



Centre for Environmental Studies

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General Information

Green Horizon is a peer reviewed e-newsletter published in English by the Centre for Environmental Studies, Yenepoya (Deemed to be University), Mangalore in two issues per year during June and December. This newsletter publishes manuscript of different categories like original articles, short communications, opinions, research communications, case study etc. We invite original contributions significantly advancing fundamental understanding and that focus on the interconnection of multiple environmental spheres of environment and nature (biodiversity, plants, animals, microbes, conservation, soil, air, water, climate, pollution, waste management, compost, environmental protection, environmental management and ecofriendly approaches). The authors, editors and reviewers need to adhere to the research and publication ethics to enhance the quality of the newsletter.

Aim and Scope

Green Horizon intends to project and share the knowledge on our environment and its protection for the benefit of society. It brings out quality and original materials exclusively on the environment and welfare of the biodiversity. Emphasis should be given to biodiversity, ecology, conservation, waste disposal, prevention of pollution and innovative ideas to protect and nurture our environment towards prolife.

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Printed at

Yenepoya Printers & Publishers, Mangalore 575 001, Karnataka, India

Published by

Yenepoya (Deemed to be University), Mangalore 575 018, Karnataka, India

Newsletter Design:

Yenepoya Printers & Publishers

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Cover page photo: Campus bird count at Yenepoya (Deemed to be University)

Photo credit: Dr. Uma Kulkarni

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EDITORIAL

We are pleased to offer the first issue of Volume 4 (2023) of *GREEN HORIZON*. This issue projects many vital environmental topics towards recent developments in environmental conservation. Contributions to this issue mainly came from the student fraternity of Yenepoya Institutions. The editorial team is happy that students showed concern about the environment and its significance. Articles in this issue could be broadly classified into two subdivisions: 1) importance of plants, animals, fungi and soil in our environment; 2) environment-friendly approaches towards producing industrial products.

The first article deals with macrofungal (mushrooms) mutualism with native tree species in the coastal scrub jungles of southwest Karnataka. Many mushrooms are edible and could be protected by conserving native tree species through social and agroforestry. The second article deals with theories of music (sound energy) on the yield of plants, plant production, and the increased yield of various metabolites. Aquatic bears (tardigrades) are an interesting fauna; their distribution, tolerance to low-oxygen habitats, osmotolerance, survival strategies, and applications are the focus of the third article. Soil is the basic resource responsible for crop production and directly connected to human wellbeing, but mismanagement causes human health problems. The fourth article provides information on the harmful effects of soil pollution on human health. Biofobia is one of the unnecessary fears of some adults and children towards nature and living organisms. This is in the fifth article by Hisana and Jamsheena. The sixth article by Kashvi emphasizes the importance of bird watching to enhance our knowledge of the contributions of birds towards environmental protection. Liquorice is a medicinal plant that has several medicinal uses owing to its bioactive properties. This is the subject matter of the seventh article. Farha, in the eighth article, draws attention to the challenges of crop breeding in space. The last two articles ventured on the environment-friendly pharmaceutical industry and the synthesis of mercaptotriazoles, respectively.

The current *GREEN HORIZON* issue tackles many value-added issues of ecosystems and the environment: environmental issues, benefits of plants, animals and fungi and environmentally friendly industrial approaches towards the green horizon. These articles are useful for various environmental issues in and around us. The *GREEN HORIZON* team anticipates more stimulating articles on the environment in future volumes.

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Ectomycorrhizal association with tree species in scrub jungles and plantations

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Abstract

This article documents the colonization of ectomycorrhizal (EM) fungi with tree species distributed in the scrub jungles of southwest Karnataka. A total of 18 EM fungi colonized the roots of 24 tree species (18 forest trees, 2 exotic trees and 4 plantation trees) distributed in and around the scrub jungles of the Mangalore University campus developed about five decades. These tree species associated with EM fungi are valuable for timber yielding, fruit yielding and fibre yielding. The colonization of 18 EM fungi with tree species ranges from 1-5. Among these, 12 species are edible and many of them have medicinal properties. Some EM fungi are extremophiles because they withstand harsh edaphic conditions. Trees of the scrub jungle serve as repositories of EM fungi and those could be inoculated to develop social forestry or agroforestry for sustainable development (forest trees, exotic trees and plantations). Besides, tree species in scrub jungles deserves conservation for their ecosystem services, especially to cater to the needs of associated flora (herbs, shrubs and lower plants), fauna (shelter, fruits, seeds and fibre) and microbes (bacteria, fungi and others).

Introduction

Among the macrofungi, the ectomycorrhizal (EM) fungi serve as essential components of the ecosystem for the development of tree species around the globe. They constitute a broad range of fungi, such as ascomycetes, basidiomycetes and mucoromycetes. It is predicted that up to 90% of plants have mycorrhizal fungal associations with their roots (Suz *et al.*, 2018). The global estimation predicts the EM fungal richness to be about 20,000-25,000 associated with up to 6,000 tree species (Rinaldi *et al.*, 2008; Tedersoo *et al.*, 2010). Their origin has been

traced back about 156 million years in association with the members of the Pinaceae (LePage, 2003; Hibbett and Matheny, 2009). According to Hawksworth (2019), about 16,000 vascular plants in the Indian subcontinent with a 1:6 ratio account for up to 96,000 EM fungi. The Himalayas and Western Ghats are the hotspots of EM fungi owing to the occurrence of a wide range of diverse and endemic tree species (Myers *et al.*, 2000; Riviere *et al.*, 2007).

The west coast of India consists of scrub jungles and plantations endowed with a variety of EM fungi (Sridhar and Karun, 2019). The campus of Mangalore University was largely grassland about five decades ago, which was initially vegetated by cashew (*Anacardium occidentale*). Subsequently, neem (*Azadirachta*), *Casuarina*, coconut (*Cocos*), *Pongamia*, tamarind (*Tamarindus*) and several native tree species (e.g., *Careya arborea* and *Terminalia paniculata*) were planted. Two botanical gardens and an arboretum on the campus consist of several endemic and endangered tree species of the Western Ghats. In five decades, the campus ecosystem has enriched the soil and supported a wide variety of flora, fauna and fungi. This article discusses the EM fungal mutualism with forest, exotic and plantation tree species, mainly in the scrub jungles of the Mangalore University campus, situated on the southwest coast of Karnataka.

Mutualism of EM fungi with tree species

The EM fungi develop extraradical hyphal biomass surrounding the tree roots and hyphal penetration of roots develops a network (known as the "Hartig Net") in the intercellular space of the cortex as well as epidermis (Smith and Read, 2008). Through positive mutualistic interactions

with tree species, the EM fungi enhance the absorptive surface through mycelia (similar to roots), acquire more moisture, nutrients and help to develop resistance against pathogens in the rhizosphere (Agerer, 2006).

The present study documents 18 EM fungi associated with the roots of 24 tree species (18 forest trees, 2 exotic trees and 4 plantations) distributed in and around the scrub jungles of Karnataka (Table 1; Fig. 1 and Fig. 2). Among the 18 EM fungi, many of them possess medicinal properties and 12 species are edible. The host tree species are economically valuable for timber yielding, fruit yielding and fibre yielding. Colonization of EM fungi with tree species ranged from 1-5; *Astraeus hygrometricus* was associated with a maximum of five tree species; followed by *Geastrum triplex*, *Lycoperdon utriforme* and *Scleroderma citrinum* (four tree species); *Amanita konajensis* and *Rubinoboletus caespitosus* (three tree species); *Amanita angustilamellata*, *Astraeus odoratus*, *Phlebopus*

marginatus and *Russula atropurpurea* colonized one of the tree species; the rest of eight EM fungi were associated with two tree species.

Among the EM fungi, many of them are capable of inhabiting extreme habitats. For example, *Scleroderma* spp. are known to inhabit ruderal habitats (e.g., mining areas, coal refuges, xeric environs, sand dunes and mangroves) and withstand drought conditions (Mark *et al.*, 2017; Karun *et al.*, 2022). *Scleroderma citrinum* and *Pisolithus albus* were found in coastal sand dunes in Karnataka, while *Lycoperdon utriforme* was seen in mangroves in Karnataka (Ghate and Sridhar, 2016a, b). Similarly, *Thelephora palmata* was an inhabitant of coastal sand dunes as well as mangroves in Karnataka.

Table 1. Ectomycorrhizal fungi recorded in the scrub jungles and plantations of Karnataka (Southwest India*, exotic species; **, plantations; trees without stars, forest trees; ?, yet to be defined).

Ectomycorrhizal fungus	Host tree species	Geographic location	Remarks
<i>Amanita angustilamellata</i> (Höhn.) Boedijn (Fig. 1a)	<i>Canarium strictum</i>	Mangalore University campus	Poisonous
<i>Amanita griseofarinosa</i> Hongo (Fig. 1b)	** <i>Cocos nucifera</i> and <i>Spathodea campanulata</i>	Mangalore University campus	?
<i>Amanita konajensis</i> K.R. Sridhar, Mahadevak., B.R. Nuthan & N.C. Karun (Fig. 1c)	* <i>Acacia auriculiformis</i> , * <i>Acacia mangium</i> and * <i>Anacardium occidentale</i>	Mangalore University campus	Edible
<i>Astraeus hygrometricus</i> (Pers.) Morgan (Fig. 1d)	<i>Artocarpus hirsutus</i> , <i>Holigarna arnottiana</i> , <i>Hopea ponga</i> , <i>Hopea parviflora</i> and <i>Syzygium cumini</i>	Mangalore University campus	Edible
<i>Astraeus odoratus</i> Phosri, Walting, M.P. Martin & halley (Fig. 1e)	<i>Hopea ponga</i>	Mangalore University campus	Edible
<i>Boletus reticulatus</i> Schaeff. (Fig. 1f)	<i>Garcinia indica</i> and <i>Hydnocarpus pentandrus</i>	Mangalore University campus	Edible

<i>Geastrum triplex</i> Jungh. (Fig. 1g)	<i>Artocarpus heterophyllus</i> , <i>Canarium strictum</i> , <i>Mangifera indica</i> and <i>Terminalia paniculata</i>	Mangalore University campus	?
<i>Lycoperdon utriforme</i> Bull. (Fig. 1h)	* <i>Acacia auriculiformis</i> , * <i>Acacia mangium</i> , <i>Cyathoxylon quadrangularis</i> and <i>Macaranga peltata</i>	Mangalore University campus	Edible
<i>Phlebopus marginatus</i> Walting & N.M. Greg. (Fig. 2a)	** <i>Bambusa burmanica</i>	Yenepoya University campus, Mangalore	Edible
<i>Pisolithus albus</i> (Cooke & Masee) Priest (Fig. 2b)	* <i>Acacia auriculiformis</i> and * <i>Acacia mangium</i>	Mangalore University campus	?
<i>Ramaria versatilis</i> Quél. (Fig. 2c)	** <i>Areca catechu</i> and * * <i>Cocos nucifera</i>	Mangalore University campus	Edible
<i>Rubinoboletus caespitosus</i> T.H. Li & Watling (Fig. 2d)	<i>Chrysophyllum roxburghii</i> , <i>Hopea parviflora</i> and <i>Hydnocarpus pentandrus</i>	Mangalore University campus	Edible
<i>Russula adusta</i> (Pers.) Fr. (Fig. 2e)	<i>Hopea ponga</i> and <i>Vateria indica</i>	Mangalore University campus	Edible
<i>Russula atropurpurea</i> Peck (Fig. 2f)	<i>Vateria indica</i>	Mangalore University campus	Edible
<i>Scleroderma areolatum</i> Ehrenb. (Fig. 2g)	* <i>Acacia auriculiformis</i> and <i>Macaranga peltata</i>	Mangalore University campus	?
<i>Scleroderma citrinum</i> Pers. (Fig. 2h)	<i>Artocarpus heterophyllus</i> , <i>Canarium strictum</i> , <i>Dysoxylum</i> <i>malabaricum</i> and <i>Schefflera</i> <i>racemosa</i>	Mangalore University campus	Edible
<i>Scleroderma verrucosum</i> (Bull.) Pers. (Fig. 2i)	<i>Canarium strictum</i> and <i>Vateria indica</i>	Mangalore University campus	Edible
<i>Thelephora palmata</i> (Scop.) Fr. (Fig. 2j)	* <i>Acacia auriculiformis</i> and * <i>Acacia mangium</i>	Mangalore University campus	?



Fig. 1. Sporocarps of *Amanita angustilamellata* (a), *Amanita griseofarinosa* (inset, developing basidium) (b), *Amanita konajensis* (inset, developing basidium) (c), *Astraeus hygrometricus* (inset, developing basidium) (d), *Astraeus odoratus* (inset, developing basidium) (e), *Boletus reticulatus* (f), *Geastrum triplex* (g) and *Lycoperdon utriforme* (h) (photocredit: snaps d and e, Dr. Mundamoole Pavithra, Department of Biosciences, Mangalore University).



Fig. 2. Sporocarps of *Phlebopus marginatus* (a), *Pisolithus albus* (b), *Ramaria versatilis* (c), *Rubinoboletus caespitosus* (d), *Russula adusta* (e), *Russula atropurpurea* (f), *Scleroderma areolatum* (g), *Scleroderma citrinum* (h), *Scleroderma verrucosum* (i) and *Thelephora palmata* (j) (photo credit: snaps b and j, Dr. Sudeep Ghate, Nitte (Deemed to be University), Mangalore; Photo Credit e and f, Dr. Mundamoole Pavithra, Department of Biosciences, Mangalore University).

