A DISSERTATION ON

"Role of Jasmonic Acid in Mitigating Mancozeb Induced Toxicity in *Vicia faba*"

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IN BIOTECHNOLOGY

BY

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DECLARATION FORM

I, **Mohd Askari Raza**, a student of **(M. Tech Biotechnology)** (IIYear/ IV Semester), Integral University have completed my six months dissertation work entitled **"Role of Jasmonic Acid in Mitigating Mancozeb Induced Toxicity in Vicia faba"** successfully from **Integral University** under the able guidance of **Dr. Aisha Kamal.**

I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

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This is to certify that **Mr. Mohd Askari Raza** (Enrollment Number 1700100415) has carried out the research work presented in this thesis entitled **"Role of Jasmonic Acid in Mitigating Mancozeb Induced Toxicity in Vicia faba"** for the award of **M.Tech Biotechnology** from Integral University, Lucknow under my supervision. The thesis embodies results of original work and studies carried out by the student himself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution. The dissertation was a compulsory part of his **M.Tech Biotechnlogy** degree.

I wish him good luck and bright future.

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TO WHOM IT MAY CONCERN

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I wish him good luck and bright future.

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Mohd Askari Raza

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LIST OF ABBREVIATIONS

NAMES	ABBREVIATIONS
Milligrams	mg
micro meter	µm
Jasmonic acid	J.A
Mancozeb	MZ
nanoparticles	nps
Fungicide Resistance Action Committee	ERAC
Grams per litre	gl^{-1}
Miligrams per litre	mgl^{-l}
Mille litre	ml
parts per million	ppm
world health organisation	WHO

1. INTRODUCTION

Pesticide is a chemical substance that is used to prevent crop damage posed by the pests such as insects, bacteria, fungi, etc. The introduction of these synthetic pesticides had been proved an effective strategy against biotic and abiotic stress coupled with adverse climatic conditions (Newton M, 1981). As a result, it has increased the crop yield and reduces post-harvest losses. These pesticides can be classified on the grounds of assignment (herbicides, fungicides), mode of application (contact, systemic, fumigants), chemical nature of the pesticide (Organochlorines, Organophosphates, etc). Insecticides contribute to the major part of the Indian crop protection trade by contributing 61.11%, next herbicides (22.22%), and fungicides (11.11%) (Kumar & Sachin., 2013).

Through their ability to effectively manage pests, illnesses, and weeds that could otherwise harm crop output and quality, pesticides play a crucial role in enhancing modern agriculture. They are chemical substances specifically designed to target and eliminate harmful organisms, ensuring the health and productivity of plants. A widely used pesticide is Mancozeb, which has its unique characteristics and applications.

Mancozeb is a multifunctional fungicide that belongs to the ethylene bisdithiocarbamate (EBDC) group. It functions as a preventative fungicide by creating a barrier of defense on the plant's surface to ward off fungal infections. (Leroux et al., 2013). Mancozeb is effective against a variety of fungal diseases, including downy mildew, blights, and leaf spots in different crops (Kosmidou et al., 2020). It prevents fungal growth by interfering with crucial metabolic processes, which ultimately results in the pathogen's death.

Vicia faba, also known as the broad bean or fava bean, is a versatile and nutrient-rich legume plant with a long history of cultivation (Smith et al., 2017). It is a member of the Fabaceae family and is widely cultivated and consumed throughout the world. *Vicia faba* is a wonderful supplement to a balanced diet because of its well-known high nutritional content. According to (Ramos et al., 2018), Protein, dietary fibre, vitamins (including vitamin C and vitamin K), and minerals (such as iron, phosphorus, and potassium) are all present in significant amounts in the seeds of *Vicia faba*. These nutritional characteristics have an impact on its standing as a wholesome food source.

The high protein content of *Vicia faba*, which ranges from 23% to 32% (dry weight) (Parks et al., 2019), is one of its main selling points. The essential amino acids lysine, leucine, and phenylalanine are abundant in the protein found in *Vicia faba*, making it a valuable source of plant-based protein for human nutrition (Siddhuraju et al., 2018). Additionally, it has been discovered that *vicia faba* protein possesses favourable qualities such a high capacity for retaining water and emulsifying capabilities, making it appropriate for use in a variety of food applications (Lestienne et al., 2007).Besides being a great source of protein, *Vicia faba* seeds are also high in dietary fiber. Because they contain both soluble and insoluble fiber, they have a positive impact on gut health and digestion (Ramos et al., 2018). In the digestive tract, soluble fiber congeals into a gel-like substance that slows down digestion and aids in controlling cholesterol and blood sugar levels (Tosh et al., 2013). According to Marlett et al. (2002), insoluble fibre gives the stool more volume, which encourages regular bowel movements and prevents constipation.

Figure 1: Seeds of *Vicia faba*

Minerals and vitamins abound in *Vicia faba*. Vitamin C, an important antioxidant that is required for collagen production, iron absorption, and immunological function, is present in significant amounts in the seeds (Ramos et al., 2018). Also beneficial for bone health and blood coagulation is vitamin K, which is found in abundance in *Vicia faba* (Booth et al., 2013). *Vicia faba* contains a variety of minerals, including

potassium, phosphorus, and iron. Potassium is involved in maintaining adequate fluid balance in the body, phosphorus is important for bone health, and iron is required for oxygen transport (Parks et al., 2019).

Figure 2: Faba Bean Gall (soil bourne fungus) disease symptoms on faba bean leaf (A), stem (B), root area (C), flower (D), and pod (E). Ref - (Bitew et al., 2021)

Moreover, *Vicia faba* exhibits environmental benefits due to its ability to repair nitrogen from the atmosphere. The nitrogen-fixing bacteria Rhizobia live in symbiotic relationships with the roots of *Vicia faba*, which convert atmospheric nitrogen into a form that plants can utilize (Duranti et al.,2017). This process, known as biological nitrogen fixation, lowers the need for synthetic nitrogen fertilisers, which require a lot of energy to produce and may have an impact on pollution. (Carlsson et al.,2019). By incorporating *Vicia faba* into crop rotations or using it as a cover crop, farmers can improve soil fertility, reduce nitrogen runoff, and promote sustainable agriculture practices. Culturally, *Vicia faba* has been cultivated and consumed for centuries in various cuisines around the world. In Mediterranean cuisine, for example, *Vicia faba* is a staple ingredient in traditional dishes like ful medames and falafel. It is also used in soups, stews, salads, and spreads, showcasing its versatility in culinary applications (Lombardi et al., 2019).

Plant growth regulators (PGRs) are substances that encourage growth and development in plants to normalize survival in stressful situations. They reduce abiotic stress-induced damage by modulating the developmental process by influencing numerous physiological and biochemical responses. Pesticide-induced toxicity is reduced by PGR, which boosts antioxidant defenses and increases stress tolerance (Parween, T, et al., 2012). The PGRs applied exogenously are brassinosteroid, cytokinins, jasmonic acid, salicylic acid, abscisic acid, ethylene, etc., they promote pesticide resistance by modulating reactive oxygen species generation, nutritional homeostasis, metabolite synthesis, and activating antioxidant systems. They are efficient at very low concentrations to control the growth, development, and mitigation of stress responses. One of these plant growth regulators used salicylic acid to overcome the stress responses induced by the excessive use of fungicides on the plant (Handford CE, et al., 2015)

The plant hormone known as a jasmonate, or jasmonic acid, is naturally occurring. It is necessary for the growth, development, and defense mechanisms of plants against array of stressors, such as herbivores and pathogens (Wasternack and Hause, 2013). Several physiological processes, together with seed germination, root growth, floral development, and senescence, are regulated by jasmonic acid. In the course of the plant's defense mechanism, it also serves as a signaling molecule, causing the creation of protective substances including phytoalexins and proteinase inhibitors to ward off herbivores and stifle pathogen growth (Creelman and Mullet, 1997).

In conclusion, *Vicia faba* is a highly nutritious and versatile legume plant with significant health benefits. Because of its high protein content and accessibility to essential amino acids, it is a valuable source of plant-based protein. *Vicia faba* plays an important role in promoting digestive health and general wellbeing thanks to the dietary fibre, vitamins, and minerals it contains. Additionally, its nitrogen-fixing ability and sustainable cultivation practices make it an environmentally friendly crop choice. With its cultural significance and culinary versatility, *Vicia faba* continues to be appreciated and incorporated into diverse cuisines worldwide.

2. AIMS AND OBJECTIVES

It seemed worthwhile to reduce and monitor the degree of intensity of pesticide stress for the best morpho-physiological gain in *Vicia faba* through priming the seeds with Jasmonic acid prior to sowing for efficient germination, seedling establishment, and morpho-physiological performance. This was done while keeping the role of phytohormones in various crop plants in mind. In this regard the present study is proposed to pursue research with the following objectives in mind

- Morphological analysis of *Vicia faba* exposed to mancozeb and jasmonic acid.
- Effect of mancozeb and jasmonic acid on physiological parameters of *Vicia faba.*
- Effect of mancozeb and jasmonic acid on biochemical parameters of *Vicia faba.*
- Effect of mancozeb and jasmonic acid on antioxidant activity of *Vicia faba.*

3. REVIEW LITERATURE

3.1 Origin and Distribution of *Vicia faba*

Vicia faba, also referred to as the wide bean or faba bean, is a member of the Fabaceae family of leguminous plants. Its origin can be traced back to the Mediterranean region and the Middle East. *Vicia faba* has been cultivated and domesticated for a very long time—dating back thousands of years. It is said to have been domesticated in the eastern Mediterranean area, especially in the contemporary countries of Turkey and Egypt. Archaeological evidence of *Vicia faba* cultivation has been found in Neolithic sites in the Near East, including Çatalhöyük in Turkey and Jericho in Palestine (Zohary and Hopf, 2000). These findings suggest that *Vicia faba* was one of the earliest domesticated crops. *Vicia faba* was introduced to Europe by ancient civilizations. The Greeks and Romans played a significant role in its spread. The Greek philosopher and botanist Theophrastus (372-287 BCE) mentioned the broad bean in his works, describing its cultivation and use (Dalby, 2003). The Romans recognized the value of *Vicia faba* as a food source and a nitrogen-fixing crop for improving soil fertility. They actively promoted its cultivation throughout Europe during the Roman Empire (Gepts et al., 2005). Additionally, historical texts provide insights into the broad bean's importance in ancient agriculture, such as De Agri Cultura by Cato the Elder, which highlights its cultivation practices.

