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Comparative cytological study of *Trigonella foenum graecum* and *Brassica compestries'*

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ABSTRACT

Thiosemicarbazides have found to contain antimicrobial, antiviral activities against various virus, bacteria and fungi strains. The objective of the study was to study the seed germination and physiological maturity stage of Trigonella foenum graecum and Brassica (L.). The effect of various concentrations of 1- γ picolinoyl-4-phenyl Thiosemicarbazides on mitotic Indices in root tip cell in Trigonella graecum and Brassica compestries was studied. Brassica plants have been the subject of much scientific interest for their agricultural importance. Seeds used in exacerbations, cancer and tumours. Roots emollient and diuretic, juice used in chronic cough and bronchial catarrh. Trigonella foenum graecum balances blood sugar levels, and contains choline which aids the thinking process. Fenugreek has been the focus of several studies concerning the treatment of diabetes and the prevention of breast cancer. Its ability to balance hormone levels aids in treating PMS and menopause. Its antioxidants slow ageing and help prevent disease.

Keywords: chromosomal anomalies, mitotic index, Plant morphology, *Trigonella graecum* and *Brassica compestries*

INTRODUCTION

Plant physiology is a sub-discipline of botany conerned with the functioning, or phsiology of plants [1] closely related fields include plant morphology (structure of plant), plant ecology (interaction with the environment), phytochemisry (biochemistry of plant), cell biology, genetics, biophysics and molecular biology. Fundamental process such as photosynthesis, respiration, seed germination, dormacy and stomata function and transpiration, both parts of plant water relations are studied by plant physiologists. The field of plant physiology includes the study of all the internal activities of plants those chemical and physical processes associated with life as they occur in plants. This includes study at many levels of scale of size and time. At the smallest scale are molecular interactions of photosynthesis and internal diffusion of water, minerals and nutrients. At the largest plant physiology includes phytochemistry (the study of the biochemistry of plants) and phytopathology (the study of disease in plants). Germination is the process by which a plant grows from a seed. The most common example of germination is the sprouting of a seedling from a seed of an angiosperm or gymnosperm. However, the growth of a sporeling from a spore, for example the growth of hyphae from fungal spores, is also germination. In a more general sense, germination can be simply anything expanding into greater being from a small existence or germ, a method that is commonly used by many seed germination. Germination is the growth of an embryonic plant contained within a seed; it results in the formation of the seedling. The seed of a vascular plant is a small package produced in a fruit or cone after the union of male and female sex cells. All fully developed seeds contain an embryo and, in most plant species some store of food reserves, wrapped in a seed coat. Some plants produce varying numbers of seeds that lack embryos these are called empty seeds [3] and never germinate. Most seeds go through a period of dormancy where there is no active growth; during this time the seed can be safely transported to a new location or survive adverse climate conditions until circumstances are favorable for growth. Dormant seeds are ripe seeds that do not germinate because they are subject to external environmental conditions that prevent the initiation of metabolic processes and cell growth. Under proper conditions, the seed begins to germinate and the embryonic tissues resume growth, developing towards a seedling. Seed germination depends on both internal and external conditions.

