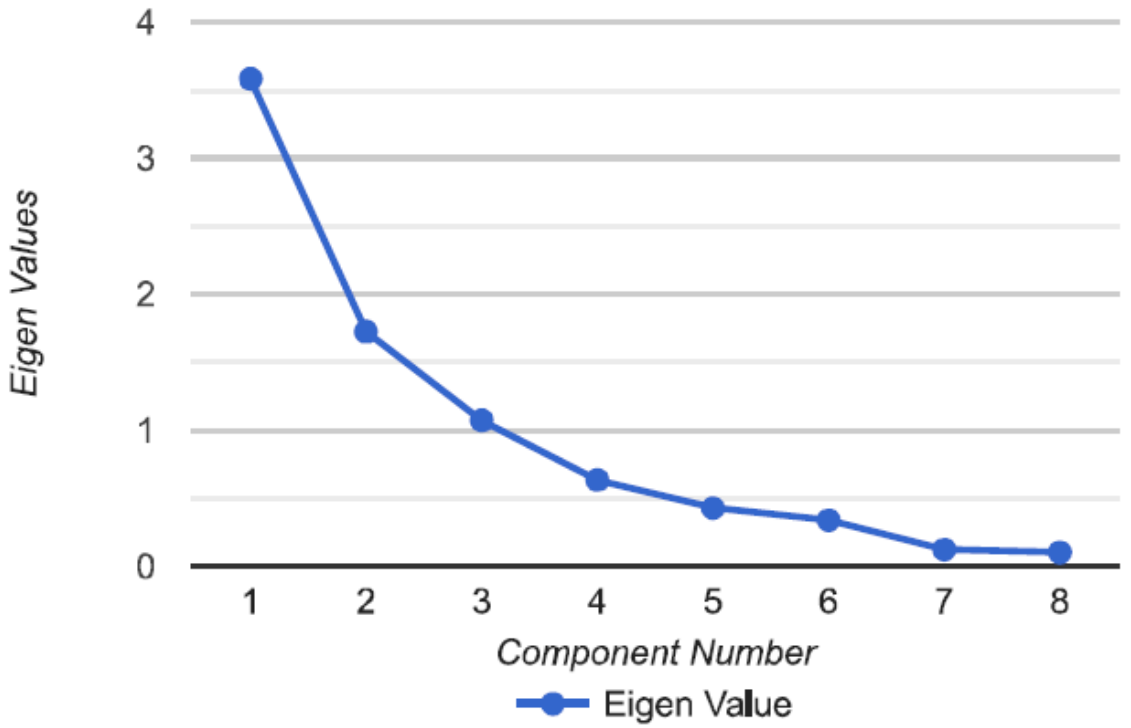


Figure 7. Scree plot showing eigen value in response to the quantitative characters of tulsi accessions

Component Number

1	Plant height	2	Primary branches per plant
3	Secondary branches per plant	4	Leaf area index
5	Days to first flowering	6	Days to 50 per cent flowering
7	Length of inflorescence	8	Biomass yield per plant

4.1.7 Biochemical parameters

4.1.7.1 Chlorophyll content at 30, 60 DAT and at harvest

There was significant difference among the accessions with respect to chlorophyll content (Table 24). At 30 DAT, accession KAU OC 28 recorded higher chlorophyll content (1.35 mg/g), which was on par with IC 583288 (1.32 mg/g), IC 583303 (1.31 mg/g), KAU OC 30 (1.28 mg/g), KAU OC 14 (1.27 mg/g), KAU OC 31 (1.25 mg/g) and KAU OC 20 (1.24 mg/g). Chlorophyll content was lower in KAU OC 26 (0.53 mg/g), whichwas on par with KAU OC 27 (0.57 mg/g), KAU OC 15 (0.58 mg/g), KAU OC 23 (0.63 mg/g), KAU OC 24 (0.65 mg/g) and KAU OC 13 (0.68 mg/g).

At 60 DAT, IC 583303 had higher chlorophyll content (1.76 mg/g), which was on par with KAU OC 28 (1.74 mg/g), IC 583288 (1.72 mg/g), KAU OC 30 (1.70 mg/g), KAU OC 17 (1.69 mg/g), IC 583304 (1.69 mg/g), IC 583278 (1.68 mg/g), KAU OC 21 (1.67 mg/g), KAU OC 31 (1.67 mg/g), KAU OC 14 (1.66 mg/g), IC 583317 (1.63 mg/g) and IC 583305 (1.63 mg/g). Lower value was recorded by KAU OC 23 (0.98 mg/g).

At harvest stage, IC 583288 had higher chlorophyll content (2.40 mg/g), which was on par with IC 583303 (2.38 mg/g), KAU OC 28 (2.37 mg/g) and IC 583304 (2.25 mg/g). Lower chlorophyll content was observed in KAU OC 13 (1.26 mg/g), which was on par with KAU OC 15 (1.27 mg/g) and KAU OC 11 (1.44 mg/g).

4.1.7.2 Carotenoid content at 30, 60 DAT and at harvest

The data on carotenoid content of tulsi accessions are given in Table 25. At 30 DAT, KAU OC 26 and KAU OC 34 recorded significantly higher carotenoid content of 0.65 mg/g and which was on par with KAU OC 32 (0.63 mg/g), KAU OC 27 (0.63 mg/g), KAU OC 35 (0.62 mg/g), IC 583314 (0.62 mg/g), KAU OC 15 (0.61 mg/g), KAU OC 11 (0.60 mg/g), KAU OC 12 (0.59 mg/g) and KAU OC 25 (0.58 mg/g). Carotenoid content was lower in IC 583303 (0.27 mg/g).

At 60 DAT, significantly higher carotenoid content was observed in KAU OC 34 (0.88 mg/g) which was on par with KAU OC 32 (0.87 mg/g), KAU OC 35 (0.86 mg/g), KAU OC 26 (0.86 mg/g), KAU OC 16 (0.84 mg/g), KAU OC 24 (0.84 mg/g), IC 583314 (0.84 mg/g), KAU OC 25 (0.83 mg/g), KAU OC 12 (0.83 mg/g), KAU OC 23 (0.82 mg/g), KAU OC 15 (0.82 mg/g), KAU OC 27 (0.82 mg/g) and KAU OC 13 (0.81 mg/g). Accession KAU OC 28 recorded lower carotenoid content of 0.49 mg/g.

At harvest stage, KAU OC 26 (0.99 mg/g), KAU OC 32 (0.98 mg/g) and KAU OC 34 (0.98 mg/g) recorded higher carotenoid content and it was on par with KAU OC 35 (0.97 mg/g), KAU OC 23 (0.97 mg/g), KAU OC 13 (0.97 mg/g), KAU OC 16 (0.96 mg/g), KAU OC 25 (0.96 mg/g), KAU OC 27 (0.96 mg/g), IC 583314 (0.94 mg/g), KAU OC 24 (0.94 mg/g), KAU OC 15 (0.93 mg/g) and KAU OC 12 (0.92 mg/g). Carotenoid content was found lower in IC 583303 (0.61 mg/g).

4.1.7.3 Essential oil content at 30, 60 DAT and at harvest

Essential oil content of different tulsi accessions is depicted in Table 26. At 30 DAT, higher essential oil content was found in KAU OC 34 (0.93 %), KAU OC 25 (0.93 %), KAU OC 32 (0.91 %), KAU OC 23 (0.89 %), KAU OC 27 (0.89 %) and KAU OC 16 (0.89 %).

At 60 DAT, higher essential oil content was recorded by KAU OC 25 (1.11 %), KAU OC 32 (1.07 %) and KAU OC 23 (1.07 %), followed by KAU OC 34 (1.02 %), KAU OC 15 (1.00 %), KAU OC 11 (1.00 %) and KAU OC 16 (1.00 %). Essential oil content was lower in IC 583288 (0.33 %) which was on par with IC 583278 (0.44 %), KAU OC 33 (0.44 %) and IC 583303 (0.44 %).

At harvest stage, essential oil content was higher in KAU OC 25 (0.93 %) and it was on par with KAU OC 15 (0.89 %), followed by KAU OC 34 (0.84 %). Lower essential oil content was observed in IC 583278 (0.22 %), KAU OC 31 (0.22 %), IC 583288 (0.22 %), IC 583304 (0.22 %), and KAU OC 28 (0.27 %).

Table 24. Chlorophyll content of tulsi accessions at 30, 60 DAT and at harvest

Accessions	Total chlorophyll (mg/g)		
	30 DAT	60 DAT	Harvest
IC 583278	1.14	1.68	2.19
IC 583288	1.32	1.72	2.40
IC 583296	1.13	1.62	1.99
IC 583303	1.31	1.76	2.38
IC 583304	1.19	1.69	2.25
IC 583305	1.18	1.63	2.18
IC 583314	0.74	1.23	1.68
IC 583317	1.18	1.63	1.90
IC 583318	1.23	1.63	2.05
IC 583322	1.12	1.51	1.89
KAU OC 11	0.80	1.10	1.44
KAU OC 12	0.74	1.20	1.49
KAU OC 13	0.68	1.01	1.26
KAU OC 14	1.27	1.66	2.03
KAU OC 15	0.58	1.08	1.27
KAU OC 16	0.70	1.18	1.55
KAU OC 17	1.18	1.69	2.00
KAU OC 18	1.17	1.59	2.08
KAU OC 19	1.20	1.63	2.16
KAU OC 20	1.24	1.59	2.08
KAU OC 21	1.11	1.67	2.10
KAU OC 22	1.23	1.51	1.96
KAU OC 23	0.63	0.98	1.59
KAU OC 24	0.65	1.10	1.63
KAU OC 25	0.71	1.11	1.65
KAU OC 26	0.53	1.04	1.61
KAU OC 27	0.57	1.13	1.58
KAU OC 28	1.35	1.74	2.37
KAU OC 29	1.19	1.43	1.76
KAU OC 30	1.28	1.70	2.07
KAU OC 31	1.25	1.67	2.19
KAU OC 32	0.72	1.07	1.46
KAU OC 33	1.05	1.37	2.01
KAU OC 34	0.75	1.09	1.48
KAU OC 35	0.74	1.18	1.46
SE m (±)	0.045	0.046	0.055
CD (0.05)	0.121	0.136	0.183

Table 25. Carotenoids of tulsi accessions at 30, 60 DAT and at harvest

Accessions	Total carotenoids (mg/g)		
	30 DAT	60 DAT	Harvest
IC 583278	0.37	0.59	0.68
IC 583288	0.28	0.52	0.62
IC 583296	0.36	0.60	0.67
IC 583303	0.27	0.51	0.61
IC 583304	0.33	0.56	0.65
IC 583305	0.35	0.61	0.66
IC 583314	0.62	0.84	0.94
IC 583317	0.33	0.57	0.69
IC 583318	0.29	0.50	0.62
IC 583322	0.37	0.62	0.70
KAU OC 11	0.60	0.81	0.86
KAU OC 12	0.59	0.83	0.92
KAU OC 13	0.56	0.81	0.97
KAU OC 14	0.33	0.62	0.71
KAU OC 15	0.61	0.82	0.93
KAU OC 16	0.55	0.84	0.96
KAU OC 17	0.29	0.60	0.69
KAU OC 18	0.36	0.59	0.73
KAU OC 19	0.34	0.56	0.65
KAU OC 20	0.37	0.55	0.69
KAU OC 21	0.34	0.58	0.71
KAU OC 22	0.39	0.66	0.73
KAU OC 23	0.53	0.82	0.97
KAU OC 24	0.55	0.84	0.94
KAU OC 25	0.58	0.83	0.96
KAU OC 26	0.65	0.86	0.99
KAU OC 27	0.63	0.82	0.96
KAU OC 28	0.28	0.49	0.63
KAU OC 29	0.39	0.60	0.71
KAU OC 30	0.37	0.54	0.63
KAU OC 31	0.32	0.59	0.66
KAU OC 32	0.63	0.87	0.98
KAU OC 33	0.37	0.61	0.69
KAU OC 34	0.65	0.88	0.98
KAU OC 35	0.62	0.86	0.97
SE m (±)	0.023	0.022	0.024
CD (0.05)	0.078	0.069	0.117

Table 26. Essential oil content of tulsi accessions at 30, 60 DAT and at harvest

Accessions	Essential oil content (%)		
	30 DAT	60 DAT	Harvest
IC 583278	0.33	0.44	0.22
IC 583288	0.22	0.33	0.22
IC 583296	0.33	0.56	0.33
IC 583303	0.27	0.44	0.40
IC 583304	0.32	0.49	0.22
IC 583305	0.22	0.78	0.56
IC 583314	0.67	0.89	0.78
IC 583317	0.36	0.67	0.44
IC 583318	0.51	0.71	0.56
IC 583322	0.22	0.62	0.56
KAU OC 11	0.78	1.00	0.78
KAU OC 12	0.78	0.89	0.67
KAU OC 13	0.44	0.67	0.56
KAU OC 14	0.56	0.78	0.58
KAU OC 15	0.67	1.00	0.89
KAU OC 16	0.89	1.00	0.78
KAU OC 17	0.44	0.82	0.67
KAU OC 18	0.69	0.84	0.56
KAU OC 19	0.33	0.60	0.40
KAU OC 20	0.44	0.56	0.33
KAU OC 21	0.57	0.78	0.44
KAU OC 22	0.67	0.84	0.44
KAU OC 23	0.89	1.07	0.71
KAU OC 24	0.56	0.80	0.67
KAU OC 25	0.93	1.11	0.93
KAU OC 26	0.78	0.78	0.67
KAU OC 27	0.89	0.93	0.71
KAU OC 28	0.22	0.51	0.27
KAU OC 29	0.47	0.67	0.56
KAU OC 30	0.33	0.56	0.44
KAU OC 31	0.24	0.56	0.22
KAU OC 32	0.91	1.07	0.78
KAU OC 33	0.33	0.44	0.33
KAU OC 34	0.93	1.02	0.84
KAU OC 35	0.67	0.78	0.73
SE m (±)	0.040	0.035	0.034
CD (0.05)	0.10	0.12	0.08

4.1.8 Diversity analysis based on biochemical characters

The thirty five accessions of tulsi were placed into five clusters at the standard Euclidean distance of 3.0 in cluster analysis (Table 27 and Fig. 8). Cluster III was the biggest cluster with 9 accessions. Cluster IV and V consisted of 7 accessions each and cluster I and cluster II included 6 accessions each. Among the clusters, Cluster IV registered higher mean value for essential oil content and lower mean value for chlorophyll content. Cluster I showed lower mean values for essential oil content and carotenoid content. Maximum divergence was observed between cluster II and cluster IV which recorded higher inter cluster distance (1.26), followed by cluster II and V (1.03). The intra cluster distance was higher in cluster II (0.06).

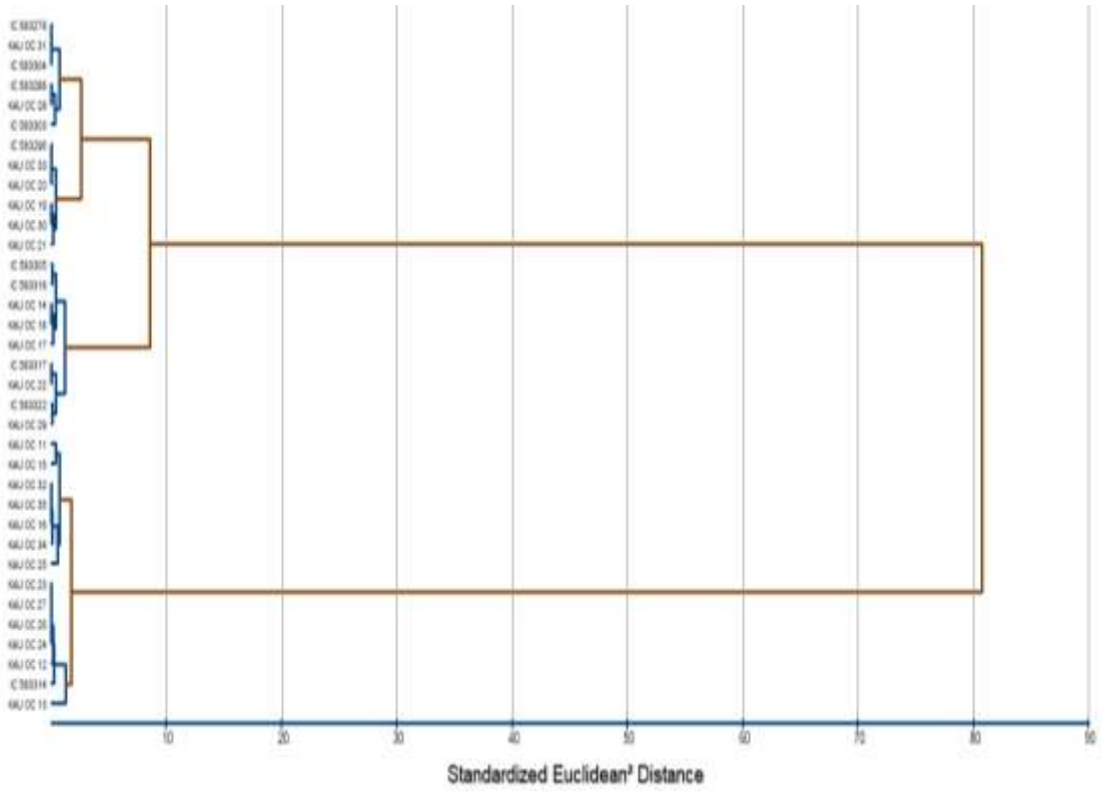


Figure 8. Dendrogram based on biochemical characters of tulsi accessions

Table 27. Clustering based on biochemical characters of tulsi accessions

Cluster No.	No. of accessions	Accessions
Cluster I	6	IC 583278, KAU OC 31, IC 583304, IC 583288, KAU OC 28, IC 583303
Cluster II	6	IC 583296, KAU OC 33, KAU OC 20, KAU OC 19, KAU OC 30, KAU OC 21
Cluster III	9	IC 583305, IC 583318, KAU OC 14 KAU OC 18, KAU OC 17, IC 583317, KAU OC 22, IC 583322, KAU OC 29
Cluster IV	7	KAU OC 11, KAU OC 15, , KAU OC 32, KAU OC 35, KAU OC 16, KAU OC 34, KAU OC 25
Cluster V	7	KAU OC 23, KAU OC 27, KAU OC 26, KAU OC 24, KAU OC 12, IC 583314, KAU OC 13

Table 28: Inter and intra cluster distance among accessions for biochemical characters

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Cluster 1	0.03				
Cluster 2	0.71	0.06			
Cluster 3	0.33	0.14	0.04		
Cluster 4	0.11	1.26	0.70	0.00	
Cluster 5	0.14	1.03	0.62	0.11	0.00

4.1.9 Principal component analysis (PCA) for biochemical variables

Principal component analysis was performed for biochemical characters to identify the contribution of each variable to the total variation. The first principal component (PC1) with eigenvalue more than 1 (Fig. 9 and Table 29) itself accounted for 89.9 per cent of the total variation and the contributing trait was total chlorophyll content (0.962), whereas total carotenoid content (-0.958) and essential oil content (-0.925) had negative effect on PC1.

Table 29. Eigen value and eigen vectors of the three principal components of biochemical characters

Variable	PC1	PC2	PC3
Eigen values	2.698	0.212	0.09
Per cent variance	89.9	7.1	3.0
Cumulative variance (%)	89.9	97.0	100
Total chlorophyl content (mg/g)	0.962	0.166	0.218
Total carotenoid content (mg/g)	-0.958	-0.2	0.206
Essential oil content (%)	-0.925	0.38	0.013

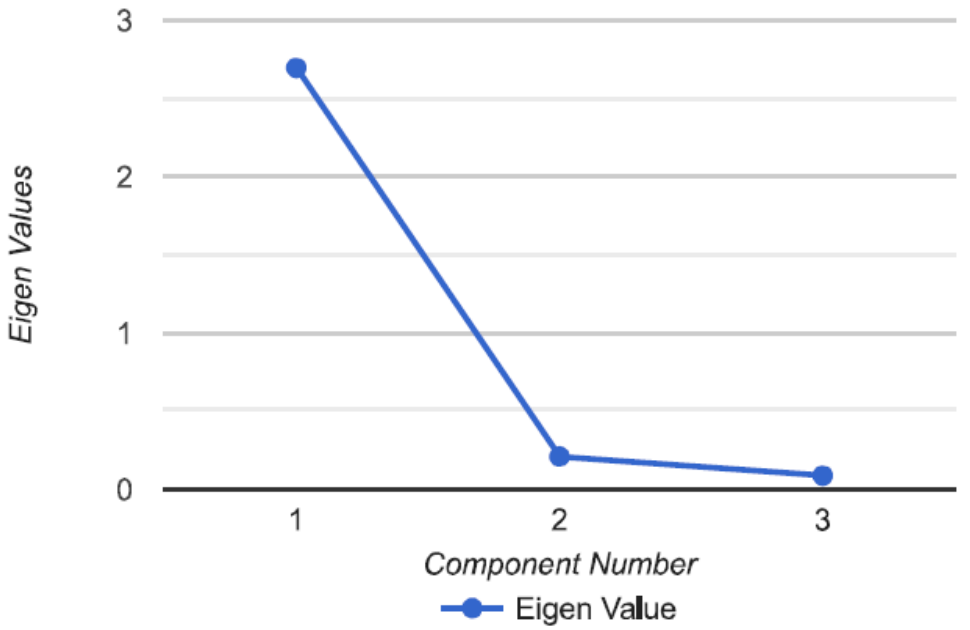


Figure 9. Scree plot showing eigen value in response to the biochemical characters of tulsi accessions

4.1.10 Correlation between growth parameters, biomass yield and biochemical parameters of tulsi at harvest

Data on correlation between growth parameters, biomass yield and biochemical characters of tulsi accessions at harvest are given in Table 30. Plant height showed significant positive correlation with leaf area index (0.808*), total chlorophyll content (0.365*) and biomass yield per plant (0.396*). Significant negative correlation was observed between plant height and total carotenoids (-0.383*). Number of primary branches per plant was positively correlated with secondary branches (0.338*), leaf area index (0.404*) and biomass yield (0.567*). Leaf area index exhibited significant positive correlation with total chlorophyll (0.397*) and biomass yield (0.611*) and negative correlation with total carotenoids (-0.401*). Total chlorophyll was negatively correlated with total carotenoids (-0.910*) and essential oil content (-0.824*). Total carotenoids and essential oil content were positively correlated (0.813*). Though non-significant, biomass yield and essential oil content were negatively correlated.

4.1.11 Path analysis with direct and indirect effects on total biomass yield of tulsi

Characters showed positive correlation with biomass yield per plant was considered for path analysis. Path analysis with direct and indirect effects on total biomass yield per plant of tulsi accessions are given in Table 31 and Figure10. Residual effect contribution on total biomass yield was 0.48.

As per Lenka and Misra (1973), the direct and indirect effects were grouped into:

> 1.00 Veryhigh
0.30 - 0.99 High
0.20 - 0.29 Medium
0.10 - 0.19 Low
0.09 - 0.00 Negligible

4.1.11.1 Direct effect

High positive direct effect on total biomass yield per plant was expressed by primary branches (0.377) and leaf area index at harvest (0.672). Plant height showed moderate negative direct effect (-0.264).

4.1.11.2 Indirect effect

Plant height

Plant height expressed positive high indirect effect on total biomass yield (0.544) through high positive direct effect of leaf area index (0.672). Low positive indirect effect (0.116) was expressed through high positive direct effect of primary branches (0.377).

Primary branches

Primary branches expressed positive medium indirect effect on biomass yield (0.272) through high positive direct effect of leaf area index (0.672). Negligible negative indirect effect (-0.082) was expressed through moderate negative direct effect of plant height (-0.264).

Leaf area index

Leaf area index expressed moderate negative indirect effect on biomass yield (-0.214) through moderate negative direct effect of plant height (-0.264). Low positive indirect effect (0.152) was expressed through high positive direct effect of primary branches (0.377).

Table 30. Correlation matrix of tulsi accessions at harvest

	Plant height	Primary branches	Secondary branches	Leaf area index	Total chlorophyll	Total carotenoid	Biomass yield	Essential oil content
Plant height	1.000							
Primary branches	0.309	1.000						
Secondary branches	-0.296	0.338*	1.000					
Leaf area index	0.808*	0.404*	-0.321	1.000				
Total chlorophyll	0.365*	0.263	0.003	0.397*	1.000			
Total carotenoids	-0.383*	-0.306	-0.042	-0.401*	-0.910*	1.000		
Biomass yield	0.396*	0.567*	0.126	0.611*	0.212	-0.158	1.000	
Essential oil content	-0.307	-0.274	0.017	-0.300	-0.824*	0.813*	-0.103	1.000

Table 31. Path analysis with direct and indirect effects on total biomass yield of tulsi

	V_1	V_2	V_3
V_1	**-0.264**	0.116	0.544
V_2	-0.082	**0.377**	0.272
V_3	-0.214	0.152	**0.672**

Residual effect: 0.48 **Direct effect: total biomass yield at harvest**

V_1: Plant height at harvest, V_2: Primary branches at harvest, V_3: Leaf area index at harvest

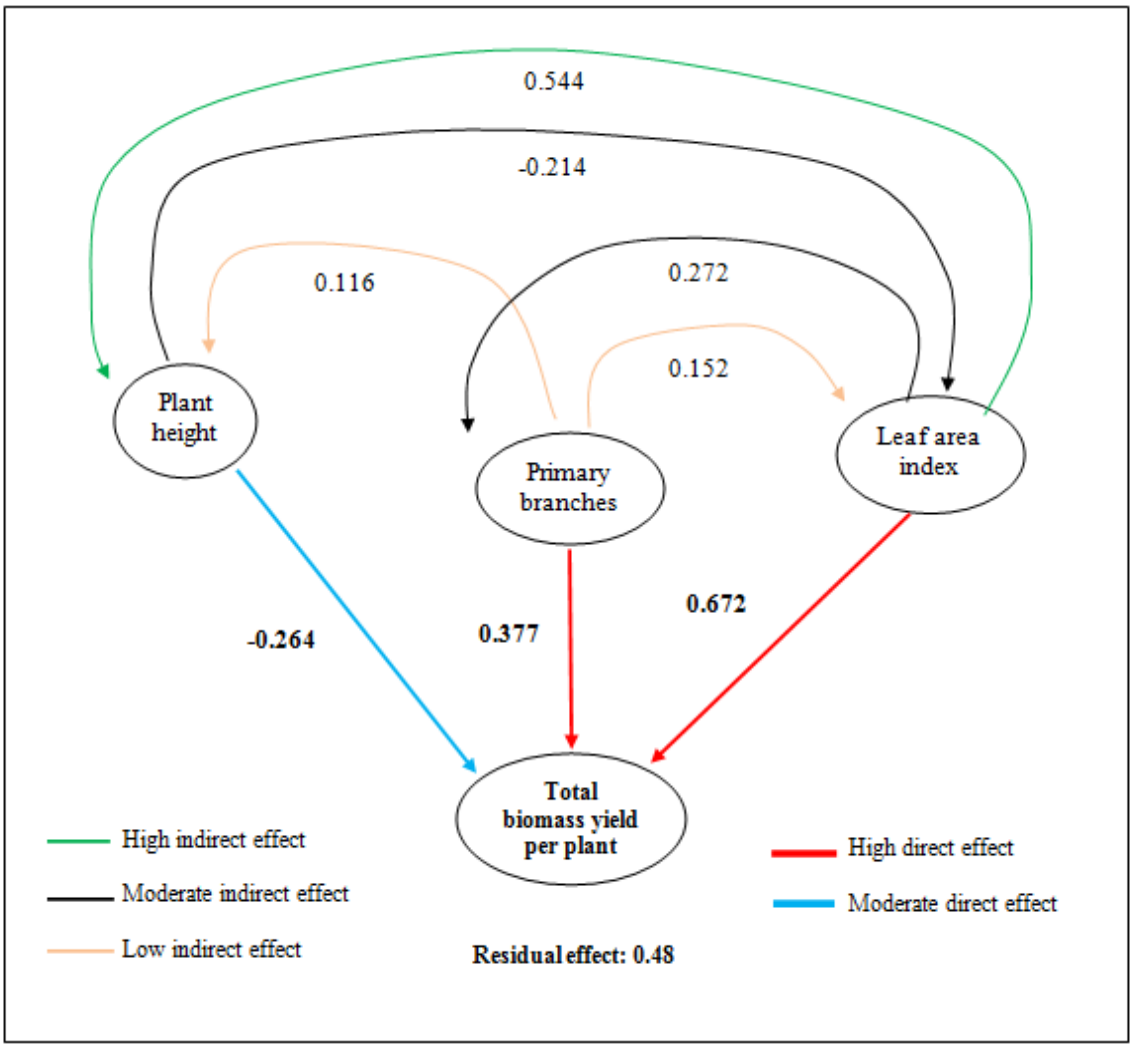


Figure 10. Path diagram for selected characters in tulsi

4.1.12 Selection of superior genotypes for biomass yield

All the accessions were scored and ranked based on total biomass yield per plant and the factors showing significant direct effect with it, *i.e.* primary branches and leaf area index and the result is given below in Table 32.

Table 32. Scoring and ranking of tulsi accessions for biomass yield and parameters

Accessions	Score for morphological parameters			Total score	Rank
	Total biomass yield per plant	Primary branches	Leaf area index		
IC 583278	29	20	9	58	18
IC 583288	1	1	1	3	1
IC 583296	4	3	5	12	3
IC 583303	26	17	10	53	17
IC 583304	15	20	8	43	13
IC 583305	16	14	4	34	10
IC 583314	5	23	3	31	8
IC 583317	18	3	7	28	6
IC 583318	3	6	2	11	2
IC 583322	12	11	7	30	7
KAU OC 11	33	15	26	74	24
KAU OC 12	20	19	12	51	16
KAU OC 13	32	24	28	84	27
KAU OC 14	34	24	28	86	28
KAU OC 15	25	18	19	62	21
KAU OC 16	11	10	21	42	12
KAU OC 17	19	7	19	45	14
KAU OC 18	27	24	25	76	25
KAU OC 19	17	22	22	61	20
KAU OC 20	30	7	27	64	22
KAU OC 21	16	15	16	47	15
KAU OC 22	28	20	23	71	23
KAU OC 23	7	5	20	32	9
KAU OC 24	24	25	22	71	23
KAU OC 25	22	19	18	59	19
KAU OC 26	31	21	26	78	26
KAU OC 27	23	4	16	43	13
KAU OC 28	8	6	13	27	5
KAU OC 29	21	16	14	51	16
KAU OC 30	2	2	17	21	4
KAU OC 31	10	9	15	34	10
KAU OC 32	13	21	24	58	18
KAU OC 33	14	13	13	40	11
KAU OC 34	6	12	12	30	7
KAU OC 35	9	8	11	28	6

Accession IC 583288 was the top rank holder with respect to biomass yield followed by IC 583318, IC 583296 and KAU OC 30 (Plate 12). Important characters of selected four high yielding accessions are given in Table 33. The biomass yield per plant at harvest was 443.7 g, 431.7 g, 427 g and 433.7 g respectively for accessions IC 583288, IC 583318, IC 583296 and KAU OC 30. Essential oil content at harvest was 0.22 per cent for IC 583288, 0.56 per cent for IC 583318, 0.33 per cent for IC 583296 and 0.44 per cent for KAU OC 30. Leaf colour, stem colour and inflorescence colour were green in all the four accessions.

4.1.13 Regression analysis

The logistic binomial estimate of colour influencing essential oil content is given in Table 34. The high value of odds ratio Exp (B) and positive coefficient indicated that colour had a positive relation with essential oil content and it expressed a significant value less than 0.005 which was the constant.

Based on Exp (B) value from the regression model, expected percentage of improvement for essential oil content over the base population was 80.41. Indicating that colour can be used as a strong morphological marker for selecting genotypes for high essential oil content.