In ancient agriculture, *Vicia faba* held great importance. Its ability to fix nitrogen in the soil made it valuable for improving soil fertility and subsequent crop yields. This quality made *Vicia faba* an essential rotational crop in the Mediterranean region (Gepts et al., 2005). Moreover, it served as a staple food source, providing a reliable and nutritious crop for ancient civilizations. The broad bean was consumed in various forms, including boiled, roasted, or ground into flour for making bread (Gepts et al., 2005). Historical records and archaeological findings shed light on the broad bean's role in the diets of ancient populations, such as the consumption of fava beans by the ancient Egyptians, Greeks, and Romans.

Over time, *Vicia faba* spread beyond its Mediterranean origins and reached other parts of the world. It was introduced to Asia, Africa, and the Americas through trade, exploration, and colonization. Arab traders likely played a crucial role in

disseminating the crop to Asia and Africa, as evidenced by its widespread cultivation in these regions (Zohary and Hopf, 2000). The Spanish conquistadors introduced *Vicia faba* to the America during the colonial era. Today, it is cultivated worldwide, with major production in countries such as China, Ethiopia, Egypt, the United Kingdom, and the United States (Maxted et al., 2009). Modern agricultural practices and genetic research have contributed to the continued cultivation and improvement of *Vicia faba* as an important crop.

3.2 Scientific Classification

Kingdom	**Plantae**
Clade	Tracheophytes
Subfamily	Faboideae
Family	Fabaceae
Class	Angiosperm
Order	Fabales
Tribe	Fabeae
Genus	*Vicia*
Species	*faba*

3.3 Habitat

Vicia faba, also referred to as the wide bean or faba bean, has a diverse habitat range suitable for its growth and cultivation. The climate requirements for *Vicia faba* cultivation include mild winters and moderate temperatures during its growing season. It thrives in temperate and subtropical regions, with an optimum temperature range of 10°C to 25°C (Jensen et al., 2017). The plant can tolerate frost to some extent, but prolonged exposure to freezing temperatures can be detrimental. In terms of soil preferences, *Vicia faba* exhibits adaptability to different soil types but performs best in well-drained soils with good water-holding capacity. It prefers fertile soils rich in organic matter. *Vicia faba* is said to grow and develop best in soils that vary from sandy loam to clay loam and have a pH between 6.0 and 7.5. (Jensen et al., 2017). Notably, studies have shown that *Vicia faba* has a higher tolerance for alkaline soils compared to other legume crops (Rubiales et al., 2017). *Vicia faba* is a sun-loving plant that requires full sunlight for optimal growth. It thrives in areas with direct exposure to sunlight for at least six to eight hours a day. Insufficient sunlight may result in reduced plant growth and lower crop yields. The geographical distribution of

Vicia faba is widespread, encompassing Europe, North Africa, Asia, and the Americas. It is commonly grown in regions with suitable climates. Major producers of *Vicia faba* include China, Ethiopia, Egypt, United Kingdom, and United States of America (FAOSTAT, 2021). The plant's genetic diversity and adaptability are also aided by the presence of wild relatives, which can be found throughout the world (Maxted et al., 2009). An important environmental factor is the *Vicia faba*'s capacity to fix atmospheric nitrogen through a symbiotic relationship with nitrogen-fixing bacteria in its root nodules. As a result, *Vicia faba* is a more environmentally friendly crop choice (Stoddard et al., 2010). This quality improves soil fertility and decreases the need for nitrogen fertilisers.

3.4 Morphological Characterisation

Fabaceae, typically exhibits an erect growth habit, reaching heights ranging from 0.5 to 2 meters (Lewington, 2013). Its stem is cylindrical, covered with fine hairs (pubescence), and may exhibit branching (Lewington, 2013).The compound leaves of *Vicia faba* are arranged alternately and consist of several pairs of leaflets, usually numbering 2 to 4 pairs, with a tendril at the termination for climbing support (Lewington, 2013; Hanelt, 2001).The leaflets have a shape that ranges from ovate to lanceolate, a noticeable midrib, and a marginal serration (Hanelt, 2001). *Vicia faba* produces large and showy flowers arranged in racemes at the end of long stalks. Each flower comprises a banner, two wings, and a keel, with white being the common color, although pink or purple hues can also be observed (Lewington, 2013).The fruit of *Vicia faba* is an elongated, slightly curved pod, ranging in length from 10 to 30 centimeters. Within the pod, several seeds, commonly known as beans, are arranged in a row (Lewington, 2013). The seeds themselves are large and oval-shaped, exhibiting a smooth or slightly wrinkled texture. They come in various colors, including white, brown, speckled, or mottled patterns, which can vary depending on the cultivar and regional characteristics (Lewington, 2013).To further explore the morphological characteristics of *Vicia faba*, a range of studies have been conducted. For instance, (Dikshit et al., 2017) investigated the diversification of Indian broad bean germplasm through morphological and biochemical characterization. (Cubero et al., 2012) focused on the identification of *Vicia faba* cultivars using isozymes, while (Zaharieva et al., 2015) delved into genetic variation and population dynamics analysis using EST-SSR markers. Other investigations have looked at the genetic variation and morphological description of Vicia faba in various habitats and

populations. Using morphological and molecular markers, (Shukla et al., 2017), (Das et al., 2014), and (El-Far et al., 2013) investigated the genetic diversity. Studies on genetic diversity and characterisation were undertaken by (Wei et al., 2016), (Amelework et al., 2016), and (Zhang et al., 2015) utilizing a variety of markers, including SSR markers. Additional research has concentrated on the genetic diversity and localization of the Vicia faba germplasm. While (Haroun et al., 2017) carried out a genetic diversity analysis using morphological and molecular markers, (El-Din et al., 2018) investigated the genetic diversity of Egyptian faba bean genotypes using ISSR markers. Through phenotypic and SSR marker analysis, (Tewelde et al., 2020) and (Ambaw et al., 2020) investigated the genetic diversity and population structure of Ethiopian faba bean accessions. Additionally, the morphological and molecular diversity of Syrian faba bean landraces was investigated by (Hajjar et al., 2008).

Figure 3: Seeds of *Vicia faba*

Figure 4: Opened *Vicia faba* pod

3.5 World Wide Production

Vicia faba is cultivated on a global scale, with production occurring in numerous countries across different continents. In Europe, it is extensively grown, with countries like the United Kingdom, France, Germany, and Spain being major producers (Rios, 2012). The Mediterranean region, including countries such as Egypt, Turkey, and Morocco, also boasts substantial production of *Vicia faba* (Stoddard, 1996). In North America, both the United States and Canada cultivate significant areas of broad beans, particularly in regions with favorable climates (Slinkard, 2000). Additionally, *Vicia faba* holds importance in Asia, with China, India, and Ethiopia being key producers (Kumar et al., 2012; Eshetu et al., 2014).

3.6 Nutrient Characterization of *Vicia faba*

3.6.1 Nutritional Properties and Health Aspects of Faba Beans

Broad beans have gained recognition for their nutritional composition and health benefits. They offer a reliable source of vitamins, minerals, dietary fibre, protein, and other nutrients. The protein content in broad beans is relatively high, with levels ranging from 25% to 35% (Riggi et al., 2018). The protein quality of *Vicia faba* is also noteworthy, due to the fact that it has a balanced amino acid profile and contains key amino acids like lysine and tryptophan. Additionally, broad beans are a reliable source of dietary fiber, which contributes to digestive health and helps regulate blood sugar levels (Tayel et al., 2020).

The nutritional profile of *Vicia faba* has been linked to several health benefits. Research suggests that the consumption of broad beans may contribute to cardiovascular protection, as they are a good source of antioxidants and bioactive compounds, including phenolic compounds, flavonoids, and phytosterols (Angelino et al., 2017). These compounds possess anti-inflammatory properties and have been linked to a lower risk of chronic illnesses, such as cardiovascular diseases and specific types of cancer.

Furthermore, the fiber content in broad beans aids in maintaining healthy cholesterol levels and promoting satiety, which may contribute to weight management and prevent obesity-related disorders (Aragonés et al., 2017). Additionally, the low

glycemic index of broad beans makes them suitable for individuals with diabetes or those seeking to regulate blood sugar levels.

3.6.2 Protein:

Vicia faba is a great option for people who follow vegetarian or vegan diets because It has a lot of plant-based protein in it. Broad beans can contain 20% to 35% of their dry weight in protein (Frias et al., 2011; Rubiales et al., 2017). This high protein content makes broad beans a valuable nutritional resource, providing essential amino acids necessary for various physiological functions. Broad beans are particularly notable for their lysine content, an essential amino acid that is limited in many other plant-based protein sources. In addition, broad beans also contain tryptophan, methionine, and other important amino acids required by the human body (Murphy et al., 2007).