Outlook

Tree species of the scrub jungle serve as repositories of several EM fungi, which could be harnessed by inoculation to establish forest trees, exotic trees and plantations under agroforestry. The development of coastal forests in association with EM fungi facilitates the provision of nutrients and preserves the soil from erosion. Besides, conservation and development of coastal forests offer several ecosystem services: enrichment of soil quality; development of other flora; offering shelter and food for fauna; scope for the development of soil-associated microbes; providing edible mushrooms; supplying health-promoting mushroom metabolites; withstanding harsh edaphic conditions. Institutions located in rural coastal regions have excellent possibilities to preserve the scrub jungles in their surroundings without human interference and to expand or harness their ecosystem services towards social forestry and agroforestry.

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Music and Plants

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Introduction

From birds tweeting and waves lapping against a coastline to cars honking in traffic, sounds are all around us. However, sounds are sometimes purposefully combined to create a specific mood or to represent ideas or feelings. Music refers to such structured sounds. Music is a grouping of coordinated sounds or noises. Though definitions of music vary greatly around the world, every known culture participates in it, making music a cultural universal.

Music has been shown to have a profound effect on humans. Plants are living things that breathe and grow. Some experts believe that because plants lack a neural system, they are unable to understand or respond to music. Several studies, however, suggest that music may have a particular influence on plants (Fig. 1). It is



Fig. 1. Music and plants
(Source: *Brittany Oxford*, 2020).

claimed that loud and unharmonious sounds might impair a plant's mood and health. Soft rhythmic music, on the other hand, improves plant protein production in plants such as wheat, spinach, horse gram, soya, and rice. Musical vibrations aided in the development of Okra and Zucchini seeds. Music not only accelerates growth but also significantly influences the concentration of various metabolites, e.g., chlorophyll and starch.

Scientific theories

One of the earliest studies of the effect of music on plants was conducted in 1962 by Dr. T. C. Singh, Head of Botany at Annamalai University. He exposed balsam plants to classical music and found that their growth rate increased by 20%, along with a 72% increase in biomass. The researchers saw positive results with flute, violin, harmonium, veena as well as to the vibrations from traditional Indian dance. Plants exposed to rock music, on the other hand, grew away from the speakers and showed signs similar to overwatering.

Various researchers have experimented with diverse styles of music, including rock and roll, jazz, classical, or light music, yielding varying outcomes. Concurrently, another group of researchers explores the impact of different sound frequencies and sound pressure levels on plant treatment. Nevertheless, the underlying mechanisms of the sound effects remain undisclosed at this point.

Evolutionarily, the reception and processing of the energy embedded in sound waves is advantageous, whether as it allows for the gain of information about the environment, close by or distant. Yet, most auditory animals lack such

external morphological features, and many also have no eardrums. As sound travels readily and far in a dense substrate like soil, it is very efficient and enables the capture of information from distant sound sources. Hearing research has shown that very different morphological structures can be functionally adapted to perform the biophysical task of sensing sounds and/or vibrations.

Do plants hear?

Plants have their own senses. Some of these can be compared to all five human senses. In addition to those, plants have some 15 other senses that are unique to the vegetal world.

Numerous studies suggest a robust correlation between sound waves and plant growth. Specific frequencies and intensities of sound waves have demonstrated substantial impacts on various biological, biochemical, and physiological processes, including the modulation of gene expression in plants. In Chrysanthemum, exposure to sound waves of particular intensities and frequencies resulted in a noteworthy increase in soluble sugar content, protein levels and amylase activity. This observation suggests that sound stimulation has the potential to augment root metabolism and promote the growth of Chrysanthemum (Michelson 2014).

The effects of music on crop yield and quality have been reported in tomato plants, barley, and other vegetables. Plants seem to react to vibrations coming from insects and other plants. The roots of the plants seem to be sensitive to a broader range of frequencies than the parts above the ground. They also produce a "clicking" sound, most probably as a byproduct of their growth. This fact about roots that both produce and perceive sound unveils a new scenario of communication networks that exist underground.

Feeling in plants

Animal and plant stimuli appear in their reactions. The plants are living organisms and they can feel and sense, as the 'touch me not' plant recoils and folds its leaves at a slight touch. Some plants jolt in pain when plucking their leaves,

while other shudder in anticipation of being axed. Protoplasm, the translucent living matter of which all animal and plant cells are composed, is in a state of perpetual movement. The vibrations picked up by the plant might speed up the protoplasmic movement in the cells. This stimulation could then affect the system and improve performance, such as through the manufacture of nutrients that develop a stronger and better plant.

Growth influenced by acoustic frequencies

Plants, being living organisms, get affected by external stimuli. The notes are designed on the basis of the quantum vibrations that occur at the molecular level as a protein is being assembled from its constituent amino acids. The length of a note corresponds to the real time that is taken by each amino acid to come after the next. Thus, on playing the appropriate tune, the production of protein increases in the plant and hence, its growth is stimulated. Sternheimer remarked that tomatoes exposed to such tunes grew two and a half times better than the control ones; even virus growth in tomato plants could be stopped by playing tunes that inhibited enzymes essential for it.

Sound energy also gets reflected and diffracted around the leaves and may thus affect the insects near the plants. Not only this, but some researchers even report that plants also emit acoustic waves. It has been reported that if the applied frequency resonates with the plant's natural frequency, then the rate of photosynthesis and cell division increases, leading to faster plant growth. (Chowdhury A.R. and Gupta A. 2015)

Influence of sound waves on seed germination

Researchers have applied sound waves, specifically utilizing natural sounds like those of birds and echoes, to okra and zucchini seeds. Remarkable, the application of these natural sounds demonstrated a notable impact on the germination process of these seeds. Further investigations into the biological effects of sound waves on paddy seeds indicated significant enhancements in various parameters such as the germination index, stem height, relative increase rate of fresh weight, root system activity, and cell

membrane penetrability. These positive effects were particularly pronounced at a sound frequency of 0.4 kHz and a sound pressure level (SPL) of 106 dB. This suggests that sound waves have the potential to bring about substantial alterations in the cell cycle of plant cells, accelerating their reproduction rate. Additionally, sound waves were found to transfer energy into the cells, contributing to the stimulation of cytoplasmic streaming.

Melodies for pests

The sound strengthens pest immunity by creating stress among plant insects. The classical music reduced the 30-day lifespan of male *Drosophila Melanogaster* and that of spider mites. The sound of 55 Hz and 120 dB for half an hour reduced the major threat to citrus fruits by 45% by killing the Chinese citrus fly. It also decreased several viruses, grey molds late blight and others. The green music decreased the aphid or plant lice damage to cabbage and sheath blight in rice by 50%. The damage by corn borer moths was reduced from 50% to 5% by the sound of 5 KHz from sunrise to sunset, while the higher frequencies controlled the insects in stored wheat (Sharma S. 2022)

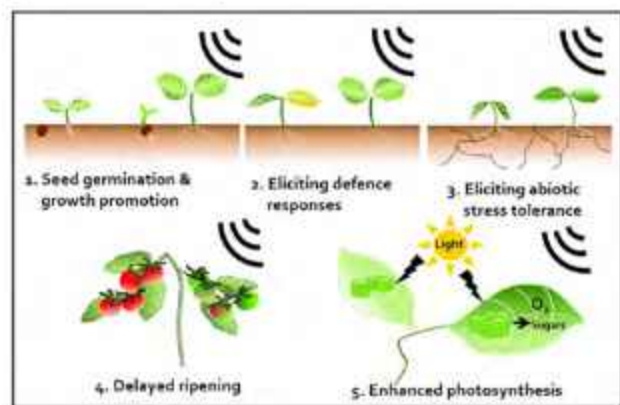


Fig. 2. Effect of music on plant
(Source: Jung et al., 2018).

What type of music?

The effect depends on plant species, type of music, and the frequency, duration, or pressure of sound. The fewer beats of higher frequencies per minute, the higher the yields. The same music on string instruments bent the plants more towards the speaker than on percussion instruments.

Rock music. The music heavy on bass like rap,

the Beatles, and noisy Rock damages the plants, as is done by excess water or strong winds. The plants lean away from such music and sometimes die.

Classical music. The plants entwined the speakers playing Hayden, Beethoven, Brahms, Schubert, Mozart, Vivaldi, Mahler, Bach, and Indian Classical music or ragas or Vedic music.

Heavy metal. Metal music increases stress in plants and decreased plant growth, comparatively.

Country and Western music. Plants remained indifferent to such music.

How music influences the plants?

The musical sounds influence plant growth in different forms and shapes as follows.

Activation. Activation of specific genes leading to increased plant cell growth has been observed in response to particular sound frequencies.

Sound Resonance. Resonance is a phenomenon where each object possesses a distinct resonant frequency, and when that frequency is played, it can induce vibrations within the object.

Effect on stomata. The growth of plants is facilitated by the exchange of carbon dioxide and oxygen with the environment. Stomata, which are the openings on the leaf surface which promote this gaseous exchange. Music has been observed to contribute to this process by keeping the stomata wide open. This opening of stomata facilitates an enhances intake of nutrients and water, ultimately supporting the growth of plant.

Effect on cell organelles. Fluid dynamics are influenced by resonance, causing more rapid and intense movement around resonating objects. In the cells, specific frequencies can induce resonance in the cell organelles. This resonance amplifies the movement of cytoplasm within cells, consequently promoting cell growth.

Cavitation. It refers to the formation of microbubbles within liquids due to specific frequencies. These microbubbles resonate with

sound waves, and when they collapse, they generate pressure. This pressure can cause damage to the contents, walls, and surrounding environment of cells.

Pressure propagation. The sound pressure could be felt by placing hands on the speaker. The compression and rarefaction of sound create a wave that travels in the medium by creating higher and lower pressure areas. Different vibrating patterns create a high or low pitch.

Movement of protoplasm. The living organisms consist of protoplasm, which is in constant motion. This motion is lowest at dawn and dusk. The vibrations produced by sounds impact the leaves, increasing the flow of protoplasm which increases the growth of healthy plants by increasing the synthesis of food and nutrients.

Plant hormone. The loud warbling sound resulted in doubling the growth of dwarf pea plants. This growth enhancement is attributed to the response of the plant hormone gibberellic acid to sound, which triggers shoot elongation or seed germination. (Oxford B. 2020)

Conclusions

The Institute of Integrated Study and Research in Biotechnology and Allied Sciences in India found that not only does music promote plant growth, but it seems that plants can actually distinguish between different types of sound, including different genres of music, nature sounds, and traffic noise. More studies need to be done to understand how this works and what it could teach us. The world population increase presents a challenge to scientists and researchers to investigate the possibilities for utilizing new

and green technologies to increase the production of food, says the team. Using sound wave technology can enhance the plant immune system, thereby avoiding many problems associated with environmental pollution and the economic costs of chemical fertilizers and herbicides.

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The Incredible Water Bears

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Introduction

Water bears, the microscopic marvels that can survive in the vacuum of space, are a cosmic enigma. They have successfully escaped man's exploitation of nature and can still be found everywhere on Earth. Water bears are short and plump microscopic animals with an oval, stout body, around back and a lumbering gait. Tardigrades are also known as water bears or moss piglets. When German biologist Johann August Ephraim Goeze first saw them in 1773, he named them as 'little bears'. Tardigrada ('Slow Steppers') was given later by the Italian biologist Lazzaro Spallanzani. Having conquered space, tardigrades may now turn their attention to saving lives on Earth. From being a 'survivor' tardigrade is now set to be a 'saviour'. The remarkable resilience of water bears to dehydration is due to a synergy between a sugar trehalose and a group of specialised proteins. They have been found from the highest mountain tops to the deepest ocean trenches and from the polar ice caps to soil. Despite their peculiar morphology and amazing diversity of habitats, relatively little is known about these unique organisms.



Fig. 1. Water bear laying eggs
(<https://www.ilabcam.com/blogs/news/researcher-capture-a-water-bear-tardigrade-laying-eggs-with-labcam>)

The Tardigrades' mouth has stylets that are used to poke holes in plant cells, algae, or small invertebrates and release fluids or cell contents. A triradiate, sucking pharynx, muscular, emerges from the mouth, pharynx extends to a short oesophagus, then to an intestine that occupies a major portion of the body. They have a buccopharyngeal apparatus, which is utilized in together with their claws to distinguish between species. Certain tardigrade species only excrete waste products when they moult, or shed their cuticles, leaving the faeces behind with the shed skin. The double ventral nerve cord, which is located in the bottom of the body cavity, is connected to a mass of nerve cells in the head that make up the nervous system. Some tardigrades have longitudinal and transverse muscles, as well as excretory organs. However, the heterotardigrade family of tardigrades does not have excretory organs at all. Instead, they remove waste products from their bodies through diffusion. A single dorsal sack-like gonad with two gono ducts characterises males, while females have only a single gono duct.

Habitat

Tardigrades are ubiquitous and common. Provided the right habitats, they are present in all marine environments, from coast to abyssal depths, that range from aquatic to arid deserts, frozen ice, and temperate to tropical conditions. While all individuals need water to be active, tardigrades inhabit three different types of environments: (1) freshwater, (2) marine and estuarine, and (3) terrestrial. Every sea, from the intertidal to the subtidal zones to the abyss (4,690 m deep in the Indian Ocean), is home to marine tardigrades. At low tide, they could be able to engage in limited cryptobiosis in the intertidal zone on sandy beaches or burrow into areas that

hold water. Marine tardigrades are found in the subtidal zone on a variety of substrates, and they have morphological adaptations in response to varying subtidal environmental requirements. Certain species inhabit the water column as semi-benthic animals, while others live in mud and deep-sea oozes or feed on subtidal algae, coral, and other creatures.