Table 34. Logistic binomial estimate of colour influencing essential oil content

Variables	Coefficient	Standard error	Wald	Significance	Exp (B)	Expected per cent of improvement over population
Colour	8.320	2.737	9.237	0.002	4.105	80.41
Constant	-4.327	1.532	7.978	0.005	0.013	

Table 33. Important characters of selected four high yielders

Sl. No.	Accessions	Characters
1	IC 583288	PH-117.6 cm, PB- 13.44 nos., SB-67.78 nos., LI- 1.19, DF-37 days, BY- 443.7 g, CH-2.40 mg/g, CR- 0.62 mg/g, EO- 0.22 %, LC- Green, SC- Green, IC- Green
2	IC 583318	PH-109.02 cm, PB- 11.78 nos., SB-57.44 nos., LI- 1.15, DF-31.67 days, BY- 431.7 g, CH-2.05 mg/g, CR- 0.62 mg/g, EO- 0.56 %, LC- Green, SC- Green, IC- Green
3	IC 583296	PH-106.77 cm, PB- 12.33 nos., SB-45.89 nos., LI- 1.08, DF-35 days, BY- 427 g, CH-1.99 mg/g, CR- 0.62 mg/g, EO- 0.33 %, LC- Green, SC- Green, IC- Green
4	KAU OC 30	PH-102.32 cm, PB- 12.45 nos., SB-64.67 nos., LI- 0.74, DF-29.67 days, BY- 433.7 g, CH-2.07 mg/g, CR- 0.63 mg/g, EO- 0.44 %, LC- Green, SC- Green, IC- Green

PH	:	Plant height at harvest	CH	:	Total chlorophyll at harvest
PB	:	Primary branches at harvest	CR	:	Total carotenoids at harvest
SB	:	Secondary branches at harvest	EO	:	Essential oil content at harvest
LI	:	Leaf area index at harvest	LC	:	Upper leaf surface colour
DF	:	Days to 50 % flowering	SC	:	Stem colour
BY	:	Total biomass yield per plant at harvest	IC	:	Infloresence colour

4.1.14 Selection of superior genotypes for essential oil content

All the accessions were scored and ranked based on essential oil content and carotenoid content and the result is given below in Table 35.

Results showed that KAU OC 25 and KAU OC 34 were the top rank holderswith respect to essential oil followed by KAU OC 32 (Plate 13). The essential oil content at harvest was 0.93 per cent, 0.84 per cent and 0.78 per cent and carotenoid content was 0.96 mg/g, 0.98 mg/g, and 0.98 mg/g respectively for accession KAU OC 25, KAU OC 34 and KAU OC 32.

4.1.15 Selection of superior genotypes for both biomass yield and essential oil content

An overall scoring and ranking was done to identify accessions with high biomass yield and essential oil content (Table 36). IC 583288 and KAU OC 34 were found most suitable for dual purpose followed by KAU OC 35 (Plate 14). These accessions could be recommended for commercial cultivation for high biomass and essential oil content.

IC 583288

IC 583318

IC 583296

KAU OC 30

Plate 12. Tulsi accessions with high biomass yield

Table 35. Scoring and ranking of tulsi accessions for essential oil and carotenoid content

Accessions	Score-carotenoid content	Score- essential oil content	Total score	Rank
IC 583278	13	14	27	14
IC 583288	18	14	32	17
IC 583296	14	12	26	13
IC 583303	19	11	30	16
IC 583304	16	14	30	16
IC 583305	15	9	24	12
IC 583314	5	4	9	4
IC 583317	12	10	22	11
IC 583318	18	9	27	14
IC 583322	11	9	20	10
KAU OC 11	8	4	12	6
KAU OC 12	7	7	14	7
KAU OC 13	3	9	12	6
KAU OC 14	10	8	18	8
KAU OC 15	6	2	8	3
KAU OC 16	4	4	8	3
KAU OC 17	12	7	19	9
KAU OC 18	9	9	18	8
KAU OC 19	16	11	27	14
KAU OC 20	12	12	24	12
KAU OC 21	10	10	20	10
KAU OC 22	9	10	19	9
KAU OC 23	3	6	9	4
KAU OC 24	5	7	12	6
KAU OC 25	4	1	5	1
KAU OC 26	1	7	8	3
KAU OC 27	4	6	10	5
KAU OC 28	17	13	30	16
KAU OC 29	10	9	19	9
KAU OC 30	17	10	27	14
KAU OC 31	15	14	29	15
KAU OC 32	2	4	6	2
KAU OC 33	12	12	24	12
KAU OC 34	2	3	5	1
KAU OC 35	3	5	8	3

Table 36. Scoring and ranking of tulsi accessions for biomass and essential oil content

Accessions	Total score for morphological parameters	Total score for biochemical parameters	Total score	Rank
IC 583278	58	27	85	18
IC 583288	3	32	35	1
IC 583296	12	26	38	3
IC 583303	53	30	83	17
IC 583304	43	30	73	16
IC 583305	34	24	58	10
IC 583314	31	9	40	4
IC 583317	28	22	50	7
IC 583318	11	27	38	3
IC 583322	30	20	50	7
KAU OC 11	74	12	86	19
KAU OC 12	51	14	65	13
KAU OC 13	84	12	96	23
KAU OC 14	86	18	104	24
KAU OC 15	62	8	70	15
KAU OC 16	42	8	50	7
KAU OC 17	45	19	64	12
KAU OC 18	76	18	94	22
KAU OC 19	61	27	88	20
KAU OC 20	64	24	88	20
KAU OC 21	47	20	67	14
KAU OC 22	71	19	90	21
KAU OC 23	32	9	41	5
KAU OC 24	71	12	83	17
KAU OC 25	59	5	64	12
KAU OC 26	78	8	86	19
KAU OC 27	43	10	53	8
KAU OC 28	27	30	57	9
KAU OC 29	51	19	70	15
KAU OC 30	21	27	48	6
KAU OC 31	34	29	63	11
KAU OC 32	58	6	64	12
KAU OC 33	40	24	64	12
KAU OC 34	30	5	35	1
KAU OC 35	28	8	36	2

KAU OC 25

KAU OC 34

KAU OC 32

Plate 13. Tulsi accessions with high essential oil content

IC 583288

KAU OC 34

KAU OC 35

Plate 14. Tulsi accessions with high biomass yield and essential oil content

4.2 Experiment 2. Effect of shade and harvesting method on the performance of tulsi

4.2.1 Growth and yield attributes

4.2.1.1 Plant height at harvests

The effect of growing condition, harvesting method and their interaction on plant height is given in Table 37. Growing condition significantly influenced plant height at first and second harvests. In general taller plants were observed under 50 per cent shade compared to open during both years of study.

Plant height at first harvest was not significantly influenced by harvesting methods, while it significantly affected the plant height at second harvest. During second harvest, taller plants were observed in S_2 (harvesting at 30 cm height from ground level at 75 and 135 DAT) during first and second years (46.50 cm and 44.80 cm) and it was statistically on par with S_4 (harvesting at 30 cm height from ground level at 90 and 150 DAT) (44.00 cm and 42.40 cm).

Significant interaction was observed between growing condition and harvesting method on plant height. During both the years of study at first harvest, irrespective of harvesting methods plants grown under 50 per cent shade recorded greater plant height. At second harvest taller plants were observed under 50 per cent shade and harvesting at 30 cm height from ground level.

4.2.1.2 Total biomass yield at harvests

Data on effect of growing condition, harvesting method and interaction effect of growing condition and harvesting method on biomass yield of tulsi is given in Table 38. Among growing conditions, plants grown under 50 per cent shade recorded greater biomass yield at first harvest (7.79 t/ha and 6.77 t/ha), second harvest (2.81 t/ha and 2.43 t/ha) and total biomass yield (10.59 t/ha and 9.19 t/ha) compared to open condition. Pooled data also showed the same trend.

Harvesting method significantly influenced biomass yield of tulsi. Biomass yield at first harvest was higher in S_1 (harvesting at 20 cm height from ground level at 75 and 135 DAT), while biomass yield at second harvest was greater in S_2 (harvesting at 30 cm height from ground level at 75 and 135 DAT) and these two treatments were statistically on par. The pooled data of total biomass yield revealed that harvesting at 20 cm height from ground level at 75 and 135 DAT (10.19 t/ha) and harvesting at 30 cm height at 75 and 135 DAT (10.24 t/ha) had greater biomass yield compared to harvesting at 20 cm height at 90 and 150 DAT (7.87 t/ha) and harvesting at 30 cm height from ground level at 90 and 150 DAT (7.76 t/ha).

Interaction effect of growing condition and harvesting method on biomass yield was significant. Greater biomass yield at first harvest was obtained under 50 per cent shade with harvesting at 20 cm height from ground level at 75 and 135 DAT (8.99 t/ha and 7.60 t/ha), while biomass yield at second harvest was greater under 50 per cent shade with harvesting at 30 cm height from ground level at 75 and 135 DAT (3.75 t/ha and 3.30 t/ha) and these two treatment combinations were statistically on par. Total biomass yield and pooled data were also greater in these two treatment combinations.

4.2.2 Chemical and biochemical observations

4.2.2.1 Chlorophyll content at harvests

The effect of growing condition, harvesting method and their interaction on chlorophyll content of tulsiat harvests is given in Table 39. Growing condition significantly influenced chlorophyll content of tulsi during first year. Plants grown under 50 per cent shade recorded greater chlorophyll content at first and second harvests (1.42 mg/g and 1.31 mg/g).

Among harvesting methods, harvesting at 20 cm height from ground level at 75 and 135 DAT and harvesting at 30 cm height from ground level at 75 and 135 DAT recorded greater chlorophyll content at first harvest during both years of study.

Chlorophyll content at second harvest was not significantly influenced by harvesting method.

The interaction effect of growing condition and harvesting method on chlorophyll content was significant except at second harvest during second year. Chlorophyll content at first harvest was greater under 50 per cent shade with harvesting at 20 cm height from ground level at 75 and 135 DAT and under 50 per cent shade with harvesting at 30 cm height from ground level at 75 and 135 DAT during both years of study. During first year at second harvest, irrespective of harvesting method plants grown under 50 per cent shade, recorded greater chlorophyll content.

4.2.2.2 Carotenoid content at harvests

Data on effect of growing condition, harvesting method and interaction of growing condition and harvesting method on carotenoid content is presented in Table 40. Growing condition significantly influenced carotenoid content of tulsi except at second harvest during second year. Plants grown under open condition recorded greater carotenoid content than 50 per cent shade during both years of study.

Harvesting method had no significant effect on carotenoid content of tulsi during both years of study. The interaction effect of growing condition and harvesting method on carotenoid content was significant except at second harvest during second year. During first year at first harvest, irrespective of harvesting method plants grown under open condition, recorded greater carotenoid content. At second harvest, irrespective of harvesting methods plants under open condition and plants under 50 per cent shade with harvesting at 20 cm height from ground level at 90 and 150 DAT recorded greater carotenoid content. During second year at first harvest, irrespective of harvesting methods plants under open condition and plants under 50 per cent shade with harvesting at 30 cm height from ground level at 90 and 150 DAT recorded greater carotenoid content.

Table 37. Direct and interaction effects of growing condition and harvesting method on plant height (cm) of tulsi

Treatments	2019 -2020		2020-2021	
	1st harvest	2nd harvest	1st harvest	2nd harvest
Growing condition				
M_1-Open	49.35	38.15	45.05	37.10
M_2- 50 % shade	64.15	43.90	57.45	40.10
CD (0.05)	5.09	4.73	4.76	2.01
Harvesting method				
S_1- Harvesting at 20 cm height from ground level at 75 and 135 DAT	56.80	37.60	48.20	33.60
S_2-Harvesting at 30 cm height from ground level at 75 and 135 DAT	52.90	46.50	50.10	44.80
S_3-Harvesting at 20 cm height from ground level at 90 and 150 DAT	58.30	36.00	53.30	33.60
S_4- Harvesting at 30 cm height from ground level at 90 and 150 DAT	59.00	44.00	53.40	42.40
CD (0.05)	NS	4.34	NS	3.92
Treatment combination				
M_1 X S_1	48.80	35.40	42.60	32.20
M_1 X S_2	45.60	42.40	43.80	43.20
M_1 X S_3	52.60	34.00	45.60	33.00
M_1 X S_4	50.40	40.80	48.20	40.00
M_2 X S_1	64.80	39.80	53.80	35.00
M_2 X S_2	60.20	50.60	56.40	46.40
M_2 X S_3	64.00	38.00	61.00	34.20
M_2 X S_4	67.60	47.20	58.60	44.80
CD (0.05)	10.09	6.14	6.41	5.54

Table 38. Direct and interaction effect of growing condition and harvesting method on biomass yield (t/ha)

Treatments	2019 -2020			2020-2021			Total yield (pooled data)
	1st harvest	2nd harvest	Total	1st harvest	2nd harvest	Total	
Growing condition							
M_1-Open	6.54	2.41	8.95	5.44	1.89	7.33	8.14
M_2- 50 % shade	7.79	2.81	10.59	6.77	2.43	9.19	9.89
CD (0.05)	0.81	0.23	1.03	1.29	0.31	1.18	1.20
Harvesting method							
S_1- Harvesting at 20 cm height from ground level at 75 and 135 DAT	8.23	3.00	11.28	7.02	2.48	9.11	10.19
S_2-Harvesting at 30 cm height from ground level at 75 and 135 DAT	7.79	3.37	11.15	6.50	2.84	9.33	10.24
S_3-Harvesting at 20 cm height from ground level at 90 and 150 DAT	6.40	1.98	8.37	5.63	1.75	7.37	7.87
S_4- Harvesting at 30 cm height from ground level at 90 and 150 DAT	6.19	2.09	8.28	5.28	1.96	7.244	7.76
CD (0.05)	1.05	0.40	1.18	0.75	0.34	0.85	0.77
Treatment combination							
M_1 X S_1	7.56	2.83	10.39	6.40	1.83	8.22	9.31
M_1 X S_2	6.99	2.98	9.96	5.59	2.35	7.94	8.95
M_1 X S_3	5.95	1.96	7.91	5.01	1.64	6.65	7.28
M_1 X S_4	5.65	1.89	7.54	4.77	1.75	6.51	7.02
M_2 X S_1	8.99	3.19	12.17	7.60	2.76	10.00	11.08
M_2 X S_2	8.59	3.75	12.34	7.40	3.30	10.72	11.53
M_2 X S_3	6.85	1.99	8.84	6.24	1.85	8.09	8.46
M_2 X S_4	6.73	2.30	9.02	5.80	2.18	7.97	8.50
CD (0.05)	1.49	0.56	1.66	1.07	0.55	1.20	1.08

Table 39. Direct and interaction effect of growing condition and harvesting method on chlorophyll content (mg/g)

Treatments	2019 -2020		2020-2021	
	1st harvest	2nd harvest	1st harvest	2nd harvest
Growing condition				
M_1-Open	1.05	1.07	1.08	1.08
M_2- 50 % shade	1.42	1.31	1.24	1.15
CD (0.05)	0.14	0.06	NS	NS
Harvesting method				
S_1- Harvesting at 20 cm height from ground level at 75 and 135 DAT	1.40	1.23	1.33	1.12
S_2-Harvesting at 30 cm height from ground level at 75 and 135 DAT	1.45	1.21	1.29	1.12
S_3-Harvesting at 20 cm height from ground level at 90 and 150 DAT	1.01	1.17	1.06	1.14
S_4- Harvesting at 30 cm height from ground level at 90 and 150 DAT	1.07	1.14	0.96	1.09
CD (0.05)	0.14	NS	0.11	NS
Treatment combination				
M_1 X S_1	1.12	1.14	1.17	1.09
M_1 X S_2	1.12	1.08	1.21	1.09
M_1 X S_3	0.91	1.05	1.02	1.13
M_1 X S_4	0.95	1.00	0.91	1.04
M_2 X S_1	1.63	1.31	1.48	1.16
M_2 X S_2	1.74	1.35	1.37	1.15
M_2 X S_3	1.12	1.29	1.09	1.15
M_2 X S_4	1.20	1.28	1.00	1.15
CD (0.05)	0.20	0.20	0.16	NS

Table 40. Direct and interaction effect of growing condition and harvesting method on carotenoid content (mg/g)

Treatments	2019 -2020		2020-2021	
	1st harvest	2nd harvest	1st harvest	2nd harvest
Growing condition				
M_1-Open	0.88	1.13	0.87	1.08
M_2- 50 % shade	0.69	1.02	0.75	0.99
CD (0.05)	0.05	0.06	0.09	NS
Harvesting method				
S_1- Harvesting at 20 cm height from ground level at 75 and 135 DAT	0.77	1.04	0.81	1.01
S_2-Harvesting at 30 cm height from ground level at 75 and 135 DAT	0.79	1.09	0.80	1.03
S_3-Harvesting at 20 cm height from ground level at 90 and 150 DAT	0.80	1.12	0.80	1.05
S_4- Harvesting at 30 cm height from ground level at 90 and 150 DAT	0.77	1.05	0.84	1.03
CD (0.05)	NS	NS	NS	NS
Treatment combination				
M_1 X S_1	0.88	1.10	0.85	1.02
M_1 X S_2	0.87	1.15	0.89	1.09
M_1 X S_3	0.90	1.16	0.86	1.09
M_1 X S_4	0.87	1.11	0.88	1.11
M_2 X S_1	0.66	0.96	0.76	1.01
M_2 X S_2	0.70	1.02	0.71	0.97
M_2 X S_3	0.70	1.09	0.74	1.02
M_2 X S_4	0.68	1.00	0.80	0.95
CD (0.05)	0.12	0.12	0.11	NS

4.2.2.3 Essential oil yield at harvests

The influence of growing condition, harvesting method and interaction of growing condition and harvesting method on essential oil yield of tulsi at harvests is given in Table 41. Among growing conditions, plants grown under 50 per cent shade recorded greater essential oil yield at first harvest (61.34 kg/ha and 49.87 kg/ha) and second harvest (20.78 kg/ha and 17.07 kg/ha) during both years of study.

During first year at first harvest, harvesting at 20 cm height from ground level at 75 and 135 DAT had greater essential oil yield (63.36 kg/ha), while essential oil yield at second harvest was greater in harvesting at 30 cm height from ground level at 75 and 135 DAT (23.13 kg/ha) and these two treatments were statistically on par. During second year at first harvest, harvesting at 20 cm height from ground level at 75 and 135 DAT recorded significantly greater essential oil yield (51.36 kg/ha) followed by harvesting at 30 cm height from ground level at 75 and 135DAT (46.29 kg/ha). At second harvest, harvesting at 30 cm height from ground level at 75 and 135DAT had significantly greater essential oil yield (19.31 kg/ha).

Interaction effect of growing condition and harvesting method on essential oil yield was significant at first harvest and second harvest. At first harvest, 50 per cent shade condition with harvesting at 20 cm height from ground level at 75 and 135 DAT recorded greater essential oil yield, at second harvest 50 per cent shade condition with harvesting at 30 cm height from ground level at 75 and 135 DAT recorded greater essential oil yield and these two treatments were statistically on par.

Table 41. Direct and interaction effect of growing condition and harvesting method on essential oil yield (kg/ha)

Treatments	2019 -2020		2020-2021	
	1st harvest	2nd harvest	1st harvest	2nd harvest
Growing condition				
M_1-Open	49.93	16.41	36.45	11.74
M_2- 50 % shade	61.34	20.78	49.87	17.07
CD (0.05)	5.64	3.01	7.12	1.77
Harvesting method				
S_1- Harvesting at 20 cm height from ground level at 75 and 135 DAT	63.36	21.92	51.36	14.21
S_2-Harvesting at 30 cm height from ground level at 75 and 135 DAT	59.29	23.13	46.29	19.31
S_3-Harvesting at 20 cm height from ground level at 90 and 150 DAT	46.74	14.72	38.27	11.37
S_4- Harvesting at 30 cm height from ground level at 90 and 150 DAT	45.17	14.62	36.72	12.74
CD (0.05)	4.62	2.33	5.02	1.88
Treatment combination				
M_1 X S_1	53.60	19.58	44.02	11.66
M_1 X S_2	50.00	20.48	38.05	14.62
M_1 X S_3	41.01	13.14	32.21	10.12
M_1 X S_4	39.11	12.43	31.49	10.56
M_2 X S_1	73.11	24.26	58.70	16.75
M_2 X S_2	68.54	25.77	54.52	24.00
M_2 X S_3	52.47	16.29	44.33	12.62
M_2 X S_4	51.23	16.81	41.94	14.91
CD (0.05)	6.54	3.30	7.01	2.65

4.2.2.4 Eugenol content at harvests

Data pertaining to the effect of growing condition, harvesting method and interaction effect of growing condition and harvesting method on eugenol content at harvests is presented in Table 42. Plants grown under open condition had significantly greater eugenol content at first harvest (65.95 % and 56.97 %) and second harvest (57.05 % and 46.97 %) during both the years of study.

Harvesting method significantly influenced eugenol content at first harvest during both years of study. At first harvest, harvesting at 20 cm height from ground level at 90 and 150 DAT recorded greater eugenol content during both years (62.17 % and 55.19 %) and it was on par with harvesting at 30 cm height from ground level at 90 and150 DAT (60.75 % and 53.92 %). At second harvest, treatments found non significant with respect to eugenol content.

Interaction effect of growing condition and harvesting method on eugenol content was significant at first harvest and second harvest. At first harvest, open condition with harvesting at 20 cm height from ground level at 90 and 150 DAT had greater eugenol content (70.77 % and 60.47 %) and it was on par with open condition and harvesting at 30 cm height from ground level at 90 and 150 DAT (69.71 % and 58.94 %). At second harvest, irrespective of harvesting methods, plants grown under open condition registered greater eugenol content.

4.2.2.5 GCMS analysis of essential oil

The GCMS analysis of tulsi essential oil showed that, the components of essential oil varied slightly under open and 50 per cent shade (Table 43). The major components identified in the essential oil of tulsi under open condition were eugenol, germacrene-D, β-elemene, β-caryophyllene , germacrene -A, copaene, β- ocimene, 4-allyl-1,2-dimethoxy benzene, elemol, delta -cadinene and endo-borneol. Essential oil of tulsi under 50 per cent shade was dominated by eugenol, β-caryophyllene, germacrene-D, β- elemene, β- ocimene, 4-allyl-1,2-dimethoxy benzene, Endo-borneol, β-linalool, β-cubebene, β-cadinene and copaene.

Table 42. Direct and interaction effect of growing condition and harvesting method on eugenol content (%) of tulsi

Treatments	2019 -2020		2020-2021	
	1st harvest	2nd harvest	1st harvest	2nd harvest
Growing condition				
M_1-Open	65.95	57.05	56.97	46.97
M_2- 50 % shade	47.79	36.45	45.06	31.90
CD (0.05)	3.77	1.68	3.73	2.84
Harvesting method				
S_1- Harvesting at 20 cm height from ground level at 75 and 135 DAT	52.63	46.73	47.99	39.61
S_2-Harvesting at 30 cm height from ground level at 75 and 135 DAT	51.93	46.56	46.97	39.70
S_3-Harvesting at 20 cm height from ground level at 90 and 150 DAT	62.17	46.30	55.19	40.01
S_4- Harvesting at 30 cm height from ground level at 90 and 150 DAT	60.75	47.41	53.92	38.42
CD (0.05)	3.43	NS	3.32	NS
Treatment combination				
M_1 X S_1	62.12	56.75	54.94	45.90
M_1 X S_2	61.21	58.11	53.55	47.19
M_1 X S_3	70.77	56.19	60.47	48.25
M_1 X S_4	69.71	57.15	58.94	46.53
M_2 X S_1	43.13	36.70	41.03	33.32
M_2 X S_2	42.65	35.02	40.39	32.22
M_2 X S_3	53.56	36.41	49.94	31.76
M_2 X S_4	51.80	37.66	48.89	30.31
CD (0.05)	4.85	4.64	4.70	3.99

Table 43. Major components identified in tulsi essential oil under open and 50 per cent shade

Open	50 % shade
Eugenol	Eugenol
Germacrene-D	β-caryophyllene
β-elemene	Germacrene-D
β-caryophyllene	β- elemene
Germacrene -A	β- ocimene
Copaene	4-allyl-1,2-dimethoxy benzene
β- ocimene	Endo-borneol
4-allyl-1,2-dimethoxy benzene	β-linalool
Elemol	β-cubebene
Delta - cadinene	β-cadinene
Endo-borneol	Copaene

4.2.3 Rhizosphere observations

4.2.3.1 Total population (cfu/g) of bacteria, fungi and actinomycetes

Data on the population of bacteria, fungi and actinomycetes at 45 DAT, first harvest and second harvest during 2019-20 and 2020-21 are presented in Table 44, 45 and 46. Growing condition, harvesting method and interaction of growing condition and harvesting method had no significant effect on population of bacteria, fungi and actinomycetes during both years. In general population of bacteria and actinomycetes were more under open condition, while population of fungus was more under 50 per cent shade.

The initial population of bacteria in the first year was 19.00 x 10^6 cfu/g. At 45 DAP, mean bacterial population increased thereafter and gradually declined at first harvest and second harvest. The same trend was followed in the second year also. The initial population of fungi was 8.00 x 10^4 cfu/g and 5.00 x 10^4 cfu/g, actinomycetes was 13.00 x 10^4 cfu/gand 7.00 x 10^4 cfu/g respectively at first and second years. Similar to bacterial population, the average population of fungi and actinomycetes increased at 45 DAP and after that it gradually declined.

4.2.3.2 Total number of plant parasitic nematodes from 250 cc soil

The influence of growing condition, harvesting method and their interaction on total population of plant parasitic nematodes at 45 DAT, first harvest and second harvest are given in table 47. Growing condition, harvesting method and interaction of growing condition and harvesting method had no significant effect on total population of plant parasitic nematodes at all stages of observation. In general the population of plant parasitic nematodes was more under 50 per cent shade compared to open.

Initial population of nematodes in the first year was 63.00 and at 45 DAP it showed a sharp increase, thereafter it showed a slight reduction at 75-90 DAT and 135-150 DAT. Similar trend was noticed in the second year also.

Table 44. Effect of treatments and interaction effect of growing condition and harvesting method on total population of bacteria (x 10^6 cfu/g)

Treatments	2019 -2020			2020-2021		
	45 DAT	1st harvest	2nd harvest	45 DAT	1st harvest	2nd harvest
Growing condition						
M_1-Open	1.43 (27.05)	1.35 (23.05)	1.27 (18.65)	1.30 (20.25)	1.22 (16.98)	1.20 (16.00)
M_2- 50 % shade	1.38 (24.30)	1.30 (20.50)	1.23 (17.35)	1.27 (18.90)	1.18 (15.20)	1.12 (13.60)
CD (0.05)	NS	NS	NS	NS	NS	NS
Harvesting method						
S_1- Harvesting at 20 cm height from ground level at 75 and 135 DAT	1.42 (26.70)	1.35 (23.00)	1.27 (18.90)	1.29 (19.90)	1.19 (15.50)	1.14 (14.20)
S_2-Harvesting at 30 cm height from ground level at 75 and 135 DAT	1.39 (24.90)	1.32 (21.20)	1.26 (16.30)	1.29 (19.80)	1.21 (16.40)	1.17 (15.10)
S_3-Harvesting at 20 cm height from ground level at 90 and 150 DAT	1.39 (25.20)	1.31 (21.10)	1.22 (16.90)	1.28 (19.40)	1.18 (15.50)	1.16 (14.80)
S_4- Harvesting at 30 cm height from ground level at 90 and 150 DAT	1.41 (25.90))	1.33 (21.80)	1.25 (17.90)	1.28 (19.20)	1.19 (15.70)	1.19 (15.80)
CD (0.05)	NS	NS	NS	NS	NS	NS
Treatment combination						
M_1 X S_1	1.46 (28.80)	1.38 (24.60)	1.28 (19.40)	1.32 (21.20)	1.21 (16.20)	1.19 (16.00)
M_1 X S_2	1.40 (25.80)	1.34 (22.40)	1.29 (19.60)	1.31 (20.40)	1.23 (17.40)	1.23 (17.20)
M_1 X S_3	1.43 (27.40)	1.37 (23.60)	1.26 (18.40)	1.30 (20.00)	1.20 (16.00)	1.18 (15.60)
M_1 X S_4	1.41 (26.20)	1.33 (21.60)	1.23 (17.20)	1.28 (19.40)	1.19 (15.80)	1.22 (16.60)
M_2 X S_1	1.39 (24.60)	1.32 (21.40)	1.26 (18.40)	1.26 (18.60)	1.16 (14.80)	1.09 (12.40)
M_2 X S_2	1.37 (24.00)	1.30 (20.00)	1.23 (17.00)	1.28 (19.20)	1.18 (15.40)	1.11 (13.00)
M_2 X S_3	1.35 (23.00)	1.26 (18.60)	1.18 (15.40)	1.27 (18.80)	1.17 (15.00)	1.14 (14.00)
M_2 X S_4	1.40 (25.60)	1.34 (22.00)	1.27 (18.60)	1.28 (19.00)	1.19 (15.60)	1.17 (15.00)
CD (0.05)	NS	NS	NS	NS	NS	NS
At sowing	19.00			13.00		

** Logarithmic transformed values, original values are in parentheses

Table 45. Effect of treatments and interaction effect of growing condition and harvesting method on total population of fungi (x 10^4 cfu/g)

Treatments	2019 -2020			2020-2021		
	45 DAT	1st harvest	2nd harvest	45 DAT	1st harvest	2nd harvest
Growing condition						
M_1-Open	1.14 (13.90)	0.93 (8.70)	0.80 (6.20)	1.10 (12.71)	0.92 (8.40)	0.77 (6.10)
M_2- 50 % shade	1.17 (14.85)	1.00 (10.20)	1.00 (6.80)	1.12 (13.10)	0.99 (10.10)	0.83 (6.90)
CD (0.05)	NS	NS	NS	NS	NS	NS
Harvesting method						
S_1- Harvesting at 20 cm height from ground level at 75 and 135 DAT	1.15 (14.10)	0.95 (9.10)	0.95 (6.40)	1.01 (12.70)	0.94 (8.90)	0.83 (6.90)
S_2-Harvesting at 30 cm height from ground level at 75 and 135 DAT	1.16 (14.70)	0.99 (9.90)	0.99 (7.20)	1.12 (13.20)	0.98 (9.60)	0.80 (6.40)
S_3-Harvesting at 20 cm height from ground level at 90 and 150 DAT	1.15 (14.10)	0.96 (9.40)	0.96 (5.90)	1.09 (12.40)	0.97 (9.50)	0.81 (6.70)
S_4- Harvesting at 30 cm height from ground level at 90 and 150 DAT	1.17 (14.60)	0.97 (9.40)	0.97 (6.50)	1.13 (13.40)	0.94 (9.00)	0.77 (6.00)
CD (0.05)	NS	NS	NS	NS	NS	NS
Treatment combination						
M_1 X S_1	1.13 (13.60)	0.92 (8.40)	0.80 (6.40)	1.12 (13.20)	0.90 (8.20)	0.81 (6.60)
M_1 X S_2	1.15 (14.20)	0.96 (9.20)	0.88 (6.80)	1.08 (12.40)	0.95 (9.00)	0.79 (6.20)
M_1 X S_3	1.14 (14.00)	0.93 (8.60)	0.78 (5.60)	1.09 (12.40)	0.92 (8.40)	0.74 (5.60)
M_1 X S_4	1.13 (13.80)	0.93 (8.60)	0.84 (6.00)	1.11 (13.00)	0.89 (8.00)	0.76 (6.00)
M_2 X S_1	1.16 (14.60)	0.98 (9.80)	0.80 (6.40)	1.07 (12.20)	0.98 (9.60)	0.85 (7.20)
M_2 X S_2	1.18 (15.20)	1.02 (10.60)	0.83 (7.60)	1.14 (14.00)	1.00 (10.20)	0.82 (6.60)
M_2 X S_3	1.15 (14.20)	1.00 (10.20)	0.74 (6.20)	1.09 (12.40)	1.02 (10.60)	0.89 (7.80)
M_2 X S_4	1.18 (15.40)	1.00 (10.20)	0.77 (7.00)	1.14 (13.80)	0.99 (10.00)	0.78 (6.00)
CD (0.05)	NS	NS	NS	NS	NS	NS
At sowing	8.00			5.00		

** Logarithmic transformed values, original values are in parentheses

Table 46. Effect of treatments and interaction effect of growing condition and harvesting method on total population of actinomycetes (x10^4 cfu/g)