Protein concentration ranged from 88% to 94% in protein isolates from alkaline and acid extraction (Eckert et al., 2019); (Singhal et al., 2016). The efficiency of wet extraction in protein purification is demonstrated by the fact that faba bean protein concentrates generated using dry fractionation had lower protein levels (51%-69%) but greater total carbs (23%-38%) and ash content (4%-5%) (Coda et al., 2015); (Vogelsang-O'Dwyer et al., 2020). Starch and fiber, which are nonprotein ingredients, can perform technologically functional roles in the food formulation process, such as thickeners, emulsifiers, and gelling agents. Less than 0.1% of protein concentrations included fat, which was higher than isolates from alkaline extraction but lower than isolates from acid extraction (Eckert et al., 2019); (Vogelsang-O'Dwyer et al., 2020). Based on the initial protein content and inherent characteristics of the faba bean genotype, compositional qualities may differ, which may affect protein extractability (Martinez et al., 2016); (Vogelsang-O'Dwyer et al., 2020).

Table 1: Nutritional makeup of faba bean proteins in relation to extraction technique

Nutritional composition (g/100g)	Proteins concentrates (dry fractionation)	Protein isolates (acid extraction)	Protein isolates (alkaline extraction)
Moisture	8-12	6	-
Total carbohydrate	23–38	0.34	-
Fiber	10	-	2
Starch	7–23	2	-
Protein	51–69	90	88–94
Ash	4–5	5	2.9–5
Fat	2–3	-4	0.1

a Acid extraction (Vogelsang-O'Dwyer et al., 2020).
b Alkaline extraction (Eckert et al., 2019),(Singhal et al., 2016).
c Dry fractionation (Coda et al., 2015),(Vogelsang-O'Dwyer et al., 2020).

3.6.3 Carbohydrates: (starch, dietary fiber, and sugars)

The amount of carbohydrates in faba bean seeds ranges from 51% to 68%; nevertheless, starch (41–58%) makes up the majority of these carbohydrates (USDA, 2021; (Vidal-Valverde et al., 1998). The main nutritional factors affecting the carbs in faba beans are their starch content, dietary fiber content, and type of sugars. Raffinose, stachyose, and verbascose, the primary soluble sugars, are oligosaccharides of the raffinose family that are thought to be the cause of flatulence and restrict faba bean intake from a digestive standpoint. According to (Vidal-Valverde et al., 1998), soluble sugars such stachyose and verbascose are abundant in faba bean seeds.

The two components amylose and amylopectin make up the majority of the starch (22-45%) in faba beans, according to (Punia et al., 2019). Faba bean starch granules have been observed in a variety of morphologies, including elliptical, circular, oval, and irregular, Studies using a scanning electron microscope have been conducted, according to (Sofi et al., 2013). Strong binding pressures caused the starch granules to integrate, which is why faba bean starch has low solubility (9.92 g/100 g) and

swelling power (12.67 g/g) (Zhang et al., 2019). Faba bean starch is resistant to enzymatic hydrolysis because of its high resistant starch (RS) content (46.7%) and low amounts of fast digested (15.3%) and slowly digestible (34.5%) starch (Bello-Pérez et al., 2007). A lab-scale technique was developed and enhanced by (Suárez-Diéguez et al., 2021) to extract RS from faba beans. An enhanced retrogradation method was used to increase the RS content of faba beans. This study showed that the reduced and delayed digestion of faba bean RS made it a potential functional component.

Faba beans are a very good source of dietary fibre, both soluble and insoluble, according to (Singh et al., 2014). FBF contains the most dietary fibre in compared to flours derived from lima, pinto, and red kidney beans (Gu et al., 2020). Whole faba beans have a dietary fibre level of 15% to 30%; the majority of this content is made up of hemicellulose, cellulose, and lign. The amount of dietary fibre contained in the faba bean seed coat has been discovered to be substantially higher (82.3%) (Karataş et al., 2017), (Vidal-Valverde et al., 1998). It is recommended to eat the faba bean with its seed coat as well because it is reported to be a rich source of dietary fibre, phenolic compounds, and minerals (Karataş et al., 2017).

3.6.4 Vitamins:

Vicia faba, especially the mature seeds, is an excellent source of several vitamins, including folate or vitamin B9. The folate content in broad beans can range from 100 to 200 micrograms per 100 grams of cooked beans (Ibrügger et al., 2020; Osman et al., 2017).The synthesis of DNA, cell division, and the creation of red blood cells are just a few biological processes that depend on folate. Due to its support for healthy foetal development and contribution to preventing neural tube defects in newborns, it is especially crucial for expectant mothers (Pfeiffer et al., 1997). Adequate folate intake is also beneficial for cardiovascular health, cognitive function, and the maintenance of a healthy immune system.

3.6.5 Minerals:

Iron, zinc, magnesium, and potassium are among the important minerals that can be found in abundance in broad beans. For the body to produce red blood cells and carry oxygen throughout, iron is necessary. The immune system, wound healing, and DNA synthesis are all significantly influenced by zinc. Magnesium is a vital component of many biochemical processes and supports strong bones and muscles in addition to ensuring proper energy metabolism. In accordance with (Riaz et al., 2018) and (Chen et al., 2020), potassium is an electrolyte that regulates blood pressure, supports nerve and muscle function, and helps keep proper fluid balance.

3.7 Bioactive Compounds:

Vicia faba contains various bioactive compounds, including polyphenols, flavonoids, and phytosterols. These substances have anti-inflammatory and antioxidant properties, which add to the potential health advantages of eating broad beans. Quercetin and kaempferol are two examples of polyphenols that have been linked to a lower risk of developing chronic illnesses like cardiovascular disease, some cancers, and neurodegenerative disorders (Duranti et al., 2008; Xu et al., 2019). Flavonoids exhibit antioxidant effects, protecting cells from oxidative damage and inflammation. Phytosterols, on the other hand, have cholesterol-lowering properties and contribute to heart health (García-Lafuente et al., 2010; Hamed et al., 2019).

3.8 Antioxidant Properties

According to (Saha et al., 2015), antioxidants are essential for humans because they snare free radicals and shield against a variety of diseases. Antioxidants have been added to the fruit *V. faba,* and they diffuse reactive free oxygen radicals by engulfing them. In order to assess the chemopreventive properties of polyphenolic compounds against topoisomerases, the substances were isolated in their purest form from the plant *V. faba*.

While the impact of nine polyphenolic compounds was assessed, only a small number of the compounds inhibited all enzymatic activities, and others inhibited specific categories of enzymes like wheat germ opoisomerase (IC 50: 120-350 g), human topoisomerase (IC 50: 240-600 g), and human topoisomerase (IC 50: 110-260 g). The ability of the polyphenolic compounds from *V. faba* to inhibit topoisomerases raises the possibility that they have anti-cancer properties (Tselepi et al., 2011).

3.9 Anti-fungal Activity

The anti-microbial activity of foods and any potential antioxidant characteristics can be combined to create a unique medicine recipe (Saha and Rajeswari, 2015). Liquid chromatography was used to isolate the 15KDa trypsin inhibitor from the *V. faba* plant. After showing potent antifungal activity against the mold Valsamali, it was given the designation Egypt trypsin inhibitor (VFTI-E1) (Fei et al., 2011). Inhibitors of chymotrypsin, chitinase, wyerone, and wyerone epoxide are only a few of the substances isolated from *V. faba* that have been discovered to be efficient anti-fungal agents (Fawcett et al., 1969); (Hargreaves et al., 1976); (Ye et al., 2002); (Wang et al., 2012).

3.10 Anti-Viral Activity

Human cytomegalovirus (HCMV), a pathogen that infects people and has an adverse effect on people with weakened immune systems as well as causing birth defects, was resistant to the *V. faba* plant. Three out of five *V. faba* plants were found to be resistant to HCMV using PCR and dot-blot hybridization, and the pp-150 protein was found to be the cause of this resistance. After being immunised with pp-150 transgenic *V. faba* seeds, mice exhibit concrete HCMV pp-150 antibody (IgG, IgA) and IFN-g engendering T cells, according to ELISA and flow cytometry analysis. According to (Yan et al., 2010), this transgenic V. faba can be used to develop an edible vaccine to prevent HCMV infection.

2.11 Anticancer Activity

The fruit derived from the *Vicia faba* plant has demonstrated potential as an effective agent against cancer. Colon cancer is among the leading causes of high mortality rates in Britain, and the *V. faba* fruit shows promising effect in combating this disease. Specifically, the lectin present in *V. faba* has been observed to induce morphological differentiation of colon cancer cells with malignant characteristics, leading to the formation of gland-like structures and ultimately halting the progression of colon cancer (Jordinson et al., 1999).

Furthermore, studies using *V. faba* protein hydrolysates on animal models at low doses (10 mg/kg body weight) have revealed significant anticancer activity. Compared to the normocholesterolemic diet group, the anticancer effects were more

prominent (Erika et al., 2016). The underlying mechanism behind this anticancer activity is believed to involve the inhibition of a subgroup of matrix metalloproteinases within *V. faba* fruit's bioactive substances. These metalloproteinases play a crucial role in the development and metastatic spread of cancer, as stated by Lima et al., 2016.

3.12 Mancozeb, the Pesticide Used in the Study:

Mancozeb, a contact fungicide is an ethylene (bis)dithiocarbamate (EBDC) fungicide. It specifically belongs to the ethylene di-thiocarbamate class of compounds. The properties of mancozeb are mentioned in the table 1. Fungicide Resistance Action Committee (FRAC) has classified mancozeb under multi-site action group. Being multi-site widens the activity spectrum against ascomycetes, basidiomycetes, oomycetes, and imperfect fungi, effective in small quantities. Although the efficacy is increased due to multisite property it also depends on the infection stage at which it is applied to the host plant (Li, P., et al., 2013). It is used for crop protection against fungal diseases in ornamental plants, fruits and crops. Mancozeb itself does not possess fungicidal action; it is a pro-fungicide which on exposure to water initiates its toxic action against fungal diseases. As mentioned in the introduction, its breakdown in EBIS and EBI also has an impact on the efficiency of the pesticide action. The breakdown rate impacts the residual activity of the compound on the crop (Teunissen-Ordelman HKG & Al E, 1993).

