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Effect of UV B Rays on the Specific Phytochemical Synthesis in Trigonella Foenum-Graecum L. And Raphanus Sativus L.

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Research Article

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1. INTRODUCTION

1.1 PLANT AS THE SOURCE OF DRUGS

The presence of a substantial array of natural product-derived compounds at various stages of clinical advancement underscores the enduring viability and importance of leveraging natural reservoirs as reservoirs for novel drug candidates (Veeresham, 2012). As per the findings of Newman and Cragg (2012) the continued relevance of natural products as reservoirs of innovative structures remains robust. Over the past three decades, up to 50% of the drugs approved have origins either directly or indirectly linked to natural products.

1.2 INCREASING TRADITIONAL MEDICINES AND NATURAL PLANT PRODUCTS

The principal phytochemical constituents found in medicinal plants encompass tannins, alkaloids, saponins, cardiac glycosides, steroids, terpenoids, flavonoids, phlobatannins, anthraquinones, and reducing sugars. As advocated by the World Health Organization (WHO), the primary healthcare needs of a significant portion of the population in developing countries rely on traditional medicines, predominantly sourced from natural plant products (Vines, 2004). Similar to global trends, communities across Ethiopia, encompassing both rural and urban areas, extensively utilize traditional medicines. The longstanding heritage of traditional practices and remedies in Ethiopia continues to offer valuable and practical tools for disease management and treatment (Helen *et al.,* 2019).

The medicinal power of traditional plants species lies in phytochemical components that cause definite pharmacological action on the human body (Naseem, 2014). Based on their metabolism activity in the plant, phytochemical components are generally can be mainly divided into two groups, which are primary which has mainly sugars, amino acids, chlorophyll and proteins, and secondary constituents while secondary constituents consist of alkaloids, flavonoids, saponins, tannins, phenolic compounds and many more (Krishnaiah, 2007).

1.3 PHYTOCHEMICALS

The analysis of phytochemical properties in medicinal plants plays a crucial role in identifying and isolating potential drug leads and key components from various plant parts.

Phytochemical properties are instrumental in pinpointing the unique biological activities exhibited by these plants. Commonly, leaves, roots, stem barks, and fruits are the plant parts subjected to phytochemical analysis (Agidew, 2022).

FLAVONOIDS

Flavonoids consist of a large group of polyphenol compounds having a benzoyl-γ-pyrone structure and are ubiquitously present in plants. They are synthesized by the phenylpropanoid pathway. Available reports tend to show that secondary metabolites of a phenolic nature including flavonoids, are responsible for the variety of pharmacological activities (Mahomoodally *et al*., 2005; Pandey, 2007). Flavonoids are hydroxylated phenolic substances and are known to be synthesized by plants in response to microbial infection (Dixon *et al.*, 1983).

TANNINS

The term tannin is widely applied to a complex large biomolecule of polyphenol nature having sufficient hydroxyls and other suitable groups such as carboxyl to form strong complexes with various macromolecules (Navarrete, 2013). Tannins were detected in most plant species like peel and *juice of Citrus medica, mango* (*Mangifera indica L.*) leaves, *Avocado fruit* (*Persea Americana*), *Dioscorea alata leaf*, of *Leucas aspera L.* leaf and root, *Ocimum gratissimum Linn* leaf, *Rhamnus prinoides* root, extract of rhizomes, *Zingiber officinale* and *Curcuma longa* and also for different solvent give different response for the same plant species like *Bersama abyssinica* leaf, Flax seeds, *Nigella sativa*, *Ruta chalepensis* leaves, and *Syzygium guineense* and not totally detected in part of plants like *Lepidium sativum* seeds and *Gilbetii* root. Tannins are generally used in the tanning process and used as healing agents in inflammation, burn, piles, and gonorrhea (Boroushaki *et al.,* 2016).

Phytochemicals are compounds primarily synthesized by plants, showcasing notable biological activities. Within the pharmaceutical sector, plants serve as the primary reservoir for a diverse range of active ingredients. These ingredients manifest pharmacological effects that are pertinent to combating bacterial and fungal infections, as well as addressing chronic-degenerative ailments like diabetes and cancer. Nonetheless, the frontier of scientific exploration now lies in innovating novel methodologies for acquiring these valuable compounds (Nadia Mendoza and Escamilla Silva, 2018).

1.4 GENERAL VIEW ON MUTATION

This nuanced perspective highlights the intricate interplay of mutations and underscores the multifaceted nature of evolutionary processes (Loewe and Hill, 2010). The population genetics of mutations: Good, bad and indifferent. A special class of mutations is caused by transposable elements that can move around in the genome and can copy, insert or excise themselves, sometimes in response to conditions such as stress (Lee and Langley, 2010). Mechanisms exist to control the frequency of transposition events to limit the damage from resulting deleterious mutations.

Mutation rates are difficult to measure because the events are so rare that it is like measuring the frequency of needles in haystacks. Historically, this has been accomplished mainly by finding single genes or groups of genes that lead to phenotypic changes that can easily be observed in populations with known descent (Drake *et al*., 1998) and extrapolating to the level of genomes. As Kondrashov and Kondrashov (2010) point out in their contribution to this issue, the recent advances in post-genomic sequencing technology have led to breakthroughs that now allow direct determination of mutation rates in species with sequenced genomes, work which Charlesworth has stimulated by his developments of theory and to which he has contributed directly (Haag-Liautard *et al*. 2007).

Mutagenesis involves exposing biological material to mutagens, which are physical or chemical agents capable of increasing the mutation rate above the spontaneous level. Both physical and chemical mutagens have been effectively utilized in plant breeding programs to create genetic diversity artificially, leading to the development of new varieties with enhanced traits like increased yield, early maturity, reduced plant height, and disease resistance. In recent times, mutation induction has emerged as a valuable tool for studying gene function and expression (Grotewold, 2024).

1.5 UV-B RADIATION ON PLANT GROWTH, ACTIVE CONSTITUENTS, AND PRODUCTIVITY

UV-B radiation causes changes in plant morphology (short stems) and leaves (short petioles, thick, and small leaves), which can affect the interception of radiation in the canopy structure and alter the spatial relationship of the plant's three-dimensional structure and physiological parameters. It was found that UV-B induces a dose-dependent biosynthesis of

antioxidant phenolic compounds (flavonoids) in leaves. The heterogeneity of the activation of biosyntheses within the whole plant is associated with the magnitude of the interception of UV-B radiation by various structural elements of plants. Yoon *et al*. (2021) analyzed the spatial interception of UV-B radiation in *Brassica oleracea* L. var. acephala inside the crown of the plant. Under UV-B radiation, phenolic compounds and flavonoids accumulated more in organs with higher UV-B uptake and in younger leaves (Golovatskaya and Laptev, 2023).

1.6 CROP CHOSEN FOR THE TREATMENT

FENUGREEK

Fenugreek belongs to Fabaceae family; it was named, Trigonella, from Latin language that means "little triangle" due to its yellowish-white triangular flowers (Flammang *et al.,* 2004). It is named as Methi (Hindi, Urdu, Punjabi and Marathi), Hulba (Arabic), Moshoseitaro (Greek), Uluva (Malayalam), Shoot (Hebrew), Dari (Persian), and heyseed in English. Fenugreek (*Trigonella foenum-graecum* L.) is one of the oldest medicinal plants from Fabaceae family originated in central Asia ~4000 BC (Altuntas *et al*., 2005). Its description and benefits had been reported in the Ebers Papyrus (one of the oldest maintained medicinal document) earlier in 1500 BC in Egypt (Betty, 2008). It is being commercially grown in India, Pakistan, Afghanistan, Iran, Nepal, Egypt, France, Spain, Turkey, Morocco, North Africa, Middle East and Argentina (Flammang *et al.,* 2004, Altuntas *et al*., 2005).

Fenugreek seeds contain a substantial amount of fiber (Montgomery, 2009, Meghwal and Goswami, 2012), phospholipids, glycolipids, oleic acid, linolenic acid, linoleic acid (Sulieman *et al.*, 2000, Chatterjee *et al*., 2010), choline, vitamin A, B1, B2, C, nicotinic acid, niacin (Leela and Shafeekh, 2008), and many other functional elements.

RADISH

One of the most commonly consumed vegetables is the Brassicaceae family, composed of leaves, roots, seasoning vegetables, and oilseeds (OECD, 2016), with approximately 310 genera and 3500 species (Al-Shehbaz, 2011). The importance of this vegetable family derives from its nutrient content, its contribution to the agricultural economy and its chemical compounds with potential health benefits (Manivannan, *et al*., 2019). Radish, known as *Raphanus sativus* L., is a species belonging to the genus *Raphanus* included in the Rapa/Oleacera lineage according

to phylogenetic studies of the *Brassicaceae* (Nishio, 2017). Radish is considered a root since its specialized structure (hypocotyls) has a full or partial subterranean habitat, its shape is close to the true roots, and it can store starch and other compounds (Radovich, 2018).

1.7 OBJECTIVE

So, the objective of the study is to

1) To treat the seeds of radish and fenugreek with UV-B rays.

2) To check for changes in germination rate of seed

3) To compare for morphological changes in physically treated plants with control

4) To compare the changes in the amount of phytochemicals (flavonoids and tannins) and

Nutrients (Carbohydrates and proteins) in physically treated plants with control

2. REVIEW OF LITERATURE

2.1 MUTATION GENERAL PERSPECTIVE:

Mutations may induce both qualitative as well as quantitative variation comparatively in a shorter period of time by altering alleles at known loci as well as at previously unknown loci, besides altering linkage groups (Konzak *et al.,* 1977). Induced mutagenesis has been used to obtain direct mutants or by using these mutants in hybridization (Ahloowalia *et al*., 2004) to overcome yield plateaus and generate desirable horticultural traits. A study of induced variability for chlorophyll and viable morphological mutations in the M2 generation was the most dependable tool to utilize useful mutations for efficient crop improvement (Kumar *et al*., 2007).

2.2 VEGETABLE CROPS

Breeding of vegetable is categorized into three sub-types as mutation breeding, recombination breeding and transgenic breeding. In case of mutation breeding, the basic fundamental and the unique feature is the generation of new mutated alleles. Hugo de Vries in 1900 used the term "mutation" to describe phenotypic changes that are inheritable. Utilization of induced mutations for crop improvement is known as mutation breeding. During the 1950s, induced mutagenesis was widely pursued in the US, Europe, Japan and China. In India, Swaminathan and his team at the Indian Agricultural Research Institute, New Delhi initiated a major programme on mutagenesis in crop plants.

The discovery of X-rays by Wilhelm Roentgen in 1895 led to the application of X-rays for inducing mutations in Drosophila melanogaster by Muller (1927) and in barley by Stadler (1928). This technique later became the most important tool for locating genes on chromosomes, studying gene structure, gene expression and regulation and for exploring genomes (Solanki *et al*. 2011). Nicotiana tabacum was the first crop in which the first commercial mutant variety "chlorine type" was induced. In general, ionizing radiations such as X-rays and gamma rays are preferred because of their easy application, good penetration, reproducibility, high mutation frequency and less disposal problem. All chemical mutagens react with DNA by alkylating the phosphate groups and also the purine and pyrimidine bases. The dose of a mutagen applied is an important consideration in any mutagenesis programme. The lethal dose-50 (LD50) gives an idea about the optimum dose of the mutagens.

Optimum dose produces the maximum mutations with minimum hazards. An overdose of mutagens will kill too many plants, while low dose will produce too less mutation spectrum and frequency. The mutagenic dose mainly depends upon the concentration, duration of treatment and temperature during the treatment. Pre-soaking, pH of solution, metallic ions, carrier agents, post washing, post drying and storage of seeds are the modifying factors for the mutagenic effect (Solanki *et al.* 2011).

2.3 EFFECT OF PHYSICAL MUTAGENS IN VEGETABLE CROPS

The goal in mutagenesis breeding is to cause maximal genomic variation with a minimum decrease in viability. Among the radiation-based methods, γ-ray and fast neutron bombardment now supersedes X-ray in most applications. Of these, γ-ray bombardment is less destructive causing point mutations and small deletions whereas fast neutron bombardment causes translocations, chromosome losses, and large deletions. Kangarasu *et al*., (2014) used different doses of gamma rays i.e. 15, 20 and 25 kR to induce flower colour and seed mutants in M2 generation mutation in dormant seeds of *Phaseolus vulgaris* L. cv. Waghya. Mutants with different flower colour and altered size, shape and seed coat colour were obtained by 20 kR dose. Micro-mutations were also scored by Mejri *et al.* (2012) for percentage of germinated seeds, pod length and photosynthetic pigment contents in faba bean.

The variation in DNA profile in responses to gamma irradiation treatments was detected by RAPD -PCR technique in variety Sabahia of okra (*Abelmoschus esculentus* (L.) Moench) (Hegazi and Hamildeldin, 2013). The relatively high doses of gamma irradiation (400 and 500 Gy) induced more changes in genomic DNA pattern than the low dose (300 Gy). Hassan and Halem (2014) studied the effectiveness of different doses of gamma rays to induce new genetic variability in some agronomic traits of canola (*Brassica napus* L.).

Nouri and Tavassoli, (2012) used one of the successful and experimented methods of mutation and irradiation techniques with use of gamma rays to identify the intensity of radiation described for understanding the changes desired phenotypic, morphological and physiological on pinto bean seeds of Khomein cultivar. Aney (2014) also studies the effect of gamma irradiation on yield attributing characters in two varieties of pea (*Pisum sativum* L.). Both the varieties showed dose dependent decrease in most of the yield attributing characters, but the genotype of var. arvense is observed more sensitive to the doses of gamma irradiation than var. hortense.