Treatments	2019 -2020			2020-2021		
	45 DAT	1st harvest	2nd harvest	45 DAT	1st harvest	2nd harvest
Growing condition						
M_1-Open	1.36 (23.10)	1.29 (20.15)	1.22 (17.10)	1.20 (16.15)	1.12 (13.30)	1.02 (10.70)
M_2- 50 % shade	1.33 (21.50)	1.24 (17.45)	1.17 (14.90)	1.17 (14.85)	1.05 (11.35)	0.90 (7.85)
CD (0.05)	NS	NS	NS	NS	NS	NS
Harvesting method						
S_1- Harvesting at 20 cm height from ground level at 75 and 135 DAT	1.33 (21.70)	1.25 (18.20)	1.19 (15.90)	1.17 (15.00)	1.08 (12.20)	0.93 (8.80)
S_2-Harvesting at 30 cm height from ground level at 75 and 135 DAT	1.38 (24.10)	1.29 (20.00)	1.22 (16.70)	1.21 (16.30)	1.11 (12.90)	0.98 (9.80)
S_3-Harvesting at 20 cm height from ground level at 90 and 150 DAT	1.32 (20.90)	1.23 (17.40)	1.17 (15.00)	1.16 (14.60)	1.05 (11.30)	0.94 (9.00)
S_4- Harvesting at 30 cm height from ground level at 90 and 150 DAT	1.35 (22.50)	1.29 (19.60)	1.21 (16.40)	1.20 (16.10)	1.10 (12.90)	0.97 (9.50)
CD (0.05)	NS	NS	NS	NS	NS	NS
Treatment combination						
M_1 X S_1	1..35 (22.80)	1.29 (19.60)	1.22 (17.20)	1.18 (15.20)	1.09 (12.60)	1.01 (10.40)
M_1 X S_2	1.39 (25.00)	1.33 (22.00)	1.27 (18.60)	1.24 (17.80)	1.15 (14.20)	1.06 (11.60)
M_1 X S_3	1.32 (21.00)	1.26 (18.80)	1.17 (15.00)	1.16 (14.60)	1.05 (11.40)	0.99 (10.00)
M_1 X S_4	1.37 (23.60)	1.29 (20.20)	1.24 (17.60)	1.23 (17.00)	1.17 (15.00)	1.03 (10.80)
M_2 X S_1	1.31 (20.60)	1.22 (16.80)	1.16 (14.60)	1.17 (14.80)	1.07 (11.80)	0.84 (7.20)
M_2 X S_2	1.36 (23.20)	1.25 (18.00)	1.17 (14.80)	1.17 (14.80)	1.06 (11.60)	0.90 (8.00)
M_2 X S_3	1.32 (20.80)	1.20 (16.00)	1.17 (15.00)	1.16 (14.60)	1.05 (11.20)	0.89 (8.00)
M_2 X S_4	1.33 (21.40)	1.28 (19.00)	1.18 (15.20)	1.18 (15.20)	1.03 (10.80)	0.91 (8.20)
CD (0.05)	NS	NS	NS	NS	NS	NS
At sowing	13.00			7.00		

** Logarithmic transformed values, original values are in parentheses

Table 47. Effect of treatments and interaction effect of growing condition and harvesting method on total population of nematodes (250 cc soil)

Treatments	2019 -2020			2020-2021		
	45 DAT	1st harvest	2nd harvest	45 DAT	1st harvest	2nd harvest
Growing condition						
M_1-Open	9.84 (97.25)	8.82 (78.15)	7.60 (57.45)	10.61 (113.30)	9.65 (93.65)	8.24 (74.50)
M_2- 50 % shade	10.37 (107.85)	9.39 (88.35)	8.23 (67.65)	11.29 (128.15)	10.26 (105.8)	9.18 (85.10)
CD (0.05)	NS	NS	NS	NS	NS	NS
Harvesting method						
S_1- Harvesting at 20 cm height from ground level at 75 and 135 DAT	10.12 (102.80)	8.84 (78.40)	7.89 (62.30)	11.06 (123.30)	9.85 (98.00)	8.01 (77.50)
S_2-Harvesting at 30 cm height from ground level at 75 and 135 DAT	10.16 (103.90)	9.28 (86.50)	7.71 (59.30)	10.74 (116.20)	9.86 (97.80)	8.81 (78.80)
S_3-Harvesting at 20 cm height from ground level at 90 and 150 DAT	10.02 (100.90)	9.04 (82.20)	7.99 (63.80)	10.19 (116.90)	10.00 (98.20)	8.95 (79.60)
S_4- Harvesting at 30 cm height from ground level at 90 and 150 DAT	10.10 (102.60)	9.25 (85.90)	8.04 (64.80)	11.21 (126.50)	10.21 (104.9)	9.04 (83.30)
CD (0.05)	NS	NS	NS	NS	NS	NS
Treatment combination						
M_1 X S_1	9.98 (100.00)	8.45 (71.60)	7.61 (57.80)	10.73 (115.60)	9.23 (86.20)	7.40 (74.80)
M_1 X S_2	9.86 (97.60)	8.88 (79.20)	7.52 (56.20)	10.16 (104.20)	9.68 (94.20)	8.71 (77.20)
M_1 X S_3	9.75 (95.60)	8.73 (76.80)	7.58 (57.40)	10.59 (112.80)	9.56 (91.60)	8.49 (72.00)
M_1 X S_4	9.77 (95.80)	9.20 (85.00)	7.65 (58.40)	10.95 (120.60)	10.11 (102.6)	8.84 (78.00)
M_2 X S_1	10.26 (105.60)	9.22 (85.20)	8.17 (66.80)	11.39 (131.00)	10.46 (109.8)	9.12 (84.20)
M_2 X S_2	10.47 (110.20)	9.68 (93.80)	7.90 (62.40)	11.31 (128.20)	10.05 (101.4)	8.92 (80.40)
M_2 X S_3	10.29 (106.20)	9.35 (87.60)	8.40 (70.20)	10.98 (121.20)	10.22 (104.8)	9.43 (87.20)
M_2 X S_4	10.43 (109.40)	9.31 (86.80)	8.45 (71.20)	11.48 (132.40)	10.31 (107.2)	9.23 (88.60)
CD (0.05)	NS	NS	NS	NS	NS	NS
At sowing	63.00			81.00		

**√x+0.5 transformed values, original values are given in parentheses

4.2.4 Observations on weeds

4.2.4.1 Weed count at 30, 60 DAT and at harvests

The effect of growing condition, harvesting method and their interaction on weed count at 30, 60 DAT, first harvest and second harvest are given in Table 48. Among growing conditions, plants grown under open condition recorded greater weed count at all stages.

Harvesting method affected weed count at first harvest and second harvest during both years. At first harvest, harvesting at 20 cm height from ground level at 90 and 150 DAT recorded greater weed count and it was on par with harvesting at 30 cm height from ground level at 90 and 150 DAT. At second harvest, harvesting at 20 cm height from ground level at 90 and 150 DAT had greater weed count and it was on par with harvesting at 20 cm height from ground level at 75 and 135 DAT.

Interaction effect of growing condition and harvesting method on weed count was significant. At 30 and 60 DAT, irrespective of harvesting method plants grown under open condition recorded greater weed count during both years of study. At first harvest, open condition and harvesting at 20 cm height from ground level at 90 and 150 DAT recorded greater weed count and it was on par with open condition and harvesting at 30 cm height from ground level at 90 and 150 DAT. At second harvest, open condition with harvesting at 20 cm height from ground level at 90 and 150 DAT registered greater weed count and it was on par with open condition and harvesting at 20 cm height from ground level at 75 and 135 DAT.

4.2.4.2 Weed dry weight at 30, 60 DAT and at harvests

Data on weed dry weight at 30 DAT, 60 DAT, first harvest and second harvest during 2019-20 and 2020-21 are presented in Table 49. Plants grown under open had greater weed dry weight at all stages during both years of study.

Harvesting method significantly influenced weed dry weight at first harvest only. During first year at first harvest, harvesting at 30 cm height from ground level at 90 and150 DAT recorded greater weed dry weight and it was on par with harvesting at 20 cm height from ground level at 90 and 150 DAT. During second year at first harvest, harvesting at 20 cm height from ground level at 90 and 150 DAT registered greater weed dry weight and it was on par with harvesting at 30 cm height from ground level at 90 and150 DAT.

Interaction effect of growing condition and harvesting method on weed dry weight was significant. Irrespective of harvesting method, plants grown under open condition recorded greater weed dry weight at 30 DAT, 60 DAT and at second harvest during both years of study. At first harvest, greater weed dry weight was observed in open condition with harvesting at 20 cm height from ground level at 90 and150 DAT and it was on par with open condition and harvesting at 30 cm height from ground level at 90 and150 DAT.

4.2.5 Soil analysis (before and after experiment)

Data on pH, EC, organic carbon and available N, P and K of soil before and after the experiment are furnished in Table 50 and Table 51. Treatments did not show any significant effect on soil pH, EC, organic carbon and available N, P and K of soil after the experiment under open and 50 per cent shade.

4.2.6 Correlation studies

Data on correlation between growth, biochemical and yield characters of tusli under open and 50 per cent shade are depicted in Table 52 and 53.

Under open growing condition, total chlorophyll expressed a significant and positive correlation with essential oil yield (0.719*) and biomass yield (0.678*). Though non-significant, total carotenoid was positively correlated with eugenol content (0.032) and negatively correlated with essential oil yield (-0.197) and biomass

yield (-0.209). Significant negative correlation was observed between eugenol content and essential oil yield (-0.676*). Biomass yield and essential oil yield are positively correlated (0.911*).

Under 50 per cent shade, total chlorophyll showed a significant and negative correlation with total carotenoid (-0.458*) and eugenol content (-0.555*) and positive correlation with essential oil yield (0.852*) and biomass yield (0.791*). Eugenol content was negatively correlated with essential oil yield (-0.732*) and biomass yield (-0.709*). Essential oil yield was positively correlated with biomass yield (0.936*).

Table 48. Effect of treatments and interaction effect of growing condition and harvesting method on weed count (nos./m^2) at different stages

Treatments	2019 -2020				2020-2021			
	30 DAT	60 DAT	1st harvest	2nd harvest	30 DAT	60 DAT	1st harvest	2nd harvest
Growing condition								
M_1-Open	36.50	46.85	15.30	30.25	59.25	69.55	27.15	45.65
M_2- 50 % shade	20.60	28.95	9.55	19.35	44.5	53.60	17.05	28.80
CD (0.05)	3.49	4.71	1.99	2.29	6.57	6.13	4.42	1.36
Harvesting method								
S_1- Harvesting at 20 cm height from ground level at 75 and 135 DAT	30.20	39.70	9.40	26.50	50.80	61.80	19.20	39.80
S_2-Harvesting at 30 cm height from ground level at 75 and 135 DAT	27.80	37.40	9.30	22.80	50.60	59.00	18.80	33.40
S_3-Harvesting at 20 cm height from ground level at 90 and 150 DAT	28.60	36.90	15.70	27.00	54.10	62.60	25.30	40.80
S_4- Harvesting at 30 cm height from ground level at 90 and 150 DAT	27.60	37.60	15.30	22.90	52.00	62.90	25.10	34.90
CD (0.05)	NS	NS	1.37	2.85	NS	NS	2.70	4.29
Treatment combination								
M_1 X S_1	38.00	49.80	12.00	32.20	55.60	69.60	24.60	50.00
M_1 X S_2	34.40	44.00	11.20	27.00	58.40	66.40	23.00	39.60
M_1 X S_3	37.40	45.40	19.80	34.00	62.60	70.00	31.00	51.20
M_1 X S_4	36.20	48.20	18.20	27.80	60.40	72.20	29.40	41.80
M_2 X S_1	22.40	29.60	6.80	20.80	46.00	54.00	13.80	29.60
M_2 X S_2	21.20	30.80	7.40	18.60	42.80	51.60	14.60	27.20
M_2 X S_3	19.80	28.40	11.60	20.00	45.60	55.20	19.00	30.40
M_2 X S_4	19.00	27.00	12.40	18.00	43.60	53.60	20.80	28.00
CD (0.05)	5.21	7.75	1.94	4.03	7.06	8.93	1.94	6.06

Table 49. Effect of treatments and interaction effect of growing condition and harvesting method on weed dry weight (g/m^2) at different stages

Treatments	2019 -2020				2020-2021			
	30 DAT	60 DAT	1st harvest	2nd harvest	30 DAT	60 DAT	1st harvest	2nd harvest
Growing condition								
M_1-Open	57.80	66.75	24.60	44.90	77.80	100.50	37.20	64.50
M_2- 50 % shade	34.30	50.20	19.50	32.90	60.75	79.25	30.45	50.45
CD (0.05)	5.38	3.75	3.63	6.23	4.40	11.24	2.63	5.36
Harvesting method								
S_1- Harvesting at 20 cm height from ground level at 75 and 135 DAT	44.80	56.50	19.50	40.40	69.80	92.10	30.30	59.40
S_2-Harvesting at 30 cm height from ground level at 75 and 135 DAT	46.00	59.80	19.20	37.30	68.60	87.90	30.70	55.60
S_3-Harvesting at 20 cm height from ground level at 90 and 150 DAT	46.10	58.10	24.40	40.30	68.70	89.60	37.50	59.10
S_4- Harvesting at 30 cm height from ground level at 90 and 150 DAT	47.30	59.50	25.10	37.60	70.00	89.90	36.80	55.80
CD (0.05)	NS	NS	2.93	NS	NS	NS	4.47	NS
Treatment combination								
M_1 X S_1	58.40	64.40	21.20	47.00	79.40	105.60	33.00	65.60
M_1 X S_2	57.20	67.60	20.00	42.60	75.80	99.00	32.60	63.40
M_1 X S_3	56.20	66.80	28.20	46.00	78.80	97.00	42.00	67.20
M_1 X S_4	59.40	68.20	29.00	44.00	77.20	100.40	41.20	61.80
M_2 X S_1	31.20	48.60	17.80	33.80	60.20	78.60	27.60	53.20
M_2 X S_2	34.80	52.00	18.40	32.00	61.40	76.80	28.80	47.80
M_2 X S_3	36.00	49.40	20.60	34.60	58.60	82.20	33.00	51.00
M_2 X S_4	35.20	50.80	21.20	31.20	62.80	79.40	32.40	49.80
CD (0.05)	7.81	9.48	4.14	6.21	7.63	13.69	6.32	9.34

Table 50. Physico-chemical characters of soil under open condition

Growing condition: Open												
Treatments	**2019 -2020**						**2020-2021**					
	pH	**EC**	**O. C (%)**	**Av. N (kg/ ha)**	**Av. P (kg/ha)**	**Av. K (kg/ha)**	**pH**	**EC**	**O. C (%)**	**Av. N (kg/ha)**	**Av. P (kg/ ha)**	**Av. K (kg/ha)**
Harvesting at 20 cm height at 75 and 135DAT	4.65	0.63	0.99	124.2	26.42	203.6	4.61	0.68	0.99	125.4	28.57	193.4
Harvesting at 30 cm height at 75 and 135DAT	4.60	0.62	0.97	130.8	24.19	196.2	4.59	0.65	0.97	122.6	27.84	199.2
Harvesting at 20 cm height at 90 and 150DAT	4.62	0.60	1.00	128.6	24.62	201.0	4.62	0.69	0.96	121.4	26.45	189.4
Harvesting at 30 cm height at 90 and 150DAT	4.66	0.59	0.98	126.4	23.61	199.0	4.59	0.67	0.97	118.4	26.59	197.6
CD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Average	4.63	0.61	0.98	127.5	24.71	200	4.6	0.67	0.97	121.9	27.36	194.9
Initial	4.68	0.66	1.02	142.1	26.96	214	4.64	0.70	1.00	132.7	31.25	209.6

Table 51. Physico-chemical characters of soil under 50 per cent shade

Growing condition: 50 per cent shade												
Treatments	**2019 -2020**						**2020-2021**					
	pH	**EC**	**O. C (%)**	**Av. N (kg/ ha)**	**Av. P (kg/ha)**	**Av. K (kg/ha)**	**pH**	**EC**	**O. C (%)**	**Av. N (kg/ha)**	**Av. P (kg/ ha)**	**Av. K (kg/ha)**
Harvesting at 20 cm height at 75 and 135DAT	4.61	0.63	1.01	135.8	26.41	206.2	4.59	0.66	0.99	124.0	29.7	195.8
Harvesting at 30 cm height at 75 and 135DAT	4.62	0.61	1.00	126.0	25.11	194.4	4.58	0.65	0.98	127.2	30.1	191.0
Harvesting at 20 cm height at 90 and 150DAT	4.63	0.59	0.99	134.6	24.26	197.4	4.57	0.68	1.00	122.2	28.0	197.8
Harvesting at 30 cm height at 90 and 150DAT	4.64	0.63	0.98	129.2	25.01	200.8	4.58	0.68	0.98	121.4	28.2	199.2
CD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Average	4.62	0.62	1.00	131.4	25.19	199.7	4.58	0.66	0.99	123.7	29	195.9
Initial	4.68	0.66	1.02	142.1	26.96	214	4.64	0.70	1.00	132.7	31.25	209.6

Table 52. Correlation between growth, biochemical and yield attributes of tulsi under open condition

	Plant height	Total chlorophyll	Total carotenoid	Eugenol content	Essential oil yield	Biomass yield
Plant height	1.000					
Total chlorophyll	-0.293	1.000				
Total carotenoid	0.241	-0.039	1.000			
Eugenol content	-0.088	-0.417	0.032	1.000		
Essential oil yield	-0.190	0.719 *	-0.197	-0.676 *	1.000	
Biomass yield	-0.168	0.678 *	-0.209	-0.438	0.911 *	1.000

Table 53. Correlation between growth, biochemical and yield attributes of tulsi under 50 per cent shade

	Plant height	Total chlorophyll	Total carotenoid	Eugenol content	Essential oil yield	Biomass yield
Plant height	1.000					
Total chlorophyll	-0.150	1.000				
Total carotenoid	0.091	-0.458 *	1.000			
Eugenol content	0.043	-0.555 *	0.071	1.000		
Essential oil yield	-0.087	0.852 *	-0.388	-0.732 *	1.000	
Biomass yield	-0.003	0.791 *	-0.357	-0.709 *	0.936 *	1.000

4.3 Experiment 3. Evaluation of allelopathic potential of tulsi on upland weeds and test crops

4.3.1 Observations on weeds

4.3.1.1 Germination count at weekly interval

Number of weeds germinated was counted at weekly intervals (Table 54). All extracts and powders of tulsi exhibited significant effects on germination count of weeds at 1st week, however, weed germination at 2nd, 3rd and 4th weeks were not statistically significant. At 1st week, control treatment recorded the highest germination of weeds (166.67 nos./m^2) which was significantly superior to all other treatments. The lowest germination count was observed in treatments which received tulsi shoot powder at 20 g/ kgsoil (3.33nos./m^2) and tulsi root powder at 20 g/kg soil (6.67nos./m^2), followed by 10 g/kg soil of tulsi shoot powder (20.00 nos./m^2) and root powder (26.67 nos./m^2).

4.3.1.2 Density of weeds at one month after application

Data on the effect of tulsi extracts and powders on weed density at one month after application are presented in Table 55. There was no significant difference in density of grass weeds. The density of broad leaved weeds was higher in control (284.00 nos./m^2) and the lowest was observed in tulsi shoot powder at 20 g/kg soil (140.33 nos./m^2) and tulsi root powder at 20 g/kg soil (144.33 nos./m^2).

The same trend was observed in case of total weeds also. The higher total weed density was observed in control treatment (359.00 nos./m^2) and was statistically on par with all other treatments except application of tulsi powders. The lowest total weed density was observed in treatment with tulsi shoot powder at 20 g/kg soil (208.67 nos./m^2) and tulsi root powder at 20 g/kg soil (211.33 nos./m^2), followed by tulsi root powder at 10 g/kg soil (248.33 nos./m^2) and tulsi shoot powder at 10 g/kg soil (260.00 nos./m^2).

Table 54. Effect of tulsi extracts and powders on weed germination (nos./m^{-2}) at weekly intervals

Germination count (nos./m^2)				
Treatments	1st week	2nd week	3rd week	4th week
T_1 - Aqueous extract (shoot 5 %)	8.59[c] (73.33)	13.40 (180.00)	7.30 (53.33)	4.14 (16.67)
T_2 - Aqueous extract (shoot 10 %)	7.76[c] (60.00)	13.42 (180.00)	6.82 (46.67)	4.02 (15.67)
T_3 - Aqueous extract (root 5 %)	8.59[c] (73.33)	13.42 (180.00)	6.57 (43.33)	4.18 (17.00)
T_4 - Aqueous extract (root 10 %)	8.19[c] (66.67)	13.16 (173.33)	6.57 (43.33)	4.22 (17.33)
T_5 - Hot water extract (shoot 5 %)	10.33[b] (106.67)	12.38 (153.33)	7.07 (50.00)	4.22 (17.33)
T_6 - Hot water extract (shoot 10 %)	9.68[b] (93.33)	11.81 (140.00)	7.41 (55.00)	4.14 (16.67)
T_7 - Hot water extract (root 5 %)	10.67[b] (113.33)	12.38 (153.33)	6.80 (46.67)	4.22 (17.33)
T_8 - Hot water extract (root 10 %)	10.33[b] (106.67)	12.28 (153.33)	6.82 (46.67)	4.13 (16.67)
T_9 - Powder (shoot 10 g/kg soil)	4.43[d] (20.00)	12.91 (166.67)	7.63 (58.33)	3.93 (15.00)
T_{10} - Powder (shoot 20 g/kg soil)	1.80[e] (3.33)	11.49 (133.33)	7.51 (56.67)	3.97 (15.33)
T_{11} - Powder (root 10 g/kg soil)	5.19[d] (26.67)	12.10 (146.67)	7.63 (58.33)	4.14 (16.67)
T_{12} - Powder (root 20 g/kg soil)	2.64[e] (6.67)	11.78 (140.00)	7.07 (50.00)	3.89 (14.67)
T_{13} - Control	12.92[a] (166.67)	11.54 (133.33)	6.70 (45.00)	3.79 (14.00)
SEm (±)	0.923	0.200	0.1008	0.039
CD (0.05)	1.062	NS	NS	NS

**√x+0.5 transformed values, original values are given in parentheses

Table 55. Effect of tulsi extracts and powders on weed density and weed dry weight at one month after application

Treatments	Weed density (nos./ m^2)			Weed dry weight (g/m^2)		
	Grasses	Broad leaved	Total weeds	Grasses	Broad leaved	Total weeds
T_1 - Aqueous extract (shoot 5 %)	8.35 (70.00)	15.89^{ab} (253.33)	17.97^{a} (323.33)	2.02 (4.10)	5.61^{abc} (31.57)	5.96^{bc} (35.67)
T_2 - Aqueous extract (shoot 10 %)	8.19 (67.33)	15.33^{b} (235.00)	17.36^{abc} (302.33)	1.98 (3.93)	5.17^{bcd} (26.73)	5.54^{cd} (30.67)
T_3 - Aqueous extract (root 5 %)	8.31 (70.00)	15.60^{ab} (243.67)	17.71^{ab} (313.67)	2.05 (4.23)	5.92^{ab} (35.10)	6.27^{ab} (39.33)
T_4 - Aqueous extract (root 10 %)	8.30 (69.00)	15.21^{b} (231.67)	17.33^{abc} (300.67)	2.00 (4.00)	5.83^{ab} (34.00)	6.16^{abc} (38.00)
T_5 - Hot water extract (shoot 5 %)	8.43 (71.33)	15.99^{ab} (256.00)	18.06^{a} (327.33)	2.05 (4.20)	6.09^{a} (37.13)	6.42^{ab} (41.33)
T_6 - Hot water extract (shoot 10 %)	8.23 (67.67)	15.40^{b} (237.33)	17.46^{abc} (305.00)	2.02 (4.10)	5.82^{ab} (33.90)	6.16^{abc} (38.00)
T_7 - Hot water extract (root 5 %)	8.54 (73.00)	16.05^{ab} (257.67)	18.17^{a} (330.67)	2.00 (4.03)	6.16^{a} (37.97)	6.48^{ab} (42.00)
T_8 - Hot water extract (root 10 %)	8.52 (72.67)	15.82^{ab} (250.67)	17.97^{a} (323.33)	1.99 (4.00)	5.97^{a} (35.67)	6.29^{ab} (39.67)
T_9 - Powder (shoot 10 g/kg soil)	8.34 (69.67)	13.79^{c} (190.33)	16.10^{bcd} (260.00)	1.99 (3.97)	4.58^{de} (21.03)	5.00^{de} (25.00)
T_{10} - Powder (shoot 20 g/kg soil)	8.26 (68.33)	11.83^{d} (140.33)	14.41^{d} (208.67)	1.97 (3.87)	4.17^{e} (17.47)	4.61^{e} (21.33)
T_{11} - Powder (root 10 g/kg soil)	8.26 (68.33)	13.39^{c} (180.00)	15.68^{cd} (248.33)	1.99 (4.00)	4.82^{cde} (23.33)	5.23^{de} (27.33)
T_{12} - Powder (root 20 g/kg soil)	8.18 (67.00)	11.96^{d} (144.33)	14.50^{d} (211.33)	1.97 (3.90)	4.40^{de} (19.43)	4.82^{de} (23.33)
T_{13} - Control	8.66 (75.00)	16.85^{a} (284.00)	18.92^{a} (359.00)	2.09 (4.37)	6.37^{a} (41.63)	6.71^{a} (46.00)
SEm (±)	0.041	0.444	0.396	0.009	0.204	0.193
CD (0.05)	NS	1.310	1.833	NS	0.787	0.855

**√x+0.5 transformed values, original values are given in parentheses

4.3.1.3 Dry weight of weeds at one month after application

The data on the effect of treatments on dry weight of weeds at one month after application are presented in Table 55. Effect on dry weight of grass weeds was found non significant. Dry weight of broad leaved weeds and total weeds were higher in control treatment (41.63 and 46.00 g/m^2). Lower dry weight of broad leaved and total weeds were found in treatment with tulsi shoot powder at 20 g/kg soil (17.47 and 21.33 g/m^2) and tulsi root powder at 20 g/kgsoil (19.43 and 23.33 g/m^2), followed by tulsi shoot powder at 10 g/kg soil (21.03 and 25.00 g/m^2) and tulsi root powder at 10 g/kgsoil (23.33 and 27.33 g/m^2).

4.3.2 Observations on rice

4.3.2.1 Speed of germination, mean germination time and final germination percentage

Speed of germination, mean germination time and final germination percentage of rice were significantly affected by the application of tulsi extracts and powders (Table 56.). Speed of germination significantly decreased in all the treatments compared to control (3.47 nos./day). Germination speed was lower in trays incorporated with tulsi shoot powder and root at 20 g/kg soil (2.49 and 2.56 nos./day), followed by 10 g/kg soil of shoot powder and root (2.72 and 2.77 nos./day).

Also, the presence of tulsi extracts and powders in soil significantly enhanced the mean germination time. Control treatments (without tulsi extracts and powders), recorded lower mean germination time (3.58 days) and was statistically on par with both aqueous and hot water extracts of shoot as well as root portion at all concentrations. However, incorporation of tulsi powder of shoot portion and root at 20 g/kg soil and powder of shoot portion and root at 10 g/kg soil showed higher mean germination time, *i.e*., 4.55, 4.31, 4.3, 4.23 days respectively.

Similarly lower germination percentage was observed in treatments received tulsi shoot portion at 20 g/kg soil (86.10 %) and tulsi root powder at 20 g/kg soil (88.90 %). However, final germination percentage was not affected by hot water extracts of different parts at all concentrations and aqueous extracts of different parts at lower concentration.

4.3.2.2 Shoot length and root length at one month after application

Data on the effect of treatments on shoot length and root length of rice are presented in Table 56. Control treatment recorded the highest shoot length of 34.77cm. Among different treatments, incorporation of tulsi root powder at 20 g/kg soil had lower shoot length (24.17 cm), which was on par with shoot powder at 20 g/kg soil (25.67 cm) and shoot powder at 10 g/kg soil (27.77 cm). Root length was not significantly influenced by treatments.

4.3.2.3 Seedling vigor index

Seedling vigor index of rice was significantly affected by the application of tulsi extracts and powders (Table 56.). Among the treatments, tulsi root powder at 20 g/kg soil recorded lower seedling vigor index (2773.9) and which was on par with tulsi shoot powder at 20 g/kg soil (2818.0). Control recorded higher seedling vigor index of 4287.3.

4.3.2.4 Fresh weight and dry weight at one month after application

Effect of treatments on fresh weight and dry weight at one month after application are furnished in Table 56. The fresh weight of rice seedlings was significantly higher in control (330 g/m^2) than other treatments. Among the treatments, powder of shoot portion of tulsi at 20 g/kg soil recorded lower value (256.67 g/m^2), and was on par with root powder at 20 g/kg soil (260.00g/m^2), aqueous extract of shoot at 10 per cent (270.00 g/m^2), and root powder at 10 g/kg soil (290.00 g/m^2).

Plate 15. Effect of tulsi shoot and root powders on seedling growth of rice

Treatments caused significant difference in dry weight of rice. Lower dry weight was observed in tulsi shoot powder at 20 g/kg soil (48.67 g/m^2) and which was on par with root powder at 20 g/kg soil (50.00 g/m^2), aqueous extract of shoot at 10 per cent (51.33 g/m^2) and aqueous extract of root at 10 per cent (53.33 g/m^2).

4.3.3 Observations on cowpea

4.3.3.1 Speed of germination, mean germination time and final germination percentage

Allelopathic effect of different extracts and powders of tulsi on germination indices and germination of the test crop cowpea are given in Table 57. Speed of germination was the highest in control (4.86 nos./day) and it was significantly superior to all treatments. Among treatments, tulsi shoot powder at 20 g/kg soil (1.87 nos./day) and powder of root at 20 g/kg soil (1.99 nos./day) recorded lower germination speed. Similarly mean germination time, an indicator of length of lag period to germination was significantly higher in all the treatments as compared to control (2.67 days). The final germination percentage was significantly lower in treatment which received tulsi shoot powder at 20 g/kg soil (80.53 %) and was on par with root powder at 20 g/kg soil (83.33 %) and shoot powder at 10 g/kg soil (88.87%). Hot water extracts had no effect on final germination percentage.

4.3.3.2 Shoot length and root length at one month after application

The shoot length and root length of cowpea seedlings were significantly inhibited by all the applied extracts and powders of tulsi as compared to control (Table 57). The lower shoot length was observed in tulsi shoot powder at 20 g/kg soil (11.00 cm) which was statistically on par with root powder at 20 g/kg soil (13.17 cm). Similarly, root length was lower in root powder at 20 g/kg soil (3.67 cm) and was statistically on par with shoot powder at 20 g/kg soil (3.83 cm) and root powder at 10 g/kg soil (4.17 cm). Control recorded the highest shoot length and root length (20.5 and 8.5 cm respectively).

4.3.2.3 Seedling vigor index

Seedling vigor index of cowpea was significantly affected by the application of tulsi extracts and powders (Table 57). Among the treatments, powder of shoot and root of tulsi at 20 g/kg soil soil recorded significantly lower value (1191.7 and 1398.6), followed by powder of shoot and root of tulsi at 10 g/kg soil (1719.4 and 1833.3). Control recorded higher seedling vigor index of 2900.0.

4.3.3.3 Fresh weight and dry weight at one month after application

Application of tulsi extracts and powders had significant effect on seedling fresh weight and dry weight (Table 57). Lower seedling fresh weight was observed in tulsi shoot portion powder at 20 g/kg soil (860.00 g/m^2), root powder at 20 g/kg soil (886.67g/m^2) and 10 g/kg soil of shoot portion (920.00 g/m^2) and root (966.67 g/m^2). Similar trend was observed for dry weight also. Control recorded maximum fresh weight and dry weight (1212 and 134.33 g/m^2 respectively).

4.2.6 Correlation studies

Correlation between content of allelochemicals in extracts and powders and germination and seedling growth upland weeds and test crops are given in Table 58. Total alkaloids expressed strong negative correlation with germination count at first week (-0.815*), density of broad leaved weeds and total weeds (-0.844* and -0.838*), and dry weight of broad leaved weeds and total weeds (-0.734* and 0.733*). Total phenols also exhibited significant negative correlation with weed germination count, dry weight of broad leaved weeds and total weeds (-0.539*, -0.634* and -0.631* respectively).

