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DECIPHERING THE MECHANISMS OF REACTIVE OXYGEN SPECIES GENERATION AND THEIR MULTIFACETED EFFECTS ON PLANT CELLULAR PROCESSES

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Abstract: Reactive Oxygen Species (ROS) play a significant role in plant development and cellular processes. ROS are molecules that are generated as by-products during various cellular reactions, including photosynthesis and respiration. While ROS are known to have harmful effects on plants in high concentrations, they are also essential signaling molecules that regulate many physiological processes in plants. ROS regulates plant development by controlling the balance between cell growth and death. For example, in roots, ROS promote cell elongation and differentiation, while in leaves, they regulate leaf size and shape. ROS also play a role in regulating the plant's response to environmental stressors, such as drought and salinity. In addition to regulating plant development, ROS also participate in various cellular processes, including hormone signalling, defence responses, and programmed cell death. ROS are involved in the synthesis and signalling of plant hormones, such as abscisic acid, which is critical in regulating plant responses to abiotic stress. ROS also play a role in the activation of defence responses, such as the synthesis of pathogenesis-related proteins and the induction of systemic acquired resistance. Furthermore, ROS are involved in programmed cell death, which is essential for plant growth and development, as well as for the defence against pathogens. In conclusion, ROS play a crucial role in plant development and cellular processes. While high levels of ROS can be harmful to plants, low levels of ROS are essential for regulating plant growth, development, and responses to environmental stressors. Understanding the role of ROS in plants is important for developing strategies to enhance plant growth and productivity in agricultural settings while also helping plants to cope with the challenges of changing environmental conditions.

Keywords: Cellular processes, Oxidative stress, Mitochondria, Developmental stages

INTRODUCTION

Genotypic and environmental factors continuously influence and drive plant growth, development, and survival. Due to their sessile nature, plants have developed mechanisms that enable them to utilise their metabolism and grow in a variety of situations. One such mechanism is the incorporation of primary metabolic products into essential processes. One example of a metabolic product that controls plant growth and development is reactive oxygen species (ROS) (Foyer and Noctor, 2009; Mittler, 2017; Noctor *et al.*, 2017). Plants, like all living organisms, are exposed to a variety of stress factors, such as high light intensity, extreme temperatures, and pathogen infection. In response to these stress factors, plants have evolved a number of physiological and biochemical mechanisms to protect themselves from damage. One of these mechanisms involves the generation of reactive oxygen species (ROS), which are highly reactive molecules that can damage cellular components, but also play important roles in signalling and defenseresponses. Several cellular organelles, including mitochondria, peroxisomes, and chloroplasts, are restricted in plants in order to produce ROS. In addition to the previously described ROS production locations, environmental stress can also cause ROS to be produced in cell walls and plasma membranes, which contain peroxidases and amine oxidases, respectively. Singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), superoxide (O^{2-}) and hydroxyl radicals are some examples of ROS (OH^-). They display a range of physiological reactions, structural alterations, and macromolecule breakdown [Huang, 2019]. According to many studies, the formation of ROS is a fundamental sign of plant toxicity under abiotic stress. According to many studies, the formation of ROS is a fundamental sign of plant toxicity under abiotic stress. The level of

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phytotoxicity rises along with increase in ROS production. Plant development and metabolism are negatively impacted in such hazardous environments, which lowers agricultural output. Although ROS play a role in the cellular metabolism of plant cells, when they are produced in excess under stressful situations, they destroy numerous cellular components like proteins, lipids, nucleic acids, and carbohydrates through photooxidative damage [Raja, 2017]. In addition to the formation of ROS, their management is primarily accomplished by protective mechanisms, such as antioxidants, both enzymatic and non-enzymatic. Superoxide, in the enzymatic system, converts hydroxyl to hydrogen peroxide, which is then catalysed by peroxidase and catalase (CAT) to produce water and dioxygen. Low-molecular-mass antioxidants including glutathione, ascorbic acid, carotenoids, flavonoids, etc. mediate the non-enzymatic scavenging mechanism by attacking hydroxyl radicals and singlet oxygen. Plant cells experience oxidative stress when there is an imbalance between the generation and scavenging of ROS, which ultimately results in oxidative modification and cell death [Gechev, 2006]. ROS take part in various processes as a secondary messenger, such as cell division, differentiation, biotic and abiotic responses as the cells are maintained in a reduced state as a result of low ROS levels [Zeng, 2017]. As a result, ROS are essential for plant growth and development, and maintaining a threshold level is key. In addition to their destructive effects, ROS are also thought of as a signalling molecule that has a significant impact on plant growth and development, the expression of stress-responsive genes, and the induction of programmed cell death. Under stressful circumstances, it aids in the development of increased tolerance in a number of cellular systems (Mansoor, 2022).

Reactive oxygen species (ROS) are thought to be by-products of plant aerobic metabolism and are produced in a number of cellular compartments, including chloroplasts, mitochondria, and peroxisomes (Dietz *et al*., 2016; Sandalio and Romero-Puertas, 2015). In addition to causing irreversible DNA damage and cell death, ROS serve as significant signalling molecules that control how plants grow normally and react to stress. This shows that depending on varied levels of reactivity, sites of production, and the ability to cross biological membranes, ROS play a dual role in vivo (Miller G. *et al.*, 2010). Some fundamental biological processes, such as cellular proliferation and differentiation, require low ROS levels to progress (Tsukagoshi *et al.*, 2010; Zafra et al., 2010). Higher levels of ROS constitute a serious risk that could eventually result in DNA damage and improper timing of PCD (Xie *et al.*, 2014). Overall, ROS generation in plants can be both a boon and a bane depending on the circumstances. While moderate levels of ROS can play beneficial roles in plant growth and stress responses, excess ROS accumulation can lead to oxidative damage and cellular dysfunction. Therefore, maintaining redox homeostasis is critical for plant survival and stress adaptation.

Types of ROS

Phototrophs are essential for maintaining life on Earth because they transform solar light energy into metabolic energy. The cost of this is that they run the risk of suffering oxidative damages as a result of the many ROS, including $^1O_2$(singlet oxygen), H_2O_2(hydrogen peroxide), $O^{\bullet-}_2$ (superoxide radical), and $OH^{\bullet}$ (hydroxyl radical), which are produced as undesirable by-products. They are produced using just 1% to 2% of the total O_2 consumed by plants (Bhattacharjee, 2005). The various ROS members produced at various sites of plants are as follows:

a) Superoxide Radical ($O^{\bullet-}_2$)

Superoxide anion is a highly reactive free radical that is generated by the incomplete reduction of molecular oxygen. It is produced in the mitochondria during respiration, as well as in the chloroplasts during photosynthesis. Superoxide anion can react with other molecules to produce other ROS, such as hydrogen peroxide and hydroxyl radical. It can also react with nitric oxide to produce peroxynitrite, a highly reactive molecule that can damage cellular components (Halliwell, 2006).

b) Singlet Oxygen ($^1O_2$)

Singlet oxygen is a non-radical ROS that is generated by the interaction of excited chlorophyll molecules with molecular oxygen during photosynthesis. It can also be produced by other photosensitizers, such as porphyrins and flavins. Singlet oxygen is highly reactive and can damage cellular components, such as lipids and proteins. It can also act as a signaling molecule, modulating cellular processes such as gene expression and cell death (Krieger-Liszkay *et al.*, 2008).

c) Hydrogen Peroxide (H_2O_2)

Hydrogen peroxide is a non-radical ROS that is generated by the dismutation of superoxide anion. It is also produced by various enzymatic reactions, such as catalase and peroxidase. Hydrogen peroxide can diffuse across membranes and into cellular compartments, where it can oxidize cellular components and modulate cellular signaling pathways. At low concentrations, hydrogen peroxide can act as a signaling molecule, whereas at high concentrations, it can cause oxidative damage and cell death(Sharma *et al.*, 2012) (Peng *et al*., 2005)(Tanou *et al.*, 2009b).

d) Hydroxyl Radical ($OH^{\bullet}$)

Hydroxyl radical is a highly reactive free radical that is generated by the reaction of superoxide anion with hydrogen peroxide. It is considered to be one of the most reactive ROS and can damage cellular components, such as DNA, proteins, and lipids. Hydroxyl radical is not produced enzymatically but is instead generated by the Fenton and Haber-Weiss reactions, which involve the reaction of iron or

copper ions with hydrogen peroxide (Pinto *et al.*, 2003).

e) Peroxyl radical (ROO•)

Peroxyl radical is a free radical that is generated by the reaction of lipid radicals with molecular oxygen. It is highly reactive and can cause lipid peroxidation, a process that damages cellular membranes and can lead to cell death. Peroxyl radical is also involved in the generation of other ROS, such as hydroxyl radical and singlet oxygen. The peroxyl radical plays an important role in the oxidation of lipids, DNA damage, changes in the protein backbone, and the degradation of food.

f) Alkoxy radical (RO•)

Alkoxyl radicals are produced during lipid oxidative degradation or peroxidization without the aid of enzymes through the Fenton reaction, electron reductions, or the fusion of two peroxyl radicals. Alkyl radical oxidation may cause apoptosis and DNA changes. Alkoxyl radicals can damage DNA and trigger apoptosis since they are potent oxidizers (Kehrer, 2010).

Sites of ROS production and ROS-scavenging

Reactive oxygen species (ROS) are produced by several cellular processes, including photosynthesis, respiration, and oxidative metabolism. While ROS are essential for several physiological processes, their overproduction can lead to oxidative stress and damage to cellular components. Therefore, plants have developed an intricate system to regulate ROS levels and protect themselves from oxidative damage. ROS can be produced in multiple sites such as, in the mitochondria, plasma membranes, chloroplasts, endoplasmic reticulum, peroxisomes, and cell wall, both under normal and stressful conditions (Choudhury, 2013; Das, 2014). Different types of ROS and its site of production along with its functions, and the factors that elicit production are presented in Table 1.

Table 1. ROS site of production, functions, and the factors that elicit production (Choudhary, 2020; Sharma, 2012)

S. no.	Site of production	ROS	Factors favoring ROS production	ROS function
1.	Plasma membrane	Superoxide anion, hydrogen peroxide	Biotic stress, mechanical stress, high salinity, low temperature	Cell wall reinforcement, defence against pathogens, programmed cell death
2.	Chloroplast	Singlet oxygen, superoxide anion, hydrogen peroxide	High light intensity, UV radiation, heat stress, high CO2, nutrient deficiency	Photosynthesis regulation, lipid peroxidation, signaling, programmed cell death
3.	Mitochondria	Superoxide anion, hydrogen peroxide	High respiratory rate, oxidative phosphorylation uncoupling, nutrient deficiency	Energy production, apoptosis, signaling
4.	Peroxisomes	Hydrogen peroxide, superoxide anion, hydroxyl radical	Fatty acid oxidation, photorespiration, abiotic stress	Detoxification, cell wall formation, signaling
5.	Apoplast	Hydrogen peroxide, superoxide anion, singlet oxygen, hydroxyl radical	Biotic stress, abiotic stress, wounding, UV radiation, heavy metals	Defense against pathogens, programmed cell death, signaling
6.	Endoplasmic reticulum	Hydrogen peroxide, superoxide anion	Protein folding stress, lipid biosynthesis, calcium homeostasis	Protein quality control, signaling
7.	Vacuoles	Hydrogen peroxide	Metabolic processes, cellular waste management, programmed cell death	Cellular waste management, defense against pathogens, signaling
8.	NADPH oxidases	Superoxide anion, hydrogen peroxide	Biotic stress, hormonal stimuli, high light intensity	Defense against pathogens, programmed cell death, signaling

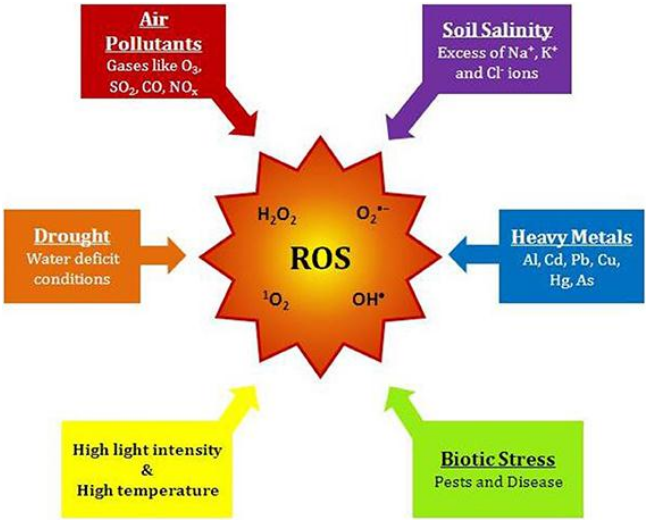

Fig. 1. Various causes responsible for the generation of ROS

Role of ROS in plant development process

ROS have been traditionally viewed as harmful molecules that cause oxidative damage to cellular components, such as lipids, proteins, and DNA, leading to cellular dysfunction and cell death. However, recent studies suggest that ROS also play important roles in plant signaling and defense responses, and that their accumulation can trigger adaptive responses to various stress conditions. Several studies have demonstrated the beneficial effects of ROS in plants. For example, ROS can act as signaling molecules to induce the expression of genes involved in stress responses, including defense genes, antioxidant genes, and genes related to stress tolerance (Mittler *et al*., 2011; Wrzaczek *et al*., 2013; Baxter *et al.,* 2014). ROS can also trigger the synthesis of secondary metabolites that can enhance plant defenses against pathogens or herbivores. The growth of the root apical meristem, shoot apical meristem, root hair cells, pollen tubes, leaves, and lateral roots are all processes in which ROS take an active part (Noctor *et al*., 2018, Mittler, 2017). For ROS to exert its regulatory activity, a careful balance between its production and scavenging must be maintained (de Pinto *et al.,* 2012; Sharma *et al*., 2012; Baxter *et al.,* 2014). Plants have developed the capacity to acquire a great degree of control over the toxicity of ROS, as evidenced by the applications of ROS as signalling molecules (Bhattacharjee, 2012; Mattila *et al*., 2015). ROS types and its cellular localization along with its role in developmental process are presented in Table 2.

Table 2. Source and type of ROS, cellular localization, and Role in Developmental Process

Type of ROS	Sources	Cellular Localization	Role in Developmental Process
Superoxide	NADPH oxidases, mitochondria, peroxisomes	Cell wall, apoplast, cytoplasm, mitochondria, peroxisomes	Regulate cell proliferation and differentiation, stimulate cell wall synthesis and lignification
Hydrogen Peroxide	NADPH oxidases, peroxisomes	Cell wall, apoplast, cytoplasm, mitochondria, peroxisomes	Regulate root hair development, stomatal closure, programmed cell death, and response to biotic and abiotic stress
Singlet Oxygen	Photooxidation	Chloroplasts, mitochondria, peroxisomes	Regulate chloroplast biogenesis and photosynthesis, and plant growth
Hydroxyl Radical	Fenton reaction, photooxidation	Nucleus, cytoplasm, mitochondria, peroxisomes	Trigger programmed cell death, regulate plant growth and development
Peroxynitrite	Reaction of nitric oxide (NO) with superoxide radical (O2-•)	Nucleus, cytoplasm, mitochondria, peroxisomes	Modulate stomatal aperture, root growth, and plant response to biotic and abiotic stress

Here are some of the roles of ROS in plant growth and development (Singh, 2016) are presented in Table 3a and 3b. The role of ROS can be broadly classified in 4 categories: -

a) ***Seed Germination:*** ROS are necessary for seed germination as they activate enzymes that help break down stored nutrients in the seed and initiate cell division. Studies have shown that ROS levels

increase during seed germination, and inhibiting ROS production in seeds leads to reduced germination rates (Bailly, 2004). ROS-mediated seed germination has been found to be regulated by multiple pathways, including gibberellin signalling, reactive nitrogen species, and ethylene signalling (Oracz, 2016).

Table 3a. ROS and its role in germination, development of root and leaf, and flowering process.

ROS Type	Role in Seed Germination	Role in Root Development	Role in Leaf Development	Role in Flowering Process
Superoxide radical	Stimulates cell elongation and division, activates antioxidant defense systems	Involved in root hair elongation, cell elongation and differentiation, lateral root formation	Involved in chloroplast biogenesis, photosynthesis, and stomatal regulation	Promotes flowering through the activation of downstream genes such as FT and SOC1
Hydrogen peroxide	Regulates seed dormancy, stimulates cell elongation and division, activates antioxidant defense systems	Stimulates root growth, regulates cell division and differentiation, involved in stress responses	Regulates stomatal closure, involved in senescence, programmed cell death, and stress responses	Regulates floral organ development, promotes the transition from vegetative to reproductive growth
Singlet oxygen	Causes oxidative damage to cellular components, regulates seed dormancy	Induces programmed cell death in roots under stress conditions	Involved in programmed cell death and regulation of photosynthesis	Regulates the expression of genes involved in flower development
Hydroxyl radical	Causes oxidative damage to cellular components, regulates seed dormancy	Involved in programmed cell death and regulation of root growth	Involved in programmed cell death and regulation of leaf growth	May play a role in floral senescence
Peroxynitrite	Causes oxidative damage to cellular components, regulates seed dormancy	Involved in root growth inhibition and programmed cell death	Involved in leaf growth inhibition and programmed cell death	Involved in the regulation of flowering time

b) ***Root Development:*** ROS signalling is involved in the formation and elongation of roots, which are critical for nutrient uptake and water absorption. ROS production is higher in the root tip, where cell division and differentiation occur. Studies have shown that ROS signalling is involved in regulating the balance between cell division and differentiation, which is necessary for proper root development. ROS also regulate the formation of root hairs, which increase the surface area of the root and enhance nutrient uptake (Schiefelbein, 2015).

c) ***Leaf Development:*** ROS play a role in leaf development by controlling cell division and differentiation. They also regulate the size and shape of leaves. Studies have shown that ROS signaling is involved in leaf vein patterning and stomatal development, which are critical for proper leaf function. ROS also regulate leaf senescence, which is the programmed death of leaves (Vainonen, 2015; Mhamdi, 2010).

d) ***Flowering:*** ROS signaling plays a critical role in the timing and coordination of flowering in plants. Studies have shown that ROS levels increase during the transition from vegetative growth to reproductive growth. ROS signaling is involved in regulating the expression of genes that control flowering, including the photoperiodic pathway, the autonomous pathway, and the vernalization pathway (Pucciariello, 2012).

e) ***Defense Against Pathogens and Pests:*** ROS production is a crucial component of plant defense against pathogens and pests. ROS can damage pathogen cell walls and activate defense genes. Studies have shown that ROS signaling is involved in the response to a wide range of pathogens, including bacteria, fungi, and viruses (Wang, 2013). ROS also play a role in the plant's response to herbivores, including insects and nematodes (Maffei, 2007).

f) ***Abiotic Stress Response:*** ROS signaling plays a vital role in the plant's response to abiotic stresses, such as drought, salinity, and high temperatures. ROS signaling helps activate stress-responsive genes and regulate stress-related physiological processes. Studies have shown that ROS signaling is involved in the response to a wide range of abiotic stresses, including drought, salinity, and high temperatures (Zhu, 2016; Chen, 2019).

g) ***Senescence and Programmed Cell Death:*** ROS play a role in senescence and programmed cell death by activating enzymes that degrade cellular components. Studies have shown that ROS signaling is involved in leaf senescence, which is the programmed death of leaves. ROS also play a role in the response to stress-induced programmed cell death, which occurs when plants are exposed to high levels of stress (Gechev, 2006).

Table 3b. ROS and its role in defense, abiotic stress, and senescence

ROS Type	Role in Defense Against Pathogens and Pests	Role in Abiotic Stress Response	Role in Senescence and Programmed Cell Death
Superoxide radical	Microbicidal activity, signal transduction	Involved in signal transduction and defense response	Triggers senescence and cell death
Hydrogen peroxide	Antimicrobial activity, signal transduction	Signaling molecule, activates MAPK cascade, activates antioxidant enzymes, and stimulates stomatal closure	Activates senescence and cell death pathways, mediates the balance between cell survival and death
Hydroxyl radical	Microbicidal activity	Damages proteins, DNA, and membranes	Causes DNA damage and protein oxidation, triggers apoptosis
Singlet oxygen	Antimicrobial activity	Inhibits photosynthesis, activates stress response genes	Causes oxidative damage, promotes programmed cell death
Peroxynitrite	Microbicidal activity	Causes nitrosative stress, activates antioxidant enzymes	Causes DNA damage and protein oxidation, triggers apoptosis

Effect of ROS generation on cellular processes

a) ROS-mediated control of the cell cycle, cell division, cell expansion and cell death

Although excessive ROS production has been linked to oxidative damage and cell death, recent research has demonstrated that ROS also play important roles in the regulation of cell cycle, cell division, cell expansion, and cell death. Although the mechanisms underlying this reaction are still largely unknown, stress in plants frequently results in reduced growth and cell cycle halt. It is understood that reductive and oxidative signals are necessary for transitions between the phases of the cell cycle and that redox cycles are conserved within the cell cycle (Menon and Goswami, 2007; Diaz-Vivancos *et al.,* 2015; de Simone *et al.,* 2017). Recent studies have clarified how ROS and changes in redox states can affect such phase-to-phase transitions and progressions, which are primarily controlled by a complex machinery of interacting cyclins (CYCs) and cyclin-dependent kinases (CDKs).

ROS have been implicated in the regulation of the cell cycle. During the G1 phase, low levels of ROS are produced, and these promote cell proliferation by activating several key signaling pathways, including the MAPK/ERK pathway and the PI3K/Akt pathway. As cells progress through the cell cycle, ROS levels increase and contribute to the regulation of the G2/M transition. ROS are known to activate the ATM/Chk2/p53 pathway, which is critical for the arrest of cells in the G2 phase and the prevention of DNA damage-induced cell death. Redox disturbances have an impact on both the activity and transcript levels of CYCs and CDKs (Reichheld *et al*., 1999; Foyer *et al*., 2018). For example, TEOSINTE BRANCHED1-CYCLOIDEA-PROLIFERATING CELL FACTOR1 (TCP) transcription factors directly influence cell cycle components as a result of redox processes (Kadota *et al.,* 2005). Moreover, CYCs levels were regulated by TCPs at transcription level, potentially through interactions with CYC promoters, and therefore, feature a conserved redox-sensitive cysteine residue that is necessary for DNA binding. This shows that the interaction between a TCP transcription factor and its promoter may be hindered under oxidising circumstances as a result of the creation of disulfide bonds (Viola *et al*., 2013, 2016).

ROS also play an important role in cell division. Studies have shown that ROS production is required for cytokinesis, the final stage of cell division. Specifically, ROS are thought to regulate the assembly and contraction of the contractile ring, which is required for the separation of daughter cells. ROS are also known to modulate the activity of microtubules, which are critical for the proper alignment and segregation of chromosomes during mitosis. Moreover, in wheat (Triticum sp.) and Arabidopsis root tip cells, pharmacological disruption of ROS homeostasis produces mostly aberrant tubulin polymer synthesis and disrupts effective cell plate assembly, ultimately leading to disturbed cytokinesis (Livanos *et al.,* 2012a, b). Similar to the genetic disruption of NADPH oxidases, which produce ROS, and mitogen-activated protein kinases, which are involved in ROS

signalling, tubulin becomes disorganised, which emphasises the need for a closely regulated ROS balance during cytokinesis (Takeda *et al*., 2008; Kosetsu *et al.,* 2010; Yao *et al.,* 2011).

ROS are also able to modulate cell expansion, via their effects on the cell wall. ROS are known to promote cell elongation and expansion by regulating cell wall remodeling and the activity of cell wall-loosening enzymes. Additionally, ROS have been shown to activate a number of signaling pathways, including the MAPK/ERK pathway, which is involved in the regulation of cell growth and differentiation. For instance, apoplastic H_2O_2, hydroxyl radicals, and superoxides alter the rigidity and relaxation of cell walls, which in turn affects the pace of cell expansion. Although several oxidant sources are acknowledged, little is known about how they are regulated. The peroxidative and hydroxylatic activities of apoplastic class III peroxidases have antagonistic effects on the stiffness of cell walls in addition to NADPH oxidases, amine, and oxalate oxidases (Passardi *et al*., 2004; Schmidt *et al.,* 2016). Peroxidases typically control H_2O_2 levels in a peroxidative mode by oxidising a variety of substrates. By doing this, they aid in the crosslinking of extensins and phenolics, which increases the stiffness and decreases the ability of cell walls to elongate. On the other hand, it has been shown that hydroxyl radical production can break pectins and xylogucans, which makes it easier to release cell walls (Fry, 1998; Passardi *et al.,* 2004). Genetic modifications of different class III peroxidases have shown that this property of peroxidases being connected with both cell elongation and growth-restricting processes is manifested in their divergent impacts on growth rates (Lu *et al*., 2014; Schmidt *et al.,* 2016).