The World Health Organization (WHO) and the United Nations Food and Agriculture Organization (FAO) recently launched an effort to enhance vegetable and fruit consumption worldwide, as low consumption is considered one of the top ten contributing factors to human mortality. It is worth mentioning that Solanaceae family plants especially are models for a number of biological studies important for agriculture. Tomato (*Solanum lycopersicum* L.), potato (*Solanum tuberosum* L.), pepper (*Capsicum annuum* L.), and eggplant (*Solanum melongena* L.) are the most important members of the Solanaceae. The members of this family have been used for pioneering research on cell wall and storage polysaccharide synthesis and metabolism, volatile production, vitamin biosynthesis, biosynthesis and action of the hormones ethylene and brassinosteroids, biosynthesis of flavonoids and carotenoids (Márkus *et al.,* 1999; Daood and Biacs, 2005; Cornelius *et al*., 2008; Tomlekova *et al.,* 2009; Guzman *et al.*, 2010; Borovsky *et al.,* 2013; Petrov *et al.,* 2013; Qu *et al.,* 2013.)

Tomato fruits are a rich source of carotenoid pigments; they are also a rich source of vitamin C. Several properties of the plant and the availability of numerous resources currently make tomato one of the most effective crop model species for basic and applied research such as transformation techniques, various mapping populations, molecular markers, a physical map, microarrays, and expressed sequence tag (EST) collections (McCormick *et al.,* 1986; Tanksley *et al.*, 1992; Livingstone *et al.,* 1999; Tanksley and Fulton 2007; Mueller *et al.,* 2008; Caicedo and Peralta, 2013).Pepper is especially high in vitamin C, and since the body neither makes nor stores vitamin C, a continuous supply must be provided in the food we eat; it also promotes the body's effective use of other nutrients, such as iron, B-vitamins, vitamins A and E, calcium and certain amino acids. Eggplant is a very good source of dietary fibre, potassium, manganese, copper and thiamine (vitamin B1). Potato is important in the human diet and is a rich source of nutrients. All potatoes have carotenoids in the flesh which are xanthophylls. Of greatest interest are lutein and zeaxanthin, components of the human retina that must be obtained from foods for proper eye health.

In plant breeding, mutation induction has become an effective way of supplementing the existing germplasm and improving varieties. For this reason mutation breeding studies have started to use an alternative breeding method. Mutations can be induced by using chemical mutagens such as EMS or physical mutagens such as gamma, X-ray, neutron and heavy ion sources (Honda *et al.,*

2006). These treatments enable the creation of a wide variation and select variants in tomato, pepper, eggplant, potato, lettuce, onion, and cucumber (Sripichitt *et al.,* 1988; Subhash *et al*., 1997; Das *et al*., 2000; Jabeen and Mirza, 2004; Menda *et al*., 2004; Honda *et al.,* 2006; Mazzucato *et al*., 2008). Furthermore, tomato's importance in agriculture has led to breeding efforts that have yielded numerous spontaneous and induced mutations, which provide a valuable resource for genetic research (Giovannoni, 2004). Induced mutations occur more or less randomly in the genome; even their target cannot be specified. Only one of the two or more alleles of a locus is affected, inheritance is almost always recessive; therefore homozygosity is normally required for new trait expression. Mutations were defined as heritable changes in the genetic material not caused by recombination or segregation. In terms of molecular genetics, a mutation, in general, is often defined as any heritable change in nucleotide sequence of a genome (Maluszynski *et al.,* 2009). Accordingly, results are more often useful in self-pollinating plant species. On the other hand, mutant heterosis has been reported and specific mutations concerning male sterility trait have proved useful in cross-pollinating species (Daskalov, 1973). Many successful examples of mutation induction have shown that mutation breeding is an effective and important approach to vegetable crop improvement.

Estimation of correlation and path analysis are useful in developing suitable selection criteria for selecting desired plant type or developing high yielding varieties. Improvement techniques like polyploidy, mutation breeding and tissue culture have helped in improving the garlic traits (Agrawal *et al.,* 2003). Induced mutation has also yielded important results in vegetatively propagated crop breeding studies on potato, sweet potato (Micke 1993; Van Harten, 1998; Maluszynski *et al*., 2009) and in garlic (Miranda, 1991; Hayward, 1993; Beşirli *et al*., 2006).

Mutants with high yield, increased vitamin C (ascorbic acid), antioxidant capacity (Öztürk *et al*., 2012), enhanced pollen viability and fruit rot resistance were also observed in Solanaceae crop mutation breeding programmes. According to IAEA's database, fifteen mutant pepper, six tomato, twenty potato, three cucumber, five eggplant, seven lettuce, four onion, one radish and three watermelon varieties that have different characteristics like high yielding with different fruit characteristics, dwarfism, semi-dwarfism, male sterility, high beta-carotene content, resistant to disease (TMV (Tm-2), *Fusarium oxysporum* f.sp. lycopersicy race 0, etc.) and drought were registered (Anonymous, 2013; Maluszynski *et al.,* 2009). Also the C.M. Rick Tomato Genetic

Resource Centre (TGRC) at the University of California; Davis has accumulated 1,017 monogenic mutant stocks throughout many years from various sources (TGRC, 2013). Seeds are also available for ~3,500 monogenic mutants in an isogenic background (Menda *et al*., 2004), along with data and images of the mutant phenotypes observed (Anonymous, 2013b). Nearly 100 cytogenetic mutant stocks, comprising translocations, trisomics, and autotetraploids are also available through the TGRC (Caicedo and Peralta, 2013). This centre and its data on mutant gene source are important for breeders who are carrying out breeding studies in vegetable crops.

Ethyl methane sulphonate and gamma irradiation were used in separate experiments to induce mutation. Observation and selection of mutant traits were carried out from M2 generation. Individuals were selected on the basis of desirable plant architecture, and specifically for improved sink capacity in the form of a larger number of pods per plant and larger number and size of seeds. Cumulative data from replicate yield trials over the past several years have isolated two mutants, A15/10 and A5/10, which are high yielding and adaptable to the local environment. A15/10 in a large seeded mutant with a high pod number, while A5/10 is an early maturing dwarf with a good yield potential (Zakri, 1986).

Two popular local varieties, Matjan and V-13 were adopted as the starting material. 3000 seeds per dose per variety were exposed to a series of gamma rays doses ranging from 0-45 krad to induce mutation of the desired traits such as high yield and resistance to *Cercospora* leaf spot (Rusli *et al*., 1992). Several mutant were selected resistance to *Cercospora* leaf spot but only 3 mutant (MJ40/42, V13/35/16 and MJ20/165-5) were out yielded check variety (Matjan and V13). These mutants were evaluate further to investigate N2 fixation as well as yield performance, and as consequences Matjan mutan MJ/40/42 consistency produced the highest pod yields, at above 41 ha"1 ,14-22% higher yields than the parent (Rusli *et al*., 1998)

Banana Novaria is a selection made from GN-60A mutant which was originated from the Seibersdorf Laboratory of The International Atomic Energy Agency (IAEA) for its vigorous growth and early flowering. In this program, Graine Naine, a popular Cavendish banana was induced by gamma irradiation using in-vitro technique. The introduced mutant was planted and evaluated at the United Plantations Bhd since 1991 (Mak *et al*., 1996). Early fruiting individuals with good bunch characteristics were identified, micropropagated and further selected in the field to ascertain stability and homogeneity of the mutant clone. This mutant clone specially selected

for the following desirable traits: early flowering, strong fruit pedicel or 'neck', good flavour and pulp texture, short stature and high yield potential.

2.4 MEDICINAL PLANT

Historically the use of mutagenesis in breeding has involved forward genetic screens and the selection of individual mutants with improved traits and their incorporation into breeding programmes (Parry *et al*., 2009). Genetic improvement work without a wide variability in the population is not possible. Intraspecific variations are prerequisite for initiating any plant improvement programme, which forms the basis for selection and improvement (Datta, 2005). The number of genes expressed during the lifetime of a particular plant is estimated to be between 16,000 and 33,000 (Gibbson and Somerville, 1993). Spontaneous and induced mutations in plants contribute for genetic dissection of the wild type genes (Datta, 2005).

Medicinal plants are used for their active principles that influence the physiological processes of an organism; mutation gives rise to a variant with secondary metabolic profile called as chemical races or chrmodemes (Lakshman Chandra De, 2017). The widespread use of induced mutants in plant breeding programme across the globe has led to the official release of 3222 plant mutant varieties from 170 different plant species in more than 60 countries throughout the world (Shu, 2009). The developed varieties increase biodiversity and provide breeding material for conventional plant breeding thus directly contributing to the conservation and use of plant genetic resource (Raina *et al*., 2016).

Pharmacognosy and Phytochemistry- medicinal and aromatic crops through mutation- Breeding as in these crops enhancement of alkaloid is prime important feature than the total crop yield. Henbane (*Hyoscyamus niger* L., 2n=34) belong to the family solanaceae, a variety Aela has been released from the CIMAP, Lucknow, which was selected from irradiated culture of H. Niger, having vigourous growth with a yield of 73q/ha, which was109 percent more than the parent (50q/ha), with the alkaloid level 0.545 per cent against 0.167 per cent in the parent plant. This mutant can be identified by its yellow flowers with slight purple tinge at the base of the petals (Farooqi and Shriramu, 2001)

In black cumin (Nigella sativa L.) mutants like lax branching, feathery leaf mutant, bushy, male sterile, crumpled leaf, early flowering, brown seed coat, dwarf and viridis mutants were developed

through X-rays and gamma radiations which were superior for many yield attributing characters stating that the parental genotypes and cross combinations identified from induced mutant progenies offer scope of improvement through proper selection in black cumin (Datta, 2005).

Kumar et al., (2007) reported a unique inflorescence bearing mutant (lli / lli) which was a monogenic recessive having leafless inflorescence architecture with increased flower frequency. This distinct plant of C. roseus was developed through chemical mutagenesis followed by salt tolerance selection (US Patent PP18315). Baskaran *et al.,* (2013) reported two EMS induced macro-mutants ('necrotic leaf' and 'nerium leaf) of periwinkle with enhanced contents of total root and leaf alkaloids and anticancer leaf alkaloids, vincristine and vinblastine than the parental variety. Their leaf and root yields were, however, significantly lower than their parental variety.

Basu *et al*., (2008) reported that mutation breeding approach has detected new breeding material exhibiting early seed maturity coupled with high seed yield, seed quality and determinate growth habit, when seeds from Tristar Fenugreek, were treated with 10–300 mM ethyl methane sulfonate (EMS) for 2–24 h and plants were selected for determinate growth habit, early maturity and high seed yield. Seeds of fenugreek (*Trigonella foenum-graecum* L.) were treated with either gamma rays (25-15 kR) or ethyl methanesulphonate (EMS 0.1-0.5%, 2h) or ethylene imine (EI, 0.01-0.1%, 2h). After repeated selection for better morphological performance from M2 to M4 generation, 11 mutant lines were identified with better yield potential and higher diosgenin content than their parent (Floria and Ichim, 2006).

A mutation breeding programme using gamma rays and ethyl methane sulphonate (EMS) was carried out for genetic conversion of narcotic 'opium poppy' into non- narcotic 'seed poppy'. The best mutant genotype, LL- 34 of the family C 1 - Comb- 113- 2, with 5.66 g/capsule seeds containing 52.6 per cent oil was designated as cv. 'Sujata'. This, perhaps the world's first opiumless and alkaloid- free seed poppy cultivar, offers a cheap and permanent (fundamental) solution to the global problem of opium- linked social abuse (Sharma *et al*., 2008). Five opium poppy mutant lines were selected from the progeny of the poppy variety, De Botosani, after treatment with either gamma rays of additionally with EMS. Most of the lines showed significant increase of both seed yield and morphine content over commercial varieties in two locations (Floria and Ichim, 2006). Further high yielding peppermint variety – multimentha: which has a high essential oil content and

resistance against *Puccinia menthae., Chamomilla recutita, Origanum majorana, Verbascum phlomoides* and *Lavandula vera* have been developed (Slavova *et at*., 2004).

Seeds are typically treated with a buffer solution containing EMS. The treatment concentrations and durations vary widely among taxa and even within the same species because different genotypes may respond differently to treatment. Deleterious effects can be caused by EMS, such as lethality, sterility, and a lower ability to regenerate plants from tissues such as floral pedicels (Latado *et al.*, 2004; Roychowdhury and Tah, 2011). Reduced fertility resulting from the treatment of some weedy or invasive species is desirable and at least as important as other phenotypic changes. Pilot experiments to determine the optimal treatment conditions, usually a combination of concentration and duration resulting in 50% survival [(median lethal dose (LD_{50})], are always advised (Napoli and Ruehle, 1996; Hohmann et al., 2005; Kim et al., 2006; Berenschot *et al*., 2008) In addition to seeds, in vitro treatments have been applied to nodal segments and ray florets in chrysanthemum (Padmadevi and Jawaharlal, 2011).

C. sativus is a valuable medicinal plant due to its medicinal apocarotenoids. The secondary metabolites represent the potential useful properties of the plant, including attributes that can treat diverse diseases such as cardiovascular diseases, anxiety, depression, and nervous disorders as well as different types of cancers (Jain *et al*., 2016). EMS had severe effects on C. sativus corms and flowers, and most of the corms did not sprout due to disintegration and crushing. According to the results, the destructive changes in most of the corm's tissues explained their failed sprouting. This could be attributed to the high production rate of mutations by EMS (Chen *et al*., 2018), which led to the biological and metabolic disruptions of corms and ultimately their death. Besides, EMS-treated plants triggered different changes in the C. sativus flowers, such as asymmetric tepals and incomplete stigmas. Compared with colchicine, the harmful effects of EMS were so severe that most of the EMS-treated corms had not grown properly or survived in the first and second year of the experiment.