Total alkaloids showed significant negative correlation with speed of germination (-0.880*, -0.802*), germination percentage (-0.858*, -0.702), shoot length (-0.841*, -0.676*), root length (-0.879*, -0.796*), seedling vigor index (-0.865*, -0.858*), fresh weight (-0.694*, -0.731*) and dry weight (-0.631*, -0.716*) of rice and cowpea respectively. Total phenols, tannins and flavanoids also showed negative relationship with germination and seedling growth, while correlation was non significant.

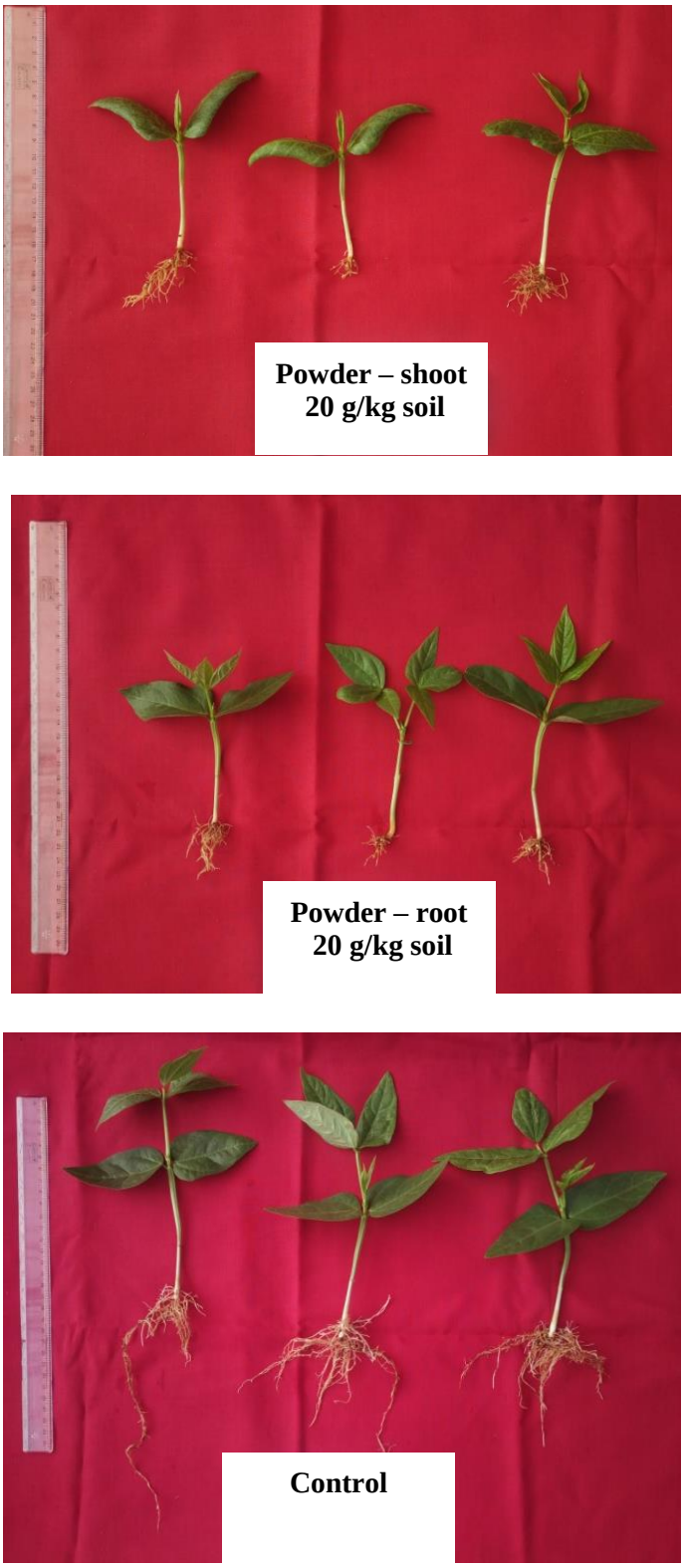


Plate 16. Effect of tulsi shoot and root powders on seedling growth of cowpea

Table 56. Effect of tulsi extracts and powders on germination and seedling growth of rice

Treatments	Speed of germination (nos./day)	Mean germination time (days)	Final germination (%)	Shoot length (cm)	Root length (cm)	Seedling vigor index	Fresh weight (g/m^2)	Dry weight (g/m^2)
T_1 - Aqueous extract (shoot 5 %)	3.30^{ab}	3.81^{c}	100.00^{a}	29.83^{bcde}	7.70	3758.3^{abc}	303.33^{abcd}	60.67^{ab}
T_2 - Aqueous extract (shoot 10 %)	3.00^{cd}	3.91^{bc}	94.47^{abc}	28.50^{def}	7.60	3412.0^{c}	270.00^{de}	51.33^{c}
T_3 - Aqueous extract (root 5 %)	3.31^{ab}	3.78^{c}	100.00^{a}	30.17^{bcde}	7.73	3790.0^{abc}	315.33^{abc}	60.00^{ab}
T_4 - Aqueous extract (root 10 %)	3.19^{bc}	3.80^{c}	97.23^{ab}	29.27^{cdef}	7.67	3607.3^{bc}	280.00^{cde}	53.33^{bc}
T_5 - Hot water extract (shoot 5 %)	3.34^{ab}	3.75^{c}	100.00^{a}	32.67^{abc}	8.20	4086.7^{ab}	323.33^{ab}	63.33^{a}
T_6 - Hot water extract (shoot 10 %)	3.29^{ab}	3.81^{c}	100.00^{a}	31.60^{abcd}	8.00	3954.3^{ab}	320.00^{ab}	61.33^{a}
T_7 - Hot water extract (root 5 %)	3.34^{ab}	3.75^{c}	100.00^{a}	33.17^{ab}	8.13	4130.0^{ab}	326.67^{ab}	64.67^{a}
T_8 - Hot water extract (root 10 %)	3.29^{ab}	3.81^{c}	100.00^{a}	32.17^{abc}	8.00	4016.7^{ab}	318.00^{ab}	62.00^{a}
T_9 - Powder (shoot 10 g/kg soil)	2.72^{de}	4.30^{ab}	94.47^{abc}	27.77^{efg}	7.33	3312.0^{cd}	300.00^{abcd}	59.33^{ab}
T_{10} - Powder (shoot 20 g/kg soil)	2.49^{e}	4.55^{a}	86.10^{c}	25.67^{fg}	7.00	2818.0^{de}	256.67^{e}	48.67^{c}
T_{11} - Powder (root 10 g/kg soil)	2.77^{de}	4.23^{ab}	94.47^{abc}	28.33^{def}	7.50	3391.7^{c}	290.00^{bcde}	60.00^{ab}
T_{12} - Powder (root 20 g/kg soil)	2.56^{e}	4.31^{a}	88.90^{bc}	24.17^{g}	7.03	2773.9^{e}	260.00^{e}	50.00^{c}
T_{13} - Control	3.47^{a}	3.58^{c}	100.00^{a}	34.77^{a}	8.23	4287.3^{a}	330.00^{a}	66.67^{a}
SEm (±)	0.093	0.081	1.302	0.841	0.114	133.58	7.161	1.612
CD (0.05)	0.284	0.385	8.577	3.615	NS	534.14	36.869	7.791

Table 57. Effect of tulsi extracts and powders on germination and seedling growth of cowpea

Treatments	Speed of germination (nos./day)	Mean germination time (days)	Final germination (%)	Shoot length (cm)	Root length (cm)	Seedling vigor index	Fresh weight (g/m^2)	Dry weight (g/m^2)
T_1 - Aqueous extract (shoot 5 %)	3.88^{de}	3.15^{de}	94.47^{ab}	18.17^{bc}	6.50^{de}	2329.2^{cd}	1023.33^{bcd}	112.00^{bc}
T_2 - Aqueous extract (shoot 10 %)	3.34^{f}	3.61^{c}	91.70^{abc}	16.67^{cde}	5.33^{fg}	2016.7^{ef}	976.67^{cde}	105.93^{bcd}
T_3 - Aqueous extract (root 5 %)	3.86^{de}	3.28^{cde}	97.23^{ab}	18.50^{abc}	7.00^{cd}	2481.9^{bc}	1033.33^{bc}	113.00^{bc}
T_4 - Aqueous extract (root 10 %)	3.56^{ef}	3.44^{cd}	94.43^{ab}	17.33^{bcd}	6.00^{ef}	2205.6^{de}	980.00^{cde}	108.00^{bcd}
T_5 - Hot water extract (shoot 5 %)	4.37^{bc}	3.06^{def}	100.00^{a}	18.33^{abc}	8.00^{ab}	2633.3^{ab}	1113.33^{ab}	117.80^{b}
T_6 - Hot water extract (shoot 10 %)	4.14^{cd}	3.22^{cde}	100.00^{a}	17.50^{bcd}	7.33^{bcd}	2483.3^{bc}	1100.00^{b}	108.60^{bcd}
T_7 - Hot water extract (root 5 %)	4.51^{b}	2.89^{ef}	100.00^{a}	19.00^{ab}	8.33^{a}	2733.3^{ab}	1126.67^{ab}	119.00^{b}
T_8 - Hot water extract (root 10 %)	4.28^{bc}	3.08^{def}	100.00^{a}	18.83^{abc}	7.67^{abc}	2650.0^{ab}	1106.67^{ab}	114.00^{bc}
T_9 – Powder (shoot 10 g/kg soil)	2.26^{h}	4.97^{ab}	88.87^{bcd}	14.50^{ef}	4.83^{gh}	1719.4^{g}	920.00^{def}	100.67^{cd}
T_{10} - Powder (shoot 20 g/kg soil)	1.87^{i}	5.34^{a}	80.53^{d}	11.00^{g}	3.83^{i}	1191.7^{h}	860.00^{f}	96.67^{d}
T_{11} - Powder (root 10 g/kg soil)	2.74^{g}	4.58^{b}	91.70^{abc}	15.83^{de}	4.17^{hi}	1833.3^{fg}	966.67^{cdef}	102.00^{cd}
T_{12} - Powder (root 20 g/kg soil)	1.99^{hi}	5.24^{a}	83.33^{cd}	13.17^{fg}	3.67^{i}	1398.6^{h}	886.67^{ef}	99.80^{cd}
T_{13} - Control	4.86^{a}	2.67^{f}	100.00^{a}	20.50^{a}	8.50^{a}	2900.0^{a}	1212.00^{a}	134.33^{a}
SEm (±)	0.277	0.262	1.831	0.732	0.480	147.61	28.933	2.794
CD (0.05)	0.322	0.419	8.388	2.308	0.861	276.34	111.040	14.645

Table 58. Correlation between total phenols, tannins, alkaloids and flavanoids in extracts and powders of tulsi and germination and seedling growth of test crops and upland weeds

	Upland weeds			
	Total phenols	Total tannins	Total alkaloids	Total flavanoids
GC-1st week	-0.539*	-0.052	-0.815*	-0.186
Density-grass	-0.482	0.100	-0.398	-0.328
Density- BLW	-0.403	-0.218	-0.844*	-0.004
Density-total weeds	-0.414	-0.207	-0.838*	-0.018
Dry weight-grass	-0.311	-0.120	-0.504	-0.075
Dry weight-BLW	-0.634*	0.007	-0.734*	-0.282
Dry weight-total weeds	-0.631*	0.005	-0.733*	-0.280

	Test crop-rice				**Test crop-cowpea**			
	Total phenols	Total tannins	Total alkaloids	Total flavanoids	Total phenols	Total tannins	Total alkaloids	Total flavanoids
SG	-0.382	-0.314	-0.880*	0.012	-0.543	-0.048	-0.802*	-0.177
G%	-0.219	-0.458	-0.858*	-0.132	-0.569	0.031	-0.702*	-0.256
Shoot length	-0.330	-0.293	-0.841*	-0.015	-0.589	0.050	-0.676*	-0.238
Root length	-0.310	-0.321	-0.879*	-0.022	-0.509	-0.098	-0.796*	-0.178
SVI	-0.311	-0.344	-0.865*	-0.024	-0.390	-0.285	-0.858*	-0.034
Fresh weight	-0.227	-0.301	-0.694*	-0.003	-0.547	0.045	-0.731*	-0.247
Dry weight	-0.070	-0.379	-0.631*	-0.107	-0.570	0.017	-0.716*	-0.238

GC: Germination count; SG: Speed of germination; MGT: Mean germination time; G%: Germination percentage; SVI: Seedling vigor index

*Significance at 5 %

4.4 Experiment 4. Evaluation of nematicidal potential of tulsi against root-knot Nematode (*Meloidogyne incognita*)

4.4.1 Mortality of second stage juveniles (J_2s)

Aqueous and hot water extracts of tulsi at different concentrations had significant effect on the mortality of second stage juveniles (J_2s) of *M. incognita* (Table 59). Aqueous extract of tulsi @ 30 per cent caused the highest mortality of J_2s after 12, 24, 48 and 72 hours of exposure (14.14 %, 23.81 %, 44.13 % and 48.46 % respectively) and it was significantly higher than all other treatments. The control (distilled water) recorded the lowest juvenile mortality (0.00 %) for all the periods of exposure and it was significantly lower than all other treatments. After 12 h of exposure, next best mortality of J_2s (7.97 %) was observed in aqueous extract of tulsi @ 20 per cent, followed by hot water extract of tulsi @ 30 per cent (6.34 %). Treatment that received aqueous extract of tulsi @ 10 per cent caused 5.47 per cent mortality of J_2s and was on par with hot water extract of tulsi @ 20 per cent (5.21 %). Control recorded the least juvenile mortality (0.00 %), followed by hot water extract of tulsi @ 10 per cent (4.25 %).

After 24 h of application, aqueous extract of tulsi @ 30 per cent exhibited significantly the highest juvenile mortality (23.81 %), followed by aqueous extract of tulsi @ 20 per cent (12.74 %) which was on par with hot water extract of tulsi @ 30 per cent (12.73 %) and aqueous extract of tulsi @ 10 per cent (11.36 %).

Aqueous extract of tulsi @ 20 per cent exhibited the second highest mortality of second stage juveniles of *M. incognita* (37.18 %) after 48 h of application, followed by treatment received hot water extract of tulsi @ 30 per cent (27.42 %) which was on par with aqueous extract of tulsi @ 10 per cent (24.28 %). Hot water extract of tulsi @ 20 per cent exhibited 16.55 per cent juvenile mortality followed by its lower concentration *i.e*., hot water extract of tulsi @ 10 per cent (8.69 %).

After 72 h of exposure, aqueous extract of tulsi @ 30 per cent recorded the highest mortality (48.46 %), followed by its lower concentration of 20 per cent (41.35 %) which was on par with hot water extract of tulsi @ 30 per cent (36.71 %). Aqueous extract of tulsi @ 10 per cent recorded 35.55 per cent mortality followed by hot water extract of tulsi @ 20 per cent (18.05 %). Hot water extract of tulsi @ 10 per cent recorded lower mortality (12.42 %) after control (0.00 %).

Table 59. Effect of tulsi extracts on mortality (%) of second juveniles of *M. incognita*

Treatments	Mortality (%) of J_2s of *M. incognita*			
	12 h	**24 h**	**48 h**	**72 h**
T_1 : Aqueous extract of tulsi @ 10 %	13.50 [d] (5.47)	19.67 [b] (11.36)	29.51 [c] (24.28)	36.58 [c] (35.55)
T_2 : Aqueous extract of tulsi @ 20 %	16.39 [b] (7.97)	20.89 [b] (12.74)	37.56 [b] (37.18)	40.01 [b] (41.35)
T_3 : Aqueous extract of tulsi @ 30 %	22.08 [a] (14.14)	29.20 [a] (23.81)	41.63 [a] (44.13)	44.11 [a] (48.46)
T_4 : Hot water extract of tulsi @ 10 %	11.87 [e] (4.25)	14.87 [d] (6.62)	17.04 [e] (8.69)	20.60 [e] (12.42)
T_5 : Hot water extract of tulsi @ 20 %	13.15 [d] (5.21)	17.51 [c] (9.08)	23.96 [d] (16.55)	24.97 [d] (18.05)
T_6 : Hot water extract of tulsi @ 30 %	14.57 [c] (6.34)	20.89 [b] (12.73)	31.54 [c] (27.42)	37.28 [bc] (36.71)
T_7 : Control (distilled water)	0.29 [f] (0.00)	0.29 [e] (0.00)	0.29 [f] (0.00)	0.29 [f] (0.00)
SE m (±)	2.485	3.336	5.269	5.745
CD (0.05)	1.018	1.314	2.149	3.00

**Arc sin transformed values, original values are in parentheses

Discussion

5. DISCUSSION

The results obtained from the field experiments and laboratory studies conducted as part of thesis work entitled 'Characterization and evaluation of tulsi (*Ocimum tenuiflorum* L.)' are discussed below based on available literature.

5.1 Experiment 1- Evaluation of *Ocimum tenuiflorum* L. types

Thirty five accessions of tulsi (10 NBPGR accessions + 25 local collections) from different phyto-geographical regions were evaluated for morpho-physiological, biochemical and yield characters. High degree of variation was observed among accessions for all the parameters studied.

Study of morphological characters in plants is an important tool for phylogenetic studies even in the modern era of molecular systematics (Lee, 2006; Bruce *et al*., 2007). Analysis of variance for morphological data (qualitative and quantitative) revealed that significant variations existed among the studied accessions of tulsi. Accessions showed different shades of green and purple colour on stem, leaf and inflorescence. The collected accessions were grouped into three clusters based on the observed colour of stem, upper leaf surface and inflorescence. (Figure 5 and Table 19). Cluster I consisted of 16 accessions and they were green types (upper leaf surface, stem and inflorescence green coloured). Cluster II consisted of 14 accessions and they shared common features such as purple coloured upper leaf, stem and inflorescence (purple types). Cluster III consisted of 5 accessions and they were intermediate types (green coloured upper leaf, stem and inflorescence purple). These results were in line with those of Malav *et al*. (2015). They distinguished 49 accessions of *Ocimum tenuiflorum* L. from four regions of India into green (Rama) types, black (Shyama) types and intermediate type based on the characteristics of leaf and inflorescence. Similarly, Das *et al*. (2020) studied morpho-metric characters of *Ocimum tenuiflorum* L. from Tripura and reported two major morphotypes *i.e*., light green-leaved (Sri or Rama tulsi) and purple or dark green-leaved (Shyama or Krishna tulsi). In *Ocimum sanctum* colour of the leaves, new branches and inflorescence

varied from green to purple-green (Saran *et al.*, 2017). According to Ahmad and Khaliq (2002), tulsi species showed variation in pigmentation and leaf shape through centuries of its cultivation.

The accessions also showed considerable variation for quantitative parameters like plant height, number of primary branches, secondary branches, number of leaves per plant and inflorescence length. In general, NBPGR collections from various parts of India were found taller than the accessions collected from various districts of Kerala. Accession IC 583288 had maximum number of primary and secondary branches per plant at all stages of crop growth, while number of leaves per plant was higher in KAU OC 12. Number of days taken to first flowering and 50 per cent flowering also varied among the accessions. Number of days from planting to first flowering ranged from 21.67 to 31.67 days, and for 50 per cent flowering from 26.67 to 39.0 days. Ahmad and Khaliq (2002) also reported significant variability in leaf area, number of racemes per plant, number of flowers per raceme, plant height and number of days to maturity in *O. tenuiflorum* accessions collected from different districts of North Himalayan regions of Pakistan. High level of variation in morphological traits of *Ocimum* species have been reported by Javanmardi *et al.* (2002).

Biomass yield per plant was recorded at 30 and 60 DAT and at harvest and it increased from 30 DAT to harvest (Table 14). Wide range of variability was observed in biomass production. At all stages of crop growth, accession IC 583288 recorded significantly higher biomass yield (205.3 g, 393.3 g and 443.7 g respectively) and accession KAU OC 14 had significantly lower biomass yield (108.0 g, 272.0 g and 319.7 g respectively).

Euclidean cluster analysis was done to classify the accessions using eight quantitative characters, and six clusters were obtained at standard Euclidean distance of 10.00 (Figure 6 and Table 21). Cluster I and Cluster III consisted of 9 accessions each and cluster V comprised of 8 accessions. Cluster VI included 5 accessions and Cluster IV included 3 accessions. Cluster II contained only one accession named IC

583288. Principal component analysis was also carried out to find out the main components which contributed genetic diversity among accessions (Table 23 and Figure 7). The first three principal components (plant height, primary branches per plant and secondary branches per plant) having eigen values greater than 1.0, contributed to 79.7 per cent of variability. The first principal component (PC1) with an eigen value of 3.583 accounted for 44.8 per cent of total variance, and the important contributing traits were leaf area index, days to 50 per cent flowering, plant height, days to first flowering and biomass yield. Similarly Malav *et al*. (2015) classified 49 accessions from different regions of India into six different clusters based on quantitative morphological characters. The first three principal components, contributed 91.55 per cent of the total variability and the first two to 85.65 per cent of the variance. The characteristics such as mature leaf length and width, plant height, petiole length, number of primary branches and leaf weight contributed the most towards the first principal component. Malav *et al*. (2020) categorized 109 accessions of *O. tenuiflorum* into seven major clusters and PCA analysis revealed that first six principal components contributed to 72.33 per cent of total variance.

In this study biomass yield per plant at harvest had showed a strong positive correlation with plant height, leaf area index and primary branches per plant (Table 30). Path analysis was done with characters that showed positive correlation with biomass yield per plant (Table31and Figure10). High positive direct effect on total biomass yield per plant was expressed by primary branches (0.377) and leaf area index at harvest (0.672). Plant height showed moderate negative direct effect (-0.264). Chang *et al*. (2008) also reported positive correlation between biomass yield, leaf area and number of branches per plant in tulsi. Correlation between biomass yield and higher leaf area index in *Ocimum* plants has also been reported by Milenkovic *et al*. (2019). All the accessions were scored and ranked based on total biomass yield per plant at harvest and the factors showing significant direct effect with it *i.e.*, primary branches and leaf area index (Table 32). Results revealed that accession IC 583288 was the top rank holder followed by IC 583318, IC 583296 and KAU OC 30.

Chlorophyll and carotenoid are important pigments that have been used to monitor photosynthetic performance and give some indication of the physiological status of the plant. Different morphotypes of tulsi *i.e*., the green, purple and intermediate types registered variation with respect to the content of chlorophyll and carotenoids at 30, 60 DAT and at harvest (Figures 11, 12 and 13). Green type accessions of tulsi recorded higher chlorophyll content and lower carotenoid at all stages of crop growth. In contrast, purple types had higher carotenoids content and lower chlorophyll value. Chlorophyll and carotenoid values of intermediate types were in between green and purple types. Halder *et al*. (2017) reported that *Ocimum* subtype Rama with green leaves had higher amount of chlorophyll A (1.95 mg/g), chlorophyll B (0.96 mg/g) and total chlorophyll (2.91 mg/g) content than subtype Krishna (1.79, 0.89 and 2.62 mg/g, respectively) with purple coloured leaves. They also found that *Ocimum* subtype Krishna had higher quantity of anthocyanin compared to subtype Rama.

Variability in essential oil content was observed among accessions (Table 26) and it ranged from 0.22 per cent to 0.93 per cent at 30 DAT, 0.33 per cent to 1.11 per cent at 60 DAT and 0.22 per cent to 0.93 per cent at harvest. Raina *et al*. (2013) reported that essential oil content of 32 accessions of *O. tenuiflorum* collected from different parts of India showed wide variation and it ranged from 0.13 to 0.45 per cent.

Based on biochemical characters (chlorophyll, carotenoid and essential oil content) 35 accessions of tulsi were placed into five clusters at the standard Euclidean distance of 3.0 in cluster analysis (Table 27 and Fig. 8). Cluster III was the biggest cluster with 9 accessions. Cluster IV and V consisted of 7 accessions each and cluster I and cluster II included 6 accessions each. Principal component analysis was performed to identify the contribution of each variable to the total variation (Fig. 9 and Table 29). The first principal component (PC1) with Eigenvalue more than 1 itself accounted for 89.9 per cent of the total variation and the contributing trait was total chlorophyll content (0.962), whereas total carotenoid content (-0.958) and essential oil content (-0.925) had negative effect on PC1.

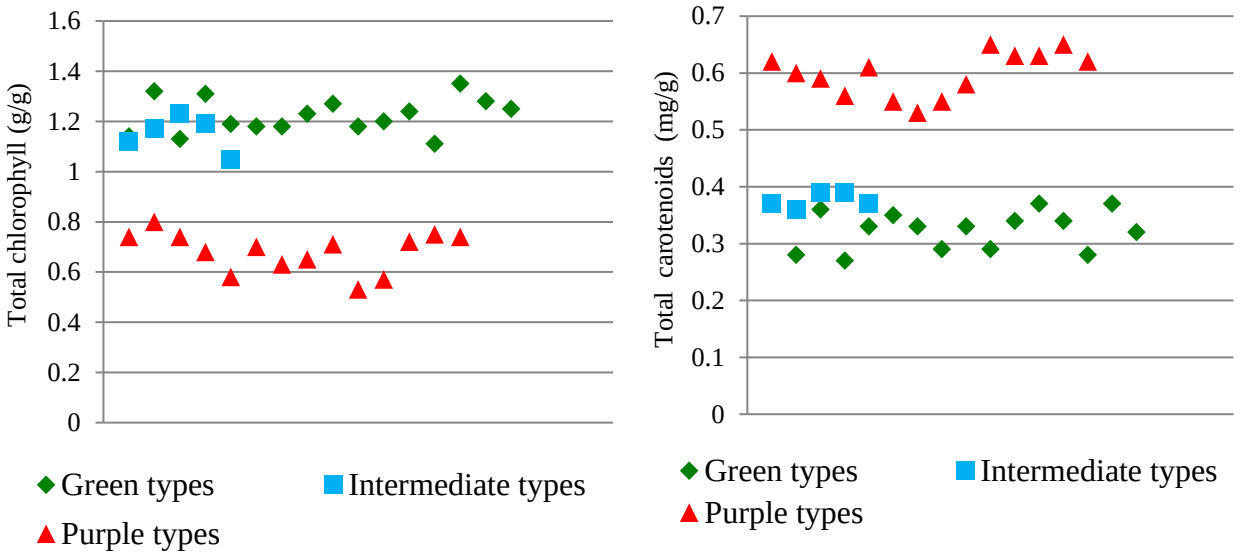


Fig. 11. Total chlorophyll and carotenoid content of green, purple and intermediate types of tulsi at 30 DAT

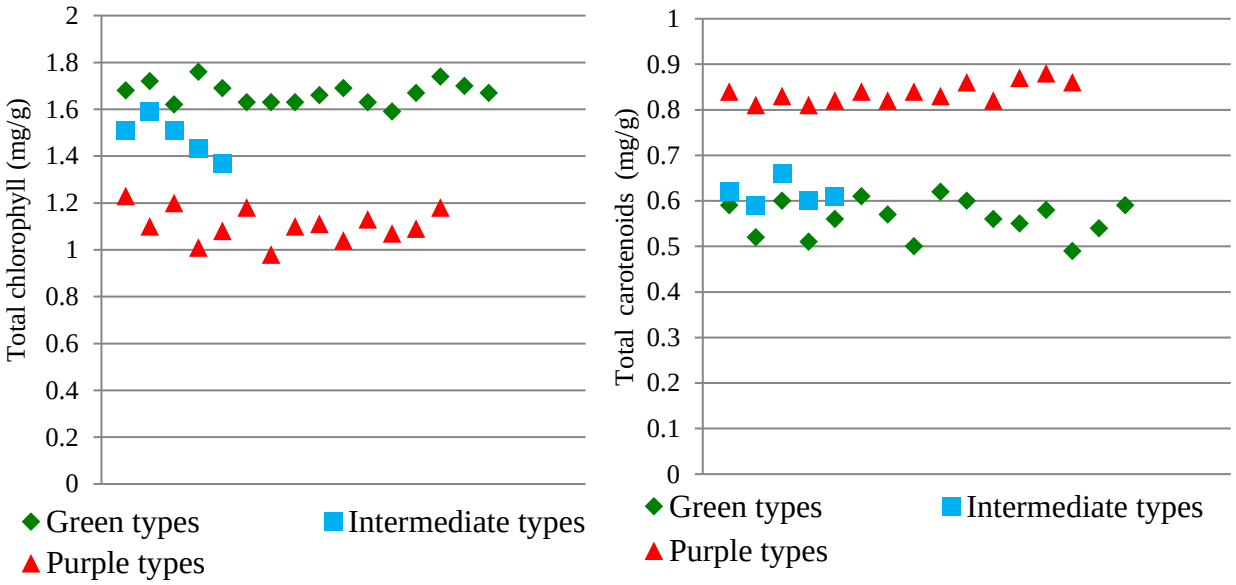


Fig. 12. Total chlorophyll and carotenoids contents of green, purple and intermediate types of tulsi at 60 DAT

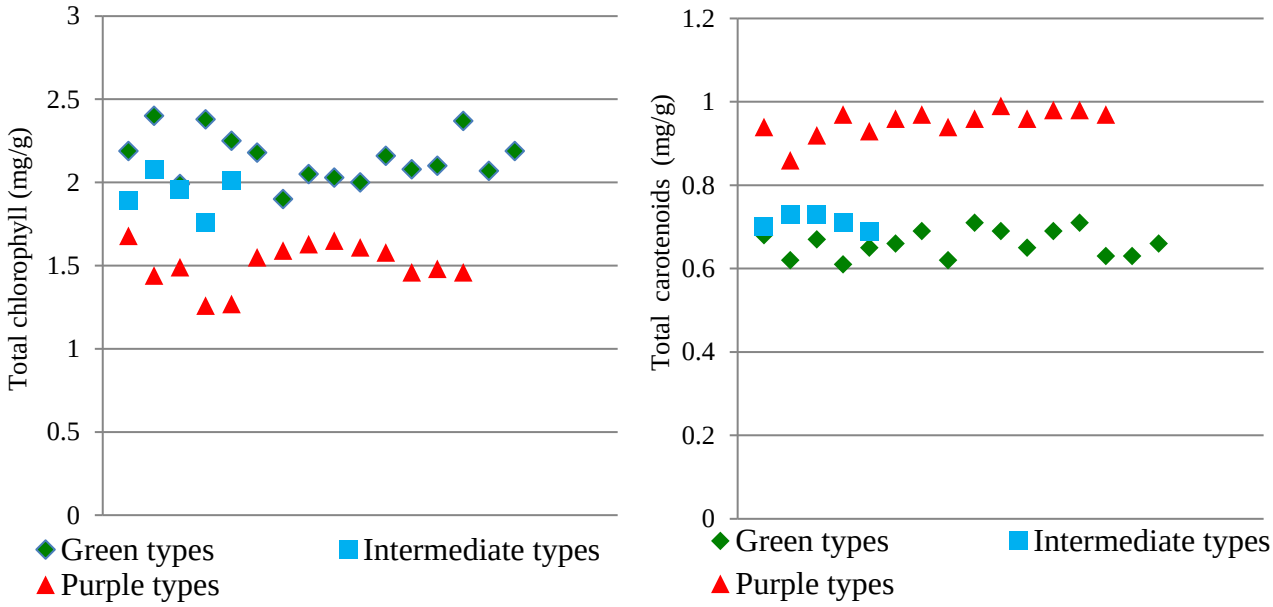


Fig. 13. Total chlorophyll and carotenoid contents of green, purple and intermediate types of tulsi at harvest