Finally, ROS play a critical role in the regulation of cell death. Excessive ROS production can lead to oxidative damage and cell death via several mechanisms, including apoptosis, necrosis, and autophagy. However, ROS can also promote cell survival by activating several signaling pathways, including the PI3K/Akt pathway and the NF-kB pathway. In response to numerous stimuli, such as development and bacterial challenges, increased ROS production happens in either a temporary or permanent manner, also known as an oxidative burst, and can start cell death signalling (Van Breusegem and Dat, 2006). The tapetum, seed coat, endosperm, and lateral root cap are a few tissues and organs where development-associated programmed cell death (PCD) occurs (Daneva *et al.,* 2016). For instance, PCD occurs in tapetal cells, which is necessary for the production of microspores. Surprisingly, the rice (Oryza sativa) mutant defective in tapetum cell death 1 (dtc1) fails to accumulate ROS and demonstrates delayed tapetum PCD resulting in male sterile plants (Yi *et al.,* 2016).

Overall, the effects of ROS on basic cellular processes – the cell cycle, cell division, cell expansion and cell death – are thought to contribute, acting in concert via interactions between plant phytohormonal pathways, to the multiple functions of ROS during the plant life cycle.

b) ROS in Cell Proliferation and Differentiation

It has been clear in recent years that plants' reaction to biotic and abiotic environmental stimuli, as well as programmed cell death (PCD), are all regulated by ROS, which are crucial signalling molecules (Alvarez et al. 1998; Blokhina *et al.* 2003; Navrot *et al*. 2007). Throughout the process of respiration and photosynthesis, ROS are continuously created. In several processes related to plant growth and development, plant cells produce ROS, including superoxide and H2O2 (Schroeder *et al*. 2001b; Foyer &Noctor 2005). Moreover, the generation of bursts of superoxide at the plasma membrane is one of the primary ways that plants communicate information about changes in the environment (Doke *et al*. 1994). Yet, the prevalent opinion continues to consider them as an unavoidable burden balanced with the advantages of using oxygen as a respiratory electron acceptor.

Cell division and cellular differentiation are the primary processes that control growth and development in multicellular organisms. Early organogenesis termination results from disruption of the balance between these two stages, which may cause aberrant growth (Zhang *et al.,* 2008). ROS homeostasis controls the change from cellular proliferation to cell elongation during the early phases of differentiation (Tsukagoshi *et al*., 2010).

The example of such process involves that, at the beginning of each stage of cell differentiation in the fungus Neurosporacrassa, a hyperoxidant condition takes place that supports the development of conidia (Gessler *et al.,* 2007). The hyperoxidant state, according to Aguirre *et al*. (2005), develops when the amount of ROS produced surpasses the antioxidant capability of the cell. The fact that antioxidants impede developmental processes is well known as an outcome of this example, which raises the possibility that ROS are also necessary for similar processes in plants. On the other hand, it is expected that a lack of antioxidant enzymes will lead to increased ROS levels and enhanced cell differentiation. ROS are significant regulators of plant development because they play crucial functions in cell proliferation. The spatial control of ROS generation is a key factor influencing plant shape. NADPH oxidases (NOXs), which use NADPH as an electron donor to create the superoxide radical ($O_{2)}$, which has been proven to have a role in development, produce ROS (Gapper & Dolan 2006). The roots of plants homozygous for loss-of-function rhd2 mutations exhibit lower levels of ROS and are 20% shorter than the wild type, showing that cell growth is faulty in these plants

(Foreman *et al*. 2003). ROS are significant regulators of plant development because they play crucial functions in cell proliferation. The spatial control of ROS generation is a key factor influencing plant shape. NADPH oxidases (NOXs), which use NADPH as an electron donor to create the superoxide radical (O_2^-), which has been proven to have a role in development, produce ROS (Gapper & Dolan 2006). The roots of plants homozygous for loss-of-function rhd2 mutations exhibit lower levels of ROS and are 20% shorter than the wild type, showing that cell growth is faulty in these plants (Foreman *et al*. 2003; Renew *et al.* 2005). Inhibitors like diphenylene iodonium (DPI) were employed by Liszkay *et al.* (2004) to demonstrate how NOX-derived ROS regulate cell growth in maize (Zea mays) roots. ROS are crucial for both wall cross-linking, which prevents cell expansion during the stoppage of growth and cell differentiation, and cell-wall loosening in developing tissues (Potikha *et al.* 1999; Liszkay *et al.* 2004; Ros-Barcelo et al. 2002).The oxidative cross-linking of cell wall glycoproteins and polysaccharides by peroxidase, which reduces cell wall extensibility, is a critical mechanism by which H2O2 modulates extension development in plants (Schopfer 1996).On the other hand, the secretory pathway's H2O2 production may encourage the development of substantial hemicellulose coagula, which will have a wall-loosening impact (Fry *et al*. 2000). OH radicals, which are found in the cell walls of elongating organs in vivo (Liszkay *et al.* 2004; Renew et al. 2005), control growth by permitting walls to extend through the scission of xyloglucan polymers (Fry 1998). Membrane-bound NAD(P)H oxidases, apoplastic oxalate oxidases, peroxidases, and amine oxidases are the primary potential ROS-delivering processes in the cell wall. Amine oxidases are ubiquitous polycationic enzymes that catalyse the oxidative de-amination of polyamines, which are engaged in essential cell-life activities (Bagni and Tassoni 2001; Seiler 2004). The polyamine oxidases that contain flavin and the copper-containing amine oxidases (CuAO) are examples of amine oxidases (PAOPlant amine oxidases are more than just a way to control cellular polyamine levels; through their reaction byproducts, aminoaldehydes, 1,3-diaminopropane (DAP), and hydrogen peroxide, they contribute to crucial physiological activities (Bouchereau *et al.* 1999; Walters 2003). As amine oxidases are widely distributed in tissues that are undergoing lignification or significant wall-stiffening processes, it is possible that these enzymes may have an impact on plant growth and development by stiffening and hardening cell walls (Cona *et al.* 2005; Paschalidis&Roubelakis-Angelakis 2005).

c) The role of ROS during germination

Reactive oxygen species (ROS) have been found to play a crucial role in various aspects of plant growth and development, including seed germination and meristem development. Seed germination is a complex process that involves the activation of various biochemical pathways and physiological changes. ROS are known to play a crucial role in seed germination by regulating cell cycle progression, cell wall remodeling, and hormone signaling. In Arabidopsis thaliana, ROS production is known to be required for germination, as inhibition of ROS production leads to reduced germination rates (Oracz, 2009). ROS are thought to regulate germination by modulating the activity of enzymes involved in the breakdown of cell wall polysaccharides and by activating signaling pathways involved in hormone responses.

Plant embryos and the surrounding endosperm in dry, dormant seeds exhibit very limited metabolic activity, and ROS generation is therefore assumed to be very low (Bailly *et al.,* 2008). However, metabolism quickly resumes after seed imbibition and throughout germination (Rajjou *et al*., 2012), and this quick metabolic start appears to be associated with enhanced ROS generation via a number of pathways and at a number of subcellular locations. This entails mitochondrial respiration, lipid catabolism, lipid -oxidation in the glyoxysomes, and synthesis via NADPH oxidases (Rajjou *et al.,* 2012; Wojtyla *et al.,* 2016; Ishibashi *et al*., 2017). Experiments showing that exogenously applied oxidants, such as H_2O_2, and a pharmacologically or genetically influenced decrease in catalase or in other antioxidant activities, were shown to positively influence the release of dormancy and the beginning of germination, have been used to support the spatiotemporal correlation of increased ROS production and accumulation during the onset of germination (Leymarie *et al*., 2012; Basbouss-Serhal *et al.,* 2017). Moreover, it has been demonstrated that overexpressing CAT in barley (Hordeum vulgare) seeds inhibits early germination (Ishibashi *et al*., 2017). Increased ROS levels are therefore essential for successful germination and serve as promising signs for the breaking of dormancy (Bailly *et al.,* 2008). After seed imbibition, ROS levels rise and serve as a primary force for germination. Yet, over a specified limit, ROS are either too strong to negatively impact embryo viability and prohibit or delay germination, or too low to allow germination (Bailly et al., 2008). The requirement to strictly regulate ROS homeostasis during germination results in the creation of an "oxidative window" for germination, which limits effective seedling development within specific limits of elevated ROS levels. Moreover, the increase in ROS concentration was also observed during the processes such as the weakening of endosperm, the cell walls loosening, and the radicle lengthening, etc. (Barba-Espin *et al.,* 2010).

After germination, the apical meristem of the seedling is responsible for the continued growth and development of the plant. ROS are known to play an

important role in meristem development by regulating cell proliferation and differentiation. In particular, ROS have been shown to regulate the balance between stem cell maintenance and differentiation in the apical meristem (Tsukagoshi, 2019). ROS are thought to activate several signaling pathways, including the MAPK/ERK pathway and the WUSCHEL-RELATED HOMEOBOX (WOX) pathway, which are involved in the regulation of stem cell fate. Recent research has also shown that ROS can modulate the activity of transcription factors involved in the regulation of meristem development. For example, the transcription factor NAC1 is known to be involved in the regulation of meristem size and differentiation. ROS have been shown to regulate NAC1 activity by inducing its degradation via the 26S proteasome pathway. Additionally, ROS have been shown to regulate the activity of other transcription factors, including MYB30 and ARF6, which are involved in the regulation of meristem development (Xie, 2000).In conclusion, ROS play a crucial role in seed germination and meristem development by regulating cell cycle progression, cell wall remodeling, hormone signaling, and stem cell fate. The modulation of ROS levels and signaling pathways may represent a potential target for the improvement of seed germination rates and the regulation of meristem development.

d) ROS homeostasis drives organ growth

Reactive oxygen species (ROS) play a crucial role in organ growth and development by regulating various cellular processes. ROS homeostasis, which refers to the balance between ROS generation and scavenging, is essential for proper organ growth and development in plants. Studies have shown that ROS homeostasis is critical for root and shoot growth in plants. For example, in Arabidopsis thaliana, the transcription factor NAC016 has been shown to regulate ROS homeostasis and promote root growth by enhancing the expression of ROS scavenging genes (Li *et al.*, 2020). Similarly, the transcription factor MYB36 has been shown to control ROS homeostasis and promote shoot growth by regulating the expression of genes involved in ROS scavenging and cell wall biosynthesis.

The majority of plant roots exhibit indeterminate growth, which involves not only ongoing primary root cell division and expansion but also the growth of lateral roots (LRs) and root hairs. Studies have demonstrated that all of these processes are impacted by altered ROS homeostasis, which inhibits primary root growth, stimulates LR emergence, and increases root hair growth. (Foreman *et al*., 2003; Orman-Ligeza *et al.*, 2016). Tsukagoshi (2016) recently given an overview of ROS function in regulating root growth and development. He emphasises that interactions between ROS and auxin signalling, which are essential in determining root architecture (Du and Scheres, 2018), partially control root growth and development.For instance, in root hairs, at least two auxin-regulated transcriptional regulators—ROOT HAIR DEFECTIVE 6-LIKE 4 (RSL4) and MEDIATOR 25 (MED25)—promote root hair elongation through the auxin-controlled transcriptional regulation of NADPH oxidases and class III peroxidases (Foreman et al., 2003; Sundaravelpandian *et al.*, 2013; Mangano et al., 2017). Also, a proper balance between H_2O_2 and superoxide levels may serve as a signal to control the development of root hair cells, according to research on ROS levels (Sundaravelpandian *et al*., 2013). The misexpression of parts of the ROS processing system has been linked to a number of mutants that exhibit growth problems, including a non-exhaustive list of mutants with leaf growth defects. Moreover, studies have shown that ROS homeostasis also plays a role in plant response to environmental stimuli, such as light and temperature. For example, in Arabidopsis thaliana, the photoreceptor cryptochrome 1 regulates ROS homeostasis and promotes seedling growth under blue light (Wang *et al.*, 2016). Similarly, in rice plants, the transcription factor OsJAZ9 regulates ROS homeostasis and controls thermotolerance by modulating the expression of ROS scavenging genes (Hu *et al*., 2019).New information has been gathered from researching cat2 mutants in particular, which exhibit growth inhibition as a result of increased photorespiratory H_2O_2availability, which causes SA aggregation and photoperiod-dependent activation of a pathogenesis-related pathway (Queval *et al*., 2007; Mhamdi *et al*., 2010a). Furthermore, it has been demonstrated that certain ROS components serve specific roles in the transmission of H_2O_2 signals and in connecting H_2O_2 to phytohormone pathways, even though they are not necessary for the normal growth and placement of leaves (i.e. into a "rosette" formation) in Arabidopsis. (Tognetti *et al.*, 2010; Han *et al*., 2013a; Rahantaniaina *et al.*, 2017).

In addition to promoting organ growth, ROS homeostasis also regulates organ size and shape. For instance, in tomato plants, the transcription factor SlCDF1 regulates ROS homeostasis and controls leaf morphology by affecting cell proliferation and expansion (Xie *et al.*, 2018). Similarly, in Arabidopsis thaliana, the transcription factor TCP4 has been shown to regulate ROS homeostasis and control leaf shape by affecting cell proliferation and differentiation (Kieffer *et al.*, 2011). In addition to influencing cell proliferation throughout development, ROS may also play a role in regulating organ number and initiation, according to Sagi et al. (2004). ROS and glutathione are among the signals that aid in the development of the legume-rhizobia symbiosis (Shaw & Long 2003; Pauly *et al.* 2006). In a semiaquatic legume, ethylene and ROS serve as positive transducers downstream of the Nod factor response and are necessary for the genesis of root nodules. Moreover, many studies have linked antioxidants and ROS to nodule senescence (Becana

et al. 2000; Matamoros *et al.* 2003). Many plant hormone responses have been linked to the involvement of ROS as second messengers (Kwak *et al.* 2006). Overall, these studies suggest that ROS homeostasis plays a critical role in plant growth and development by regulating various cellular processes, including cell proliferation, expansion, and differentiation. Further research is needed to elucidate the molecular mechanisms underlying ROS homeostasis and its regulation of organ growth and development in plants.

e) Redox signalling in flower development

Reactive oxygen species (ROS) play a crucial role in the redox signaling pathway, which regulates various cellular processes including flower development. The redox signaling pathway involves the generation of ROS as a result of metabolic processes and environmental stresses, which are then rapidly detoxified by the antioxidant defense system to maintain ROS homeostasis. However, in certain cases, ROS can act as signaling molecules and trigger downstream signaling events by oxidizing and modifying proteins, lipids, and nucleic acids (Han, 2019). In flower development, ROS have been shown to regulate various processes such as cell proliferation, differentiation, and senescence. For example, ROS are involved in the regulation of floral organogenesis, which is the process by which floral organs such as petals and sepals are formed. ROS signaling also plays a role in regulating the timing of floral transition and in the development of floral organs (Mittler, 2017; Hu, 2017).

Recent studies have addressed the critical role that ROS play in the development of plant reproductive organs and tissues (Jiménez-Quesada *et al*., 2016; Schippers *et al*., 2016). The buildup of ROS at the tip of pollen tubes is required for their successful development towards the female gametophyte, according to pharmacological methods and ROS-staining tests (Potock *et al*., 2012). The activation of these NADPH oxidases by calcium and phosphorylation is required for appropriate growth, and genetic evidence for the involvement of two RBOH genes (RBOHH and RBOHJ) in pollen tube growth has been reported (Duan *et al.*, 2014; Lassig et al., 2014). Recent studies have identified several transcription factors and signaling pathways that are regulated by ROS during flower development. For instance, the transcription factor AGAMOUS-LIKE 15 (AGL15) is involved in the regulation of floral meristem identity and floral organogenesis, and its expression is modulated by ROS levels. Similarly, ROS have been shown to regulate the expression of several genes involved in flower development, including those encoding MADS-box transcription factors, which are critical regulators of flower development. Several studies have also shown that ROS are involved in regulating pollen development, fertilization, and seed formation in plants. For example, ROS are required for pollen tube growth and guidance towards the ovule during fertilization. In addition, ROS signalling has been shown to play a role in the regulation of seed dormancy and germination (Mhamdi, 2018).Overall, these studies demonstrate the important role of ROS in flower development through their involvement in redox signalling pathways.

CONCLUSIONS

Reactive oxygen species (ROS) are generated in different organelles of plant cells, including chloroplasts, mitochondria, and peroxisomes, as by-products of various metabolic processes or under stress conditions. ROS play a dual role in plant development, acting as signalling molecules that regulate various cellular processes, such as growth, differentiation, and programmed cell death, and as oxidative stress factors that can damage cellular components and impair plant development. During germination, ROS are essential for the activation of various enzymes and signalling pathways that promote seed germination and seedling growth. However, excessive ROS accumulation can lead to oxidative damage and inhibit germination. In flower development, ROS play a crucial role in regulating the balance between cell proliferation and differentiation, as well as in the formation and differentiation of floral organs. ROS also participate in the signal transduction pathways that control the expression of genes involved in flower development.

In conclusion, the site of ROS generation in plants, and the amount of ROS produced, can significantly affect plant development and cellular processes, including cell proliferation and differentiation, seed germination, organ growth, and flower development, etc. Understanding the molecular mechanisms underlying ROS production and signalling in plants is essential for developing strategies to enhance plant growth and productivity under stress conditions.

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RESEARCH ARTICLE

AURICULARIA SINODELICATA (*AURICULARIACEAE*) Y.C. DAI & F. WU, A NEW RECORD FOR THE INDIAN MYCOBIOTA

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Abstract: *Auricularia*, a genus of macrofungi with broad geographical distribution, is highly valued for its edible and medicinal properties. It ranks as the third most extensively cultivated mushroom worldwide due to its remarkable nutritional and bioactive attributes. In the Present communication *Auricularia sinodelicata* is reported first time from the India with detailed descriptions and colour illustrations.

Keywords: Arunachal Pradesh, *Auricularia*, India, New Record, Taxonomy

INTRODUCTION

Arunachal Pradesh (the land of the dawn-lit mountains) is a state in North-east India with an area of 83,743 km^2. It is having the largest area among the other states of North-east India and the largest mountainous state of India. Arunachal Pradesh, is famous for its rich and diverse flora, because of to its unique geographical position and varying climatic conditions. Numerous rivers and streams traverse its landscape, contributing to its hydrological diversity. The region is regarded as one of the important hotspots of extraordinary biodiversity, hosting a wide array of flora and fauna. Additionally, Arunachal Pradesh is home to multiple indigenous communities, whose cultural practices and sustainable interactions with the environment have contributed to the preservation of its ecological balance. Among the various wild resources utilized by these communities, mushrooms hold significant importance. Many species of wild mushrooms are harvested and consumed as food, reflecting both the ecological knowledge and dietary habits of the indigenous populations of this wonderful state. Many species of the Genus *Auricularia*, commonly known as "Ear Fungi" are widely used as a food by the local people of Arunachal Pradesh.

Members of the genus *Auricularia* (Auriculariaceae, Auriculariales), typified by *Auricularia mesenterica* (Dicks.) Pers. are widely distributed and recognized for their ecological, economic, and medicinal properties (Wu et al. 2021). These species play a crucial role in the degradation of forest ecosystems, particularly in tropical forests, typically colonizing angiosperm wood, such as dead trees, stumps, fallen trunks, branches, and decayed wood, with a few species also inhabiting gymnosperm wood (Dai & Bao, 2007; Baldrian & Lindahl, 2011). Several *Auricularia* species are highly valued as edible and medicinal mushrooms, particularly in China and other East Asian countries, where they play a significant role in traditional diets and medicine (Wu et al., 2021). Morphologically, *Auricularia* is distinguished by its gelatinous, resupinate to substipitate basidiomata with pilose upper surfaces, cylindrical to clavate and transversely three-septate basidia containing oil guttules, and hyaline, thin-walled, allantoids basidiospores (Lowy, 1951; Kobayasi, 1981). As per as per the morphological examinations and phylogenetic analyses conducted by Wu et al. 2021, 37 species of *Auricularia* are recognized worldwide. Although *Auricularia* can be differentiated from other genera within the family *Auriculariaceae*, but species identification based on macromorphological features, such as size of abhymenial hair, size of basidiospores, and the presence or absence of a medulla, remains challenging (Lowy 1952; Kobayasi 1981), because of overlapping morphological charecters. In Asian

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context many workers significantly contributed on the taxonomical and phylogenetical studies of the Genus *Auricularia* (Kobayasi 1981, Bandara et al. 2017, Wu et al. 2015, Wu et al. 2021). During the routine survey of Arunachal Pradesh, India several specimens of Genus *Auricularia* were collected by the author. After thorough macro and micromorphological characterization of these samples one specimen is identified as *Auricularia sinodelicata* Y.C. Dai & F. Wu. This species is earlier reported from China only and this article reports it first time for the India.

MATERIALS AND METHODS

Routine Survey and exploration tours of different districts of Arunachal Pradesh were conducted during the monsoon seasons of 2023–2024 and specimens of macro fungi were collected. Specimens were photographed with the help of a Camera (Nikon P950) and also with the mobile showing important morphological features in the field and also in the base camp before drying. After macro-morphological charecterization, specimens were dried with the help of drier for future study. Colour codes and terminology follows Kornerup and Wanscher (1978). To study the microscopic features, first, a piece of basidiomata was soaked in water. Detailed observations of micromorphological structures, like basidiospores, cross-sections of the basidiomata, abhymenial hairs, hymenium were done using a light microscope (Olympus CX43 and Olympus BX 52). Photographs of these structures were obtained through an attached dedicated camera, with the free-hand sections of desiccated samples mounted in a solution comprising 5% potassium hydroxide (KOH), 30% glycerol, phloxine, and cotton blue either separately or together. Measurements of basidiospores were done for twenty basidiospores. The dimensions of the basidiospores, along with their length/width ratios (Q), are presented as minimum, mean, and maximum values. The nomenclature of herbaria is

RESULTS AND DISCUSSION

Auricularia sinodelicata Y.C. Dai & F. Wu

Basidiomata 18–55×16–45, Gelatinous, fleshy, soft, caespitose, imbricate sessile or substipitate, light orange (5A5) to orange gray (6B2) to grayish orange (6B3); pileus projecting up to 55 mm, 1–2 mm thick, discoid or auriculate, margin entire, and brownish orange (6C4–6C5) when dry; upper surface scantly pilose, azonate; hymenophore surface conspicuously reticulate orange white (5A2) to pale orange (5A3). Thickness 1140–1460 µm when dry; medulla indistinctly present near the hymenium; crystals absent; abhymenial hairs 45–70 × 6–8 µm, with slightly swollen base or centre, thick-walled, with a wide or narrow septate lumen, apical tips acute or obtuse, single, hyaline; hyphae 2–4 µm in diam., with clamp connections.; basidia 40–50 × 4–4.5 µm, clavate, transversely 3-septate, with oil guttules; cystidioles absent. Basidiospores 9–(11.10)–12.50 × 4–(5.20)–6.0, Q = 1.83–(2.10)–2.25, allantoid, thin-walled, smooth, usually with one to two large guttules, hyaline.

Specimens Examined: India, Arunachal Pradesh, Tirap District, Khonsa, Lazo basti, alt. 1179 m 26º57' 51.62" N, 95º30' 31.10" E, 15.06.2024, on a dead wood log, A. Parihar AP 24-48 (ARUN F 41).

Notes: The macro- and micromorphological characteristics of the examined specimens align closely with the original description of *Auricularia sinodelicata* Y.C. Dai & F. Wu (Wu et al., 2021). Presence of morphological characters like porose-reticulate hymenophore surface, absence of crystals, small abhymenial hairs (<100 µm), small basidiospores, confirm the placement of these specimens within the *Auricularia delicata* complex, as defined by Wu et al. (2021). While the present specimens showing some similarities to *Auricularia delicata*, the presence of a pilose surface, along with slightly longer basidia and abhymenial hairs, distinguishes it from *Auricularia sinodelicata* additionally, *Auricularia delicate* has a distribution in Africa. *Auricularia tremellosa*, another morphologically similar species, differs from the present specimens due to its pilose abhymenial surface, poroid hymenophore, and geographic distribution, in the America. In contrast, *A. sinodelicata*, with its distribution in China, seems more closely related to present specimen. Based on these distinct macro- and micro-morphological characters and geographic considerations, the present specimen is identified and treated as *Auricularia sinodelicata*.

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Legends for Figures:

Fig. 1: *Auricularia sinodelicata* Y.C. Dai & F. Wu A. Habitat of the basidiomata; B. Basidiomata with scale showing abhymenial surface and strongly reticulate hymenial surface; C.Habit of the basidiomata; D. Colour change of basidiomata after collection and drying; E. Cross section of the basidiomata; F. Abhymenial hairs; G. Basidiospores. Scale Bars: E = 100 µm; F & G = 10 µm.