The structure of mancozeb has zinc surrounding the central nucleus of the ethylene (bis) dithiocarbamate forms a stable structure. The low solubility of zinc favors sustained and controlled release of the EBDC towards the leaf surface but this barrier of zinc constantly gets diluted by weathering effects. To acquire an effective control this continual barrier has to exist or else it might lead to toxicity (Gullino ML, et al., 2010). Anitha and Savitha (2013) reported that mancozeb has morphotoxic and phytotoxic effects in rice seedlings. The ability of the pesticide to cause temporary or long-lasting damage to plants as a whole or some parts is called morphotoxicity. These morphotoxic effects include abnormal growth and leaf drop, bronzing, chlorosis, mottling, and necrosis. Adverse effects of mancozeb, such as drying, necrosis, and shredding of leaves and wilting of plants are also observed in chilli (Saxena, A., et al., 2016).

Table 2: Details of Mancozeb

Properties	**Description**
Chemical formula	$C_4H_6N_2S_4Mn$, $C_4H_6N_2S_4Zn$ or $C_8H_{12}MnN_4S_8Zn$
Molar mass	541.01g/mol
Appearance	Yellow color
Texture	Powdery
Odour	Odorless
IUPAC name	(1,2-Ethanediylbis (carbamodithioato))2- manganese zinc salt
Common names	Dithane-M,Carmazine, Kascade, Mancomix
Mode of Action	Inhibits fungal growth

3.13 Pesticide and its effect on plant species.

Pesticides that play a critical role in modern agriculture by successfully managing pests, diseases, and weeds, may also negatively affect crop yields (Oerke & Dehne, 2004). These chemical compounds have been developed and utilized to protect crops and ensure food security. However, the excessive use of pesticides has also raised concerns about their potential impacts on human health, non-target organisms, and the environment (Pimentel, 2005). This introduction will provide an overview of pesticides, their types, and the current understanding of their effects, highlighting recent research findings.

Pesticides are substances specifically designed to kill, repel, or control pests. They cover a broad range of substances, such as pesticides, fungicides, herbicides, rodenticides, and others. Each type of pesticide targets a particular group of pests or weeds, and their effectiveness in pest management has revolutionized agricultural practices. While pesticides are essential tools for crop protection, their use comes with potential risks. Concerns have been raised regarding their impact on human health, including the potential for pesticide residues in food and the potential development of pesticide resistance in pests. Additionally, there is growing awareness of their effects on non-target organisms, such as beneficial insects, birds, aquatic life, and soil microorganisms (Pimentel, 2005). The environmental impacts of pesticides include pollution of water bodies through runoff, contamination of soil, and the potential for long-term ecological disturbances. Recent research has focused on understanding the

effects of pesticides and developing sustainable approaches to minimize their negative impacts. For example, studies have investigated the effects of neonicotinoid insecticides on pollinators, highlighting the potential harm to bees and other beneficial insects crucial for pollination (Goulson, 2013; Woodcock et al., 2017). Research has also examined the impact of herbicides on weed resistance and the development of strategies to manage herbicide-resistant weed populations (Duke, 2012; Délye et al., 2013). Additionally, investigations into the persistence, degradation, and movement of pesticides in soil and water systems contribute to our understanding of their environmental fate (Capel et al., 2019; Coupe et al., 2012).Efforts are being made to develop alternative pest management strategies that reduce reliance on pesticides. Integrated Pest Management (IPM) practices, for instance, promote a holistic approach by combining various methods, including biological controls, crop rotation, and cultural practices, to minimize the need for chemical pesticides (Oerke & Dehne, 2004; Elliott et al., 2017). This approach focuses on long-term prevention and pest management while reducing potential risks associated with pesticide use.

Furthermore, the advent of precision agriculture and the use of digital technologies have enabled more targeted and efficient application of pesticides. This allows for reduced pesticide usage and minimizes the potential for unintended environmental impacts (Fawcett, 2015; Slaughter et al., 2019).

3.14 Role of Plant growth regulators in amelioration of stress in plants

Plant growth regulators (PGRs) are natural or synthetic organic substances that are used to regulate the growth of plants or plant parts. They have the power to influence plant growth by accelerating or delaying it. Plants produce a hormone called phytohormone, also referred to as plant hormone. Phytohormone is a term used to describe an organic molecule produced spontaneously in higher plants that regulates growth or other physiological activities at a location far from its source and is active at very low levels. Plant hormones are a class of chemical compounds found in nature that regulate physiological processes at low doses (Sajjad et al., 2017).

According to (Shakirova et al., 2010), each phytohormone is known to actively interact with other hormones in order to influence vital physiological processes such plant growth, development, and differentiation in both normal and abnormal circumstances.

For instance, Abscisic acid (ABA) serves as a critical phytohormone responsible for regulating plant growth and development. throughout the entirety of the ontogenesis period, including ripening, senescence, seed dormancy and germination, lateral root development, stomata function, and the change from the vegetative to reproductive phase. ABA also protects plants from oxidative stress produced by unfavorable environmental circumstances by up-regulating genes and activity of relevant antioxidant enzymes. The rise in saccharopine, most amino acids, and organic acids levels is regulated by stress-induced endogenous ABA buildup (Urano et al., 2009).

In accordance with (Shakirova et al., 2010), ABA is necessary for the initiation of biosynthesis and the accumulation of proline, an osmoprotectant that contributes to the stability of biopolymers and cell membranes as well as defence against the negative effects of stress generated reactive oxygen species (ROS).

Scientists have made a significant discovery regarding the role of the phenolic compound salicylic acid (SA) in both the development and immune responses of plants. SA, derived from t-cinnamic acid, plays a vital part in the plant's ability to cope with adverse environmental conditions. Additionally, various SA analogues, particularly its precursors like benzoic acid or O-coumaric acid, as well as 2,6-dichloro-isonicotinic acid, exhibit antioxidative properties similar to SA, leading to increased resistance against metal and salt-induced stress. To respond effectively to unfavorable environmental factors, SA acts as a direct hydroxyl radical scavenger, which is crucial for the plant's stress tolerance. As demonstrated by Asgher et al. (2015), supplementation of SA enhances the plant's ability to withstand abiotic stressors such as osmotic stress, drought, salinity, metal toxicity, and heat.

In higher plants, only a specific subset of the diverse group of GAs (Gibberellins) act as growth hormones, with GA1 and GA4 being the most bioactive among them. GAs belongs to a class of tetracyclic diterpenoid carboxylic acids. Throughout the plant's life cycle, they play a crucial role in facilitating various developmental phase transitions, such as shifts between vegetative and reproductive development, juvenile and adult growth stages, and other important phases. GAs exert their influence by promoting cell division and cell elongation, thereby contributing to the growth of most organs. Healthy growth and development in plants largely depend on the activity

of GAs. Seedlings lacking the ability to synthesize or sense GAs exhibit limited growth and may even fail to bloom under specific lighting conditions.

Environmental influences, particularly abiotic stress, have an impact on the GA signalling system's active hormone synthesis, perception, signal transduction, and inactivation (Colebrook et al. 2014). Significant effects on plant growth and development result from the dynamic interaction between GAs and environmental stimuli.

Jasmonates (JAs), the other important phytohormone, are lipid-derived chemicals that serve as crucial signaling agents in plants during times of stress (Noor et al., 2022). Jasmonic acid (JA) was initially identified as a stress-related hormone in higher plants, and it functions as an endogenous growth-regulating chemical. Moreover, the effects of exogenous JA administration also regulate plants. When plants face abiotic stress, they often endure significant damage. However, it has been established that JA does not act as a stand-alone regulator; rather, it is part of a complex signaling network that includes various other phytohormone pathways (Wang, Jia et al., 2020).

3.15 Jasmonic Acid

In response to biotic and abiotic challenges, jasmonates, a class of stress-responsive phytohormones produced from polyunsaturated fatty acids, are essential. Along with jasmonic acid, this also refers to its precursors and derivatives. Jasmonates, which act as the primary immune hormone, take part in several signaling pathways that include enzymes, compounds that shield cells from abiotic stressors, regulatory proteins, signaling intermediates, gene networks, and signaling proteins. Jasmonates are crucial in lowering a variety of environmental challenges, including salt stress, drought stress, heavy metal toxicity, micronutrient toxicity, freezing stress, ozone stress, CO2 stress, and light stress. They function as crucial cellular hubs for processing environmental stimuli. Jasmonates also have an impact on a variety of physiological and developmental processes in plants. The study carried out(Ali et al., 2020), thoroughly explores the production of JAs, their signaling routes, and their involvement in plants' reactions to abiotic stressors.

Abiotic stressors are the primary cause of crop losses globally. If they are to live and grow, plants must be able to recognize and set up crucial processes in response to

abiotic challenges. A recent study found that phytohormones, also known as plant growth regulators (PGRs), particularly jasmonic acid (JA), have enhanced our knowledge of how plants transmit hormones in challenging environments. In several physiological and biochemical processes related to plant growth and development as well as defense mechanisms against pathogen and insect assaults, jasmonic acid takes part. According to recent study, JA may be able to facilitate plants' abiotic stressor-mediated adaptation to severe conditions. (Raza et al., 2021)

Jasmonic acid (JA) is a phytohormone that is essential for plants to defend themselves against herbivore attacks. When a plant is injured, JA and its bioactive derivatives build up at the site and interact with COI1 and JAZ proteins, which serve as jasmonate co-receptors. Among these derivatives, (+)-7-iso-jasmonoyl-L-isoleucine (JA-Ile), an active JA form, plays a key role in regulating how vascular plants react to herbivores. Other endogenous JA-amino acid conjugates (JA-AAs) might, however, also play a role in the defensive responses induced by herbivores. Researchers have looked at the role of herbivore-induced JA-AAs in a study that concentrated on rice crop plants. In response to assaults by the armyworm, leaf folder, and brown planthopper, the levels of five JA-AAs considerably rose (Fu, Wenjie, et al., 2022).