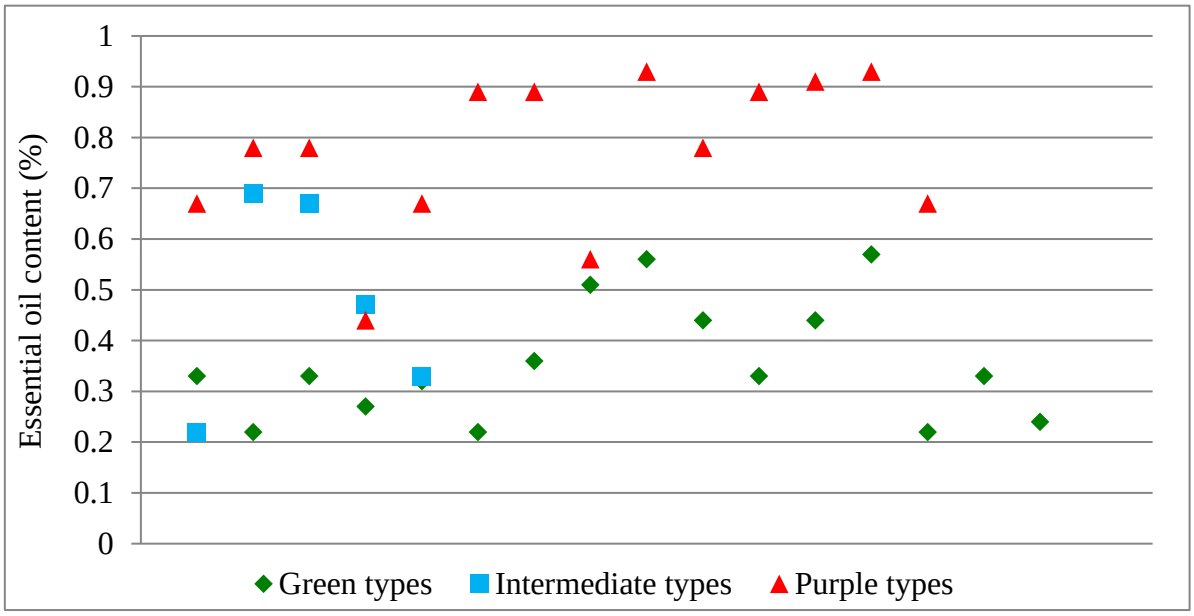


Fig.14 Essential oil content of green, intermediate and purple types of tulsi at 30 DAT

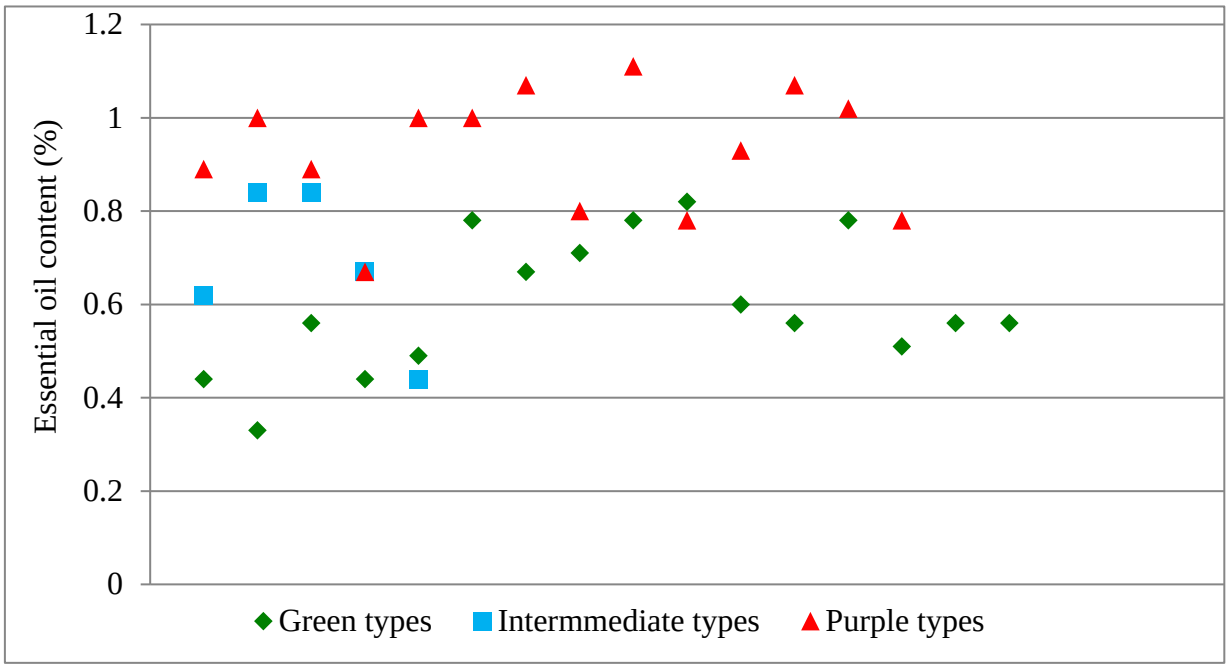


Fig.15 Essential oil content of green, intermediate and purple types of tulsi at 60 DAT

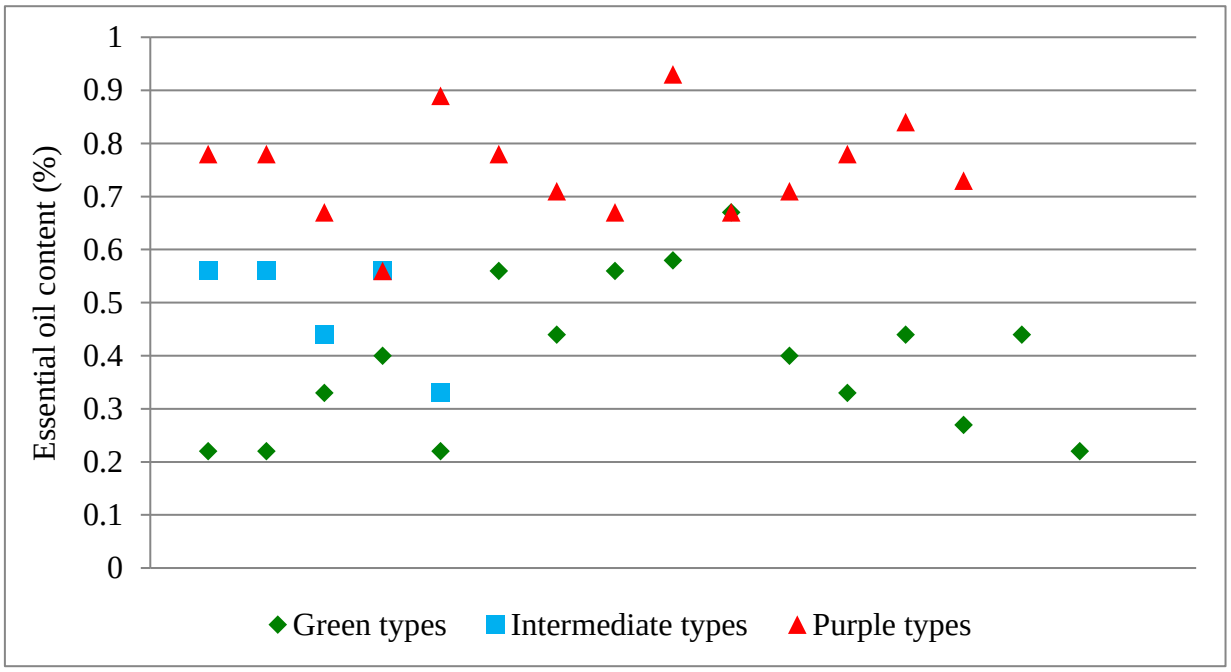


Fig.16 Essential oil content of green, intermediate and purple types of tulsi at harvest

Essential oil content at harvest had showed a strong positive correlation with carotenoid content (0.813*), and chlorophyll showed a strong negative correlation with essential oil content (-0.824*) (Table 30). Though non significant at all stages of growth biomass yield per plant was negatively correlated with essential oil content. While comparing essential oil content of three morphotypes, purple types recorded higher essential oil content (%), followed by intermediate types, and then green types (Figures 14, 15 and 16). High carotenoid content might have contributed to the increased essential oil content in purple type accessions. Purple types were found more suitable for essential oil production than green types. According to Dharmadasa *et al*. (2015), the essential oil yield obtained from *Ocimum tenuiflorum* purple and purple green morphotypes were 1.51±0.02 per cent (v/w) and 1.45±0.01 per cent (v/w), respectively. According to Sharma *et al*. (2014), black types of *O. sanctum* L. from Himachal Pradesh registered higher essential oil yield (1.3 %) than white types (0.8 %). The logistic binomial estimate of colour influencing essential oil content indicated that colour had a positive relation with essential oil content and the expected percentage of improvement for essential oil content over the base population was 80.41 per cent. The results revealed that colour could be used as a strong morphological marker for selecting genotypes for high essential oil content.

All the studied accessions were scored and ranked based on essential oil content and carotenoid content (Table 35) and best performing accessions for essential oil content were KAU OC 25, KAU OC 34 and KAU OC 32. An overall ranking was done to identify accessions suitable for both biomass yield and essential oil content (Table 36) and IC 583288, KAU OC 34 and KAU OC 35 were found best suitable for dual purpose. In the present study, all the 35 accessions displayed great diversity in all the parameters screened. The high degree of variation among the studied accessions indicated rich diversity represented within the populations from different phytogeographical regions (Malav *et al.,* 2015). Inter and intra-specific hybridization among basil species had resulted in morphotypic and chemotypic variation (Carovic *et al.,* 2006; Tilwari *et al*., 2013). Variability found in *Ocimum* spp was the due to the combined influence of genotype and environment (Verma *et al*., 1989).

5.2 Experiment 2. Effect of shade and harvesting method on the performance of tulsi

The influence growing condition (open and 50 per cent shade), harvesting method (stage of harvest and height of harvest) and their interactions on the performance of tulsi were studied during 2019-20 and 2020-21.

Growing condition significantly influenced plant height of tulsi. Plants grown under 50 per cent shade was taller compared to open condition (Figure 17). This might be due to the fact that plants under shade shows phototropism response and increases height to capture more light (Kumar *et al*., 2014). Milenkovic *et al*. (2019) reported that sweet basil plants under shade were taller than those grown under open due to increase in internodal length. As per Costa *et al*. (2010), *Ocimum selloi* plants raised under coloured shade net were taller than those under full sunlight. Thakur *et al*. (2019) and Huang *et al*. (2016) also reported higher plant height due to shade in aromatic and medicinal plants.

Plant height of tulsi during second harvest was higher in the treatments in which first harvest was done at 30 cm height from ground level (T_2 and T_4) compared to first harvest at 20 cm height from ground level (T_1 and T_3) (Figure 18). Retaining more shoot portion in plants harvested at 30 cm from ground level compared to those harvested at 20 cm from ground level helped might have helped them to retain more food reserves. This will have enhanced crop re-growth and resulted in taller plants during second harvest. Singh *et al*. (2010) reported that plant height in ratoon crop of *Ocimum basilicum* L. was significantly affected by cutting height in first harvest.

While comparing biomass yield between open and shade, it was clear that compared to open condition, plants grown under 50 per cent shade had higher biomass yield at all harvests (Figure 19). Suvera *et al*. (2015) reported higher biomass yield in *Ocimum* grown under silvi medicinal systems compared to sole cropping. Shaded plants of *Ocimum basilicum* registered significantly higher plant biomass yield than sun-exposed plants (Milenkovic *et al*., 2019).

Harvesting method significantly influenced the biomass yield of tulsi. Biomass yield at first harvest, second harvest and total biomass yield were higher when harvesting was done at 20 cm height from ground level at 75 DAT (T_1) and harvesting at 30 cm height from ground level at 75 DAT (T_2) (Figure 20). Early first harvesting (75 DAT) either at 20 or 30 cm height from ground was found significantly superior to late harvesting (90 DAT) and production of biomass was higher in first harvest and it gradually decreased in the second harvest. As per Gupta (1996) biomass yield of *Ocimum* was maximum when the plants were harvested between full flowering and initiation of seed formation stage. According to Corrado *et al.* (2020), basil could be harvested more than once and yield was significantly affected by the cut factor. They observed the higher biomass yield at the first harvest because the average size of leaf and total leaf area per plant was significantly higher at the first harvest. Kothari *et al*. (2004) reported that harvesting methods affected biomass yield of *Ocimum tenuiflorum* grown in south India and harvesting of shoot biomass at 30 cm from ground gave maximum herbage yield and harvesting of secondary branches had less biomass yield. According to them irrespective of method of harvest, biomass production was higher in first harvest and declined gradually in second, third and fourth harvests.

Growing condition also influenced photosynthetic pigments. Total chlorophyll content was higher for plants grown under 50 per cent shade, and in contrast total carotenoid content was higher in open condition. Stagnari *et al*. (2015) also reported higher chlorophyll content and lower carotenoid content under shade in lettuce. In this study, chlorophyll was negatively correlated with total carotenoid and eugenol content and positively correlated with essential oil yield and biomass yield. In contrast, total carotenoid was positively correlated with eugenol content and negatively correlated with essential oil yield and biomass yield (Table 52 and 53).

Chlorophyll content at first harvest was significantly higher in T_1 (harvesting of tulsi plants at 20 cm height from ground level at 75 and 135 DAT) and T_2 (harvesting at 30 cm height from ground level at 75 and 135 DAT). In these two treatments plants were harvested early (75 DAT) compared to other treatments (90

DAT), hence age of the leaf and plant might have affected the chlorophyll content. In plants, chlorophyll content increased from youngest leaf to photosynthetically mature leaf and thereafter decreased (Sestak, 1963). Mauromicale *et al*. (2006) also indicated the importance of plant age on chlorophyll content. Carotenoid contents were not affected by harvesting methods.

Among growing conditions, plants grown under 50 per cent shade recorded higher oil yield than open grown plants (Figure 21) and this might have been due to higher biomass yield under shade. African basil recorded comparatively high essential oil yield per plant when grown under natural shade compared to full sunlight (Ade-Ademilua *et al*., 2013). According to Suvera *et al*. (2015), *Ocimum* spp cultivated under silvi-medicinal systems produced higher oil yield than those raised under sole cropping.

Essential oil yield of tulsi was higher in T_1 (harvesting of tulsi plants at 20 cm height from ground level at 75 and 135 DAT) and T_2 (harvesting at 30 cm height from ground level at 75 and 135 DAT) (Figure 22). This might have due to higher biomass production in above treatments. The correlation studies also showed that essential oil yield and biomass yield were positively correlated (Tables 46 and 47). Essential oil yield (kg/ha) is obtained by multiplying biomass yield with essential oil content. Results also revealed that, oil yield of tulsi were higher in first harvest as compared to second harvest (Figures 21 and 22). Tansi and Nacar (2000) stated that in lemon basil, essential oil yield was maximum at the first harvest. According to Moghimipour *et al*. (2017), essential oil yield of *O. sanctum* at first harvest was slightly higher than at second harvest.

The GCMS analysis of tulsi essential oil showed that the components varied slightly under open and 50 per cent shade (Table 43). The principal constituent of tulsi essential oil under open as well as 50 per cent shade was eugenol. The second and third major component under open was germacrene-D and β-elemene respectively. However under 50 per cent shade, second major constituent was β-caryophyllene, germacrene-D was third major component. According to Costa *et al*. (2010), the compositions of essential oils of *Ocimum selloi* plants varied according to

the quality of light. Padalia and Verma (2011) reported that essential oil of *O. sanctum* was dominated by eugenol (67.4 % - 72.8 %). Awasthi and Dixit (2007) conducted GC and GC-MS analysis of *O. sanctum* oils and found that eugenol as the major constituent.

In this study, higher eugenol content was recorded in tulsi plants grown under open condition, compared to 50 per cent shade (Figure 23). According to Chang *et al*. (2008), essential oils composition of basil was strongly affected by light intensity and eugenol concentration and was higher under higher light integrals. In basil oil, the concentration of volatile compounds as well as their composition was affected by solar irradiance and the relative content of eugenol was high under shade (50 % shading) (Milenkovic *et al*., 2019). Similarly, in *O. gratissimum* L., eugenol content was maximum under open conditions compared to shade (Pillai, 1990).

Harvesting method influenced the eugenol content of tulsi. The eugenol content of tulsi at first harvest was higher in T_3 (harvesting at 20 cm height from ground level at 90 and 150 DAT) and T_4 (harvesting at 30 cm height from ground level at 90 and 150 DAT) (Figure 24). Harvesting of tulsi at 90 DAT (T_3 and T_4) produced significantly higher eugenol content than harvesting at 75 DAT (T_1 and T_2). This might be due to increase in the maturity of leaves in above treatments. *Ocimum* spp exhibited a high degree of variation in the essential oil composition with respect to the ontogenetical stage of the plant at the time of harvest (Gupta, 1996). According to Sims *et al*. (2013), eugenol content of tulsi increased with a delay in harvest time. Increase in eugenol concentration of *O. sanctum* with the advancement of harvesting stage was reported by Saran *et al*. (2017). It was also evident from the study that eugenol was higher in the first harvest than in the second. Similarly Tsasi *et al*. (2017) observed higher eugenol concentration in tulsi in the the first harvest under field conditions.

The growing condition and harvesting method had no significant influence on the population of soil microorganisms. In general population of bacteria and actinomycetes were more under open condition, while population of fungi was more under 50 per cent shade (Figure 25, 26 and 27). The growth and development of

microorganisms is affected by temperature, humidity, pH, litter inputs, light intensity, plant root exudates, nutrient content and proportion, and hydrostatic pressure (Bardgett *et al*., 2013). Fungi prefer a relative air humidity of 40 per cent or more and less light intensity for proper growth and functioning (Curling *et al*., 2002) and this might have been the reason for higher population of fungi under 50 per cent shade. Compared to initial count, average population of rhizosphere micro organisms showed increases at 45 DAT and thereafter it gradually declined. Data on rhizospere population of microbes clearly proves that tulsi plants do not have any inhibitory effect on the rhizospere micro organisms.

Growing condition and harvesting method had no significant effect on population of plant parasitic nematodes. In general nematode population was more under 50 per cent shade and compared to initial count plant parasitic nematode population was higher during crop growth period (Figure 28). Shukla *et al*. (1986) reported the association of plant parasitic nematodes with *Ocimum tenuiflorum* L. Even though tulsi extracts caused mortality of second stage juveniles of *M. incognita* (Table 52 and Figure 24), the cultivation of tulsi could not bring down the population of plant parasitic nematodes in soil. This showed that growing tulsi as a trap crop is not effective to control plant parasitic nematodes.

As compared to 50 per cent shade, weed growth was more under open condition. Plant height of tulsi was higher under 50 per cent shade, the reduced light availability to weeds due to taller crop plants could be the reason for less weed growth under shade. According to Vidhu (2019), weed growth in medicinal plant sida hemp was more under open compared to 50 per cent shade.

In this study, weed count and dry weight at first harvest were higher in T_3 (harvesting at 20 cm height from ground level at 90 and 150 DAT) and T_4 (harvesting at 30 cm height from ground level at 90 and 150 DAT). In these two treatments first harvest was late (90 DAT) compared to T_1 and T_2 (75 DAT) and this might have been the reason for higher weed growth at first harvest, while, at second harvest weed growth was higher in T_3 (harvesting at 20 cm height from ground level at 90 and 150 DAT) and T_1 (harvesting at 20 cm height from ground level at 75 and 135 DAT). In

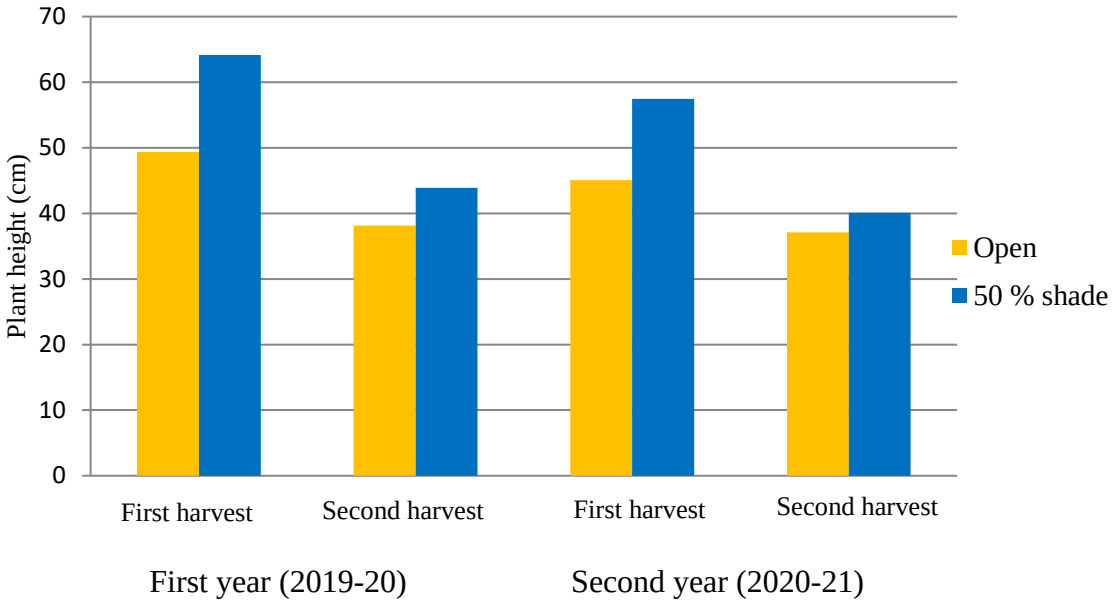
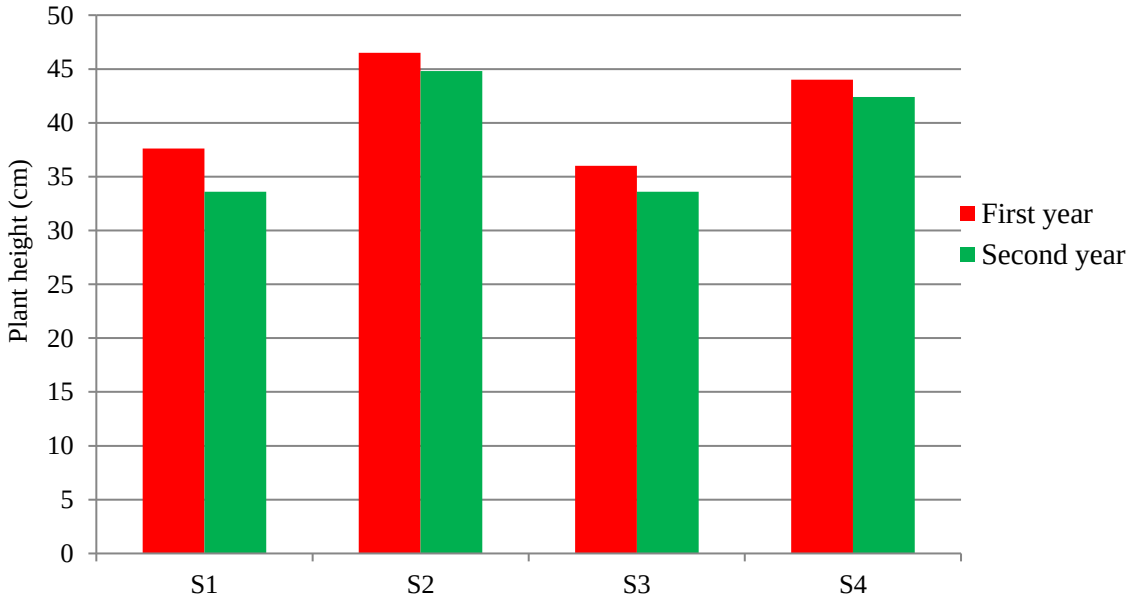


Figure 17. Effect of growing condition on plant height of tulsi at first harvest and second harvest during first year and second year



S1. Harvest at 20 cm height at 75 and 135 DAT
S2. Harvest at 30 cm height at 75 and 135 DAT
S3. Harvest at 20 cm height at 90 and 150 DAT
S4. Harvest at 30 cm height at 90 and 150 DAT

Figure 18. Effect of harvesting method on plant height of tulsi at second harvest during first year and second year

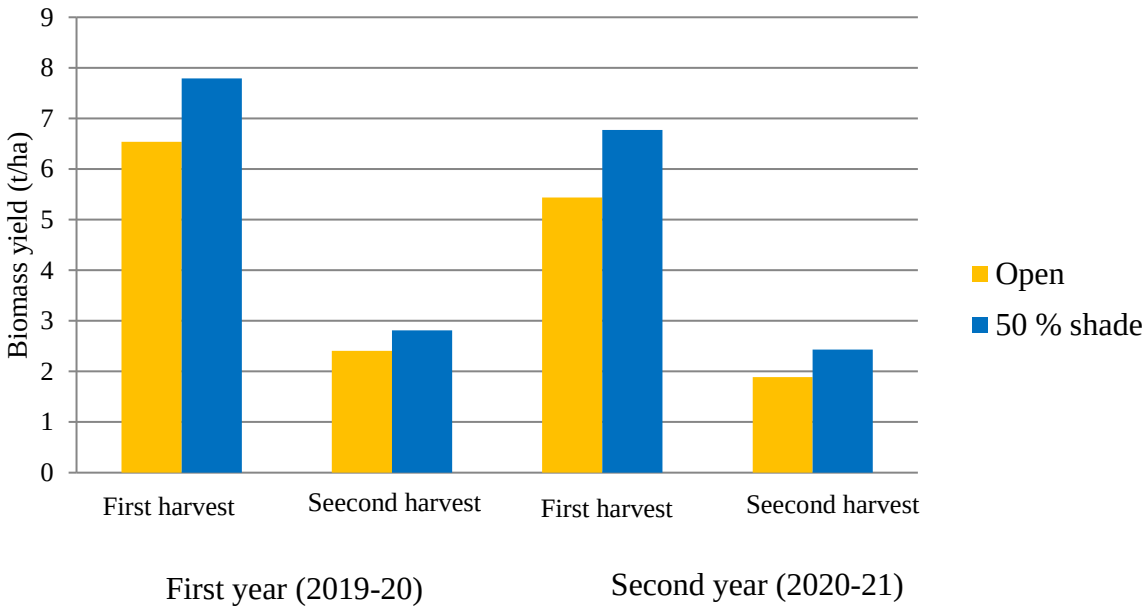
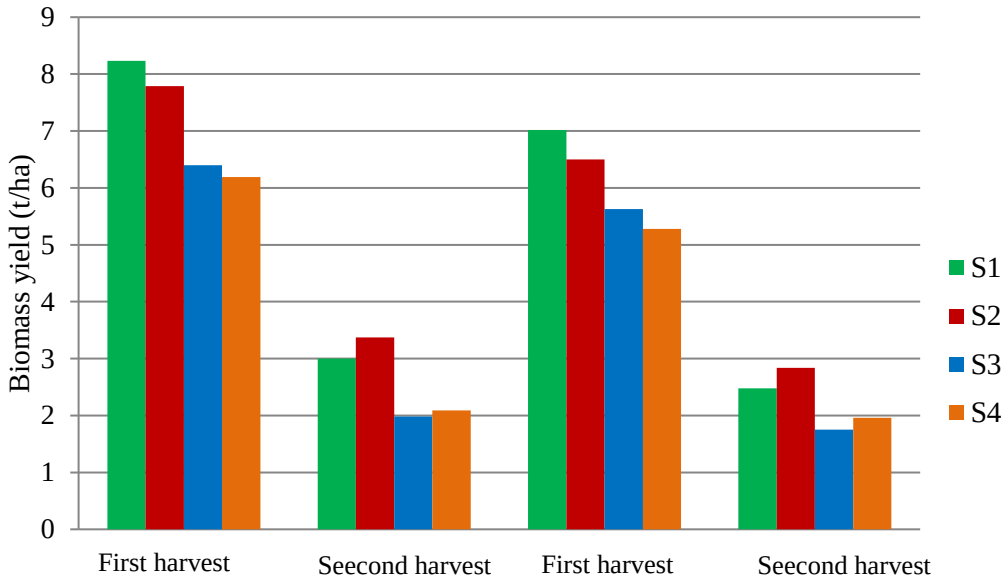


Figure 19. Effect of growing condition on biomass yield of tulsi at first harvest and second harvest during first year and second year



S1. Harvest at 20 cm height at 75 and 135 DAT
S2. Harvest at 30 cm height at 75 and 135 DAT
S3. Harvest at 20 cm height at 90 and 150 DAT
S4. Harvest at 30 cm height at 90 and 150 DAT

Figure 20. Effect of harvesting method on biomass yield of tulsi at first harvest and second harvest during first year and second year

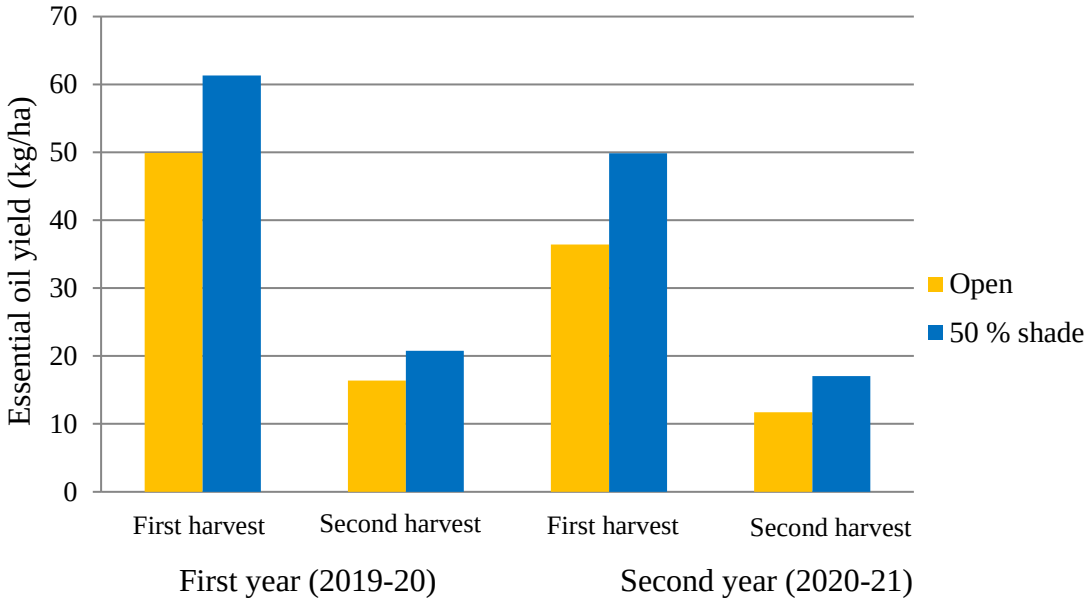
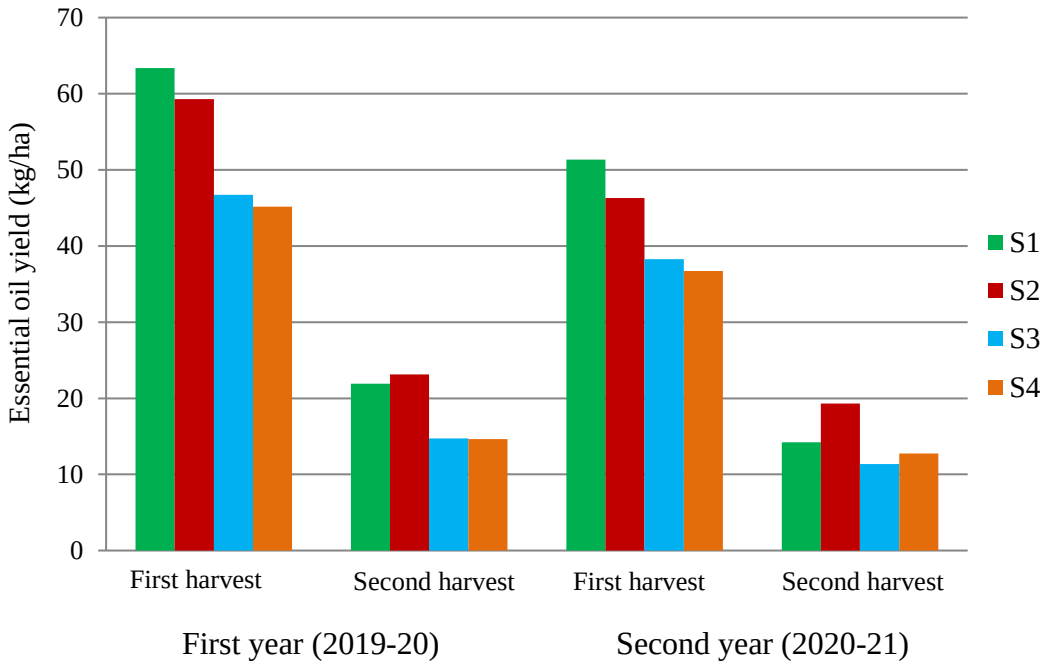


Figure 21. Effect of growing condition on essential oil yield (kg/ha) of tulsi at first harvest and second harvest during first year and second year



S1. Harvest at 20 cm height at 75 and 135 DAT
S2. Harvest at 30 cm height at 75 and 135 DAT
S3. Harvest at 20 cm height at 90 and 150 DAT
S4. Harvest at 30 cm height at 90 and 150 DAT

