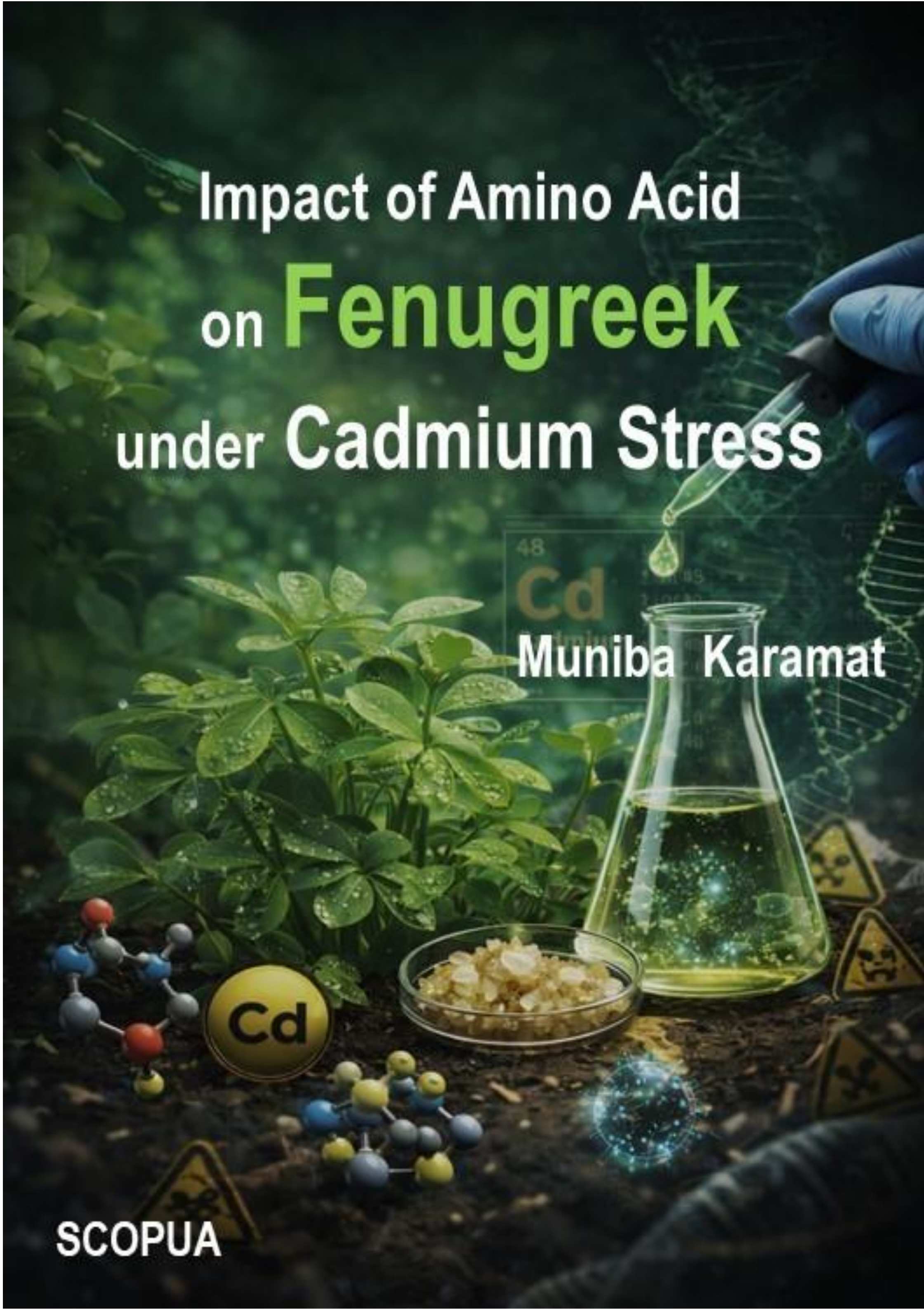

Impact of Amino Acid on Fenugreek under Cadmium Stress

Muniba Karamat

SCOPUA

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BY

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May ALLAH give me strength and opportunity to utilize my knowledge for the betterment of mankind (Ameen).

ABSTRACT

Fenugreek (Trigonella foenum-graecum) belongs to the Leguminosae family and is an annual plant. It is extensively grown in Egypt, India, and other Middle Eastern nations. Cadmium is a major source of heavy metal stress. Cadmium (Cd) contamination in the environment poses a significant threat to plant growth and development, leading to reduced crop productivity. Production of sorghum is reduced every year due to abiotic stresses as well as heavy metals. In this study, the potential of Amino acid (proline), a naturally occurring plant organic compound, was explored to mitigate the detrimental effects of Cd stress in fenugreek (Trigonella foenum-graecum L.). While the amino acid (proline) acts as a buffer which helps to maintain a favorable PH level within plant cells and promotes cell growth, it also regulates the function of gibberellin biosynthesis as well as affects the vegetative growth. Plants treated with AA (proline) exhibited improved growth parameters, including increased shoot and root length, enhanced biomass accumulation sand higher chlorophyll content compared to non-treated Cd-stressed plants. This study was conducted to investigate the effect of amino acid (proline) (0kg /ha,12kg/ha, 20kg/ha) on fenugreek grown under cadmium stress. Different levels of cadmium (0ppm,15ppm,30ppm) were applied. Fenugreek seeds were obtained from Ayub Agriculture Research Institute, Faisalabad. Seeds were sown in pots filled with soil in the research area of the Botanical Garden. PARS. Data for growth, yield (biological, economic, yield), and quality parameters (chlorophyll a, chlorophyll b, carotene content) were recorded and statistically analyzed by the ANOVA and Tukey's HSD Test

at 5% probability level. According to our results, the toxic effects of cadmium stress levels (15ppm and 30 ppm) decreased the morphophysiological and biochemical attributes of the fenugreek plant. However, the application of amino acid (Proline) at a level mitigated the cadmium stress and improved the growth of the fenugreek plant. While the amino acid (proline) acts as a buffer which helps to maintain a favorable PH level within plant cells and promotes cell growth, it also regulates the function of gibberellin biosynthesis as well as affects the vegetative growth.

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Chapter 1: Introduction

As an annual plant, fenugreek (*Trigonella foenum-graecum*) belongs to the Leguminosae family (Çamlýca and Yaldýz, 2019). Fenugreek is grown all over the world and it thrives in warm temperate and tropical conditions. It can withstand mild salt and is semi-arid. India, known as the "Land of Spices,"(Sharangi *et al.*, 2018). Fenugreek is a crop renowned for its nutritional, medicinal and culinary significance. Widely cultivated as a spice, it has gained popularity across continents, from Africa to Australia. Its distinctive yellow-to-amber seeds, used whole or ground, are often roasted to enhance flavor and reduce bitterness, making them a common ingredient in Indian cuisine.

Besides spicing up pickles and vegetable dishes, fenugreek seeds are increasingly utilized as a flavor enhancer in diverse culinary creations. Its aromatic fragrance and sweet taste are highly esteemed, enriching the dining experience (Olaiya and Soetan, 2014). Beyond its seeds, fenugreek's leaves, sprouts and microgreens are also valued as vegetables and feature prominently in Indian curries. The widespread adoption of fenugreek worldwide underscores its adaptability and the recognition of its myriad benefits, including its medicinal properties. As it continues to integrate into global cuisines, fenugreek's versatility and nutritional value are likely to be further appreciated, solidifying its status as a prized crop (Olaiya and Soetan, 2014). Salads are made with the sprouted seeds and microgreens, which add natural dietary fiber and other nutrients that the human body needs.

Fenugreek helps with digestion due to higher dietary fiber and it can change the texture of food. It appears from a literature search that there is not enough data on fenugreek's health advantages from a nutritional, biochemical and pharmacological standpoint. This article examines the nutritional and nutraceutical components of fenugreek, including its effects on digestion, modulatory activity on enzymes and metabolic syndrome and application in food processing, with the goal of promoting the health and wellbeing of both people and animals. Fenugreek nutrients and nutritional constituents. A special kind of useful food crop is fenugreek. In addition to being a rich supply of nutrients (both macro- and micronutrients), the chemical components of both seeds and leaves have made them valuable as food and medicine. Fenugreek seeds are frequently utilized as a seasoning in curry mixes and sauces. They are known to enhance appetite and alleviate conditions like anemia, weakness and various chest ailments (Olaiya and Soetan, 2014).

Widely employed in both culinary and medicinal applications, fenugreek seeds are generally considered safe for human and animal consumption, owing to their nutritional richness and low toxicity potential (FAO, 1998). With a notable protein content and nutritional value, fenugreek holds significant dietary importance. *T. foenum-graecum* has several medicinal effects, hypoglycemic, stimulant, laxative, stomachic, antibacterial, antioxidant and diuretic action (Olaiya and Soetan, 2014) . It has a good favorable effect for raising hemoglobin is present in the blood. Fenugreek has anti-diabetic, antifertility, anti-cancer, antibacterial, antiparasitic and hypocholesterolemia properties. In addition to having in addition to 22% protein, fenugreek seeds have a healthy dose of phosphorus, sulfur and lecithin which is made up of globulin, histidine and albumin. They also have 55% insoluble and soluble fiber, which is important for overall health (Feyzi *et al.*, 2015).

Oleic, linoleic and linolenic acids were the most abundant unsaturated fatty acids, accounting for 13.3%, 52% and 23.4% of total fatty acids, respectively. But fenugreek seed lipids contain the fatty acids that are most abundant. The contents of fenugreek seeds are high in fatty acids, fiber, protein and saponins. These studies indicate that research on the oil properties and nutrients, specifically amino acids, of *T. foenum-graecum* seeds is still scarce. Finding out about the chemical characteristics, sugar levels, amino acids and fatty acid composition was the current study's goal. combinations of oils and seeds from fenugreek (*T. foenum-graecum* L). Gujarat and Rajasthan combined account for nearly 80% of the nation's total production of seed spices, which is a group that includes annuals whose seeds are utilized as seasonings. Fenugreek is an essential third-highest seed spice in Indian agriculture, behind cumin and coriander. It has been a part of Indian cuisine for more than 3000 years.

Fenugreek seeds, widely used in herbal medicine for their antidiabetic effects in India and Iran, are also valued for their high protein content, ranging from about 250.00 to 386.00 grams per kilogram. Among the main protein components of these seeds are albumins (433.00 g/kg), globulins (273.00 g/kg), glutelin's (175.00 g/kg) and prolamin's (72.00 g/kg). Research has shown that a combination of 53 to 220 grams of fenugreek flour for every kilogram of wheat flour increases the protein content and improves the sensory and rheological properties of a wide range of foods, including bread, biscuits, noodles, macaroni and macaroni (Altuntaş *et al.*, 2005).