Aside from its role in defense, JA also acts as a phytohormone regulating various aspects of plant root development. Throughout vegetative and reproductive development, senescence phases, and in response to desiccation, salt, and cold stress, different expression patterns of JA biosynthesis genes have been observed (Deepika et al., 2022). This indicates the diverse and important roles that JA plays in plants' growth and response to environmental cues.
Absolutely, hormones play a crucial role in controlling plant development and responses to different environmental conditions, including both normal and stressful environments. These phytohormones act as chemical messengers within plants, coordinating various physiological and developmental processes. When plants encounter stress, such as from abiotic factors like temperature fluctuations, drought, salinity, or biotic factors like herbivore attacks, hormones initiate the adaptation process to help the plant cope with these challenges.

Hormones are involved in regulating key processes, such as growth, flowering, fruiting, root development, leaf senescence, and responses to external stimuli. They

function in complex signaling networks, where different hormones often interact and modulate each other's actions to fine-tune the plant's responses. For example, jasmonic acid (JA) and abscisic acid (ABA) are crucial hormones involved in stress responses, while gibberellins (GAs) and auxins play significant roles in growth and development.

Overall, hormones act as central regulators, orchestrating the plant's adaptive responses to changing environments, thereby ensuring its survival and successful growth in different conditions.

4. MATERIAL AND METHODS

4.1 Test Chemicals:

The pesticide employed in this study is mancozeb (fungicide). It was bought from an agricultural store in Lucknow, India. The treatment doses applied were both above and below the suggested concentration range.

4.2 Procedure Adopted for *Vicia faba* Treatment:

Vicia faba seeds were obtained from a nearby agricultural store in Lucknow, India. Seeds that were healthy and consistent in size were chosen, and they were surface sterilized by washing them with distilled water before being treated with sodium hypochlorite for 10 minutes and then 70% ethanol for 30 seconds. Finally, rinsed with distilled water thrice to completely remove the traces of sterilizing chemicals. The treated seeds were split into two groups: one was soaked in distilled water, while the other was soaked in 25 and 50 µL of JA for 24 hrs keeping in dark. Following that, the seeds were placed in petridish on double-layered filter papers that had been wetted with distilled water and different doses of mancozeb (i.e 10ppm , 30ppm , 50ppm, 70ppm, 90ppm, 110ppm,130ppm) and observe for 21 days with constant monitoring in controlled conditions (Temperature 25°C; humidity 50-60%; photoperiod 16/8 hrs; light intensity 4000Lux). Three replicates of the culture were kept, each with ten seeds per petridish, as well as a control for comparison.

4.3 Treatment of seeds during Germination:

Mancozeb treatment was given at different concentration at interval of 7 days during the experimental period. Following combinations are used for this study-

Table 3: Different sets of seeds used in study

Treatment	SET 1 DW primed Seeds	SET 2 25µl JA primed Seeds	SET 3 50µl JA primed Seeds
Control	DW	DW	DW
T1	10 ppm mancozeb	10 ppm mancozeb	10 ppm mancozeb
T2	30 ppm mancozeb	30 ppm mancozeb	30 ppm mancozeb
T3	50 ppm mancozeb	50 ppm mancozeb	50 ppm mancozeb
T4	70 ppm mancozeb	70 ppm mancozeb	70 ppm mancozeb
T5	90 ppm mancozeb	90 ppm mancozeb	90 ppm mancozeb
T6	110 ppm mancozeb	110 ppm mancozeb	110 ppm mancozeb
T7	130 ppm mancozeb	130 ppm mancozeb	130 ppm mancozeb

4.4 Measurement of Various Parameters:

Morphological, Physiological, biochemical, and antioxidant analyses were performed with standardized protocols.

4.4.1 Morphological Analysis:-

Seed germination percentage (G%), survival percentage (S%), root length (RL), shoot length (SL), seedling vigour index (SVI), percentage of phytotoxicity (P%), and tolerance index (TI) were all evaluated throughout the morphological examination.

4.4.1.1 Germination percentage:-

The germination percentage was calculated on the third day by counting the number of seeds that germinated out of the total number of plated seeds by using formula-

$$G\% = \frac{\text{No. of seeds germinated}}{\text{Total No. of seeds plated}} * 100$$

4.4.1.2 Survival percentage:-

After 7 days, S% was computed by dividing the total number of seedlings that survived by the total number of treated seeds.

$$S\% = \frac{\text{Number of seedling survived}}{\text{Total number of seeds plated}} * 100$$

4.4.1.3 Percentage of Phytotoxicity and Tolerance Index:-

The percentage of phytotoxicity and TI was calculated through the methods proposed by Chou et al. (1978) and Turner and Marshal (1972) respectively with some modifications.

$$P\% = \frac{\text{Radicle length of control plant} - \text{Radicle length of treated plant}}{\text{Radicle length of control plant}} * 100$$

$$TI = \frac{\text{Mean length of five longest roots in treatment}}{\text{Meanlength of five longest roots in control}}$$

4.4.1.4 Growth Parameters:-

Shoot and root length, shoot/root ratio, and fresh and dry weights were measured on 15 Day of Germination.

4.4.1.5 Seedling Vigour Index:-

The SVI is a seed feature that measures the seed's activity level during germination and seedling emergence. Abdul-Baki and Anderson in 1973 described that SVI is calculated on the 7th and 15th days.

$$SVI = \frac{\text{Germination percentage}}{\text{length of seedlings}}$$

4.4.2 Biochemical Analysis:-

4.4.2.1 Assessment of Photosynthetic Pigments:-

The chlorophyll pigment was estimated by Arnon's method (1949) with some modification. 0.5g of a fresh plant sample was harvested and then homogenised in 20 ml of 80% ice-chilled acetone in the dark. A trace amount of $MgCO_3$ powder was added. Whatman No. 1 filter paper was used to filter the homogenate, and the volume was made up to 100 ml. Absorbance was measured at a spectrophotometer (2202, Systronics, India) at 645 nm and 663 nm wavelengths for chlorophyll 'a' and chlorophyll 'b, respectively, by using 80% acetic acid as a blank. Chlorophyll 'a' and chlorophyll 'b' content were calculated using the following equation:

$$\text{Chlorophyll } 'a' = 12.7(A663) - 2.69(A645)$$

$$\text{Chlorophyll } 'b' = 22.9(A645) - 4.68(A663)$$

The carotenoid content was estimated by Kirk and Allen method,(1965), for which the absorbance was taken at 480nm.

$$\text{Carotenoid} = \text{A480} + (0.114 * \text{A663}) - (0.638 * \text{A645})$$

4.5 Assessment of Enzymatic Anti-Oxidants:-

4.5.1 Extraction of Antioxidant Enzymes

The samples were prepared as described by Mukherjee and Choudhuri (1983) with some modifications. A sample (0.5 g) was finely ground by pestle in a chilled motor; 10 mL of 100 mM phosphate buffer (KH_2PO_4/K_2HPO_4) pH 7.0, containing 0.1 mM Na_2EDTA and 0.1 g of polyvinylpyrrolidone (PVP) was added to the sample. The homogenate was filtered through cheese cloth, centrifuged at 15000×g for 10 min at 4°C. The supernatant was recentrifuged at 18000×g for 10 min; the supernatant was stored at 4°C for enzyme assay.

4.5.2 Assessment of CAT Activity

Catalase activity was estimated by the method of Aebi (1984).

Reagents:-

A. Potassium Phosphate Buffer (50mM, pH 7.0)

B. H_2O_2 (30%)

C. Plant Extract

Preparation of aliquots:

Pipette (in milliliters) the following reagents into suitable cuvettes.

Reagent	Sample	Blank
Reagent A	-------	3.0 ml
Reagent B	2.5 ml	------
Reagent C (Plant extract)	0.5 ml	------

The reaction was started by the addition of enzyme extract. The activity of catalase was estimated by the decrease of absorbency at 240 nm for 1 min as a consequence of H_2O_2 consumption. The extinction coefficient for H_2O_2 was 4.32 $cm^2/\mu mol$.

Calculation:

$$\text{Activity}\left(\frac{\text{unit}}{\text{ml}}\right) = \frac{(\Delta A0 - \Delta A5) * 3 * \text{Df}}{\text{extinction coefficient of H2O2} \times \text{ amount of enzyme in aliquots}}$$

ΔA_5=Absorbance at 5 min.

ΔA_o= Absorbance at 0 min.

Df=Dilution factor

4.5.3 Assessment of POD Activity:-

POD activity was determined according to Maehly and Chance, (1954). The reaction solution (3ml) contained 10 mM (KH_2PO_4/K_2HPO_4) pH 7.0, 10 mM H_2O_2, 20 mM pyrogallol and 0.5 mL enzyme extract. Hydrogen peroxide and pyrogallol should be kept in the dark. The aliquots of the test tube were transferred into the cuvette and hydrogen peroxide is added immediately before obtaining absorbance, as the reaction is very rapid and also hydrogen peroxide is light sensitive. The increase in absorbance due to formation of purpurogallin was recorded at 420 nm.

Table 4: Preparation of different aliquots of reaction mixture

Reagents	**Quantity**	**Exp.**	**Blank**
Double distilled water	2.1 ml	√	√
Potassium Phosphate Buffer	0.32 ml	√	√
H_2O_2	0.32 ml	√	√
Pyrogallol	0.16 ml	√	√
Enzyme extract	0.5 ml	√	X

Calculation:

$$\text{Activity}\left(\frac{\text{unit}}{\text{ml}}\right) = \frac{(\Delta A5 - \Delta A0) * 3 * \text{Df}}{\text{extinction coefficient of H2O2} \times \text{ amount of enzyme in aliquots}}$$

ΔA5=Absorbance at 5 min.