Freshwater tardigrades live in a variety of freshwater habitats, including lakes, ponds, streams, springs, and temporary ponds. Freshwater tardigrades that are hydrophilous are limited to permanent freshwater environments.

They are benthic organisms, crawling on vegetation and in the interstitial spaces of sandy substrates. Most tardigrades inhabit moist terrestrial environments, such as soil, leaf litter, mosses, lichens, liverworts, and cushion-shaped flowering plants.

Survival tactics of tardigrades

A cryptobiotic state known as anoxybiosis is brought on by low oxygen tension in environment. Tardigrades are sensitive to oxygen levels. Prolonged anoxia can result in failure of the osmoregulatory system, causing the tardigrade to swell up and float around. Strictly aquatic species can survive in an anoxic state for

a few hours to three days. When the tardigrade's habitat dries out, it will resume active life.

Limno-terrestrial tardigrades are widespread in polar regions and high mountains because they undergo a sort of cryptobiosis called cryobiosis, which is triggered by low temperatures and allows tardigrades to withstand extreme cold and thaw back to life.

Increased osmotic pressures can cause a type of cryptobiosis called osmobiosis. The salinity tolerance of some intertidal marine and euryhaline limno-terrestrial species allows them to survive in a variety of salinity conditions. However, the majority of terrestrial and freshwater tardigrades develop contracted tuns, or resistant forms structured like barrels.

When most terrestrial eutardigrades and echeineidids undergo anhydrobiosis, a form of cryptobiosis brought on by evaporative water loss, it affects the eggs, juveniles, and adults. Trehalose and glycerol are essential for membrane preservation during desiccation. Although there isn't much scientific support for the theory that true freshwater tardigrades can't go through anhydrobiosis or withstand dehydration, this is usually accepted.

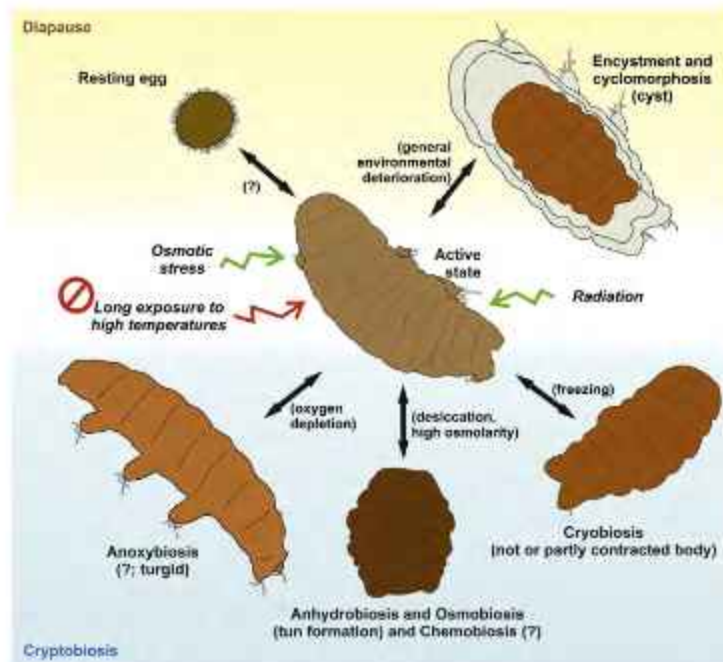


Fig. 2. A glimpse into the resilient realm of tardigrades

In September 2007, tardigrades were carried into low Earth orbit on the FOTON-M3 mission, where they were subjected to the vacuum of space for a period of ten days. Upon being rehydrated back on Earth, over 68% of the specimens that had been shielded from high-energy UV radiation survived, and many of these went on to produce viable embryos. In contrast, when dehydrated samples were exposed to the combined effects of vacuum and cosmic radiation, just three *Milnesium tardigradum* subjects survived, indicating a sharp decline in survival.

On STS-134, the last trip of the Space Shuttle Endeavour, in 2011, Italian scientists delivered tardigrades and other extremophiles to the International Space Station (ISS). The findings of this experiment were that radiation and microgravity had no discernible impact on the tardigrades' ability to survive in flight, thus confirming that these remarkable creatures are indeed valuable creatures for studies in space.

Applications

- Researchers dehydrated blood platelets after infusing them with trehalose produced by tardigrades. The dehydrated platelets were able to maintain their viability for up to two years.
- Research is also underway using tardigrade sugars and proteins to create dry vaccines that will not require refrigeration, resulting in a longer shelf life and improved portability. These dry vaccines are expected to be more stable and less likely to degrade than traditional vaccines.
- The Defence Advanced Research Projects Agency (DARPA) created the Biostasis program to support research on a wide range of technologies that could be used to preserve life. The Biostasis program is also investigating the use of tardigrade-inspired technologies to extend the lifespan of human cells and tissues

- Based on the tardigrade's ability to dehydrate and suspend its bodily processes for decades, it is possible that inducing a state of suspended animation in humans could be used to treat a variety of conditions, such as bleeding, sepsis, or damage from stroke or heart attack.
- Tardigrades in cancer research: They can maintain their genomic integrity. Hence, their peculiar property of DNA repair and protection of DNA damage has been stealing the interest of cancer researchers as well as those who are studying the effects of body ageing. Transfer of the DSUP (DNA associating protein) gene to human embryonic kidney cells can increase tolerance to radiation. The DSUP gene has been shown to protect cells from a variety of DNA-damaging agents, including radiation, chemotherapy drugs, and ultraviolet light. So eventually it will benefit those who are undergoing radiation therapy.
- Cryopreservation: Trehalose, a naturally occurring sugar found in tardigrades, helps protect cells from dehydration and other forms of stress. It is used in transplantology and organ preservation.
- Radiation Resistance: To enhance human survivability, researchers bound tardigrade proteins to human cells. The resulting cells exhibited a 40-50% reduction in X-ray damage compared to normal human cells. The tardigrade proteins responsible for conferring radiation resistance are believed to do so by aiding in DNA repair.
- Tardigrades as bio-indicators: Tardigrades are able to detect a wide range of environmental stressors, such as heavy metals, organic pollutants, and radiation. They sense changes in their environment, such as temperature, osmotic pressure, and oxygen levels. This can be used as bioindicators to monitor environmental pollution and assess the health of ecosystems.

Conclusions

The unique abilities of tardigrades suggest that they may have a role to play in a wide range of fields, from astrobiology to medicine. As we learn more about these resilient creatures, we may find that they are the key to unlocking some of the most profound mysteries of the universe.

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Soil and Human Health

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Introduction

Soil is a fundamental natural resource that strengthens a variety of ecosystem goods and services for the benefit of mankind (Joylata Laishram, et.al., 2012). It is a provider of water, nutrient, and supplies mechanical support to crop plants and is considered dynamic in nature (Lal, R. 2016) Soil health is known as “the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals and humans (Guo, 2021). To a large extent, human health depends on soil health and evidently, there is a connection between soil and human health through crop production (Lehmann et.al., 2020).

The condition of soil significantly impacts the health and well-being of individuals, with effects that can be either positive or negative, direct or indirect. The influence varies based on the state of the soil and its interactions. Natural soils with minimal anthropogenic contamination tend to have a more favorable impact on individual health. Conversely, soils in agroecosystems, urban areas, mines, oil and gas extraction sites, landfill areas, and similar locations are more likely to exhibit human-induced contamination. Occupational groups that work closely with soil, such as farmers, construction workers, or miners, face a higher risk of health issues related to soil exposure. However, the health of the general population is also affected to a lesser extent. It is noteworthy that soil serves as a source of essential nutrients for humans and can convey harmful substances through the food we consume (Steffan et.al., 2013). The nutritive value of a crop can be improved by properties like type of soil, its nutritional content, soils with greater micronutrient availability and higher soil organic matter. Additionally, it also depends on robust soil biodiversity, which increases

micronutrient bioavailability for crops and suppresses soil borne plant disease (Lehmann et.al., 2020). Soil generated dust can also travel thousands of miles and affect people's health. (Steffan et.al., 2018).

Healthy soil is fundamental for human health

Soil is requisite for human health in a variety of ways. Worldwide, around 78% of the average per capita calorie consumption comes from crops that are grown directly on soil and the remaining 20% comes from terrestrial food sources that rely indirectly on soil. Soil is a significant source of nutrients, along with this, it also acts as a natural filter to remove contaminants from water.

Soil is the foundation of the agri-food system and after the oceans, it is the largest active carbon store. One cubic metre of soil can store up to 600 litre of water, which makes crops grow during dry periods. Biodiversity, which occurs above and below ground, is important to make sure that the soil is healthy. Soil biodiversity contributes to the cycling of nutrients and carbon, regulates the appearance of pests and diseases and also supplies pharmaceuticals that boost our health. Soil also serves as building materials, fuel and fibre which are the basis for human infrastructure and protect cultural heritage. Microplastics, deforestation, pesticides, over fertilization and heavy metals are considered the main threats to healthy soil (Thomas Münzel et. al., 2022)

Exposure routes of soil:

There are three main ways of being exposed to soil materials:

- (i) Ingestion
- (ii) Inhalation (respiration)
- (iii) Skin absorption or penetration (dermal)

Ingestion can happen intentionally, known as

geophagy, or incidentally, like hand-to-mouth contact, which can be seen particularly in children, or when raw vegetables or fruits are eaten without proper washing. This is commonly seen in children and pregnant women. Ingested soil can supply necessary nutrients, but it also leads to exposure to heavy metals, organic chemicals, or pathogens and if in large amounts cause intestinal obstruction (Brevik 2013, Henry and Cring 2013)

Inhalation includes inhaling the soil substances. Serious conditions that are related to respiration are acute inflammation of the bronchial passages, chronic bronchitis, emphysema, fibrotic changes from breathing in soil-derived dust and others (Buck et.al., 2016)

Dermal exposure to soil can expose humans to pathogens and soil chemicals. It can cause *podoconiosis* (endemic non-filarial elephantiasis), which is a non-infectious disease seen in subsistence farmers who frequently walk barefoot. This may be due to long term contact with volcanically-derived clay in the soil, which affects the lymph system (Deribe et al., 2013)

Adverse effect of soil on human health

Soil can adversely affect human health in many ways. The contamination of soil can occur either naturally or through anthropogenic activities with chemical elements and other substances that are toxic in nature. A supply of any element can lead to human toxicity, if the element is in the optimal range. Thus, the degree of any essential element in an individual can be deficient, adequate or toxic depending upon the concentrations of these elements in the soil and the degree of exposure. Both deficiency and toxicity can lead to morbidity and sometimes mortality (Brevik and Burgess 2015).

Heavy metals

Heavy metal exposure through soil contact is a major human health concern. They are Arsenic, Lead, Mercury, Cadmium, Chromium, Copper, Nickel, and Zinc. These metals enter soils naturally through the weathering of rocks, and additionally, they also enter through human activity. Heavy metals are regarded as by-

products of mining ores and they are also found in mine spoils and the surroundings of metal processing plants. They are released into the soil at landfills which contain industrial and household wastes and from sewage sludge that takes place at waste water treatment plants. Electronic appliance waste is a major source of heavy metals like mercury, lead, cadmium in the soil. In agriculture, the usage of pesticides, fertilizers, leads to the accumulation of heavy metals in the soil. The heavy metals that are more toxic to humans are cadmium, lead, mercury, arsenic, which don't have any biological function and disrupt enzymatic activities that commonly affect the brain and kidneys (Brevik et.al., 2014 Hu, 2002)

Soil pathogens

The organisms that are present in soil are not harmful. But soil does serve as home for many pathogenic agents. It is a reservoir for many microorganisms like bacteria, fungi, protozoa, and viruses, which can be pathogenic to humans and animals. There are four types of human and animal pathogens in soil based on their degree of residency in soil: permanent, periodic, transient and incidental. Permanent pathogens are soil inhabitants and they spend their life cycle in soil, which become infectious to humans and animals. For example, organisms like *Clostridium botulinum* or *Clostridium tetani* produce neurotoxins when contaminated food is ingested or through contaminated wounds, respectively. A pathogen's ability to colonize and establish itself in soil or ultimately infect a potential host, will be influenced by the soil environment and its indigenous communities (Samaddar et. al., 2021).

Conclusions

Soil is a natural resource and healthy soils are the foundation of the food system, they are the basis for agriculture, and the medium for plant growth. Healthy soil provides healthy crops, which influence the nourishment of humans. Healthy soil should have low populations of pathogenic organisms and heavy metals. Since soil is a vital source of nutrients, nutrient imbalances and the presence of pathogens in the soil can have negative effects on health.

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Biophobia

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Introduction

People can express irrational fears and disgust responses towards certain wild organisms. This so-called 'biophobia' can be useful and indeed necessary in some circumstances. Yet, biophobia can also cause very distressing and anxious feelings, which can make people avoid being in nature. Here, we draw attention to the worry that this decline in contact with the environment can cause a gradual uptick in biophobia, further solidifying it across people and throughout society. We put out the idea of the "vicious cycle of biophobia," which describes how an overwhelming fear of nature may spread throughout society. The vicious circle of biophobia has the possibility of hastening the extinction of experience, which would have long-term negative effects on biodiversity preservation (Soga *et al.*, 2023).