Protein digestibility, crude fiber and lysine levels are all improved when germinated fenugreek seed flour is used in place of wheat flour at a ratio of 140 grams per kilogram. Nevertheless, some have voiced worries about the possible effects of fenugreek seed flour on the volume and consistency of bread (Altuntaş *et al.*, 2005) . To address these issues, it is necessary to do more research on the nutritional and functional qualities of fenugreek protein isolate. This will help us understand how to use fenugreek seed flour in culinary recipes. Because of their high vitamin and mineral content, fenugreek leaves are eaten raw as a vegetable. *Trigonella* is a genus named for the triangular shape of its white-yellowish blooms, while "Greek hay" refers to the species' name (Riaz *et al.*). Fenugreek contains N-chemicals, volatile components, amino acids, and polyphenolic compounds, indicating its therapeutic usefulness. *T. foenum-graecum* has several medicinal effects, including hypoglycemia, function as a diuretic, laxative, stimulant, carminative, antibiotic and stomachic agent It has a good favorable impact for raising hemoglobin is found in blood. Fenugreek seeds are high in protein, lysine, soluble and insoluble fiber as well as calcium, iron and beta-carotene, making them an excellent cereal addition (Hooda and Jood, 2005).

The presence of saponins makes the seeds bitter, rendering them unfit for culinary use. Fenugreek can be used to make biscuits or as a cereal additive (PHYTOCHEMICALS, 2012). Cadmium (Cd) is a hazardous substance for crop plants, entering the environment through various human activities like electroplating, industrial waste and battery production(Younis *et al.*, 2015). It contaminates agricultural lands mainly through sewage irrigation water and sludge. When taken up by plant roots, Cd accumulates in above-ground parts, causing metabolic, physiological and growth problems. Cd toxicity disrupts photosynthesis, damages membranes and triggers oxidative damage by generating free radicals and lipid peroxidation. Overall, Cd contamination poses a significant threat to agriculture and ecosystems, emphasizing the need for preventive measures. Black carbon, or biochar, is a byproduct of biomass pyrolysis that may reduce the accumulation of heavy metals like Cd in plants. Biochar helps stabilize soil, which in turn assists in heavy metal entrapment. Biochar, sourced from different materials, enhances soil characteristics (like texture, structure, PH) and fertility levels and also helps in the management of organic waste and the capture of carbon, thus decreasing greenhouse gas emissions (Younis *et al.*, 2015). Soil heavy metals may be efficiently bound by biochar's organic functional groups and high surface area indicated reduced copper uptake by Chenopodium quinoa after applying biochar at a 4% rate, suggesting that copper was adsorbed onto biochar surfaces. In regions like Pakistan, where vegetables are often cultivated using sewage water, posing a risk of heavy metal accumulation there is an urgent need for methods to reduce metal availability to crops, especially vegetables. This study aimed to explore the effects of Mercury and biochar on the metabolic pathways of *Trigonella corniculata*, the plant known as fenugreek. The working hypothesis was that biochar might reduce the availability of Cd to *T. corniculata*, thereby reducing Cd stress. Since cotton sticks were cheap and readily available in rural regions, they were used to make biochar. The study looked at how biochar affected the development of fenugreek seedlings, levels of chlorophyll and MDA (malondialdehyde), glucose and protein concentrations, amino acid composition and ion and Cd accumulation in soil that had been polluted with Cd (Younis *et al.*, 2015). The investigated objective to assess how the application of biochar (BC) affects spinach plants grown in soil containing cadmium (Cd). Different levels of BC (0%, 3% and 5%) and Cd concentrations (ranging from 10 to 200 mg Cd per kg of soil) were tested. The findings revealed that Cd exposure negatively impacted spinach growth, photosynthesis and biochemical composition. However, BC application mitigated these effects by improving development, chlorophyll and reducing Cd intake of stress. The study suggests that BC could be a valuable tool for alleviating Cd harmful in spinach sowing of plant into contaminated soil (Younis *et al.*, 2016).

Cadmium (Cd), mercury (Hg) and lead (Pb) are heavy metals that pose a serious threat to both humans and other organisms, as they can mimic essential minerals and exploit existing uptake mechanisms to penetrate cells. For instance, cadmium exposure can result in serious health issues such as emphysema and osteoporosis in humans. Similarly, plants, unable to move away from contaminated areas, suffer reduced growth, leaf discoloration, inhibited root development and even death when exposed to excessive heavy metals. Thus, plants must evolve mechanisms with heavy metal stress, underscoring the importance of understanding these adaptations for ecosystem health and management (Lin and Aarts, 2012). Cadmium, in its elemental form, displays characteristics of being pliable and possessing a shiny silvery-white or bluish-white appearance. It's relatively scarce in nature and isn't typically found in its pure state. Its presence in the Earth's crust ranges from 0.2 to

0.6 mg per kg. Naturally, cadmium is primarily found alongside zinc, lead and copper in sulfide ores.

The process of refining cadmium began with Stromeyer in 1817, who isolated it from an ore primarily composed of zinc carbonate (Nordberg *et al.*, 2018). Cadmium finds utility in various applications such as plating steel, stabilizing plastics, in addition to its use as a semiconductor fabrication component and electrode material in nickel-cadmium batteries. However, its extraction, processing and industrial use have contributed to an increase in its presence in the ecosystem. Human activities pose the mostly risk to both environmental integrity and human health. Exposure to cadmium (Cd) can harm the reproductive systems of both men and women. It negatively affects male fertility by disrupting sperm production, semen quality and hormone levels. Similarly, in females, Cd exposure can disturb hormone balance and menstrual cycles. Even low concentration of Cd can cause the significant impacts in living life style reproduction and pregnancy outcomes. Maternal exposure to Cd during pregnancy may affect male and female offspring differently, potentially leading to more adverse effects in females (Kumar and Sharma, 2019).

It's crucial to take steps to reduce Cd exposure. This may involve implementing regulations to limit Cd emissions, raising awareness about sources of Cd exposure and adopting practices to minimize exposure in workplaces. Protecting reproductive health from harmful environmental toxins like Cd is vital for overall well-being and the health of future generations (Kumar and Sharma, 2019).

This review offers a thorough examination of how cadmium (Cd) toxicity affects plants from ecological, physiological and biochemical perspectives, particularly at context of soil science and plant nutrition. Cd stands out among heavy metals due to its potential harm to humans and its ability to move within the soil-plant system. The review covers various aspects of Cd's impact on plants, including growth, photosynthesis, enzymatic activities, water balance and more. It also explores how plants defend against Cd toxicity and discusses the role of oxidative stress in this process. By taking an interdisciplinary approach, the review contributes to understanding the ecological implications of Cd stress, which is valuable for both researchers and policymakers concerned with environmental health (Andresen and Küpper, 2013).

The passage delineates the ramifications of cadmium (Cd) build-up in plants, elucidating its diverse impacts on their physiological, biochemical and structural aspects, ultimately impeding their growth and productivity. It details how Cd accumulation hampers essential plant functions like stomatal operation, photosynthesis and antioxidant activity, consequently inducing oxidative stress. Furthermore, it highlights the interplay between Cd and mineral nutrients, underscoring their role in mitigating Cd-induced toxicity. The passage also underscores recent advancements in understanding how different nutrients interact to alleviate Cd stress in plants, emphasizing the necessity to optimize nutrient levels for effective Cd stress alleviation (Nazar *et al.*, 2012).

Calcium (Ca) serves as a vital macronutrient for plants, influencing essential physiological processes like growth, cell division and photosynthesis. Due to its similarity to cadmium (Cd), Ca can counteract Cd-induced changes in plant function. Recent research indicates that applying Ca externally can shield plants from Cd stress by modifying signal transmission, lowering oxidative damage, improving photosynthesis, relieving growth inhibition and controlling metal intake. This suggests that plants with higher Ca levels demonstrate improved resilience against Cd toxicity, highlighting the significance of understanding how nutrients interact with harmful substances in plant systems (Huang *et al.*, 2017).

Cadmium (Cd) toxicity profoundly affects the physiological processes and physical characteristics of plants, including both their vegetative and reproductive structures. Cd, a harmful pollutant, disrupts essential functions vital for plant health and development. Even at concentrations as low as 5–10 mg Cd per gram of leaf dry weight, plants can experience severe consequences, including death. This underscores the potent toxicity of Cd and its ability to interfere with crucial plant processes. In rice, for instance, Cd is transported from the soil to grains during grain-filling stages via specialized transfer nodes within the vascular system. Proteins such as OsHMA2 and OsLCT1 play critical roles in facilitating this transport across vascular bundles. Studies have shown that altering or inhibiting the function of these genes can significantly reduce Cd accumulation in grains, demonstrating the importance of these transport mechanisms in Cd toxicity. Similarly, in wheat, genes like TaHMA2 are involved in Cd transfer to leaves and grains. Strategies such as the application of zinc (Zn), either to the soil or directly onto leaves, have shown promise in decreasing Cd concentrations in grains, potentially mitigating its toxic effects. Moreover, the use of rare earth elements like lanthanum as microelement fertilizers has been found to enhance crop yield while simultaneously reducing Cd concentration into plant tissues (Haider *et al.*, 2021).

This suggests that agricultural practices could have a critical ability in mitigating Cd toxicity in plants. Understanding the mechanisms of Cd transport, gene regulation and the effectiveness of mitigation strategies is essential for developing approaches to alleviate Cd toxicity in crops, thereby ensuring food safety and security. All three parts of the AOAC Official Method 980.30 E (AOAC International) were used to determine the amino acid content. Acid hydrolysis, performic acid oxidation and alkaline hydrolysis were the three primary phases in the procedure that broke down the material for examination. To perform acid hydrolysis, the sample was subjected to a vacuum treatment with 5 M HCl at 100 °C for 24 hours. The resulting solution was filtered and rinsed multiple times. For performic acid oxidation, a portion of the sample was treated with cold performic acid overnight, followed by further chemical treatment and drying. Alkaline hydrolysis involved mixing the sample with NaOH and other compounds before subjecting it to high temperature for 22 hours. After hydrolysis, the concentration of different amino acids was determined by analyzing the samples using an amino acid analyzer. This comprehensive method ensures accurate assessment of the amino acid profile in the sample (Aljuhaimi *et al.*, 2018).

The study investigated the oils, fatty acid and sterol content of fenugreek seeds collected from three separate regions. The oil content of the seeds was found to be between 6.01% and 7.82% after solvent extraction. Unsaturated fatty acids, including linoleic acid (35.10–36.19 percent by weight), made up the bulk of the fenugreek seed oils, according to gas chromatography research. The oils included a total of 7,681.54 to 8,591.70 ppm of sterols, with β-sitosterol making up between 55.94% and 65.24% of those sterols (Kıralan *et al.*, 2017).

This research investigated how Iron sulfate ($FeSO_4$), ethylenediaminetetraacetic acid (EDTA), and indole-3-acetic acid (IAA) were used to test how fenugreek plants affected by elevated lead (Pb) levels in their phytochemical processes. The findings indicated that adding EDTA, IAA and $FeSO_4$ improved various germination aspects in fenugreek. Specifically, IAA treatment notably enhanced radicle length and amylase activity treated with $FeSO_4$ and EDTA. Overall growth characteristics were significantly improved by $FeSO_4$. Furthermore, levels of hydrogen peroxide (H2O2) and malondialdehyde (MDA) were lowered by all additions, with IAA showing the most improvement, suggesting that oxidative stress was reduced. In addition, when it came to raising levels of catalase, glutathione (GSH), ascorbate peroxidase (APX), flavonoids and phenols, IAA was more effective than EDTA and $FeSO_4$. Interestingly, EDTA led to a more pronounced increase in superoxide dismutase (SOD) production in Pb-stressed fenugreek compared to other chelators (Mnafgui *et al.*).