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RESEARCH ARTICLE

EFFECT OF BLENDING RATIO OF BANANA FRUIT WITH PSEUDOSTEM CENTRAL CORE AND STORAGE PERIOD ON NUTRITIONAL AND PHYSICOCHEMICAL PROPERTIES OF JAM

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Abstract: The experiment was carried out to investigate the nutritional and physicochemical properties of blended jam made from banana fruit and pseudostem central core, focusing on various blending ratios and storage periods. Treatments ranged from 0:100 (banana fruit: pseudostem central core) to 100:0. Each combination were analyzed for key parameters such as iron, potassium, carbohydrates, protein, calorific value, and acidity, were analyzed at three-month intervals starting from immediately after processing and at three and six months of storage. Significant variation in nutrients was observed across the treatments, where T_1 (0:100) showing the highest iron content (3.13 mg/100g) and T_{11} (100:0) exhibiting the highest calorific value (277.74 KCal). A general decreasing trend in protein, iron, and potassium was noted over the storage period, while slight increases in calorific value and carbohydrates were observed. The study concludes that the blending ratio significantly affects the nutritional composition, with the pseudostem central core contributing to higher mineral content, while banana fruit enhances caloric value. These findings offer valuable insights into the potential of utilizing banana by-products in food processing, contributing to the development of nutritious, sustainable food products.

Keywords: Banana, Pseudostem, Central core, Blended Jam, Storage

INTRODUCTION

Banana (*Musa paradisiaca* L.) is a monocarpic, herbaceous, monocotyledonous plant that belongs to the family Musaceae, originating from the tropical regions of South-East Asia through the hybridization of *Musa acuminata* and *Musa balbisiana*. India is also recognized as a secondary center of origin. Banana is one of the oldest cultivated fruits, with references in ancient Indian texts like the Ramayana (2020 BC) and Kautilya's Arthashastra (300-400 BC). It holds immense nutritional, economic, and cultural significance, serving as a staple and versatile crop across tropical and subtropical regions, particularly between 30°N and 30°S latitudes.

Banana (*Musa paradisiaca* L.) is one of the most popular and refreshing fruits globally, offering nourishment and a well-balanced diet to millions. It plays a crucial role in livelihoods through its production, processing and marketing. Commercially, banana ranks as the fourth most important global food commodity after paddy, wheat and milk in terms of gross value of production, highlighting its significant socio-economic impact. In India, banana is a highly predominant and beloved crop, enjoyed equally by both the rich and the poor.

Banana is often referred to as the "Apple of Paradise" in countries like Uganda, Bakauba, and Tanzania, it serves as a staple food and is among the most widely traded tropical fruits globally (Radha and Mathew, 2007). In India, banana is deeply woven into cultural traditions, with its leaves, pseudo-stem, and fruits regarded as highly auspicious during festivals and celebrations. Beyond its role as a food crop, the banana provides fibre, fodder, beverages, fermented sugars, medicines, flavorings, silage, rope, cordage,

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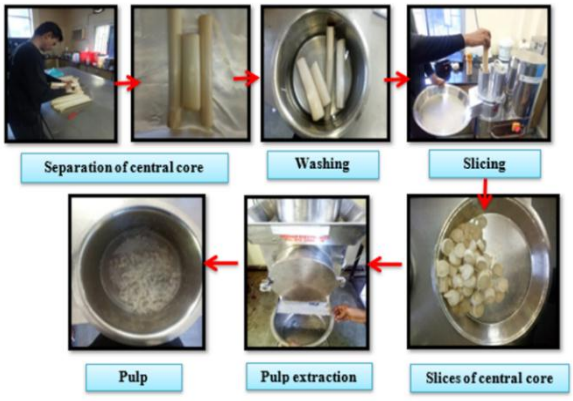
garlands, shelter materials and even roofing, and wall linings, earning it the title *Kalpatharu* (Tree of life). Banana pseudostem, a major agro-waste, contains significant amounts of dietary fiber and bioactive compounds. Its incorporation into food products like jam can enhance nutritional value while promoting waste utilization. Banana fruit, rich in sugars and micronutrients, offers a suitable base for blending. However, the effects of varying pseudostem-to-fruit ratios and storage duration on jam quality remain underexplored. This study evaluates the nutritional and physicochemical properties of banana fruit and pseudostem central core blended jam across different blending ratios and storage periods, aiming to develop a functional product with improved shelf life and sustainability potential. Hence, the present experiment was conducted to study the nutritional and physicochemical properties of blended jam made from banana fruit and pseudostem central core.

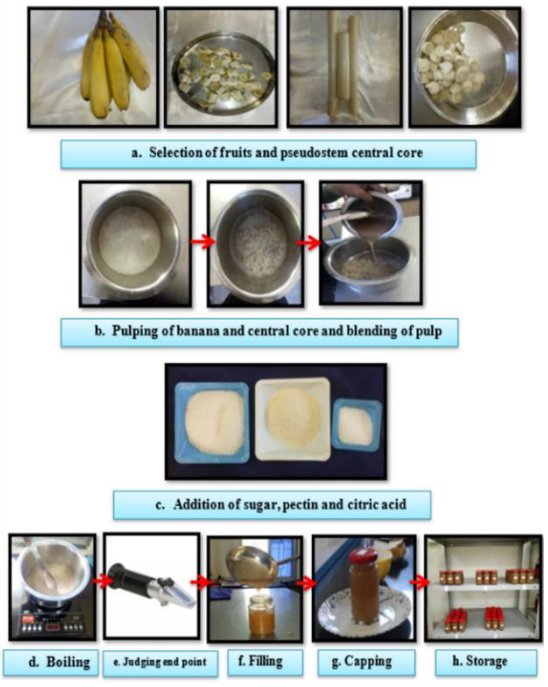
MATERIALS AND METHODS

The experiment was conducted at the Banana Pseudostem Processing Unit, Soil and Water Management Research Unit and Post Harvest Technology Laboratory, N.A.U., Navsari. Uniformsized three-quarter mature banana fruits (cv. Grand Naine) were harvested along with pseudostem immediately after bunch harvest in February. The pseudostems were washed, split and the central core was sliced and pulped. The experiment consisted of eleven treatments (T1 to T11), each varying in the proportion of banana fruit pulp to pseudostem central core. The proportion of banana fruit (BF) increased gradually from T_1 to T_{11}, while the proportion of pseudostem central core (PCC) decreased correspondingly. In Treatment T_1, the formulation contained 0% banana fruit and 100% central core. This was followed by T_2 with 10% banana fruit and 90% central core, T_3 with 20% banana fruit and 80% central core. The trend continued with T_4 (30% BF :70% PCC), T_5 (40% BF:60% PCC), T_6 (50% BF:50% PCC), T_7 (60% BF:40% PCC), T_8 (70% BF:30% PCC), T_9 (80% BF:20% PCC), and T_{10} (90% BF:10% PCC). Finally, Treatment T_{11} consisted of 100% banana fruit and 0% central core. This systematic variation allowed for the assessment of changes in quality parameters and acceptability as the proportion of banana fruit increased in the formulation. With each treatment maintaining a 1:1 pulp to sugar ratio, constant acidity (0.5%), pectin (0.75%) and TSS (68%). The principal steps followed for preparation of blended jam is given below and schematically presented in Figure 1.

Principal steps used for extraction of banana pulp



Principal steps used for extraction of central core pulp



Principal steps used for blended jam preparation

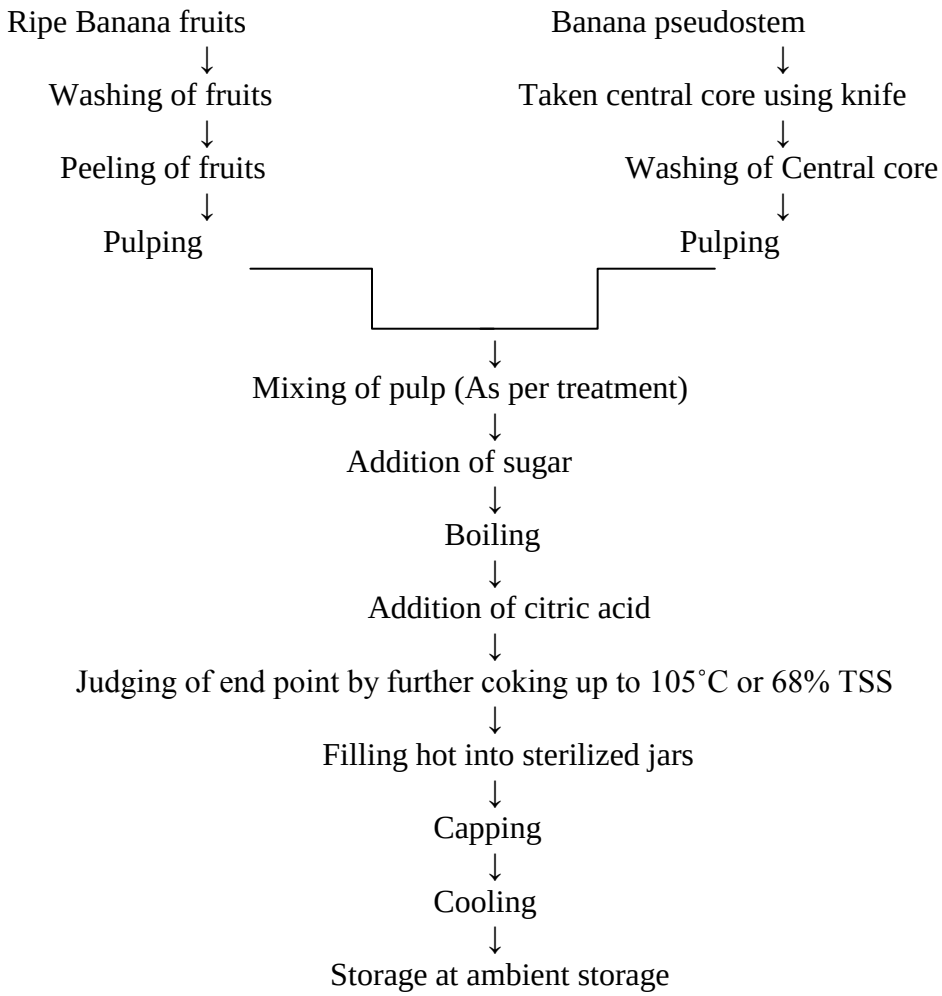


Figure 1. Principal step used for preparation of blended Jam

The 200g jam was stored in glass jars at room temperature (20 to 35°C) and evaluated for chemical and organoleptic properties at 0, 3, and 6 months (Feb–July 2018). Parameters like carbohydrates, protein (Sadasivam and Manickam, 1991), iron, potassium (Ranganna, 1986), calorific value (Eneche, 1991) and microbial load (Ranganna, 1986) were analyzed. Treatment differences were analyzedusing the 'F' test based on the null hypothesis. The standard error of mean (S.Em) was calculated for each parameter, and the critical difference (CD) at the 5% probability level was determined to assess statistical significance.

RESULTS AND DISCUSSION

Carbohydrates (%)

Carbohydrate content in blended jam recorded over 6 months is presented in Table 1. Significant variation was observed among treatments, ranging from 53.95% to 68.08% with a general increasing trend with increasing the ratio of banana flesh. The highest carbohydrate content was in T_{11} (68.08%) and the lowest in T_1 (53.95%). Over the storage period, the period mean carbohydrates slightly increased from 61.60% to 62.14% overall, with a marginal upward trend (Table 1). The increase in carbohydrates over storage might be due to the hydrolysis of polysaccharides into simple sugars, as noted by Oyleke et al. (2013). The non-significant interaction effects were observed between the treatment of blending and storage intervals (Table 1).

Protein (%)

Protein content in blended jam varied significantly across treatments and storage periods (Table 2). Treatment mean protein values ranged from 0.47% (T_1: 00:100) to 1.11% (T_{11}: 100:00), with T_{10} (1.02%) and T_2 (0.52%) following. A significant decrease in period mean protein was observed, from initially 0.80% to 0.74% after 6 months of storage. Significant treatment × period (T×P) interaction effects were noted. The highest protein was in $T_{11}P_1$ (1.16%) followed by $T_{11}P_2$ (1.14%), while the lowest was in T_1P_3 (0.46%) followed by T_1P_2 (0.47%) (Table 2). Protein declined over storage, with the least reduction in T_1 (0.49 to 0.46%) and higher in T_{11} (1.16 to 1.03%). Overall, protein content decreased gradually over 6 months. This trend was also reported by Reddy and Chikkasubbanna (2009) in aonla jam storage, Karanjalker *et al*. (2013) in

guava nectar with soy milk, and in banana central core jam.

Iron (mg/100 g)

The Iron content of banana fruit and pseudostem central core blended jam showed significant variation across treatments and storage periods (Table 3), with a decreasing trend over 6 months. Treatment means ranged from 0.75 mg/100g (T_{11}: 100:00) to 3.13 mg/100g (T_1: 00:100), with the lowest iron in T_{11} (0.75 mg/100gm) and the highest in T_1(3.13mg/100gm). Over storage period, mean iron content decreased significantly from 1.78 to 1.51 mg/100g. Among the interactions, highest iron was recorded in T_1P_1 (3.24 mg/100g), while the lowest was in $T_{11}P_3$ (0.63 mg/100g). Over period of storage, minimum decrease in iron was occurred in T_{10} (0.87 to 0.69 mg/100g), while the maximum was observed in T_2 (2.85 to 2.42 mg/100g). The decrease in iron content in storage might be due to higher iron content pseudostem central coredecline during storage. Similar results were found by Kumar *et al*. (2012).

Potassium (mg/100 g)

Potassium content of blended jam varied significantly across treatments, ranging from 34.03 to 42.37 mg/100g (Table 4). T_1 (42.37 mg/100g; 00:100) had the highest, while T_{11} (34.03 mg/100g; 100:00) had the lowest. Storage and treatment × period interactions were non-significant, but a general decrease in potassium was observed (Table 4). Similar trends were reported by Patel (2016). The non-significant interaction effects were observed between the treatment of blending and storage intervals (Table 4).

Calorific value (Kcal)

Calorific values of blended jam ranged from 218.79 to 277.74 KCal (Table 5), with the highest in T_{11} (277.74 KCal; 100:00) and the lowest in T_1 (218.79 KCal; 00:100). A slight increase in period mean calorific value was observed, from 250.60 to 252.51 KCal after 6 months. The treatment × period interaction was non-significant, and a stable, slight increase in calorific value during storage was noted (Table 5). The findings align with those of Anonymous (2015) in banana central core jam.

Microbial growth

No microbial growth was observed in any of the treatments throughout the storage period up to 6 months, and the microbial test results consistently yielded a nil outcome. The Total Plate Count (TPC) test revealed that, quality of blended jam did not deteriorate during the storage period at room temperature. There was no bacterial growth was found in the product up to 6 months. These kinds of observations were also recorded by Reddy and Chikkasubbanna (2009) in aonla jam, Sharma (2014) in mango-jamun blended jam.

Table 1. Effect of banana fruit and pseudostem central core blended jam on carbohydrates (%) during storage.

Treatments	Storage periods (P)			Mean (T)
	P_1 (Initial)	**P_2 (3 month)**	**P_3 (6 month)**	
T_1(0% BF:100% PCC)	53.68	53.96	54.22	**53.95**
T_2(10% BF:90% PCC)	55.71	55.99	56.25	**55.98**
T_3(20% BF:80% PCC)	56.83	57.14	57.40	**57.12**
T_4(30% BF:70% PCC)	59.39	59.67	59.99	**59.68**
T_5(40% BF:60% PCC)	61.00	61.28	61.54	**61.27**
T_6(50% BF:50% PCC)	62.35	62.63	62.83	**62.60**
T_7(60% BF:40% PCC)	64.45	64.72	64.98	**64.72**
T_8(70% BF:30% PCC)	64.81	65.10	65.34	**65.08**
T_9(80% BF:20% PCC)	65.48	65.72	65.98	**65.72**
T_{10}(90% BF:10% PCC)	66.06	66.37	66.64	**66.36**
T_{11}(100% BF:0% PCC)	67.81	68.09	68.33	**68.08**
Mean (P)	**61.60**	**61.88**	**62.14**	
	Treatment (T)	**Period (P)**	**T×P**	
S.Em±	0.156	0.078	0.334	
CD at 5%	0.459	0.222	**NS**	
CV % (a)	0.437	**CV % (b)**	1.10	

Table 2. Effect of banana fruit and pseudostem central core blended jam on protein (%) during storage.

Treatments	Storage periods (P)			Mean (T)
	P_1 (Initial)	**P_2 (3 month)**	**P_3 (6 month)**	
T_1(0% BF: 100% PCC)	0.49	0.47	0.46	**0.47**
T_2(10% BF: 90% PCC)	0.55	0.51	0.50	**0.52**

T_3(20% BF: 80% PCC)	0.66	0.63	0.59	**0.63**
T_4(30% BF: 70% PCC)	0.68	0.67	0.64	**0.66**
T_5(40% BF: 60% PCC)	0.73	0.69	0.68	**0.70**
T_6(50% BF: 50% PCC)	0.82	0.80	0.77	**0.80**
T_7(60% BF: 40% PCC)	0.85	0.83	0.81	**0.83**
T_8(70% BF: 30% PCC)	0.88	0.84	0.80	**0.84**
T_9(80% BF: 20% PCC)	0.93	0.91	0.89	**0.91**
T_{10}(90% BF: 10% PCC)	1.05	1.03	0.98	**1.02**
T_{11}(100% BF:0% PCC)	1.16	1.14	1.03	**1.11**
Mean (P)	**0.80**	**0.77**	**0.74**	
	Treatment (T)	**Period (P)**	**T×P**	
S.Em±	0.005	0.003	0.009	
CD at 5%	0.014	0.007	0.024	
CV % (a)	1.74	**CV % (b)**	1.85	

Table 3. Effect of banana fruit and pseudostem central core blended jam on iron (mg/100 g) during storage.

	Storage period (P)			
Treatments	**P_1 (Initial)**	**P_2 (3 month)**	**P_3 (6 month)**	**Mean (T)**
T_1(0% BF: 100% PCC)	3.24	3.19	2.95	**3.13**
T_2(10% BF: 90% PCC)	2.85	2.81	2.42	**2.69**
T_3(20% BF: 80% PCC)	2.57	2.39	2.32	**2.43**
T_4(30% BF: 70% PCC)	2.10	2.01	1.83	**1.98**
T_5(40% BF: 60% PCC)	1.85	1.79	1.46	**1.70**
T_6(50% BF: 50% PCC)	1.62	1.54	1.31	**1.49**
T_7(60% BF: 40% PCC)	1.42	1.23	1.14	**1.26**
T_8(70% BF: 30% PCC)	1.18	1.07	0.97	**1.07**
T_9(80% BF: 20% PCC)	1.07	1.01	0.84	**0.97**
T_{10}(90% BF: 10% PCC)	0.87	0.84	0.69	**0.80**
T_{11}(100% BF:0% PCC)	0.83	0.79	0.63	**0.75**
Mean (P)	**1.78**	**1.70**	**1.51**	
	Treatment (T)	**Period (P)**	**T×P**	
S.Em±	0.012	0.005	0.018	
CD at 5%	0.035	0.015	0.051	
CV % (a)	1.90	**CV % (b)**	1.86	

Table 4. Effect of banana fruit and pseudostem central core blended jam on potassium (mg/100 g) during storage.

Treatments	Storage period (P)			Mean (T)
	P_1 (Initial)	P_2 (3 month)	P_3 (6 month)	
T_1(0% BF: 100% PCC)	42.49	42.37	42.24	**42.37**
T_2(10% BF: 90% PCC)	41.63	41.53	41.43	**41.53**
T_3(20% BF: 80% PCC)	40.50	40.12	39.69	**40.10**
T_4(30% BF: 70% PCC)	39.37	39.24	39.12	**39.24**
T_5(40% BF: 60% PCC)	39.35	39.25	39.14	**39.25**
T_6(50% BF: 50% PCC)	38.27	38.25	38.18	**38.22**
T_7(60% BF: 40% PCC)	37.59	37.46	37.24	**37.43**
T_8(70% BF: 30% PCC)	36.35	36.26	36.14	**36.25**
T_9(80% BF: 20% PCC)	35.00	34.60	34.47	**34.69**
T_{10}(90% BF: 10% PCC)	34.44	34.29	34.22	**34.32**
T_{11}(100% BF:0% PCC)	34.05	34.02	34.00	**34.03**
Mean (P)	**38.10**	**37.95**	**37.80**	
	Treatment (T)	**Period (P)**	**T×P**	
S.Em±	0.278	0.102	0.337	
CD at 5%	0.808	**NS**	**NS**	
CV % (a)	2.18	**CV % (b)**	1.54	

Table 5. Effect of banana fruit and pseudostem central core blended jam on calorific value (KCal) during storage.

Treatments	Storage periods (P)			Mean (T)
	P_1 (Initial)	P_2 (3 month)	P_3 (6 month)	
T_1(0% BF: 100% PCC)	217.76	218.80	219.80	**218.79**
T_2(10% BF: 90% PCC)	226.12	226.90	227.99	**227.00**
T_3(20% BF: 80% PCC)	230.95	232.07	232.95	**231.99**
T_4(30% BF: 70% PCC)	241.27	242.35	243.51	**242.38**
T_5(40% BF: 60% PCC)	247.91	248.87	249.87	**248.88**
T_6(50% BF: 50% PCC)	253.67	254.71	255.39	**254.59**
T_7(60% BF: 40% PCC)	262.19	263.19	264.15	**263.18**
T_8(70% BF: 30% PCC)	263.75	264.75	265.55	**264.68**
T_9(80% BF: 20% PCC)	266.63	267.51	268.47	**267.54**
T_{10}(90% BF: 10% PCC)	269.43	270.59	271.47	**270.50**
T_{11}(100% BF:0%	276.87	277.91	278.43	**277.74**

PCC)				
Mean (P)	**250.60**	**251.60**	**252.51**	
	Treatment (T)	**Period (P)**	**T×P**	
S.Em±	0.555	0.423	1.40	
CD at 5%	1.638	1.22	**NS**	
CV % (a)	0.38	**CV % (b)**	1.12	

CONCLUSION

In conclusion, the study on banana fruit and pseudostem central core blended jam revealed significant variations in nutritional and physicochemical properties based on blending ratios and storage periods. The iron, potassium, carbohydrate, and protein content showed a general decrease over 6 months. The findings highlight that blending ratios and storage conditions significantly impact the nutritional profile, suggesting that optimized combinations can enhance specific components in the jam. Additionally, the study emphasizes the importance of balancing the fruit and pseudostem central core ratios for maximizing nutrient retention, while also contributing valuable insights into the effects of storage on product quality. These results can guide future research and product development in the field of food processing using banana and pseudostem resources.

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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING OF 70% HYDROALCOHOLIC AND ACETONE EXTRACT OF RHIZOME OF *CURCUMA LONGA*

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Abstract: The genus *Curcuma* belongs to the family Zingiberaceae. *Curcuma longa*, commonly known as turmeric, rhizomatous herb is indigenous to the Indian subcontinent and Southeast Asia. The rhizomes can be utilized fresh or boiled, and when dried, they are ground into a vibrant orange-yellow powder that serves as both a coloring and flavoring agent in various Asian cuisines, particularly in curries. *C. longa* is frequently used in traditional medicine to treat a wide range of ailments, including diarrhoea, diabetes, skin infections and wounds. An experimental study was undertaken to assess the phytochemical constituents of *C. longa* by using 70% ethanol and acetone as solvent for the extraction of rhizomes. The sieved plant powder was soaked in 70% ethanol and acetone in glass containers for 48 hrs. and then filtered (maceration method). Finally obtained extract was used for phytochemical screening tests. All the tests were performed as per standard protocol using the chemicals and reagents procured from authenticated chemical manufacturers and results were recorded. The percent yield was 10.81% and 7.31%, dark brown and yellowish orange color and gummy-sticky and granular consistency of extract were also found respectively. The phytochemical screening of 70% hydroalcoholic extract of rhizome of *C. longa* revealed that the presence of phytoconstituents *viz.*: alkaloids, flavonoids, phenolic compounds, tannins, saponins, phytosterols, resins and triterpenoids. Preliminary phytochemical analysis of acetone extract of rhizome of *C. longa* revealed that the presence of phytoconstituents *viz.*: alkaloids, flavonoids, phenolic compounds, tannins, saponins, phytosterols, diterpenes, carboxylic acid, resins, triterpenoids, fixed oils and fat. Primary metabolites are absent in both the extracts of *Curcuma longa*. These phytoconstituents of the plant might play an important role in therapeutic properties.