Fig. 1. Earachnophobia

Source: <https://static.standard.co.uk/s3fs-public/thumbnails/image/2014/11/04/09/33/spider0411a.jpg?width=968&auto=webp&quality=50&crop=968:645%2Csmart>

Mitigate against increasing biophobia among children

The "extinction of experience," or the disappearance of direct encounters between humans and nature, has the potential to worsen attitudes towards the natural world (also known as "biophobia"). The protection of biodiversity is impacted by an increase in biophobia since it may result in a decline in the desire to defend wild animals and their habitats. Future biodiversity conservation strategies and results could be significantly impacted if biophobia among today's youth persists into adulthood. In order to better understand the various elements impacting schoolchildren's degrees of biophobia (dislike, disgust, fear, and perceived threat towards common invertebrates), we performed a large-scale questionnaire survey in Japan. The degree of biophobia in children was inversely correlated with how frequently they encountered nature and how much they knew about invertebrates. It was strongly correlated with the level of urbanisation around the children's school and the biophobia of the family members (Soga *et al.*, 2020).

The vicious cycle of biophobia

We suggest a novel idea we refer to as the "vicious cycle of biophobia," which captures how extreme biophobia could develop and spread in society. We outline the cycle's mechanics, go over potential repercussions and mitigation strategies, identify significant information gaps, and make suggestions for additional research. We concentrate primarily on urbanised and high-income nations and regions, where excessive biophobia is suggested to be prevalent and because there is still a dearth of research on this phenomenon in other areas (Soga *et al.*, 2023).

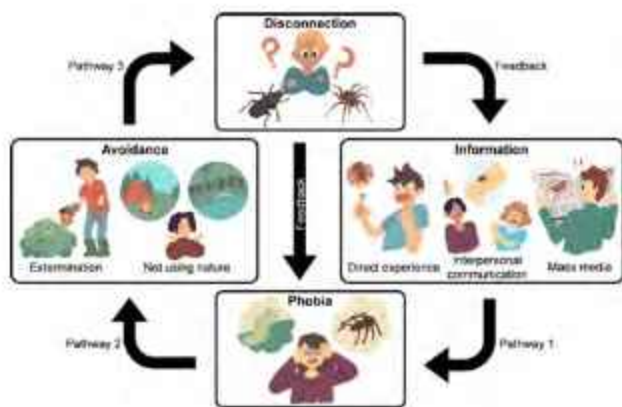


Fig. 2. The vicious cycle of Biophobia

Source: https://hakaimagazine.com/wp-content/uploads/gr2_lrg-1536x1026.jpg

What can you do to fix biophobia?

Overcoming biophobia starts with a willingness to learn and the courage to abandon even long-held assumptions and beliefs about nature. Where children need an ecological education, adults often need reeducation to overcome ingrained assumptions and behaviors. Consider taking quick action to stop the reported loss of exposure to nature. Louv advises keeping some of your yard "untamed" so that children can explore it and discover intriguing weeds and rocks. So also, he advises parents to let their kids help out in the garden, play in a nearby park, go on hikes and camping trips, and explore.

1. Other suggestions include going on outdoor and indoor safaris for bugs or animals. Apps like iNaturalist by National Geographic can help you identify and learn about various plants, animals and bugs near your home.
2. The attitudes and perspectives of friends, family and the community are such powerful influencers on ourselves and our children's perceptions. These all help to reject popular misperceptions of nature that feed biophobia.
3. Prioritise understanding, especially of creatures that often inspire fear or disgust.

Like people, the value of the natural world cannot be accurately appraised based on appearances.

4. Learn how your actions, even at home, are improving or degrading the environment and take action to improve your impact and the impact of your community on the environment.
5. Be patient, but don't procrastinate. Frequent and positive experiences in nature during childhood greatly influence lifelong behaviors and concern for the environment. Early and consistent investment in ecological literacy and repeated efforts to dissipate biophobia have the ability to pay remarkable dividends and greatly improve personal health along with the health of the planet (<https://www.ksl.com/article/50265489/are-you-a-biophobe-if-so-your-children-probably-are-too>).

Conclusion

Biophobia refers to the fear of the natural world, including living organisms. It is negative emotions towards nature. It can be fear of animals, insects, or plants, as well as discomfort in natural settings. Many factors contribute to biophobia such as lack of exposure to nature during childhood, negative experiences with animals and cultural influences.

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Bird Watching

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Introduction

Bird watching is the observation of live birds in their natural habitat, a popular pastime and scientific sport that developed almost entirely in the 20th century. Watching the birds is a treat for the human eye. The color of their feathers, their flight (ability to fly) and the different sounds (vocalisation) produced by different birds are just wonderful to watch. People enjoy watching birds. Bird watching is one of the hobbies of this generation. As it costs very little money, even a student studying in college can engage in such kind of pleasant experience as watching birds. As a hobby, it provides pleasure, gives aesthetic delight and at the same time creates a scientific attitude.

Bird watching can be done anywhere, from your back garden to the local park and various other natural areas. Basic equipment includes a binocular, a field book to aid identification, and a notebook for recording the time and place of sighting (Fig. 1). We can also setup of feeding station that will attract the birds. Most preferably,

early morning is one of the best times to observe birds. We can hear different kinds chirping of birds which are pleasant to hear. The activities of the bird are greatest in the early morning. So if one has to watch the bird, it is better to start early in the morning. So rising early is the most important pre-requisite for successful watching.

It should be kept in mind that not only forests are places for bird watching. This can be done anywhere in the surrounding area. The banyan trees and the peepal trees are the best sites to observe because, when these trees are laden with fruits, they attract a large number of birds of many species. Initially, for bird watching, one must have a guide who will tell them what to observe and how to observe the bird within their habitat (Fig. 2). Once we have understood the technique, we can easily make notes on birds behaviour. When identifying the bird, it is important to know the colour, size, general shape of the bird, the beak, legs and feet, its behaviour and the habitat in which it is living. All these will help in identifying the bird accurately.

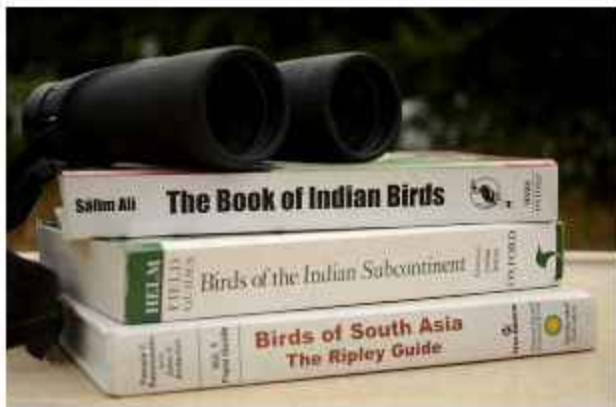


Fig. 1. Birding gear
(Source: <http://birding-world.com/>)



Fig. 2. Team discussion

Birds are also referred to as “FEATHERED SINGERS”, because birds can produce complex varieties of notes that are melodious to hear. Not all birds sound the same. Some sounds will be melodious, whereas some birds will not have that feature. Most poets and musicians are inspired by the songs of bird. The 'syrinx' is a sound producing organ in birds. The bird with more vocal muscles produces more complex sounds. The sound is generally produced when the air from the lungs vibrates the membrane, which generates sound waves.

Birds are going extinct faster due to human interference

Globally, about 500 species of birds have gone extinct due to human activities and climate change. This extinction of bird species is mainly due to human activities, particularly habitat loss or degradation resulting from unsustainable and often illegal logging, and land or wetland clearance for agriculture or exotic timber plantations. Habitat destruction, selective hunting, invasive alien species and global warming are all adversely affecting natural populations of plants and animals.

According to Bombay Natural History Society (BNHS) ornithologists, 99% of globally threatened species are at risk from human activities such as agriculture, logging, hunting, trapping and industry. Species extinction is also an indication and result of major human induced changes in the world's ecosystem. The ecosystem provides vital services such as maintaining the global climate pattern, safeguarding watersheds, mediating the carbon cycle and stabilizing soil. In order to prevent this extinction of bird species, we must have knowledge of these species and

also about legal and social actions and sustainable use of natural resources. We have to take great care (caution) while bird watching, one must see to it that no birds are harmed. We must remember that the aim is not only to study and watch birds but also to protect them. We should never examine the nest unless the bird has abandoned it. It is not possible to make new discoveries about the bird for every bird watcher. But this may bring the source of delight and provide a lifelong hobby.

Conclusion

Bird watching, also known as birding, is a popular and rewarding hobby enjoyed by people of all ages around the world. It involves observing and identifying birds in their natural habitats. Bird watchers, known as birders, often use binoculars, field guides, and sometimes cameras or notebooks to document their sightings. Whether you're a beginner or an experienced birder, bird watching offers a lifelong learning experience, allowing you to explore the diversity of bird life and appreciate the beauty of the natural world.

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Glycyrrhiza glabra (Liquorice) and its health benefits

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Introduction

The medicinal plant *Glycyrrhiza glabra* Linn. is commonly known as liquorice, sweet wood, Mulahatti, or Yashtimadhu and belongs to the family Leguminosae (Table 1). In Ayurveda, it is one of the most utilized therapeutic herbs. The plant is predominantly found in China, India, the United States, and Russia, among other places. It grows wild but is also cultivated in sub-tropical and warm regions in many parts of the world, including India.

Table 1. Taxonomy of liquorice

Kingdom :	Plantae
Division :	Magnoliophyta
Class :	Magnoliopsida
Order :	Fabales
Family :	Fabaceae
Genus :	<i>Glycyrrhiza</i>
Species :	<i>glabra</i>

Roots of *G. glabra* possess medicinal value (Fig. 1). The use of liquorice root extract can be dated to 200 BC. It contains sugars, starch, bitters, resins, essential oils, tannins, inorganic salts, and others (Table 2). Triterpene saponins and flavonoids are the major pharmacologically important compounds found in these root extracts. Liquorice owes its ulcer-healing properties to glycyrrhizin and glycyrrhetic acid. However, these substances have unwanted mineralocorticoid-like effects. Excessive consumption can cause some adverse effects. For example, hypokalemia and hypertension have been associated with the use of liquorice-flavored chewing gum. Liquorice is sometimes present in laxatives, for example, those prepared by herbalists, and hyperaldosteronism can occur unexpectedly in such cases.

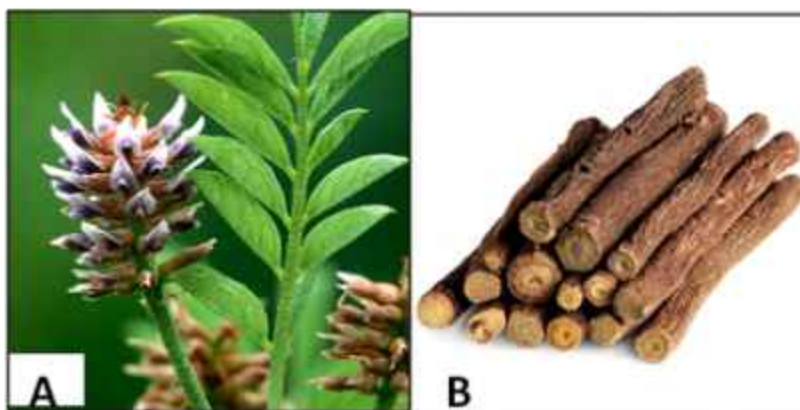


Fig. 1. Liquorice plant (A) and its roots (B) (Source: <https://www.nccih.nih.gov/>)

Table 2. Phytochemical constituents of Liquorice (Source: Hasan *et al.*, 2021)

Groups	Bioactive compounds
Triterpenoid	Glycyrrhizin
Saponins	Glycyrrhizic acid and 18- β -glycyrrhetic acid
Flavonoids	Liquiritin, isoliquiritin, liquiritigenin, glabrene, glabridin, rhamnoliquiritin, glucoliquiritinapioside, prenyllicoflavone A, shriniflavonone, shrinpterocarpin, 1-methoxy-phaseolin, glisoflavone, kanzonol R, licochalcone A, hispaglabridin A and B, licuraside, and glyzaglabrin
Coumarins	Licocoumarin, glycy coumarin, licopyranocoumarin, and glabrocoumarone A and B
Isoprenoid substituted phenols	Semilicoisoflavone B, 1- methoxyficifolinol, and isoangustone A, licoriphenone
Alcohols (volatile)	Pentanol, hexanol, and 2,3 Butanediol
Acids (volatile)	Propionic acid, benzoic acid, and ethyl linoleate Acetic acid, malic acid, butyric acid, fumaric acid, and citric acid
Terpenoids	Alpha terpineol, Geraniol
Aldehyde	Furfuraldehyde

Medicinal Uses

- *Glycyrrhiza glabra* helps to pacify vata and pitta dosha due to its sweet taste and cold potency, respectively. Hence, it is effectively used in various diseases like chronic cough, breathing difficulties, burning sensations, headaches and so on.
- It contains different chemical constituents like glycyrrhine, prenylated bioflavone, licoagron and isoflavone, which play an important role in antimicrobial, antiviral, and anti-inflammatory activities. Due to these properties, it is effectively used as an anti-allergic drug.
- Due to its cold potency, its paste can be applied effectively to skin allergies. As it helps in alleviating pitta dosha, skin diseases like paittika kushta can be treated effectively by using *Glycyrrhiza glabra*.