Compared to untreated samples the treated samples are significantly affected the vincristine and vinblastine content by physical and chemical mutagenic treatments. Based on High performance Liquid Chromatography method vincristine and vinblastine content are analysed in M2 generation treated plants. The vincristine sulphate ranged from 0.03 to 0.37 similarly the vinblastine sulphate ranged from 0.037 to 0.085 .The maximum vincristine sulphate percentage was observed in T1

(40 kR) i.e., 0.37 followed by T5 (30 mM) i.e., 0.31. The minimum vincristine sulphate percentage was observed T3 (40 kR) i.e., 0.03 followed by control. The maximum vinblastine sulphate percentage was observed in T1 (40 kR) i.e., 0.085 followed by T4 (30 mM) i.e., 0.077. The minimum vinblastine sulphate percentage was observed T6 (30 mM) i.e., 0.037 followed by control. The experiment has exposed to presence of phytochemical constituents from periwinkle extracted leaves the graphical presentation of nine selected phytochemical viz., alkaloids, terpenoids, phenol, sugar, saponins, flavonoids, quinines, protein, steroids. The phytochemical constituents from periwinkle M2- 30mM generation extracted leaves the 30mM terpenoids, saponins, alkaloids were higher in concentration while phenol and protein in medium concentration and sugar, flavonoids, quinines, steroids in lowest concentration or absent. Extracted from leavesM2-40 Kr periwinkle leaves quinines, alkaloids were higher concentration, while saponins, phenol in medium concentration and steroids, flavonoids, sugar in lowest concentration or absent

Higher doses of mutagen were directly proportional to the quality parameters of periwinkle. Enzymes involved in different stages biosynthesis of vincristine might be due to promotive or inhibiting influence exerted by gamma and ems at different doses causing altered physiological and biochemical reactions in enzymes biosynthesis. The mutants would have enhanced protein synthesis and synthesis of cellulose hydrolyzing enzymes which might have triggered the secondary metabolites involved in different stages of biosynthesis of phytochemical compounds, similar results were obtained by Anandhi *et al.,* (2013) and Barnett *et al.,* (1978). The selection of plant types rich in alkaloids and phytochemical can be easily achieved by simple selection on the basis of a per se performance of the M2 generation, selection in the early generation followed by confirmation of the enhanced trait in M3 has been reported by many researchers (Mishra and Momin, 2004). Similar trend was also revealed by Baskaran *et al.,* (2013) in which two EMS induced macro-mutants (‘necrotic leaf’ and ‘nerium leaf) of periwinkle with enhanced contents of total root and leaf alkaloids and anticancer leaf alkaloids, vincristine and vinblastine than the parental variety was being reported. Increasing in alkaloid biosynthesis in plant can be stimulated by various means, including radio-mutagenesis confirmation of results needs to be drawn from field experiments by inducing mutations and then making a survey of subsequent generations to select mutant lines richest in respective alkaloids content and other desired characteristics (Benslimani and Khelifi, 2014).

Physical and chemical mutagenesis in *Stevia rebaudiana*: variant generation with higher UGT expression and glycosidic profile but with low photosynthetic capabilities has been done with a positive result of increase in the glycosidic level (Khan *et al,* 2015).

2.5 ORNAMENTAL PLANTS

EMS has been used for several annual and biennial ornamental genera. Seeds were treated in every case except for *Begonia*, concentrations have ranged from 0.10% to 1.20%, with an outlier .at 40%, and treatment durations are commonly between 4 and 24 h. Kashikar and Khalatkar (1981) observed flower color changes in the M1 and M2 generations of white flowering *Petunia ×hybrida*. Several different shades of violet were found in the M1 generation, and colors ranging from pink to pale blueish magenta were found in the M2 generation. M1 mutants displaying dwarfism and abnormal leaf morphology were found in *Antirrhinum majus* (Heffron *et al.,* 2006). In addition to changing flower color and other morphological traits, EMS has also been used to create mutants with resistance against pathogens, as shown by Chen et al. (2014) who obtained *Begonia ×hiemalis* mutants that were resistant to stem rot caused by *Rhizoctonia solani*.

Gamma and X-rays have been used to study mutagenesis in a few annual and biennial ornamental genera, with *Petunia* being the most common. For gamma irradiation, the total absorbed doses have ranged from 0.5 to 320 krad. A dose of 320 krad is exceptionally high, however; the median maximum dose was 12.5 krad. For X-rays, the doses have ranged from 0.22 to 20 krad. Seeds are the most commonly irradiated tissues, but others such as leaf discs and cuttings have been used. Many traits were affected. Berenschot *et al.* (2008) identified a *Petunia* mutant with a higher density of trichomes and a distinct leaf shape. Venkatachalam and Jayabalan (1997) found zinnias with novel flower colors such as yellow, magenta, and red with white spots in mutants of the cultivar Crimson Red. A *Zinnia* mutant showing a larger number of whorls in its flowers was also found. Doorenbos and Karper (1975) identified *Begonia ×hiemalis* mutants displaying dwarfism, petaloid stamens, and varying leaf colors. Fertility, characterized as the number of seed capsules produced after manual pollination, was reduced in *Petunia ×hybrida* (Berenschot *et al.,* 2008).

Ion: a few annual or biennial genera have been treated with ion beams, and neutron beams have not been used at all . The heavy ion doses used during these studies have ranged from 0.1 to 8 krad at LETs from 22.5 to 76 keV/μm. The tissues used for ion beam irradiation vary. Shoot cultures

and apical meristems have been used, but cuttings with nodes and leaves are suitable irradiation targets as well. Flower color is often affected. Ogawa *et al.* (2014) found *Limonium* mutants showing lighter, darker, or more reddish shades of purple compared with the original purple flowers. Miyazaki et al. (2006) found similar *Torenia* mutants showing pale or dark pink flowers compared with blue in the wild type. They also revealed that the pink color likely resulted from the inhibition of dihydromyricetin biosynthesis, thus preventing build-up of the anthocyanidins delphinidin, petunidin, and malvidin. Other traits such as variegation in *Petunia* and sterility in *Verbena* were observed (Miyazaki *et al.*, 2002; Kanaya *et al.*, 2008).

TABLE-1

Effect of physical mutagens on plant's different parts

Genus	Mutagen	Dose	LD_{50}	Material	Reference
Acer	Neutrons	2–14 krad	—	Seeds	Smith and Noyszewski, 2018
Achimenes	Neutrons	0.75–2 krad	—	Leaves	Broertjes, 1976
Berberis	Neutrons	2–14 krad	—	Seeds	Smith and Noyszewski, 2018
Chrysanthemum	Ion beam	1 krad, unknown, 23 keV/μm	—	Scions	Hisamura et al., 2016
	Ion beam	0.1–0.8 krad, 220 MeV, 122 keV/μm	—	Ray florets and leaf explants	Matsumura et al., 2010
	Ion beam	0.1–0.5 krad, 446 MeV/u, 93 keV/μm	—	Leaf segments and ray florets	Okamura et al., 2015
	Ion beam	0.3–0.6 krad, unknown, 22.6 keV/μm	—	Cuttings	Sakamoto et al., 2016
	Ion beam	0.1–2 krad, 135 MeV/u, 23 keV/μm	—	Stem segments	Suzuki et al., 2005
	Ion beam	0.2–0.5 krad, unknown, 23/62/280 keV/μm	—	Cuttings	Tanokashira et al., 2014
	Ion beam	0.01–0.3 krad, unknown, 23/310 keV/μm	—	Leaf blades	Tanokashira et al., 2016
	Ion beam	0.3–0.6 krad, 135 MeV/u, 22.5 keV/μm	—	Stem segments	Wakita et al., 2008
Cyclamen	Ion beam	0–5 krad, 220/320 MeV, unknown	—	Petioles	Ishizaka et al., 2012
	Ion beam	0.05–1.6 krad, 320 MeV, unknown	—	Petioles	Kondo et al., 2009
	Ion beam	1–8 krad, 1.62 GeV, 23 keV/μm	—	Callus, somatic embryos, plantlets, tubers	Sugiyama et al., 2008a
Dahlia	Ion beam	0.5–10 krad, unknown, unknown	—	Shoots	Hamatani et al., 2001
Dianthus	Ion beam	0.5–3 krad, 220 MeV, unknown	—	Leaf segments	Okamura et al., 2003
	Ion beam	0.7–2 krad, 320 MeV, 76 keV/μm	—	Petals	Okamura et al., 2013
	Ion beam	2–8 krad, 135 MeV/u, 23 keV/μm	—	Stem segments	Sugiyama et al., 2008b
Hydrangea	Ion beam	1–5 krad, 100 MeV, unknown	—	Seeds	Kudo et al., 1998
Limonium	Ion beam	0.5–3 krad, unknown, 23 keV/μm	—	Shoot cultures	Ogawa et al., 2014
Pelargonium	Ion beam	1–4 krad, 80 Mev/u, 34 keV/μm	—	Buds	Yu et al., 2016
Petunia	Ion beam	0–3 krad, 320 MeV, 76 keV/μm	—	Shoot apical meristem	Hase et al., 2010
	Ion beam	0.1–2 krad, 135 MeV/u, unknown	—	Nodal cuttings	Miyazaki et al., 2002
Populus	Ion beam	0–3 krad, unknown, unknown	—	Explants	Biswas et al., 2013
Prunus	Ion beam	0.5–2 krad, 135 MeV/u, 23 keV/μm	—	Scions	Hayashi et al., 2019
	Ion beam	1–2 krad, 135 MeV/u, 22.6 keV/μm	—	Scions	Ishii et al., 2009
Rosa	Ion beam	1–5 krad, 135 MeV/u, unknown	—	Buds	Hara et al., 2003
	Ion beam	0.5–10 krad, 100/220 MeV, unknown	20, 5–7.5 krad	Stem cuttings with bud	Yamaguchi et al., 2003
Saintpaulia	Ion beam	0.5–8 krad, 960 MeV, unknown	2.52, 2.32 krad	Leaf explants	Zhou et al., 2006
Sandersonia	Ion beam	2–10 krad, unknown, unknown	—	Seeds	Horita et al., 2002
Spiraea	Ion beam	0.5–14 krad, 220 MeV, unknown	—	Seeds	Iizuka et al., 2001
Torenia	Ion beam	0.5–5 krad, unknown, unknown	—	Leaves and internodes	Miyazaki et al., 2006
	Ion beam	0.5–8 krad, 1.62/2.70 GeV, 22.5/61.5 keV/μm	—	Leaf disks	Sasaki et al., 2008
Tricyrtis	Ion beam	0.5–5 krad, 135 MeV/u, 23 keV/μm	—	Callus	Nakano et al., 2010
Verbena	Ion beam	0.1–1 krad, 1.89 GeV, 31 keV/μm	—	Nodes	Kanaya et al., 2008

LD_{50} = median lethal dose.

X-rays and gamma rays have been widely used for several herbaceous perennials, with *Chrysanthemum* being the most commonly treated genus by far. The X-ray treatments have consisted of doses ranging from 0.44 to 13 krad, with one exceptionally high dose at 50 krad. For gamma irradiation, the doses ranged from 0.15 to 15 krad, with two outliers at 40 krad. Many different tissues were used during these studies. For *Chrysanthemum* alone, the tissues ranged from cuttings and whole plants to individual cells and ray florets. For other genera, corms, leaf cuttings, and bulbs were used. Surprisingly, seeds were not used in the reviewed studies. Regarding annuals and biennials, many morphological traits were affected. Preil *et al.* (1983) selected two *Chrysanthemum* mutants that were tolerant to low temperatures. Lee *et al*. (2010) irradiated cuttings of the *Chrysanthemum* cultivar Beakma and found a mutant that did not form a hollow stem when grown in high summer temperatures, leading to plants with stronger stems that are easier to handle. A *Gerbera jamesonii* mutant that was tolerant to powdery mildew was found by Ghani and Sharma (2019). X-ray irradiation restored male fertility in *Dianthus caryophyllus* (Sagawa and Mehlquist, 1959); however, Biswas and Biswas (2006) were able to induce partial sterility in *Ornithogalum virens*. Oates *et al.* (2013) noticed that treating *Rudbeckia subtomentosa* callus with relatively low levels of gamma radiation resulted in high survival and a good number of mutations, including reduced height and good flower form. A large variety of changes in flower color as well as flower morphology were found in most of the other genera, and traits like vase life can also be improved, as shown by Shukla *et al*. (2018).

Many diverse woody genera have been treated with gamma or X-rays. Sparrow *et al.,* (1968) determined the gamma radiation LD_{50} doses for 28 woody plant species and predicted the LD_{50} for another 190 species based on their interphase chromosome volumes, although the authors concede there exists wide variations because of timing and other factors involved with their method. They did not describe any visible phenotypes resulting from mutations, but the LD_{50} values provide starting points when trying to determine the optimal dose for any of these species. The aforementioned studies used doses ranging from 0.1 to 40 krad with outliers at 140 and 225 krad for gamma rays and 2.5 to 6 krad for X-rays. Seeds and cuttings were often the choice of tissue to treat, but whole plants and explants were also treated. Different traits were affected by the mutations. A jasmine-like fragrance was found in a *Vitex agnus-castus* mutant by Ari *et al*. (2015), who showed that complicated traits like fragrance can also be improved by inducing mutations. Dwarfism was found by Kukimura *et al*. (1976) in *Cryptomeria* and in *Jasminum* (Ghosh *et al.,*

2019). Shorter internodes resulting in lower plant height were also observed in *Populus* and *Rosa* (Baig *et al.*, 2012; Nishiguchi *et al.*, 2012). Other variations in *Rosa* were restoration of fertility and changes in color (orange, pink, etc. compared with red in the original cultivar), possibly because of changes in cyanidin and pelargonidin content (Smilansky *et al*., 1986).

X-ray irradiation have been very effective mutagens in a large number of plant species, they require more expensive equipment such as gamma sources and X-ray machines. They provide good penetration of tissues and seeds, making it possible to treat material with a higher consistency. They also provide ways to treat fragile tissue, such as pollen grains, that could be damaged by soaking them in chemicals (Schum, 2003; Virgilio, 2007; Oladosu *et al.,* 2016). Their mutagenic efficiency, however, seems to be relatively low (Kaul , 1977; Gautam *et al*., 1992; Girija *et al*.2009; Wani, 2009).