High-performance liquid chromatography the primary phenol generated with all chelator treatments, particularly with IAA, according to HPLC analysis, was gallic acid. Only by using exogenous IAA could syringic acid be generated; in contrast, EDTA was the only method for detecting quercetin. In general, the findings indicate that when it comes to reducing Pb stress in fenugreek, IAA outperforms EDTA and FeSO4 by activating antioxidant mechanisms, which in turn lower ROS and enhance the formation of particular phenols. The effects of a combination of amino acids and algal extract on fenugreek plants grown in different soil types were studied in this experiment. Results revealed that spraying the amino acids mixture notably boosted plant growth, particularly at a concentration of 1.25 g/L and enhanced nitrogen levels, essential oil content and yield. Similarly, applying algae extract led to significant growth improvements such as increased plant height, leaf and branch numbers and fresh and dry weights, especially with a concentration of 5 g/L. This treatment also elevated nitrogen, phosphorus and potassium levels in the plants. Notably, these effects were consistent across both clay and sandy soils. In essence, both treatments showed promise in enhancing fenugreek growth and nutrient content, each with its own optimal concentration (Tarraf *et al.*, 2015).

Fenugreek seeds had high concentrations of glutamic and aspartic acids before they germinated, with leucine, arginine, lysine and proline close behind. But once the seeds germinated, certain amino acids like valine, phenylalanine, tyrosine, threonine, tryptophan and aspartic acid were more abundant, while others like proline and glutamic acid were thinner (El-Mahdy and El-Sebaiy, 1985). With the exception of glycine, which saw a drop, all free amino acids rose. Importantly, upon germination, cystine, tryptophan and proline were discovered in their liberated forms. After germination, the chemical score, which is derived from the essential amino acid pattern of whole egg protein, dropped 21%, suggesting that the quality of the protein had declined. The C-PER went down, indicating a decline in protein quality after germination, even though in vitro digestion with pancreatin and pepsin was somewhat improved for germinated seeds. The purpose of this three-day experiment was to examine the effects of salt stress on the nutritional analysis and amino acid content of two different types of germinated fenugreek seeds, namely Giza 2 and Giza 30. Both tap water and a sodium chloride solution (1,000 ppm) were used to germinate the fenugreek seeds; however, the germination process was hindered by greater salt concentrations. Germinating the Giza 2 and Giza 20 cultivars in plain water or brine resulted in higher crude protein levels. Carbohydrates (40.3%), fiber (7.10%) and ash (8.60%) were more abundant in Giza 2 seeds, but crude protein (32.60%), fat (8.20%) and energy (313.13 kcal/g) were greatest in Giza 20 sprouts. Amino acid profiles showed that fenugreek sprouts were rich in aspartic acid and proline and low in cysteine and methionine, two types of amino acids that contain sulfur. All amino acid levels were lower in the Giza 2 and Giza 20 genotypes when germinated in salt water as opposed to tap water (Elgebaly *et al.*, 2022).

This method involves using agricultural techniques to boost plant growth and yield efficiency. By applying small amounts of plant growth regulators like GA3, essential processes such as photosynthesis, flowering and root development can be enhanced. GA3 functions by counteracting proteins like DELLA, which can hinder cell expansion, thus fostering healthier plant growth. Furthermore, the process of spraying amino acids, which are essential for protein synthesis, hormone production and other metabolic processes, can also improve plant vigor. In summary, these practices aim to maximize nutrient uptake and stimulate plant growth, ultimately increasing agricultural productivity. Hence, amino acids are crucial as they directly or indirectly influence plant physiological functions. They play a role in energy storage, enzyme synthesis and the production of crucial organic molecules for protoplasm development. Among the twenty-one amino acids included in proteins, arginine has the highest nitrogen-to-carbon ratio. Arginine serves as a reservoir of organic nitrogen, crucial for plant growth as it's essential for building nucleic acids and proteins. It plays a pivotal role in promoting vegetative growth by stimulating physiological processes that enhance cell development and carbohydrate production, thus fostering overall plant growth. Moreover, arginine contributes to reducing ABA levels while boosting GA3 and IAA synthesis, leading to increased cell division. Previous research has demonstrated significant enhancements in plant height, branch number and dry weight with leaf-applied arginine. This investigation objective to explore the impact of GA3, arginine and their interaction on various growth indicators of fenugreek plants (Naser and Mheidi, 2021).

Objectives

- To observed the effect of cadmium on fenugreek growth, yield and productivity.
- To observed the impact of amino acid on fenugreek under cadmium stress and their economic benefit.

Chapter 2: Review of Literature

Zayneb *et al.* (2015) research investigated that the fenugreek responds to varying levels of cadmium (Cd) exposure. While Cd did not significantly affect germination except at high concentrations, it hindered early seedling growth even at lower levels. Enzyme activity, particularly amylase, decreased with Cd treatment, indicating disruption in vital metabolic processes. Cd accumulation was higher in roots, leading to reduced root biomass. Chlorophyll content decreased, causing chlorosis at certain Cd concentrations. Elevated Cd levels induced oxidative stress, evidenced by increased hydrogen peroxide and malondialdehyde levels. Fenugreek responded by boosting antioxidant enzyme activity and accumulating phenolics and flavonoids, potentially aiding in scavenging reactive oxygen species. Despite Cd's adverse effects, fenugreek's ability to maintain oxidative balance suggests its potential for Cd hyperaccumulation and phytoremediation in heavily contaminated soils.

Younis *et al.* (2015) study investigated that the impact of different levels of CSB application on photosynthetic pigments, in combination with varying amounts of cadmium in soil. Chlorophyll (a, b, total), carotenoids and anthocyanin levels increased with a 5% increase in CSB application compared to a 0% increase in lycopene levels. Chlorophyll b, total chlorophyll, carotenoids and anthocyanin exhibited comparable trends at 40 and 80 mg Cd/kg soil with 5% CSB, however chlorophyll a and lycopene levels were noticeably higher at 100 mg Cd/kg soil. Protein and sugar concentrations declined significantly with varying levels of Cd; a trend also evident when Cd levels were combined with different biochar percentages. Protein levels increased with higher CSB application rates, particularly rising from 3% to 5%. However, protein levels decreased with increasing Cd concentrations, especially noticeable in treatments combining CSB and Cd. Similarly, soluble amino acid levels decreased under Cd stress, albeit to a lesser extent in soil rejuvenated using biochar. In terms of plant Cd content, *T. corniculata* showed a significant decrease in Cd levels after being treated with 5% CSB.

Naeem *et al.* (2020) study investigated that in the modern industrial period, soil pollution by heavy metals, one of the most pressing environmental concerns, in particular cadmium (Cd). Depending on its characteristics and application rates, biochar can be a great ameliorating agent. The pot size research examined the physiological impacts, grain production, Cd buildup, and tolerance of quinoa with possible health concerns of both untreated (WSB) and acid-treated (AWSB) wheat straw biochar. We treated the soil with different amounts of Cd (0, 20, 55 and 70 mg kg^{-1}), AWSB, and WSB (3% and 4% (w/w)). Increased lipid peroxidation was one indicator of oxidative stress brought on by Cd accumulation in control plant tissues. However, the application of biochar reduced the oxidative damage, as seen by the decreased levels of TBARS and H_2O_2 generation.

Izadi *et al.* (2021) studied, it was founded that heavy metal stress significantly decreased dry weight, RWC, chlorophyll, total soluble carbohydrate, protein and proline contents. However, the effects of heavy metal stress were mitigated by inoculation with fungus spores and mycelium, which affected proline, total soluble carbohydrate and protein. Membrane instability is brought on by heavy metal stress and rising electrolyte leakage, while lipid peroxidation was reduced by *P. indica* inoculation, particularly with the spore. One major way to combat the damaging effects of heavy metals is by using biotic techniques. These results lend credence to the idea that P. indica, being an endophytic fungus that can be cultivated axenically on many types of medium, might be useful in cleaning up soil that has been polluted with heavy metals.

Javad *et al.* (2022) study examined that due to cadmium (Cd) toxicity, the research found that crops grown in metal-polluted regions had slower growth and yield. H_2S was tested for its potential to mitigate the harmful effects of copper (Cd) on fenugreek seedlings (*Trigonellafoenum-graecum* L.). Various concentrations of $CdCl_2$ (Cd1—1 mM, Cd_2—1.4 mM and Cd_3—2 mM) and H_2S (HS1—80 µM, HS_2—140 µM and HS_3—100 µM) were tested in the research. To prepare the fenugreek seeds, sodium hydrosulfite (NIHs) was used as a hydrogen sulfite (H_2S) donor. The seedlings were harvested after two weeks of growth in medium that had been treated with H_2S and spiked with Cd. The growth of fenugreek seedlings was impaired when exposed to cadmium. Relative water content of leaves (LRWC), intercellular CO2 concentration, net photosynthesis, stomatal conductance and transpiration were all negatively impacted by toxicological levels of Cd. However, by increasing the activity of antioxidant enzymes such as APX, CAT and SOD, the application of H2S greatly enhanced the morphological characteristics of seedlings.

Javad *et al.* (2022) research investigated that the ability of hydrogen sulphide (H_2S) to counteract the harmful effects of cadmium (Cd) toxicity in fenugreek seedlings. Various concentrations of $CdCl_2$ and H_2S were tested, with fenugreek seeds treated with sodium hydrosulphide (NIHs) to provide H_2S. The findings showed that Cd stress hindered seedling growth and physiological functions, while H_2S treatment notably improved morphological attributes and boosted the antioxidant performance. H_2S also regulated the levels of phenolic and flavonoid compounds. The study highlighted the role of H_2S-induced spermidine and putrescine synthesis in enhancing seedling performance under Cd stress and demonstrated a reduction in oxidative stress markers with H_2S treatment. It suggests further exploration of H_2S's potential in mitigating heavy metal toxicity in different crop plants.

Gladysh *et al.* (2023) study investigated that increasing cadmium concentration affects the phosphorus levels in fenugreek seeds and plant development. Root and shoot length and fresh mass were reported to decrease significantly as cadmium levels increased. Furthermore, when cadmium levels rose, the majority of the shoot's phosphorus fractions declined; nevertheless, lipid and nucleic acid phosphorus increased at certain phases. During the vegetative stage, protein phosphorus levels only rose. Lipid phosphorus rose in post-flowering seed samples, with the exception of those with low cadmium concentrations, while protein phosphorus decreased. These results indicate that cadmium negatively impacts fenugreek growth and alters phosphorus content, potentially affecting seed quality and stress resilience.