Keywords: Hydroalcoholic extract, Acetone extract, *Curcuma longa*, Phytochemical, Rhizomes, Therapeutic

INTRODUCTION

The Zingiberaceae family comprises approximately 50 genera and around 1,600 recognized species of aromatic perennial herbs characterized by creeping horizontal or tuberous rhizomes. These plants are predominantly found in tropical regions of Africa, Asia, and the Americas. Numerous species within this family hold significance as ornamental, spice, or medicinal plants (Christenhusz, 2016). *Curcuma longa*, commonly known as turmeric, is a flowering plant belonging to the Zingiberaceae family. This perennial, rhizomatous herb is indigenous to the Indian subcontinent and Southeast Asia. The rhizomes can be utilized fresh or boiled, and when dried, they are ground into a vibrant orange-yellow powder that serves as both a coloring and flavoring agent in various Asian cuisines, particularly in curries.

The primary component of turmeric, curcumin, is also noted for its dyeing properties (Curcumin from PubChem). Turmeric has been an integral part of Asian culture for centuries and plays a significant role in Ayurveda, Siddha medicine, traditional Chinese medicine and Unani practices (Chattopadhyay, 2004).

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A. Organic farm turmeric B. Rhizomes C. Powder

PLATE 1: *Curcuma longa* (Turmeric)

MATERIALS AND METHODS

Fresh rhizomes of Curcuma *longa* were collected from organic farms in the Udgir region. The taxonomic identification of the plant was certified by the botanist from the Department of Botany, Maharashtra Udaygiri Mahavidyalaya, Udgir. The fresh rhizomes of C. *longa* were collected, washed under running tap water and boiled for thirty minutes and then rinsed with distilled water. The rhizomes were cut into small pieces (for early drying) and dried in the shade for three weeks. Completely dried small pieces were pulverized using an electric mixer grinder. The coarse powder was then passed through a mesh screen, and the fine powder obtained was kept in an airtight container for its further phytochemical analysis.

Chemicals & Reagents

The chemicals employed in the current investigation were commercially purchased from authenticated dealers. Absolute ethanol, acetone, copper acetate, 1% glacial acetic acid, resorcinol, dil. HCl, copper sulphate (CuSO4), potassium sodium tartrate, 10% 1N Sodium hydroxide (NaOH), concentrated nitric acid (Conc. HNO3), Ninhydrin, saturated aqueous solution of picric acid, mercuric chloride, potassium Iodide (KI), iodine, bismuth carbonate, sodium iodide, sodium acetate, ethyl acetate, acetic acid, anhydrous ferric chloride (An. FeCl3), concentrated sulphuric acid (Conc.H2SO4), lead acetate, sodium bi-carbonate (NaHCO3), sulphur powder, Chloroform, sodium chloride (NaCl), and Conc. HCl were purchased from Hi-media Laboratories Pvt. Ltd.

Reagents

Commercially available reagents which included Folin Wu copper reagent, Benedict's reagent, Biuret reagent, Fehling's reagents 1 & 2 were purchased from Hi-media Laboratories Pvt. Ltd.

Preparation of the extract

Preparation of plant extract for the experimental study is the first step, before moving further with the desired biological testing as it entails the extraction and assessment of the quality and amount of bioactive elements. The 70% hydroalcoholic and acetone extract of rhizome of *curcuma longa* was prepared by maceration method.

70% hydroalcoholic extract of rhizome of *Curcuma longa:*

Fifty gm powder of *Curcuma longa* rhizome and was placed in a stoppered conical flask with 300 ml of 70% ethanol (70 ml of ethanol & 30 ml of distilled water) and kept at room temperature for a maceration period of 48hrs with frequent agitation at an interval of two hrs. The mixture was then filtered through muslin cloth. The residue left in the flask was washed twice taking a little quantity of 70% ethanol and filtered through a muslin cloth. After that, the filtrate was again filtered through Whatman filter paper No. 1. The filtrate was transferred to a sterilized evaporating dish and then kept under a fan to allow the solvent to evaporate. The extract left in the dish were taken in the airtight screw cap vials, labeled and stored at 4^0C.

Acetone extract of rhizome of *Curcuma longa:*

Fifty gm powder of *Curcuma longa* rhizome and was placed in a stoppered conical flask with 300 ml of acetone and kept at room temperature for a maceration period of 48hrs with frequent agitation at an interval of two hrs. The mixture was then filtered through muslin cloth. The residue left in the flask was washed twice taking a little quantity of acetone and filtered through a muslin cloth. After that, the filtrate was again filtered through Whatman filter paper No. 1. The filtrate was transferred to a sterilized evaporating dish and then kept under a fan to allow the solvent to evaporate. The extract left in the dish were taken in the airtight screw cap vials, labeled and stored at 4^0C.

Calculation of yield (Extractability percentage)

The percent yield (dry weight of extract) of 70% hydroalcoholic and acetone extract of rhizome of *Curcuma longa* (w/w) was calculated using the formula as mentioned below:

$$\% \text{ yield} = \frac{\text{Final weight of the extract}}{\text{Initial weight of the powder}} \times 100$$

Phytochemical screening

The preliminary qualitative phytochemical analysis of acetone & 70% hydroalcoholic extract of rhizome of *Curcuma longa* were done to detect presence or absence of various phytoconstituents namely alkaloids, carbohydrates, glycosides, proteins, reducing sugar, tannins, sterols, phenolic compounds and saponins (Raaman, 2006; Silva *et al*., 2017; Shaikh & Patil, 2020).

Qualitative phytochemical analysis

Organic analysis of primary metabolites

The 70% hydroalcoholic and acetone extract of rhizome of *curcuma longa* was subjected for qualitative phytochemical analyses using the tests and techniques as described by previous authors for the presence or absence of various phytoconstituents.

Test for carbohydrates:

Solvent free extract (100mg) was dissolved in 5mL of distilled water and filtered. To the filtrate, a few drops of each of the following reagents were added and thoroughly mixed. The appearance of turbidity or any alteration in color during the test signifies the presence of carbohydrates.

a) Barfoed's test:

To a 1 mL sample of filtrate, 1 mL of Barfoed's reagent was added and subsequently heated in a boiling water bath for a duration of 2 minutes.

Barfoed's reagent:

Copper acetate (30.5gm) was dissolved in 1.8mL glacial acetic acid.

b) Seliwanoff's Test:

To a 1mL extract solution, 3mL seliwanoff's reagent was added and heated in a boiling water bath for 1 min.

Seliwanoff's reagent:

Resorcinol (0.05 gm) was dissolved in 100 mL of dilute HCl.

Test for Reducing Sugar:

Solvent free extract (100mg) was dissolved in 5mL of distilled water and filtered. A few drops of each of the following reagents were added to the filtrate and mixed well, the appearance of turbidity or any changes in color to the test indicates the presence of reducing sugar.

a) Benedict's test:

To a 0.5mL filtrate, 0.5mL Benedict's reagent was added and heated on a boiling water bath for 2 min.

b) Fehling's test:

To a 1mL filtrate, 1mL each of Fehling's solution A & B was added and boiled in a water bath.

Fehling's solutions:

Solution A: 34.66gm copper sulphate + distilled water to make final volume 100mL.

Solution B: 173 gm potassium sodium tartrate + 50 gm NaOH + distilled water to make 100mL.

Test for Proteins:

a) Biuret test:

A few amounts of the extracts were added to 4% sodium hydroxide solution followed by a drop of 1% copper sulphate solution.

b) Xanthoprotein test:

A small amount of the extract was added with 0.5 mL of concentrated HNO_3.

Test for amino acids

A test for amino acids was performed on the extract filtrate prepared by dissolving the 100 mg of extract in 10 mL of distilled water and filtering it through Whatman filter paper No. 1.

Ninhydrin test:

Two drops of ninhydrin solution (10 mg of ninhydrin in 200 mL of acetone) are added to two mL of filtrate.

Qualitative analysis of secondary metabolites

Test for Alkaloids:

Solvent free extract (50 mg) was mixed with a few mL dil. HCl and then filtered. A few drops of each of the following reagents were added to the filtrate and mixed well, the appearance of turbidity or any changes in color to the test indicates the presence of alkaloids.

a) Hager's test:

To a few mL of filtrate, 1-2 mL Hager's reagents (saturated aqueous solution of picric acid) were added.

b) Mayer's test:

To a few mL of filtrate, a drop or two of Mayer's reagent were added by the side of the test tube.

Mayer's reagent:

Mercuric chloride (1.358gm) was dissolved in 60mL distilled water and 5 gm potassium iodide was dissolved in 10mL distilled water. The two solutions were mixed and made upto 100mL with distilled water.

c) Wagner's test:

To a few mL of filtrate, a drop or two of Wagner's reagent were added by the side of the test tube.

Wagner's reagent:

Iodine (1.27gm) and potassium iodide (2gm) were dissolved in 100mL of distilled water.

d) Dragendorff's test:

To a few mL of filtrate, 1-2 mL Dragendorff's reagents were added.

Dragendorff's reagent:

Stock solution: Bismuth carbonate (5.2 g) and sodium iodide (4 g) were boiled for a few min with 50 mL glacial acetic acid. After 12 h, the precipitated sodium acetate crystals were filtered off using a sintered glass funnel. Clear, red-brown filtrate, 40 mL was mixed with 160 mL ethyl acetate and 1 mL water and stored in an amber - coloured bottle.

Working solution: Ten mL of stock solution was mixed with 20 mL of acetic acid and made up to 100 mL with water.

Test for Glycoside:

a) Folin Wu copper reagent test:

A few drops of Folin Wu copper reagent were added to a small amount of extract.

b) Benedict's test:

To a 0.5mL filtrate, 0.5mL Benedict's reagent was added and heated on a boiling water bath for 2 min.

c) Concentrated H_2SO_4 test:
To a 5ml plant extract, 2mL glacial acetic acid, a drop of 5% $FeCL_3$ and conc. H_2SO_4 were added along the side of test tube.

Test for cardiac glycosides:

Keller-Killani test:
To 1 ml of an extract solution, 1.5 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Carefully few drops of concentrated sulphuric acid were added to the sides of the test tube.

Test for flavonoids:

a) Lead acetate test:
To 1 ml of plant extract solution, a few drops of 10% lead acetate solution was added in a test tube.

b) Ferric chloride test:
To extract aqueous solution, a few drops of 10% ferric chloride solution was added in a test tube.

c) Conc. H_2SO_4 test:
To a plant extract solution, a few drops of conc. H_2SO_4 was added in a test tube.

Test for Phenolic Compounds:

a) Iodine test:
To 1 ml of plant extract solution, a few drops of dil. Iodine solution was added in a test tube.

b) Ferric chloride test:
To extract aqueous solution, a few drops of 5% ferric chloride solution was added in a test tube.

c) Lead acetate test:
Plant extract was dissolved in 5mL distilled water and added 3 mL of 10% lead acetate solution in a test tube.

Test for Tannins:

10% NaOH test:
To a 0.4mL plant extract, 4mL 10% NaOH was added and shaken well.

Test for Saponins:

a) Foam test:
A small amount of extract was treated with 2 mL of distilled water, the mixture shaken vigorously.

b) Sodium bicarbonate test:
A small amount of extract was treated with 2 mL of sodium bicarbonate and added with distilled water, the mixture shaken vigorously.

Test for Phytosterols:

a) Salkowski test:
A small amount of filtrate (Equal quantity of chloroform is treated with plant extract and filtered) was treated with few drops of conc. H_2SO_4, Shaken well and allowed to stand.

b) Sulphur test:
A small amount of extract solution was added with a pinch of sulphur powder.

Test for Diterpenes:

Copper acetate test:
Plant extract was dissolved in distilled water and added 3-4 drops of copper acetate solution.

Test for Quinones:

Conc. HCl test:
A small amount of plant extract was treated with conc. HCl.

Test for Carboxylic acid:

Effervescence test:
Plant extract (1mL) was added with 1mL sodium bicarbonate solution.

Test for Coumarins:

Sodium hydroxide test:
A few amounts of the extracts were added to 10% sodium hydroxide solution followed by chloroform.

Test for Resins:

Turbidity test:
Plant extract was dissolved in acetone and poured in distilled water.

Test for Fixed Oils and Fat:

Spot test / Stain test:
Little quantity of plant extract was pressed in between two filter papers.

Test for Triterpenoids:

Salkowski test:
A small amount of filtrate (Equal quantity of chloroform is treated with plant extract and filtered) was treated with few drops of conc. H_2SO_4, Shaken well and allowed to stand.

RESULTS

Phytochemical analysis

Physical nature and pH of the extract

Physical nature and percentage yield of 70% hydroalcoholic and acetone extract of rhizome of *C. longa* is presented in table 1.

Table 1. Physical nature and *percent* yield of 70% hydroalcoholic and acetone extract of rhizome of *C. longa*

Sr. no.	Details	Results	
		70% hydroalcoholic extract of *Curcuma longa*	**acetone extract of *Curcuma longa***
1	Solvent used	70% ethanol	acetone
2	Extract colour	dark brown	yellowish orange
3	Consistency	gummy-sticky (semisolid)	granular (semisolid)
4	Percent Yield (w/w)	10.81%	7.31%
5	pH	6.5	6.5

Organic analysis

The 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was subjected to preliminary phytochemical analysis and the results were illustrated in table 2 and Plate 2, 3, 4 and 5.

Qualitative analysis of primary metabolites

Test for carbohydrates

a. Barfoed's test

There was no formation of red precipitate indicating the absence of carbohydrates (monosaccharides) in 70% hydroalcoholic and acetone extract of rhizome of *C. longa*.

b. Seliwanoff's test

The non appearance of rose red color indicated that the result was negative for the presence of carbohydrate in 70% hydroalcoholic and acetone extract of rhizome of *C. longa*.

Test for Reducing Sugar:

a. Benedict's test

Absence of the formation of characteristic red precipitate with the test revealed the absence of sugars in 70% hydroalcoholic and acetone extract of rhizome of *C. longa*.

b. Fehling's test

The absence of carbohydrates was indicated by the lack of formation of a brick red color precipitate in 70% hydroalcoholic and acetone extract of rhizome of *C. longa*.

Test for proteins

a. Biuret test

The test was negative as indicated by lack of formation of pink/violet color in the ethanolic layer indicating the absence of protein in 70% hydroalcoholic and acetone extract of rhizome of *C. longa*.

b. Xanthoproteic test

The absence of brownish-yellow or reddish orange color revealed that the 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was negative for proteins.

Test for amino acids

Ninhydrin test

The 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was negative for amino acids as indicated by absence of blue or violet color.

Qualitative analysis of secondary metabolites

Test for alkaloids

a. Hager's test

The formation of yellow precipitate indicated that the test was positive for the presence of alkaloids in 70% hydroalcoholic and acetone extract of rhizome of *C. longa*.

b. Mayer's test

Development of cream coloured precipitate indicated that 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was positive for the presence of alkaloids.

c. Wagner's test

The reddish brown coloured precipitate observed indicated the presence of alkaloids in 70% hydroalcoholic and acetone extract of rhizome of *C. longa*.

d. Dragendorff's test

The formation of reddish-brown precipitate indicated that, 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was positive for the presence of alkaloids.

Test for Glycoside:

a. Folin Wu copper reagent test:

The 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was negative for glycosides as indicated by absence of red color.

b. Benedict's test:

The 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was negative for glycosides as indicated by absence of green, red or yellow color.

c. Concentrated H_2SO_4 test:

The 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was negative for glycosides as indicated by absence of A brown ring.

Test for cardiac glycosides:

Keller-Killani test:

The 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was negative for cardiac glycosides as indicated by absence of formation of blue color in the acetic acid layer.

Test for flavonoids:

a. Lead acetate test:

The 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was negative for flavonoids as indicated by absence of a yellow precipitate.

b. Ferric chloride test:

The 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was positive for flavonoids as indicated by presence of a green precipitate.

c. Conc. H_2SO_4 test:

The 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was positive for flavonoids as indicated by the appearance of an orange color.

Test for Phenolic Compounds:

a. Iodine test:

The 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was positive for phenolic compounds as indicated by the appearance of a transient red color.

b. Ferric chloride test:

The 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was positive for phenolic compounds as indicated by the appearance of dark green or bluish black color.

c. Lead acetate test:

The 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was negative for phenolic compounds as indicated by absence of a white precipitate.

Test for Tannins:

10% NaOH test:

The 70% hydroalcoholic and acetone extract of rhizome of *C. longa* were positive for tannins as indicated by the formation of emulsion.

Test for Saponins:

a. Foam test:

The 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was positive for saponins as indicated by development of froth.

b. Sodium bicarbonate test:

The 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was positive for saponins as indicated by development of froth.

Test for Phytosterols:

a. Salkowski test:

Development of the red layer at the bottom indicated that 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was positive for the presence of phytosterols.

b. Sulphur test:

Sulphur sinks to the bottom indicated that 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was positive for the presence of phytosterols.

Test for Diterpenes:

Copper acetate test:

The 70% hydroalcoholic extract of rhizome of *C. longa* was negative for diterpenes as indicated by absence of emerald green color. Development of the emerald green color indicated that acetone extract of rhizome of *C. longa* was positive for the presence of diterpenes.

Test for Quinones:

Conc. HCl test:

The 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was negative for quinones as indicated by absence of green color.

Test for Carboxylic acid:

Effervescence test:

The 70% hydroalcoholic extract of rhizome of *C. longa* was negative for carboxylic acid as indicated by absence of Effervescence. Appearance of Effervescence indicated that acetone extract of the rhizome of *C. longa* was positive for the presence of carboxylic acid.

Test for Coumarins:

Sodium hydroxide test:

The 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was negative for coumarins as indicated by absence of yellow color.

Test for Resins:

Turbidity test:

The 70% hydroalcoholic and acetone extract of rhizome of *C. longa* was positive for resins as indicated by formation of turbidity.

Test for Fixed Oils and Fat:

Spot test / Stain test:

The 70% hydroalcoholic extract of rhizome of *C. longa* was negative for fixed oils and fat as indicated by absence of oil staining on filter paper. Acetone extract of rhizome of *C. longa* was positive for the presence of fixed oils and fat as indicated by oil staining on filter paper.

Test for Triterpenoids:

Salkowski test:

Development of the red layer at the bottom indicated that 70% hydroalcoholic and acetone extract of the rhizome of *C. longa* was positive for the presence of triterpenoids.

Table 2. Preliminary phytochemical analysis of 70% hydroalcoholic and acetone extract of rhizome of *C. longa*

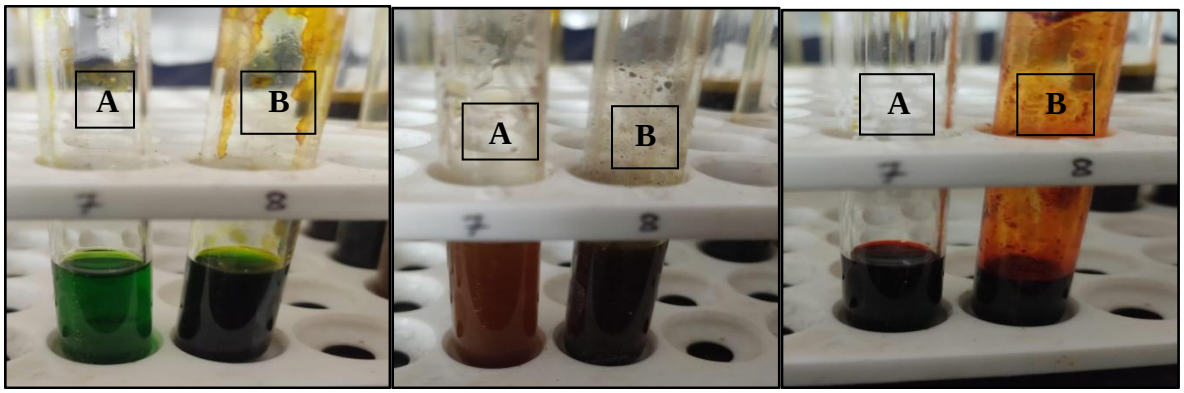
Sr. no.	Phytochemical (active constituent)	Tests	Positive reaction	70% hydroalcoholic extract of *Curcuma longa*	acetone extract of *Curcuma longa*
Primary metabolites					
1	Carbohydrates	Barfoed's test	red precipitate	- ve	- ve
		Seliwanoff's Test	rose red color	- ve	- ve
2	Reducing sugars	Benedict's test	green, red or yellow color	- ve	- ve
		Fehling's test	red precipitate	- ve	- ve
3	Proteins	Biuret test	A pink colored solution	- ve	- ve
		Xanthoproteic test	A yellow color solution	- ve	- ve
4	amino acids	Ninhydrin test	A purple colored solution	- ve	- ve

Secondary metabolites					
1	Alkaloid	Hager's test	yellow precipitate	+ ve	+ ve
		Mayer's test	white or creamy precipitate	+ ve	+ ve
		Wagner's test	reddish brown precipitate	+ ve	+ ve
		Dragendorff's test	reddish brown precipitate	+ ve	+ ve
2	Glycosides	Folin Wu copper reagent test	red color	- ve	- ve
		Benedict's test	green, red or yellow color	- ve	- ve
		Concentrated H_2SO_4 test	A brown ring	- ve	- ve
3	Cardiac glycosides	Keller-Killani test	blue color in the acetic acid layer	- ve	- ve
4	Flavonoids	Lead acetate test	A yellow precipitate	- ve	- ve
		Ferric chloride test	A green precipitate	+ ve	+ ve
		Conc. H_2SO_4 test	An orange color	+ ve	+ ve
5	Phenolic compounds	Iodine test	A transient red color	+ ve	+ ve
		Ferric chloride test	Dark green/ bluish black color	+ ve	+ ve
		Lead acetate test	A white precipitate	- ve	- ve
6	Tannins	10% NaOH test	Formation of emulsion	+ ve	+ ve
7	Saponins	Foam test	Persistent foam for 10 min.	+ ve	+ ve
		$NaHCO_3$ test	Stable honeycomb like froth	+ ve	+ ve
8	Phytosterols	Salkowski test	Red/ golden yellow layer (at the bottom)	+ ve	+ ve
		Sulphur test	Sulphur sinks to bottom	+ ve	+ ve

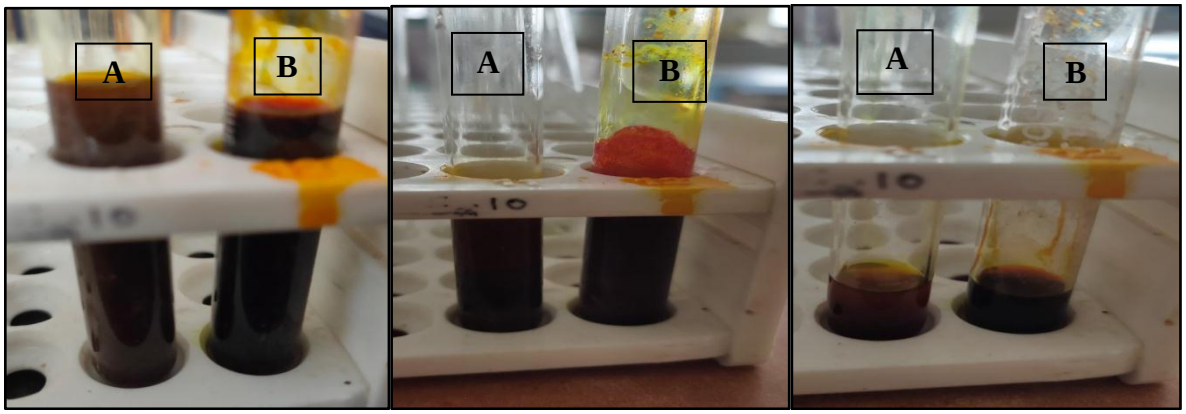
9	Diterpenes	Copper acetate test	Emerald green color	- ve	+ ve
10	Quinones	Conc. HCl test	A green color	- ve	- ve
11	Carboxylic acid	Effervescence test	Appearance of effervescence	- ve	+ ve
12	Coumarins	NaOH test	Yellow color	- ve	- ve
13	Resins	Turbidity test	Turbidity	+ ve	+ ve
14	Fixed oils and fat	Spot test/ stain test	Oil stain on filter paper	- ve	+ ve
15	Triterpenoids	Salkowski test	Red/ golden yellow layer (at the bottom)	+ ve	+ ve

Inference

Preliminary phytochemical analysis of 70% hydroalcoholic extract of rhizome of *curcuma longa* revealed that the presence of phytoconstituents *viz.*: alkaloids, flavonoids, phenolic compounds, tannins, saponins, phytosterols, resins and triterpenoids. Preliminary phytochemical analysis of acetone extract of rhizome of *Curcuma longa* revealed that the presence of phytoconstituents *viz.*: alkaloids, flavonoids, phenolic compounds, tannins, saponins, phytosterols, diterpenes, carboxylic acid, resins, triterpenoids, fixed oils and fat. Primary metabolites are absent in both the extracts of *Curcuma longa*. The results of phytochemical tests are as presented in table 2 and Plate 2, 3, 4 and 5.