The total absorbed dose is described in treatments and relatively little attention is focused on the dose rate and/or duration of radiation treatment. Acute irradiation (high dose rate/short duration) has been favored over chronic irradiation (low dose rate/long duration) for a long time, but the efficiency of both methods has been debated (Kodym and Afza, 2003). Nagatomi and Degi (2009) showed that chronic gamma irradiation more effectively induced flower color mutants in *Chrysanthemum*, and Broertjes (1972) showed a similar effect of chronic X-ray irradiation in *Saintpaulia*. Numerous plant species have been successfully treated with chronic irradiation (Nagatomi, 1993; Richter and Singleton, 1995; Nagatomi *et al.,* 2000 Datta, 2012;). Therefore, experimentation with different dose rates and durations may lead to more efficient irradiation protocols for many ornamental species.

A much larger number of herbaceous perennial genera have been treated with ion beam and neutron irradiation, with *Chrysanthemum* again being the most common. Treatment conditions included ion doses ranging from 0.01 to 10 krad at LETs ranging from 22.5 to 310 keV/μm. Only *Achimenes* was treated with neutrons at doses ranging from 0.75 to 2 krad. Tissues such as ray florets, leaf segments, callus, petioles, and buds were used as targets. Seeds were used in one case, too. Basic morphological traits such as plant size (dwarfism), leaf shape, flower color, flower size, and flower shape were affected in the majority of mutants. Sugiyama *et al*. (2008a) found

sterile *Cyclamen* mutants. A *Chrysanthemum* mutant that flowered early and under low temperatures was also identified (Sakamoto *et al.*, 2016).

Relatively few woody genera were treated with neutron or ion beam irradiation . Ion beam doses ranging from 0.5 to 14 krad were used. LETs were mostly not reported, except for two cases in which the LET was ≈23 keV/μm. Neutron irradiation doses ranged from 2 to 14 krad. Seeds were most commonly used as irradiation targets, but scions, stem cuttings, and buds have been used. Among the affected traits were flower color in *Prunus* and *Rosa* and plant size (dwarfism) in *Spiraea* and *Hydrangea*. Hayashi *et al.* (2019) found that a *Prunus* mutant, which flowered twice during a single year, did not require a cold period for flowering and produced three-times the number of flowers of the original variety if the mutant was exposed to cold winter temperatures. This mutant was named 'Nishina Otome' and commercially released in 2010. Smith and Noyszewski (2018) found several *Acer* mutants that did not flower and, thus, produced no seeds. They also found a *Berberis* mutant that did flower; nonetheless, it produced nonviable seeds. These mutants are still being evaluated but could provide solutions for mitigating the invasiveness of non-native *Acer* and *Berberis* species in North America.

2.6 EFFECT OF RADIATION ON PLANT CELLS

The response of plants to radiation is more or less linear with the dose used (Yamaguchi, *et al*. 2008), which can cause changes in cell structure, such as physical-chemical lesions, small lesions on chromosomes or the combination of both effects (Ramesh, *et al.* 2013). Primary lesions delay or inhibit cell division, affect mitotic activity, growth rate or habit, metabolism (such as dilation of thylakoid membranes), photosynthesis, modulation of the antioxidant system and accumulation of phenolic compounds (Wi *et al.*, 2005), cause modifications in lipids, enzymes and other cellular constituents (Kodym *et al*. 2003) and induce cell death (Wi *et al.*, 2005).

The use of induced mutations for rice improvement in Malaysia started in 1972. In the project, several varieties including Jaya, Malinja and Mahsuri were treated with two mutagen, viz. Gamma rays and EMS, and screened for blast. More than 100 mutants resistant to blast were identified. Most of the mutants do not have good plant type but showed changes in many other traits. However, a more concerted effort was begun in 1979 to look for blast (BI) resistance (Arasu *et.al.,* 1983). To date, 48 varieties and elite breeding lines have been treated with ethyl methane sulfonate (1.5-2.5%) and gamma radiation (15-40 krad). Mutant line with short stature, early maturity,

TABLE-2

Effect of different mutagens on various parts of a plant

Genus	Mutagen	Dose (krad)[z]	LD_{50} (krad)	Material	Reference
Acer	Gamma rays	0.1–5	—	Cuttings	Smith and Noyszewski, 2018
	Gamma rays	50–225	—	Seeds	Smith and Noyszewski, 2018
Achimenes	X-rays	2–4	—	Leaves	Broertjes, 1976
Agave	Gamma rays	1–40	—	Bulbs	Navabi et al., 2016
Antirrhinum	Gamma rays	10–320	—	Seeds	Gupta and Matsuo, 1969
	Gamma rays	0.5–60	—	Plants	Sekiguchi et al., 1971
Begonia	X-rays	1.5–2.5	—	Leaves	Doorenbos and Karper, 1975
	X-rays	0.5–3	—	Leaves	Roest et al., 1981
Berberis	Gamma rays	0.1–5	—	Cuttings	Smith and Noyszewski, 2018
	Gamma rays	50–225	—	Seeds	Smith and Noyszewski, 2018
Bougainvillea	Gamma rays	0.5–1	—	Cuttings	Anitha et al., 2017
	Gamma rays	0.5–2	—	Cuttings	Swaroop et al., 2015
Calendula	Gamma rays	2.5–7.5	—	Seeds	Tiwari and Kumar, 2011
Chrysanthemum	Gamma rays	1–4	—	Cuttings	Dowrick and El Bayoumi, 1966
	X-rays	0.44–1.75	—	Cuttings	Dowrick and El Bayoumi, 1966
	Gamma rays	0.5–1	—	Ray florets	Hossain et al., 2006b
	Gamma rays	3–10	—	Plantlets	Kang et al., 2013
	Gamma rays	0.5–2.5	—	Cuttings	Kapadiya et al., 2014
	Gamma rays	1–2	—	Cuttings	Kumari et al., 2013
	Gamma rays	1–5	—	Cuttings	Lee et al., 2010
	Gamma rays	2.5–15	—	Plants	Nagatomi and Degi, 2009
	Gamma rays	0.5–1.5	—	Unknown	Padmadevi and Jawaharlal, 2011
	X-rays	2.5	—	Callus	Preil et al., 1983
	X-rays	0.5–2	—	Cuttings	Tanokashira et al., 2014
	Gamma rays	1.5	—	Cuttings	Zalewska et al., 2011
Cryptomeria	Gamma rays	0.51–3.51	—	Plants	Kukimura et al., 1976
Dahlia	Gamma rays	1–3	—	Cuttings	Sundar et al., 2017
Datura	X-rays	1.3–2.6	—	Protoplasts	Krumbiegel, 1979
Dianthus	Gamma rays	5.26–10.5	—	Cuttings	Buiatti and Ragazzini, 1965
	X-rays	2–50	—	Nodes	Cassells et al., 1993
	X-rays	4–13	—	Leaf segments	Okamura et al., 2003
	Gamma rays	3–10	—	Leaf segments	Okamura et al., 2003
	Gamma rays	2–8	2—4	Shoots	Paramesh and Chowdhury, 2005
	X-rays	2.2–4.4	—	Plants	Sagawa and Mehlquist, 1959
	Gamma rays	2–8	—	Petal explants	Simard et al., 1992
Gerbera	Gamma rays	0.15–3	0.65	Shoots	Ghani and Sharma, 2019
	Gamma rays	0.15–1	0.6	Shoots	Ghani et al., 2014
	Gamma rays	2	—	Shoots	Laneri et al., 1990
	X-rays	0.5–5	—	Microshoots	Walther and Sauer, 1990
Gladiolus	Gamma rays	1.5–5.5	—	Corms	Sathyanarayana et al., 2019
	Gamma rays	1.5–6	—	Corms	Shukla et al., 2018
	Gamma rays	1.75–10.5	—	Corms	Tirkey and Singh, 2019
	Gamma rays	5–15	—	Corms	Tiwari et al., 2010
Hebe	Gamma rays	1.5–6	3.3, 3.7, 4.8, 5.6	Nodal explants	Gallone et al., 2012
Iris	X-rays	0.5–1.1	—	Bulbs	Hekstra and Broertjes, 1968
Jasminum	Gamma rays	1–2.5	—	Cuttings	Ghosh et al., 2019
Lonicera	Gamma rays	1–6	2.1, 3.5	Microcuttings	Cambecèdes et al., 1992
Ornithogalum	Gamma rays	20–40	—	Seeds	Biswas and Biswas, 2006
Pelargonium	Gamma rays	1.5	—	Leaves	Grunewalt, 1983
Petunia	Gamma rays	2–10	10	Seeds	Berenschot et al., 2008
	Gamma rays	5–10	—	Leaf disks	Donà et al., 2013
	Gamma rays	0.87–5.26	—	Seeds	Kashikar and Khalatkar, 1981
	X-rays	0.22–2.6	—	Protoplasts	Krumbiegel, 1979
	X-rays	0.28–2.2	—	Flower buds	Moore and Haskins, 1935
Pinus	Gamma rays	0.18–3.5	—	Plants	Donini, 1967
Plectranthus	Gamma rays	1.5–6	3.762–6.52	Cuttings	Aisyah et al., 2015
Populus	Gamma rays	1–30	—	Plantlets	Nishiguchi et al., 2012
Portulaca	Gamma rays	2–6	—	Unknown	Raghuvanshi and Singh, 1979
	Gamma rays	1–4	—	Stem cuttings	Wongpiyasatid and Hormchan, 2000
Pseudotsuga	Gamma rays	0.5–10	—	Seeds	El-Lakany and Sziklai, 1970
Rosa	Gamma rays	1–6	—	Shoot tips	Aamir et al., 2016
	Gamma rays	1–12	3.3–5.4	Shoot tips	Baig et al., 2012
	Gamma rays	0.5–8	4	Stem cuttings with bud	Bala and Singh, 2013
	Gamma rays	5–20	—	Seeds	Giovanni et al., 2015
	Gamma rays	0.88–11.4	—	Stem cuttings with buds	Smilansky et al., 1986
	X-rays	2.5–6	—	Microshoots	Walther and Sauer, 1986a, 1986b
Rudbeckia	Gamma rays	0.5–4	—	Callus	Oates et al., 2013
Saintpaulia	Gamma rays	1–10	5.6	Leaf cuttings	Wongpiyasatid et al., 2007
	X-rays	0.5–10	5.69	Leaf explants	Zhou et al., 2006

3. MATERIALS AND METHODS

3.1 MATERIALS

Seeds of ***Trigonella foenum-graecum*** L. and ***Raphanus sativus*** L. were purchased from an online platform www.all that grows.in.

3.1.1 SYSTEMATIC POSITION OF *Raphanus sativus* L.

Kingdom : Plantae

Clade : Tracheophytes

Clade : Angiosperms

Clade : Eudicots

Clade : Rosids

Order : Brassicals

Family : Brassicaceae

Genus : *Raphanus*

Species : *sativus*

3.1.2 DESCRIPTION

Radishes are brassicaceous crops that are grown either annually or biennially for their swollen tap roots, which can be globular, tapering, or cylindrical in shape. The color of the root skin varies from white to pink, red, purple, yellow, green, and even black, although the flesh is typically white, deriving its color from anthocyanins. Red varieties utilize the anthocyanin pelargonidin as a pigment, while purple cultivars get their color from cyanidin. Smaller radish types have leaves measuring around 13 cm (5 in) long and round roots up to 2.5 cm (1 in) in diameter, or more elongated, slender roots up to 7 cm (3 in) long. These are commonly consumed raw in salads. On the other hand, longer root forms such as oriental radishes, daikon or mooli, and winter radishes can grow up to 60 cm (24 in) in length, with foliage reaching about 60 cm (24 in) in height and spreading up to 45 cm (18 in). Radish flesh is crisp and sweet when harvested at the right time but

tends to become bitter and tough if left in the ground for too long. The leaves of radishes are arranged in a rosette and have a lyrate shape, characterized by pinnate division with an enlarged terminal lobe and smaller lateral lobes. The white flowers are borne on a racemose inflorescence, while the fruits are small pods that are edible when young. Radishes are a diploid species with 18 chromosomes (2n=18), and it is estimated that their genome ranges between 526 and 574 Mb in size. (Gupta *et al.*, 2003; U.S. Department of Agriculture. Agricultural Research Service)

Radish is considered a root since its specialized structure (hypocotyls) has a full or partial subterranean habitat, its shape is close to the true roots, and it can store starch and other compounds (Radovich, 2018). The surface color of radish can vary from white in Asia to red in Europe through purple green and black (Nishio, 2017), while its flesh is white in most European and Asian crops (Hadley & Fordham, 2003). Certain characteristics of radish are due to its phytochemical content. Anthocyanin pigments provide the red color of roots, while a high potential to form isothiocyanates contributes to the pungent flavor and distinctive taste, which is highly popular in countries like Japan, the Philippines and Hawai (Gupta, Talwar, Jain, Dhawan, & Jain, 2003; Nishio, 2017). The edible part of radish is mainly the root. However, the consumption of leaves and sprouts is increasing (Xiao, Lester, Luo, & Wang, 2012; Radovich, 2018; Butt, *et al.* 2018; Manivannan *et al*., 2019;). The root is usually consumed in salads, but it can also be cooked or salted together with other vegetables (Hadley & Fordham, 2003; Roy & Chakrabarti, 2003; Kaneko, Kimizuka-Takagi, Bang, & Matsuzawa, 2007; Nishio, 2017). Radish roots can also be processed as dried or canned pickles (Manivannan *et al.*, 2019; Nishio, 2017), which are most commonly consumed in Asia (Roy & Chakrabarti, 2003). Leaves and sprouts are usually eaten raw as part of salads. Overall, radish is low in calories and a good source of calcium, magnesium, copper, manganese, potassium, vitamin B_6, vitamin C, and folate (Gupta *et al.*, 2003; U.S. Department of Agriculture. Agricultural Research Service). According to data from the EPIC cohort in Europe, radish intake was 6% of the total of cruciferous or *Brassicaceae* vegetables, being fifth after cauliflower, broccoli, and white and normal cabbage (IARC, 2004).