Pharmacological activity

Liquorice contains various chemical constituents in its root. The major constituents of liquorice are glycyrrhine, prenylated bioflavone, licoagron, isoflavone, licofamin, glyzaglabrin, quercetin, kaempferol, steragaion, liquiritigenin, glycyrrhizin acid, liconicone, glabridin, and glabrene. The above-mentioned constituents help yashtimadhu act as a hepatoprotective, antimicrobial, anti-viral, spasmolytic, anti-ulcer, anti-mutagenic, anti-pyretic, antioxidant, anti-inflammatory, and expectorant drug. Some of these biological activities are discussed below.

Antibacterial activity

The methanol extract of *Glycyrrhiza glabra* showed a bactericidal effect against *Streptococcus mutans* in 2 min at a 50 g/ml concentration. The active component of *Glycyrrhiza glabra* (i.e., glabridin), exhibited antimicrobial activity against different strains of

bacteria, including *Mycobacterium tuberculosis*, at a 500 mg/ml concentration. These results indicated that licorice can be used as a potential antitubercular agent.

Antifungal activity

The active component of *Glycyrrhiza glabra*, 18-beta glycyrrhetic acid (18-beta GA), was reported to inhibit *Candida albicans* growth at relatively low doses (6.2 µg/mL).

Dermatological effect

Ethanol extract of *Glycyrrhiza glabra* is reported to have a positive effect on skin, i.e., improvement in the viscoelastic and hydration properties of the skin. Licorice extract also has synergistic effects on UV protection and antioxidant and anti-inflammatory properties. Hence, it has a beneficial effect on the skin.

Antithrombotic effect

The compound isolated from licorice, GU-7, which is a 3-aryl coumarin derivative, is shown to possess antiplatelet activity. In addition, the anti-thrombotic effect of *Glycyrrhiza glabra* extract, along with vitamin K and heparin, was reported in Sprague-Dawley rats.

Anti-inflammatory activity

Licorice root extract has been used in the healing of ulcers of the stomach and mouth for 2000 years. According to studies, glycyrrhetic acid is responsible for this anti-inflammatory

effect. Glycyrrhetic acid inhibits cyclooxygenase activity and prostaglandin formation, and it also indirectly inhibits platelet aggregation.

Conclusion

The licorice plant has many more constituents and potential activities that are yet to be discovered. Accordingly, more studies have to be carried out to examine the biological activities of different parts of licorice plant for more beneficial utilization of the plant.

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Crop Breeding In Space

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Introduction

Agriculture specialists from all over the world are searching for cutting-edge equipment and methods to boost food production as a result of the demand from an ever-growing global population. The only viable option left for increasing crop production is to increase crop productivity per unit area since horizontal expansion of agriculture through the inclusion of more areas under cultivation has essentially reached a dead end and threat to agricultural land has increased due to rapid industrialization and urbanisation (FAO, 2017).

The development of short-statured, fertiliser-responsive, high yielding crop types has been made possible by the steadfast efforts of agricultural scientists, and the resulting bumper crop production (The Green Revolution) has revolutionised agriculture. Application of chemical fertilisers, water supply via irrigation, chemical crop protection from numerous pests and diseases, etc., revolutionised the agricultural landscape of the nation, but it was unable to last for an extended period of time. This is as a result of the major crops' yield status having essentially reached a plateau. Therefore, finding other methods to increase agricultural output in order to fulfil the rising demand is urgently needed.

In one of their most recent efforts, scientists have focused on using space to boost food productivity. A new section in agricultural sciences dubbed "Space-Breeding" has been added as a result of these efforts.

Breeding in space

Space breeding is the process of developing new genetic variants in seeds by launching them into space in a recoverable spaceship. Such space-

returned seeds, also known as space-bred seeds, go through extensive testing on Earth to find beneficial varieties. The eligible variations are released for commercial production after being assessed for yield and other quality indicators. In order to create hybrids, the beneficial space variants that are not acceptable for release as varieties are crossed with the standard seed plants.

On spacecraft, seeds are placed in orbit and held there for a set amount of time. The very energetic, densely ionising parts of heavy ions cause biological stress and injury in orbital circumstances in space. In zero gravity, seeds in space are exposed to a variety of high-energy cosmic radiation. Additionally, the seeds experience mutation under conditions like super-vacuum, microgravity, a variable magnetic field, prolonged space travel, etc. Genetic variants are present in the offspring of such altered seeds, some of which may be advantageous to us. Such variations can be used in breeding strategies since they are stable and heritable.

Evolution of space breeding

Crop improvement technology from space is already being used in nations like China, the US, and Russia. China is using space breeding techniques to improve terrestrial farming, in contrast to the USA and Russia, which are primarily focused on learning how plants grow in space and generating food for humans in orbit. The advancement of space breeding technologies has been astounding. It was claimed that rice seeds reproduced in outer space. The plants yield 10-15% more seeds than usual and mature early (by more than 5–20 days). The seeds exposed to space grew more quickly than seeds grown on earth, according to several test results. Additionally, the space-exposed seeds grew

more quickly than regular seeds did. The epidermal layer of the tomato seeds that were exposed to space was observed using scanning electron microscopy (SEM) and X-ray analysis to be more porous than the control seeds that were grown on earth. The rise in the growth pattern of the space seeds may be caused by this peculiar trait.

The vegetables grown in space are said to be more flavourful, vitamin-rich, and resistant to a variety of diseases and insect pests. According to reports, the common names for these plants, space pepper and space tomato, can reach weights of 500g and 800g, respectively. More minerals, trace elements, vitamin C, and soluble carbohydrates are present in space pepper. Similarly, muskmelon that was grown in space was altered to be bigger, sweeter, and had gorgeous skin colour (Workshop proceedings, China, 2005).

China has also included forestry in its space breeding technology. More than 30 different varieties of space-travelled herb seeds have reportedly been found to have larger leaves, stronger stems, superior quality, stable heredity, and increased disease resistance. Poplar tree seeds that spent more than 160 hours in "Shenzhou" in orbit had a greater germination rate and a quicker growth pattern (Chinadaily, 2023).

The success of space-bred crops has delighted China, which hopes that it will enable it to increase food production while improving crop quality to feed its expanding population. It has now focused on the International Space Station (ISS) in order to send some rice seeds there for extended periods of time and assess how the seeds fared upon returning to Earth.

Are crops bred in space safe?

Will food that has been exposed to unexplained quantities of cosmic rays be safe to consume? Why wouldn't the seeds from space evolve into plants that might cause ecological catastrophe? These are a few of the pointed inquiries that have

been made regarding the eating of crops that have been grown in space. However, according to scientists, foods grown in space are both eco-friendly and safe to eat. Because, unlike GM crops, space-bred crops don't contain any alien genes; any changes that take place are solely the result of mutations in their own genes. Therefore, neither the environment nor human health are threatened by the space-bred crops.

The long-term impacts of the space-bred crops have not yet been determined, despite the fact that they currently seem to be safe for the environment and human health. We know from experience that several drugs that were once believed to be safe for human intake eventually turned out to be lethal. Therefore, more thorough research must be started to prevent our future generations from suffering as a result of our rashness.

Conclusion

Crop breeding in space refers to the process of developing and adapting plant varieties for cultivation in the unique conditions of space environments, such as those found on space stations, spacecraft, or future extra-terrestrial colonies. The goal is to create crops that can thrive in microgravity, limited space, and controlled atmospheric conditions, allowing for sustainable food production during long-duration space missions or for the establishment of human settlements on other celestial bodies.

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Environmental Sustainability in Pharmaceutical Industry

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Introduction

The pharmaceutical industry has a substantial impact on the environment, especially when the materials used to make them and the chemicals that comprise them make their way directly into the environment. High energy usage, complex supply chains, and environmental contamination are some of the environmental challenges that the industry must solve (Mohit Mishra et al., 2021). The pharmaceutical industry at large as well as the average consumer can take steps to make the use of medicine more sustainable through both significant and relatively minor changes. The industry can increase value while simultaneously ensuring environmental sustainability by enhancing production processes and streamlining supply chains.

Pharmaceuticals have been a focus of research for many years among the enormous number of organic compounds that may infiltrate the environment due to their biological action and evidence of their existence in the environment, albeit in low quantities. Biological medications, such as vaccinations, antibodies, insulin, vitamins, and other protein-based drugs, are not considered hazardous to the environment because they are almost always digested by people and/or are easily biodegradable.

Non-biological medicinal chemicals may wind up in the environment in a variety of ways. Patients' usage of medications is thought to be the primary source. Pharmaceuticals are excreted by patients in the same form in which they were absorbed or supplied, or they are converted by the body into metabolites, which may be discharged into the environment via sewers and sewage treatment facilities. Additional sources of pollution include manufacturing facility

emissions and the improper disposal of old or expired medications.

Medicines and the environment

The drugs that we consume naturally enter our environment as our bodies turn them into waste. This issue becomes exacerbated when people intentionally dispose of unused medicine by flushing it down the drain. Although our water treatment systems are designed to take contaminants out of our wastewater before we re-introduce it to the natural environment, some still get through. These contaminants, which include those in medications, can damage the ecosystems they end up in.

High levels of oestrogen in water due to birth control pills, for example, can hamper the ability of fish to reproduce by altering their genes and reducing their population size. Once those chemicals find their way into the water, they enter the food chain and eventually impact animals that live on land too, including humans.

When we take medicine, a portion of it is expelled from our systems via pee or stools, which then travel a great distance to the sewage system. Human excretions are routed through a treatment plant in the sewage system, where many contaminants are eliminated. This cleaner water is then returned to rivers, which eventually reach the sea. River water is generally used for crop irrigation as well as drinking and other purposes. Groundwater is also used by humans for crop irrigation and drinking water production. Once again, abandoned pharmaceuticals can endanger both the environment and human health. Plants will absorb the chemicals from medications. Animals then eat these plants or drink the water and ingest the contaminants.

Humans might drink the water or eat the plants or animals, making pollution from pharmaceuticals a human health hazard as well. This problem becomes worse in the summer when livestock such as cattle require two to three times as much water as they do during other times of the year.

Proper disposal of medicines

If you have unused medications that you need to get rid of, don't flush them down the drain or throw them straight into the trash. The U.S. Food and Drug Administration (FDA) recommends one of several other options for the safe and sustainable disposal of medicines (FDA, 2020).

1. The easiest approach to disposing of most types of unused or expired drugs (including prescription and OTC) is to drop them off at a drug take back site, location, or program as soon as possible.

2. If you can't get to a drug-take-back location quickly, or there aren't any near you and your medicine is on the FDA's flush list, your next best option is to flush these potentially harmful medications down the toilet.

If the medication is not on the list, you should follow these guidelines to dispose of it in your home garbage.

Some communities have drug take-back programs that the Drug Enforcement Administration (DEA) approves. Some pharmacies also allow you to mail in or dispose of unused medications at kiosks. The DEA also organizes a national drug take-back day. The nationwide prescription medicine take-back day is scheduled for April 22, 2023, from 10 a.m. until 2 p.m. Its goal is to provide a secure, convenient, and responsible way to dispose of prescription drugs while simultaneously educating the general public about the dangers of medication abuse.

Although certain medications have recommendations on the label to flush them, you can dispose of the majority of them in your regular trash at home. The FDA recommends mixing them with something unpalatable, such

as dirt, kitty litter, or coffee grounds, in a plastic bag that you can seal. This disguises the drugs and prevents pets from getting into them. You can then throw the bag away. If you are throwing away a prescription medication container, be sure to scratch out all potentially identifying information to protect your privacy and identity.

Using medicines more sustainably

Another option for reducing the impact your use of medicine has on the environment is to use less of it or use more environmentally friendly medications. To use less medicine, only use it when you truly need it, and try substituting natural remedies for pharmaceuticals. Reach for naturally derived medications such as essential oils, vitamins, herbs, or a cup of hot tea. Always consult with your doctor before changing your medication regimen. As a long-term strategy, regular exercise and a healthy diet can do wonders for improving your overall health and decreasing your need to take medicines.

Sustainability from the industry's perspective

Making the pharmaceutical industry more sustainable isn't the sole responsibility of the consumer. The industry can also change its practices to manage pharmaceuticals in an eco-friendly fashion. One aspect of this involves energy use. The manufacturing and transportation of medications can be extremely energy-intensive. By using energy more efficiently and using cleaner energy, drug companies can reduce their environmental impact. Some companies adopt a high-level approach, establishing very explicit corporate standards. These requirements could include things like which ventilation system to buy, whether a system should have heat recovery, or how efficient a pump should be to save energy.

Some companies examine their current facilities and processes to determine how much energy is consumed per ton of medicine. They then attempt to lower this by setting energy consumption targets during operation. Similarly, when it comes to water, some businesses set particular goals for an entire site. As a result, if water consumption increases in one building or a new facility is constructed, they must find savings

elsewhere. This is especially common in water-stressed areas.

Expired medicines management

The Pharmaceutical industry can change its practices to manage pharmaceuticals in an eco-friendly manner. These corporations can also make an effort to include eco-friendly substances in their medications. While they may not be able to remove every non-natural chemical from their products, they can offer greener alternatives to consume and look into reducing the presence of damaging substances as much as possible (Pharmanewsintel 2023).

This applies not only to the organizations closest to consumers but to the entire supply chain. Medications are often vital to our health, but they can also have a negative impact on the health of our environment. Taking steps to manage pharmaceuticals more sustainably can enable us to protect our own well-being as well as that of our environment.