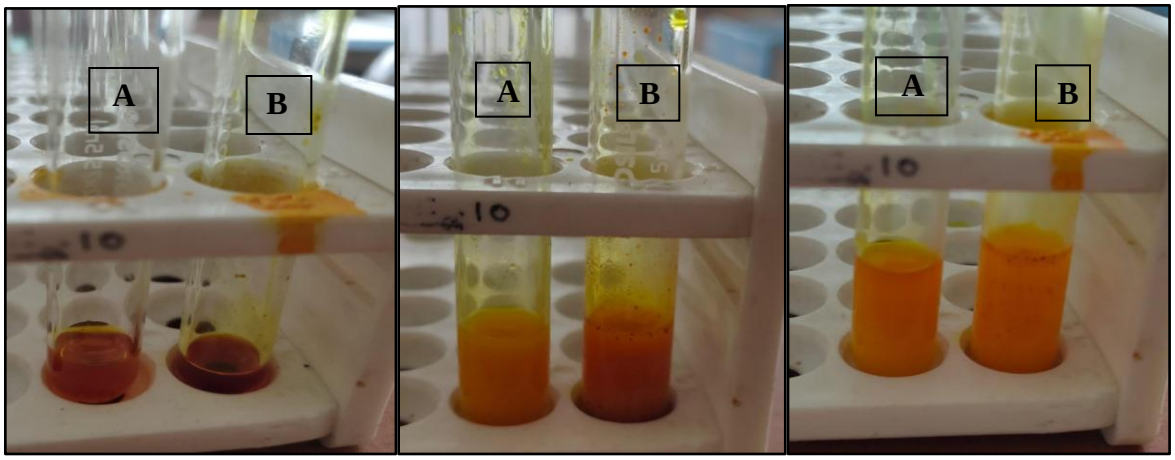


Barfoed's test: both -ve Seliwanoff's Test: : both -ve Benedict's test: both -ve

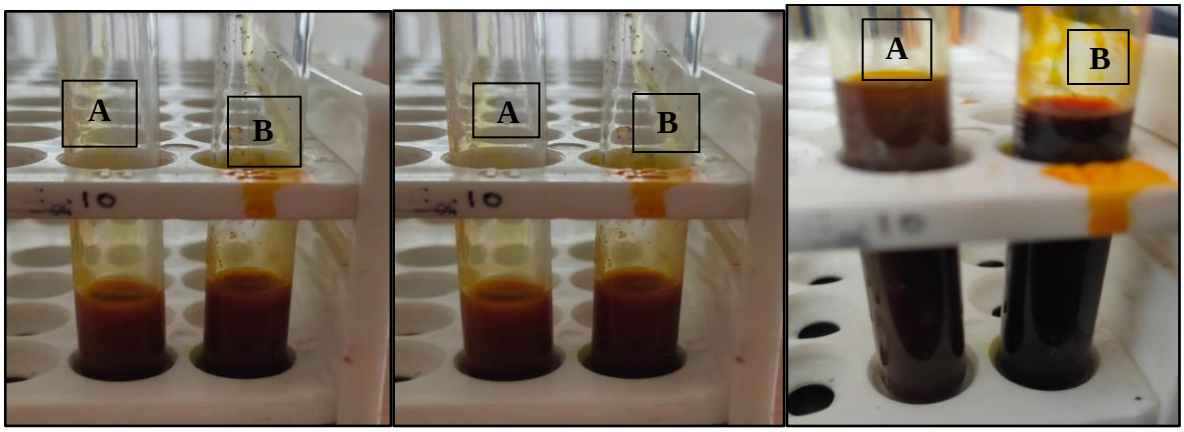


Fehling's test: both -ve Biuret test: both -ve Xanthoproteic test: both -ve

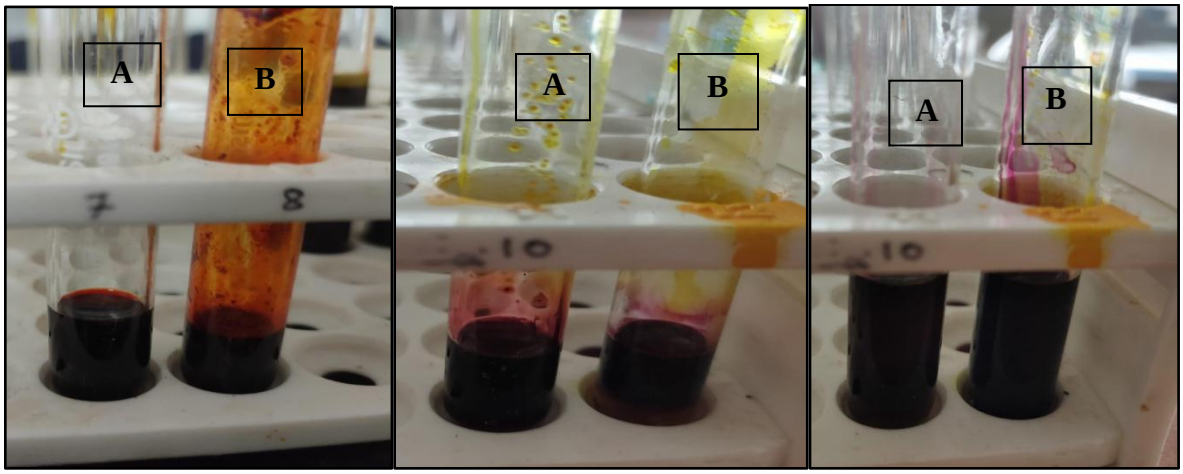
PLATE 2: Results of Phytochemical analysis of 70% hydroalcoholic and acetone extract of the rhizome of *Curcuma longa*



Ninhydrin test: both -ve | Hager's test: both +ve | Mayer's test: both +ve

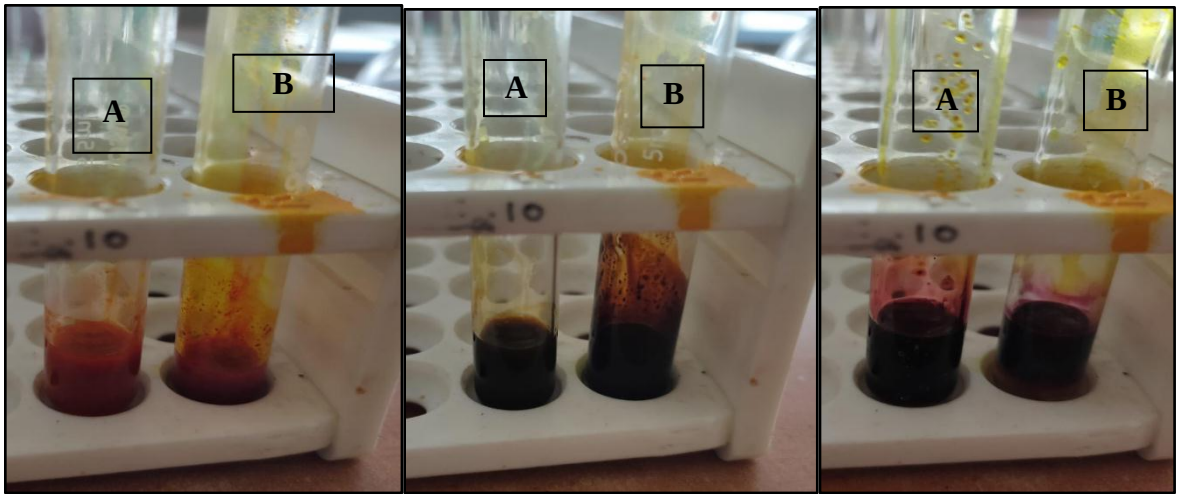


Wagner's test: both +ve | Dragendorff's test: both +ve | Folin Wu test: both -ve

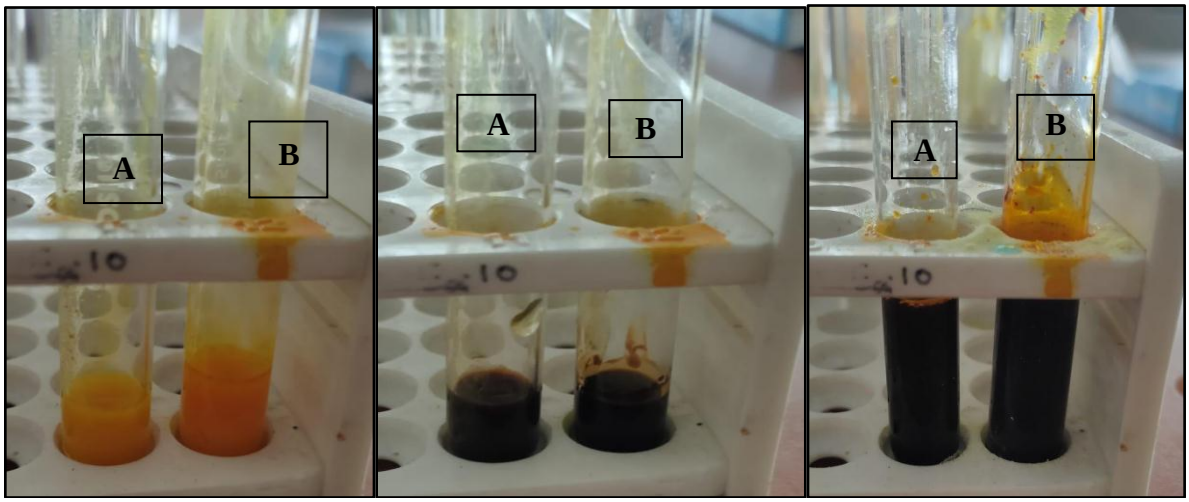


Benedict's test: both -ve | Concentrated H_2SO_4 test: both -ve | Keller-Killani test: both -ve

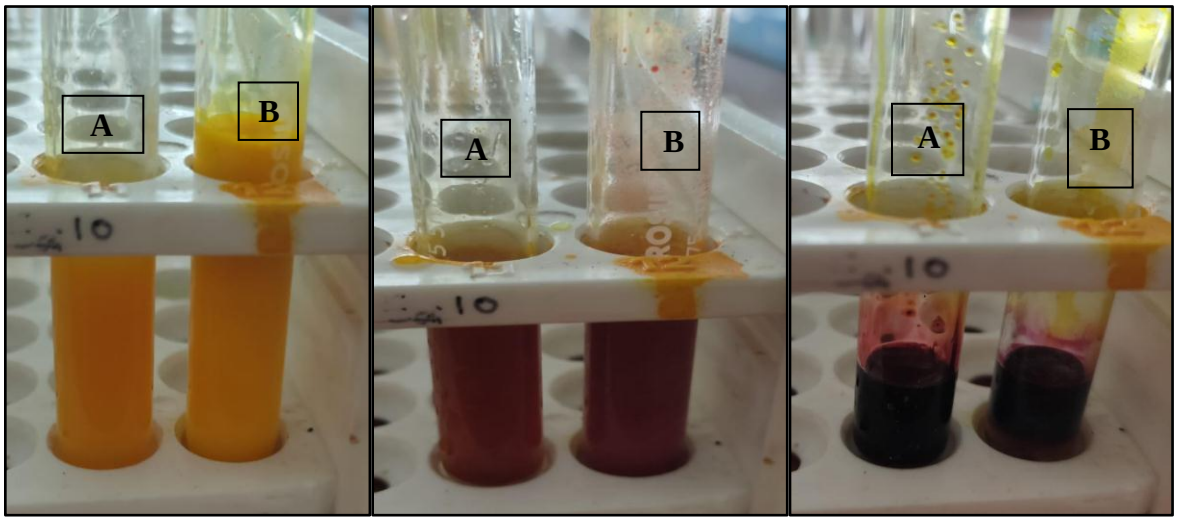
PLATE 3: Results of Phytochemical analysis of 70% hydroalcoholic and acetone extract of the rhizome of *Curcuma longa*



Lead acetate test: both -ve | Ferric chloride test: both +ve | Conc. H_2SO_4 test: both +ve



Iodine test: both +ve | Ferric chloride test: both +ve | 10% NaOH test: both +ve



Foam test: both +ve | $NaHCO_3$ test: both +ve | Salkowski test: both +ve

PLATE 4: Results of Phytochemical analysis of 70% hydroalcoholic and acetone extract of the rhizome of *Curcuma longa*

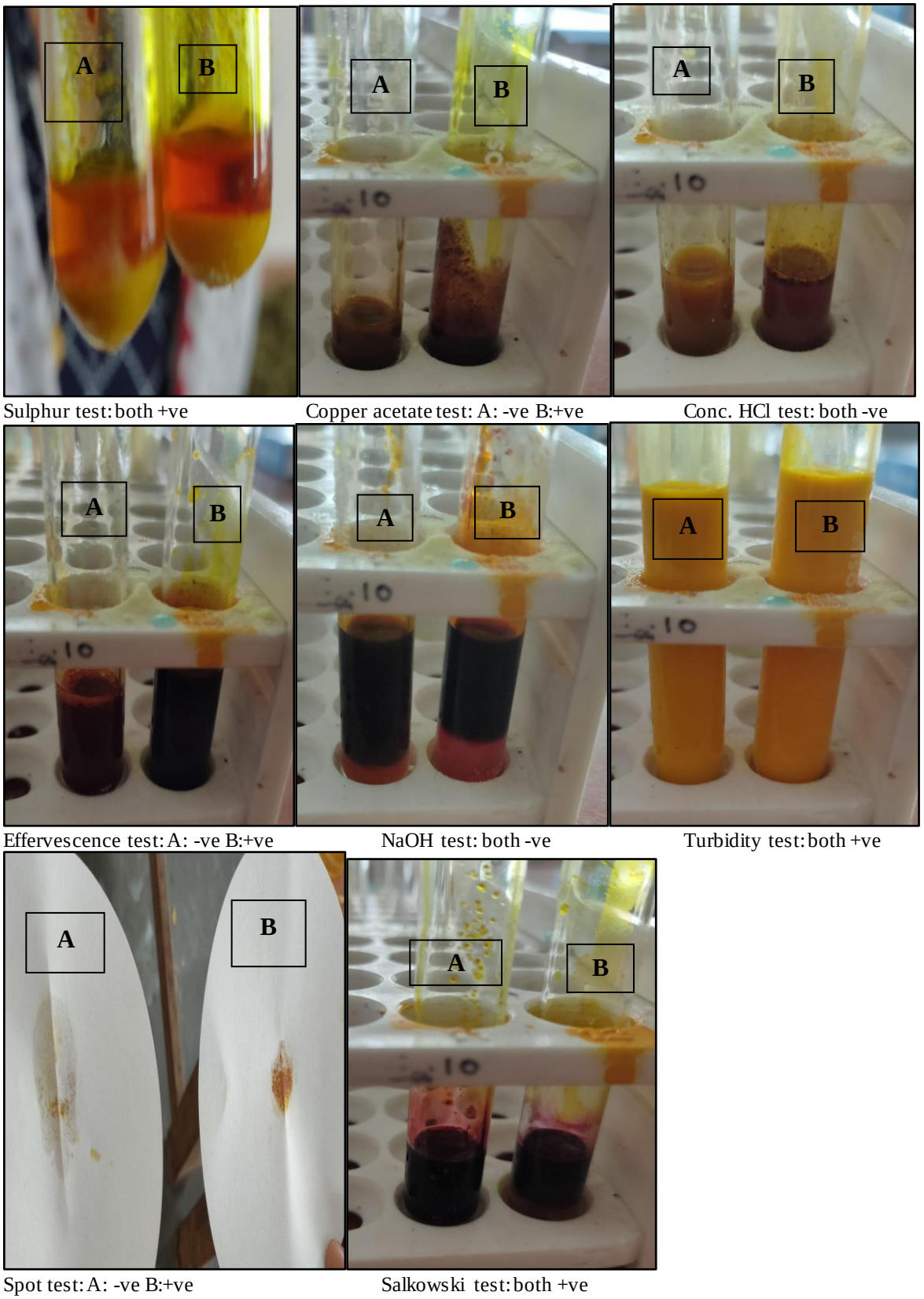


PLATE 5: Results of Phytochemical analysis of 70% hydroalcoholic and acetone extract of the rhizome of *Curcuma longa*

DISCUSSION

Curcuma longa contains various phytochemical constituents with therapeutic properties and has been previously reported to be used in traditional medicine to treat various ailments due to various therapeutic effects.

The physical nature of 70% hydroalcoholic extract of the rhizome of *C. longa* was dark brown with gummy-sticky (semisolid) consistency. A percent yield of 10.81% w/w was estimated in the present study. The pH of the extract was 6.5. The physical nature of acetone extract of the rhizome of *C. longa* was yellowish orange with granular (semisolid) consistency. A percent yield of 7.31% w/w was estimated in the present study. The pH of the extract was 6.5.

The extractability percentage of 70% hydroalcoholic and acetone extract of rhizome of *C. longa* is presented in Table no. 1. In the present study, the extractability percentage was higher in 70% ethanol than the acetone. Whereas, Arawande *et al.*, (2018) observed the same results i.e. extractive value for turmeric was highest in ethanol and this was followed by acetone.

Primary metabolites viz.: carbohydrates, reducing sugars and proteins were absent in acetone and 70% hydroalcoholic extract of rhizome of *Curcuma longa*. These findings were not in agreement with Arawande *et al.*, (2018) reported presence of reducing sugar in acetone extract of rhizome of *Curcuma longa*; Mehra and jain (2019) and Pawar and Kadu (2020) recorded presence of carbohydrates, absence of protein in acetone extract of *Curcuma longa*. Santhosh *et al.*, (2011), Oghenejobo and Bethel (2017) and Nazir and Chauhan (2019), reported the presence of similar primary metabolites, in distilled water and ethanol.

Preliminary phytochemical analysis of acetone extract of rhizome of *Curcuma longa* revealed that the presence of secondary metabolites viz.: alkaloids, flavonoids, phenolic compounds, tannins, saponins, phytosterols, diterpenes, carboxylic acid, resins, triterpenoids, fixed oils and fat presented in table no. 4.6. The findings of phytochemical analysis of *Curcuma longa* in the present study were in agreement with the reports of Arawande *et al.*, (2018), Mehra and jain (2019) and Pawar and Kadu (2020), reported the presence of similar phytoconstituents, in acetone extract of rhizome of *Curcuma longa*.

Preliminary phytochemical analysis of 70% hydroalcoholic extract of rhizome of *Curcuma longa* revealed that the presence of secondary metabolites viz.: alkaloids, flavonoids, phenolic compounds, tannins, saponins, phytosterols, resins and triterpenoids presented in table no. 4.7. The findings of phytochemical analysis of *Curcuma longa* in the present study were in agreement with the reports of Santhosh *et al.*, (2011), Oghenejobo and Bethel (2017) and Nazir and Chauhan (2019), reported the presence of similar phytoconstituents, in distilled water and ethanol. Whereas, Singh *et al.*, (2011) reported presence of alkaloids, saponins, flavonoids, terpenes and steroids in 70% hydroalcoholic extract of rhizome of *Curcuma longa*.

Acetone and 70% hydroalcoholic extract of rhizome of *Curcuma longa* revealed the presence of secondary metabolites viz.: alkaloids, flavonoids, phenolic compounds, tannins, saponins, phytosterols, resins and triterpenoids; in addition to above phytoconstituents diterpenes, carboxylic acid, fixed oils and fat were present in acetone extract of rhizome of *Curcuma longa*.

CONCLUSION

The acetone extract of *Curcuma longa* rhizome was yellowish-orange and granular (semisolid) consistency, yielding 7.31% w/w with a pH of 6.5. In contrast, the 70% hydroalcoholic extract was dark brown and gummy- sticky consistency, yielding 10.81% w/w, also with a pH of 6.5. Preliminary phytochemical analysis of acetone and 70% hydroalcoholic extract of rhizome of *Curcuma longa* revealed that the presence of phytoconstituents *viz.*: alkaloids, flavonoids, phenolic compounds, tannins, saponins, phytosterols, resins and triterpenoids. While diterpenes, carboxylic acid, fixed oils and fat were present in acetone extract of rhizome of *Curcuma longa*. Primary metabolites *viz.*: carbohydrates, reducing sugars and proteins and secondary metabolites *viz.*: glycosides, cardiac glycosides, quinones, coumarins were absent in acetone and 70% hydroalcoholic extracts of *Curcuma longa*.

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RESEARCH ARTICLE

5-HYDROXYMETHYL FURFURAL FROM THE FRUIT PART OF *GREWIA ASIATICA* POSSESSES ANTI-COLON CANCER ACTIVITY

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Abstract: The present research work aims to investigate the *in vitro* cytotoxic potential of *Grewia asiatica* (phalsa) fruit against human cancer cell lines and further isolation of active ingredient. The methanolic extract was prepared and further fractionated with hexane, chloroform and butanol in the order of increasing polarity. The extract and fractions were evaluated against eight human cancer cell lines (A-549, HCT-116, HT-29, SW-620, PC-3, MCF-7, MDAMB-231, MIAPACA) from five different tissues (lung, colon, prostate, breast, pancreatic) respectively at the conc. of 100 µg/ml *via* SRB assay. The results revealed that chloroform fraction of *G. asiatica* exhibited *in vitro* cytotoxicity against colon cancer cell line (SW-620) with growth inhibition of 70% whereas hexane fraction also inhibited the growth of same cell line by 76%. A compound namely 5-hydroxy methyl furfural (5-HMF) was isolated from chloroform fraction through column chromatography and characterized *via* NMR (^{1}H and ^{13}C) and mass spectroscopy (HRMS). It was tested against SW-620 cancer cell line and the IC_{50} value was calculated that showed growth inhibitory potential of the component against colon cancer cells.

Keywords: *Grewia asiatica*, 5-HMF, SRB assay, *In vitro* cytotoxicity, Cancer cell lines

INTRODUCTION

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells that is the common cause of death in humans, worldwide (Wand *et al*., 2014; Goyal, 2012; Ghali *et al*., 2014). Despite of developments in the tools of disease diagnosis and treatment, it is one of the leading causes of death and only modest progress has been made in reducing the morbidity / mortality of this disease (He *et al*., 2014; Hail, 2005). *Grewia asiatica*, locally known as phalsa, is well-known for its nutritional and therapeutic attributes. The fruits are claimed to be beneficial for heart, blood / liver disorders, anorexia, indigestion, thirst, toxemia, stomatitis, hiccough, asthma, spermatorrhoea, fever, diarrhea and are used for treating throat, tuberculosis, sexual debility troubles (Sharma and Sisodia, 2009; Pallavi *et al*., 2011; Mishra *et al*., 2012). The root bark is used for the treatment of rheumatism and urinary tract problems while the stem bark is used in sugar refining (Sisodia and Singh, 2009; Muhammad *et al*., 2006; Haq *et al*., 2012). Some phalsa species have free radical scavenging activities which may be responsible for therapeutic action against tissue damage (Kshirsagar and Upadhyay, 2009). *Grewia*'s extracts are also supposed to be helpful in curing hepatitis and used as herbal antidiabetic (Tripathi *et al*., 2011). *In vitro* cytotoxic activity was determined by methylthiazolyltetrazolium (MTT) assay using epidermal kidney (HEK-293), breast (MCF-7), cervical (HELA), lung (NCI-H522) and laryngeal (Hep-2) cancer cell lines. The fruit extract was found to be active on lung (IC_{50} = 59.03 µg/mL) and breast (IC_{50} = 58.65 µg/mL) cancer cell lines, while the leaf extract was active against breast (IC_{50} =50.37 µg/mL) and Hep-2 (IC_{50} = 61.23 µg/mL) cancer cell lines (Marya *et al*., 2011). Therefore, the fruits of *G. asiatica* are reported for their antitumor and cytotoxic activity (Kakoti *et al*., 2011). The search for novel drugs is still a priority target for cancer therapy due to the fact that chemotherapeutic drug resistance is becoming more and more frequent and very little work has been reported on *Grewia asiatica*

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(phalsa) fruit regarding medicinal application and biological activity of its phytochemicals. In view of the above, *in vitro* cytotoxic potential of methanolic extract / fractions of *Grewia asiatica* fruit was tested against human cancer cells for further isolation of bioactive compound.

MATERIALS AND METHODS

Chemicals

RPMI-1640 medium, dimethyl sulfoxide (DMSO), EDTA, fetal bovine serum (FBS), sulphorhodamine blue (SRB) dye, phosphate buffer saline (PBS), trypsin, gentamycin, penicillin and 5-flurouracil were purchased from Sigma Chemical Co., USA. All other chemicals were of high purity and obtained locally with the brand Sigma-Aldrich Chemicals Pvt. Ltd. and S.D. Fine Chemicals Pvt. Ltd. from Ramesh Traders, Panjthirthi-Jammu, J&K.