Finally, radish extracts have been employed to treat several organ disorders (Hadley & Fordham, 2003; Manivannan *et al.,* 2019). The potential use of radish as a source of bioactive compounds with clinical and health implications in diseases such as hypertension, cardiometabolic

disorders and as an antimicrobial and antioxidant agent has become a field of interest for researchers and the pharmaceutical industry (T. K. Lim, 2012; Manivannan *et al.,* 2019). Therefore, a comprehensive and systematic assessment of the nutrient and phytochemical composition of radish will allow a better understanding of its role in human nutrition and health, besides providing insights to prioritize future biological and pharmacological research about the wide range of phytochemicals contained in this vegetable. Compared to previous efforts, which are mainly narrative or expert reviews (Manivannan *et al.,* 2019) and, consequently, may not capture the overall evidence on the topic, the present work systematically evaluated the nutrient and bioactive composition of *Raphanus sativus* (radish) and reported the concentration of its bioactive components for the first time.

3.1.3 SYSTEMATIC POSITION OF *Trigonella foenum-graecum* L.

Kingdom : Plantae

Clade : Tracheophytes

Clade : Angiosperms

Clade : Eudicots

Clade : Rosids

Order : Fabales

Family : Fabaceae

Tribe : Trifolieae

Genus : *Trigonella*

Species : *T. foenum-graecum*

3.1.4 DESCRIPTION

Fenugreek is categorized as an annual herbaceous plant. Its growth pattern involves spreading, moderate branching, and a delicate structure. Initially, the plant develops a taproot, followed by a profusion of secondary roots. These roots form nodules and establish a symbiotic relationship with the Rhizobium bacterium. The stem is upright, branched, green, smooth, and herbaceous in nature. Its leaves are petiolate, alternate, compound, trifoliolate, and emit a pleasant fragrance, featuring stipules as well (Subramanian 1996; Malhotra and Rana 2008). The genus Trigonella encompasses mainly annual and perennial plants, often characterized by strong scents.

The pods exhibit a sickle shape, transitioning in color from green or light purple to brown or yellow-brown as they reach maturity. They are characterized by a beak, measuring 9.5–18.6 cm in length and 0.2–10.4 cm in width, curved in form, and occasionally straight, adorned with transient hairs. Each pod typically contains around 10–20 seeds. The seeds themselves vary in shape, ranging from rectangular to round, featuring a deep groove between the radical and cotyledons, with dimensions spanning 3.5–6 mm in length and 2.5–4 mm in width. The seeds may appear light grayish, brown, olive green, or cinnamon in color, depending on the type and variety, showcasing a pronounced radical that is half the length of the cotyledons. The minute hilum is partly concealed by a deep notch (Petropoulos 1973; Basu 2006). Surrounding the seeds is the seed coat, distinctly separated from the embryo by a well-developed endosperm, serving as the primary storage organ. Most endospermic cells within the seeds are nonliving, housing cytoplasmic contents filled with store reserves known as galactomannan (Bewley *et al.* 1993), which facilitate seedling growth. This tissue is enclosed by a single-cell layer of living tissue—the aleuronic layer—where cells are small, thick-walled, and contain aleurone grains that diminish during seed germination (Reid and Meier 1972).

Following fertilization, a series of transformations occur within the ovule, leading to the formation of the seed. In fenugreek, the process of seed development from initiation to mature, well-filled grains typically spans approximately 120 days post-anthesis. The pivotal role of galactomannan within the endosperm becomes evident around 30 days post-anthesis, reaching its peak activity at approximately 55 days post-anthesis, coinciding with a decrease in seed fresh weight. Galactomannan plays a crucial role in supporting seedling growth during germination and plays a vital role in regulating the water balance of the embryo (Campbell and Reid 1982). The versatile

utility of galactomannan extends to various industries such as food, pharmaceuticals, cosmetics, and paper products, thereby attracting considerable research interest in understanding its biochemistry, synthesis, and mobilization (Rosser 1985; Scherbukhin and Anulov 1990). The comprehensive review by Petropolous (2002) has consolidated many of these findings. Among the Trigonella species studied, it has been observed that the mannose-to-galactose ratio is approximately 1:1 in most species, except for T. erata, which displays a ratio of 1.6:1 (Reid and Meier 1970). Studies on galactomannan biosynthesis have employed techniques such as cell-free extracts and whole endosperm tissue (Edwards *et al*. 1989, 1992). Furthermore, advancements in genetic engineering offer the potential to modify fenugreek to produce galactomannan with specific mannose-to-galactose ratios, such as 4:1, catering to industrial applications.

3.1.5 MEDICINAL PROPERTIES

The green leaves of fenugreek are renowned as one of the oldest medicinal herbs, rich in β-carotene (19 mg/100 g) and ascorbate (220 mg/100 g) (Thomas *et al*., 2011), along with significant amounts of fiber, iron, calcium, and zinc, surpassing the nutritional content of many common food items (Muralidhara *et al.,* 1999). Among its plant parts, the seeds, which are biologically endosperm, hold the highest value. When raw, these seeds exhibit a golden color and possess a distinctive maple flavor, albeit with a bitter taste. However, this bitterness can be mitigated through roasting. The seeds are characterized by their fibrous, sticky, and gummy texture (Jani *et al*., 2009).

Nutritional constituents and associated functionality

In terms of nutritional factors, saponins and alkaloids are regarded as anti-nutritional components within the seeds. Nonetheless, defatted seeds are devoid of these compounds, making them suitable for consumption by individuals with fat-related dietary concerns (Altuntas *et al*., 2005).

TABLE-3

Lipid profile of fenugreek seeds (adopted from Chatterjee et al., 2010)

Lipid Species Identified	Amount (g/100 g)
Triacylglycerols	4.330 ± 0.011
Diacylglycerols	0.280 ± 0.008
Monoacylglycerols	0.180 ± 0.005
Phosphotidylcholine	0.110 ± 0.002
Phosphotidylethanolamine	0.036 ± 0.003
Phosphotidylinositol	0.009 ± 0.001
Free fatty acids	0.160 ± 0.001

TABLE-4 Crude or proximate composition of fenugreek seeds and leaves

Particulars	Contents (g/100 g)	References
Carbohydrates	42.3	El Nasri and El Tinay (2007)
Gum (seeds)	20.9	Kakani et al. (2009)
Ash (seeds)	3.38	Sowmya and Rajyalakshmi (1999)
Fiber (seeds)	50.0	Montgomery (2009)
Soluble		
Raw	21.7	Muralidhara et al. (1999)
Germinated	10.3	Muralidhara et al. (1999)
Insoluble		
Raw	26.8	Muralidhara et al. (1999)
Germinated	23.9	Muralidhara et al. (1999)
Fiber (leaves)		
Soluble	0.7	Altuntas et al. (2005)
Insoluble	4.2	Altuntas et al. (2005)
Dietary fiber	48.0	Brummer et al. (2003)
Fats (seeds)	7.9	El Nasri and El Tinay (2007)
Fats (leaves)	1.0	Montgomery (2009)
Protein (seeds)	25.4	El Nasri and El Tinay (2007)
Protein (leaves)	4.4	Montgomery (2009)
Moisture (seeds)	7.49	Sowmya and Rajyalakshmi (1999)
Moisture (leaves)	86.0	Sowmya and Rajyalakshmi (1999)

3.1.6 BIOLOGICALLY ACTIVE COMPOUNDS

The antioxidant properties of fenugreek are associated with its numerous health benefits. It's noteworthy that germinated seeds offer more advantages in this aspect compared to un-germinated dry seeds. Additionally, the aqueous fraction of fenugreek exhibits significant antioxidant activity compared to flavonoids and phenolics (Balch, 2003; Meghwal and Goswami, 2012; Khole *et al*., 2014). Fenugreek is rich in flavonoids, alkaloids, saponins, and other antioxidants. It contains notable quantities of phenolics such as gallic acid (1.7), protocatechuic acid (4.0), catechin (0.4), gentisic acid (35.8), chlorogenic acid (0.7), vanillic acid (58.5), and syringic acid (0.3) per 100 grams of seed extract (Rababah *et al.,* 2011). The endosperm of fenugreek contains around 35% alkaloids, primarily trigonelline (Jani *et al*., 2009). Flavonoids contribute more than 100 mg/g to the fenugreek seed's composition (Naidu *et al.,* 2011). These compounds are considered biologically active due to their pharmacological effects on the human body when consumed. Therefore, their inclusion in the daily diet is recommended for managing conditions like hypercholesterolemia, cancer, and diabetes mellitus, as they possess hypoglycemic, antilipidemic, anticarcinogenic, and cholagogic properties (Meghwal and Goswami, 2012). However, volatile oils and alkaloids are the primary constituents responsible for the characteristic bad odor and bitter taste of fenugreek, and thus, they may be removed before consumption.

3.2 PHYSICAL TREATMENT

UV TREATMENT

Physical treatment was done at DST-FIST lab of the Department of botany, St. Joseph's College (Autonomous), Tiruchirappalli.

PRINCIPLE

The utilization of ultraviolet (UV) light lamps in the curing, bonding, and coating industries is widely recognized, although the technical application of UV light varies across these operations. This discrepancy arises from factors such as the specific type of UV light employed, the distance and duration of exposure, and the nature of the ink, adhesive, or coating being used. Depending on the application and the materials involved, the response to UV light differs, thereby influencing

the efficacy of the overall system. This not only affects the required exposure times for curing, bonding, or coating but also impacts the long-term effectiveness of the treatment.

UV light encompasses a range of wavelengths from 250 nm to 400 nm, with most of it being invisible to the human eye, though a small portion is visible at longer wavelengths. UV-C possesses the shortest wavelengths and highest energy, while UV-B and UV-A occupy the middle spectrum, and UV-V exhibits the longest wavelengths and lowest energy. For this project, UV-B with a wavelength of 280-320 nm has been selected. When an electrical current passes through the lamp, it excites the elements inside, generating UV light as a result. (A.T. Black, in Encyclopedia of Toxicology (Third Edition), 2014)

CHEMICAL USED

Sodium buffer.

3.3 PROTOCOL (Article in Revista Brasileira de Ciências Agrárias - Brazilian Journal of Agricultural Sciences · June 2023)

PRE TREATMENT OF SEEDS :

- The seeds of ***Raphanus. sativus*** and ***Trigonella foenum-graecum*** are soaked in NaCl buffer 0.1N for 24 hours before the treatment as shown in the figure 1 & 2.
- After the time period they are rinsed thoroughly with distilled water for 2-3 times.
- The seeds are then dried on a filter paper and 50-60 seeds are isolated for the treatment purpose as in figure 3

UV TREATMENT:

- The chamber is cleaned and sanitized using ethanol and brought to an aseptic condition.
- The UV-B light is turned on and kept as such for around 10 min.
- After the timeline they are turned off and prepared to keep the seeds inside.

INNOCULATION

- The rinsed seeds are then taken in a Petri dish and spread in such a way that one doesn't overlap the other, as shown in Figure-4.
- These petri dishes are kept inside the UV chamber and closed as shown in Figure-5.
- Now the lights are turned on and let the seeds to get exposed to the UV radiation.
- The seed are treated in different time gap
- One set of seed were treated for 3 hours and another for 6 hours.
- After the treatment of seeds, they were dropped in growing bags for the plants to grow as shown in Figure-6

3.4 PHYTOCHEMICAL ANALYSIS

Quantitative phytochemical studies were done in the Department of Chemistry, St. Joseph's College (Autonomous) , Tiruchirappalli.

The extraction technique is done based on the texture and components that must be extracted. For the separation of Flavanoids, Tanins, protein and carbohydrates the plant parts are extracted sequentially with a broad variety of solvents, starting with chloroform and ethanol. Extraction was done by repeated process of maceration and extraction using soxhlet apparatus. Each time, before extracting with the next solvent, the powder plant material was dried in hot air oven below 50°C. Each extract was concentrated by distilling off the solvents and then evaporating to dry at room temperature. The extract of each solvent was weighed and it's percentage was calculated in terms of air dried weight of plant materials (Harborne, 1983).

3.4.1 PRELIMINARY IDENTIFICATION

The plant extract may contain along with desire compounds, some other compounds viz chlorophyll, or other kinds of pigments, inorganic and organic acids, resins, fatty substances etc. depending upon the types of impurities present the method of purification varies, and large separation constitutes of partitioning between two immiscible solvents in which the compound dissolves preferentially or precipitation of either the desired product or the impurities by certain reagents are quickly widely used. However the most common method of purification is

chromatography. Here purification of desired compounds was done by the precipitation of impurities by Hydrocloric acid (Harborne, 1983)

3.4.1.1. QUANTITATIVE ANALYSIS

Determination of Tannins

The quantitative analysis of tannins was conducted using Swain's method. Firstly, finely powdered sample were placed in a beaker containing 20 mL of 50% methanol, covered with parafilm, and heated at 80°C in a water bath for 1 hour with continuous stirring. The resulting extract was then filtered quantitatively using a double-layered Whatman Number 1 filter paper and rinsed with 50% methanol. Next, 1 mL of the sample extract was mixed with 20 mL of distilled water, 2.5 mL of Folin-Denis reagent, and 10 mL of 17% Na2CO3 to develop a bluish-green color, and the mixture was allowed to stand for 20 minutes.as shown in Figure-7. The absorbance was measured at 760 nm, and the tannin content was determined by comparing it with a standard curve prepared in the range of 0–10 ppm (Swain, 1995).

Determination of Flavonoids

The flavonoids content was also determined by Harborne method (Harborne, 1983). Briefly, 5 gm of leaves was boiled in 2 M HCl for 30 mins under reflux and filtered after cooling. Equal volume of ethyl acetate was then added drop wise in filtrate, as shown in the figure-8 The weight of precipitated flavonoid was determined and reported as mg/g.