The most important external factor include temperature, water, oxygen and sometimes light or darkness [4]. Various plants require different variables for successful seed germination. Often this depends on the individual seed variety and is closely linked to the ecological conditions of a plant's natural habitat. For some seeds, their future germination response is affected by environmental conditions during seed formation; most often these responses are types of seed dormancy. Water is required for germination. Mature seeds are often extremely dry and need to take in significant amounts of water, relative to the dry weight of the seed, before cellular metabolism and growth can resume. Most seeds need enough water to moisten the seeds but not enough to soak them. The uptake of water by seeds is called imbibition which leads to the swelling and the breaking of the seed coat. When seeds are formed, most plants store a food reserve with the seed, such as starch, proteins, or oils. This food reserve provides nourishment to the growing embryo. When the seed imbibes water, hydrolytic enzymes are activated which break down these stored food resources into metabolically useful chemicals [5]. After the seedling emerges from the seed coat and starts growing roots and leaves, the seedling's food reserves are typically exhausted; at this point photosynthesis provides the energy needed for continued growth and the seedling now requires a continuous supply of water, nutrients, and light. Oxygen is required by the germinating seed for metabolism [6]. Oxygen is used in aerobic respiration, the main source of the seedling's energy until it grows leaves [7] .Oxygen is an atmospheric gas that is found in soil pore spaces; if a seed is buried too deeply within the soil or the soil is waterlogged, the seed can be oxygen starved. Some seeds have impermeable seed coats that prevent oxygen from entering the seed, causing a type of physical dormancy which is broken when the seed coat is worn away enough to allow gas exchange and water uptake from the environment. Temperature affects cellular metabolic and growth rates. Seeds from different species and even seeds from the same plant germinate over a wide range of temperatures. Seeds often have a temperature range within which they will germinate, and they will not do so above or below this range. Many seeds germinate at temperatures slightly above 60-75 F (16-24˚C) [room-temperature if you live in a centrally heated house], while others germinate just above freezing and others germinate only in response to alternations in temperature between warm and cool. Some seeds germinate when the soil is cool 28-40 F (2 - 4˚C), and some when the soil is warm 76-90 F (24-32˚C). Some seeds require exposure to cold temperatures (vernalization) to break dormancy. Seeds in a dormant state will not germinate even if conditions are favorable. Seeds that are dependent on temperature to end dormancy have a type of physiological dormancy. For example, seeds requiring the cold of winter are inhibited from germinating until they take in water in the fall and experience cooler temperatures. Four degrees Celsius is cool enough to end dormancy for most cool dormant seeds, but some groups, especially within the family Ranunculaceae and others, need conditions cooler than -5 C. Some seeds will only germinate after hot temperatures during a forest fire which cracks their seed coats; this is a type of physical dormancy. Most common annual vegetables have optimal germination temperatures between 75-90 F (24-32˚C), though many species (e.g. radishes or spinach) can germinate at significantly lower temperatures, as low as 40 F (4˚C), thus allowing them to be grown from seed in cooler climates. Suboptimal temperatures lead to lower success rates and longer germination periods.

Thiosemicarbazone derivatives have found application in drug development for the treatment of central nervous system disorders, of bacterial infection, as well as analgesic and antiallergic agent. Thiosemicarbazones are potent intermediates for the synthesis of pharmaceutical and bioactive materials and thus, they are used extensively in the field of medicinal chemistry. Moreover, thiosemicarbazones have found their way into almost every branch of chemistry; commercially they are used as dyes, photographic films, plastic and in textile industry. Over the years, thiosemicarbazone derivatives have demonstrated wide range of biological activity *viz.* antimicrobial [8-9], antitumor[10-11], sodium channel blocker [12], antibacterial [13], anti-malarial [14]. Thiosemicarbazides posses useful pharmacological and corrosion inhibition properties. They have been frequently employed for the quantitative determination of inorganic ions [15-16]. Determination of adulteration and characterization are based on the analysis of major compounds of the oils [17] . Thiosemicarbazides have occupied an important place in drug industry. Use of these compounds in organic synthesis has become a classical strategy for the synthesis of several heterocycles. Their reactions with compounds containing C=O and C=N groups is an important method for the synthesis of biologically active compounds, viz triazoles and thiazoles. A better understanding of their biological activity can be derived from their oxidation mechanisms. It is widely accepted that the prerequisite for thio compounds to express their physiological effects is through S-oxygenation. The chemistry of hydrazine derivatives, such as thiosemicarbazide and its hydrazones is of immense interest owing to their wide synthetic and analytical applications and biological activities [18]. Thiosemicarbazides and their derivatives display interesting biological activities, including anticancer, anti-HIV, antibacterial, antiviral and antifungal. owing to their ability to diffuse through the semi-permeable membrane of celllines [19-25]. Due to their abundance in plants and ease of synthesis, this class of compounds has generated great interest for possible therapeutic uses. These sulfur and nitrogen donor ligands and their coordination complexes have gained special attention due to their activity against protozoa, influenza, small pox virus, fungi and cancer. Some industrially important activities, such as anticorrosion and antifouling effects have also been observed for these compounds. Thiosemicarbazide is a useful structural moiety that has the potential to display chemical functionality in biologically active molecules and optimization of this structure can result in ground breaking discovery of new class of therapeutic agents. Thiosemicarbazide (NH2-NH-CSNH2) is the simplest hydrazine derivative of thiocarbamic acid. The chemical behavior of thiosemicarbazide is similar to its analogue semicarbazide, however is of greater chemical versatility of thione group as compared with that of keto group and is responsible for more varied behavior of thiosemicarbazide. Among the increasing number of heterocyclic sulfur and nitrogen containing compounds, being pursued in both industry and academia, thiosemicarbazide derivatives are also interesting targets for drug design. During the past few decades, interest has been rapidly growing in gaining insight into the properties and transformations of thiosemicarbazides and their derivatives due to their appreciable pharmacological activities . Significant biological activities exhibited by thiosemicarbazides and their derivatives are discussed below. Derivatives of thiosemicarbazides and thiosemicarbazones have been found to have excellent antibacterial activities.