Lindi *et al.* (2024) study investigation to explored that the utilization of a novel bio-nanocomposite, FSM/Sic, for efficiently removing cadmium ions from water. The fenugreek mucilage extraction process was optimized using ultrasound, with ideal conditions identified. Characterization techniques confirmed the composite's suitability for adsorption. Through systematic adjustments of various factors, optimal conditions for maximum adsorption were determined. Kinetic and thermodynamic analyses revealed the adsorption process's nature, while the composite showed excellent regeneration capabilities. The study highlights the potential of FSM/Sic to address cadmium pollution, according to its impressive maximum adsorption capacity of 40.6 mg/g.

FAIZY *et al.* (2022) research carried out At the Kurdistan region, at the University of Duhok's College of Agricultural Engineering Science, aimed to explore how different concentrations of cadmium (Cd) and nickel (Ni) fertilizers affect (*Trigonellafoenum-graecum* L.) seed germination, photosynthetic pigments and vegetative growth. The findings indicated that interactions between certain levels of nickel and cadmium significantly impacted plant height and root weight. Additionally, specific concentrations of these heavy metals, with and without combination, influenced the dry mass and fresh mass of the plant's vegetative parts. Seed germination was notably affected by the interaction between a moderate level of cadmium and the absence of nickel. Moreover, the concentrations of various photosynthetic pigments were found to be influenced by both cadmium and nickel, individually and in combination.

Younis *et al.* (2015) study investigated that the effects of cadmium (Cd) contamination on fenugreek soil-grown plants, in addition to the potential mitigating effects of cotton-sticks derived biochar (CSB). Results revealed that high Cd concentrations caused plants to exhibit markedly diminished physiological traits, photosynthetic pigments and growth. However, the presence of CSB alleviated these negative impacts. Furthermore, increasing levels of CSB application resulted in lower concentrations of Cd in both roots and shoots. Overall, higher CSB application rates led to increased processing of carbohydrates, proteins and amino acids, indicating a decrease in Cd toxicity. And thus, the use of cotton-sticks biochar as soil amendment not only provides benefits in plant growth but also helps in reducing Cd toxicity in crop plants.

Albasha and Azab (2014) study objective explored the effective fenugreek seeds, rosemary and cinnamon are in protecting against liver damage caused by cadmium exposure in guinea pigs. Forty-eight guinea pigs were divided into control and experimental groups, with some receiving cadmium alone and others receiving cadmium along with extracts of the mentioned plants. Liver tissues and blood samples were examined for structural and biochemical changes. Results revealed severe liver damage in cadmium-exposed animals, including abnormal cell structure and elevated liver enzymes. However, animals given the plant extracts alongside cadmium showed improved liver health. These findings suggest that fenugreek seeds, rosemary, and cinnamon may help alleviate cadmium-induced liver damage and could be beneficial for individuals at risk of cadmium exposure.

Albasha and Azab (2014) study investigated that the fenugreek plants are affected by exposure to cadmium (Cd) and lead (Pb). It notes that both Cd and Pb negatively influence fenugreek growth, causing a gradual reduction in shoot and root length as well as in certain photosynthetic functions. However, Cd has a more noticeable impact on fenugreek seedlings compared to Pb. The text also mentions a tolerance index indicating that the fenugreek variety being studied shows relative resilience to both Cd and Pb. Despite the stress from these heavy metals, fenugreek manages to maintain stable water levels. Notably, high concentrations of Cd significantly decrease various physiological processes in fenugreek, including CO2 assimilation and chlorophyll production. Under Cd stress, the plant's proline content notably increases, whereas Cd and Pb both occur and mitigate in magnesium and phosphorus concentration but an enhance in iron levels in fenugreek shoots. Additionally, fenugreek tends to accumulate Cd in its tissues, especially in parts that are harvested, while Pb is primarily stored in the roots.

Alle *et al.* (2019) study investigated that focused on exploring the soil contamination with cadmium and lead affects the development and morphological characteristics of field beans. It conducted experiments under controlled conditions, gradually increasing Cd and Pb levels in the substrate. Various analytical methods were employed to assess parameters like proline content and chlorophyll fluorescence. Results indicated that while Cd and Pb influenced bean growth and physiology, their effects were more evident towards the experiment's end. Cd exposure led to increased proline levels in leaves, indicating stress, while Pb treatments also triggered a stress response. Notably, Pb's negative impact on chlorophyll was less severe compared to Cd. Overall, the study suggests that field beans can withstand substantial Cd and Pb levels until flowering, provided optimal conditions are maintained, emphasizing the need for ongoing soil contamination monitoring.

Okutu Jackson and Enebrayi (2022) research conducted that the aimed to explore the potential protective benefits of combining the effects of garlic and chili pepper water extracts on female albino rats' livers and oxidative stress caused by cadmium. A total of twenty-five rats were used in the investigation one group served as a control, while the other four groups were treated with cadmium chloride. Different groups were given extracts of *Allium sativum*, *Justicia carnea*, or a mix of the two. After treatment, blood samples were obtained and analyzed for cadmium levels and various liver function and oxidative stress markers. The finding showed that the combined extracts exhibited a protective impact, improving liver function parameters and reducing markers of oxidative stress compared to cadmium-exposed rats without treatment. These findings suggest a potential therapeutic use for the combined extracts in counteracting rats exposed to cadmium developed oxidative stress and liver damage. To completely comprehend their workings and their uses, further research is necessary.

Jiang *et al.* (2022) study investigated that the efficacy of biochar in mitigating cadmium (Cd) stress on two mint species, 'Chocolate' *Mentha piperita* with *Mentha spicata*. The effects of Cd damage were greatly reduced in both species after applying biochar at several rates (45 g Kg^{-1}, 85 g Kg^{-1} and 165 g Kg_{-1})., with 165 g Kg^{-1} showing the most promising results. Biochar treatment enhanced various growth parameters and physiological functions such as plant height, leaf dimensions, biomass, photosynthetic rate, while decreasing the levels of antioxidant enzymes and non-enzymatic antioxidants and chlorophyll content. Additionally, biochar effectively mitigates Cd uptake by plants and improved soil properties including increased availability of nitrogen, phosphorus and potassium as well as soil PH. These findings underscore biochar's potential as an effective solution to mitigate Cd contamination in mint species while enhancing plant health and soil quality.

Gill *et al.* (2011) study investigated that the different mustard cultivars respond to cadmium (Cd) toxicity in agricultural soils.

It involved a greenhouse experiment using pots filled with soil containing varying levels of CdCl2. As the Cd concentration increased all cultivars showed reduced growth, photosynthesis and yield, with the most significant effects seen at the highest Cd level. Among the cultivars, Alankar exhibited the highest tolerance to Cd stress, displaying greater plant mass, photosynthesis and lower Cd levels in leaves, indicating less damage. Conversely, RH30 was the most sensitive cultivar, experiencing the most severe damage. Alankar also had a higher tolerance index compared to others, suggesting better resilience to Cd toxicity. This was attributed to its superior coordination of antioxidants, which protected it from oxidative damage. In contrast, RH30 had lower antioxidant activity, leading to more damage. Overall, Alankar ranked highest in Cd tolerance among the cultivars tested, followed by Varuna, Pusa Bold, Sakha and RH30, respectively. These findings underscore the importance of selecting tolerant cultivars for Cd-contaminated soils and highlight Alankar as a promising option for such environments.

T Abbas *et al.* (2019) researched into the potential of fenugreek (*Trigonella foenum-graecum* L.) to protect Nile tilapia (*Oreochromis niloticus* L.) against cadmium (Cd) poisoning was the primary goal of this research. For 40 days, 450 fish were fed either raw or extracted fenugreek seeds while being exposed to Cd in one of ten groups. Results indicate that Cd exposure enhanced certain serum biochemical markers and oxidative stress indicators, while also affecting gene expression and tissue Cd levels. Fenugreek extract, particularly at 1% concentration, helped mitigate some Cd-induced effects, though it also altered certain biochemical parameters. Overall, fenugreek extract showed potential in reducing Cd toxicity in

Nile tilapia and decreasing Cd accumulation in fish tissues, with implications for public health.

Rizwan *et al.* (2018) research investigated that the lasting effect in rice straw biochar on rice growth and cadmium accumulation. Different levels of biochar were added to the soil, resulting in improved rice growth and decreased cadmium levels in the plants. The study found that biochar treatments not only enhanced plant growth and reduced cadmium uptake but also affected soil properties like pH and electrical conductivity, ultimately reducing cadmium bioavailability in the soil. However, the study suggests that further research is necessary to fully understand the long-term impacts of biochar from various sources on soil health and crop metal uptake in real-world agricultural settings.

ESPANANY *et al.* (2015) study, conducted at Shahrekord University's Agriculture College in Iran in 2014, studied the effect of various amounts of cadmium on the germination of fenugreek seeds treated with salicylic acid (SA), potassium nitrate (KNO_3) and potassium chloride (Kcl). Using a factorial experiment design with complete randomization and four replications, it explored four levels of seed priming (Kcl, KNO_3, SA) and four cadmium concentration levels (0, 20, 30, 40 mg/L). NaCl greatly reduced the proportion of seeds that germinated, the length of the roots and shoots and the dry weight of the seedlings. The effects of cadmium concentrations on shoot length were more pronounced than on root length. Nevertheless, as compared to the control treatment, seeds that were primed with SA (at 80 mg/L) demonstrated protection against cadmium stress, leading to improved germination percentage, root and shoot elongation and seedling dry weight. SA-primed seeds were particularly effective in counteracting the toxic impact of cadmium on germination parameters. In summary, the study suggests that employing SA seed priming can effectively enhance fenugreek crop growth in fields with high cadmium toxicity. It sounds like you're summarizing the findings of a study on the impact of nickel (Ni) accumulation in fenugreek plants grown in soil with varying levels of Ni contamination.

Parida *et al.* (2003) experiment involved using alkaline sandy loam soil with varying Ni values, from zero to three hundred milligrams per kilogram of soil. At first, fenugreek plants showed a little uptick in green and dry matter yields up to 30 mg Ni kg^{-1} soil. Nonetheless, yields dropped significantly when the Ni concentration surpassed 50 mg kg^{-1} soil. This indicates that low levels of Ni may have some beneficial effects on growth, but higher concentrations are detrimental. Plants exhibited toxicity symptoms such as interveinal chlorosis when the soil Ni concentration reached or exceeded 40 mg kg−1 soil. This indicates that fenugreek plants are sensitive to high levels of Ni contamination. As expected, as the amount of Ni added to the soil grew, the total Ni content in plant tissues also rose. Interestingly, Ni was deposited preferentially in the root system, since the root system had a much greater accumulation rate than the shoots. As the concentration of Ni given to plants grew, their iron (Fe) content also rose. However, the content of copper (Cu) and zinc (Zn) decreased as Ni concentration increased. This suggests that Ni accumulation in soil may affect the uptake or distribution of other essential micronutrients in fenugreek plants. Overall, the study highlights the value of observing the impact of heavy metal contamination such as Ni on plant growth, nutrient uptake and overall plant health. Such insights are crucial for assessing the potential risks associated with poisoning of heavy metal in agricultural soils and for developing strategies to mitigate its toxic impact on cultivar production.