Conclusion

The pharmaceutical industry plays a pivotal role in global healthcare, but its activities also have significant environmental implications. Achieving environmental sustainability in the pharmaceutical sector is a multifaceted challenge that involves addressing issues such as resource consumption, waste generation, and the environmental impact of drug manufacturing. Companies are increasingly recognizing the importance of adopting eco-friendly practices to minimize their carbon footprint. Initiatives include the implementation of green chemistry

principles to reduce the use of hazardous materials and energy consumption during drug synthesis, as well as the development of more sustainable packaging materials. Additionally, there is a growing emphasis on responsible waste management, with efforts to minimize pharmaceutical residues in water systems and the promotion of recycling programs for packaging. Collaboration between industry stakeholders, regulatory bodies, and research institutions is essential to drive innovation and establish standards that prioritize both public health and environmental preservation in the pharmaceutical realm. As the industry continues to evolve, the integration of environmentally sustainable practices not only aligns with global efforts to combat climate change but also contributes to a healthier and more sustainable future.

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Efficient and Environment Friendly Synthesis of Mercapto Triazoles

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Abstract

Green chemistry is a contemporary method of chemical synthesis that strives to work at the molecular level to achieve sustainability. This consolidated set of twelve principles simultaneously targets environmental and economic goals. This article covers the synthesis of a library of 4-[4-(4-nitrophenoxy) phenyl]-5-substituted-2H-1,2,4-triazole-3-thiones containing different substituents at the 5-position. The mechanochemical treatment is followed to synthesize the target compounds **8 (a-p)** and the yields are compared with the conventional outcome. The title compounds are characterized by spectral and elemental analyses. *Keywords: Green Chemistry, mercapto triazoles, grinding synthesis*

Introduction

Green chemistry has played a pivotal role in maintaining global equilibrium by delineating fundamental principles for chemists and chemical engineers (1). As a consequence of organic synthetic conversions, a significant number of chemical substances, namely solvents, are wasted. Therefore, the examination of solvents represents a crucial endeavour in the field of green chemistry (2). Moreover, the customary solvents employed in practice are deemed toxic, flammable, and corrosive, which contribute to pollution of soil, water, and air due to their volatility and solubility. The exposure of workers to certain substances may pose a threat to their well-being, resulting in both minor and major accidents. Such substances are susceptible to cross-contamination and necessitate energy-intensive distillation for recovery and reprocessing. To address these shortcomings, chemists have embarked on a quest for safer alternatives, including solventless systems (3), water (4) supercritical fluids (5) and, more

recently, ionic liquids. Solventless systems are particularly appealing as they eliminate the need for removing solvents from reaction mixtures, thereby reducing the effort and energy required. This realization has led to a revamped approach that allows reactions to occur without the use of solvents.

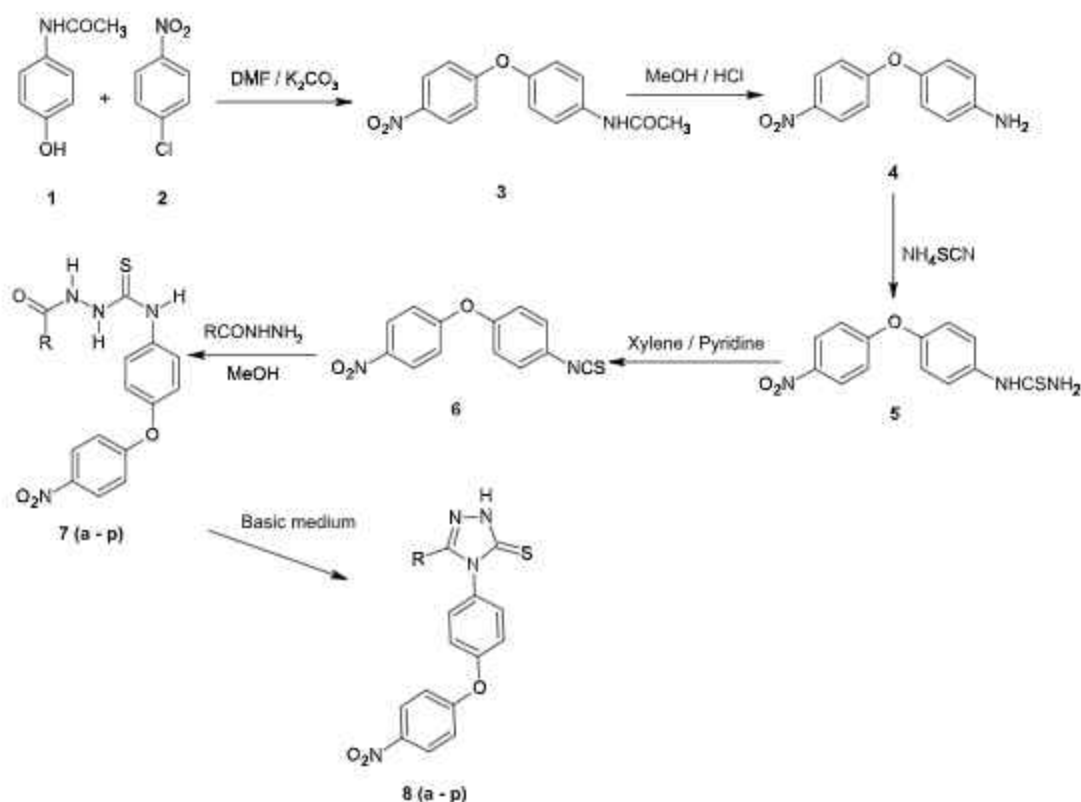
In accordance with microwave-assisted synthesis, the methodology of grinding-accelerated synthesis is gaining significant recognition within the field. This particular method involves the utilization of mechanical force during chemical synthesis. Notably, the salient features of grinding synthesis include shorter reaction times, higher yields, environmental friendliness, and operational simplicity. The present study outlines the grinding synthesis of novel 4[4-(4-nitrophenoxy) phenyl]-5-substituted-2H-1,2,4-triazole-3-thiones and their subsequent characterization.

Methodology

Synthesis of a library of 4-[4-(4-nitrophenoxy) phenyl]-5-substituted-2H-1,2,4-triazole-3-thiones (Scheme 1) (6) can be illustrated as follows-

Scheme 1, Where R = (a) phenyl, (b) 4-methylphenyl, (c) 4-methoxyphenyl, (d) 4-nitrophenyl, (e) 4-bromophenyl, (f) 4-chlorophenyl, (g) 2-furyl, (h) 2-thiophenyl, (i)3-methoxyphenyl, (j)2,4-dimethoxyphenyl, (k)3,4-dimethoxyphenyl, (l) 4-fluorophenyl, (m)2-benzofuryl, (n)3-chlorophenyl, (o)3-bromophenyl, (p)2,4-dichlorophenyl.

The target compounds are synthesized as per **scheme 1**. *p*-hydroxy acetanilide **1** is condensed



with *p*-chloronitrobenzene **2** in DMF at 135 °C-140 °C in presence of anhydrous K_2CO_3 . An excess amount of unreacted *p*-hydroxyacetanilide **1** is removed by washing with hot water. The second step involves the hydrolysis of *N*-[4-(4-nitrophenoxy)phenyl]acetamide **3** with hydrochloric acid in methanol. 4-(4-nitrophenoxy)aniline **4** is converted to 4-(4-nitrophenoxy)phenyl thiourea **5** in an environmentally benign method by heating **4** with ammonium thiocyanate in water at 100 °C. 4-(4-nitrophenoxy)phenyl thiourea **5** is refluxed in a nonpolar solvent, xylene, in presence of pyridine to afford isothiocyanato-4-(4-nitrophenoxy)benzene **6**. Thus, use of hazardous chemicals like thiophosgene, CS_2 , and NaOCl for the conversion of 4-(4-nitrophenoxy)aniline **4** to isothiocyanato-4-(4-nitrophenoxy)benzene **6** can be avoided. Isothiocyanato-4-(4-nitrophenoxy)benzene **6** is then treated with different synthesized aryl carbohydrazides to yield *N*-[4-(4-nitrophenoxy)phenyl]-2-(phenylcarbonyl)hydrazinecarbothioamide **7 (a-p)**. Cyclodehydration of **7 (a-p)** in basic medium affords 4-[4-(4-nitrophenoxy)phenyl]-5-substituted-2H-1,2,4-triazole-3-thiones **8 (a-p)**. This reaction is carried out by both the grinding

method and the conventional method. The grinding method is a solid-phase synthesis that requires a shorter duration (12 min) and occurs at room temperature, resulting in better yields. On the other hand, conventional synthesis needs heating in a solvent at 80 °C for about an hour.

Results

The melting points are ascertained on the Thomas Hoover apparatus and are uncorrected. IR (KBr) spectra are recorded on a Shimadzu 8300 Fourier transform infrared spectrometer. 1H NMR spectra are recorded on Bruker AM 400 MHz spectrometer and ^{13}C NMR spectra are recorded on a Bruker AM 100 MHz spectrometer, using DMSO- d_6 as a solvent and TMS as an internal standard (chemical shift in ppm). Elemental analyses are obtained on a Vario-EL instrument. Thin layer chromatography (TLC) is conducted on 0.25 mm silica gel plates (60F₂₅₄, Merck). Visualization is done with ultraviolet light. The solvents are evaporated with a BUCHI rotary evaporator.

N-[4-(4-nitrophenoxy)phenyl]acetamide (**3**): *p*-hydroxyacetanilide **1** (6.00 g, 0.039 mol) is dissolved in DMF (20 ml) under room

temperature (r.t.) stirring. *p*-chloronitrobenzene (6.26 g, 0.039 mol) **2** and anhydrous K_2CO_3 (2.75 g, 0.0198 mol) are then added to the reaction mixture. This brown reaction mass is stirred at r.t. for 30 min. The temperature of the reaction mass is slowly raised to 135-140C under stirring. This temperature is maintained at 135-140C for 1 hr as monitored by TLC (Hexane: EtOAc; 1:1). Cool the reaction mass and wash the yellow precipitate formed with a 25 % NaOH solution. Suck dry the solid upon a hot water wash and crystallized from methanol to afford *N*-[4-(4-nitrophenoxy)phenyl] acetamide **3** as yellow solid (10.17 g, 94 %); mp 146-148 °C; R_f 0.81; IR (KBr): ν_{max} 3291, 1656, 1342, 1235 cm^{-1} ; 1H NMR (DMSO-*d*₆, 400 MHz): δ = 8.32-8.24 (4H, m, ArH), 7.64-7.36 (4H, m, ArH), 3.22 (1H, s, NH), 2.20 (3H, s, CH₃). ^{13}C NMR (DMSO-*d*₆, 100 MHz): δ = 186.7(C, CO), 162.4 (C, C-6), 153.6(C, C-5), 143.5(C, C-9), 128.2(CH, C-3, C-3'), 126.9(C, C-2), 126.1(CH, C-8, C-8'), 121.3(CH, C-4, C-4'), 117.5(CH, C-7, C-7'); Anal. Calcd. for C₁₄H₁₂N₂O₄: C, 61.76; H, 4.44; N, 10.29. Found: C, 61.72; H, 4.49; N, 10.27.

4-(4-nitrophenoxy) aniline (**4**): Reflux *N*-[4-(4-nitrophenoxy)phenyl]acetamide **3** (8.00 g, 0.029 mol) and Conc. HCl (18 ml) in methanol (16 ml) for 20 minutes. Upon completion of the reaction as marked by TLC (Hexane: EtOAc; 1:1), the reaction mass is cooled down, and the yellow solid formed is washed with chilled water. This solid is crystallized from methanol to yield 4-(4-nitrophenoxy) aniline **4** (6.94 g, 89 %); mp 228-230 °C; R_f 0.76; IR (KBr): ν_{max} 3120, 1451, 1335, 1244 cm^{-1} ; 1H NMR (DMSO-*d*₆, 400 MHz): δ = 8.26-7.58 (4H, m, ArH), 7.25-7.14 (4H, m, ArH), 3.71 (2H, bs, NH₂); ^{13}C NMR (DMSO-*d*₆, 100 MHz): δ = 162.2(C, C-6), 154.6(C, C-5), 143.7(C, C-9), 128.4(CH, C-3, C-3'), 127.3(C, C-2), 126.5(CH, C-8, C-8'), 121.1(CH, C-4, C-4'), 118.1(CH, C-7, C-7'); Anal. Calcd. for C₁₂H₁₀N₂O₃: C, 62.60; H, 4.38; N, 12.17. Found: C, 62.55; H, 4.33; N, 12.13.

4-(4-nitrophenoxy) phenyl thiourea (**5**): Ammonium thiocyanate (4.27 g, 0.056 mol) is stirred in water (20 ml) for 15 min at r.t. to get a clear solution. Add 4-(4-nitrophenoxy) aniline **4** (5.00 g, 0.018 mol) and reflux at 100 °C for 2h as indicated by TLC (Hexane: EtOAc; 1:1). Cool

and filter off the yellow solid, wash with hot water. Recrystallize from methanol to offer 4-(4-nitrophenoxy)phenyl thiourea **5** (4.60 g, 85 %); mp 190-192C; R_f 0.4; IR (KBr): ν_{max} 3352, 3280, 1428, 1351, 1328, 1212 cm^{-1} ; 1H NMR (DMSO-*d*₆, 400 MHz): δ = 8.26-7.44 (4H, m, ArH), 7.26-7.13 (4H, m, ArH), 2.60 (1H, bs, NH), 2.48 (2H, bs, NH₂). ^{13}C NMR (DMSO-*d*₆, 100 MHz) δ 172.5 (C, CS), 166.4(C, C-6), 153.7(C, C-5), 143.5(C, C-9), 127.7(CH, C-3, C-3'), 127.3(C, C-2), 126.1(CH, C-8, C-8'), 121.9(CH, C-4, C-4'), 117.3(CH, C-7, C-7'); Anal. Calcd. for C₁₃H₁₁N₃O₃S: C, 53.97; H, 3.83; N, 14.52. Found: C, 53.99; H, 3.80; N, 14.56.