Brassica is a genus of plants in the mustard family (Brassicaceae). The members of the genus are informally known ascruciferous vegetables, cabbages, or mustard plants. Crops from this genus are sometimes called *cole crops*—derived from the Latin*caulis*, denoting the stem or stalk of a plant. The genus is known for its important agricultural and horticultural crops and includes a number of weeds, both of wild taxa and escapees from cultivation. *Brassica* plants have been the subject of much scientific interest for their agricultural importance. Six particular species (*B. carinata*, *B. juncea*, *B. oleracea*, *B. napus*, *B. nigra* and *B. rapa*) evolved by the combining of chromosomes from three earlier species. In the division of a large number of plants in the plant kingdom into the groups "monocotyledon" and "dicotyledon" all members of *Brassica*are dicotyledons. Seeds used in exacerbations, cancer and tumours. Roots emollient and diuretic, juice used in chronic cough and bronchial catarrh.

Trigonella foenum graecum balances blood sugar levels, and contains choline which aids the thinking process. Fenugreek has been the focus of several studies concerning the treatment of diabetes and the prevention of breast cancer. Its ability to balance hormone levels aids in treating PMS and menopause. Its antioxidants slow ageing and help prevent disease. The plant has also been employed against bronchitis, fevers, sore throats, wounds swollen glands, skin irritations, diabetes, ulcers, and in the treatment of cancer. Some evidence suggests that fenugreek may also have other medical uses. It may reduce the amounts of calcium oxalate in the kidneys. Calcium oxalate often contributes to kidney stones. In animal studies, fenugreek also appeared to lessen the chance of developing colon cancer by blocking the action of certain enzymes.

EXPERIMENTAL SECTION

The solutes used in the present investigation were synthesized by standard methods [26]. Taking each of 50 healthy seed of Trigonella foenum graecum and brassica compestries in petridishes. These selected seed were chemically treated with various concentration like 0.01 mol kg^{-1}, 0.006 mol kg^{--1}, 0.008 mol kg^{-1} and Control. The seeds were soaked in chemical for 24 hrs. After that seed were washed with bavistine and Dried on filter paper. 25 seeds of both variety of were soaked on filter Paper and kept under observation for 48 hrs. for seed germination. Remaining 25 seed of both plant were sowed in soil pot and kept under observation for 48 hrs. for seed germination in soil and various stages of plant growth. Seeds of two varieties of *Trigonella foenum -graecum* and *Brassica compestries* procured. Chemical treatment of different concentration were grow. For mitosis study root tips were collected in carnoy's fluid (glacial acetic acid 25% and absolute alcohol 75%) After 24 hours of fixation root tips were transferred to 70% alcohol separately and stored in a refrigerator. Acetocarmine squashes of root Tips were prepared after hydrolyzing with 1N HCl, at 40˚C for chromosomal studies. Mitotic index was calculated using the following formula:

$$\text{Mitotic Index} = \frac{\text{Total number of dividing cell}}{\text{Total number of cells studied (Dividing + undividing)}} \times 100$$