Figure 22. Effect of harvesting method on essential oil yield (kg/ha) of tulsi at first harvest and second harvest during first year and second year

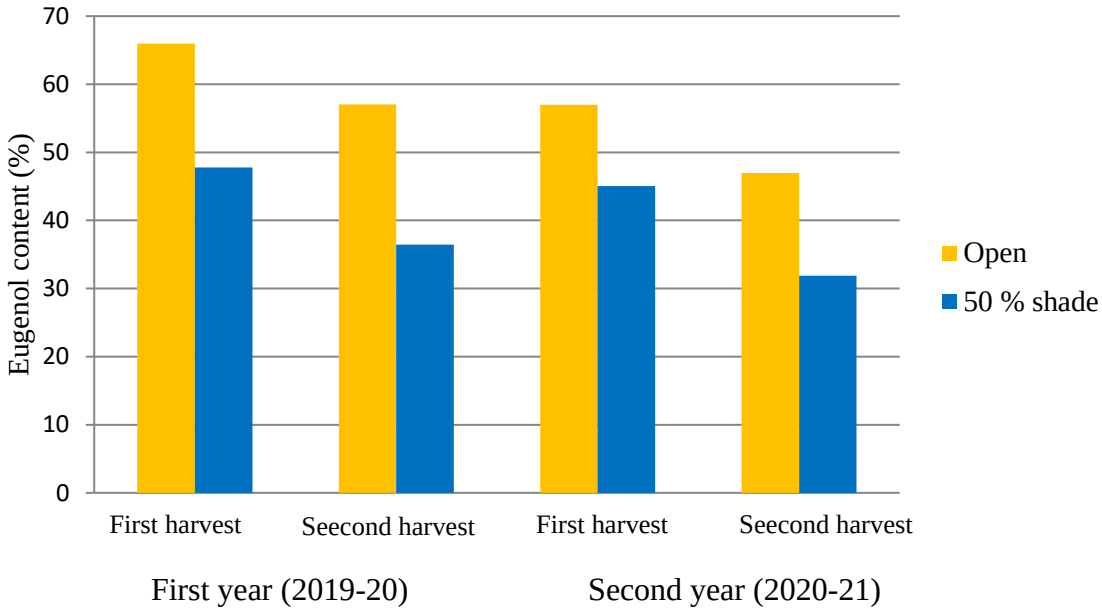
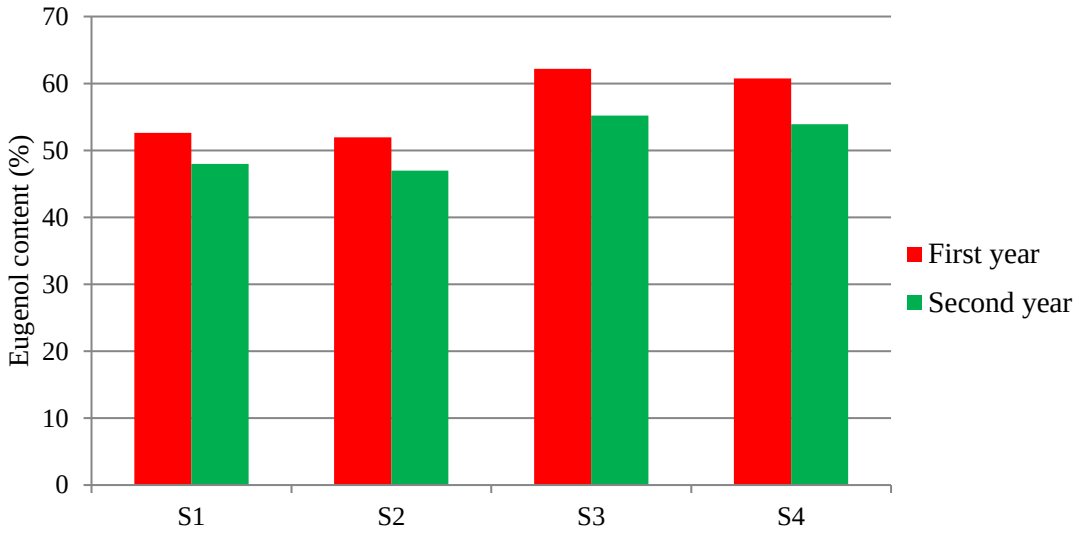


Figure 23. Effect of growing condition on eugenol content (%) of tulsi at first harvest and second harvest during first year and second year



S1. Harvest at 20 cm height at 75 and 135 DAT
S2. Harvest at 30 cm height at 75 and 135 DAT
S3. Harvest at 20 cm height at 90 and 150 DAT
S4. Harvest at 30 cm height at 90 and 150 DAT

Figure 24. Effect of harvesting methods on eugenol content (%) of tulsi at first harvest during first year and second year

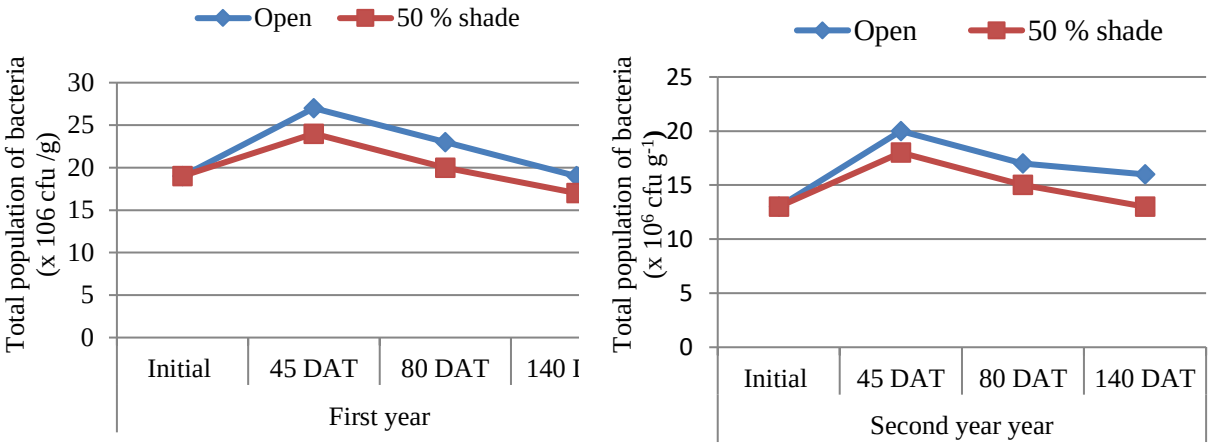


Figure 25.Total population of bacteria under open and 50 % shade at different growth stages during first year and second year

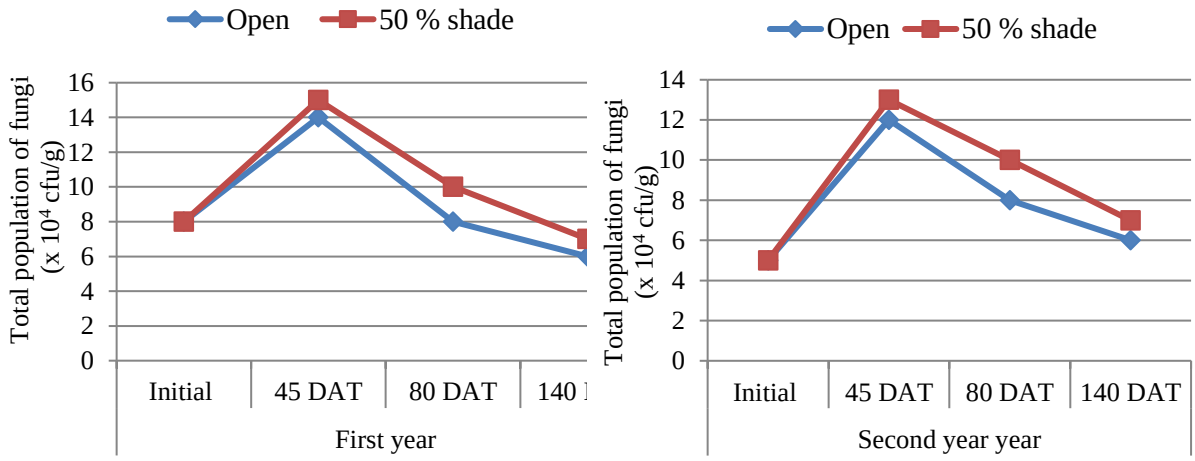


Figure 26.Total population of fungi under open and 50 per cent shade at different growth stages during first year and second year

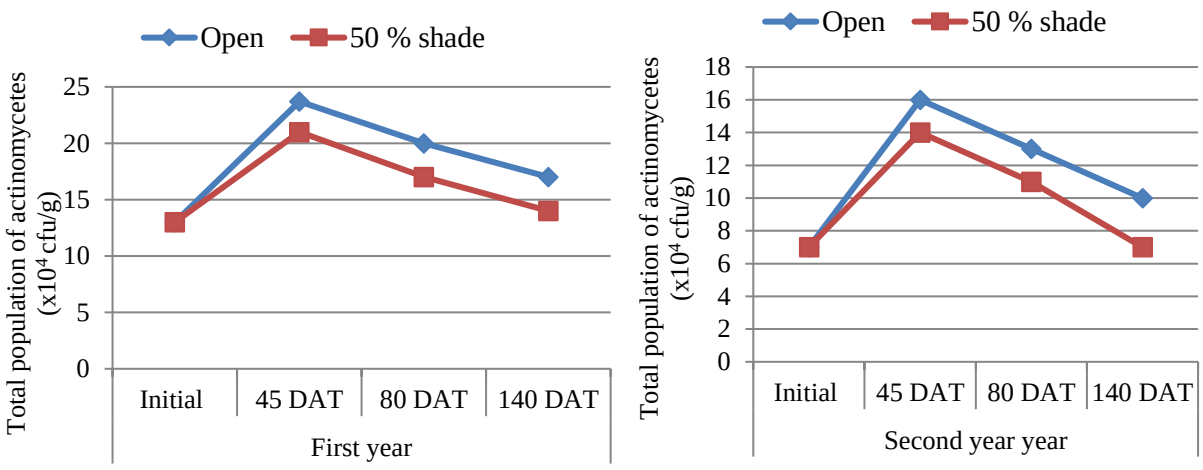


Figure 27.Total population of actinomycetes under open and 50 per cent shade at different growth stages during first year and second year

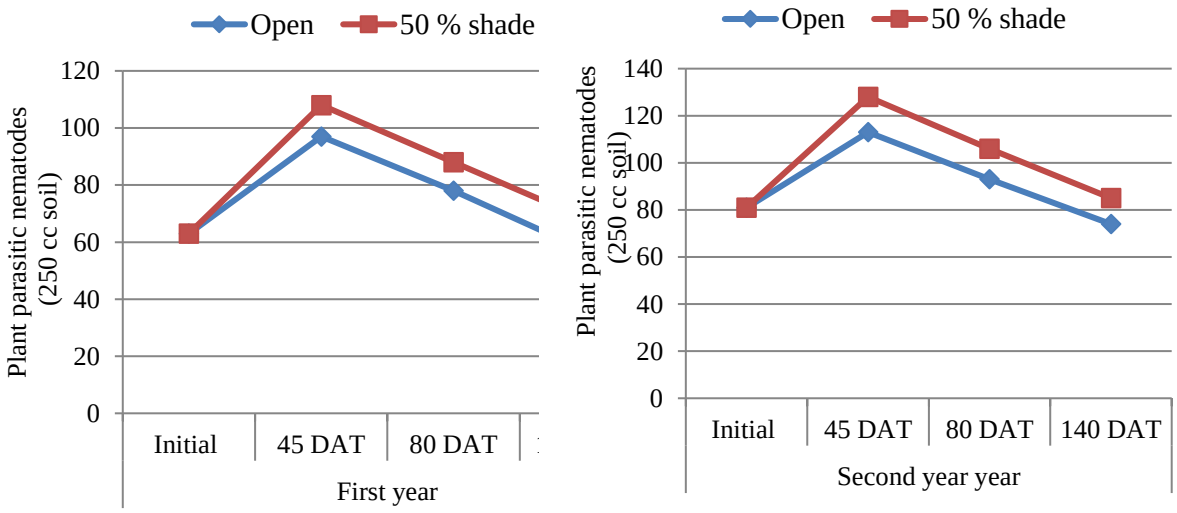


Figure 28.Total population of plant parasitic nematodes from 250 cc soil under open and 50 per cent shade at different growth stages during first year and second year

these two treatments first harvest was done at lower height (20 cm), and this might have reduced ground cover by the crop and increased light availability to weeds and thereby enhanced weed growth at second harvest.

Growing condition and harvesting method significantly influenced the performance of tulsi. Plants grown under 50 per cent shade and harvesting either at 20 or 30 cm height from ground level at 75 and 135 DAT recorded higher biomass yield. Plants grown under open condition with harvesting either at 20 or 30 cm height from ground level at 90 and 150 DAT recorded higher eugenol content.

5.3 Allelopathic potential of tulsi on upland weeds and test crops

5.3.1 Effect on upland weeds

Major weeds germinated from trays were *Trianthema portulacastrum*, *Alternanthera philoxeroides*, *Boerhavia diffusa*, *Cleome viscosa, Euphorbia hirta*, *Amaranthus spinosus* and *Panicum repens*. Tulsi extracts and powders of different parts at all concentrations exhibited phytotoxic activity against upland weeds. In the 1st week, the highest weed germination of 166.67 nos./m^2 was observed in control (without tulsi extracts and powders), while the germination of weeds in treatments which received tulsi shoot powder at 20 g/kg soil and root powder at 20 g/kg soil were only 3.33 and 6.67 nos./m^2 (Table 54 and Fig.29). Weed germination at 2nd, 3rd and 4th weeks were not statistically significant. There was a notable delay or inhibition in germination of upland weeds. As per Sharma and Singh (2003), germination of redroot pigweed, hairy beggarticks and guineagrass were completely inhibited with incorporation of 7.5 g basil leaf powder as compared to control. According to them, this significant reduction in germination of weed species could be the result of the presence and/or release of phenolic compounds from basil leaf powder. Similarly Dhima *et al*. (2009) found that aqueous extracts of shoot portion of sweet basil decreased germination of barnyard grass (*Echinochloa crusgalli* L.). The inhibition of weed seed germination due to *Ocimum sanctum* was also reported by Knox *et al*. (2010).

Weed density and dry weight were recorded at one month after application (Table 55 and Fig. 30). As compared to control, the presence of all extracts and powders of tulsi decreased density as well as dry weight of grasses, broad leaved weeds and total weeds. However, there was no significant difference in density and dry weight of grass weeds. In this experiment, broad leaved weeds showed more inhibitory response than grass weeds. These results were in line with Mekky and Hassanein (2021). According to them fresh weight of grassy, broad leaved and total weeds were significantly reduced by aqueous extracts of sweet basil, but the reduction of grassy weeds was not statistically significant.

All treatments had adverse effect on weed growth and among these, powders of shoot portion and roots of tulsi exhibited the highest allelopathic activity, followed by aqueous extracts and hot water extracts. The lower density of broad leaved weeds and total weeds were observed when shoot portion powder of tulsi at 20 g/kg soil (140.33 and 208.67 nos./m^2) and root powder at 20 g/kg soil (144.33 and 211.33 nos./ m^2) were incorporated into the soil. Similar trend was observed in the case of weed dry weight also. The powder form of shoot and root at 20 g/kg soil decreased total weed density by 41.87 per cent and 41.13 per cent and total weed dry weight by 53.63 per cent and 49.2 per cent respectively. Among plant parts used, shoot portion had more allelopathic activity. Allelopathic effects of basil powder were probably due to the release of allelochemicals after decaying (Chou and Patrick, 1976). Similar results were reported by Verma *et al*. (2012). According to them, all the extracts of *Ocimum* exhibited inhibitory effect on weeds with maximum effect by leaf followed by roots and seeds. Mekky *et al*. (2019) also observed allelopathic activity of *Ocimum* extracts against weeds.

The *Ocimum* plants have been investigated as potential allelopathic plants (Balicevic *et al*., 2015). Tusli extracts and powders of different parts contain allelochemicals such as phenols, tannins, alkaloids and flavanoids (Table 5). Phytotoxic effects of tulsi on upland weeds might be due to different allelochemicals present in the extracts and powders and in which total alkaloids contributed maximum to the phytotoxicity, followed by total phenols (Table 58). Allelochemicals influences the cell division, cell elongation, membrane permeability and enzyme activity of receiver plants (Dragoeva *et al*., 2015). The growth reduction induced by allelochemicals might be either due to the prevention of cell division and enlargement or by the inhibition of IAA and GA_3 (Tomaszewski and Thimann, 1966).

5.3.2 Effect on test crops (rice and cowpea)

Both weeds and crops are sensitive to allelochemicals. In this study, germination indices and seedling growth of test crops were adversely affected by the application of extracts and powders of tulsi (*O. tenuiflorum*) (Table 56, Table 57, Fig.31 and Fig. 32). As compared to control (without tulsi extracts and powders) speed of germination and final germination percentage were significantly decreased and mean germination time increased.

In rice, final germination percentage was lower in the treatment with shoot powder at 20 g/kg soil (86.10 %), followed by root powder at 20 g/kg soil (88.90 %), whereas hot water extract of different parts at all concentrations and aqueous extract of different parts at lower concentration did not affect germination percentage of rice. Similarly, the final germination percentage of cowpea was significantly lower in the treatment which received tulsi shoot powder at 20 g/kg soil (80.53 %) and was on par with all other treatments except control and hot water extracts. Tulsi is a great source of allelochemicals, the easy absorption of these chemicals by crop seeds might have delayed or inhibited their germination.

The effects of extracts and powders of tulsi were not limited to germination inhibition alone, it also caused impairment in the metabolic activities leading to decrease in their root and shoot length, fresh and dry weight (Table 56 and 57 and Fig.32). Compared to control, shoot length, root length and seedling vigor index of test crops were lower in all extracts and powders of tulsi. Similarly fresh weight and dry weight at one month after application also was significantly reduced due to the presence of tulsi. As per Rice (1984), reduced seed germination, coleoptile and radical elongation and root or shoot growth inhibition were the most common allelopathic symptoms.

In this study, both rice and cowpea were found sensitive to all extracts and powders of tulsi. Among test crops, cowpea was more sensitive. Plants responded to various *Ocimum* extracts through morphological, anatomical and physiological adjustments and this vary from one species to another (Mekky *et al*., 2019) and this

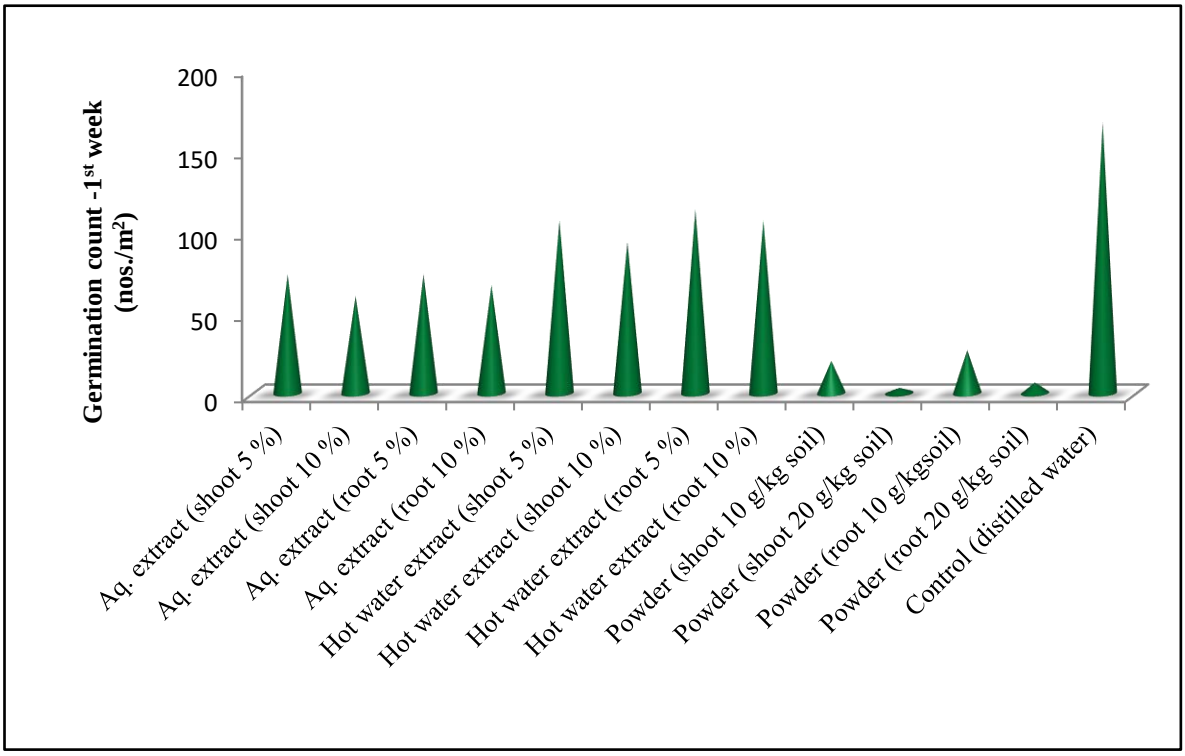


Fig. 29. Effect of tusli extracts and powders on weed germination count at 1st week

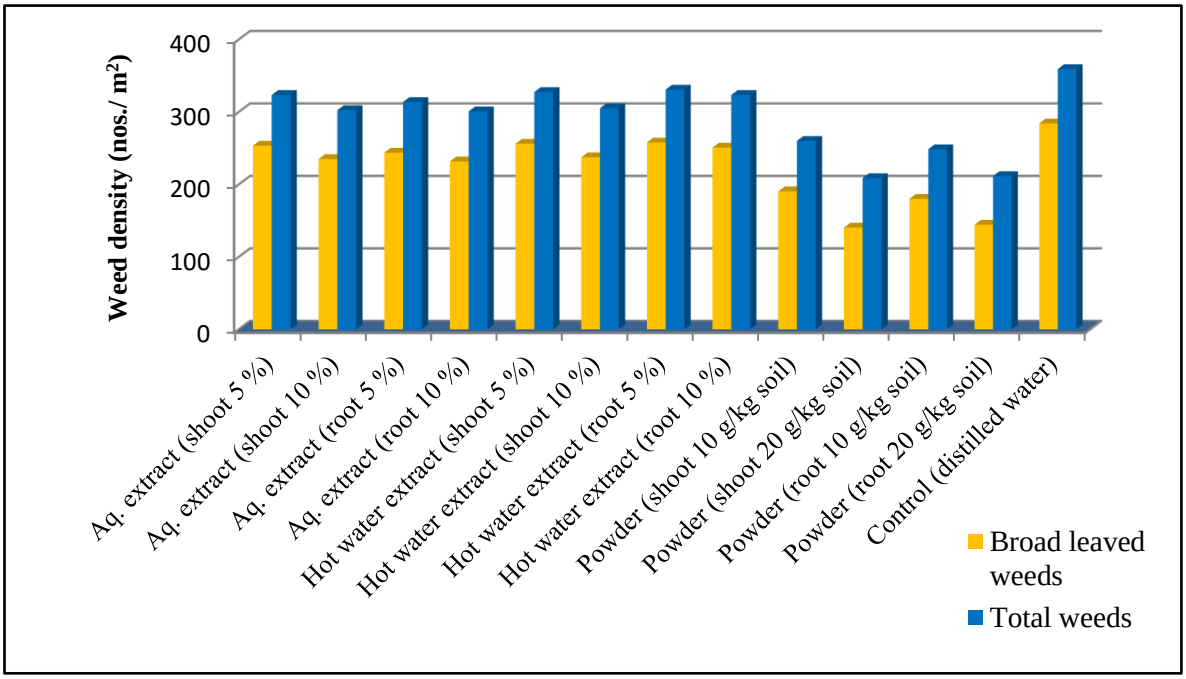


Fig. 30. Effect of tusli extracts and powders on broad leaved weeds and total weeds density

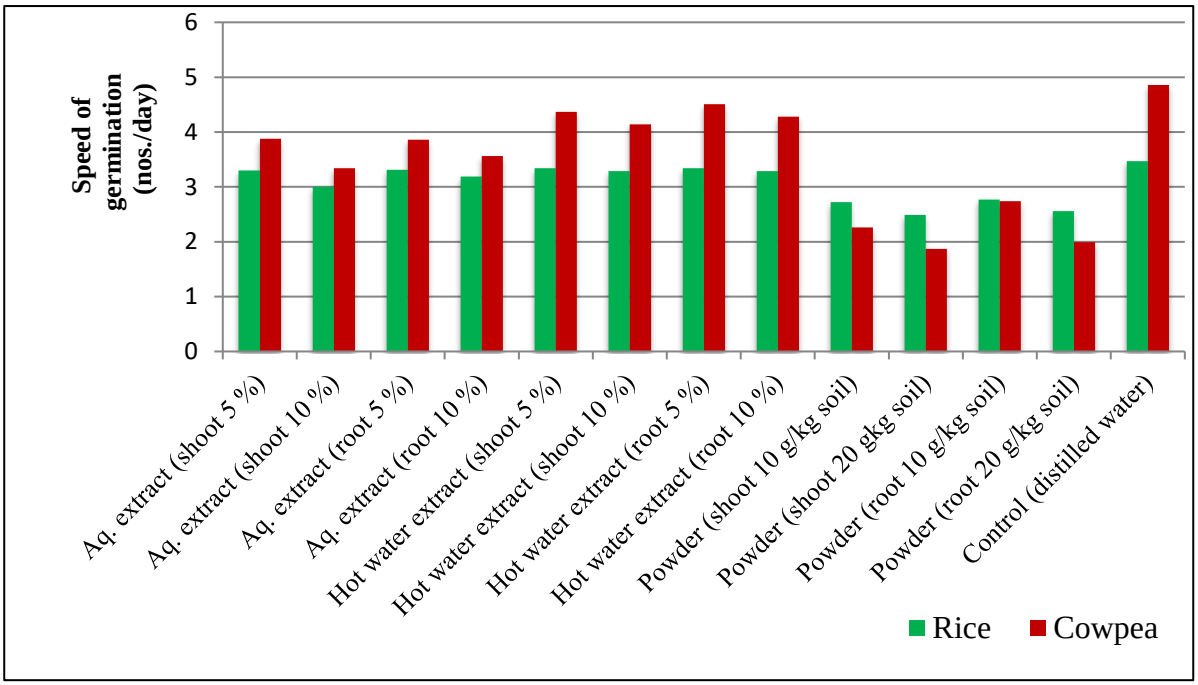


Fig. 31. Effect of tusli extracts and powders on speed of germination of rice and cowpea

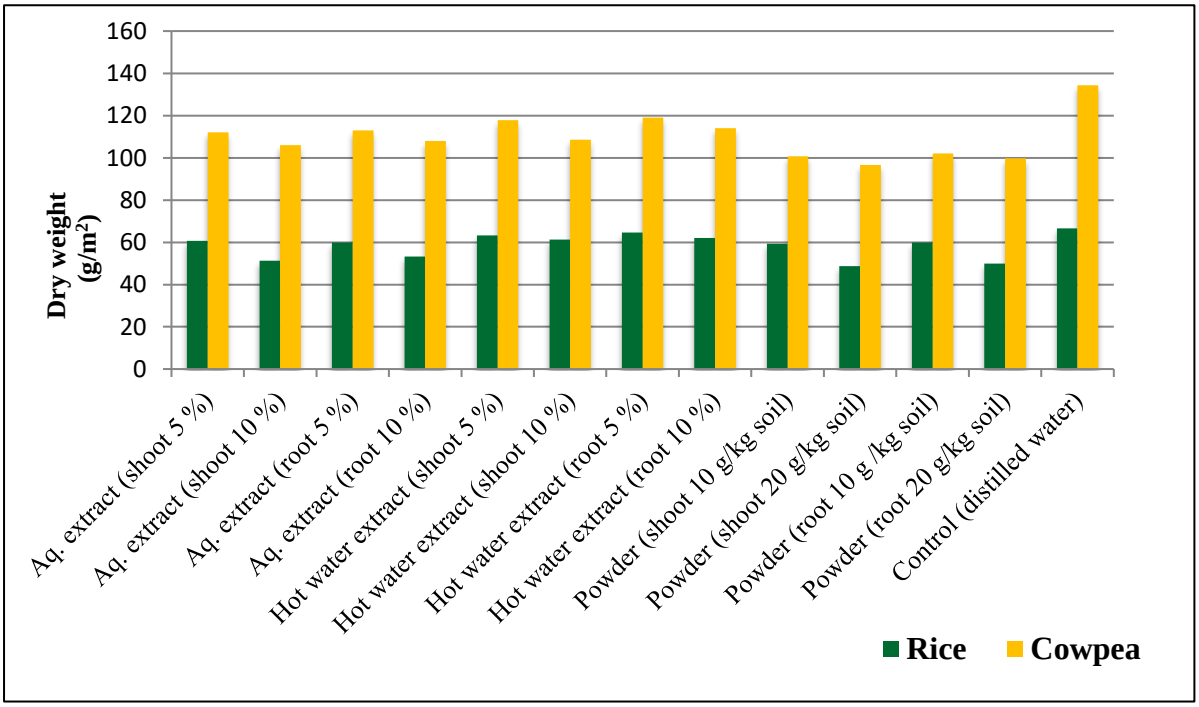


Fig. 32. Effect of tusli extracts and powders on dry weight of rice and cowpea at one month after application

might be the reason for differential response of test crops rice and cowpea in this study. The inhibition was related to method of extraction, concentration and plant part used. Among plant parts used, shoot portion had more allelopathic activity. It was observed from the study that incorporation of tulsi powder showed greatest inhibitory effect, followed by aqueous extracts. The powder form of shoot and root at 20 g/kg soil inhibited germination percentage of rice by 13.9 per cent and 11.1 per cent and cowpea by 19.47 per cent and 16.67 per cent respectively. Hot water extracts exhibited least effect. The inhibition increased with increase in concentration also.

The observed reduction in germination and seedling growth of test crops could be due to the presence of allelopathic phytochemicals such as alkaloids, flavanoids, phenols and tannins in extracts and powders. Allelochemicals influences the cell division, cell elongation, membrane permeability and enzyme activity of receiver plants (Dragoeva *et al*., 2015). The correlation studies showed that among different allelochemicals, total alkaloids contributed maximum to the phytotoxicity (Table 58).

According to Verma *et al*. (2012), aqueous extracts derived from *Ocimum* plants inhibited seed germination of crops like wheat, gram, lentil, mustard, barley, okra and pea. In addition, *Ocimum* extracts significantly affected the root and shoot elongation of all the test crops. Dafaallah and Ahmed (2019) also screened phytotoxic activity of sweet basil on cereal crops and reported that aqueous extract of above ground parts of sweet basil inhibited seed germination of sorghum, millet, maize and wheat. Among tested cereal crops, maize was most sensitive followed by millet, wheat and sorghum. Tulsi plants contain various biologically active compounds that might have contributed to its phytotoxic effect (Nahak *et al*., 2011). The allelopathic effects of sweet basil on seed germination and seedling growth of poaceous crops were reported by Dafaallah and Ahmed (2017). Suwitchayanon *et al.* (2017) evaluated phytotoxic activity of fourteen medicinal plants on test crop lettuce and reported that *Ocimum tenuiflorum* inhibited lettuce radicle and hypocotyls growth by 74.0 per cent and 31.8 per cent respectively. The inhibitory activity of *O. tenuiflorum* plant extracts on the germination and seedling growth of other plant species was also reported by Islam and Noguchi (2014).

5.4 Nematicidal potential of tulsi against root-knot nematode (*Meloidogyne incognita*)

Evaluation of nematicidal action of tulsi against root-knot nematode proved significant effect of tulsi extracts on the mortality rate of second stage juveniles (J_2s) of *M. incognita* (Table 59 and Figure 33). The highest mortality of J_2s was observed in aqueous extract of tulsi @ 30 per cent for all the periods of exposure, whereas control (distilled water) recorded 0.00 per cent mortality. The second best treatment was 20 per cent aqueous extract of tulsi followed by hot water extract @ 30 per cent concentration. Hot water extract of tulsi @ 20 per cent and 10 per cent showed less effect on mortality of juveniles. However, all the extracts of tulsi tested exhibited some level of toxicity toward second stage juveniles (J_2s) of the root-knot nematode.