Isothiocyanato-4-(4-nitrophenoxy)benzene (**6**): Dissolve 4-(4-nitrophenoxy)phenyl thiourea **5** (4.0 g, 0.013 mol) in xylene (20 ml) and add a catalytic amount of pyridine (0.1 ml), reflux at 140C for 45 min as indicated by TLC (Hexane: EtOAc; 1:1). Gradually cool the reaction mass and stir at r.t. for 30 min. Filter off the insoluble solid and remove the xylene *in vacuo*. The yellow solid thus obtained is crystallized from methanol to provide isothiocyanato-4-(4-nitrophenoxy)benzene **6** (3.08 g, 82 %); mp 120-122 °C; R_f 0.73; IR (KBr): ν_{max} 2115, 1489, 1358, 1238, 842 cm^{-1} ; 1H NMR (DMSO-*d*₆, 400 MHz): δ = 8.26-7.51 (4H, m, ArH), 7.25-7.14 (4H, m, ArH); ^{13}C NMR (DMSO-*d*₆, 100 MHz): δ = 162.4 (C, C-6), 153.9(C, C-5), 143.1(C, C-9), 134.1(C, NCS), 128.4 (CH, C-3, C-3'), 127.2 (C, C-2), 126.3 (CH, C-8, C-8'), 121.1 (CH, C-4, C-4'), 117.7 (CH, C-7, C-7'); Anal. Calcd. for C₁₃H₈N₂O₃S: C, 57.35; H, 2.96; N, 10.29. Found: C, 57.30; H, 2.99; N, 10.22.

Representative procedure for *N*-[4-(4-nitrophenoxy) phenyl]-2-(phenylcarbonyl) hydrazinecarbothioamide **7 (a-p)**: Reflux an equimolar proportion of compound **6** and aryl carbohydrazide in methanol (10 vol.) for 1 h. Upon cooling, the resulting solid is filtered off and crystallized from methanol to afford pure *N*-[4-(4-nitrophenoxy) phenyl]-2-(phenylcarbonyl) hydrazinecarbothioamide **7 (a-p)**.

4-[4-(4-nitrophenoxy) phenyl]-5-substituted-2*H*-1,2,4-triazole-3-thiones **8 (a-p)**: typical procedure

Conventional method

Reflux hydrazinecarbothioamide **7 (a-p)** (0.3 mmol) with aqueous 5% NaOH solution (10 mL) for 1 h; check the clear solution with TLC (Toluene: EtOAc; 2:1). Cool and acidify with 6 N HCl and crystallize from ethanol.

Grinding method

Grind hydrazinecarbothioamide **7 (a-p)** (0.3 mmol) and NaOH pellets (0.6 mmol) in a porcelain mortar with a pestle for 12 min at r.t. Monitor the pasty reaction mixture by TLC (Toluene: EtOAc; 2:1), pour into ice cold water and acidify with 6 N HCl. Filter off the crude solid and crystallize it with ethanol.

4-[4-(4-nitrophenoxy)phenyl]-5-phenyl-2*H*-1,2,4-triazole-3-thione (**8a**): *R*_f 0.57; IR (KBr): ν_{\max} 3474, 3142, 1554, 1542, 1358, 1224 cm^{-1} ; ¹H NMR (DMSO-*d*₆ 400 MHz): δ = 14.07 (1H, s, NH), 8.36-7.68 (4H, m, ArH), 7.64-7.40 (4H, m, ArH), 7.36-6.92 (5H, m, ArH); ¹³C NMR (DMSO-*d*₆, 100 MHz): δ = 167.4(C, C-5), 160.2(C, C-3), 156.7(C, C-14), 149.3(C, C-13), 143.1(C, C-17), 131.6(C, C-6), 130.6 (CH, C-8, C-8'), 129.1(C, C-9), 128.6(CH, C-7, C-7'), 128.5(CH, C-11, C-11'), 128.3(C, C-10), 126.9(CH, C-16, C-16'), 122.6(CH, C-12, C-12'), 118.7(CH, C-15, C-15'); Anal. Calcd. for C₂₀H₁₄N₄O₃S: C, 61.53; H, 3.61; N, 14.35. Found: C, 61.57; H, 3.63; N, 14.38.

4-[4-(4-nitrophenoxy) phenyl]-5-(4-methylphenyl)-2*H*-1,2,4-triazole-3-thione (**8b**): *R*_f 0.68; IR (KBr): ν_{\max} 3452, 3052, 1526, 1502, 1326, 1240 cm^{-1} ; ¹H NMR (DMSO-*d*₆ 400 MHz): δ = 14.12 (1H, s, NH), 8.32-7.32 (4H, m, ArH), 7.25-7.12 (4H, m, ArH), 6.98-6.88 (4H, m, ArH), 2.68 (3H, s, CH₃); ¹³C NMR (DMSO-*d*₆, 100 MHz): δ = 168.5(C, C-5), 161.4(C, C-3), 160.7(C, C-14), 155.2(C, C-13), 143.4(C, C-17), 138.3(C, C-6), 132.7(CH, C-11, C-11'), 130.2(C, C-10), 129.6(CH, C-7, C-7'), 128.7(C, C-9), 126.1(CH, C-16, C-16'), 122.6(CH, C-12, C-12'), 118.2(CH, C-8, C-8'), 116.8(CH, C-15, C-15'), 21.5(CH₃, CH₃); Anal. Calcd. for C₂₁H₁₆N₄O₃S: C, 62.36; H, 3.99; N, 13.85. Found: C, 62.35; H, 3.91; N, 13.80.

4-[4-(4-nitrophenoxy)phenyl]-5-(4-methoxyphenyl)-2*H*-1,2,4-triazole-3-thione

(**8c**): *R*_f 0.62; IR (KBr): ν_{\max} 3448, 3247, 1596, 1504, 1334, 1249 cm^{-1} ; ¹H NMR (DMSO-*d*₆ 400 MHz): δ = 14.07 (1H, s, NH), 8.34-7.46 (4H, m, ArH), 7.33-7.26 (4H, m, ArH), 7.23-6.95 (4H, m, ArH), 3.71(3H, s, OCH₃); ¹³C NMR (DMSO-*d*₆, 100 MHz): δ = 168.8(C, C-5), 162.4(C, C-3), 161.1(C, C-6), 155.3(C, C-14), 150.9(C, C-13), 143.2(C, C-17), 131.9(C, C-9), 131.5(CH, C-8, C-8'), 130.2(CH, C-11, C-11'), 126.8(C, C-10), 121.2(CH, C-16, C-16'), 118.5(CH, C-12, C-12'), 118.3(CH, C-7, C-7'), 114.5(CH, C-15, C-15'), 55.7(CH₃, OCH₃); Anal. Calcd. for C₂₁H₁₆N₄O₄S: C, 59.99; H, 3.84; N, 13.33. Found: C, 59.91; H, 3.87; N, 13.39.

4-[4-(4-nitrophenoxy)phenyl]-5-(4-nitrophenyl)-2*H*-1,2,4-triazole-3-thione (**8d**): *R*_f 0.61; IR (KBr): ν_{\max} 3409, 3070, 1596, 1504, 1334, 1242 cm^{-1} ; ¹H NMR (DMSO-*d*₆ 400 MHz): δ = 14.21 (1H, s, NH), 8.34-8.16 (4H, m, ArH), 7.54-7.42 (4H, m, ArH), 7.26-6.99 (4H, m, ArH); ¹³C NMR (DMSO-*d*₆, 100 MHz): δ = 170.1(C, C-5), 164.8(C, C-3), 155.5(C, C-14), 150.2(C, C-13), 148.6(C, C-6), 145.3(C, C-17), 135.2(C, C-9), 130.7(CH, C-11, C-11'), 130.1(C, C-10), 129.9(CH, C-8, C-8'), 126.4(CH, C-16, C-16'), 123.7(CH, C-7, C-7'), 122.8(CH, C-12, C-12'), 119.9(CH, C-15, C-15'); Anal. Calcd. for C₂₀H₁₃N₅O₅S: C, 55.17; H, 3.01; N, 16.08. Found: C, 55.12; H, 3.08; N, 16.03.

4-[4-(4-nitrophenoxy)phenyl]-5-(4-bromophenyl)-2*H*-1,2,4-triazole-3-thione (**8e**): *R*_f 0.71; IR (KBr): ν_{\max} 3389, 1589, 1514, 1366, 1241 cm^{-1} ; ¹H NMR (DMSO-*d*₆ 400 MHz): δ = 14.14 (1H, s, NH), 8.30-7.60 (4H, m, ArH), 7.40-7.21 (4H, m, ArH), 7.03-6.94 (4H, m, ArH); ¹³C NMR (DMSO-*d*₆, 100 MHz): δ = 167.2(C, C-5), 163.6 (C, C-9), 161.9(C, C-3), 154.6(C, C-14), 148.3(C, C-13), 143.6(C, C-17), 134.5(CH, C-11, C-11'), 133.6 (C, C-6), 132.0(C, C-10), 128.1(CH, C-16, C-16'), 123.5(CH, C-12, C-12'), 122.1 (CH, C-7, C-7'), 118.6(CH, C-15, C-15'), 114.2 (CH, C-8, C-8'); Anal. Calcd. for C₂₀H₁₃BrN₄O₃S: C, 51.18; H, 2.79; N, 11.94. Found: C, 51.14; H, 2.75; N, 11.89.

4-[4-(4-nitrophenoxy)phenyl]-5-(4-chlorophenyl)-2*H*-1,2,4-triazole-3-thione (**8f**): *R*_f 0.73; IR (KBr): ν_{\max} 3415, 3072, 1591, 1507, 1340, 1251, 745 cm^{-1} ; ¹H NMR (DMSO-*d*₆ 400

MHz): δ = 14.21 (1H, s, NH), 8.34-7.81 (4H, m, ArH), 7.68 -7.54 (4H, m, ArH), 7.48 -7.28 (4H, m, ArH); ^{13}C NMR (DMSO- d_6 , 100 MHz): δ = 169.6(C, C-5), 162.1(C, C-3), 154.8(C, C-14), 149.7(C, C-13), 143.4(C, C-17), 135.4(C, C-6), 132.6(CH, C-7, C-7'), 131.8(CH, C-11, C-11'), 130.2(CH, C-8, C-8'), 129.9(C, C-10), 127.4(C, C-9), 126.4(CH, C-16, C-16'), 121.5(CH, C-12, C-12'), 116.7(CH, C-15, C-15'); Anal. Calcd. for $\text{C}_{20}\text{H}_{13}\text{ClN}_4\text{O}_5\text{S}$: C, 56.54; H, 3.08; N, 13.19. Found: C, 56.60; H, 3.06; N, 13.10.

4-[4-(4-nitrophenoxy)phenyl]-5-(furan-2-yl)-2H-1,2,4-triazole-3-thione (**8g**): R_f 0.63; IR (KBr): ν_{max} 3438, 3042, 1591, 1504, 1334, 1244 cm^{-1} ; ^1H NMR (DMSO- d_6 400 MHz): δ = 14.24 (1H, s, NH), 8.26-7.68 (4H, m, ArH), 7.62-7.36 (4H, m, ArH), 7.33-7.22 (3H, m, ArH); ^{13}C NMR (DMSO- d_6 , 100 MHz): δ = 168.2(C, C-5), 162.5(C, C-3), 158.8(C, C-14), 151.6(C, furan C-6), 149.5(C, C-13), 143.7(C, C-17), 140.9(CH, furan C-9), 132.3(CH, C-11, C-11'), 130.6 (C, C-10), 127.9 (CH, C-16, C-16'), 122.8(CH, C-12, C-12'), 117.5(CH, C-15, C-15'), 110.3(CH, furan C-8), 106.1(CH, furan C-7); Anal. Calcd. for $\text{C}_{18}\text{H}_{12}\text{N}_4\text{O}_4\text{S}$: C, 56.84; H, 3.18; N, 14.73. Found: C, 56.80; H, 3.11; N, 14.75.

4-[4-(4-nitrophenoxy)phenyl]-5-(thiophen-2-yl)-2H-1,2,4-triazole-3-thione (**8h**): R_f 0.63; IR (KBr): ν_{max} 3432, 3241, 1573, 1512, 1339, 1245 cm^{-1} ; ^1H NMR (DMSO- d_6 400 MHz): δ = 14.18 (1H, s, NH), 8.14-7.76 (4H, m, ArH), 7.68-7.46 (4H, m, ArH), 7.38-7.16 (3H, m, ArH). ^{13}C NMR (DMSO- d_6 , 100 MHz): δ = 169.1(C, C-5), 162.6(C, C-3), 158.2(C, C-14), 149.9(C, C-13), 144.7(C, C-17), 133.3 (CH, C-11, C-11'), 130.4(C, C-10), 129.3(C, thiophene C-6), 126.6(CH, C-16, C-16'), 126.0(CH, thiophene C-9), 124.3(CH, thiophene C-8), 122.8(CH, C-12, C-12'), 118.3(CH, C-15, C-15'), 117.6(CH, thiophene C-7); Anal. Calcd. for $\text{C}_{18}\text{H}_{12}\text{N}_4\text{O}_3\text{S}_2$: C, 54.53; H, 3.05; N, 14.13. Found: C, 54.59; H, 3.01; N, 14.16.