Bhardwaj *et al.* (2009) research on (*Phaseolus vulgaris* L.) plants examined that the effect of various levels of lead (Pb) and cadmium (Cd) on several physiological and biochemical aspects. Here's a condensed version of the findings low metal levels didn't affect germination, but high Cd concentrations stopped it entirely. Growth decreased with rising metal concentrations. Pigment levels dropped with increasing metal concentrations. Reduced compared to control plants.

Bhardwaj *et al.* (2009) investigated that the effect of increasing cadmium (Cd^{2+}) concentration on the development and phosphorus amount of fenugreek plants and seeds. Here's a breakdown of the findings. Root length ranged from 11.63 to 27.72 cm. Shoot lengths varied from 9.70 and 54.78 cm. There was a significant decline in plant fresh mass, root and shoot length and Cd2+ content with increasing concentration. With the exception of lipid P and nucleic acid P, the concentration of all other phosphorus components in the shoot dropped as the Cd^{2+} concentration increased. By 60- and 90-days post-sowing, lipid P and nucleic acid P levels had risen, respectively. It was only during the vegetative stage when protein P levels rose. Seed Phosphorus Content. Except for 3.5 μg (Cd^{2+}) g^{-1} (soil), lipid P increased in seeds 40 days after blooming. But when the quantity of Cd^{2+} in the seeds increased, the protein P content declined. Decreases in root and shoot length as well as fresh mass are indicators that an increase in cadmium content has a deleterious effect on fenugreek plant growth. Additionally, the study indicates alterations in phosphorus content, with some fractions increasing (lipid P and nucleic acid P) while others decrease (protein P), depending on the stage on plant growth and the concentration of Cd^{2+}.

El Rasafi *et al.* (2022) investigated that the presentation of Cd in crops and its potential entry into the food chain pose serious health concerns. The analysis explores the toxic impacts of cadmium in cultivar and the mechanisms they employ to tolerate and resist its toxicity. Cadmium adversely affects various plant mechanism like that seed growing, growth and physiological functionality like photosynthesis and water regulation. The generation of reactive oxygen species, which cause DNA damage, is also set off by it. To combat the harmful effects of cadmium, plants have evolved. including excluding or storing cadmium in specific plant parts and activating signaling pathways for survival and growth under cadmium stress. Additionally, they produce hormones, activate antioxidant systems and synthesize compounds like phytochelatins and proline to enhance cadmium tolerance. Soil microorganisms also play a significant role in cadmium tolerance by reducing its availability and improving plant health. Understanding these mechanisms is crucial for devising strategies to mitigate cadmium contamination in agriculture, thus protecting both plant and human health.

Lindi *et al.* (2024) investigated that the utilization of new bio-nanocomposite, FSM/Sic, for removing cadmium ions from water. The process of extracting fenugreek mucilage is optimized by it and confirms the composite's adsorption properties through various tests. By adjusting several factors like pH and temperature, the study identifies the best conditions for maximum adsorption. It finds that adsorption follows a specific model and occurs in a single layer. The research also shows that the process is spontaneous and can be reversed effectively. FSM/Sic demonstrates significant potential for water purification, with a maximum capacity to adsorb cadmium ions at 41.6 mg/g.

Parida *et al.* (2003) studied found that the impacts of different concentration of nickel (Ni) in soil on fenugreek plants, focusing on growth and micronutrient distribution. It was found that as soil Ni levels increased, fenugreek accumulated more Ni in both roots and shoots. Initially, there was a slight growth improvement with low Ni levels, but higher concentrations led to significant growth decline due to Ni toxicity symptoms like interveinal chlorosis. Additionally, while iron (Fe) content increased with Ni application, copper (Cu) and zinc (Zn) decreased. These findings emphasize the importance of understanding heavy metal effects on crop growth and nutrient uptake for better agricultural management in contaminated areas.

Abd-Elmoniem and El-Tayeh (2017) study aimed to explored the various nutrients, including chemical, organic and bio-options, affect the growth, phosphorus levels and presence of heavy metals in Jew's mallow plants. Conducted in Egypt over two summers (2013 and 2014), both A variety of fertilizers were tested in both controlled laboratory settings and outdoor settings, either alone or in combination with organic additions such as filter mud cake (FMC) and phosphate dissolving bacteria (PDB). The results indicated that while subsequent cuts of plants generally showed better growth, only Reduced heavy metal levels were seen in the first cut treated with rock phosphate fertilizer, whether applied alone or in conjunction with PDB and FMC. Specifically, these treatments resulted in the lowest Zn levels below the permissible limit, while Ni levels were borderline acceptable. Cobalt levels were below the limit for all phosphate fertilizers and Cu levels remained within acceptable ranges. Moreover, using natural rock phosphate with PDB was recommended for cultivating Jew's mallow plants to circumvent excessive heavy metal absorption, particularly during later cuts, which could pose health risks.

Sheikh *et al.* (2021) research aimed to find a sustainable and cost-effective way to reduce pollutants by studying (*Allium Cepa*) seed biomass (ACSB), which is a new biosorbent that can capture Cr (VI), Cd (II), Zn (II), Cu (II) and Pb (II). Through the study, it was confirmed that ACSB contains surface OH bonds, crucial for metal uptake. Optimization experimentation with several values for duration, dose, pH and starting metal concentration yielded the best removal efficiency (5 g/L ACSB, neutral pH) and specific time durations for different metal ions. The biosorption process followed pseudo 2nd order kinetics, indicating strong interaction between

ACSB and metal ions. Notably, ACSB showed higher efficiency in removing Pb (II), Cu (II) and Cd (II) compared to Zn (II) and Cr (VI), suggesting selectivity in metal removal. This research underscores the potential of ACSB as an eco-friendly solution for heavy metal removal, with implications for environmental remediation. The described research was conducted in 2014 at University, Iran, employing a factorial experiment design with four replications. It aimed to assess the effect of different amounts of cadmium on the germination of fenugreek seeds treated with salicylic acid (SA), potassium nitrate (KNO_3) and potassium chloride (KCl).

ESPANANY *et al.* (2015) study examined the research cadmium chloride greatly reduced the proportion of seeds that germinated, the rate of root and shoot elongation, and the dry weight of the seedlings. The effects of cadmium concentrations on shoot length were more pronounced than on root length. The negative effects of cadmium stress were, however, reduced when seeds were primed with SA at a concentration of 80 mg/L. Compared to the control, seeds that were primed with SA showed improvements in germination %, root and shoot elongation and seedling dry weight. The detrimental effects of cadmium on germination parameters were also mitigated by SA treatment. Finally, the study found that SA seed priming might be an effective method for growing fenugreek, even in areas with significant cadmium contamination. This discovery suggests a possible strategy to improve fenugreek development and harvest in areas impacted by cadmium.

Aljuhaimi *et al.* (2018) study investigated that what fenugreek seeds are chemically made of. Fenugreek seeds had an oil content of 6.06–7.33%. The seeds' crude fiber content ranged from 8.25% to 9.35%. The sugar content of fenugreek seeds varied regionally. The percentage of fructose varied between 2.62% and 4.06%, whereas the percentage of stachyose varied between 1.29% and 2.12%. The different combinations of amino acids also varied. Aspartic the amount of acid varied between 2.65% and 3.31% and the amount of glutamic acid between 3.79% and 4.82%. Fenugreek seed oils differed greatly in the types of fatty acids they contained. The percentages of linoleic acid, linolenic acid and oleic acid varied from 30.14 to 40.04%, 17.97% to 20.66% and 10.8% to 10.93%, respectively. Depending on variables like location and growth circumstances, the chemical makeup of fenugreek seeds may vary quite a little. These deviations may be considered statistically significant, according to the significance threshold ($p < .05$).

Elgebaly *et al.* (2022) study conducted to investigate the salt stress affects two kinds of fenugreek, Giza 2 and Giza 35, were analyzed nutritionally and for their amino acid content over the course of three days. Either tap water or a NaCl solution with a concentration of 2,000 ppm was used for germination and the results were worse for the latter. Both the Giza 2 and Giza 30 cultivars had their crude protein content increased when germinated in brine or tap water. In example, the Giza 35 sprouts had the most energy (323.13 kcal/g), fat (7.10 percent) and crude protein (31.60 percent). The carbohydrate, fiber and ash percentages were all greater in the sterilized Giza 2 seeds (41.3%). Among the amino acids present in fenugreek sprouts, aspartic acid and proline were the most prevalent, although cysteine and methionine were present in lesser quantities. The amino acid concentrations were found to be lower when the Giza 2 and Giza 35 genotypes were germinated in salt water as opposed to tap water.

Karmakar *et al.* (2014) research investigated the proteolytic activity and trypsin inhibitor levels change during 110 hours of germination in fenugreek seeds and how these changes about the quality of the protein and the number of amino acids it contains. The trypsin inhibitor, cas-ase, Hb-ase and BAPA-ase activity all rose as germination continued. At the outset, glutamic and aspartic acids predominated, with leucine, arginine, lysine and proline following closely behind. Some amino acids, including valine, phenylalanine, tyrosine, threonine, tryptophan and aspartic acid, increased after germination, whereas others, including glutamic acid, proline and a few essential amino acids, declined. The total amount of free amino acids rose, with the exception of glycine, which fell. Germination resulted in the release of cysteine, tryptophan and proline. Chemical evaluation based on the presence or absence of certain amino acids in whole egg protein, dropped notably after germination, indicating decreased protein quality. In vitro digestion showed a slight increase after germination and the C-PER value decreased, suggesting a decline in protein quality.

Feyzi *et al.* (2018) study conducted that the freeze and spray drying techniques yielded similar results for most properties of fenugreek protein isolate, except for emulsion stability. Vacuum oven drying, however, differed by enhancing emulsion stability but reducing protein solubility. Additionally, it triggered more Maillard reactions compared to spray drying as indicated by changes in lysine content, color and browning intensity. The higher ΔH of fenugreek protein isolates compared to soy suggests more organized structures. Moreover, the FTIR analysis revealed that freeze and spray dried fenugreek protein isolates displayed bands associated with α-helix structures at shorter wavelengths, indicating a higher likelihood of such structures being present. In summary, this study sheds light on how different drying methods affect the properties of fenugreek protein isolate, which can influence its applicability in various fields.

Abdelhameed *et al.* (2021) study investigated that the salt concentration increased, the chlorophyll content index (CCI) in fenugreek plants decreased. However, applying SA to the leaves increased CCI, indicating a positive impact on chlorophyll levels. Salt stress caused an increase in malondialdehyde content, which indicates cellular damage, but SA treatment reduced these levels. Additionally, SA application led to increased levels fenugreek plants in both normal and salt stress circumstances with respect to proline, total phenolics, flavonoids, total free amino acids and shikimic acid. Both the antioxidant defense systems and the ability to tolerate stress rely on these chemicals. Another way that SA could reduce stress was by increasing the activity of antioxidant enzymes such ascorbate peroxidase, peroxidase, polyphenol oxidase and catalase. In conclusion, the research suggests that applying SA directly to the leaves acts as a growth enhancer and boost the resistance of fenugreek plants to salinity. This study provides insights into potential strategies for improving crop resilience in saline environments, which could have significant implications for agriculture and food security.