Determination of protein

BSA is used as standard reagent for preparing the standard curve against which the unknown concentration of proteins was estimated. 4.5 ml of reagent 1 (48 ml of 2% sodium carbonate in 0.1N sodium hydroxide + 1ml of 1% sodium potassium tartrate 1ml of 0.5% copper sulphate) was added to the sample extracts and incubated for 15 min. After this, 0.5 ml of freshly prepared reagent 2 (1 part Folin- Ciocalteau: 1 part water) was mixed with the each sample and left for 30 min of dark incubation. After that the absorbance was measured at 660 nm and the amount of protein is expressed as mg BSAE/ g of fresh weight.

Determination of carbohydrates

The samples were analysed using FTIR spectroscopy (Perkin Elmer Spectrum Version 10.03.09, at Bharathidasan University, Trichy, Tamil Nadu, India). To establish the presence of unique functional groups, we identifed the biomolecular functional groups widespread in 10 prominent rice varieties, a resolution of 4 cm−1 in the frequency range of 400 to 4000 cm−1.

PRETREATMENT OF SELECTED SEEDS

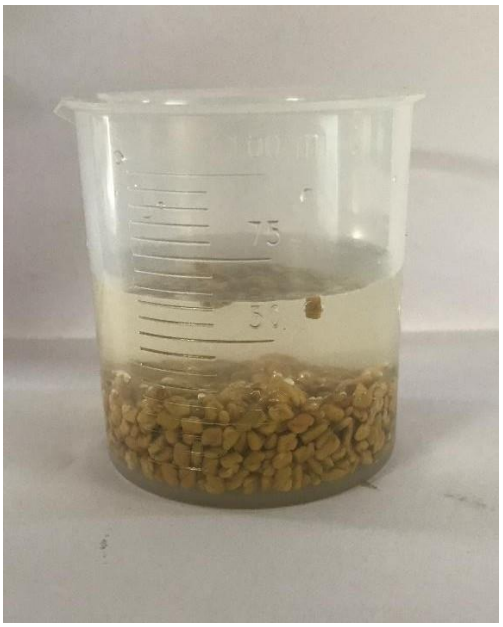
FIGURE-1

RADISH SEEDS SOAKED IN BUFFER (NaCl-0.1N)



FIGURE-2

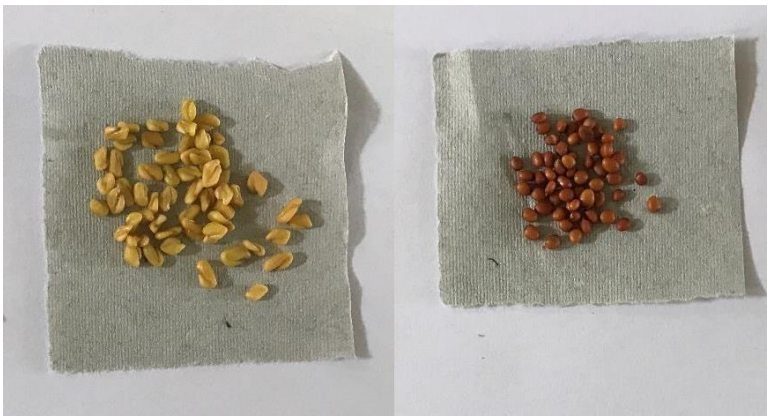
FENUGREEK SEEDS SOAKED IN BUFFER, (NaCl-0.1N)



PREPARATION OF SEEDS

FIGURE-3

SEEDS DRIED ON A FILTER-PAPER



PREPARATION OF PLATES TO BE PLACED IN THE UV CHAMBER

FIGURE -4

PETRIPLATES WITH SEEDS.

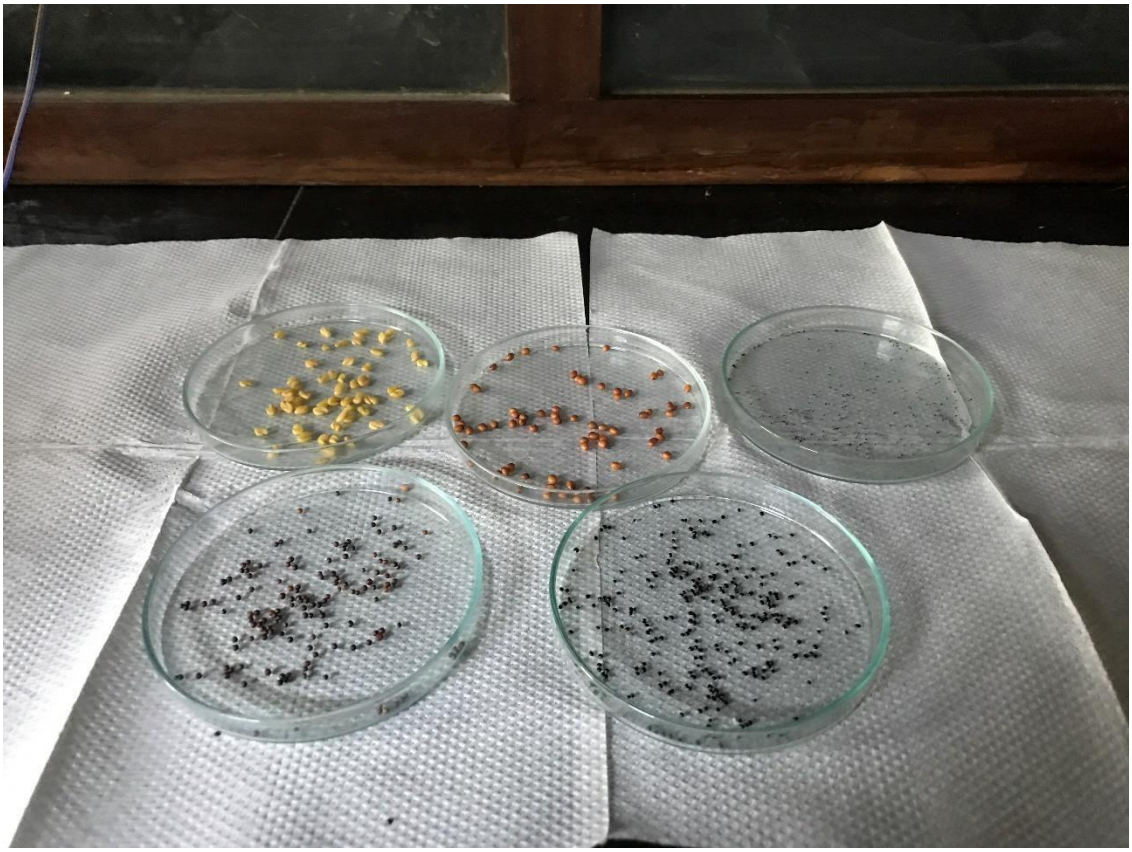


FIGURE-5

INNOCULATION OF PREPARED SEEDS IN THE UV CHAMBER

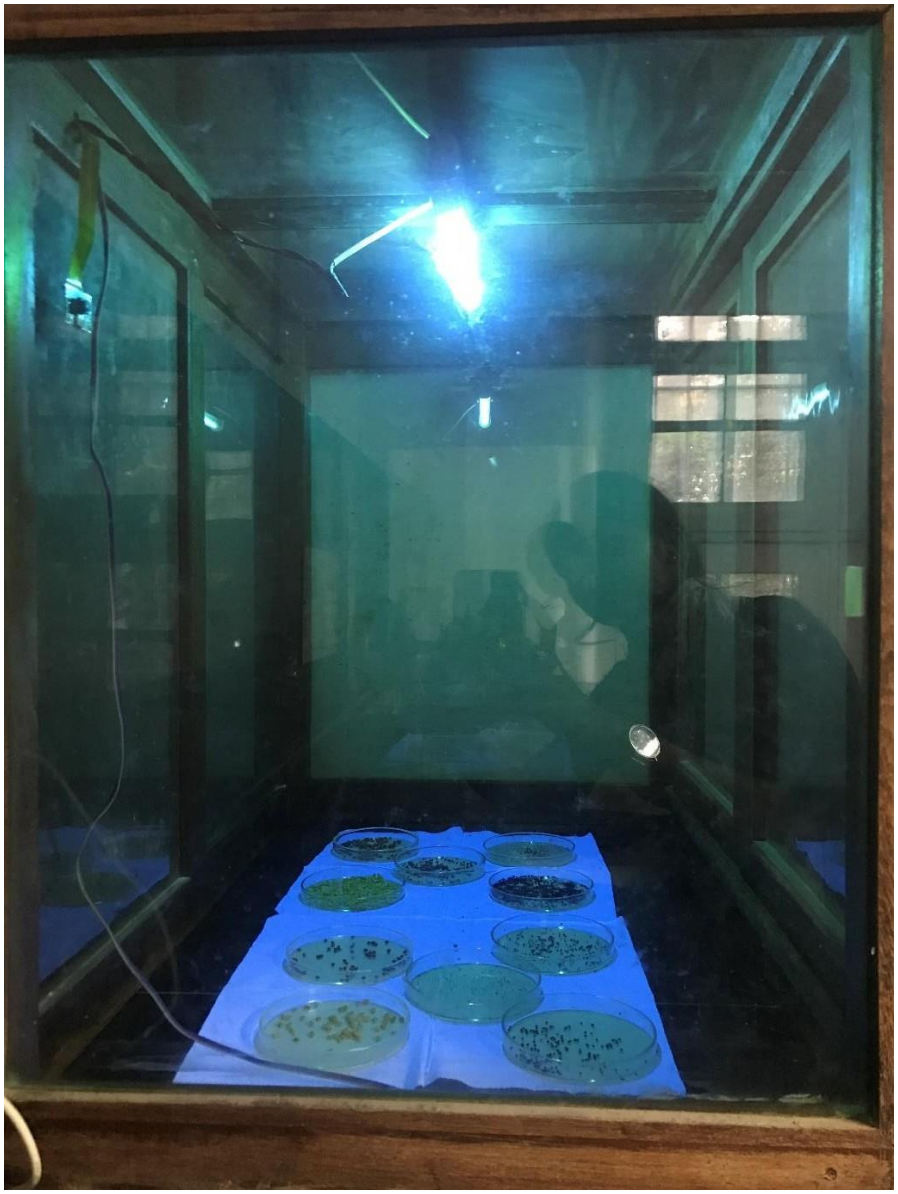


Figure-6

GROWTH SETUP OF THE TREATED SEEDS



QUANTITATIVE PHYTOCHEMICAL ANALYSIS

FIGURE- 7
DETERMINATION OF TANNIN IN FENUGREEK

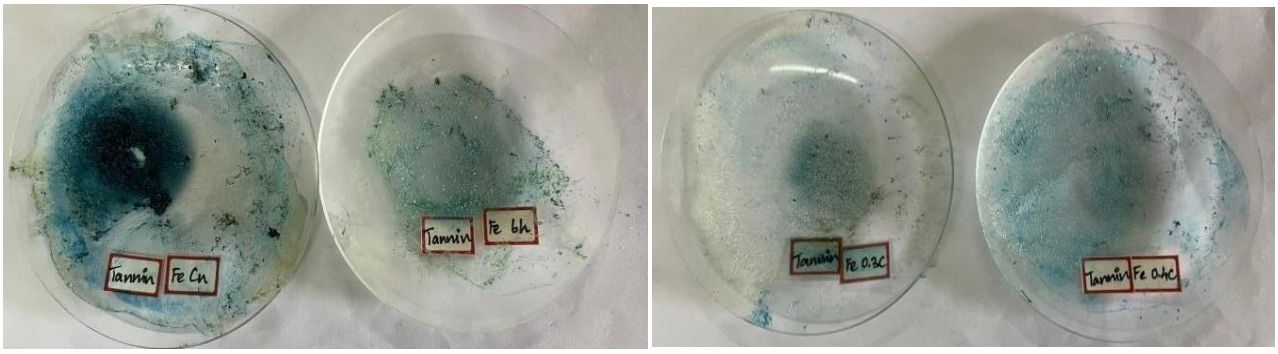


FIGURE-8

DETERMINATION OF FLAVONOID IN FEUGREEK:

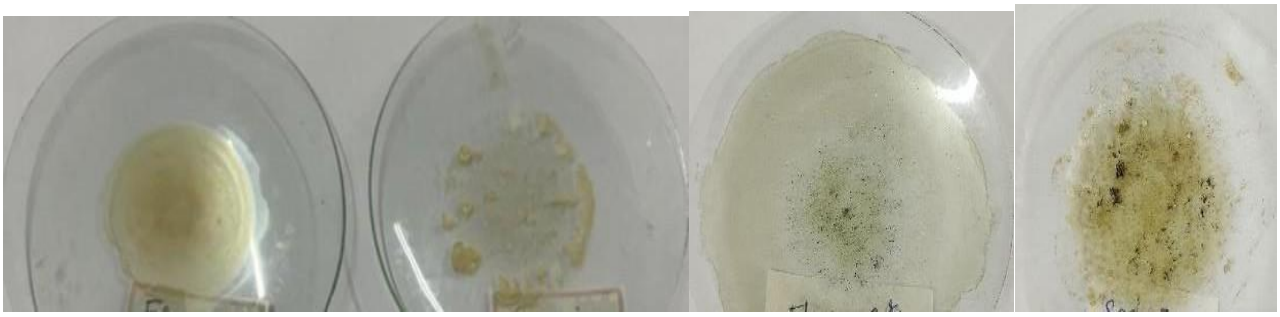


FIGURE-9
DETERMINATION OF PROTEIN IN RADISH:



4. RESULTS & DISCUSSION

4.1. EFFECT OF UV-B RADIATION ON THE GERMINATION OF *Trigonella foenum-graecum* L. and *Raphanus sativus* L.

Two sets of physically treated seeds of *Trigonella foenum-graecum* L. and *Raphanus sativus* L. were treated under UV-B rays for different time frames. In the first set, 60 seeds of both species were treated for 3 hours and in the second set, another 60 seeds were treated for 6 hours. The germination rates of these seeds were noted and compared with a control group of 60 untreated seeds of each species. The effects of UV-B radiation on the germination of Trigonella foenum-*graecum* L. *and Raphanus sativus* L. were analyzed and the results are presented in a table (5&6).