Fruit material and preparation of extract

Grewia asiatica, fruit part, was authenticated at site by Professor Vijay Bahadhur Singh and enough quantity of fresh fruits were collected from Rainfed Research Sub-station for Sub-tropical Fruits, Raya, SKUAST-Jammu. The freshly collected fruits were chopped, shade-dried and ground into powdered form. The methanolic extract of the fruit was prepared by percolating the dried ground plant material (400 g) with 95% methanol and then concentrating it to dryness under reduced pressure to yield a crude extract of 101 g (Kandil *et al.*, 1994). Stock solutions of 20 mg/ml were prepared by dissolving 95% methanolic extract in DMSO. Stock solutions were prepared at least one day in advance and were not filtered. The microbial contamination was controlled by addition of 1% gentamycin in complete growth medium *i.e.*, used for dilution of stock solutions to make working test solutions of 200 µg/ml.

Fractionation, isolation and characterization

The crude (methanolic) extract was fractionated with hexane, chloroform and butanol solvents. For fractionation, water was added to dissolve the extract and the same volume of hexane was used for solvent-solvent partitioning, and the upper phase (hexane) was removed (3.85 g). The residue was further partitioned using chloroform (3.38 g) and *n*-butanol (37.18 g). Chloroform fraction was purified by column chromatography (silica gel, 100-200 mesh), eluted with a gradient of hexane-ethyl acetate (250 mL collected volumes of each fraction) and concentrated, giving sixty fractions based on TLC profile. Fractions (Fr. 35-39) showed same pattern in TLC were pooled and concentrated. Obtained compound was subjected to TLC using 50% ethylacetate: hexane as mobile phase and detected a single spot after spraying with anisaldehyde reagent and heating the plate at 100 ^{0}C for 5 min. Spot on TLC obtained was dark blackish green in colour and the nature of compound was oil type (yellow colour).

All NMR spectral data were recorded on a Bruker 400 MHz spectrometer. Chemical shifts (δ) were referenced internally to the residual solvent peak ($CDCl_3$: ^{1}H-7.26, ^{13}C-77.0 ppm) and the reference point was TMS (δ_H and δ_C: 0.00 ppm). HR-ESIMS spectra were recorded on an Agilent 1100 LC-Q-TOF mass spectrometer and HRMS-6540-UHD machines. HPLC purifications were performed on an Agilent 1260 Infinity II HPLC system with UV detector. HPLC solvents like MeOH and ACN were procured from Merck chemicals and water for extractions and HPLC analysis was obtained from high-purity Milli-Q Advantage A10 water system (Millipore, Molsheim, France). Column chromatography was done using silica gel (100-200 mesh). Thin layer chromatography (TLC) analysis were performed using Merck Kieselgel (Aufoilen) 60 F_{254} plates.

Cell lines / cultures and positive controls

The human cancer cells-A-549 (lung), HCT-116, HT-29 & SW-620 (colon), MCF-7 & MDAMB-231 (breast), MIAPACA (pancreatic), PC-3 (prostate) were obtained from National Centre for Cell Science, Pune, India and National Cancer Institute, Frederick, USA. These human cancer cells were further grown and maintained in RPMI-1640 medium.Doxorubicin, 5-Fluorouracil, Mitomycin-C and Paclitaxel were used as positive controls.

In vitro assay for cytotoxic activity

Extract, fractions and the compound were subjected to *in vitro* anticancer activity against various human cancer cell lines (Monks *et al.*, 1991). In brief, the cells were grown in tissue culture flasks in growth medium at 37 ^{o}C in an atmosphere of 5% CO_2 and 90% relative humidity in a CO_2 incubator (Hera Cell, Heraeus; Asheville, NCI, USA). The cells at sub-confluent stage were harvested from the flask by treatment with trypsin (0.05% trypsin in PBS containing 0.02% EDTA) and suspended in growth medium. Cells with more than 97% viability (trypan blue exclusion) were used for determination of cytotoxicity. An aliquot of 100 µl of cells (10^5 cells/ml) was transferred to a well of 96-well tissue culture plate. The cells were allowed to grow for 24 h. Extracts (100 µl/well) were then added to the wells and cells were further allowed to grow for another 48 h.

The anti-proliferative SRB assay which estimates cell number indirectly by staining total cellular protein with the dye SRB was performed to assess growth inhibition. The SRB staining method is simpler, faster and provides better linearity with cell number. It is less sensitive to environmental fluctuations and does not require a time sensitive measurement of initial reaction velocity (Skehan *et al.*, 1990). In brief, the cell growth was stopped by gently layering 50 µl of 50% (ice cold) trichloroacetic acid on the top of growth medium in all the wells. The plates were incubated at 4 ^{o}C for 1 h to fix the cells attached to the bottom of the wells.

Liquid of all the wells was then gently pipetted out and discarded. The plates were washed five-times with distilled water and air-dried. SRB 100 µl (0.4% in 1% acetic acid) was added to each well and the plates were incubated at room temperature for 30 min. The unbound SRB was quickly removed by washing the cells five-times with 1% acetic acid. Plates were air-dried, tris buffer (100 µl, 0.01 M, pH 10.5) was added to all the wells to solubilize the dye and then plates were gently stirred for 5 min on a mechanical stirrer. The optical density (OD) was recorded on ELSIA reader at 540 nm. Suitable blanks (growth medium and DMSO) and positive controls (prepared in DMSO and distilled water) were also included. Each test was done in triplicate and the values reported were mean values of three experiments.

Calculations

The cell growth was determined by subtracting average absorbance value of respective blank from the average absorbance value of experimental set. Percent growth in presence of test material was calculated as under:

OD Change in Presence of Control = Mean OD of Control – Mean OD of Blank

OD Change in Presence of Test Sample = Mean OD of Test sample – Mean OD of Blank

% Growth in Presence of Control = 100/OD change in presence of control

% Growth in Presence of Test Sample = (% growth in presence of control) × OD change in presence of test sample

% Inhibition by Test Sample = 100 – % growth in presence of test sample

Statistical Analysis

The experiments were done in triplicates and each data represents the average of at least three independent experiments. The data was expressed as mean±S.D. IC_{50} value was calculated by Graph PAD Prism software version 5.0.

RESULTS AND DISCUSSION

The phalsa fruit extract did not exhibit any significant cytotoxicity (growth inhibition of 70% or more) against any of the human cancer cell lines mentioned above and the overall growth inhibition was seen in the range of 0-54%. For colon (HCT-116 & HT-29) and lung (A-549) cancer cell line, the extract was completely considered as inactive as growth inhibition was 0% whereas the results produced in fractions were quite interesting as the n-hexane fraction and chloroform fractions were found to be active specifically on colon cancer cell line *i.e.*, SW-620 with 76% and 70% growth inhibition respectively. The butanol fraction was not found to be active on any of the human cancer cell lines as the growth inhibition was in the range of 0-35% (Table 1). Chloroform fraction was further taken up and a compound namely 5-hydroxy methyl furfural (5-HMF) was isolated *via* column chromatography and characterized by NMR (^{1}H and ^{13}C) and mass spectroscopy (HRMS). It was tested against SW-620 cell line at different conc. of 50, 30, 10 and 1 µM. The compound exhibited 40% (50 µM) and 0% growth inhibition (30, 10 and 1 µM) with IC_{50} value calculated as > 50 ±0.77 µM for SW-620 (Table 2).

Yellowish oil; ^{1}H NMR ($CDCl_3$, 400MHz); 9.50 (1H, s, H-7), 7.19 (1H, d, *J*=3.6 Hz, H-3), 6.47 (1H, d, *J*=3.2 Hz, H-4), 4.65 (2H, d, *J*=3.2 Hz, H-6); ^{13}C NMR ($CDCl_3$, 100MHz); 178.1 (C-7), 161.4 (C-5), 151.8 (C-2), 124.2 (C-3), 110.0 (C-4), 56.8 (C-6), ESIMS *m/z*: 127.1000 $[M+H]^+$ (calcd for $C_6H_6O_3$, 126.10). The structural feature of 5-HMF contributes to its anticancer potential. The ^{1}H NMR spectrum exhibited an aldehyde group at δ_H 9.50 (1H, s, H-7), an aromatic group at δ_H 7.19 (1H, d, *J*=3.6 Hz, H-3) & 6.47 (1H, d, *J*=3.2 Hz, H-4) and CH_2 group at δ_H 4.65 (2H, d, *J*=3.2 Hz, H-6). The ^{13}C NMR spectrum exhibited total six carbon signals including CHO group, two aromatic carbon, two quartnery carbon and CH_2 group. The compound obtained was profiled by HPLC (High-performance liquid chromatography) with an intense peak at R_t= 14.56 and confirming its presence in chloroform fraction of *Grewia asiatica* at same R_t (Fig. 1, 2, 3, 4 & 5)

Grewia asiatica provides a unique source of various phytochemicals, is known to be a versatile genus of medicinal plants. 5-HMF isolation from chloroform fraction of fruit part of *G. asiatica* possess anticancer potential specifically on colon tissue. A growing interest is arising around phytochemicals role in cancer prevention and treatment. Recent research suggests that the investigation of "new" phytochemicals and related molecular targets can be exploited to identify novel anti-cancer drugs, following sequential steps. This approach includes preliminary selection of phytochemical candidates for cancer prevention or therapy, based on the pre-clinical results related to cell-transformation and antitumorigenic activity assays. Further *in vivo* studies are required for exploring the mechanism of action of 5-HMF, responsible for cytotoxicity and thereby helps in the managment of colon carcinoma.

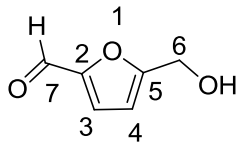


Table 1. Growth inhibitory effect of *Grewia asiatica* (extract & fractions) against different human cancer cell lines

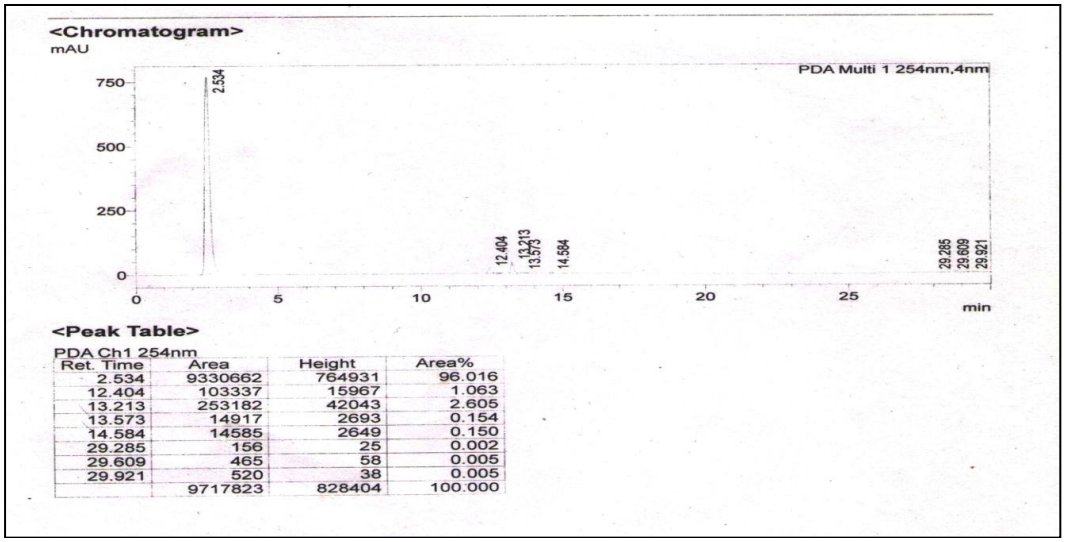
Generic name of the fruit	**Conc. (µg\ml)**	**Human cancer cell lines from five different tissues**							
Grewia asiatica		Breast	Breast	Colon	Colon	Colon	Lung	Pancreatic	Prostate
		MCF-7	MDAMB-231	HCT-116	HT-29	SW-620	A-549	MIAPACA	PC-3
		Growth Inhibition (%)							
Extract (Methanolic)	100	31	34	0	0	54	0	19	0
Fractions (n-Hexane)	100	23	44	0	0	**76**	0	17	0
Chloroform	100	19	29	0	0	**70**	0	0	0
Butanol	100	29	35	0	0	32	0	1	0
Positive controls (standard drugs)	Conc. (µM)								
Doxorubicin	1	65	65	-	-	-	-	-	-
5-Fluorouracil	20	-	-	52	52	65	-	-	-
Mitomycin-C	1	-	-	-	-	-	-	-	66
Paclitaxel	1	-	-	-	-	-	78	-	-
Paclitaxel	50	-	-	-	-	-	-	87	-

Growth inhibition of 70% or more in case of fractions has been indicated in bold numbers
Mark (-) indicates that particular human cancer cell line was not treated with that particular positive control

Table 2. Growth inhibitory effect of 5-HMF from *Grewia asiatica* against colon cancer cell line

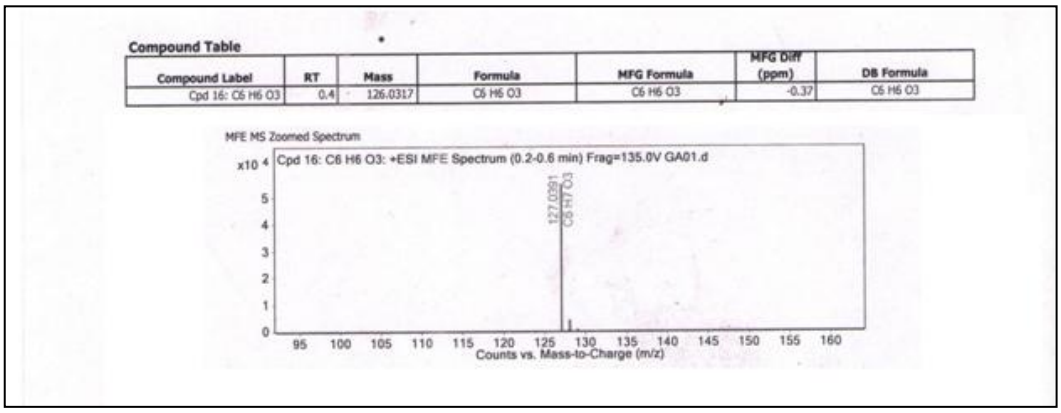
Compound	**Conc.**	**Colon cancer cell line**
		SW-620
	Growth Inhibition (%)	
5-HMF	50	40
	30	0
	10	0
	1	0
IC_{50} (µM)		> 50±0.77
Positive control (standard drug)		
5- Fluorouracil	20	65

Fig. 1. TLC profile of 5-hydroxy methyl furfural isolated (35 & 36) and crude extract of *Grewia asiatica* with solvent system of 50% EtoAc/hex.



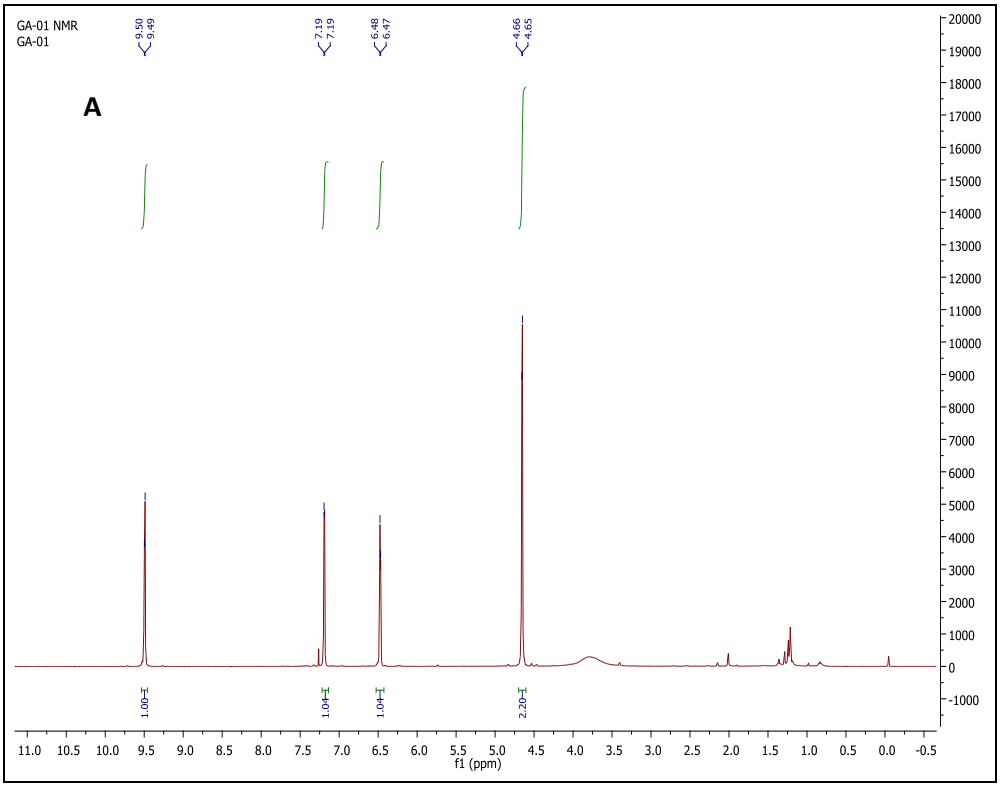
Ret. Time	Area	Height	Area%
2.534	9330662	764931	96.016
12.404	103337	15967	1.063
13.213	253182	42043	2.605
13.573	14917	2693	0.154
14.584	14585	2649	0.150
29.285	156	25	0.002
29.609	465	58	0.005
29.921	520	38	0.005
	9717823	828404	100.000

Fig 2. HPLC chromatogram of 5 - hydroxy methyl furfural isolated from *Grewia asiatica*



Compound Label	RT	Mass	Formula	MFG Formula	MFG Diff (ppm)	DB Formula
Cpd 16: C6 H6 O3	0.4	126.0317	C6 H6 O3	C6 H6 O3	-0.37	C6 H6 O3

Fig. 3. HRMS chromatogram of 5 - hydroxy methyl furfural isolated from *Grewia asiatica*



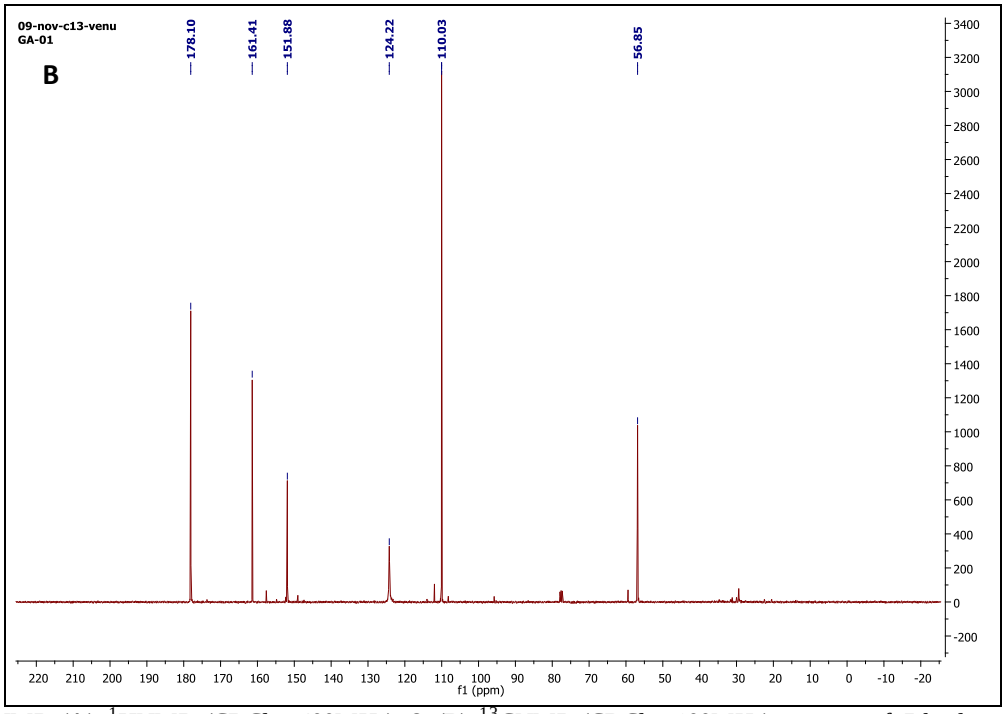


Fig 4. NMR (A) ^{1}HNMR ($CDCl_3$; 400MHz) & (B) ^{13}CNMR ($CDCl_3$; 100MHz) spectra of 5-hydroxymethyl furfural isolated from *Grewia asiatica*

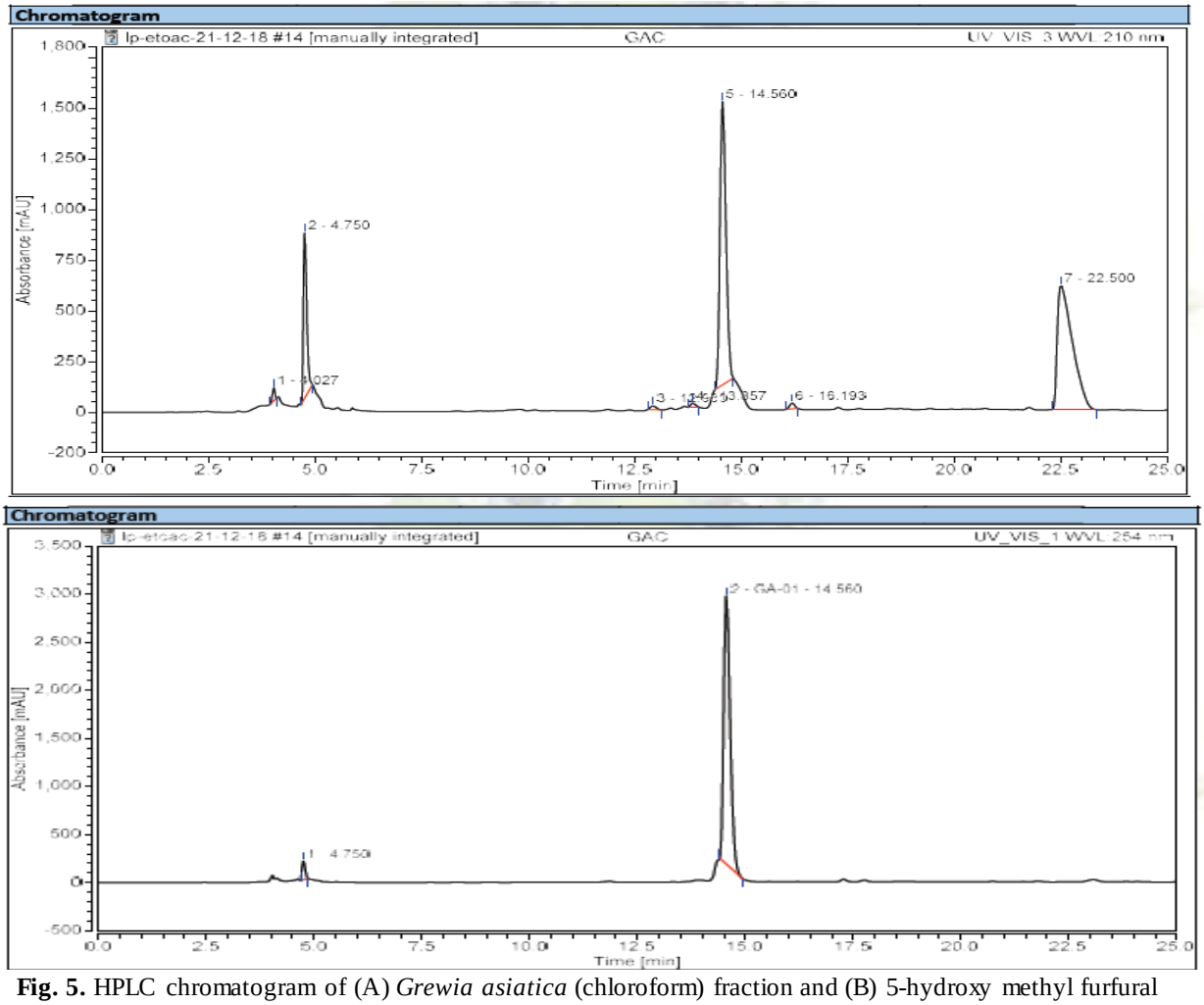


Fig. 5. HPLC chromatogram of (A) *Grewia asiatica* (chloroform) fraction and (B) 5-hydroxy methyl furfural isolated from *Grewia asiatica*

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RESEARCH ARTICLE

EFFECT OF INSECTICIDAL SEED TREATMENT AND FOLIAR SPRAY ON EARLY SEASON INSECT PESTS OF SOYBEAN [*GLYCINE MAX* (L.) MERRILL] AND THEIR NATURAL ENEMIES

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Abstract: A field trial was conducted in RBD to know the efficacy of insecticidal seed treatment and foliar spray on insect pests of soybean and their natural enemies at Breeder Seed Production Farm, JNKVV, Jabalpur (MP) during *Kharif* season. All the insecticidal treatments were sprayed on 35 days old crop (DOC). Observations of insect pests and natural enemies was recorded on 35, 55 and 75 DOC. Observation of whitefly was taken by caging the individual plant, while larval (*S. litura* and *C. acuta*) and adult (spider and lady bird beetle) population was recorded in per meter row length (mrl) basis. Incidence of YMD, stemfly and girdle beetle was recorded in percent basis. The results revealed that T_7, T_8 and T_{11} on 35, 55 and 75 DOC was significantly better than other treatments over the control. Seed treatment with Imidacloprid 48FS @1.25 ml / kg seed (T_1) and with Thiamethoxam 30FS @ 10 ml / kg seed (T_2) were effective in checking the YMD incidence during the early stage of the crop. Highest grain yield (2062.13 kg/ha) was registered in the treatment T_7- seed treatment with Imidacloprid 48FS @1.25 ml / kg seed followed by foliar spray of Triazophos 40EC @ 800 ml / ha. The next insecticidal treatments also yielded better than the control were T_{11}> T_8> T_{12}> T_1>T_2>T_6>T_5>T_9>T_{10}>T_{13}>T_{14}>T_3>T_4. Whereas, the lowest seed yield (8.54.70 kg/ha) was recorded from T_{15} (control). Maximum net return (Rs. 33045.35/ha) was obtained from T_7-seed treatment with Imidacloprid 48 FS @ 1.25 ml / kg seed + Triazophos 40EC @ 800 ml / ha followed by T_{11}-seed treatment with Imidacloprid 48FS @1.25 ml / kg seed + Flonicamid 50WG @ 200 g / ha (Rs. 25088.94/ha. Highest ICBR was recorded from T_1 (1:0.97) followed by T_7 (1:0.96) and T_5 (1:0.94).