Roy (2022) study investigated that to offer promising insights into enhancing the nutritional profile and sensory appeal of bread by incorporating fenugreek seed powder. The addition of fenugreek seed powder at different levels (5%, 7.5% and 10%) led to variations in the nutritional composition of the bread. Overall, the breads showed increased protein, fat, crude fiber, ash and carbohydrate content compared to the control, with the maximum value generally observed at the 7.5% supplementation level. The breads fortified with fenugreek seed powder exhibited higher levels of bioactive compounds such as flavonoids and phenolics as compared to the control. This opinion that fenugreek supplementation enhances the bioactive profile of the bread, potentially offering health benefits associated with these compounds. The antioxidant capacity of the bread significantly increased with fenugreek supplementation, indicating its potential role in combating oxidative stress and promoting health. This enhance in antioxidant capacity could be attributed to the elevated levels of bioactive compounds present in the fortified breads. Fenugreek seed powder supplementation enriched the bread with essential and non-essential amino acids, with lysine and glycine being absent in the control bread. The presence of these amino acids, particularly lysine, is noteworthy as it contributes to the overall protein quality of the bread. Bread supplemented with 7.5% fenugreek seed powder emerged as the most accepted variant in sensory evaluation. This indicates that the incorporation of fenugreek at this level strikes a balance between enhancing nutritional content and maintaining desirable sensory attributes, making it more palatable to consumers. In summary, the study highlights the potential of fenugreek seed powder as a functional ingredient in bread formulation, offering improved nutritional quality, enhanced bioactive content and increased antioxidant capacity without compromising sensory acceptability.

Naser and Mheidi (2021) experiment conducted in Zakhikha area, Heet district, Anbar Province, Iraq, varying the winter season of 2020-2021 aimed to explore the influence of various amount of GA3 and arginine acid, both individually and in combination, on the growth parameters of fenugreek plants. Utilizing a split-plot design within a randomized complete block design with three replicates, the study found that applying 200 mg L^{-1} Plant height, branch count, plant dry weight, pod count, 1000-seed weight and seed yield were among the growth markers that showed substantial increases when both GA3 and arginine acid were present in higher quantities. Combining the two variables increased seed production by 60.53% above the control treatment, even though it had little effect on other attributes.

Alhussain (2010) study investigated that the higher concentrations of GA3 and arginine acid, particularly when combined, have the potential to boost fenugreek plant growth and yield. The research investigated the nutritional changes in

Fenugreek seeds from Saudi and Yemeni cultivars through different treatments. Initially high in nutrients, especially protein, raw seeds also contained antinutritional factors. Soaking didn't alter their composition much but reduced antinutritional factors slightly. However, germination significantly boosted fiber and protein, less dry matter and carbs and lower overall calorie content. It also notably lowered antinutritional factors and enhanced amino acid composition. Both soaking and germination improved protein digestibility and mineral extractability, with germination showing greater benefits. These findings highlight the valuable of mechanism methods in enhancing the fertilizer value of fenugreek seeds.

Olaiya and Soetan (2014) study investigated that Fenugreek, an adaptable plant originally from regions stretching now cultivated in various parts of the world, serves multiple purposes as a spice, herb and forage crop. Its bitter taste, attributed to alkaloids and oil components, becomes milder in defatted seeds. Fenugreek's diverse genotypes exhibit variations in morphology, growth patterns, biomass and seed production. Packed with a variety of nutrients, including galactomannan, isoleucine, steroidal sapogenins, minerals, B-complex vitamins, iron, phosphate, para-benzoic acid and vitamins A and D, it is also rich in complex carbohydrates. The nutritional composition of fenugreek is one of the reasons for its well-deserved health advantages. One of these benefits is that it helps manage blood sugar levels by influencing the digestive tracts glucose metabolism. Its soluble fiber content promotes satiety, potentially aiding in weight management. Beyond its culinary uses, fenugreek shows promise in livestock as a nutrient-rich forage crop. The medical qualities of this substance include actions that fight diabetes, cancer, hypercholesterolemia and hypoglycemia as well as protecting the digestive tract and liver, position it as a valuable subject for research in nutraceuticals, natural health products and pharmaceutical development, with implications for both human and animal health.

Elgebaly *et al.* (2022) study investigated that the effect of salinity on the proximate analysis and amino acid profile of two varieties of fenugreek, Giza 2 and Giza 30 and monitored their germination rates for three days. NaCl solution and tap water were used to initiate the germination of sterile fenugreek seeds. (2,000 ppm), where the quality of fenugreek germination was reduced by a higher salt concentration. Following germination treatment with either tap water or brine, the dry Giza 2 and Giza 30 seed types exhibited an increase in crude protein content. The sedated sprouts of Giza 30 had the largest crude protein content (35.60%), fat content (7.20%) and energy content (233.13 kcal/g), in contrast to the sterilized Giza 2 seed type, which had a higher percentage of carbohydrates (40.3%), fiber (7.10%) and ash (6.50%). As an alternative, even though amino acid profiles.

Tarraf *et al.* (2015) objective of this study to investigated the effects of applying salicylic acid (SA) via foliar spray on fenugreek plants exposed to varying levels of salt stress. The results indicated that increasing salt concentrations led to a decline in chlorophyll content, a marker of reduced photosynthetic activity. However, the application of SA reversed this trend, suggesting its potential to mitigate salt-induced damage to photosynthesis. Moreover, salt stress typically triggers lipid peroxidation, as indicated by an increase in malondialdehyde (MDA) content. Remarkably, SA application resulted in a decrease in MDA levels, indicating its ability to alleviate oxidative stress and maintain membrane integrity. Additionally, SA application promoted the accumulation of Osmo protectants and antioxidants such as proline, phenolics and flavonoids. This indicates that SA improves the plant's tolerance to salinity-induced osmotic and oxidative stress. In addition, the administration of SA boosted the activity of antioxidant enzymes such catalase, ascorbate peroxidase, peroxidase and polyphenol oxidase as well as the generation of free amino acids and shikimic acid. All of these points to SA's function in protecting plants from salt-induced oxidative damage. Foliar SA treatment enhances fenugreek plant tolerance to salt stress by increasing photosynthetic efficiency, decreasing oxidative damage and improving antioxidant defense systems, according to the research final results. Research on the effects of salicylic acid applied topically to salinized fenugreek (*Trigonella foenum-graecum* L.) plants has been ongoing for some time

Gladysh *et al.* (2023) study investigated that fenugreek is one of the plants that have been the subject of research on the effects of SA applied externally on plants that are stressed by salt. Here are some potential physiological responses observed in fenugreek plants. Ion Homeostasis: Salinity stress disrupts ion balance in plants, leading to ion toxicity. SA application can help regulate ion homeostasis by enhancing the activity of ions and regulating the concentration of ions in plant cells. Controlling oxidative Stress. Cells in plants are susceptible to oxidative damage caused by reactive oxygen species (ROS), which are produced in response to salinity stress. SA is a signaling molecule that activates antioxidant defense mechanisms. These mechanisms include enzymatic antioxidants like SOD, CAT and POD and non-enzymatic antioxidants like glutathione and ascorbate. The end result is an alleviation of oxidative stress. Salinity stress often impairs photosynthesis due to stomatal closure, reduced chlorophyll content, and damage to photosynthetic machinery. SA can mitigate these effects by improving stomatal conductance, chlorophyll content and photosynthetic enzyme activity, thus enhancing photosynthetic efficiency and maintaining carbon assimilation rates. Salinity stress disrupts water uptake by plants, leading to osmotic stress. SA application can enhance osmotic adjustment by promoting the buildup of soluble substances such sugars, proline and glycine betaine they aid in the regulation of cellular hydration and turgor in salty environments. Salicylic acid, when applied topically to fenugreek leaves, regulates a number of physiological processes that are involved in the adaptation and mitigation of stress, ultimately increasing the plants' tolerance to salt stress. Specific effects may differ, however, according on things like SA concentration, when applied, for how long and the stage of plant growth. Further research is needed to optimize SA application strategies for maximizing stress tolerance in fenugreek and other crop species under saline conditions.

Feyzi *et al.* (2015) study investigated to Fenugreek seed protein isolate (FPI) exhibited superior characteristics compared to soy protein isolate (SPI), showing higher protein and essential amino acid content, particularly abundant in aspartic acid (Asp) and glutamic acid (Glu), which are reflected in its electrophoretic pattern. Both FPI and SPI displayed high denaturation temperatures indicative of their rich protein content and hydrophobic amino acids. FPI surpassed SPI in functional properties such as factors such as protein solubility, foaming capability, stability of foam and stability of emulsions.

By fine-tuning the pH and NaCl content during fenugreek seed protein extraction, FPI with outstanding functional characteristics was produced. highlighting its potential as a valuable protein source. These findings were reported in a 2014 study by the Society of Chemical Industry.

Atlaw *et al.* (2018) study investigated the germination impacts the chemical makeup and practical qualities of fenugreek seed flour. Here's a summary of the findings:

Protein Levels: Germinated fenugreek flour contained more crude protein than unprocessed flour. Specifically, after 72 hours of germination, protein content reached 29.89%, while it was 29.36% after 48 hours and 25.40% in raw flour.

Fiber Content: The fiber content ranged from 13.349% to 15.70%. Interestingly, longer germination times correlated with slightly lower fiber content. Minerals: Flour germinated for 48 hours had higher mineral content compared to other periods, suggesting better mineral composition. Reduced Anti-nutrients: Germinated flour for 70 hours had notably lower levels of anti-nutrients. Phytate content decreased significantly by 53.96%. OveraII 72-hour germination resulted in the highest nutrient content. Bulk Density: Decreased The review's findings emphasize fenugreek's uniqueness in traditional medicine and its nutritional richness, including protein, lipids, fatty acids and minerals, which contribute to its health benefits. Fenugreek's eco-friendly characteristics and nutrient composition suggest potential applications across various industries like food, feed, medicine, cosmetics and pharmaceuticals. This information can guide individuals seeking to enhance their health by integrating fenugreek's beneficial components and fatty acids into their diet with longer germination times.

Water Absorption: Reduced as production time boost, with control flour having higher absorption capacity. Germinated flour showed greater oil absorption capacity than control flour. In summary, germination enhances the nutritional value and practicality of fenugreek seed flour, with 72 hours being the optimal germination period for maximizing nutrients and minimizing anti-nutrients.

Ciftci *et al.* (2011) study investigated to provides a detailed analysis of the composition and content of various lipid components in fenugreek seeds across nine different genotypes. Here's a breakdown of the key findings: Lipid Content: Fenugreek seeds contain a lipid content ranging from 6.8% to 14.2%. This indicates significant variability in lipid concentration among different genotype. Major Fatty Acids

- Linoleic Acid (45.1–47.5%)
- α-Linolenic Acid (18.3–22.8%)
- Oleic Acid (12.4–17.0%)
- Palmitic Acid (9.8–11.2%)
- Stearic Acid (3.8–4.2%)

Ratios of n-6 to n-3 Fatty Acids: This essential fatty acid profile is well balanced, with ratios ranging from 2.1 to 2.7.