Fenugreek:

Out of the total of 60 seeds sown, 55 seeds showed germination without any physical treatment, resulting in a growth rate of 92%. However, when 3 hours of UV-B rays were applied, only 28 seeds showed germination, resulting in a growth rate of 47%. On the other hand, when the seeds were treated with UV-B radiation for 6 hours, 37 seeds showed germination, resulting in a growth rate of 62%.

There was a gradual decline in germination, survival, and pollen fertility percentage of fenugreek plants induced from caffine treatment. Therefore, the germination was highly affected at higher doses. There was a progressive decline in the percentage of survival of plants at maturity with 88% (control) decreasing from 85.35% (0.2%) to 71.26% (1.0%) in treatments (Naaz *et al*., 2022). The seeds of soybeans and wheat presented minimal sensitivity to UV-B radiation, which increases as the dose of radiation applied increases. UV-B inhibited the growth and nutrient metabolism of soybeans during germination (Rahim *et al.,* 2019).

Raddish

Out of the total 60 seeds that were planted, 55 seeds germinated without any physical treatment resulting in a growth rate of 92%. However, when 3 hours of UV-B rays were applied, only 47 seeds showed germination, resulting in a growth rate of 78%. On the other hand, when the seeds were treated with UV-B radiation for 6 hours, only 30 seeds showed germination, resulting in a growth rate of 50%.

Sugimoto (2013) reported that UV light suppresses the gibberellin in lettuce seeds which hindered the germination. These results were in agreement with the previous available literature (*Kakani et al.*, 2003; Shetta, 2008; Liu *et al.*, 2009; Shetta and Areaf, 2009). Seeds radiated with UV light at germination of seed and appearance of seedling stage have more affect than other stage (Saile-Mark and Tenivi, 1997; Li *et al.*, 2009). Hence in my work the decline in the germination rate meets with the vegetable crop species.

EFFECT OF UV-B RADIATION ON THE GERMINATION OF

Trigonella foenum graecum l..

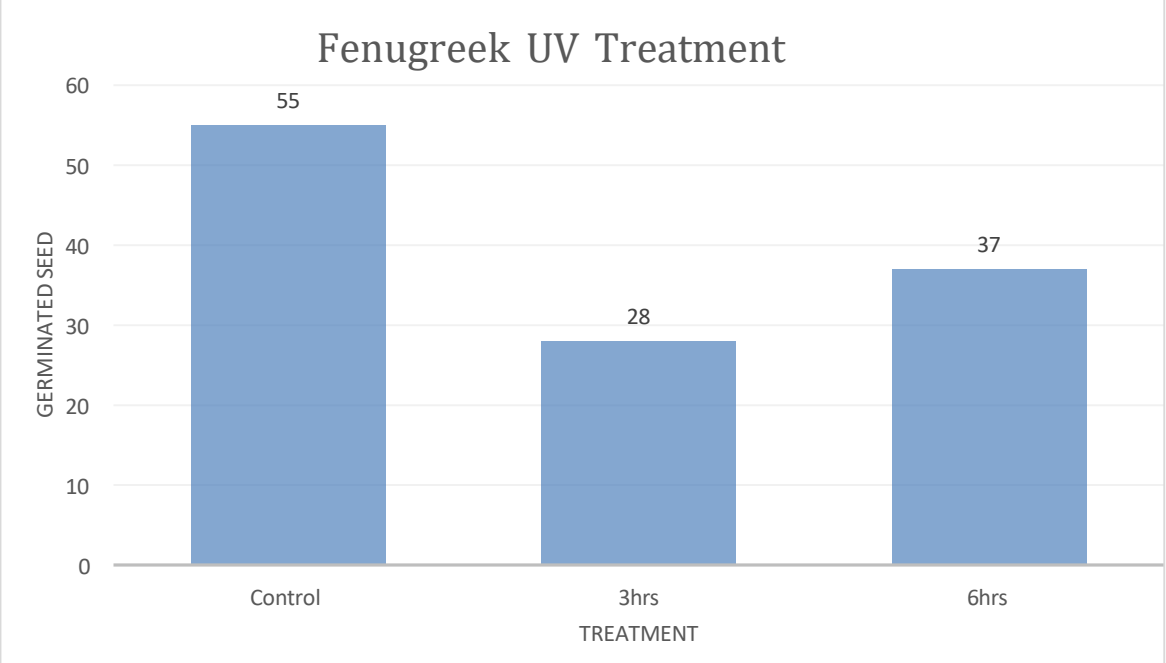
TABLE-5

FENUGREEK		
Treatment	Number	Percentage
Control	55	92%
3 hrs	28	47%
6 hrs	37	62%

EFFECT OF UV-B RADIATION ON THE GERMINATION OF

Trigonella foenum graecum l.

FIGURE-10



EFFECT OF UV-B RADIATION ON THE GERMINATION OF

***TRIGONELLA FOENUM-GRAECUM* L.**

FIGURE-11

GERMINATION RATE OF FENUGREEK IN CONTROL



FIGURE-12

GERMINATION RATE OF FENUGREEK SEEDS IN 3HRS OF UV RAYS



FIGURE –13

GERMINATION RATE OF FENUGREEK IN 6HRS OF UV RAYS



EFFECT OF UV-B RADIATION ON THE GERMINATION OF

Raphanus sativus L

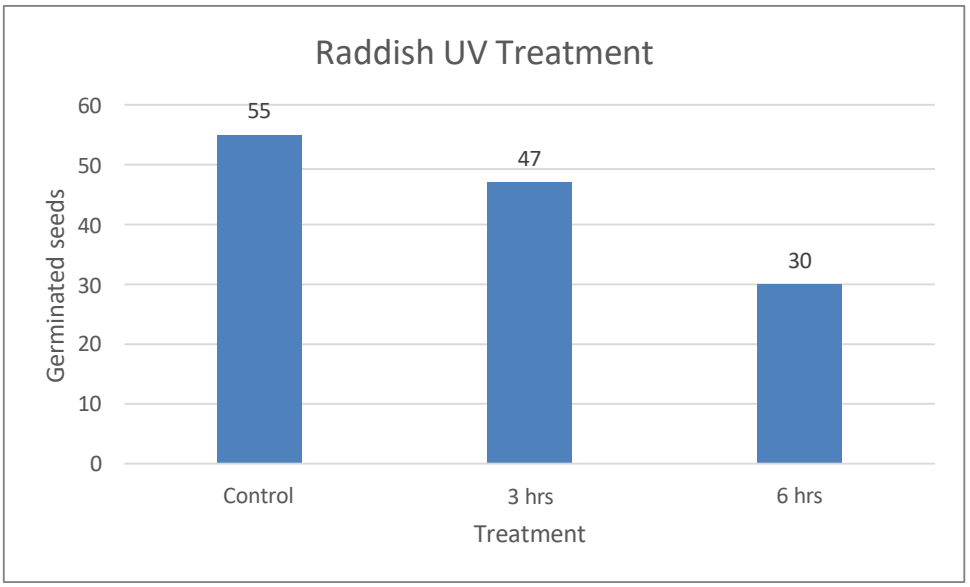
TABLE-6

RADDISH		
Treatment	Number	Percentage
Control	55	92%
3 hrs	47	78%
6 hrs	30	50%

EFFECT OF UV-B RADIATION ON THE GERMINATION OF

Raphanus. sativus L

FIGURE-14



EFFECT OF UV-B RADIATION ON THE GERMINATION OF *RAPHANUS RAPHANUS SATIVUS* L.

FIGURE-15

GERMINATION RATE OF RADISH IN CONTROL



FIGURE-16

GERMINATION RATE OF RADISH IN 3HRS OF UV RAYS



FIGURE-17

GERMINATION RATE OF RADISH IN 6HOURS OF UV RAYS



4.2 PHYTOCHEMICAL ANALYSIS.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Phytochemicals are non nutritive plant chemicals with protective or disease prevention properties. Such compounds are found in plants to protect themselves, but current research suggests that they can also defend humans and animals from many diseases and infections (Ngbede *et al* 2008; Kubmarawa *et al*, 2008). The presence and change in the phytochemical constituents after treatment of physical mutagen UV-B rays shows variation in the amount of phytochemicals.

FENUGREEK.

The fenugreek seed has been found to contain a number of chemical compounds, They contain various types of alkaloids, flavonoids, and tannins, with the tannin showing the highest concentration with 4.63 g per 10. Fenugreek seeds also contain more than 10 mg of flavonoid per gram of seed, (Visuvanathan *et al* 2022). Hence further treatment of UV-B rays were induced to know the change in the highest produces secondary metabolites. The results show a fine defense the phytochemical amout present in the treated seeds as they were compared with the control as in table-7&8

With 1 gram of sprout sample the total weight of flavonoid present were 0.0958g while the fenugreek seed treated with UV-B radiation for 3 hours had 0.4987g of flavonoid which shows an increased percentage. On the other hand the 6 hours UV-B treated seeds show 0.4013g of flavonoids which is a increase from the control but shows a slight variation of decreased value from the 3 hours treated seed's flavonoid content as in Figure-12. For Tannin The untreated seeds shows 0.2g in them while the 3hours treated seeds show 0.24g of Tannin content in them. On the other hand, the 6 hours UV-B treated seeds show 0.1707g of tannin which is a decline of amount from the control as shown in Figure-13

The results of means show that plant phytochemical increased along with increasing supplemental UV-B dose. Compared to the reference plants, supplementary UV-B light significantly increased the growth of assimilating leaf area of *Ocimum basilicum* L. (Jurga *et al.* 2012). Hence has found to meet the same interpretation in the plant choosen for this project.

QUANTITATIVE ANALYSIS OF SELECTIVE PHYTOCHEMICALS

IN *Trigonella foenum-graecum L.*

TABLE-7

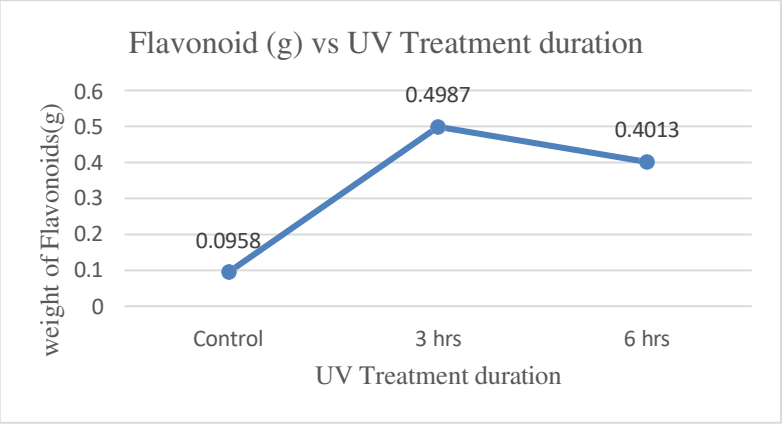
FLAVONOID

FENUGREEK		
Sample name	Sample weight in grams	Weight of Flavonoids in grams
Control	1	0.0958
3 hrs	1	0.4987
6 hrs	1	0.4013

QUANTITATIVE ANALYSIS OF FLAVANOID IN

Trigonella foenum-graecum L.

FIGURE-18



QUANTITATIVE ANALYSIS OF SELECTIVE PHYTOCHEMICALS

IN *Trigonella foenum-graecum L.*

TANNIN

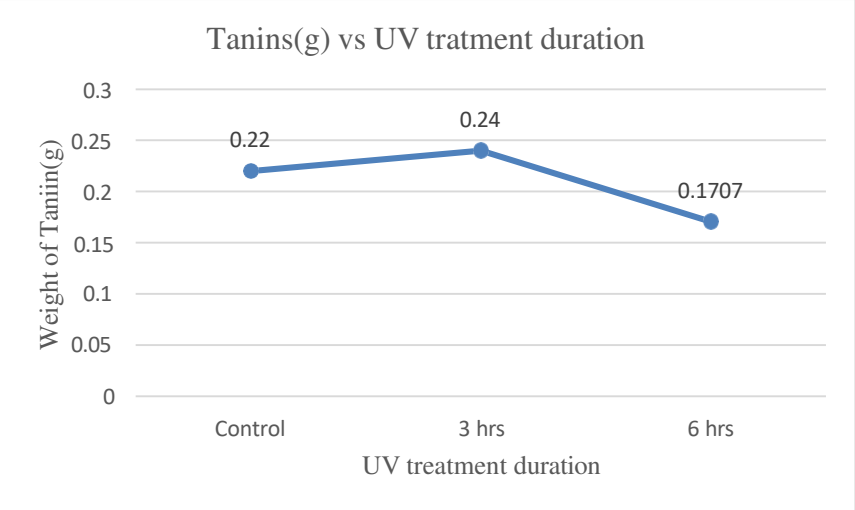
TABLE-8

FENUGREEK		
Sample name	Sample weight in grams	Weight of Tannin in grams
Control	1	0.22
3 hrs	1	0.24
6 hrs	1	0.1707

QUANTITATIVE ANALYSIS OF TANNIN IN

Trigonella foenum-graecum L.

FIGURE-19



RADISH

Papers reported quantitative information on the proximate composition of radish (Mahmood, & Hameed, 2013; Ankita & Prasad, 2015; Goyeneche *et al*., 2015; Ashraf, Sultana, Iqbal, & Mushtaq, 2016; Ashraf *et al.,* 2018; Azam, Khan, Chihoub *et al*., 2019;). The total content of carbohydrates, fibers, lipids, proteins, and ash was reported. Amongst them carbohydrate and protein content marks the larger composition. The threated seeds were compared with the control and shows fine differences and recorded in the table (9&10). Untreated seeds of radish sprouts contained 0.1217 g of carbohydrate while the 3hours treated seed's sprouts contained 0.4987g of carbohydrate which shows an increased amount of this nutrient. On the other hand the 6 hours treated seed sprouts contained 0.4013g of carbohydrate, it has a increased variant from the control but decreased by 0.09 value whish interprets they have reached a saturation level and decreased at a point. The protein content in the untreated seeds of radish contains 0.1794g of protein in their sprouts. Whine in the 3 hours treated seeds the amout of proteins were 0.67g which showed a greater variant. On the other hand the 6 hours treated seeds show 0.543 g.