Keywords: Soybean, Whitefly, Stemfly, Girdle beetle, Infestation, Grain yield

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] occupies prominent place among the important legume crops in the world and known as miracle bean. It contains about 40 percent protein, 20 percent oil with balanced essential amino acids, rich in poly-unsaturated fatty acids, specially omega 6 and omega 3 fatty acids, 6-7 percent minerals, 5-6 percent crude fibre and 17-19 percent carbohydrates and it has many uses in the food industries (Chauhan and Joshi, 2005; Roopa and Kambrekar, 2019). In the world, the soybean is cultivated in area about 136.82 million ha with production of 353.47 million tones.

World soybean production in 2020-21 is being estimated as 353.47 million tones from a total area of 136.82 million hectares.

Brazil occupies first rank in soybean production with 121.80 million tones followed by USA, Argentina, China and India. India ranks fourth in area with 12.12 million hectares accounting for 8.86 percent of the world area and fifth in production with 11.23 million tonnes in 2020-21. The present area and production of soybean in the country is 114.48 lakh ha and production 11.20 MMT with the productivity 882 kg/ha. It is the main *Kharif* legume crop of Madhya Pradesh in which the area occupies in 50.645 lakh ha with production and productivity of 53.25 lakh tones and 1051 kg/ha, respectively (Anonymous, 2021), owing to this MP is well known for Soya state. It has been noticed that the production and productivity of soybean varieties is relatively yielding less than that of potential yield. The biotic and abiotic stresses are the major constraints for soybean production. Among the biotic stresses, insect pests are the major factors in reducing the soybean yield. About 275 different insect species has been reported which cause detrimental effect on soybean and of them a dozen of pests have been most accountable for serious damage from sowing to harvesting stages (Babu, 1984 and Netam *et al.*, 2013). Soybean crop is a luxuriant in phenologically which in turn offers many insect pests to feed and shelter (Lal *et al.*, 1981). This crop is mainly damaged by stemfly (*Ophiomyia phaseoli* Tryon), girdle beetle (*Obereopsis brevis* Swed.), gram pod borer (*Helicoverpa armigera* Hubner), tobacco caterpillar (*Spodoptera litura* Fabricious), green semilooper (*Chrysodeixis acuta* Walker), grey semilooper (*Amyna octo* Guenee), whitefly (*Bemisia tabaci* Gennadius) and jassid (*Empoasca kerri*

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Pruthi) (Ahirwar *et al*., 2015, Marabi *et al*., 2017 and Kumar and Yadav, 2022).

Chemical insecticides are most commonly used to control the insect pests among the farmers. Although, several insecticides have been identified and recommended for management of major insect pests through seed treatment, soil application and foliar sprays. Applications of non-selective insecticide sprays in early growth stage destroy natural bio-agents. This led to problem like insect resistance, outbreak or resurgence of unexpected insect pests. In agro-ecosystem, several bio-agents such as parasitoids, predators and insect pathogens influence the population of the insect pests. Activities of such bio-agents are hampered due to indiscriminate use of chemical insecticides (Sharma and Ansari, 2007). Hence, looking to the above facts an attempt was made to know the efficacy of insecticides by means of seed treatment followed by foliar spray against the early stage of insect pests and impact on their natural enemies.

MATERIALS AND METHODS

The experiment was carried out to evaluate the efficacy of insecticides against early stage of insect pests and their natural enemies during *Kharif* season 2016 at experimental field of Breeder Seed Production Farm, JNKVV, Jabalpur (MP), India. Soybean variety JS 97-52 was sown in three replications having plot size of 8.0 m x 4.05 m with row to row distance of 45 cm in randomized block design (RBD). The treatments were comprised of seed treatment and foliar spray alone and combination of both of them. The treatments details were: T_1: Imidacloprid 40FS @1.25 ml/kg seed, T_2: Thiamethoxam 30FS @ 10 ml/kg seed, T_3: Fipronil 5 SC @ 4 ml/kg seed, T_4: Bifenthrin10 EC @ 4 ml/kg seed, T_5: Foliar spray of Triazophos 40EC @ 800 ml/ha, T_6: Foliar spray of Flonicamid 50WG @200 g/ha, T_7: T_1 + followed by foliar spray of Triazophos 40EC @ 800 ml/ha, T_8: T_2 + followed by foliar spray of Triazophos 40EC @ 800 ml/ha, T_9: T_3 + followed by foliar spray of Triazophos 40EC @ 800 ml/ha, T_{10}: T_4 + followed by foliar spray of Triazophos 40EC @ 800 ml/ha, T_{11}: T_1 + followed by foliar spray of Flonicamid 50WG @ 200 g/ha, T_{12}: T_2 + followed by foliar spray of Flonicamid 50WG @ 200 g/ha, T_{13}: T_3 + followed by foliar spray of Flonicamid 50WG @ 200 g/ha, T_{14}: T_4 + followed by foliar spray of Flonicamid 50WG @ 200 g/ha and T_{15}: Control (untreated). All the application of foliar sprays was done at 35 days old crop (DOC). Observations on incidence of insect pests, natural enemies and yellow mosaic disease (YMD) infestation were recorded on 35, 55 and 75 DOC. The counts of adult whiteflies were recorded by caging individual plant with the help of cage and observations were taken on 10 randomly selected plants per plot. Defoliator insect pests and natural enemies were recorded by ground shaking on soybean survey sheet (Size: L x W; 1m x 0.5m) from 10 randomly selected spots from one meter row length (mrl) per plot. Stemfly and girdle beetle infestation was recorded from 10 randomly selected plants from each plot. The infected soybean plants caused by YMD were recorded from per mrl from 5 different sites in per plot. Grain yield from different treatments were recorded after harvesting of the crop and finally converted in to kg/ha.

Data analysis on occurrence of insect pests and their natural enemies and yield data after appropriate transformations were tested by ANOVA followed by an appropriate significant difference tests. The data collected during the course of study were subjected to statistical analysis with appropriate transformation for interpretation of results following RBD in order to test the level of significance among the various treatments (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Effect of insecticides on whitefly

At 35 days old crop (DOC) after application of insecticides, the data presented in Table 1 indicated that the differences in the adult whitefly population among different treatments were significant. Among the treatments T_7, T_8 and T_{11} were found to be most effective as they recorded significantly lowest whitefly population (0 adult whitefly / plant). These was followed by treatments T_{12} (0.13 adult whitefly / plant), T_1 (0.20 adult whitefly / plant) and T_2 (0.33 adult whitefly / plant) and they differed significantly from each other. The next effective treatments were T_5 (0.47 adult whitefly / plant) and T_6 (0.47 adult whitefly / plant), but were at par with each other. The next effective treatments were T_9 and T_{10} (0.60 and 0.60 adult whitefly / plant, respectively), but were found to be non-significant. The next effective treatments were T_{13} and T_{14} (0.80 and 0.80 adult whitefly / plant, respectively), but they did not differ significantly from each other. The least effective treatments were T_3 and T_4 (0.93 and 1.13 adults whitefly / plant, respectively), however, they differed significantly from each other. Highest whitefly population (3.20 adults' whitefly / plant) was recorded in T_{15} (Control).

At 55 DOC, the differences in the adult whitefly population among different treatments were significant (Table 2). Among the treatments T_7 was found to be the most effective as it recorded significantly lowest whitefly population (0.93 adult whitefly / plant). This was followed by treatments T_{11}, T_8, T_{12}, T_1, T_2, T_5, T_6, T_9, T_{10}, T_{13}, T_3, T_{14} and T_4 (1.47, 1.80, 2.00, 2.33, 2.40, 2.80, 2.87, 3.13, 3.33, 3.60, 3.67, 3.80 and 4.00 adult whitefly / plant, respectively which were differed significantly from each other. Highest whitefly population (6.90 adults' whitefly / plant) was recorded in T_{15} (Control).

At 75 DOC, the data presented in Table 3 showed

that the differences in the adult whitefly population among different treatments were found to be significant. Among the treatments, T_7 was found to be the most effective with recorded significantly lowest whitefly population (0.60 adult whitefly / plant). This was followed by treatments T_{11}, T_8 and T_{12} (0.80, 1.00 and 1.00 adult whitefly / plant, respectively) and they differed significantly from each other. The next effective treatments were T_1 (1.13 adults whitefly / plant) and T_2 (1.20 adults whitefly / plant), but they were at par with each other. The next effective treatments were T_6, T_5 and T_9 (1.33, 1.40 and 1.40 adults whitefly / plant, respectively) but they did not differ significantly from each other. The next effective treatment was T_{10} (1.60 adults whitefly / plant) and it differed significantly from T_{13} and T_{14} (1.73 and 1.80 adults whitefly / plant, respectively). The least effective treatments were T_3 and T_4 (2.00 and 2.20 adults whitefly / plant, respectively) and they differed significantly from each other. Highest whitefly population (3.73 adults whitefly / plant) was recorded in T_{15} (Control).

Overall effect of insecticides on whitefly

The perusal of data presented in Table 4 revealed that the differences in the adult whitefly population among different treatments were found to be significant. Among the treatments, T_7 was found to be the most effective as it recorded significantly lowest whitefly population (0.51 adult whitefly / plant). This was followed by treatments T_{11}, T_8 and T_{12} (0.76, 0.93 and 1.04 adult whitefly / plant, respectively) and they were significantly differed from each other. The next effective treatments were T_1 and T_2 (1.13 and 1.20 adults whitefly / plant, respectively) and they also were at par with each other. Further, the next effective treatments were T_5, T_6 and T_9 (1.56, 1.56 and 1.40 adults whitefly / plant, respectively), but they did not differ significantly from each other. Moreover, the next effective treatment was T_{10} (1.84 adults whitefly / plant) and it was significantly differed from T_{13} and T_{14}. The least effective treatments were T_3 and T_4 (2.24 and 2.44 adults whitefly / plant, respectively) and they were differed significantly from each other. The highest whitefly population was recorded in T_{15} (control) with 4.60 adults whitefly / plant. Present results are corroborated with the findings of Netam *et al*. (2013) as they revealed that Imidacloprid 600 FS @ 0.75 g a.i./kg seed as seed treatment was found most effective against the sucking pests of soybean up to 4th week of seed germination followed by Imidacloprid 600 FS @ 0.60 g a.i./ kg and Thiamethoxam 70 WS @ 2.1 g a.i./kg seed.

Effect of insecticides on YMD infection

At 35 DOC, perusal of the data in Table 1 revealed that the differences in the YMD infection among different treatments were significant. Among the treatments T_1, T_2, T_5, T_7, T_8, T_{11} and T_{12} were found to be most effective as they recorded significantly no YMD incidence. These were followed by treatments T_9 (0.09%), T_{10} (0.13%), T_{13} (0.16%), T_{14} (0.16%), T_3 (0.19%), T_6 (0.23%) and T_4 (0.25%) but all were at par with each other. While, highest incidence (2.68%) was recorded in T_{15} control.

At 55 DOC, the differences in the YMD incidence among different treatments were significant (Table 2). Among the treatments, T_7 was found to be most effective as it recorded significantly lowest YMD infection (2.25%). The next effective treatments were T_{11} (2.97%), T_8 (3.33%), T_{12} (3.74%), T_1 (3.95%) and T_2 (4.23%) but all were at par with each other. These were followed by treatments T_5 (5.22%), T_6 (5.42%) and T_{10} (7.42%), but all were at par with each other. The next effective treatments were T_9 (7.68%), T_{13} (7.80%), T_{14} (7.92%), T_3 (8.64%) and T_4 (8.84%), but they did not differ significantly from each other. While highest infection was recorded in T_{15} control (43.73%).

Further, at 75 DOC it is revealed that the differences in the YMD infection among different treatments were significant (Table 3). Among the treatments, T_7 was found to be most effective as it recorded significantly lowest YMD incidence (2.58%) followed by T_{11} (3.48%), but they were at par with each other. The next effective treatments were T_8 (4.01%) and T_{12} (4.48%), they did not differ significantly from each other. The next effective treatments were T_1 (4.97%) and T_2 (5.28%), but they were at par with each other. These were followed by treatments T_5 (6.38%) and T_6 (6.39%), but they did not differ significantly from each other. The next effective treatments were T_9 (7.84%) and T_{10} (8.63%), but both were at par with each other. The next effective treatments were T_{13} (10.53%) and T_{14} (10.86%), but non-significant differences were found between them. The least effective treatments were T_3 (13.02%) and T_4 (13.75%), but they did not differ significantly from each other. While highest infection (61.31%) was recorded in T_{15} control.

Overall effect of insecticides on YMD infection

The perusal of data prasented in Table 4 revealed that the differences in the YMD incidence among different treatments were significant. Among the treatments, T_7 was found the significantly most effective in reduction of YMD infection (1.61%) followed by T_{11} (2.15%), but they were at par with each other. The next effective treatments were T_8 (2.45%) and T_{12} (2.74%) which were did not differ significantly from each other. The next effective treatments were T_1 (2.97%) and T_2 (3.17%), but they were at par with each other. These were followed by treatments T_5 (3.85%) and T_6 (4.01%), but they did not differ significantly from each other. The next effective treatments were T_9 (5.20%) and T_{10} (5.39%), but both were at par with each other. The next effective treatments were T_{13} (6.16%) and T_{14} (6.31%), but non-significant differences were found between them. The least effective treatments were T_3 (7.28%) and T_4 (7.61%), but they did not differ

significantly from each other, while the highest incidence (35.91%) was recorded in T_{15} (control).

Effect of insecticides on stemfly

At 35 DOC, the data in Table 1 revealed that the differences in the stemfly infestation among different treatments were non-significant. However, no infestation was recorded in treatment T_7 (0.00%) followed by treatments T_8 (3.33%), T_{11} (3.33%), T_1 (6.67%), T_2 (6.67%), T_3 (6.67%), T_4 (6.67%), T_5 (6.67%), T_6 (6.67%), T_9 (6.67%), T_{10} (6.67%), T_{12} (6.67%), T_{13} (6.67%), T_{14} (6.67%) and T_{15} (6.67%), but all were at par with each other.

At 55 DOC, it is revealed that the differences in the stemfly infestation among different treatments were non-significant (Table 2). However, lowest infestation was recorded in treatment T_7 (6.67%) followed by treatments T_4 (8.33%), T_8 (8.33%), T_{11} (8.33%), T_2 (10.0%), T_6 (10.0%), T_9 (10.0%), T_{10} (10.0%), T_{13} (10.0%), T_{14} (10.0%), T_1 (11.67%), T_3 (11.67%), T_5 (11.67%), T_{12} (11.67%) and T_{15} (11.67%), but all were at par with each other.

Further, the differences in the stemfly infestation among different treatments were found to be non-significant at 75 DOC (Table 3). However, lowest infestation was recorded in treatment T_7 (6.67%) followed by treatments T_8, T_1, T_5, T_{11}, T_{14}, T_2, T_3, T_4, T_6, T_9, T_{10}, T_{12}, T_{13} and T_{15} (6.67, 10.00, 10.00, 10.00, 10.00, 13.33, 13.33, 13.33, 13.33, 13.33, 13.33, 13.33 and 13.33, respectively), but all were at par with each other.

Overall effect of insecticides on stemfly

The perusal of data given in Table 4 showed that the differences in the stemfly infestation among the treatments were found to be significant. Among the treatments, T_7 was found the significantly most effective in reduction of stemfly (4.45%) followed by T_8 (6.11%), T_{11} (7.22%), T_{14} (8.89%), T_4 (9.44%), T_5 (9.45%), T_1 (9.45%), T_2 (10.00%), T_6 (10.00%), T_9 (10.00%), T_{10} (10.00%), T_{13} (10.00%), T_3 (10.56%) and T_{12} (10.56%). The highest whitefly population was found in T_{15} (10.57%), but all were at par with each other. Manjanaik *et al*. (2022) reported that seed treatment with Thiamethoxam 30FS @ 10 ml/kg seed and foliar application of Thiamethoxam 25WG 0.40 g/L at 30 days after germination were registered most effective against stemfly in soybean. Further, it was found at par with Imidacloprid 48FS @ 1.25 ml/ kg seed as seed treatment and foliar application of Imidacloprid 17.8 SL @ 0.50 ml/ L at 30 DAG. The spraying of Chlorantraniliprole 18.5 SC @ 0.30 ml/ L was recorded high seedling mortality. Similar results were also reported by Gopali *et al*. (2007), Prabhu and Patil (2016) and Shreedhara *et al*. (2017).

Effect of insecticides on girdle beetle

The perusal of the data in Table 1 revealed that all the treatments were found to be free from girdle beetle infestation at 35 DOC while, at 55 DOC, it was revealed that the differences in the girdle beetle infestation among different treatments were non-significant (Table 2). However, no infestation was recorded in treatments T_1, T_3, T_4, T_7, T_9, T_{10}, T_{11}, T_{12}, T_{13} and T_{14}. These were followed by treatments T_2 (6.67%), T_5 (6.67%), T_6 (6.67%) and T_8 (6.67%), but all were at par with each other. While highest infestation was recorded in T_{15} control (13.33%).

At 75 DOC, the differences in the girdle beetle infestation among different treatments were found non-significant (Table 3). However, no infestation was recorded in treatments T_2, T_3, T_4 and T_6. These were followed by treatments T_5 (6.67%), T_{11} (6.67%), T_{12} (6.67%), T_1 (13.33%), T_7 (13.33%), T_8 (13.33%), T_9 (13.33%), T_{10} (13.33%), T_{12} (13.33%), T_{13} (13.33%), T_{14} (13.33%) and T_{15} control (13.33%), but all were at par with each other.

Overall effect of insecticides on girdle beetle

The perusal of the data in Table 4 revealed that all the insecticidal treatments were found to be significant in reducing the girdle beetle infestation. It was revealed that the differences in the girdle beetle infestation among different treatments were non-significant (Table 4). However, no infestation was recorded in T_3 and T_4. These were followed by treatments T_2, T_6, T_{11} and T_{12}. These were followed by treatments T_1, T_7, T_9, T_{10}, T_{13}, T_{14}, T_5, T_8, but all were at par with each other. While, the highest infestation (8.89%) was recorded in T_{15} (control). Chaudhary and Tripathi (2011) reported that girdle beetle infestation was varied from 12.49 to 22.16 per cent in the soybean crop among various treatments. The treatment with Triazophos registered comparatively less infestation than other treatments but it was statistically at par with Quinalphos, Chlorpyriphos and Ethofenprox.

Effect of insecticides on *S. litura*

Perusal of the data in Table 1 revealed that all the treatments were free from *S. litura* infestation at 35 DOC. While, at 55 DOC, it was revealed that the differences in the tobacco larval population among different treatments were found significant (Table 2). However, no infestation was recorded in treatment T_5. This was followed by treatments T_7, T_8, T_9 and T_{10}, but they did not differ significantly from each other. The next effective treatments were T_{14}, T_1, T_2, T_3, T_4 and T_6 (0.40, 0.47, 0.47, 0.47, 0.47 and 0.47 larva/mrl, respectively), but they were at par with each other. The least effective treatments were T_{11}, T_{12} and T_{13} (0.53, 0.53 and 0.53 larva/mrl, respectively), but they did not differ significantly from each other. While, the highest infestation (1.07 larvae/mrl) was observed in T_{15} (control).

Further, at 75 DOC, the differences in the larval population of *S. litura* among different treatments were also found significant (Table 3). However, no infestation was recorded in treatments T_5, T_7, T_8, T_9 and T_{10}. These were followed by treatments T_4, T_6, T_{11}, T_1, T_2 and T_3, but they did not differ significantly from each other. The least effective treatments were T_{12} (0.33 larva/mrl), T_{13} (0.33 larva/mrl) and T_{14} (0.33 larva/mrl), but they did

not differ significantly from each other. While, the highest infestation was recorded in T_{15} (control) (1.73 larvae/mrl).

Overall effect of insecticides on *S. litura*

The overall mean larval population of *S. litura* were found to be significant different among the treatments over the control (Table 4). However, no infestation was recorded in treatments T_5, T_{10}, T_9, T_8 and T_7. These were followed by treatments T_6, T_4 T_{14} T_{11} T_3, T_2 and T_1 (0.22, 0.22, 0.24, 0.24, 0.25, 0.25 and 0.25 larva/mrl), but they did not differ significantly from each other. The least effective treatments were T_{12} (0.29 larva/mrl) and T_{13} (0.29 larva/mrl) which did not differ significantly from each other. While, the highest infestation (0.93 larvae/mrl) was recorded in T_{15} (control).

The lowest larval population of *S. litura* in soybean was recorded from second spray of Chlorantraniliprole followed by Teflubenzuron, Alphacypermethrin, Profenophos and Indoxacarb. Matcha *et al*. (2021) reported that among the insecticides, Spinetoram 11.7 SC @ 0.7ml/L and also at 0.5ml/L safely protected the soybean crop with lowest larval population 0.17 and 0.25 larvae/mrl, respectively.

Effect of insecticides on *C. acuta*

Perusal of the data in Table 1 expressed that all the treatments were found free from *C. acuta* infestation at 35 DOC. At 55 DOC, the data presented in Table 2 revealed that the differences in the larval population among different treatments were significant. However, no infestation was recorded in treatment T_5, T_7, T_8, T_9 and T_{10}. These were followed by treatments T_3 (0.4 larva/mrl), T_1 (0.47 larva/mrl), T_2 (0.47 larva/mrl), T_4 (0.47 larva/mrl), T_6 (0.47 larva/mrl), T_{11} (0.47 larva/mrl), T_{12} (0.47 larva/mrl), T_{13} (0.53 larva/mrl) and T_{14} (0.53 larva/mrl), but all were at par with each other. While, highest infestation (1.87 larvae/mrl) was recorded in T_{15} (control).

At 75 DOC, it was revealed that the differences in the larval population among different treatments were found significant (Table 3). However, no infestation was recorded in treatment T_5, T_7 and T_8. These were followed by treatments T_9 (0.07 larva/mrl), and T_{10} (0.07 larva/mrl), they were at par with each other. The next effective treatments were T_1, T_4, T_6, T_2, T_3, T_{11}, T_{12} and T_{13}, but they did not differ significantly from each other. The least effective treatment was T_{14} (1.40 larvae/mrl). While, the highest infestation (3.20 larvae/mrl) was recorded in T_{15} (control).

Overall effect of insecticides on *C. acuta*

The overall mean larval population of *C. acuta* were found to be significant different among the treatments over the control (Table 4). However, no infestation was recorded in treatments T_8, T_7 and T_5. These were followed by treatments T_9, T_{10}, T_1, T_3, T_4, T_6, T_2, T_{11} and T_{12} (0.02, 0.02, 0.56, 0. 56, 0. 56, 0. 56, 0.58, 0.58 and 0.58 larva/mrl), but they did not differ significantly from each other. The least effective treatments were T_{13} (0.60 larva/mrl) and T_{14} (0.64 larva/mrl) which did not differ significantly from each other. While, the highest infestation (1.69 larvae/mrl) was recorded in T_{15} (control).