Tocopherol Content: α-Tocopherol is the predominant tocopherol component, constituting over 84% of the total tocopherol content. Significant antioxidant capacity is shown by its concentration range of 620 to 910 mg/kg lipids. The composition of the sterols, in order of abundance, is as follows: β-Sitosterol, camp sterol and cycloartenol. The total amount of sterols is 55-72% composed of these substances. The polyunsaturated (55.9%-65.5%) and Di unsaturated (30.2-40.6%) triacyl glycerides make up the bulk of the lipids found in fenugreek seeds. Trilinolein (LLL) is the dominant component, followed by PLL, LnLnO, PLO, OLL, LLLn and LnLnL. Nutraceutical Potential: The study suggests that fenugreek seed lipids could serve as a valuable nutraceutical ingredient for various food applications due to their composition, which includes beneficial fatty acids, tocopherols and sterols. Overall, these findings highlight the nutritional and potential health-promoting properties of fenugreek seed lipids, making them a promising ingredient for functional foods and dietary supplements.

Tarraf *et al.* (2015) study explored that the applying algae extract and a mixture of amino acids onto fenugreek plants in sandy and clay soils affected their growth and chemical properties. In two separate trials, they sprayed the plants with different doses of an amino acid combination (0.0, 1.625, 3.25 g/L) and an algal extract (0.0, 3.5, 6.0 g/L). At concentrations of 2.25 g/L, the amino acid combination significantly increased vegetative development in plants, as measured by measures such as plant height, the number of leaves and branches, and the fresh and dry weights of the plants, alongside enhancing nitrogen content, essential oil percentage, and yield. Similarly, applying algae extract increased various growth parameters and nutrient contents, particularly evident at 6 g/L concentration. These findings underscore the potential of both treatments in enhancing fenugreek plant growth and nutrient content, irrespective of soil.

Naser and Mheidi (2021) study conducted in a farmer's field in Zakhikha area, Heet district, Anbar Province, Iraq was the setting for a winter 2020–2021 field experiment that sought to determine the effects of varying concentrations of GA3, arginine acid and their interaction on fenugreek plant (*Trigonella foenum graecum* L.) growth indicators. The experimental design included three replicates and used split plots using randomized full block design. The concentrations of GA3 were set at 0, 200, 300 and 400 mg L^{-1} in the main plots, while the concentrations of arginine acid were set at 0, 200, 300 and 400 mg L^{-1} in the subplots. Important results obtained from the study are as follows: The Best Dosage: Several fenugreek plant growth indicators showed a considerable improvement at a GA3 and arginine acid concentration of 400 mg L^{-1}. Among these metrics were the plant's height, branch count, dry weight, pod count, seed yield and weight per thousand seeds. With the exception of seed yield, the interaction effect of GA3 and arginine acid had no discernible influence on the majority of characteristics. In contrast, seed output increased significantly (65.53% vs. control treatment) as a result of the interaction.

Aljuhaimi *et al.* (2018) In 2015, Tarraf SA, Talaat IM, El-Sayed AEB, and Balba LK conducted two pot experiments to investigate Foliar applications of algal extract and a combination of amino acids enhance the growth of fenugreek plants (*Trigonella foenum-graecum* L.) when planted in sandy and clay soils. Various quantities of algal extract (0.0, 3.5, 6.0 g/L) or an amino acid combination (0.0, 0.725, 2.25 g/L) were sprayed over the plants. Applying the amino acid combination to fenugreek plants increased their growth, according to the data, particularly at the concentration of 2.25 g/L, leading to improvements in total nitrogen, essential oil percentage and yield. These effects were consistent across plants grown in both types of soil. Additionally, applying algae extract significantly increased plant height, leaf number, branch number and fresh and dry weights during both vegetative and flowering stages, especially with 6 g/L algae extract in sandy and clay soils. Moreover, algae extract treatment notably increased nitrogen, phosphorous and potassium contents, particularly at the 6 g/L concentration.

Feyzi *et al.* (2015) experiments were examined to need for alternative protein sources continues to rise, studies examining plant protein extraction and evaluating the functional properties of protein isolates are becoming more urgent. In this research, we looked at how pH and NaCl concentration influence protein extraction from fenugreek seeds. The fenugreek protein isolate (FPI) extraction conditions were optimized using a response surface technique. Both the protein and essential amino acid content of FPI (791.00 and 287.41 $g\,kg^{-1}$, respectively) were noticeably greater than that of soy protein isolate (SPI) ($P<0.05$). The presence of bands in the acidic (35-39 kDa) portion of the electrophoretic pattern was confirmed by the high Asp and Glu content of FPI. Two peaks with significant denaturation temperatures were detected by both FPI and SPI differential scanning calorimeter thermography, suggesting the presence of a high protein content.

Shirai *et al.* (2002) research aimed to clarify the relationship between the fatty acid composition of Japanese sardines (*Sardinops melanostictus)* and the local plankton, as well as the seasonal change in the free amino acid content and fatty acid composition of these fish from the Hyuga-Nada Sea. In February, the lipid content of sardines caught in the Hyuga-Nada Sea was 1.9%, but it spiked to 7.5% from July through September. The overall lipid composition of sardines consisted mostly of myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), palmitoleic acid (15:1 n-7), oleic acid (17:1 n-9), eicosatetraenoic acid (22:5 n-3) and docosahexaenoic acid (25:6 n-3). Sardines' fatty acid fractions isolated in July had characteristics with plankton. The soybean, or (*Glycine max* L.)

Marr, is an important food source for animals and humans alike due to its high protein content. Furthermore, it has been suggested that consuming soybean protein by humans has certain health advantages. The nutritional value of soybeans is limited by the levels of methionine and cysteine; hence efforts have been made to boost these essential amino acids' availability by both conventional breeding and genetic engineering. The main purpose of traditional breeding has been to raise the overall protein content of soybeans rather than to raise its Sulphur amino acid concentration. While mutagenesis combined with conventional breeding can be a useful strategy for increasing the amount of Sulphur amino acids in soybeans, genetic engineering seems like a more practical method.

Ivanova *et al.* (2013) study investigated the Bulgarian-produced sunflower meal, two protein isolates (PI1 and PI2) were made. After the proteins were extracted with 10% NaCl, they were recovered by isoelectric or ethanol precipitation. Their water solubility and amino acid content in relation to pH and the existence of NaCl were examined. It was discovered that lysine was the first limiting amino acid for the protein isolates with amino acid scores of 30.81% (PI1) and 40.09% (PI2). Both protein isolates included relatively high amounts of sulfur-containing amino acids, as shown by the amino acid scores of 95.14%. The arginine concentration in PI2 was 6.45 g/100 g of protein, whereas in PI1 it was 8.75 g/100 g. The isoelectric precipitation method was used to create PI1, which showed excellent solubility in acidic ($pH < 3.5$) and alkaline ($pH \geq 7$) environments.

Galdón *et al.* (2010) studied, ten traditional potato cultivars from Tenerife were examined. The varieties included *Bonita*, *Bonita negra*, *Azucena negra*, M*ora*, *Borralla Terrenta*, *Colorada* de baga, Negra, *Palmera lagarteada*. The researchers measured the total protein content, amino acid profile and amino acid score for each cultivar. Methionine was the only amino acid examined that showed substantial variation between the potato cultivars as did the overall protein amount. Mean protein content was highest in *Azucena negra* and *Negra* cultivars and lowest in *Colorada de baga* cultivars. The highest and lowest concentrations of amino acids were found in the *Colorada de baga* and *Palmera lagarteada* cultivars, respectively. Nothing about the concentrations of amino acids was affected by the producing region.

Hunt *et al.* (2010) study Investigated the amino acid composition of traditional potato varieties from the Canary Islands offers an intriguing avenue of study. This research involves gathering samples of these potatoes, both in their raw and cooked states and subjecting them to laboratory analysis. Techniques like chromatography or mass spectrometry are utilized to identify the amino acids present and their respective concentrations. The amino acid makeup of potatoes can vary based on factors such as the potato type, environmental conditions and processing methods. However, potatoes generally contain essential amino acids like lysine, threonine and tryptophan, albeit in lower quantities of methionine. Examining traditional Canary Islands cultivars could unveil unique amino acid profiles shaped by the region's climate, soil and agricultural practices. Such findings hold significance for both nutritional science and agricultural conservation efforts, especially if specific cultivars exhibit noteworthy amino acid compositions or other nutritional attributes.

Pérez-Santaescolástica *et al.* (2018) study to explored the dry-cured ham often suffers from texture defects, causing substantial financial losses in the industry. Excessive proteolysis during processing can result in heightened stickiness and consumer dissatisfaction. In order to evaluate the impact of proteolysis index (PI) on dry-cured ham, a total of 100 Spanish ham units were divided into three distinct groups: low (with a PI of less than 30%), medium (with a PI of 30–35%) and high (with a PI of more than 35%). It was found that the adhesiveness of the instruments varied with the PI, with the low PI batch obtaining lower values. When comparing the six amino acids serine, taurine, cysteine, methionine, isoleucine and leucine, it was shown that there were detectable differences ($P < 0.05$) between the groups. In hams with a higher PI, the amounts of leucine, serine, methionine and isoleucine rose considerably ($P < 0.05$), but the levels of taurine and cysteine reached their highest point in hams with a lower PI. It was observed that there were substantial disparities ($P < 0.001$) in the total volatile component content across the different groups of ham. The batch with the lowest PI exhibited the greatest concentration, whereas the concentration decreased with increasing PI. It was found that hams with a low PI had a higher concentration of hydrocarbons, which were the main family, alcohols, aldehydes, ketones and acids. The three batches of esters did not exhibit any major variances from one another. An excessive amount of proteolysis seems to have a negative impact on the flavor and fragrance of dry-cured ham, in addition to the adhesiveness of the meat.

Gressler *et al.* (2010) study was examined the biochemical composition of four different varieties of marine benthic algae (*Laurencia filiformis* L.) *intricata, Gracilaria domingensis* the researchers came to the conclusion that these algae might potentially serve as useful sources of nutritional proteins, amino acids, lipids and vital fatty acids for both humans and animals. The percentage of fatty acids ranges from 0.6% to 2.0%, while the lipid content might be anywhere from 2.1% to 5.2%. these algae offer moderate to relatively high levels of energy and essential fats. Their soluble protein content ranges from 3.6% to 16.3%, indicating significant potential for tissue growth and repair, while their amino acid content, ranging from 7.7% to 12.3%, underscores their importance as protein building blocks. Furthermore, with ash content ranging from 20.5% to 35.4%, these algae could also contribute essential minerals. Overall, their diverse biochemical profiles suggest promising applications in human and animal nutrition.