. Various studies have compared the phytochemical content of radish with other Brassicaceae vegetables. Bhandari *et al.* (2015) compared total glucosinolate content (TGC) in radish with broccoli, cabbage, and cauliflower, consistently finding that radish had the lowest values. Sun and Chen (2019) found that black radish ranked third in TGC values when compared to arugula, broccoli, cauliflower, and turnip. Lastly, Shehata *et al.* (2014) analyzed total flavonoid content (TFC) in radish leaves compared to cabbage and cauliflower, noting that radish ranked second after cauliflower.

The diversity of varieties within these vegetables makes it difficult to pinpoint exact contributions of phytochemicals. Nevertheless, radish has emerged as a significant source of various nutrients and bioactive compounds within the Brassicaceae family. In a separate study, the phenolic content in spinach was found to vary based on species and UV-B radiation exposure. "Matador" spinach exposed to 4 kJ UV-B radiation showed a higher amount of phenolic compounds, but exposure to 6 kJ UV-B radiation led to a significant decrease in phenolic content (Algirdas *et al*., 2018). This finding aligns with observations in radish plants.

QUANTITATIVE ANALYSIS OF SELECTIVE NUTRIENTS

IN *Raphanus. sativus L.*

CARBOHYDRATES

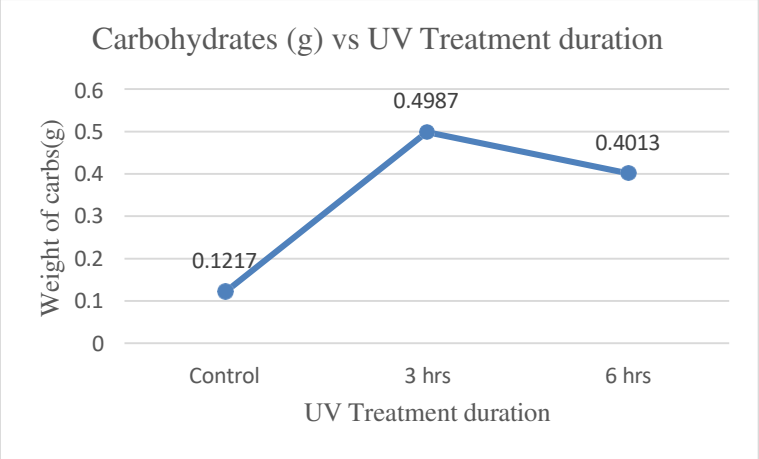
TABLE-9

Raddish		
Sample name	Sample weight	Weight of Carbohydrates
Control	1	0.1217
3 hrs	1	0.4987
6 hrs	1	0.4013

QUANTITATIVE ANALYSIS OF CARBOHYDRATES

IN *Raphanus. sativus L.*

FIGURE-20



QUANTITATIVE ANALYSIS OF SELECTIVE NUTRIENTS

IN *Raphanus. sativus L.*

PROTEIN

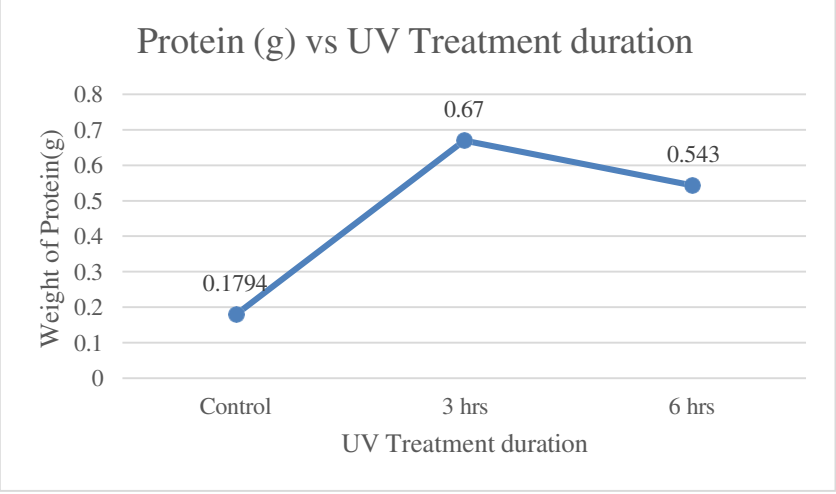
TABLE-10

Raddish		
Sample name	Sample weight	Weight of Protein
Control	1	0.1794
3 hrs	1	0.67
6 hrs	1	0.543

QUANTITATIVE ANALYSIS OF PROTEIN

IN *Raphanus sativus L.*

FIGURE-21



4.3 MORPHOLOGICAL CHANGES IN THE PHYSICALLY TREATED PLANTS

The treated seeds with UV-B radiation shows a vast difference as they continued to grow. The interpretation is clearly shown in the table (11 & 12)The untreated plants in the control group displayed an average height of 7cm, while the batch of seeds exposed to 3 hours of UV-B radiation during germination showed a slightly reduced average height of 6.8cm. Conversely, the batch treated with 6 hours of UV-B radiation exhibited a more significant reduction in growth, with an average height of 6.2cm.

The untreated plants in the control group displayed an average height of 8.5cm, while the batch of seeds exposed to 3 hours of UV-B radiation during germination showed a slightly reduced average height of 8cm. Conversely, the batch treated with 6 hours of UV-B radiation exhibited a more significant reduction in growth, with an average height of 7.8cm.

The UV-B-induced phenotype is diverse, with many apparently contradictory reports of UV-B effects on plant architecture. The diversity of effects at the organismal level is echoed by similar diversity at the cellular level, with reports of increased or decreased cell numbers, increased or decreased cell size, and changes or no changes in cellular differentiation(Mathew *et al*. 2014). A highly significant UV-B-induced reduction in plant height was observed, with plants exposed to high levels of UV-B being 47 % shorter than control plants. Exposure to ambient UV-B radiation (8 kJ m^{-2} d^{-1}) reduced plant height compared with that of controls (Kakani *et al.* 2003).Hence this was met with the current study.

MORPHOLOGICAL CHANGES IN THE PHYSICALLY TREATED PLANTS

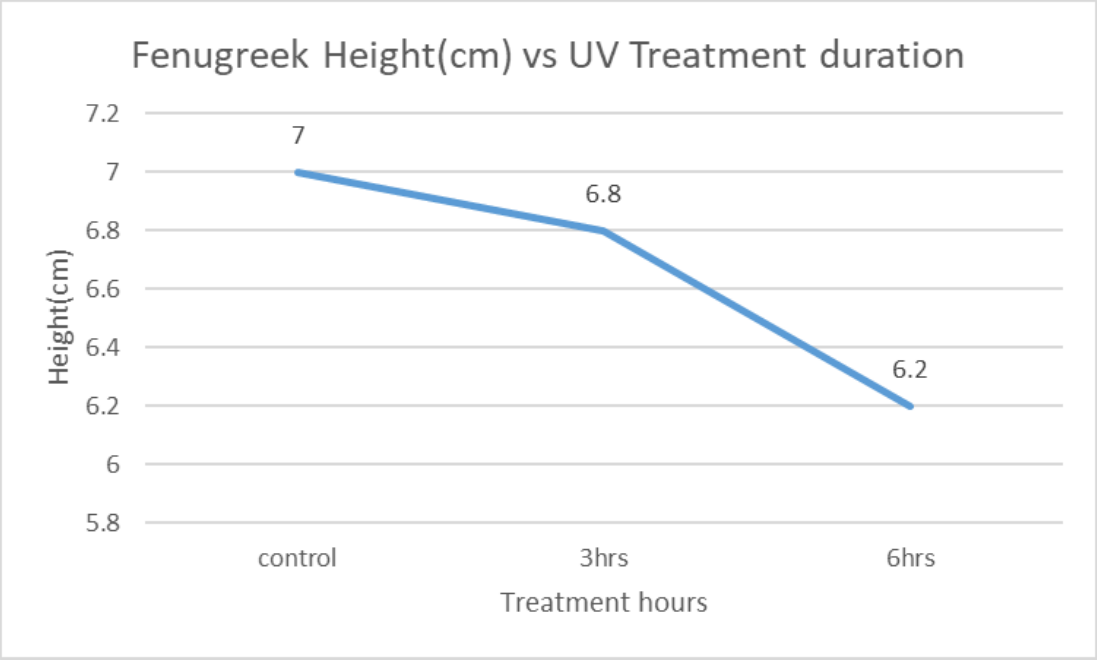
TABLE-11

HEIGHT OF THE SPROUTS MEASURED IN FENUGREEK IN (cm)

Sample name:	Average height of the sprout in cm
Control	**7**
3hrs	6.8
6hrs	6.2

FIGURE-22

HEIGHT OF THE SPROUTS MEASURED IN FENUGREEK IN (cm) OF DIFFERENT DURATION.



MORPHOLOGICAL CHANGES IN THE PHYSICALLY TREATED PLANTS

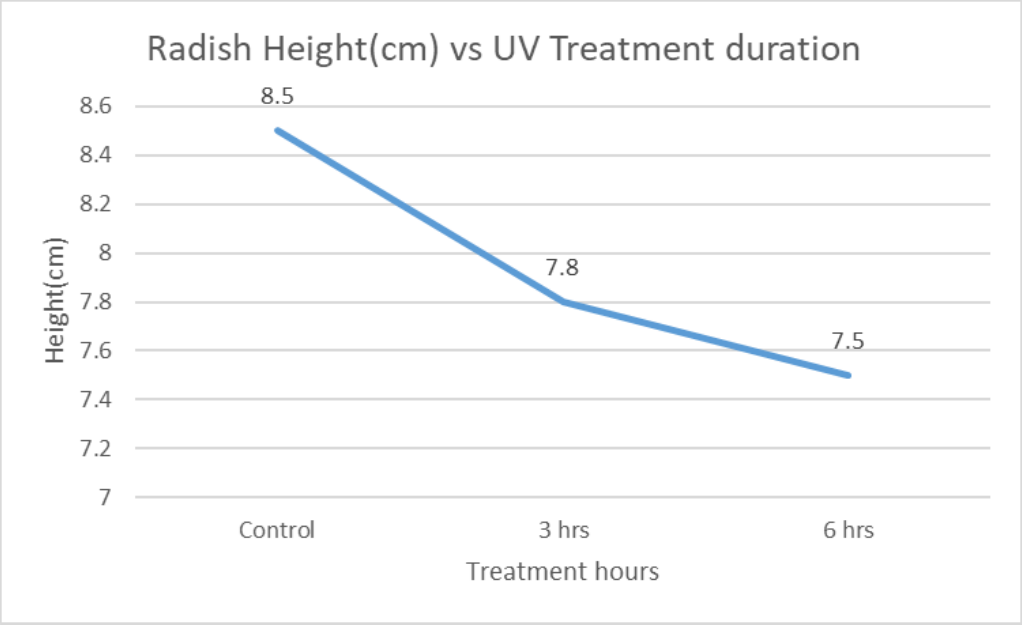
TABLE-12

HEIGHT OF THE SPROUTS MEASURED IN RADISH

Sample name	Average height of the sprout in cm
Control	8.5
3hrs	7.8
6hrs	7.5

FIGURE-23

HEIGHT OF THE SPROUTS MEASURED IN RADISH



5. SUMMARY AND CONCLUSION

The present study of "effect of UV B rays on the specific phytochemical synthesis in *Trigonella foenum-graecum* L. and *Raphanus sativus* L*."* is a positive response in comparison to the control. The germination rate of fenugreek and radish was shown to be a gradual decline and survival percentage. As the germinated seeds proceeds to grow they show a variation in their morphological appearance. The height of the plant declines when compared with the control. The control plants were 7cm tall, while those exposed to 3 hours of UV-B were 6.8cm tall. The 6-hour group was 6.2cm tall, indicating reduced growth. The control group was 8.5cm tall, with UV-B groups at 8cm (3-hour) and 7.8cm (6-hour), showing decreased heights with longer UV-B exposure.

The main purpose of this project was to check the phytochemical variation and it had met an positive response to the treatment. In a sprout sample, 1 gram contained 0.0958g of flavonoids. Fenugreek seeds treated with UV-B radiation for 3 hours showed an increased flavonoid content of 0.4987g, while those treated for 6 hours had 0.4013g. However, the 6-hour treatment resulted in a slightly lower flavonoid content compared to the 3-hour treatment. Untreated seeds had 0.2g of flavonoids, and 3-hour treated seeds had 0.24g of tannins, while 6-hour treated seeds had 0.1707g of tannins, showing a decline from the control. And in radish, Untreated radish sprout seeds had 0.1217g of carbohydrates, while 3-hour treated seeds had 0.4987g, indicating an increase. The 6-hour treated seeds had 0.4013g, slightly lower than the 3-hour treatment, suggesting a saturation point. Untreated seeds had 0.1794g of protein, whereas 3-hour treated seeds had 0.67g, and 6-hour treated seeds had 0.543g, showing a varied protein content.

CONCLUSION

Extensive research has delved into the multifaceted effects of UV radiation on plants, examining various facets such as alterations in photomorphogenesis, primary metabolism—especially photosynthesis—secondary metabolism, and germination rate. Model plants like *Trigonella foenum-graecum L.* and *Raphanus sativus L.* have been pivotal in these investigations, shedding light on numerous aspects, yet certain avenues remain unexplored. The advent of cutting-edge technologies like omics tools has revolutionized plant studies, enabling researchers to analyse plants within their natural ecosystems, encompassing their intricate complexities. This

advancement promises a deeper comprehension of how UV radiation impacts plants, a pivotal component of the evolving climate scenario.

Looking ahead, a holistic understanding of plant responses to UV radiation holds immense significance across agriculture and conservation realms, serving as a cornerstone for forthcoming international regulations.

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