4-[4-(4-nitrophenoxy)phenyl]-5-(3-methoxyphenyl)-2H-1,2,4-triazole-3-thione (**8i**): R_f 0.61; IR (KBr): ν_{max} 3436, 3240, 1588, 1503, 1368, 1226 cm^{-1} ; ^1H NMR (DMSO- d_6 400 MHz): δ = 14.03 (1H, s, NH), 8.36-7.44 (4H, m,

ArH), 7.33-7.26 (4H, m, ArH), 7.11-6.83 (4H, m, ArH), 3.75(3H, s, OCH_3); ^{13}C NMR (DMSO- d_6 , 100 MHz): δ = 167.9 (C, C-5), 162.1(C, C-3), 160.9(C, C-6), 155.3(C, C-14), 150.6(C, C-13), 145.7(C, C-17), 131.8(C, C-9), 131.5(CH, C-8, C-8'), 130.5(CH, C-11, C-11'), 126.9(C, C-10), 122.4(CH, C-16, C-16'), 119.1(CH, C-12, C-12'), 118.2(CH, C-7, C-7'), 114.5(CH, C-15, C-15'), 53.2(CH_3 , OCH_3); Anal. Calcd. for $\text{C}_{20}\text{H}_{13}\text{BrN}_4\text{O}_5\text{S}$: C, 51.18; H, 2.79; N, 11.94. Found: C, 51.11; H, 2.77; N, 11.97.

4-[4-(4-nitrophenoxy)phenyl]-5-(2,4-dimethoxyphenyl)-2H-1,2,4-triazole-3-thione (**8j**): R_f 0.62; IR (KBr): ν_{max} 3413, 3245, 1587, 1513, 1345, 1268 cm^{-1} ; ^1H NMR (DMSO- d_6 400 MHz): δ = 14.01 (1H, s, NH), 8.15-7.25 (4H, multiplet, ArH), 7.16-7.08 (4H, multiplet, ArH), 6.88-6.72 (3H, multiplet, ArH), 3.02 (6H, s, OCH_3); ^{13}C NMR (DMSO- d_6 , 100 MHz): δ = 164.8 (C, C-5), 161.4(C, C-3), 160.9 (C, C-9), 159.0 (C, C-11), 154.2 (C, C-16), 150.6(C, C-15), 147.9(C, C-19), 130.7(CH, C-13, C-13'), 127.6 (CH, C-7), 126.8(C, C-12), 124.0(CH, C-18, C-18'), 123.7 (C, C-6), 119.3(CH, C-14, C-14'), 116.1(CH, C-17, C-17'), 115.1 (CH, C-8), 114.8 (C, C-10), 54.7(CH_3 , OCH_3); Anal. Calcd. for $\text{C}_{22}\text{H}_{18}\text{N}_4\text{O}_5\text{S}$: C, 58.66; H, 4.03; N, 12.44. Found: C, 58.60; H, 4.05; N, 12.49.

4-[4-(4-nitrophenoxy)phenyl]-5-(3,4-dimethoxyphenyl)-2H-1,2,4-triazole-3-thione (**8k**): R_f 0.62; IR (KBr): ν_{max} 3455, 3211, 1579, 1498, 1328, 1214 cm^{-1} ; ^1H NMR (DMSO- d_6 400 MHz): δ = 14.01 (1H, s, NH), 8.15-7.25 (4H, m, ArH), 7.18-7.10 (4H, m, ArH), 6.88-6.73 (3H, m, ArH), 3.11 (6H, s, OCH_3); ^{13}C NMR (DMSO- d_6 , 100 MHz): δ = 165.2 (C, C-5), 162.4(C, C-3), 157.9(C, C-16), 153.3 (C, C-15), 150.2 (C, C-10), 146.2(C, C-19), 145.5 (C, C-9), 144.5 (C, C-6), 133.7(CH, C-31, C-13'), 127.6 (CH, C-7), 125.5 (C, C-12), 121.8(CH, C-18, C-18'), 119.1(CH, C-14, C-14'), 114.8 (CH, C-8), 113.6(CH, C-17, C-17'), 113.2 (CH, C-11), 51.1(CH_3 , OCH_3); Anal. Calcd. for $\text{C}_{22}\text{H}_{18}\text{N}_4\text{O}_5\text{S}$: C, 58.66; H, 4.03; N, 12.44. Found: C, 58.62; H, 4.09; N, 12.41.

4-[4-(4-nitrophenoxy)phenyl]-5-(4-fluorophenyl)-2H-1,2,4-triazole-3-thione (**8l**):

R_f 0.69; IR (KBr): ν_{\max} 3448, 3078, 1596, 1504, 1342, 1242 cm^{-1} ; ¹H NMR (DMSO-d₆ 400 MHz): δ = 14.23 (1H, s, NH), 8.34-7.64 (4H, m, ArH), 7.50-7.31 (4H, m, ArH), 7.29-7.20 (4H, m, ArH); ¹³C NMR (DMSO-d₆, 100 MHz): δ = 169.4(C, C-5), 162.6(C, C-3), 154.8(C, C-14), 149.9(C, C-13), 143.1(C, C-17), 132.9(CH, C-7, C-7'), 132.0(CH, C-11, C-11'), 131.3(CH, C-8, C-8'), 130.1(C, C-10), 126.9(C, C-9), 126.6(CH, C-16, C-16'), 123.4(C, C-6), 121.1(CH, C-12, C-12'), 118.4(CH, C-15, C-15'); Anal. Calcd. for C₂₀H₁₃N₄O₃S: C, 58.82; H, 3.21; N, 13.72. Found: C, 58.86; H, 3.21; N, 13.79.

4-[4-(4-nitrophenoxy)phenyl]-5-(benzofuran-2-yl)-2H-1,2,4-triazole-3-thione (**8m**): R_f 0.66; IR (KBr): ν_{\max} 3481, 3042, 1565, 1492, 1313, 1269 cm^{-1} ; ¹H NMR (DMSO-d₆ 400 MHz): δ = 14.20 (1H, s, NH), 8.24-7.66 (4H, m, ArH), 7.56-7.30 (4H, m, ArH), 7.77-7.28 (5H, m, ArH); ¹³C NMR (DMSO-d₆, 100 MHz): δ = 167.9 (C, C-5), 161.5(C, C-3), 156.0(C, C-18), 155.7 (C, C-12), 149.1(C, C-17), 143.2(C, C-21), 134.6(CH, C-15, C-15'), 130.9 (C, C-14), 127.9 (CH, C-20, C-20'), 127.8 (CH, C-7), 127.3 (C, C-6), 126.9 (CH, C-8), 125.4(CH, C-16, C-16'), 124.7 (C, C-13), 123.2 (CH, C-9), 117.3(CH, C-19, C-19'), 113.5 (CH, C-11), 111.7 (CH, C-10). Anal. Calcd. for C₂₂H₁₄N₄O₃S: C, 61.39; H, 3.28; N, 13.02. Found: C, 61.38; H, 3.24; N, 13.05.

4-[4-(4-nitrophenoxy)phenyl]-5-(3-bromophenyl)-2H-1,2,4-triazole-3-thione (**8n**): R_f 0.71; IR (KBr): ν_{\max} 3476, 1586, 1504, 1342, 1242, 667 cm^{-1} ; ¹H NMR (DMSO-d₆ 400 MHz): δ = 14.26 (1H, s, NH), 8.64-7.91 (4H, m, ArH), 7.68-7.48 (4H, m, ArH), 7.18-7.08 (4H, m, ArH); ¹³C NMR (DMSO-d₆, 100 MHz): δ = 168.1(C, C-5), 164.8(C, C-3), 154.8(C, C-16), 148.3 (C, C-15), 146.1(C, C-19), 140.2 (C, C-6), 136.7(CH, C-13, C-13'), 132.9(C, C-12), 130.2 (C, C-10), 127.6 (CH, C-7), 126.6(CH, C-18, C-18'), 123.9(CH, C-14, C-14'), 120.9(CH, C-17, C-17'), 114.8 (CH, C-8), 114.1 (CH, C-9), 112.2 (CH, C-11); Anal. Calcd. for C₂₀H₁₃BrN₄O₃S: C, 51.18; H, 2.79; N, 11.94. Found: C, 51.19; H, 2.70; N, 11.92.

4-[4-(4-nitrophenoxy)phenyl]-5-(3-chlorophenyl)-2H-1,2,4-triazole-3-thione (**8o**): R_f 0.73; IR (KBr): ν_{\max} 3428, 3065, 1587, 1413, 1244, 781 cm^{-1} ; ¹H NMR (DMSO-d₆ 400 MHz): δ = 14.23 (1H, s, NH), 8.34-7.64 (4H, m, ArH), 7.50-7.31 (4H, m, ArH), 7.29-7.20 (4H, m, ArH); ¹³C NMR (DMSO-d₆, 100 MHz): δ = 169.4(C, C-5), 161.8(C, C-3), 159.7(C, C-16), 150.3(C, C-15), 143.4(C, C-19), 140.9(CH, C-13, C-13'), 139.0(C, C-6), 135.6 (C, C-10), 130.2 (CH, C-8), 128.7 (CH, C-9), 128.5 (C, C-12), 126.9 (CH, C-11), 126.7(CH, C-18, C-18'), 124.6 (CH, C-7), 121.5(CH, C-14, C-14'), 117.0 (CH, C-17, C-17'); Anal. Calcd. for C₂₀H₁₃ClN₄O₃S: C, 56.54; H, 3.08; N, 13.19. Found: C, 56.56; H, 3.10; N, 13.13.

4-[4-(4-nitrophenoxy)phenyl]-5-(2,4-dichlorophenyl)-2H-1,2,4-triazole-3-thione (**8p**): R_f 0.73; IR (KBr): ν_{\max} 3430, 3012, 1515, 1489, 1246, 730 cm^{-1} ; ¹H NMR (DMSO-d₆ 400 MHz): δ = 14.19 (1H, s, NH), 8.31-7.72 (4H, m, ArH), 7.58-7.39 (4H, m, ArH), 7.22-7.14 (3H, m, ArH); ¹³C NMR (DMSO-d₆, 100 MHz): δ = 167.4(C, C-5), 161.4(C, C-3), 154.9(C, C-16), 146.1(C, C-15), 143.2(C, C-19), 136.3 (C, C-6), 135.9 (C, C-9), 135.0(CH, C-13, C-13'), 134.0 (C, C-11), 129.7(C, C-12), 129.5 (CH, C-10), 128.8 (CH, C-8), 128.6 (CH, C-7), 126.9(CH, C-18, C-18'), 123.5(CH, C-14, C-14'), 116.6(CH, C-17, C-17'); Anal. Calcd. for C₂₀H₁₂Cl₂N₄O₃S: C, 52.30; H, 2.63; N, 12.20. Found: C, 52.28; H, 2.60; N, 12.26.

Discussion

In the IR spectra of compounds **8 (a-p)**, carbonyl absorption around 1750 cm^{-1} and SH stretching in the characteristic region of 2540 cm^{-1} are absent. Their ¹H NMR signals for NH emerging downfield around δ 14.0 authenticates the existence of the ring in thione form. In the ¹³C NMR spectra, C-3 carbons have shown up around δ 168 and C-5 carbons around δ 162, denoting the formation of target compounds. Other signals are observed in the respective regions. A comparison of the yields obtained by grinding and conventional methods is given in Table I.

Table 1 Physical data of synthesized compounds **8 (a-p)**

Compound	Melting point	Yield	
		Conventional	Grinding
8a	192 -194	(0.093 g, 80 %)	(0.096 g, 82 %)
8b	226 -228	(0.107 g, 89 %)	(0.113 g, 93 %)
8c	248 -250	(0.109 g, 87 %)	(0.112 g, 89 %)
8d	268 -270	(0.096 g, 74 %)	(0.097 g, 75 %)
8e	230 -232	(0.114 g, 81 %)	(0.118 g, 84 %)
8f	254 -256	(0.101 g, 79 %)	(0.105 g, 83 %)
8g	202 -204	(0.093 g, 82 %)	(0.096 g, 85 %)
8h	212 -214	(0.099 g, 84 %)	(0.105 g, 89 %)
8i	236-238	(0.107 g, 85 %)	(0.109 g, 87 %)
8j	250-252	(0.110 g, 82 %)	(0.114 g, 85 %)
8k	260-262	(0.110 g, 82 %)	(0.113 g, 84 %)
8l	218-220	(0.104 g, 85 %)	(0.107 g, 88 %)
8m	206-208	(0.108 g, 84 %)	(0.113 g, 88 %)
8n	244-246	(0.096 g, 76 %)	(0.096 g, 76 %)
8o	240-242	(0.115 g, 82 %)	(0.116 g, 83 %)
8p	218-220	(0.101 g, 74 %)	(0.101 g, 74 %)

In conventional synthesis, there is an unceasing exchange of protons between the solvent and the reactants until the product is realised. The product formation thus needs a longer reaction time. On the other hand, grinding synthesis is solvent-free. Hence, there is no exchange of protons. In contrast to traditional methods, solid-state organic reactions are more efficient, selective, and require less time to accomplish. Higher concentrations of reactants in the absence of solvents paved the way for more favourable kinetics than in the solution phase. Such grinding syntheses can be explored further for other molecules to have greener organic conversions (7, 8)

The use of environmentally benign green solvents remains ancillary, as they must be separated from desired products. This matter of separation must be taken into consideration when choosing the appropriate solvent, if not using solventless systems.

Statement of conflict of interest

The author declares no conflict of interest with regard to the materials discussed in this manuscript.

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