RESULTS AND DISCUSSION

Table 1: Effect of different concentration of 1- γ picolinoyl-4-phenyl Thiosemicarbazides on seed germination

Sr No.	Concentration (moles kg^{-1})	Plants under investigation	No of seeds taken	No of seed germinated	% germination
1.	0.01	Trigonella	25	14	56
		Brassica	25	15	60
2.	0.006	Trigonella	25	16	64
		Brassica	25	18	72
3.	0.008	Trigonella	25	23	92 *
		Brassica	25	24	96 *
4.	Control	Trigonella	25	22	88
		Brassica	25	20	80

Mitotic Index

Table 2: The effect of various concentrations of 1- γ picolinoyl-4-phenyl Thiosemicarbazides on mitotic Indices in root tip cell in *Trigonella graecum* and *Brassica compestries*

Chemical compound	Plant	Dose Mol kg^{-1}	Root Tip Cell: Number of cells observed	Number of dividing cells	Number of non-dividing cells	Mitotic Index
1- γ picolinoyl-4-phenyl thiosemicarbazides	Trigonella	Control	152	30	122	19.73
		0.01	142	19	123	13.38
		0.006	140	21	119	15
		0.008	172	36	136	26.47 *
	Brassica compestries	Control	156	32	124	20.51
		0.01	145	23	122	15.86
		0.006	147	29	118	19.72
		0.008	180	38	143	21.11*

Early attempts have been made by Bera *et al.*[27] to study the effect of tannery effluent on seed germination, seedling growth and chloroplast pigment content in mungbean. Adhikari *et al.*[28] have observed the effect of raw sewage water on wheat. Poonam Varshney et.al have observed the effect of cultural filtrates of certain fungal species on seed germination of *Trigonella* .[29]. The present study was conducted to evaluate the effect of 1- γ picolinoyl-4-phenyl thiosemicarbazides on the chlorophyll, percent germination, of *B. campestris* (L.) and *Trigonella* have been studied.

Chlorophyll:

Chlorophyll biosynthetic mutants have always been utilized in plant breeding due to the easily observed phenotype (white, yellow or pale green color due to lack/reduced amount of chlorophyll pigments). Basically, among the smallest group of coordinating pigment molecules necessary to affect a photochemical act, the most important pigments involved in photosynthesis are chlorophyll and carotenoid. There are five types of chlorophyll viz. a, b, c, d and e amongst which only a and b are present in higher plants. Chlorophyll a appears blue green in transmitted light but reddish in reflected light and is the principal pigment involved in trapping the light of wavelength 670 nm. Chlorophyll b is yellowish green in transmitted light but reddish in reflected light and traps the light of wavelength 645 nm.

Percent Germination

The effect of different concentrations of 1- γ picolinoyl-4-phenyl Thiosemicarbazides on germination percentage, is furnished in Table 1. The development of majestic oak tree from a small acorn requires a precise and highly ordered succession of events. Starting from a single fertilized egg, plant cells divide, grow and differentiate into increasingly complex tissues and organs. These events along with their underlying biochemistry and many factors that either impose or modulate on unfailing an orderly progression through the life cycle constitute development, i.e., seed germination. If any cycle can be said to have a beginning in plants, the beginning would be germination of seed. The seed is a convenient place to begin because seeds are quiescent or resting organs that represent a normal hiatus in life cycle. When the conditions are appropriate, the seed will renew its growth and germinate. Such an important phenomenon will be affected by different conditions[30]. In case of Trigonella germination percentage for soil is increased at 0.008 mol kg^{-1}upto 92 % and also brassica compestries germination percentage for soil is increased at 0.008 mol kg^{-1} upto 96%. Pre-treatment enhanced early germination of seeds in *Brassica and Trigonella*.