ΔAo= Absorbance at 0 min.

Df=Dilution factor

4.5.4 Assessment of SOD Activity:

The method for determining SOD activity was devised by Beauchamp and Fridovich (1971).13 mM methionine, 0.025 mM nitroblue tetrazolium (NBT), 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.8), 50 mM sodium bicarbonate, and 0.5 mL of the enzyme extract were all present in 3.0 mL of the mixture. After adding 0.002 mM riboflavin, the reaction was initiated. The tubes were then shaken and placed beneath two 15-W fluorescent lights. At 30°C, illumination was turned on to start the reaction. After allowing the reaction to last for 15 minutes, it was stopped by turning off the lights and covering the tubes with black material. While the non-irradiated reaction mixture served as a control, the enzyme-free reaction medium produced the most colour. At 560 nm, absorbance was measured.The amount of enzyme generating a 50% inhibition of the photochemical reduction of NBT was used to define one unit of SOD activity.

Table 5: Preparation of different aliquots of reaction mixture

Reagents	Quantity	Exp.	Blank
Methionine	390 µl	√	√
NBT	225 µl	√	√
EDTA	300 µl	√	√
Riboflavin	6 µl	√	√
Enzyme extract	100 µl	√	X
Potassium Phosphate buffer	1979 µl	√	√

Calculation of Percentage Inhibition:

K'[SOD] equals one when the test reaction is 50% inhibited. SOD activity can be calculated directly from the V/V ratio using the equation if the SOD unit is redefined as the amount of enzyme for which K' [SOD] = unity..

$$\text{SOD}\,\frac{\text{units}}{\text{ml}} = \left[\left(\frac{\text{V}}{\text{v}}\right) - 1\right] * \text{Df}$$

v= absorbance at 5 min

V= absorbance at 15 min

Df= dilution factor

4.6 Determination of Non-Enzymatic Antioxidant:-

4.6.1 Total Phenol Estimation

About 0.1 g of plant material was crushed in 5 ml of methanol to obtain the enzyme extract. After centrifugation, the enzyme extract (supernatant) was used. The enzyme extract was added to a test tube along with Folins reagent in a 1:10 ratio and Na_2Co_3 and then D.W. The solutions were kept for 15 minutes, followed by measurements of absorbance at 765nm. The phenol content of the sample was calculated from the standard graph of Gallic acid.

4.6.2 Total Flavonoid Estimation

0.1gram plant sample was crushed in 5 ml of methanol to obtain the enzyme extract. After centrifugation, the enzyme extract (supernatant) was used. Now the addition of methanol and aluminium chloride is done in test tubes. After this, CH_3COOK and D.W. were added in an appropriate order, and the tubes were incubated at room temperature for 30 min, followed by recording absorbance at 415nm. The flavonoid content of the samples was calculated from the standard graph of Quercetin.

4.7 Statistical Analysis:-

Each treatment and experiment was carried out twice, and the findings were averaged over three different analyses. The mean and standard deviation (SD) of the results were presented.s

5. RESULTS

5.1 Morphological Analysis and Physiological Analysis:-

Both Analysis was done on 10th day and 21st day of plant germination.

Table 5.1: Effect of, D.W on the morphological parameters of *Vicia faba* evaluated on 10th day.

Mancozeb Concentration	Root Length (Cm)	Shoot Length (Cm)	Germination (%)	Survival (%)	Phytotoxicity (%)
CONTROL	2	2	83.3	83.3	0
10ppm	1	2	83.3	83.3	50
30ppm	1	2	83.3	66.6	50
50ppm	1	2	66.6	66.6	50
70ppm	1	2	66.6	66.6	50
90ppm	0.5	1	66.6	66.6	70
110ppm	0.5	1	66.6	33.3	70
130ppm	0.5	1	33.3	33.3	70

Table 5.2: Effect of, D,W on the morphological parameters of *Vicia faba* evaluated on 21th day.

Mancozeb Concentration	Root Length (Cm)	Shoot Length (Cm)	Germination (%)	Survival (%)	Phytotoxicity (%)
CONTROL	3	8	83.3	83.3	0
10ppm	2	3	83.3	83.3	50
30ppm	1	2	83.3	66.6	50
50ppm	1	2	83.3	66.6	70
70ppm	2	5	83.3	66.6	70
90ppm	1	3	66.6	83.35	70
110ppm	1	3	66.6	50	70
130ppm	1	2	50	66.6	70

Table no 5.3: Effect of, 25µl on the morphological parameters of *Vicia faba* evaluated on 10th day.

Mancozeb concentratons	**ROOT LENGTH(cm)**	**SHOOT LENGTH(cm)**	**GERMINATION PERCENTAGE**	**SURVIVAL PERCENTAGE**	**PHYTOTOXICITY PERCENTAGE**
CONTROL	2	3	83.3	83.3	0
10ppm	2	3	83.3	83.3	25
30ppm	2	3	83.3	66.6	50
50ppm	2	2	66.6	66.6	50
70ppm	1	1	66.66	66.6	75
90ppm	1	2	66.6	66.6	75
110ppm	1	1	66.6	33.3	75
130ppm	1	1	33.3	33.3	75

Table no 5.4: Effect of, 25 µl on the morphological parameters of *Vicia faba* evaluated on 21th day.

Mancozeb concentratons	**ROOT LENGTH(cm)**	**SHOOT LENGTH(cm)**	**GERMINATION PERCENTAGE**	**SURVIVAL PERCENTAGE**	**PHYTOTOXICITY PERCENTAGE**
CONTROL	2	8	83.3	83.3	0
10ppm	2	6	83.3	100	50
30ppm	1	5	83.3	83.3	50
50ppm	1.5	4	83.3	66.6	75
70ppm	1	5	66.6	66.6	75
90ppm	1	3	66.6	66.6	75
110ppm	1	3	50	66.6	50
130ppm	1	1	50	50	50

Table no 5.5: Effect of,50µl of jasmonic acid on the morphological parameters of *Vicia faba* evaluated on 10th day.

Mancozeb concentratons	**ROOT LENGTH(cm)**	**SHOOT LENGTH(cm)**	**GERMINATION PERCENTAGE**	**SURVIVAL PERCENTAGE**	**PHYTOTOXICITY PERCENTAGE**
CONTROL	2	3	66.6	66.6	0
10ppm	0.5	2	66.6	66.6	75
30ppm	0.5	2	83.3	83.3	50
50ppm	0.5	1	83.3	83.3	75
70ppm	0.5	2	83.3	50	50
90ppm	0.5	1	66.6	50	75
110ppm	0.5	1	50	50	75
130ppm	0.5	1	50	50	75

Table no 5.6: Effect of 50µl of jasmonic acid, on the morphological parameters of *Vicia faba* evaluated on 21th day.

Mancozeb Concentratons	**ROOT LENGTH(cm)**	**SHOOT LENGTH(cm)**	**GERMINATION PERCENTAGE**	**SURVIVAL PERCENTAGE**	**PHYTOTOXICITY PERCENTAGE**
CONTROL	2	5	83.3	83.3	0
10ppm	2	3	66.6	83.3	25
30ppm	2	2	66.6	83.3	75
50ppm	1	2	83.3	66.6	50
70ppm	1	3	66.6	66.6	50
90ppm	1	2	66.6	66.6	75
110ppm	1	1	50	66.6	50
130ppm	0.5	1	50	50	50

5.2 BIOCHEMICAL ANALYSIS

5.2.1 Analysis results of chlorophyll A content in the sample

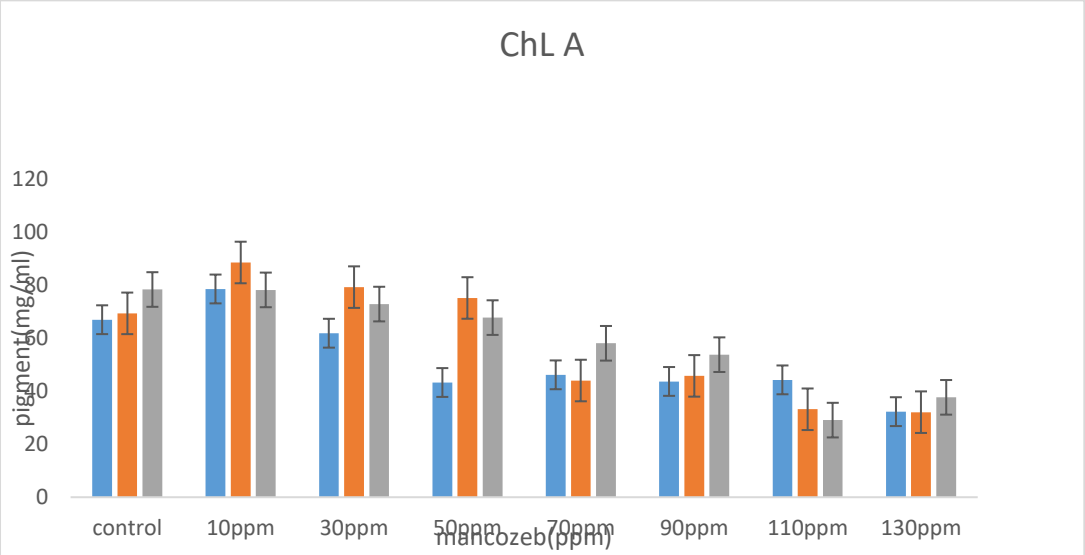

Figure 5: Effect of different concentrations of mancoeb, 25µl and 50µl JA on chlorophyll A content of *Vicia faba.*

It was observed that Chlorophyll A content was continuously decreasing with increasing concentration of Mancozeb. Priming with JA enhaced the chlorophyll content significantly till the 70 ppm of mancozeb dose. At higher doses its effect is not found significant. The maximum chlorophyll A content was observed in 25 µl JA primed seeds in control set. It can also be concluded that 25 µl of JA is more effective in retaining the chlorophyll.