Chaudhary and Tripathi (2011) recorded larval population of *C. acuta* varied from 0.55 to 3.55/mrl in different treatments. The lowest larval population was recorded with treatment of Triazophos followed by Ethofenprox, Quinalphos and Chlorpyriphos, respectively and however, all the insecticidal treatments were statistically at par to each other. Wagh and Budhvat (2020) also found that foliar application of Profenophos 50EC @ 0.185% was most effective against larval population of *C. acuta* followed by Profenophos 50EC @ 0.125%, Quinalphos25EC @ 0.075%, Quinalphos 25 EC @ 0.05%, Chlorpyriphos 20EC @ 0.06%, Chlorpyriphos 20 EC @ 0.04%, Triazophos 40 EC @ 0.06% and Triazophos 40 EC @ 0.04% over the control in soybean crop.

Effect of insecticides on spider

At 35 DOC, the data in Table 1 revealed that the differences in the spider population among different treatments were non-significant. However, highest spider population was recorded in treatments T_2 (0.13 spider/mrl) and T_5 (0.13 spider/mrl) followed by T_8 (0.20 spider/mrl), T_9 (0.20 spider/mrl), T_{11} (0.27 spider/mrl), T_{14} (0.27 spider/mrl), T_{13} (0.33 spider/mrl), T_1 (0.40 spider/mrl), T_3 (0.47 spider/mrl), T_7 (0.47 spider/mrl), T_7 (0.47 spider/mrl), T_{12} (0.53 spider/mrl), T_4 (0.60 spider/mrl), T_{10} (0.60 spider/mrl) and T_{15} (0.60 spider/mrl), but all were at par with each other.

At 55 DOC, it was revealed that the differences in the spider population among different treatments were non-significant (Table 2). However, highest spider population was recorded in treatment T_{15} (0.8 spider/mrl). This was followed by treatments T_5 (0.73 spider/mrl), T_{12} (0.73 spider/mrl), T_4 (0.67 spider/mrl), T_6 (0.67 spider/mrl), T_1 (0.60 spider/mrl), T_3 (0.60 spider/mrl), T_{11} (0.60 spider/mrl), T_{14} (0.53 spider/mrl), T_8 (0.47 spider/mrl), T_7 (0.40 spider/mrl), T_9 (0.40 spider/mrl), T_{10} (0.33 spider/mrl), T_2 (0.27 spider/mrl) and lowest in T_{13} (0.07 spider/mrl), but they did not differ significantly from each other.

Further, at 75 DOC, the differences in the spider population among different treatments were non-significant (Table 3). However, highest spider population was recorded in treatment T_{15} (1.27 spider/mrl). This was followed by treatments T_6 (1.16 spider/mrl), T_5 (0.87 spider/mrl), T_{10} (0.73 spider/mrl), T_{13} (0.73 spider/mrl), T_{14} (0.73 spider/mrl), T_4 (0.67 spider/mrl), T_8 (0.67 spider/mrl), T_9 (0.67 spider/mrl), T_{12} (0.67 spider/ mrl), T_2 (0.53 spider/mrl), T_{11} (0.53 spider/mrl), T_1 (0.33 spider/mrl), T_3 (0.33 spider/mrl) and lowest in T_7 (0.27 spider/mrl), but they did not differ significantly from each other. The populations of spider were coincided with the occurrence of

host insects on the crop during *kharif* season.

Overall effect of insecticides on spider

The overall mean population of spider were found to be significant different among the treatments (Table 4). The spider population was evenly distributed in all the treatments, but they did not differ significantly from each other. Moreover, the highest spider population was (0.89 spider/mrl) was recorded in T15 (control). Present results are supports the findings of Nage *et al*. (2013). Similar results were also reported by Kushram *et al*. (2021), as they stated that Lynx spider (*Oxyopes* sp.) and Orb weaver spider (*Neoscona* sp.) were found preying upon Lepidopteran caterpillars and sucking insect pests, respectively. The spider populations were ranged from 0.10 to 1.30 spiders/plant. The spiders (0.20 spiders / plant) were first occurred on the crop during last week of July and available up to third week of October.

Effect of insecticides on lady bird beetle

Perusal of the data in Table 1 revealed that all the treatments did not harbor lady bird beetle population at 35, 55 and 75 DOC. Nage et al. (2013) reported that highest population of coccinellids was observed in untreated control and was at par with Imidacloprid 70 WS @ 12 g/kg seed while remaining seed treatments recorded natural enemies population in the range of 1.26 to 0.46/plot. Similar findings was also recorded by More *et al*. (2014) who revealed that population of lady bird beetles were observed, but it was very low at negligible level. Matcha *at al*. (2021) stated that the highest population of natural enemies in soybean crop was recorded with the treatments of Flubendiamide and Chlorantraniliprole.

Grain yield and economics of treatments

The mean grain yield data presented in Table 4 revealed that it was significantly ranged between 854.70 (T_{15}) to 2062.13 kg/ha (T_7). All the insecticidal treatments recorded significantly higher grain yield than untreated check. Treatment T_7 recorded highest grain yield, which was followed by treatments T_{11} (1831.50 kg/ha), T_8 (1566.95 kg/ha) and T_{12} (1526.25 kg/ha) and they differed significantly from each other. The next effective treatments were T_1 (1451.63 kg/ha) and T_2 (1410.93 kg/ha), but they were at par with each other. The next effective treatments were T_6 (1345.81 kg/ha), T_5 (1343.10 kg/ha), T_9 (1322.75 k /ha) and T_{10} (1309.18 kg/ha), but they did not differ significantly from each other. The next effective treatments were T_{13} (1234.57 kg/ha) and T_{14} (1221.00 kg/ha), but non-significant differences were observed between them. The least effective treatments were T_3 (1126.03 kg/ha) and T_4 (1119.25 kg/ha) and they differed significantly from each other. The result supports the findings of Nage *et al*. (2013) who tested the insecticides as seed treatment against early stage of insect pests of soybean. Their results revealed that the treated seed with Imidacloprid 70 WS @ 12 g/kg seed registered highest grain yield (1300 kg/ha) followed by Thiamethoxam 25 WG @ 1.50 g/kg seed (1000 kg/ha). Singh *et al*. (2000) also recorded higher grain yield of soybean over control plot when the seed treated with Thiamethoxam 70 WS @ 3 and 5 g/ kg seed. Similarly, Taware *et al*. (2000) studied the efficacy of some insecticides as seed treatment, soil application and foliar spray against insect pests of soybean. They revealed that the highest grain yield was obtained in Chlorpyriphos spray @ 1.5 a.i. kg/ha at 8-10 days after germination (3548 kg/ha) followed by Thiamethoxam seed treatment @ 3 g and 5 g/ kg seed and Phorate 10 G @ 10 kg/ha as soil application before sowing.

Maximum net return per hectare was obtained from T_2 (Rs. 33045.35 with BC ratio 0.96) followed by T_{11} (Rs. 25088.94 with B:C ratio 0.90) , T_8 (Rs. 17764.11 with B:C ratio 0.87), T_1 (Rs. 16491.26 with B:C ratio 0.97), T_{12} (Rs. 15235.90 with B:C ratio 0.79) , T_2 (Rs. 14199.05 with B:C ratio 0.89), T_5 (Rs. 13064.47 with B:C ratio 0.94), T_9 (Rs. 11883.87 with B:C ratio 0.89), T_6 (Rs. 11776.92 with B:C ratio 0.84) , T_{10} (Rs. 11468=04 with B:C ratio 0.88), T_{13} (Rs. 7998.68 with B:C ratio 0.74), T_{14} (Rs. 7582.85 with B:C ratio 0.72), T_3 (Rs. 7155.61 with B:C ratio 0.92) , while it was minimum in T_{14} (Rs. 6993.84 with B:C ratio 0.92). Chaudhary and Tripathi (2011) reported that Quinalphos had registered highest seed yield, however, it was statistically at par with Triazophos, Ethofenprox and Chlorpyriphos. The maximum net return was obtained with Quinalphos whereas, highest ICBR (7.26) was observed in spray of Chlorpyriphos. Manjanaik *et al*. (2022) was also recorded higher C:B ratio (1: 4.22) from the soybean seeds treated with Thiamethaxom 30FS @ 10 ml/ kg seed and foliar spray of Thiamethoxam 25WG 0.40 g/L at 30 DAG followed by alone foliar spray of Chlorantraniliprole 18.5SC @ 0.30 ml/L at 10 and 30 DAG (1: 3.94).

CONCLUSION

Seed treatment with Imidacloprid 48 FS @1.25 ml/kg seed and with Thiamethoxam 30 FS @ 10 ml/kg seed were effective in checking the YMD infestation during the early stage of the crop. Natural enemy population was very low during the entire cropping season. Grain yield of seed treatment with Imidacloprid 48 FS @ 1.25 ml/kg seed followed by foliar spray of Triazophos 40 EC @ 800 ml/ha at 35 DOC and seed treatment with Thiamethoxam 30 FS @ 10 ml/kg seed followed by foliar spray of Triazophos 40 EC @ 800 ml/ha at 35 DOC were significantly higher than the untreated check. Maximum net return was obtained from seed treatment with Imidacloprid 48 FS @ 1.25 ml/kg seed + Triazophos 40 EC @ 800 ml/ha at 35 DOC followed by seed treatment with Imidacloprid 48 FS @1.25 ml/kg seed + Flonicamid 50 SWG @ 200 g/ha at 35 DOC and seed treatment with Thiamethoxam 30 FS @ 10 ml/kg seed followed by

foliar spray of Triazophos 40 EC @ 800 ml/ha at 35 DOC. The seed treatment practice may be effective for about 35 days and no insecticide spray in early growth stage is required. It will also enhance the activities of natural enemies and thus low insect pest occurrences may be expected even after the expiry period of the effectiveness of the systemic insecticidal seed treatments.

Table 1. Effect of insecticides against insect pests, natural enemies and YMD incidence on soybean (at 35 DOC)

Tr. Code	Adult whitefly /plant*	YMD incidence (%)**	% Infestation		Larval population /mrl		Adult population /mrl	
			Stem fly**	Girdle beetle**	*S. litura**	*C. acuta**	Spider*	Lady bird beetle
T_1	0.20 (0.84)	0.00 (4.05)	6.67 (11.67)	0	0	0	0.40 (0.94)	0
T_2	0.33 (0.91)	0.00 (4.05)	6.67 (11.67)	0	0	0	0.13 (0.79)	0
T_3	0.93 (1.20)	0.19 (4.76)	6.67 (11.67)	0	0	0	0.47 (0.98)	0
T_4	1.13 (1.28)	0.25 (4.97)	6.67 (11.67)	0	0	0	0.60 (1.04)	0
T_5	0.47 (0.98)	0.00 (4.05)	6.67 (11.67)	0	0	0	0.13 (0.79)	0
T_6	0.47 (0.98)	0.23 (4.80)	6.67 (13.96)	0	0	0	0.47 (0.98)	0
T_7	0.00 (0.71)	0.00 (4.05)	0.00 (4.05)	0	0	0	0.47 (0.97)	0
T_8	0.00 (0.71)	0.00 (4.05)	3.33 (9.00)	0	0	0	0.20 (0.83)	0
T_9	0.60 (1.05)	0.09 (4.39)	6.67 (11.67)	0	0	0	0.20 (0.83)	0
T_{10}	0.60 (1.05)	0.13 (4.55)	6.67 (11.67)	0	0	0	0.60 (1.04)	0
T_{11}	0.00 (0.71)	0.00 (4.05)	3.33 (9.00)	0	0	0	0.27 (0.86)	0
T_{12}	0.13 (0.79)	0.00 (4.05)	6.67 (13.96)	0	0	0	0.53 (1.01)	0
T_{13}	0.80 (1.14)	0.16 (4.67)	6.67 (11.67)	0	0	0	0.33 (0.89)	0
T_{14}	0.80 (1.14)	0.16 (4.66)	6.67 (11.67)	0	0	0	0.27 (0.87)	0
T_{15}	3.20 (1.92)	2.68 (10.16)	6.67 (11.67)	0	0	0	0.6 (1.04)	0
SEm±	0.03	0.26	4.25	-	-	-	0.09	-
CD at 5%	0.09	0.75	NS	-	-	-	NS	-

* Figures in parentheses are in square root transformed values √x+0.5; **Arc sin transformed values (x+0.5)

Table 2. Effect of insecticides against insect pests, natural enemies and YMD incidence on soybean (at 55 DOC)

Tr. Code	Adult whitefly /plant*	YMD incidence (%)**	% Infestation		Larval population /mrl		Adult population /mrl	
			Stem fly**	Girdle beetle**	*S. litura**	*C. acuta**	Spide*	Lady bird beetle
T_1	2.33 (1.53)	3.95 (11.39)	11.67 (19.15)	0.00 (4.05)	0.47 (0.98)	0.47 (0.98)	0.60 (1.05)	0
T_2	2.40 (1.55)	4.23 (11.78)	10.00 (15.98)	6.67 (11.67)	0.47 (0.98)	0.47 (0.98)	0.27 (0.85)	0
T_3	3.80 (1.95)	8.64 (17.05)	11.67 (19.15)	0.00 (4.05)	0.47 (0.98)	0.40 (0.94)	0.60 (1.05)	0

T_4	4.00 (2.00)	8.84 (17.26)	8.33 (17.13)	0.00 (4.05)	0.47 (0.98)	0.47 (0.98)	0.67 (1.07)	0
T_5	2.80 (1.67)	5.22 (13.13)	11.67 (19.15)	6.67 (11.67)	0.00 (0.71)	0.00 (0.71)	0.73 (1.09)	0
T_6	2.87 (1.69)	5.42 (13.44)	10.00 (18.55)	6.67 (11.67)	0.47 (0.98)	0.47 (0.98)	0.67 (1.05)	0
T_7	0.93 (0.96)	2.25 (8.34)	6.67 (15.34)	0.00 (4.05)	0.20 (0.84)	0.00 (0.71)	0.40 (0.95)	0
T_8	1.80 (1.34)	3.33 (10.41)	8.33 (13.56)	6.67 (11.67)	0.20 (0.84)	0.00 (0.71)	0.47 (0.98)	0
T_9	3.13 (1.77)	7.68 (16.04)	10.00 (18.01)	0.00 (4.05)	0.20 (0.84)	0.00 (0.71)	0.40 (0.95)	0
T_{10}	3.33 (1.83)	7.42 (15.79)	10.00 (18.01)	0.00 (4.05)	0.20 (0.84)	0.00 (0.71)	0.33 (0.90)	0
T_{11}	1.47 (1.21)	2.97 (9.87)	8.33 (16.77)	0.00 (4.05)	0.53 (1.01)	0.47 (0.98)	0.60 (1.04)	0
T_{12}	2.00 (1.41)	3.74 (11.04)	11.67 (19.98)	0.00 (4.05)	0.53 (1.01)	0.47 (0.98)	0.73 (1.10)	0
T_{13}	3.60 (1.90)	7.80 (18.18)	10.00 (18.01)	0.00 (4.05)	0.53 (1.01)	0.53 (1.01)	0.07 (0.75)	0
T_{14}	3.67 (1.91)	7.92 (16.29)	10.00 (18.01)	0.00 (4.05)	0.40 (0.95)	0.53 (1.01)	0.53 (1.01)	0
T_{15}	6.87 (2.62)	43.73 (41.33)	11.67 (19.98)	13.33 (19.30)	1.07 (1.25)	1.87 (1.53)	0.80 (1.13)	0
SEm±	0.03	0.79	3.23	4.27	0.03	0.04	0.09	-
CD at 5%	0.08	2.29	NS	NS	0.08	0.13	NS	-

*Figures in parentheses are square root transformed values; ** Figures in parentheses are arcsin transformed values.

Table 3. Effect of insecticides against insect pests, natural enemies and YMD incidence on soybean (at 75 DOC)

Tr. Code	Adult whitefly /plant*	YMD incidence (%)**	% Infestation		Larval population /mrl		Adult population /mrl	
			Stem fly**	Girdle beetle**	*S. litura**	*C. acuta**	Spider*	Lady bird beetle
T_1	1.13 (1.06)	4.97 (12.83)	10.00 (16.63)	13.33 (15.87)	0.27 (0.86)	1.20 (1.30)	0.33 (0.91)	0
T_2	1.20 (1.10)	5.28 (13.22)	13.33 (21.58)	0.00 (4.05)	0.27 (0.86)	1.27 (1.33)	0.53 (0.99)	0
T_3	2.00 (1.41)	13.02 (21.13)	13.33 (21.58)	0.00 (4.05)	0.27 (0.86)	1.27 (1.33)	0.33 (0.91)	0
T_4	2.20 (1.48)	13.75 (21.72)	13.33 (18.83)	0.00 (4.05)	0.20 (0.83)	1.20 (1.30)	0.67 (1.07)	0
T_5	1.40 (1.18)	6.38 (14.55)	10.00 (18.91)	6.67 (11.67)	0.00 (0.71)	0.00 (0.71)	0.87 (1.17)	0
T_6	1.33 (1.15)	6.39 (14.62)	13.33 (21.59)	0.00 (4.05)	0.20 (0.83)	1.20 (1.30)	1.13 (1.27)	0
T_7	0.60 (0.77)	2.58 (9.15)	6.67 (13.96)	13.33 (19.30)	0.00 (0.71)	0.00 (0.71)	0.27 (0.86)	0
T_8	1.00 (1.00)	4.01 (11.49)	6.67 (13.96)	13.33 (19.30)	0.00 (0.71)	0.00 (0.71)	0.67 (1.08)	0
T_9	1.40 (1.18)	7.84 (16.22)	13.33 (21.58)	13.33 (19.30)	0.00 (0.71)	0.07 (0.75	0.67 (1.07)	0
T_{10}	1.60 (1.26)	8.63 (17.05)	13.33 (21.58)	13.33 (19.30)	0.00 (0.71)	0.07 (0.75)	0.73 (1.10)	0

T_{11}	0.80 (0.89)	3.48 (10.67)	10.00 (18.91)	6.67 (11.67)	0.20 (0.83)	1.27 (1.33)	0.53 (1.01)	0
T_{12}	1.00 (1.00)	4.48 (12.11)	13.33 (19.30)	6.67 (11.67)	0.33 (0.91)	1.27 (1.33)	0.67 (1.07)	0
T_{13}	1.73 (1.31)	10.53 (18.88)	13.33 (21.58)	13.33 (15.89)	0.33 (0.91)	1.27 (1.33)	0.73 (1.10)	0
T_{14}	1.80 (1.34)	10.86 (19.22)	10.00 (16.63)	13.33 (19.30)	0.33 (0.91)	1.40 (1.37)	0.73 (1.11)	0
T_{15}	3.73 (1.93)	61.31 (51.49)	13.33 (21.58)	13.33 (18.11)	1.73 (1.49)	3.20 (1.90)	1.27 (1.30)	0
SEm±	0.03	0.33	4.25	7.20	0.04	0.05	0.10	-
CD at 5%	0.08	0.94	NS	NS	0.12	0.15	NS	-

*Figures in parentheses are square root transformed values; ** Figures in parentheses are arcsin transformed values.

Table 4. Effect of insecticides against insect pests, natural enemies and YMD incidence on soybean (overall mean)

Tr. Code	Adult whitefly/ plant*	YMD incidence (%)*	% Infestation		Larval population /mrl		Adult population /mrl	
			Stem fly**	Girdle beetle**	*S. litura**	*C. acuta**	Spider*	Lady bird beetle
T_1	1.22 (1.50)	2.97 (9.93)	9.45 (17.90)	4.44 (12.16)	0.25 (1.12)	0.56 (1.25)	0.44 (1.20)	0
T_2	1.31 (1.51)	3.17 (10.26)	10.00 (18.41)	2.22 (8.57)	0.25 (1.12)	0.58 (1.26)	0.31 (1.15)	0
T_3	2.24 (1.80)	7.28 (15.65)	10.56 (18.96)	0.00 (0.00)	0.25 (1.12)	0.56 (1.25)	0.47 (1.21)	0
T_4	2.44 (1.85)	7.61 (16.01)	9.44 (17.89)	0.00 (0.00)	0.22 (1.11)	0.56 (1.25)	0.65 (1.28)	0
T_5	1.56 (1.60)	3.87 (11.35)	9.45 (17.90)	4.45 (12.17)	0.00 (1.00)	0.00 (1.00)	0.58 (1.26)	0
T_6	1.56 (1.60)	4.01 (11.55)	10.00 (18.37)	2.22 (8.57)	0.22 (1.10)	0.56 (1.25)	0.76 (1.33)	0
T_7	0.51 (1.23)	1.61 (7.29)	4.45 (12.17)	4.44 (12.16)	0.07 (1.03)	0.00 (1.00)	0.38 (1.18)	0
T_8	0.93 (1.39)	2.45 (9.01)	6.11 (14.31)	6.67 (14.96)	0.07 (1.03)	0.00 (1.00)	0.45 (1.20)	0
T_9	1.71 (1.65)	5.20 (13.18)	10.00 (18.41)	4.44 (12.16)	0.07 (1.03)	0.02 (1.01)	0.42 (1.19)	0
T_{10}	1.84 (1.69)	5.39 (13.42)	10.00 (18.30)	4.44 (12.16)	0.07 (1.03)	0.02 (1.01)	0.55 (1.25)	0
T_{11}	0.76 (1.33)	2.15 (8.43)	7.22 (15.58)	2.22 (8.57)	0.24 (1.11)	0.58 (1.26)	0.47 (1.21)	0
T_{12}	1.04 (1.42)	2.74 (9.52)	10.56 (18.96)	2.22 (8.57)	0.29 (1.14)	0.58 (1.25)	0.64 (1.28)	0
T_{13}	2.04 (1.73)	6.16 (4.37)	10.00 (18.37)	4.44 (12.16)	0.29 (1.14)	0.60 (1.27)	0.38 (1.18)	0
T_{14}	2.09 (1.76)	6.31 (14.55)	8.89 17.34)	4.44 (12.16)	0.24 (1.11)	0.64 (1.28)	0.51 (1.23)	0
T_{15}	4.60 (2.37)	35.91 (36.80)	10.57 (18.96)	8.89 (17.34)	0.93 (1.39)	1.69 (1.64)	0.89 (1.38)	0
SEm±	0.06	0.07	0.51	0.02	0.01	0.01	0.004	-
CD at 5%	0.18	0.22	1.78	0.06	0.02	0.02	0.01	-

*Figures in parentheses are square root transformed values; ** Figures in parentheses are arcsin transformed values.

Table 4. Economics of insecticidal treatments on yield of soybean crop

Treatment Code	Yield kg/ha	Additional yield over control kg/ha	Cost of additional yield (Rs./ha)	Cost of treatment (Rs./ha)	Net return (Rs./ha)	ICBR
T_1	1451.63	596.93	17060.26	569	16491.26	1:0.97
T_2	1410.93	556.23	15897.05	1698	14199.05	1:0.89
T_3	1126.03	271.33	7754.611	599	7155.61	1:0.92
T_4	1119.25	264.55	7560.839	627	6933.84	1:0.92
T_5	1343.10	488.4	13958.47	894	13064.47	1:0.94
T_6	1345.81	491.11	14035.92	2259	11776.92	1:0.84
T_7	2062.13	1207.43	34508.35	1463	33045.35	1:0.96
T_8	1566.95	712.25	20356.11	2592	17764.11	1:0.87
T_9	1322.75	468.05	13376.87	1493	11883.87	1:0.89
T_{10}	1309.18	454.48	12989.04	1521	11468.04	1:0.88
T_{11}	1831.50	976.8	27916.94	2828	25088.94	1:0.90
T_{12}	1526.25	671.55	19192.9	3957	15235.90	1:0.79
T_{13}	1234.57	379.87	10856.68	2858	7998.68	1:0.74
T_{14}	1221.00	366.3	10468.85	2886	7582.85	1:0.72
T_{15}	854.70	0	-	-	-	-
SEm ±	15.59	-	-	-	-	-
C.D. at 5%	45.14	-	-	-	-	-

Note: Soybean @ Rs. 2858/q, Imidacloprid 40FS Rs. 350 per 100 ml, Thiamethoxam 30FS Rs. 1025 per 500 ml, Fipronil 5SC Rs. 600 per 500 ml, Bifenthrin 10 EC Rs. 130 per 100 ml, Triazophos 40 EC Rs. 230 per 500ml, Flonicamid 50WG Rs. 260 per 30 g, Labour Charges - Rs. 263/day.

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