Plant morphology

It is the study of the physical form and external structure of plants[31]. This is usually considered distinct from plant anatomy, which is the study of the internal structure of plants, especially at the microscopic level[32]. Plant morphology is useful in the visual identification of plants.

Morphological variation

Plants exhibit natural variation in their form and structure. While all organisms vary from individual to individual, plants exhibit an additional type of variation. Within a single individual, parts are repeated which may differ in form and structure from other similar parts. This variation is most easily seen in the leaves of a plant, though other organs such as stems and flowers may show similar variation. There are three primary causes of this variation: positional effects, environmental effects, and juvenility.

Positional effects

Variation in leaves from the giant ragweed illustrating positional effects. The lobed leaves come from the base of the plant, while the unlobed leaves come from the top of the plant. Although plants produce numerous copies of the same organ during their lives, not all copies of a particular organ will be identical. There is variation among the parts of a mature plant resulting from the relative position where the organ is produced. For example, along a new branch the leaves may vary in a consistent pattern along the branch. The form of leaves produced near the base of the branch will differ from leaves produced at the tip of the plant, and this difference is consistent from branch to branch on a given plant and in a given species. This difference persists after the leaves at both ends of the branch have matured, and is not the result of some leaves being younger than others.

Mitotic Index

Cell population growth occurs as cells pass through interphase and mitosis to complete the cell cycle. Many cells lose the capacity to divide as they mature or divide only rarely. Other cells are capable of rapid cell division. For example, as plant roots grow, cells near the tip of the root, in the apical meristem, divide rapidly to push the root through the soil. The root cap detects the pull of gravity and directs the rapid growth of cells near the tip. In general, the mitotic index decreases with increasing distance from the root cap junction. Cells of the root cap protect the root and must be constantly replaced as they are damaged or scraped away. The apical meristem, just beneath the root cap, contains most of the root's dividing cells. Therefore, cells in this area must complete the cell cycle often. Some daughter cells become part of the root cap, others differentiate and elongate into primary tissues of the root. If we allow growing roots to take up a radioactive precursor of DNA, almost all of the labelled cells lie in the meristem. The mitotic index can also be used to quantify differences in cell division when an environmental parameter is changed. Plants grown in space in microgravity had a greater mitotic index than control plants grown on the ground. In zero-gravity, the gravity sensing cells in the root cap are unable to send the proper orientation signals. These signals normally inhibit growth in cells that are more distant from the root cap junction, and direct elongation of the primary root. In the absence of these signals, cells begin dividing to produce secondary roots, leading to a greater number of cells in mitosis. The nucleus is the major site of injury caused by chemical compound in a wide variety of plant. Thus cytological anomalies are considered as one of the dependable measure for estimating the effect of chemical on immediate generation. Results in present investigation revealed that higher doses of all three chemical concentration agent were significantly effective for causing the chromosomal anomalies.

1] In Trigonella foenum graceum highest mitotic index 0.008 mol kg^{-1} was 26.47 and the lowest mitotic index observed under 0.01 mol kg^{-1} was 13.38.
2] In case of Brassica compestries highest mitotic Index 0.008 mol kg^{-1} was 21.11, while lowest mitotic Index 0.01 mol kg^{-1} was 15.86 as the dose of three chemical concentration is increased mitotic Index was found to decrese.

CONCLUSION

From the present research work, it has been found that the1- γ picolinoyl-4-phenyl thiosemicarbazides acts as a good plant growth regulator for germination of seed and mitotic Index.

1] In case of Trigonella germination percentage for soil is increased at 0.008 mol kg^{-1} was 92 % and also brassica compestries germination percentage for soil is increased at 0.008 mol kg^{-1} was 96%.
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