5.2.2 Analysis results of chlorophyll B content in the sample

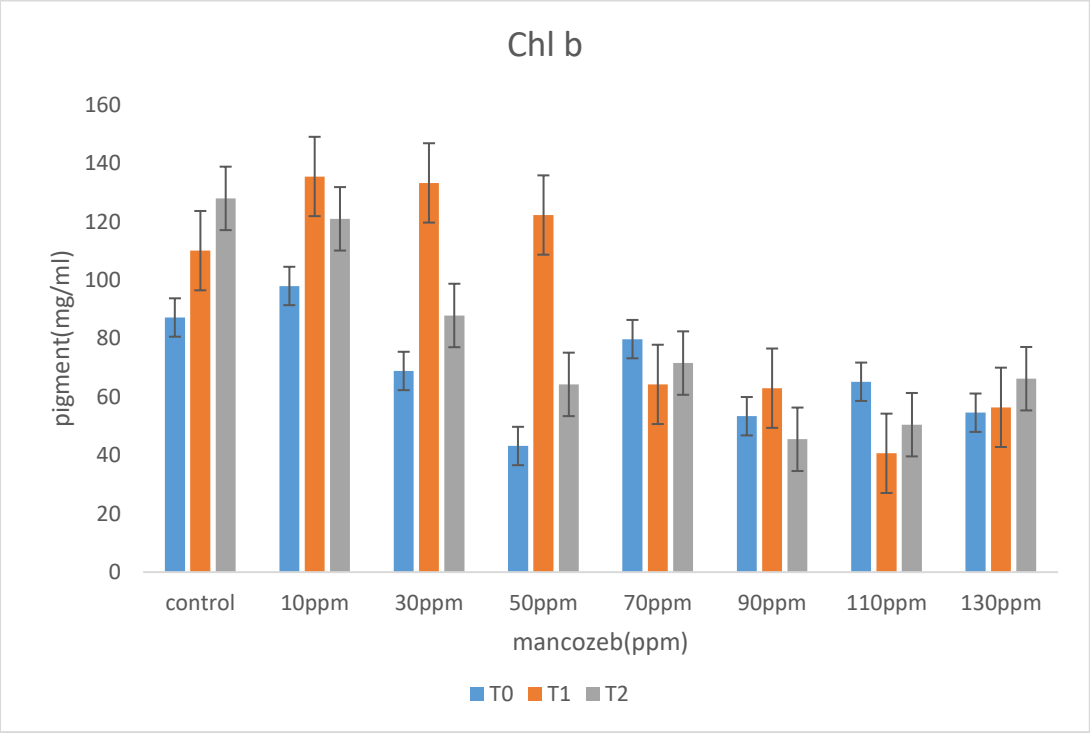

Figure 6: Effect of different concentrations of mancozeb, 25 µl and 50 ul JA on Chi B content of *Vicia faba.*

Similarly Chlorophyll B content was also decreasing with increasing concentration of Mancozeb. As well as JA priming enhanced the chlorophyll content significantly even at higher concentration of mancozeb. The maximum chlorophyll B content was observed in 50 µl JA primed seeds in control set. It can also be concluded that 50 µl of JA is more effective in retaining the chlorophyll B content.

5.2.3 Analysis results of carotenoid content in the sample

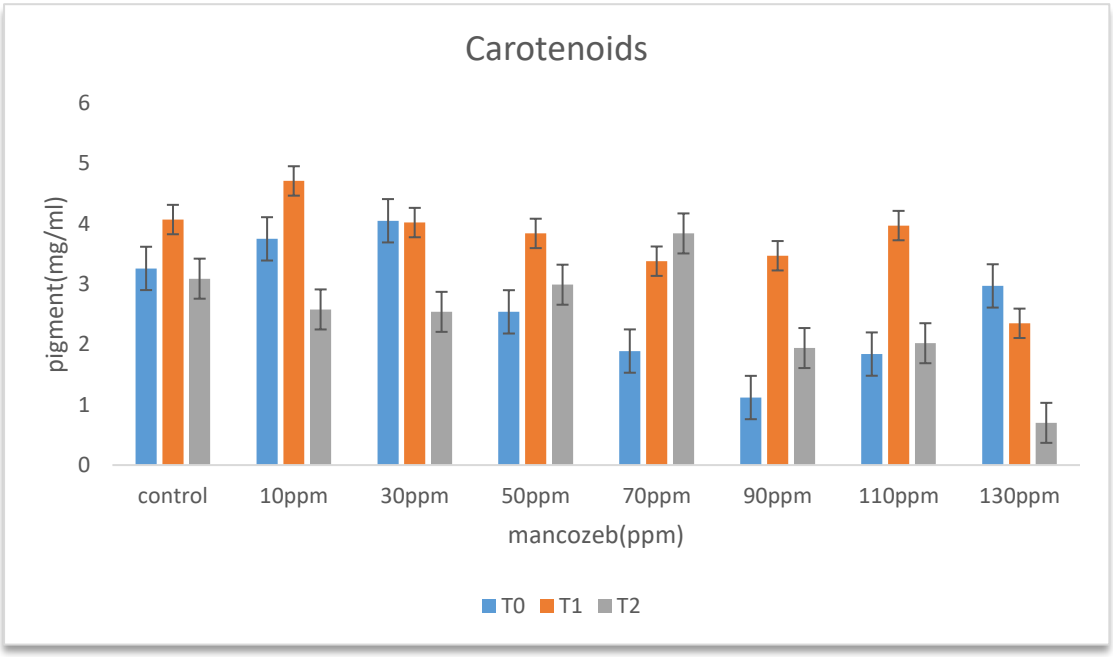

Figure 7: Effect of different concentrations of mancozeb, 25 µl and 50 ul JA on Carotenoid content of *Vicia faba.*

The carotenoid content also had a similar declining pattern with increasing doses of mancozeb, i.e.; an inverse relation relationship was observed with mancozeb concentration. The concentration of carotenoid reduced. The concentration of carotenoid was fund to be improved in JA primed sets. Although the effect of JA is not found so prominent as in case of chlorophyll content.

5.3 ANALYSIS RESULTS OF ENZYMATIC ANTI-OXIDANTS

5.3.1 CATALASE

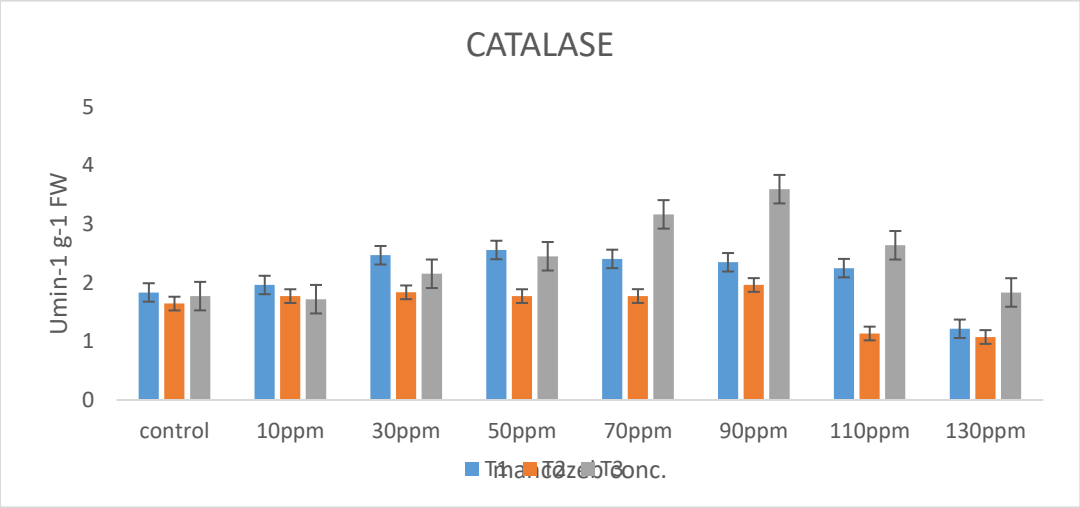

Figure 8: Effect of different concentrations of mancozeb, 25 ul and 50 ul JA on Catalase enzymatic antioxidant analysis of *Vicia faba*.

The catalase activity was measured after 21 days of treatment. The lowest activity was observed in control and it increases with the increasing mancozeb concentration. It showed that plant trying to cope up with the stress generated by the mancozeb application. Priming seeds with jasmonic acid significantly improved the catalase activity. The increase in CAT activity was dose-dependent. The results exhibited that JA strengthen the resistance potential of *Vicia faba* in stressful conditions.