Egg hatch inhibition and high juvenile mortality of the root-knot nematode by the application of leaf extracts of tulsi was reported by Claudius-cole *et al*. (2010). As per Pandey *et al*. (2000), essential oil of *O. basilicum* was highly toxic to *M. incognita* even at the lower concentrations. According to Asimiea *et al.* (2015), aqueous extract of *O. gratissimum* leaves at 20 ml/kg of soil was comparable to carbofuran in the management of *M. incognita* in okra. Neeraj *et al*. (2017) also observed mortality in juveniles of root-knot nematode due to the presence of aqueous extracts of tulsi.

In this study, when second stage juveniles were exposed to tulsi extracts for a longer period (72 h) higher mortality compared to shorter periods (48, 24 and 12 h) were observed. Hasabo and Noweer (2005) and Ranjitsingh and Sucheta (2009) reported that botanicals at longer exposure time were more effective in decreasing egg-hatch inhibition and increasing juvenile morality of *M. incognita* compared to the control. According to Pavaraj *et al*. (2012), plant extracts exhibited highly promising mortality against *M. incognita* after 72 h exposure.

Mortality of J_2s was influenced by concentration of extracts also. In general, juvenile mortality increased with increase in concentration. Both aqueous and hot water extracts of tulsi at higher concentration (30 %) showed higher mortality of J_2s,

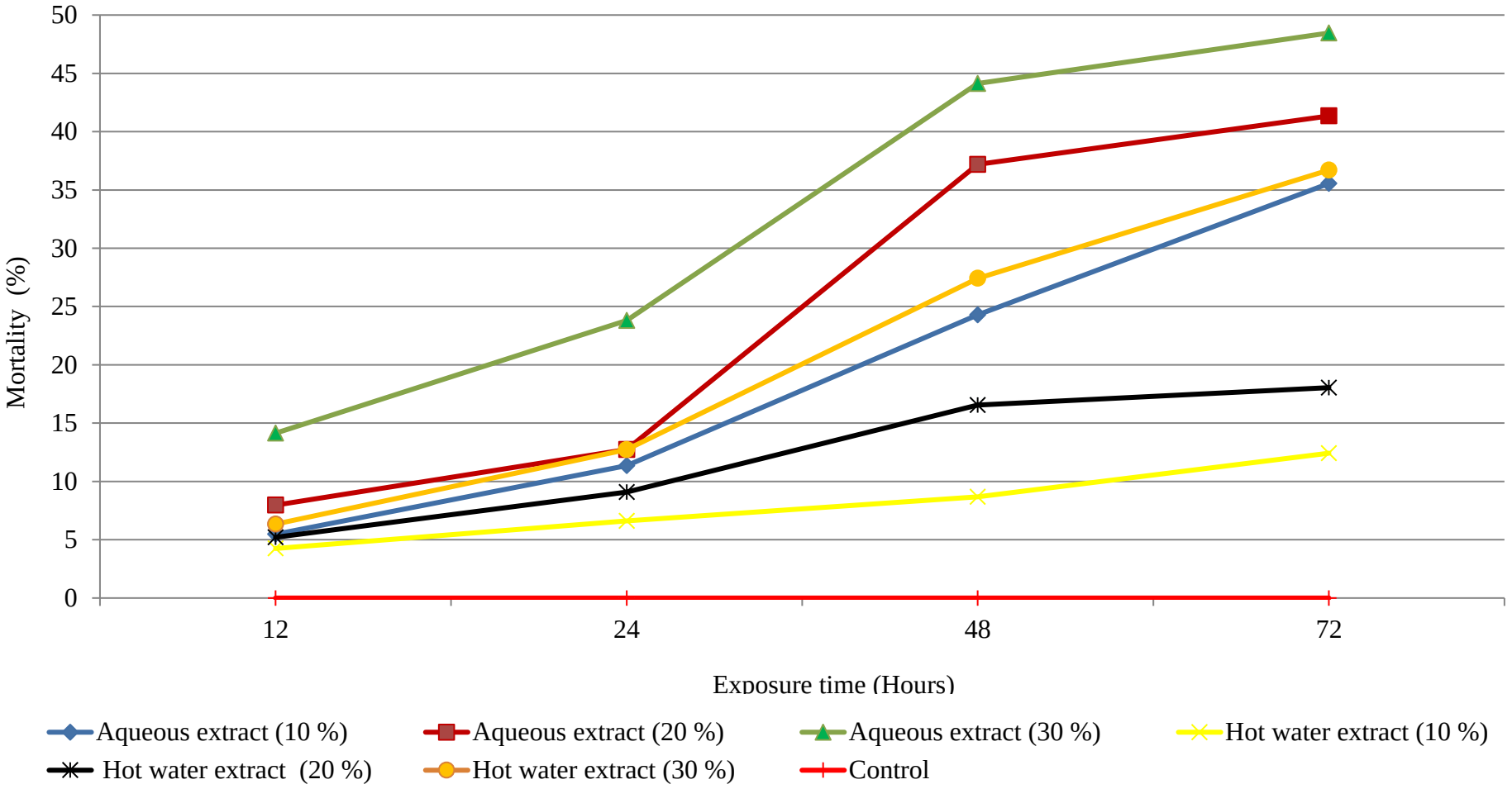


Fig.33 Effect of treatments on mortality of second stage juveniles after 12, 24, 48 and 72 hours of incubation

followed by its lower concentration (20 % and 10 %). This agrees with the findings of Jidere and Oluwatayo (2018), who indicated that increase in concentration and exposure time led to decrease in egg hatching ability and increase in mortality rate of *M. incognita* and vice versa. Compared to hot water extracts, aqueous extract of tulsi gave better results at all the periods of exposure. Results also showed that after 24, 48 and 72 h of application, mortality caused by hot water extract of tulsi at higher concentration (30 %) was statistically on par with mortality caused by aqueous extract of tulsi at lower concentration (10 %).

Many plants possessing insecticidal activity are known today. The inhibitory activity of plant extracts might be due to the presence of chemicals that possessed ovicidal and larvicidal properties. *Ocimum* spp. contains phenolic constituents such as eugenol, methyl eugenol, iso eugenol, methyl chavicol and traces of terpenoids and traces of acids (Vasudevan *et al.*, 1999). Some of the bio active compounds such as eugenol and methyl eugenol in *O. sanctum* exhibited insecticidal and antimicrobial properties (WOI, 1991; Bhavya *et al*., 2018). Secondary metabolites such as phenols, tannins, alkaloids and flavanoids that were identified in the extracts (Table 5) could be responsible for the observed nematicidal effect in the present study. Direct contact of plant extracts might have effectively delivered the active ingredients to the juveniles of the root-knot nematodes.

The present study proved the nematicidal property of tulsi extracts on second stage juveniles of root-knot nematode (*M. incognita*). Among the treatments, 30 per cent aqueous extract of tulsi exhibited highest inhibitory effect and could be utilized for nnematode control. However, further studies on identification of the exact active compound and its mode of action have to be conducted.

Summary

6. SUMMARY

The research programme entitled 'Characterization and evaluation of tulsi (*Ocimum tenuiflorum* L.)' was carried out during 2018-2021 at the Department of Agronomy, College of Agriculture, Vellanikkara. The research work consisted of four experiments.The objectives of the study were evaluation of *Ocimum tenuiflorum* L. accessions, standardisation of shade requirement and method of harvesting, investigation of allelopathic effect on upland weeds and crops, and also assessment of the effect of tulsi on root-knot nematode (*Meloidogyne incognita*). The salient findings are summarised and presented here.

Experiment 1: Evaluation of *O. tenuiflorum* L. accessions

- High degree of variation was observed among the accessions for all the parameters studied.
- Thirty five accessions of tulsi were grouped into three morphotypes based on the colour of stem, upper leaf surface and inflorescence. 16 accessions were identified as green types, 14 were purple types and 5 were intermediate.
- The Euclidean cluster analysis grouped the accessions into six clusters using eight quantitative characters and first three principal components (plant height, primary branches and secondary branches) contributed for 79.7 per cent of variability.
- Biomass yield per plant showed strong positive correlation with leaf area index and primary branches per plant.
- Accessions were scored and ranked based on total biomass yield, number of primary branches and leaf area index. IC 583288, IC 583318, IC 583296 and KAU OC 30 were the best performing accession with respect to biomass yield.
- The essential oil content of the accessions also exhibited wide variation.
- Accessions were grouped into 3 clusters based on biochemical characters and the first principal component (chlorophyll content) accounted for 89.9 per cent of the total variation.

- Colour can be used as a strong morphological marker for selecting genotypes for high essential oil content and expected percentage of improvement for essential oil content over the base population is 80.41 per cent.
- The best performing accessions with respect to essential oil content were KAU OC 25, KAU OC 34 and KAU OC 32.
- Overall ranking was done to identify accessions with high biomass yield and essential oil content. IC 583288, KAU OC 34 and KAU OC 35 were found most suitable for dual purpose.

Experiment 2: Effect of shade and harvesting method on the performance of tulsi

Effect of growing condition

- Plant height, biomass yield and essential oil yield of tulsi was higher under 50 per cent shade.
- Total chlorophyll content was higher under 50 per cent shade, while total carotenoid was higher in open condition.
- The principal constituent of tulsi essential oil under open as well as 50 per cent shade was eugenol, with higher eugenol content under open condition.
- Population of bacteria and actinomycetes were higher under open condition, while population of fungi and plant parasitic nematodes were higher under 50 per cent shade.
- As compared to 50 per cent shade, weed growth was more under open condition.

Effect of harvesting method

- Harvesting at 20 cm or 30 cm height from ground level at 75 and 135 DAT recorded higher biomass yield, essential oil yield and total chlorophyll content.
- Delay in harvest increased eugenol content of tulsi.
- Harvesting at 20 cm or 30 cm height from ground level at 90 and 150 DAT recorded higher eugenol content.
- Eugenol content of tulsi was higher in the first cut than second cut.

- The harvesting methods had no significant influence on the population of soil micro organisms and plant parasitic nematodes.

Interaction effect of growing condition and harvesting method

- Plants grown under 50 per cent shade with harvesting either at 20 or 30 cm height from ground level at 75 and 135 DAT recorded higher biomass yield.
- Plants grown under open condition with harvesting either at 20 or 30 cm height from ground level at 90 and 150 DAT recorded higher eugenol content.

Experiment 3: Evaluation of allelopathic potential of tulsi on upland weeds and test crops

Effect of tulsi on upland weeds

- Tulsi is rich in allelochemicals and exhibited phytotoxic activity against upland weeds.
- A notable delay or inhibition in germination of upland weeds observed.
- Weed density and dry weight decreased by the application of tulsi extracts and powders.
- Broad leaved weeds showed more inhibition than grass weeds.
- Powdered form of tulsi had the highest allelopathic activity, followed by aqueous extracts and hot water extracts.
- Among plant parts used, shoot had more allelopathic activity than roots.
- Tulsi shoot and root powder at 20 g/kg showed the highest allelopathic activity against weeds.

Effect of tulsi on test crops (rice and cowpea)

- Germination indices and seedling growth of rice and cowpea were adversely affected by the application of extracts and powders of tulsi.
- Lower speed of germination, germination percentage and higher mean germination time was observed with the application of tulsi extracts and powders.

- The shoot and root length, seedling vigor, fresh weight and dry weight of test crops were also reduced by the application of tulsi extracts and powders.
- Cowpea was more sensitive than rice.
- The inhibition was related to method of extraction, concentration and plant part used.
- Tulsi shoot powder and root powder at 20 g/kg soil showed the highest phytotoxicity.

Experiment 4: Evaluation of nematicidal potential of tulsi against root-knot nematode (*Meloidogyne incognita*)

- Tulsi extracts had significant effect on mortality rate of second stage juveniles (J_2s) of *M. incognita.*
- The highest mortality of J_2s was observed in aqueous extract of tulsi @ 30 per cent for all the periods of exposure.
- J_2s exposed to tulsi extracts for a longer period (72 h) had higher mortality compared to shorter periods (48, 24 and 12 h).
- Mortality of J_2s was influenced by concentration of extracts. Juvenile mortality increased with increase in concentrations.
- 30 per cent aqueous extract of tulsi exhibited maximum inhibitory effect and can be utilized for the nematode control.

Conclusion

- Wide variability observed among the accessions of tulsi. The best performing accessions for biomass yield were IC 583288, IC 583318, IC 583296 and KAU OC 30, and for essential oil production the best accessions were KAU OC 25, KAU OC 34 and KAU OC 32. Accessions IC 583288, KAU OC 34 and KAU OC 35 were found most suitable to cultivate for both biomass yield and essential oil content.

- Growing of tulsi under shade and harvesting at 20 - 30 cm height from ground level at 75 and 135 DAT can be recommended for higher biomass. Whereas , open growing condition and harvesting at 20 - 30 cm height at 90 and 150 DAT can be recommended for higher eugenol content.

- Tulsi shoot and root powder at 20 g/kg soil showed the highest allelopathic activity against weeds and test crops.

- Aqueous extract of tulsi @ 30 per cent can be recommended for the nematode control.

Future line of work

- Evaluation of best performing accessions under multi locations and seasons for varietal development.

- Identification of exact compound responsible for the allelopathic and nematicidal activity of tulsi.

References

REFERENCES

Ade-Ademilua, E.O., Obi, H.O., and Craker, L.E. 2013. Growth and essential oil yield of African basil, *Ocimum gratissimum*, under light and water stress. *J. Med. Aromat. Plants* 1(4): 143-149.

Adhvaryu, M.R., Reddy, N., and Vakharia, B.C. 2008. Prevention of hepatotoxicity due to anti tuberculosis treatment: a novel integrative approach. *World J. Gastroenterol*. 14: 4753-4762.

Agarwal, C., Sharma, N.L., and Gaurav, S.S. 2013. An analysis of basil (*Ocimum* sp.) to study the morphological variability. *Indian J. Fundam. Appl. Life Sci.* 3(3): 521-525.

Agarwal, G.P. and Hasija, S.K. 1986. *Microorganisms in Laboratory*. Print House India Ltd., Lucknow, 155p.

Ahmad, S.D. and Khaliq, I. 2002. Morphomolecular variability and heritability in *Ocimum sanctum* genotypes from northern Himalayan regions of Pakistan. *Pakistan J. Biol. Sci.* 5(10): 1084-1087.

Ali, A. and Ali, M. 2012. New fatty acid derivatives from *Ocimum sanctum* L. leaves. *Indian Drugs* 49(11): 112-123.

Allan, R.E., Vogel, O.A., and Peterson, C.J. 1962. Seedling emergence rate of fall-sown wheat and its association with plant height and coleoptile length. *Agron. J.* 54(4): 347- 353.

Amrin, S. and Abhijit, K. 2019. Studies on allelopathic effect of *Ocimum sanctum* on a common weed *Cassia uniflora*. *Int. J. Res. Anal. Rev.* 42: 40-45.

Asimiea, O.A., Tanimola, A.A., and Osaronwi, M.O. 2015. Nematicidal effect of aqueous extract of *Ocimum gratissimum* (L.) leaves on *Meloidogyne incognita* on okra. *J. Appl. Sci. Agric.* 10(1): 22-28.

ASTA (American Spice Trade Association). 1968. *Official Analysis Methods* (2nd Ed.). American Spice Trade Association, New York, 53p.

Awasthi, P.K. and Dixit, S.C. 2007. Chemical compositions of *Ocimum sanctum* shyama and *Ocimum sanctum* rama oils from the plains of Northern India. *J. Essent. Oil- Bear. Plants* 10(4): 292 -296.

Bahl, J.R., Garg, S.N., Bansal, R.P., Naqui, A.A., Singh, V., and Kumar, S. 2000. Yield and quality of shoot essential oil from vegetative, flowering and fruiting stage crops of *Ocimum basilicum*. *J. Med. Plant Sci.* 22: 743-746.

Balicevic, R., Ravlic, M., and Ravlic, I. 2015. Allelopathic effect of aromatic and medicinal plants on *Tripleurospermum inodorum* (L.) C.H. Schultz. *Herbologia* 15(2): 41-53.

Balyan, S.S. and Pushpangadan, P. 1988. A study on the taxonomic status and geographical distribution of genus *Ocimum*. *PAFAI* 10(2): 13-19.

Bardgett, R.D., Manning, P., Morrien, E., and De Vries, F.T. 2013. Hierarchical responses of plant-soil interactions to climate change: consequences for the global carbon cycle. *J. Ecol*. 101: 334–343.

Basra, S.M.A., Farooq, M., Tabassam, R., and Ahmad, N. 2005. Physiological and biochemical aspects of pre-sowing seed treatments in fine rice (*Oryza sativa* L.). *Seed Sci. Technol.* 33(3): 623-628.

Bast, F., Pooja, R., and Devendra, M. 2014. Chloroplast DNA phylogeography of holy basil (*Ocimum tenuiflorum*) in Indian subcontinent. *Sci. World J*. 84: 45-53.

Bharadwaj, A. and Sharma, S. 2007. Effect of some plant extracts on the hatch of *Meloidogyne incognita* eggs. *Int. J. Bot.* 3(3): 312-316.

Bhavya, M.L., Chandu, A.G.S., and Devi, S.S. 2018. *Ocimum tenuiflorum* oil, a potential insecticide against rice weevil with anti-acetylcholin esterase activity. *Indian Crops Prod.* 126: 434-439.

Bhavya, M.L., Obulaxmi, S., and Devi, S.S. 2020. Efficacy of *Ocimum tenuiflorum* essential oil as grain protectant against coleopteran beetle, infesting stored pulses. *J. Food Sci. Technol.* 70: 25-33.

Blackman, V.H. 1919. The compound interest law and plant growth. *Ann. Bot.* 33(3): 353-360.

Bruce, K.K., Scott, J.R., and David, L.R. 2007. Characters as groups: a new approach to morphological characters in phylogenetic analysis. *Taxon*. 56(2): 479-492.

Bunrathep, S., Palanuvej, C., and Ruangrungsi, N. 2007.Chemical compositions and antioxidative activities of essential oils from *Ocimum* species endemic to Thailand. *J. Health Res.* 21(3): 210- 206.

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in food-A review. *Int. J. Food Microbiol*. 94: 223-253.

Carovic, K., Liber, Z., Javornik, B., Kolak, I., and Satovic, Z. 2006. Genetic relationships within basil (*Ocimum*) as revealed by RADP and ALP markers. *Acta Hortic.* 760: 171-178.

Carovic-Stanko, K., Liber, Z., Besendorfer, V., Javornik, B., Bohanec, B., Kolak, I., and Satovic, Z., 2010. Genetic relations among basil taxa (*Ocimum* sp.) based on molecular marker nuclear DNA content, and chromosome number. *Plant Syst. Evol*. 285: 13-22.

Castronuovo, D., Russo, D., Libonati, R., Faraone, I., Candido, V., Picuno, P., Andrade, P., Valentao, P., and Milella, l. 2019. Influence of shading treatment on yield, morphological traits and phenolic profile of sweet basil (*Ocimum basilicum* L.). *Sci. Hortic*. 254: 91-98.

Chang, C.L., Cho, I.K., and Li, Q.X. 2009. Insecticidal activity of basil oil, trans-anethole, estragole, and linalool to adult fruit flies of *Ceratitis capitata*, *Bactrocera dorsalis*, and *Bactrocera cucurbitae*. *J. Econ. Entomol*. 102: 203-209.

Chang, X., Alderson, P.G., and Wright, C.J. 2008. Solar irradiance level alters the growth of basil (*Ocimum basilicum* L.) and its content of volatile oils. *Environ. Exp. Bot*. 63(3): 216-223.

Chedekal, A.N. 2013. Effect of four leaf extracts on egg hatching and juvenile mortality of root knot nematode (*Meloidogyne incognita*). *Int. J. Adv. Life Sci.* 6(1): 68-74.

Chitwood, D.J. 2002. Phytochemical based strategies for nematode control. *Ann. Rev. Phytopathol*. 40: 221-249.

Chou, C.H. and Patrick, Z.A. 1976. Identification and phytotoxic activity of compounds produced during decomposition of corn and rye residues in soil. *J. Chern. Eca*. 2: 369-387.

Claudius-cole, A.O., Aminu, A.E., and Fawole, B. 2010. Evaluation of plant extracts in the management of rootknot nematode *Meloidogyne incognita* on cowpea [*Vigna unguiculata* (L)]. *Mycopath* 8(2): 53-60.

Cobb, N.A. 1918. Estimating the nematode population of the soil. *Agric. Tech. Cir*. 1: 43-48p.

Corrado, G., Chiaiese, P., Lucini, L., Moreno, B.M., Colla, G., and Rouphae, Y. 2020. Successive harvests affect yield, quality and metabolic profile of sweet basil. *Agron*. 10(830): 2-13.

Costa, L.C.B., Pinto, J.E.P., Castro, E.M., Alves, E., Rosal, L.F., Bertolucci, S.K.V., Alves, P.B., and Evangelino, T.S. 2010.Yield and composition of the essential oil of *Ocimum selloi* cultivated under colored netting. *J. Essent. Oil Res.* 22(1): 34-39.

Curling, S.F., Clausen, C.A., and Winandy, J.E. 2002. Experimental method to quantify progressive stage of decay of wood by basidomycetes fungi. *Int. Biodeterior. Biodegrad.* 49: 13-19.

Dafaallah, A.B. and Ahmed, S.A. 2017.Allelopathic effects of sweet Basil (*Ocimum basilicum* L.) on seed germination and seedling growth of some poaceous crops. *Int. J. Environ. Agric. Biotechnol.* 2(5): 2629-2635.

Dafaallah, A.B. and Ahmed, S.A. 2019. Phytotoxic effects of Basil (*Ocimum basilicum* L.) aqueous extract on seed germination of some cereal crops. *Libyan J. Agric*. 24(2): 63-72.

Das, A., Kar, S., and Datta, B.K. 2020. Morpho-metric study of four *Ocimum* taxa from Tripura. *J. Adv. Plant Sci*. 10(1): 36-41.

Devi, P.U. 2001. Radioprotective, anticarcinogenic and antioxidant properties of the Indian holy basil, *Ocimum sanctum* (Tulasi). *Indian J. Exp. Biol*. 39: 185-190.

Dewey, D.R. and Lu, K.H. 1959. A correlation and path coefficient analysis of components of crested wheat grass seed production. *Agron. J*. 51: 515-518.

Dhar, A.K., Dhar, R.S., Rekha, K., and Koul, S. 1996. Effect of spacings and nitrogen levels on herb and oil yield, oil concentration and composition in three selections of *Cymbopogon jwarancusa* (Jones) Schultz. *J. Spices Arom. Crops* 5(2): 120-126.

Dhar, A.K., Sharma, P.R., and Agarwal, S.G. 2006. Dynamics of essential oil biosynthesis in relation to inflorescence and glandular ontogeny in *Salvia sclarea*. *Flav. Frag. J.* 21: 817-821.

Dharmadasa, R.M., Siriwardhane, D.A.S., Samarasinghe, K., Rangana, L., Nugaliyadda, S., and Aththanayake, A.M. 2015. Screening of two *Ocimum tenuiflorum* L. (Lamiaceae) morphotypes for their morphological characters, essential oil composition and fruit fly attractant ability. *World J. Agric. Res*. 3: 1-4.

Dhima, K.V., Vasilakoglou, I.B., Gatsis, D., and Eleftheroh, I.G. 2009. Effects of aromatic plants incorporated as green manure on weed and maize development. *Field Crops Res.* 110: 235-241.

Douda, O., Zouhar, M., Mazakova, J., Novakova, E., and Pavela, R. 2010.Using plant essences as alternative mean for northern root-knot nematode (*Meloidogyne hapla*) management. *J. Plant Sci*. 83: 217-221.

Dragoeva, A.P., Koleva, V.P., and Stoyanovea, Z.D. 2015. Allelopathic effects of *Adonis vernalis* L.: root growth inhibition and cytogenetic alterations. *J. Agric. Chem. Environ*. 4(2): 48-55.

Durrani, M.K.A. and Prasad, C. 2009. Allelopathic influence of basil extracts on *Brassica rapa* L.: II. root weight and days to flower. *Dev. Rep. Biol*. 28: 34-37.

El-Rokiek, K.G., and El-Nagdi, W.M. 2011. Dual effects of leaf extracts of eucalyptus citriodora on controlling purslane and root-knot nematode in sunflower. *J. Plant Prot. Res.* 51(2): 121-129.

El-Rokiek, K.G., El-Din, S.A.S., El-Wakeel, M.A., Dawood, M.G., and El-Awadi, M.E. 2018. Allelopathic effect of the two medicinal plants *Plectranthus amboinicus* (Lour.) and *Ocimum basilicum* L. on the growth of *Pisum sativum* L. and associated weeds. *Middle East J. Agric. Res.* 7(3): 1146-1153.

Fanaei, M., Aboutalebi A., and Hasanzadeh, H. 2013.Allelopathic effects of Sweet basil (*Ocimum basilicum*) extract and essence on chlorophyll content of three weed species. *Int. Res. J. Appl. Basic Sci.* 4(6): 1511-1513.

Fernandes, V.F., de Almeid, L.B., Feijo, E.V.R., Silva, D.C., de Oliveira, R.A., Mielke, M.S., and Costa, L.C. 2013. Light intensity on growth, leaf micromorphology and essential oil production of *Ocimum gratissimum*. *Brazilian J. Pharmacogn.* 23(3): 419-424.

Filho, S., Bayma, A.P. S., Guilhon, J. C., and Zoghbi, M. G. B. 2009. Potential allelopathic activity of the essential oil of *Ocimum americanum*. *Planta Daninha* 27(3): 499 -505.

Gniazdowska, A. and Bogatek, R. 2005. Allelopathic interaction between plants. Multi site action of allelochemicals. *Acta Physiol. Plant* 27: 395-407.

Gould, W.D., Hagedorn, C., Bardinelli, T.R., and Zablotowics, R.M. 1985. New selective media for enumeration and recovery of fluorescent pseudomonas from various habitats. *Appl. Environ. Microbiol*. 49: 28-32.

Guerra, A.M., Silva, M.G.M., and Evangelista, R.S. 2020. Growth environment and pot volume affect biomass and essential oil production of basil1. *Rev. Caatinga* 33(1): 135-141.

Gupta, S., Mediratta, P.K., Singh, S, Sharma, K.K., Shukla, R. 2006. Antidiabetic, antihypercholesterolaemic and antioxidant effect of *Ocimum sanctum* (Linn) seed oil. *Indian J. Exp. Biol.* 44: 300-306.

Gupta, S.C. 1996. Variation in herbage yield, oil yield and major component of various *Ocimum* species varieties (chemotypes) harvested at different stages of maturity. *J. Essent. Oil Res*. 8: 275-279.

Halder, J., Kushwaha, D., Deb, D., Karmakar, P., Rai, A.B., and Singh, B. 2017. Assessment of susceptibility of *Ocimum tenuiflorum* to lace wing bug [*Cochlochila bullita* (Stal.)]: host's physico-morphic and biochemical properties. *J. Entomol.* 5(3): 814-821.

Harborne, A.J. 1973. *Phytochemical methods a guide to modern techniques of plant analysis*. Chapman and Hall, London, 295p.

Hasabo, S.A. and Noweer, E.M.A. 2005. Management of root-knot nematode *Meloidogyne incognita* on eggplant with some plant extracts. *Egypt. J. Phytopathol*. 33(2): 65-72.

Hiscox, J.D. and Israelstam, G.F. 1979.A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot*. 57(12): 1332-1334.

Huang, C.J., Wei, G., Jie, Y.C., XU, J.J., Anju, S.A., and Tanveer, M. 2016. Effect of shade on plant traits, gas exchange and chlorophyll content in four ramie cultivars. *Photosynthetica* 54(3): 390-395.

Islam, A.K.M. and Noguchi, H.K. Phytotoxic activity of *Ocimum tenuiflorum* extracts on germination and seedling growth of different plant species. *Sci. World J*. 10: 1-8.

Jackson, M.L. 1958. *Soil Chemical Analysis* (Indian Reprint, 1967). Prentice Hall of India, New Delhi, 498p.

Javanmardi, J., Khalighi, A., Kashi, A., Bais, H.P., and Vivanco, J.M. 2002. Chemical characterization of *Ocimum basilicum* L. found in local accessions and used in traditional medicines in Iran. *J. Agric. Food Chem.* 50: 5878-5883.

Jensen, H.L. 1955. Non-symbiotic nitrogen fixation. In: Barthdlomew, W.V. and Clark, F.E. (eds), *Soil Nitrogen*. Proceeding of the 12th International Congress of Soil Science, New Delhi, India. American Society of Agronom*y*, Madison, pp. 436-480.

Jidere, C.I. and Oluwatayo, J.I. 2018. Efficacy of some botanical extracts on root-knot nematode (*Meloidogyne incognita*) egg- hatch and juvenile mortality *in vitro. Asian J. Agrl. Hort. Res.* 2(3): 1-13.

Kamaraj, C., Rahuman, A. A., and Bagavan, A. 2008. Antifeedant and larvicidal effects of plant extracts against *Spodoptera litura* (F.), *Aedes aegypti* L. and *Culex quinquefasciatus. Parasitol. Res*. 103: 325-331.

Kathiresan, K., Guanasekan, P., Rammurthy, N., and Govidswami, S. 1999. Anticancer activity of *Ocimum sanctum. Pharm. Biol.* 37: 285-290.

Kayesth, S., Gupta, K.K., Kumar, S., and Shazad, M. 2018. Effects of *Ocimum sanctum* hexane extract on survival and development of *Dysdercus koenigii* (Heteroptera: Pyrrhocoriedae). *Arch. Phytopathol. Plant Prot*. 51: 17-18.

Khalid, K.A., Hendawy, S.F., and Gezawy, E.E. 2006. *Ocimum basilicum* L. production under organic farming. *Res. J. Agric. Biol. Sci*. 2: 25-32.

Kirtikar, K.R. and Basu, B.D. 1984. *Indian medicinal plants* (2nd Ed.). Narosa Publishing House, Dehradun, India, 1968p.

Kitchlu, S., Bhadauria, S., Ram, G., and Bindu, K. 2013. Chemo-divergence in essential oil composition among thirty one core collections of *Ocimum sanctum* L. grown under sub-tropical region of Jammu, India. *Am. J. Plant Sci.* 4(2): 10-14.

Knox, J., Jaggi, D., and Paul, M.S. 2010. Evaluation of allelopathic potential of selected plant species on *Parthenium hysterophorus*. *Egypt. J. Biol.* 12: 57-64

Kothari, S.K., Bhattacharya, A.K., and Ramesh, S. 2004. Essential oil yield and quality of methyl eugenol rich *Ocimum tenuiflorum* L. (syn. *O. sanctum* L.) grown in south India as influenced by method of harvest. *J. Chromatogr. A.* 1054: 67-72.

Kothari, S.K., Bhattacharya, A.K., Ramesh, S., Garg, S.N., and Khanuja, S.S. 2005. Volatile constituents in oil from different plant parts of methyl eugenol-rich *Ocimum tenuiflorum* L.f. (syn. *O. sanctum* L.) grown in south India. *J. Essen. Oil Res.* 17(6): 656-658.

Kumar, M., Thakur, N.S., Bardhan, K., and Bhusara, J.B. 2017. Effect of teak (*Tectonagrandis* L)- *Ocimum* spp-based silvi-medicinal systems on growth and physiological parameters of *Ocimum* spp. *Int. J. Farm Sci.* 7(1): 8-14.

Kumar, V., Swamy, K.N., Vardhini, B.V., and Rao, S.S.R. 2014. Effect of light curtailment on growth, biochemical response and essential oil content of rose scented geranium. *Int. J. Multidiscip. Curr. Res*. 2: 322-326.

Laskar, S. and Majumdar, S.G .1988. Variation of major constituents of essential oil of the leaves of *Ocimum Sanctum* Linn. *J. Indian Chem. Soc.* 65: 301-302.

Lawrence, B.M. 1985. A review of the world production of essential oil. *Perfum. Flavor*. 10: 2-16.

Lawrence, B.M. 1998. A world perspective. *Proceedings of the 10th international congress of essential oils, fragrances and flavors*, Washington, USA, Elsevier Science Publisher, pp. 161-179.

Lee, B.S., Seo, B.S., and Chung, S.J., 1994. Shading effects on growth and essential oil content of hydroponically grown sweet basil (*Ocimum basilicum* L.). *Korean J. Hortic. Sci. Technol*. 35(2): 95-102.

Lee, M.S.Y. 2006. Morphological phylogenies and the universe of useful characters. *Taxon.* 55: 5-7

Lenka, D. and Misra, B. 1973. Path-coefficient analysis of yield in rice varieties. *Indian J. Agric. Sci*. 43(4): 376-379.

Maboko, M.M. and Plooy, C.P.D. 2013. High-plant density planting of basil (*Ocimum basilicum*) during summer/fall growth season improves yield in a closed hydroponic system. *Soil Plant Sci.* 63(8): 748-752.

Mafini, C. and Omoruyi, O. 2013. Logistics benefits and challenges: The case of SMEs in a South African local municipality. *South. Afr. J. Entrepreneurship Small Business Manag*. 6(1): 61-77.

Malav, P.K., Pandey, A., Bhatt, K.C., Krishnan, G., and Bisht, I.S. 2015. Morphological variability in holy basil (*Ocimum tenuiflorum* L.) from India. *Genet. Resour. Crop Evol*. 15: 72-77.

Malav, P.K., Pandey, A., Gupta, V., Bhatt, A.K., Agaikwad, A., Ahlawat, O.S., and Dhaka, R. 2020. The diversity in holy basil (*Ocimum tenuiflorum*) germplasm from india. *Indian J. Agric. Sci.* 90(10): 1937-1945.

Mauromicale, G., Ierna, A., and Marchese, M. 2006. Chlorophyll fluorescence and chlorophyll content in field-grown potato as affected by nitrogen supply, genotype, and plant age. *Photosynthetica* 44(1): 76-82.

Mekky, M.S. and Hassanein, A.M.A., 2021. Allelopathic Effect of Sweet Basil (*Ocimum basilicum* L.) as phytotoxic extract on Vegetative growth of commercial crop plants and its associated weed. *Int. J. Life Sci.* 10(1): 1-7.

Mekky, M.S., Hassanien, A.A. Kamel, E.M., and Ismail, A.E.A. 2019. Allelopathic effect of *Ocimum basilicum* L. extracts on weeds and some crops and its possible use as new crude bio-herbicide. *Ann. Agric. Sci.* 12(5): 1-11.

Milenkovic, L., Stanojevic, J., Cvetkovic, D., Stanojevic, L., Fallik, E., and Ilic, Z.S. 2019. New technology in basil production with high essential oil yield and quality. *Ind. Crops Prod.* 140: 1-11.

Mishra, D., Awasthi, A., and Mishra, P. 2014. Phylogenetic evolution studies on different varieties of genus *Ocimum* with special reference to Rewa district of Madhya Pradesh. *Sci. Secure J. Biotech* 3(2): 188-197.

Moghimipour, Z., Sourestani, M.M., Ansari, N.A., and Ramezani, Z. 2017. The effect of foliar application of zinc on essential oil content and composition of holy

basil (*Ocimum sanctum*) at first and second harvests. *J. Essent. Oil-Bear. Plants* 20(2): 449-458.

Mondal, S., Mirdha, B.R, and Mahapatra, S.C. 2009. The science behind sacredness of Tulsi (*Ocimum sanctum* L.). *Indian J. Physiol. Pharmacol*. 53: 291-306.

Mukherjee, R., Das, P.K., and Ram, G.C. 2005. Immuno therapeutic potential of *Ocimum sanctum* L. Bovine subclinical mastitis. *Res. Vet. Sci.* 79: 37-43.

Mukhtar, T., Arooj, M., Ashfaq, M. and Gulzar, A. 2017, Resistance evaluation and host status of selected green gram genotypes against *Meloidogyne incognita*. *Crop Prot*., 92: 198-202.

Nahak, G., Mishra, R.C., and Sahu, R.K. 2011. Taxonomic distribution, medicinal properties and drug development potentiality of *Ocimum* (tulsi). *Drug Invent. Today* 3(6): 95-113.

Nazim, K., Ahmed, M., and Uzair, M. 2009. Growth potential of two species of basil in sandy soil of Karachi. *Pak. J. Bot*. 41(4): 1637-1644.

Neeraj, N., Goel, S.R., Singh, K.A., and Madan, V.K. 2017. Effect of plant extracts on hatching and mortality of root-knot nematode, *Meloidogyne incognita* larvae (*in vitro*). *Biosci. Biotechnol. Res. Asia* 14(1): 114-120.

Nelkin, J.B. and Schuch, U.K. 2004. Retractable roof greenhouse production of basil (*Ocimum basilicum*) and lemon grass (*Cymbopogon citrates*) in a semi-arid climate. *Acta Hortic.* 659: 113-120.

Nguemtchouin, M.G.M., Ngassoum, M.B., Chalier, P., and Cretin, M. 2013. *Ocimum gratissimum* essential oil and modified montmorillonite clay, a means of controlling insect pests in stored products. *J. Stored Products Res.* 52: 57-62.

NMPB [National Medicinal Plants Board]. 2020. *High Demanded Medicinal Plants*. [on-line]. Available: https://www.nmpb.nic.in/content/demand-and-supply-position medicinal plants [05 November 2020].

Olabiyi, T.I. 2008. Pathogenisity study and nematoxic properties of some plant extracts on root-knot nematode pest of tomato *Lycopersicon esculentum* L. *Plant Pathol. J.* 7(1): 45-49.

Padalia, R.C. and Verma, R.S. 2011. Comparative volatile oil composition of four *Ocimum* species from northern India. *Nat. Product Res*. 25(6): 40-49.

Padalia, R.C., Verma, R.S., Chauhan, C.S., and Chanotiya, C.S. 2013. Changes in aroma profiles of 11 Indian *Ocimum* taxa during plant ontogeny. *Acta Physiol. Plant* 10: 90-93.

Pandey, R., Kalra, A., Tandon, S., Mehrothra, N., and Kumar, S. 2000. Essential oils as potent sources of nematicidal compounds. *J. Phytopathol.* 148: 501-503.

Patel, S., Singh, C.P., and Yadav, S.K. 2016. Effect of *Lantana camera* and *Ocimum sanctum* extracts on the fecundity of mustard aphid, *Lipaphis erysimi*. *J. Plant Dev. Sci.* 8(2): 83-85.

Pavaraj, M., Bakavathiappan, G., and Baskaran, S. 2012. Evaluation of some plant extracts for their nematicidal properties against root-knot nematode, *Meloidogyne incognita*. *J. Biopest.* 5: 106-110.

Paxton, J. and Hereman, S. 1868. *Paxton's Botanical Dictionary*. Bradbury Evans Ltd., London, 1865p.

Pikovskya, R.I. 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Microbiologiya* 17: 362-370.

Pillai, R.R. 1990. Quality of oil of clocimum (*Ocimum gratissimum* L.) as influenced by stages of harvest and shade. M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 158p.

Pistrick, K. 2001. Labiatae. In: Hanelt, P. (ed.), *Mansfeld's encyclopedia of agricultural and horticultural crops*. Mir Publishers, Berlin, pp. 1954-1960

Popovic, Z., Kostic, M., Popovic, S., and Skoric, S. 2006. Bioactivities of essential oils from basil and sage to *Sitophilus oryzae* L. *Biotechnol. Equip.* 20: 36-40.

Prakash, P. and Gupta, N. 2005. Therapeutic uses of *Ocimum sanctum* Linn (Tulsi) with a note on eugenol and its pharmacological actions: A short review. *Indian J. Physiol. Pharmacol.* 49:125-31.

Prashar, R., Kumar, A., Banerjee, S., and Rao, A. R. 1994. Chemopreventive action by an extract from *Ocimum sanctum* on mouse skin papillomagenesis and its enhancement of skin glutathione-S-transferase activity and acid soluble sulfhydryl level. *Anticancer Drugs* 5: 567-572.

Purohit, S. and Pandya, N. 2013. Allelopathic activity of *Ocimum sanctum* L. And *Tephrosia purpurea* (L.) Pers. leaf extracts on few common legumes and weeds. *Int. J. Res. Plant Sci.* 3(1): 5-9.

Raina, A.P., Kumar, A., and Dutta, C. 2013. Chemical characterization of aroma compounds in essential oil isolated from ‘‘holy basil’’ (*Ocimum tenuiflorum* L.) grown in India. *Genet. Resour. Crop Evol.* 60: 1727-1735.

Ranjitsingh, K.N. and Sucheta, K.R. 2009. Effect of plant root extract to control root-knot nematode (*Meloidogyne* spp) of soybean (*Glycine max*). *Biol. Forum Int. J.* 1(1):65-68.

Rao, M.R., Palada, M.C., and Becker, B.N. 2004. Medicinal and aromatic plants in agroforestry systems. *Agrofor. Syst*. 61: 107-122.

Rice, E.L. 1974. *Allelopathy*. Academic Press, New York, 602p.

Rice, E.L. 1984. *Allelopathy*. Academy Press Inc. Ltd., London, 422p.

Rizvi, S.J.H., Haque, H., Singh, V.K., and Rizvi, V. 1992. A discipline called allelopathy. In: Rizvi, S. and Rizvi, V. (eds). *Allelopathy: Basics and Applied Aspects,* Chapman and Hall, London, pp. 1-8.

Rosado, L.D.S., Rodrigues, H.C.A., Pinto, E.P., T.N., Pinto, L.B.B., Bertolucci, S.V. 2009. Allelopathy of aqueous extract and essential oil of leaves from basil “Maria Bonita” on lettuce, tomato and Melissa germination. *Rev. Bras. Plantas Med.* 11(4): 422-428.

Runyoro, D., Ngassapa, O., Vagionas, K., Aligiannis, N., Graikou, K., and Chinou, I. 2010. Chemical composition and antimicrobial activity of the essential oils of four *Ocimum* species growing in Tanzania. *Food Chem*. 119(1): 311-316.

Sajjadi, S.E. 2006. Analysis of the essential oils of two cultivated basil (*Ocimum bas*ilicum L.) from Iran. *J. Fac. Pharma.* 14: 128-130.

Saran, P.L., Tripathy, V., Saha, A., Kalariya, A., Suthar, M.K., and Kumar, J. 2017. Selection of superior *Ocimum sanctum* L. accessions for industrial application. *Indian Crops Prod.* 108: 700–707.

Schindler, A.F. 1961. A simple substitute for a Baermann funnel. *Plant Dis. Reptr*. 45: 747-748.

Sestak, Z. 1963. Changes in the chlorophyll content as related to photosynthetic activity and age of leaves. *J. Photochem. Photobiol.* 2: 101 -110.

Shahak, Y., Gussakovsky, E.E., Cohen, Y., Lurie, S., Stern, R., Kfir, S., Naor, A., Atzmon, I., Doron, I., and Greenblat, Y. 2004. Color nets: a new approach for light manipulation in fruit trees. *Acta Hortic.* 636: 609-616.

Sharma, S.D. and Singh, M. 2003.Allelopathic effect of basil *(Ocimum sanctum)* materials on the germination of certain weed seeeds. *Indian J. Weed Sci.* 36(2): 99-103.

Sharma, T. 2010. Toxic effect of *Ocimum sanctum* plant extract against *Acrida exaltata* (Orthoptera: Acrididae) adults. *J. Environ. Res. Dev.* 4(4): 1008-1012.

Sharma, V., Gupta, R.C., Singh, B., and Dhaliwal, H.S. 2014. Volatile oil composition and antimicrobial activity of essential oil of two varieties of *Ocimum sanctum* (l.) from Dhameta (Kangra)-a North Indian region. *Int. J. Recent Sci. Res.* 5(1): 142-147.

Shukla, R.C., Dwiedi, V.K., and Misra, S.L. 1986. Some new records of the plant parasitic nematodes associated with some important medicinal plants at Allahabad [abstract]. In: *Abstracts, National Conference of Plant Parasitic*

Nematodes of India, *Problems and Progress*; 17-20, December, 1986, New Delhi, p.52. Abstract No. 5.1.5.

Siddiqui, I.A. and Shaukat, S.S. 2004. *Trichoderma harzianum* enhances the production of nematicidal compounds in vitro and improves biocontrol of *Meloidogyne javanica* by *Pseudomonas fluorescens* in tomato. *Lett. Appl. Microbiol.* 38: 169-175.

Simon, J.E., Quinn, J., Murray, R.G. 1990. Basil: a source of essential oils. In: Janick, J. and Simon, J.E. (eds), *Advances in new crops*. Timber Press, Portland, pp. 484-989.

Simpson, B.B. and Ogorzaly, M.C. 1986. *Economic Botany: Plants in our World*. McGraw-Hill Book Company, Hamburg, 152p.

Sims, C.A., Juliani, H.R., Mentreddy, S.R., and Simon, J.E. 2013. Essential oils in holy basil (*Ocimum tenuiflorum* L.) as influenced by planting dates and harvest times in North Alabama. *J. Med. Active Plants* 2(3): 33-37.

Singh, E., Sheel, S., Jaya D., and Swapnil, S. 2012. Diversified potentials of *Ocimum sanctum* L. (Tulsi): an exhaustive survey. *J. Nat. Prod. Plant Resour*. 2(1):39 - 48.

Singh, N. and Sharma, R.K. 1980. The morphology and floral biology of tulsi (*Ocimum sanctum* L.). *Plant Sci.* 12: 26-27.

Singh, N., Hotte, Y., and Miller, R. 2010. *Tulsi: the mother medicine of nature* (2nd Ed.). International Institute of Herbal Medice, Lucknow, 47p.

Singh, S. and Singh, M. 2009. Interactions of Basil (*Ocimum sanctum* L.) with some weed species-competition or allelopathy? *Indian J. Weed Sci.* 41(2): 12-22.

Singh, S., Singh, M., Singh, A.K., Kalra, A., Yadav, A., and Patra, D.D. 2010.Enhancing productivity of Indian basil (*Ocimum basilicum* L.) through harvest management under rainfed conditions of subtropical north Indian plains. *Indian Crops Products* 32: 601-606.

Sirkar, N.N. 1989. Pharmacological basis of ayurvedic therapeutics. In: Atal, C. K. and Kapoor, B. M. (eds), *Cultivation and utilization of medicinal plants*. New Delhi: PID, CSIR, 168p.

Smitha, G.R. 2019. Influence of pre and post-harvest factors on yield and quality of medicinal and aromatic plants. *In: Medicinal and Aromatic Crops Conservation & Cultivation,* AkiNik Publications, Rohini, Delhi, pp.116-143.

Sokal, R.R. and Rohlf, T.J. 2003. *Biometry: the principles and practice of statistics in biological research*. W H Printing, San Francisco, 585p.

Stagnari, F., Galieni, A., and Pisante, M. 2015. Shading and nitrogen management affect quality, safety and yield of greenhouse-grown leaf lettuce. *Sci. Hort.* 192: 70-79.

Stagnari, F., Mattia, C., Galienia, A., Santarellia, D., Egidioa, S., and Pisante, M. 2018. Light quantity and quality supplies sharply affect growth, morphological, physiological and quality traits of basil. *Indian Crops Prod*. 122: 277-289.

Subbiah, B.V. and Asija, G.A. 1956. A rapid procedure for the estimation of available nitrogen in soil. *Curr. Sci.* 25: 259-360.

Suvera, A.H., Thakur, N.S., and Jha, S.K. 2015. Herbage and essential oil yield of *Ocimum* spp intercropped under *Pongamia pinnata* based silvi medicinal systems in Gujarat, India. *The Bioscan* 10(1): 81-85.

Suwitchayanon, P., Kunasakdakul, K., and Kato-noguchi, H. 2017. Screening the allelopathic activity of 14 medicinal plants from Northern Thailand. *Environ. Control Biol.* 55(3): 143 -145.

Tansi, S. and Nacar, S. 2000. First cultivation trials of lemon basil (*Ocimum basilicum* var. citriodorum) in Turkey. *Pak. J. Biol. Sci*. 3(3): 395-397.

Thakur, M., Bhatt, V., and Kumar, R. 2019. Effect of shade level and mulch type on growth, yield and essential oil composition of damask rose (*Rosa damascena* Mill.) under mid hill conditions of Western Himalayas. *PLoS ONE* 14(4): 1-14.

Tilwari A., Tamrakar, K., and Sharma, R. 2013. Use of random amplified polymorphic DNA (RAPD) for assessing genetic diversity of *Ocimum sanctum* (Krishna tulsi) from different environments of Central India. *J. Med. Plant Res.* 7(24):1800-1808.

Tomaszewski, M. and Thimann, K.V. 1966. Interactions of phenolic acids, metallic ions and chelating agents on auxin-induced growth. *Plant Physiol*. 41: 1443-1454.

Toncer, O., Karaman, S., Diraz, E., and Tans, S. 2017. Essential oil composition of *Ocimum basilicum* L. at different phonological stages in semi-arid environmental conditions. *Fresenius Environ. Bull.* 26(8): 54441-5449.

Tsasi, G., Mailis, T., Daskalaki, A., Sakadani, E., Razis, P., Samaras, Y., and Skaltsa, H. 2017. The effect of harvesting on the composition of essential oils from five varieties of *Ocimum basilicum* L. cultivated in the island of Kefalonia,. *Plants* 6(3): 41-48.

Tucker, A.O. 1986. Botanical nomenclature of culinary herbs and pot herbs. In: Craker, L. E. and Simon, J. E. (eds), *Herbs spices and medicinal plants*. Haworth Press Inc., Binghampton, New York, pp. 33-80.

Udupa, S.L., Shetty, S, Udupa, A.L., Somayaji, S.N. 2006. Effect of *Ocimum sanctum* L. on normal and dexamethasone suppressed wound healing. *Indian J. Exp. Biol.* 44:49-54.

van Staden, J., Sparg, S.G., Kulkarni, M.G., and Light, M.E. 2006. Post-germination effects of the smoke-derived compound 3-methyl-2H-furo [2, 3-c] pyran-2-one, and its potential as a preconditioning agent. *Field Crops Res*. 98: 98-105.

Vasudevan, P., Kashyap, S., and Sharma, S. 1999. Bioactive botanicals from basil (*Ocimum* sp.). *J. Sci. Indian Res.* 4: 332-338.

Verma, P.K., Punia, M.S., Sharma, G.D., and Talwar, D. 1989. Evaluation of different species of *Ocimum* for their herb and oil yield under Haryana conditions. *Indian Perfumer* 2:79-83.

Verma, R.S., Rahman, L., Verma, R.K., Chanotiya, C.S., Chauhan, A., Singh, A. K. 2010.Changes in the essential oil content and composition of *Origanum vulgare* L. during annual growth from Kumaon Himalaya. *Curr. Sci.* 98: 1010-1012.

Verma, S.K., Kumar, S., Pandey, V., Verma, R.K., and Patra, D.D. 2012. Phytotoxic effects of sweet basil (*Ocimum basilicum* L.) extracts on germination and seedling growth of commercial crop plants. *Eur. J. Exp. Biol.* 6: 2310-2316.

Vidhu, P.P.T. 2019. Phytosphere variations of Sida hemp [*Sida alnifolia* L.] under varying agronomic management. M.Sc. (Ag) thesis, Kerala Agricultur al University, Thrissur, 189p.

Wachira, P.M., Kimenju, J.W., Okoth, S.A. and Miley, R.K. 2009, Stimulation of nematode destroying fungi by organic amendments applied in management of plant parasitic nematode. *Asian J. Plant Sci*., 8 (2): 153-159.

Walkley, A.J. and Black, C.A. 1934.Estimation of soil organic carbon by the chromic acid titration method. *Soil Sci*. 37: 29-38.

Warrier, P.K. 1995. *Indian Medicinal Plants*. Orient Blackswan Private Limited, New Delhi, 168p.

Watanabe, P.S. and Olsen, S.R. 1965. Test of an ascorbic acid method for determining phosphate in water and NH_4HCO_3 extracts from soil. *Proc. Soil. Sci. Am*. 29: 677-678.

Watson, D.J. 1952. The physiological basis of variation in yield.*Adv. Agron*. 4: 101-145.

Weber, C.R. and Moorthy, B.R. 1952. Heritable and nonheritable relationships and variability of oil content and agronomic characters in the F2 generation of soyabean crosses. *Agron. J*. 44: 202-209.

Willis J.C. 1919. *A dictionary of the flowering plants and ferns*. The University Press, London, 954p.

WOI [The Wealth of India]. 1991. *The Wealth of India. A Dictionary of Raw materials and Industrial Products.* CSIR, New Delhi, 89p.

Wollum, A.G. 1982. *Methods of Soil Analysis.* Madison, Wisconsin, USA, pp. 781-801.

Wright, S. 1921. Correlation and causation. *J. Agric. Res*. 20: 557-585.

CHARACTERIZATION AND EVALUATION OF TULSI (*Ocimum tenuiflorum* L.)

by

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ABSTRACT

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ABSTRACT

Medicinal and aromatic plants (MAPs) have been ingredients of traditional medicine since time immemorial. *Ocimum tenuiflorum* L. (synonym *Ocimum sanctum*), commonly known as holy basil or tulsi, is an aromatic and medicinal plant native to Indian subcontinent. This plant is known to produce essential oils comprising of a number of aromatic compounds and has immense use in traditional system of medicine, perfumery, and pharmaceutical industry and hence known as the "queen of herbs".

Tulsi is a valuable herb with diverse potential. Almost all parts such as leaves, stem, flower, roots and seeds of tulsi have been used in numerous formulations of Ayurvedha, Sidha, Unani and Homeopathy. However, as a commercial crop it is less exploited in Kerala. Hence the present study entitled "Characterization and evaluation of tulsi (*Ocimum tenuiflorum* L.)" was taken up in the Department of Agronomy, College of Agriculture, Vellanikkara.

The objectives of the study included evaluation of *Ocimum tenuiflorum* L. accessions for yield and quality, standardization of shade requirement and method of harvesting, investigation of allelopathic effect on upland weeds and crops, and also assessment of the effect of tulsi on root-knot nematode (*Meloidogyne incognita*).

Thirty five accessions of tulsi (10 NBPGR accessions + 25 local collections) from different locations were evaluated for morpho-physiological, biochemical and yield characters. High degree of variation was observed among accessions for all the parameters studied. The collected accessions were grouped into three morphotypes based on the colour of stem, upper leaf surface and inflorescence. Out of the 35 accessions, 16 were identified as green types, 14 were purple types and 5 were intermediate. The collected accessions were grouped into six clusters using eight quantitative characters. Principal component analysis revealed that first three principal components (plant height, mumber of primary branches and secondary branches)

contributed for 79.7 per cent of variability. Based on biochemical characters (chlorophyll, carotenoid and essential oil content), 35 tulsi accessions were placed into five clusters and the first principal component (chlorophyll content) accounted for 89.90 per cent of the total variation. The logistic binomial estimate revealed that colour had a positive relation with essential oil contentand the expected percentage of improvement for essential oil content over the base population was 80.41 per cent. Scoring and ranking based on total biomass yield, number of primary branches and leaf area index revealed accession IC 583288 to be the top rank holder, followed by IC 583318, IC 583296 and KAU OC 30. Based on essential oil content and carotenoid, the best performing accessions were KAU OC 25, KAU OC 34 and KAU OC 32. Accessions IC 583288, KAU OC 34 and KAU OC 35 were found best suited for biomass yield and essential oil content.

Field experiments were conducted to assess the influence of growing condition (open and 50 per cent shade) and harvesting method (stage of harvest and height of harvest) on the performance of tulsi. Growing of tulsi at 50 per cent shade was superior to open condition with respect to fresh biomass yield and oil yield. However, eugenol content of tulsi was higher when grown under open condition. Further, harvesting of tulsi at 20 cm or 30 cm height from ground level at 75 and 135 DAT resulted in higher biomass yield, essential oil yield and total chlorophyll. In contrast, eugenol content was higher when plants were harvested at 20 or 30 cm height from ground level at 90 and 150 DAT.

Tulsi extracts, shoot and root powders were evaluated for allelopathic effect on upland weeds and test crops (rice and cowpea). The results established the presence of allelochemicals in tulsi and phytotoxic activity on upland weeds and test crops (rice and cowpea). Incorporation of tulsi powder recorded greatest inhibitory effect, followed by aqueous extracts. Application of shoot and root powders of tulsi at 20 g/kg soil caused weed germination inhibition of 86.06 per cent and 79.56 per cent respectively in the first week. The powder form of shoot and root at 20 g/kg soil

inhibited germination percentage of rice by 13.9 and 11.1 per cent and cowpea by 19.47and 16.67 per cent respectively.

Evaluation of nematicidal action of tulsi against root-knot nematode (*Meloidogyne incognita*) proved significant effect of tulsi extracts on the mortality rate of second stage juveniles (J_2s). The highest mortality of J_2s was observed with aqueous extract of tulsi @ 30 per cent at all the periods of exposure such as 12, 24, 48 and 72 hours. Studies indicated the possibility of exploiting tulsi as a cheap and safe nematicide for the control of root-knot nematode.

Appendix

Appendix 1

Monthly weather data during experimental period (2019 and 2020)

2019 (June- December)							
	Max temp (0 ˚C)	Min temp (0 ˚C)	RH (%)	Sunshine hours (Hrs)	Rainfall (mm)	Rainy days	Total Evaporation (mm)
June	32.2	23.5	83	3.7	324.4	15	84.4
July	30.4	22.8	86	2.6	654.4	21	73.5
Aug	29.5	21.9	89	1.5	977.5	24	59.0
Sep	31.2	22.0	85	3.3	419	19	75.2
Oct	32.4	21.4	80	5.5	418.4	16	84
Nov	32.9	21.7	72	7.5	205.0	5	101.5
Dec	32.3	22.1	63	6.7	4.4	1	141
2020 (June- December)							
	Max temp (0 ˚C)	Min temp (0 ˚C)	RH (%)	Sunshine hours (Hrs)	Rainfall (mm)	Rainy days	Total Evaporation (mm)
June	31.1	23.7	85	2.5	427.2	20	69.9
July	30.5	23.2	87	2.8	563.0	21	76.7
Aug	30.2	23.1	86	3.1	607.7	17	78.3
Sep	30	22.4	88	2.4	587.6	21	62.3
Oct	31.0	21.5	82	5.5	310.3	12	75.5
Nov	33	22	70	6.6	56.1	2	107.4
Dec	32	21.9	65	6.3	7.7	1	135.2

സംഗ്രഹം

വൈവിധ്യങ്ങളായ ഗുണവിശേഷങ്ങളുള്ള ഒരു ഔഷധസസ്യമാണ് തുളസി. ഒസിമം ടെനുയിഫ്ലോറം എന്ന ശാസ്ത്ര നാമത്തിൽ അറിയപ്പെടുന്ന തുളസിയുടെ വിവിധ ജനിതകശേഖരങ്ങളുടെ വിലയിരുത്തൽ, തുളസി കൃഷിക്ക് അനുയോജ്യമായ തണൽ ക്രമീകരണവും വിളവെടുപ്പ് രീതിയും മാനദണ്ഡപ്പെടുത്തൽ, കളകളിലും വിളകളിലും നിമാവിരകളിലും ഉള്ള തുളസിയുടെ രാസ പ്രവർത്തനം തെളിയിക്കൽ എന്നീ ഉദ്ദേശങ്ങളോടുകൂടി 2018 മുതൽ 2021 വരെയുള്ള കാലയളവിൽ വെള്ളാനിക്കര അഗ്രിക്കൾച്ചറൽ കോളേജിൽ പഠനങ്ങൾ നടത്തുകയുണ്ടായി.

ഈ പഠനങ്ങളുടെ ഭാഗമായി തുളസിയുടെ 35 ജനിതകങ്ങൾ ശേഖരിക്കുകയും നട്ടുവളർത്തി അവയുടെ പ്രകടനം വിലയിരുത്തുകയും ചെയ്തു. തണ്ട്, ഇലയുടെ ഉപരിതലം, പൂങ്കുല എന്നിവയുടെ നിറത്തെ അടിസ്ഥാനമാക്കി ഇവയെ മൂന്ന് വിഭാഗങ്ങളായി വേർതിരിച്ചു. ഇവയിൽ 16 എണ്ണം പച്ചയും 14 എണ്ണം പർപ്പിളും 5 എണ്ണം പച്ചയും പർപ്പിളും ഇട കലർന്നവയും ആയിരുന്നു. 35 തുളസി തരങ്ങളെ താരതമ്യം ചെയ്തപ്പോൾ IC 583288, IC 583318, IC 583296, KAU OC 30 എന്നിവയാണ് മികച്ച തോതിൽ ഇലകളുടെ വിളവ് നൽകുന്നത് എന്ന് കണ്ടെത്തി. എന്നാൽ ബാഷ്പ തൈല ഉൽപാദനത്തിന് കൂടുതൽ അനുയോജ്യമായത് KAU OC 25, KAU OC 34, KAU OC 32 എന്നിവ ആയിരുന്നു. IC 583288, KAU OC 34, KAU OC 35 എന്നിവ മികച്ച തോതിലുള്ള വിളവും ഗുണമേന്മയും ഉള്ളവയാണ്.

തുളസി കൃഷിക്ക് ഏറ്റവും അനുയോജ്യമായ തണൽ ക്രമീകരണവും വിളവെടുപ്പ് രീതിയും കണ്ടെത്തുന്നതിനായി

മറ്റൊരു പരീക്ഷണം നടത്തുകയുണ്ടായി. ഈ പഠനത്തിൽ നിന്നും തുളസി 50 ശതമാനം തണലിൽ കൃഷി ചെയ്യുന്നതാണ് കൂടുതൽ വിളവ് ലഭിക്കുന്നതിന് ഉത്തമം എന്ന് കണ്ടെത്തി. എന്നാൽ ഗുണ മേന്മ നിശ്ചയിക്കുന്ന യൂജിനോൾ എന്ന രാസഘടകം കൂടുതൽ ലഭിച്ചത് തുറന്ന അവസ്ഥയിൽ കൃഷിചെയ്തപ്പോഴാണ്. വിവിധ വിളവെടുപ്പു രീതികളിൽ 75 ദിവസവും 135 ദിവസവും പ്രായമാകുമോൾ 20 സെ. മീ അല്ലെങ്കിൽ 30 സെ. മീ ഉയരത്തിൽ മുറിച്ചപ്പോഴാണ് കൂടുതൽ വിളവ് ലഭിച്ചത്. എന്നാൽ 90 ദിവസവും 150 ദിവസവും പ്രായമാകുമോൾ 20 സെ. മീ അല്ലെങ്കിൽ 30 സെ. മീ ഉയരത്തിൽ വിളവെടുക്കുന്നതിലൂടെ താരതമ്യേന കൂടുതൽ യൂജിനോൾ ലഭിക്കുന്നു.

കളകളിലും, നെല്ല്, പയർ എന്നീ വിളകളിലും തുളസിയുടെ രാസ പ്രഭാവം പഠന വിധേയമാക്കി. തുളസിയുടെ സത്തും ഉണക്കിയ പൊടിയും വിവിധ അളവിൽ ഉപയോഗിച്ചു നടത്തിയ ഈ പഠനത്തിൽ നിന്നും തുളസിയുടെ ഉണക്കിയ പൊടിക്ക് തുളസി സത്തിനേക്കാൾ കൂടുതൽ രാസ പ്രഭാവം ഉണ്ടെന്ന് മനസ്സിലായി. തുളസിയുടെ സത്തും, ഉണക്കിയ പൊടിയും മണ്ണിൽ ചേർക്കുന്നത് ഒരു പരിസ്ഥിതി സൗഹൃദ കള നിയന്ത്രണ മാർഗ്ഗമായി കൃഷിയിൽ അവലംബിക്കാം. അതോടൊപ്പം മെലോയ്ഡോഗൈനെ ഇൻകോഗ്നിറ്റ എന്ന ശാസ്ത്ര നാമത്തിൽ അറിയപ്പെടുന്ന വേരുബന്ധ നിമാവിരകളിൽ തുളസി സത്തിന്റെ പ്രഭാവം പഠിച്ചു. ഈ പഠനത്തിൽ നിന്നും കൃഷിക്ക് ഭീഷണിയായ ഈ നിമാവിരകളെ നശിപ്പിക്കുന്നതിനുള്ള സുരക്ഷിതമായ ഒരു മാർഗ്ഗമാണ് തുളസി എന്നു വ്യക്തമായി.