5.3.2PEROXIDASE

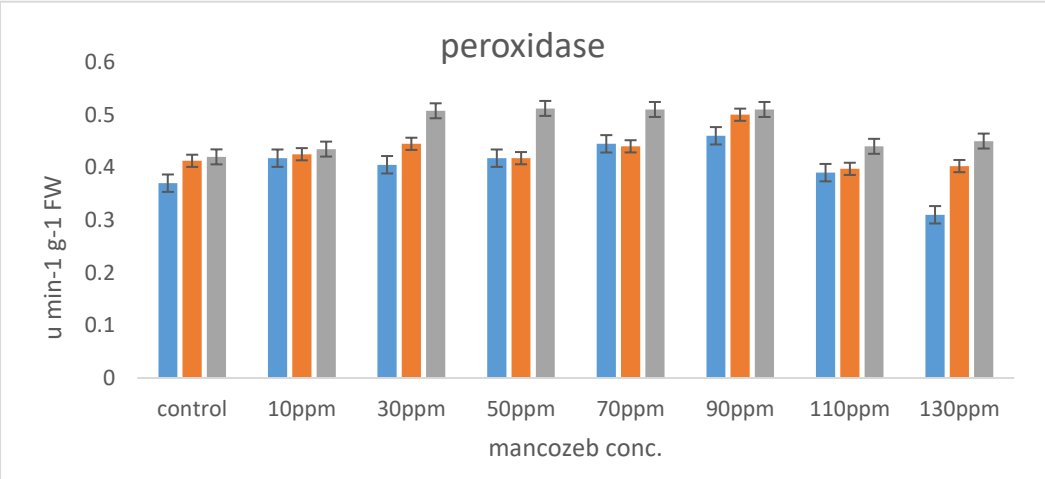

Figure 9: Effect of different concentrations of mancozeb, 25 ul and 50 ul JA on Peroxidase enzymatic antioxidant analysis of *Vicia faba*.

Similar observatioin was found in case of peroxidase activity. It was exibited that jasmonic acid enhanced the antioxidant potential 4 to 5 folds as compared to control sets.

5.3.3 SUPEROXIDE DISMUTASE

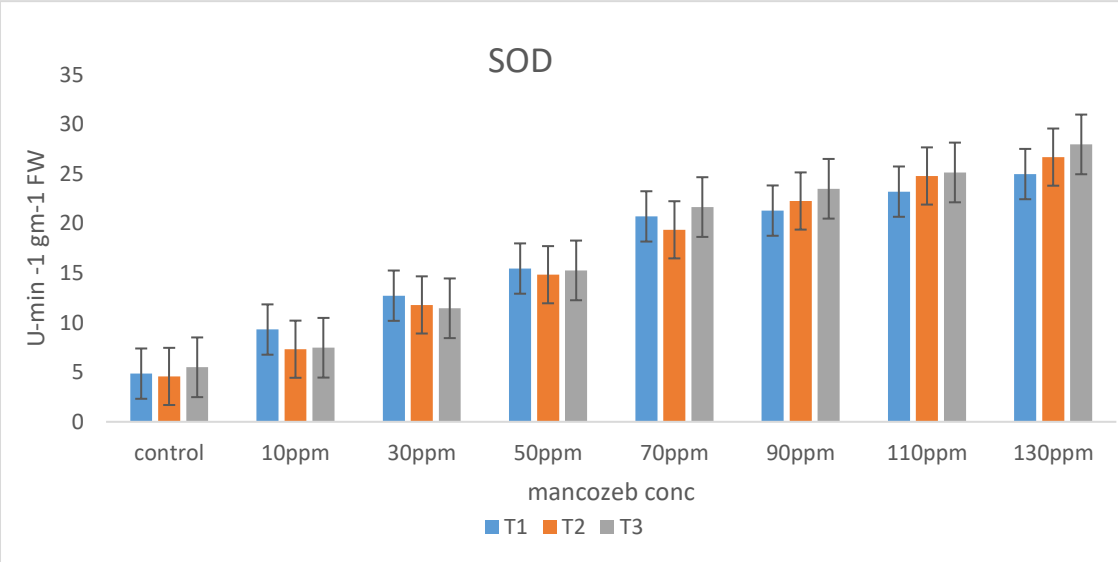

Figure 10: Effect of different concentrations of mancozeb, 25 ul and 50 ul JA on Superoxide dismutase enzymatic antioxidant analysis of *Vicia faba*.

Similar observatioin was found in case of peroxidase activity. It was exibited that jasmonic acid enhanced the antioxidant potential 1 to 3 folds as compared to control sets.

5.4 ANALYSIS RESULTS OF NON ENZYMETIC ANTIOXIDANTS

5.4.1 TOTAL PHENOL

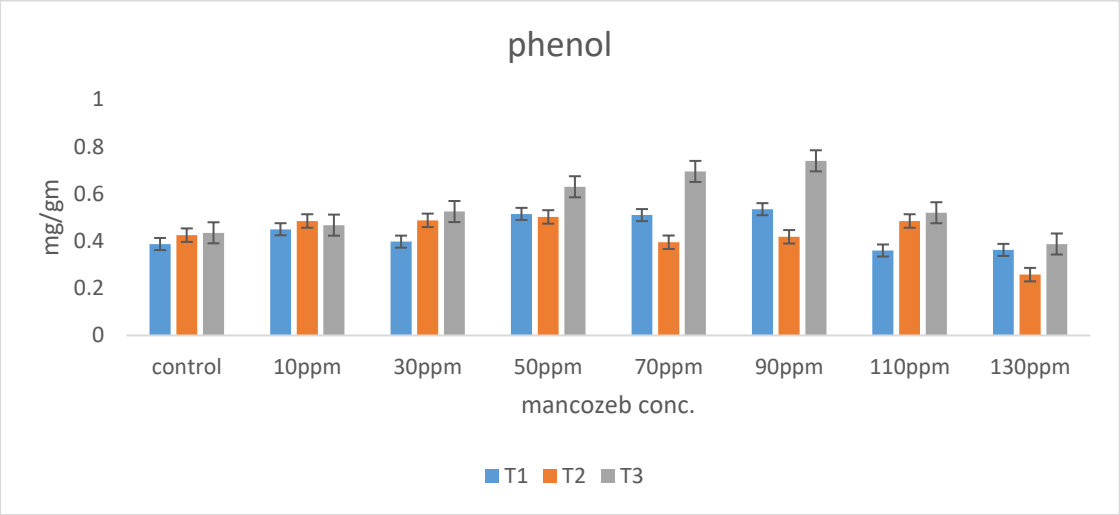

Figure 11: Effect of different concentrations of mancozeb, 25 µl and 50 µl JA on Total Phenol Non-enzymatic antioxidant analysis of *Vicia faba*.

The Total Phenol was measured after 21 days of treatment. The concentration of phenol content also found to be increased with increasing stress. JA treatment was not found significant in changing the content of phenols I tested plants.

5.4.2 TOTAL FLAVONOID

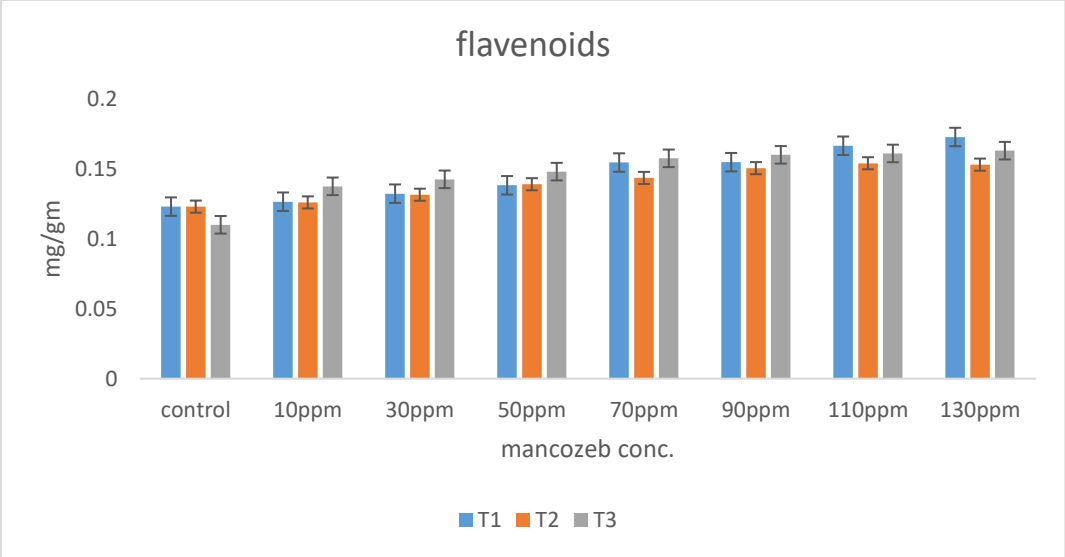

Figure 12: Effect of different concentrations of mancozeb, 25 µl and 50 µl JA on Total Flavonoid Non-enzymatic antioxidant analysis of *Vicia faba*.

The Total Flavonoid was measured after 21 days of treatment. The lowest activity was observed in control which was increased in dose dependent manner. JA further enhaced the flavonoid concentration. It was well documented that flavonoid also play an important role in combating various abiotic stresss.

6. CONCLUSION

Jasmonic acid, a phenolic growth regulator has a potential role in ameliorating the adverse effects of mancozeb-induced toxicity on *Vicia faba*. Extensive study has been done recently on the exogenous delivery of JA to plants, which has been shown to improve abiotic conditions such drought, cold, heavy metal toxicity, heat, and osmotic stress. As a result, JA looks to be a "promising therapeutic drug" for plants. For the study different parameters like morphology, physiology, tolerance index, phytotoxicity, pigment concentration, was taken in consideration. The toxic effects on crop plants increased significantly with the concentration of mancozeb. It influenced improving the seed germination, survival percentage, root length, shoot length, tolerance index, phytotoxicity of root and shoot, pigment content and anti-oxidant activities.

From the present research, it can also be concluded that exogenous application of jasmonic acid was able to ameliorate the toxic effect exerted by mancozeb on the tested plant. Further, in this research it was found that priming of seeds with JA has significantly improved tolerance towards stress in test plants through strengthening the antioxidative defense system. Henceforth it can be recommended that JA has great agronomic potential to improve the stress tolerance ability of agriculturally important crops. Henceforth, in order to improve the stress tolerance ability of agriculturally important crop, JA has a great agronomic potential.

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