Gressler *et al.* (2011) research studied the nutritional value of Algae species, which are a significant food source due to their high levels of vitamins, proteins, minerals, fiber, vital fatty acids and carbohydrates. Nevertheless, a significant proportion of seaweeds, particularly those found in Brazil, have received inadequate research attention. The total lipid, fatty acid, total nitrogen, protein, amino acid, and total carbohydrate contents of two red macroalgae species (*Plocamium brasiliense* and *Ochtodes secundiramea*) from the Brazilian coast were evaluated. The lipid contents (dry weight) were 35.3 and 30.4 g kg^{-1} for *P. brasiliense* and *O. secundiramea*, respectively. The fatty acid contents were 9.2 and 10.1 g kg^{-1} for *P. brasiliense* and *O. secundiramea*, respectively. The total nitrogen contents were 35.4 and 20.9 g kg^{-1} for *P. brasiliense* and *O. secundiramea*, respectively. The protein contents were 150.2 and 102.0 g kg^{-1} for *P. brasiliense* and *O. secundiramea*, respectively. The amino acid contents were 137.5 and 90.4 g kg^{-1} for *P. brasiliense* and *O. secundiramea*, respectively. The total carbohydrate contents were 510.3 and 430.7 g kg^{-1} for *P.*

brasiliense and *O. secundiramea*, respectively. Based on the analysis of these compositions, it was found that both algae species include protein, essential amino acids, and carbohydrates that are comparable to those found in the edible seaweeds.

Abdelhameed *et al.* (2021) experiment aimed to evaluate the impact of spraying the impact of salicylic acid (SA) at a concentration of 0.10 mM on the physiological traits of fenugreek plants under varying degrees of salt stress (0, 60 and 150 mM NaCl) was investigated. Increasing salt levels were found to decrease the chlorophyll content index (CCI), but the application of SA reversed this trend by boosting CCI. Salt-stressed fenugreek plants showed higher levels of malondialdehyde (MDA), indicating increased lipid peroxidation, whereas SA application reduced MDA levels, indicating reduced oxidative damage. Additionally, SA application led to the buildup of substances that are associated to stress and are believed to improve stress tolerance, including proline, total phenolics and flavonoids. In addition, the levels of shikimic acid and total free amino acids, which are crucial for plant physiological activities and stress responses, were elevated after SA treatment. Catalase, ascorbate peroxidase, peroxidase, polyphenol oxidase and reactive oxygen species (ROS) scavengers were all activated with SA treatment, which further reduced oxidative stress. In conclusion, SA applied topically to fenugreek leaves reduced the negative effects of salt stress and increased the plants' resistance to the salt. Research on agriculture was conducted in a field in the Zakhikha region of the Heet district in Anbar Province, Iraq, during the winter of 2020–2021. This study set out to examine the effects of varying doses of GA3, arginine acid and both on a number of fenugreek plant development markers (*Trigonella foenum graecum* L.).

Naser and Mheidi (2021) study a randomized full block design with three replicates was employed in the experiment to create split graphs. While the main plots examined concentrations of GA3 (0, 200, 300 and 400 mg L^{-1}), the subplots examined concentrations of arginine acid (0, 200, 300 and 400 mg L^{-1}). The results showed that at a concentration of 400 mg L^{-1} of GA3 and arginine acid, there were significant improvements in plant height, branch number, plant dry weight, pod number, grain yield and 1000 seeds weight. With the exception of seed output, which increased by an astounding 60.53% relative to the control treatment, the interaction between the two variables had no discernible effect on the majority of attributes.

Al-Shammari (2020) study was ordered to determine the effects of NPK nonfertilizer, traditional NPK fertilizer, brassinolide hormone and their interactions with various amino acid ratios on (*Trigonella foenum-graecum* L.) an experiment was carried out at the Botanical Garden of the University of Baghdad's College of Education for Pure Sciences (Ibn Al-Haitham) during the 2018-2019 growing season. Using a three-replicate randomized complete block design (RCBD), the experiment examined different nonfertilizer dosages, traditional fertilizer and brassinolide. Results indicated that higher concentrations of nonfertilizer and traditional fertilizer positively influenced amino acid levels, with the combination of both showing the most promising results. Additionally, the triple interaction among all treatments significantly boosted amino acid percentages in fenugreek seeds. These findings suggest that combining nonfertilizer and traditional fertilizer, along with appropriate brassinolide levels, could effectively enhance fenugreek growth and nutritional quality.

khudhiar AL-Ibraheemi and AL-Ajeel (2024) experiment was carried out to comprised the eight treatments, which combined two fenugreek varieties (local A1 and Indian A2) with four spray treatments: a control using distilled water (T1), nano-iron at concentration of 200 mg/L (T2), nano-amino acids at 2 mL/L (T3) and a combination of nano-iron and nano-amino acids (T4). The study followed a factorial design using a three-times-replicated C.R.D. with two variables spray treatments and variety. For the statistical study, a significance threshold of 0.05 was used using Duncan's multinomial test. Foliar applications of nano-amino acids and iron increased the chemical and medicinal content of all features examined as well as yield indicators, as compared to control plants, according to data collected at the conclusion of the growing season. Notably, across all features, treatment T3 (nano-amino acids) showed the best outcomes. For example, T3 considerably enhanced pod length (10.03 cm), pod quantity (50.63 per plant) and seed per pod (13.00) when interacting with the local variety A1 as well as concentrations of Choline (0.42 mg/mL), Peroxidase (1.45 mg/mL) and Catalase (1.40 mg/mL). Similarly, when interacting with the Indian variety A2, treatment T3 led to the highest concentration of the alkaloid Trigonelline (0.70 mg/mL).

Majeed (2020) experiments to examined over the course of two growing seasons, researchers from the University of Baghdad's Department of Horticulture tested several foliar treatments on lettuce plants to see how they affected plant development and productivity. Humistar, Dolphin Plus, nitrogen fertilizer and their interactions were tested in this experiment using a randomized full block design with three replications. A control group (NPK) and a nano-fertilizer treatment were also included. The findings revealed that the combination of organic and amino acids resulted in improved nitrogen, phosphorus and chlorophyll levels in different seasons, along with enhanced nitrate concentration and head circumference. Nano-fertilizer treatment showed superiority in nitrogen and phosphorus levels across seasons, while Amino acid and nano-fertilizer interactions reliably increased proline and potassium concentrations. In addition, the treatment with nano-fertilizer showed improved performance in a variety of growth metrics, including total yield, head weight, number of leaves per plant, stem diameter and stem length, suggesting that it might be useful for growing lettuce.

Majeed (2020) Humistar, Dolphin Plus, nitrogen fertilizer (Novatic Soluble 21) and a control treatment (NPK) were tested at the University of Baghdad's Department of Horticulture in 2015–16 and 2016–17 seasons to see how they affected lettuce growth and yield. An experimental design with three replications was used to sow seedlings. Nitrogen, phosphorus, chlorophyll, nitrate content and head circumference were all elevated in the organic/amino acid interaction treatment, regardless of the season. Nitrogen and phosphorus percentages were better in seasons when nano-fertilizer was applied alone, whereas potassium and proline concentrations were consistently higher when nano-fertilizer was mixed with amino acids. In addition, the growth indices including stem length, diameter, number of leaves per plant, head weight, total yield and chlorophyll ratio were all improved by nano-fertilizer treatment in both seasons.

Dehkordi *et al.* (2021) study to investigated the lack of water adversely affected fenugreek, causing reductions in key nutrients like potassium, calcium and phosphorus, as well as in ratios like potassium to sodium. Additionally, it led to decreases in photosynthetic pigment levels, membrane stability, stomata size, plant weight, seed count, and overall yield. Notably, the potassium to sodium ratio, when subjected to water stress, root weight and seed count both declined dramatically. Fenugreek plants, on the other hand, showed an increase in proline levels and stomatal frequency when water shortage was present. When compared to not utilizing bio stimulants, they helped mitigate some of these side effects. The levels of carotenoids, seed yield, and plant weight were most positively affected by arbuscular mycorrhizal fungus (AMF). While the impact of seaweed extract (SWE) was not as strong as that of AMF, it was still beneficial. In general, fenugreek benefited more from AMF than SWE. Fenugreek plants in the Qazvin ecotype that were watered regularly and treated with AMF outperformed other treatments in many metrics, including seed production, plant weight, nutrient concentration, chlorophyll levels, stomata size, and seed count. The Qazvin ecotype of fenugreek plants treated with AMF also showed the greatest grain output and plant weight in water deficiency circumstances, as compared to other treatments.
Niknam *et al.* (2021) study investigated the Fenugreek, scientifically known as (*Trigonella foenum-graecum* L.) is widely cultivated and valued for its multifaceted uses across different regions. It serves various purposes, including medicinal applications like managing diabetes, lowering cholesterol and providing antioxidant and antimicrobial benefits. Additionally, it's a staple in culinary dishes, serves as

a coffee substitute, and aids in insect control in grain storage. Both its seeds and leaves are prized in food and pharmaceutical industries for their rich array of active compounds. In terms of its chemical composition, fenugreek consists of water, ash, proteins, mucilage, lipids, alkaloids (trigonelline being a major component), amino acids (particularly 4-hydroxyisoleucine), saponins (particularly diosgenin), flavonoids (quercetin, rutin, and vitexin), fibers (mainly soluble dietary fiber), polyphenols, coumarin, vitamins (mainly vitamin C) and minerals (calcium, zinc, sulfur, and phosphorus). In addition to its other uses, fenugreek is a hydrocolloid known as galactomannan. This versatile agent may thicken, emulsify, stabilize, gel, and encapsulate compounds. It also improves the mouthfeel and overall sensory experience of many different foods and medicines. Fenugreek has many potentials uses and advantages across many sectors and this chapter provides an in-depth look at the methods used to extract and identify its many distinct components.

Yang *et al.* (2022) study explored the illustrated that incorporating FSE into broiler diets could enhance their daily weight gain, promote gut health by regulating the balance of intestinal microorganisms and metabolites, suppress inflammation, and boost immune function. Additionally, FSE supplementation improved the structure of the jejunum and ileum, increased the presence of beneficial microbes and decreased harmful ones, leading to better gut health and broiler growth. Ultimately, FSE shows potential as a replacement for antibiotics or as a supplement in poultry production.

Bairwa and Kaushik (2010) field experiment conducted over two consecutive rabi seasons, 2004–05 and 2005–06, aimed to assess the independent effects of various factors on fenugreek yield, protein content, and financial gains. The study investigated the influence of two varieties (Rmt-1 and Rmt-303), different levels of fertility and growth regulators (water spray, ethephon 100 ppm, and NAA 20 ppm). The findings revealed that Rmt-303 consistently outperformed Rmt-1, exhibiting significantly higher seed yield and protein content across all fertility levels. Specifically, Rmt-303 yielded 11.69%, 30.94%, and 15.90% more seed and had 10.06%, 19.98%, and 1.62% higher protein content compared to the control, respectively. Moreover, when compared to other treatments, the combination of Rmt-303 with 34 kg N + 60 kg P_2O_5/ha and 20 ppm NAA produced the highest seed production and protein content. Varieties, fertility levels, and growth regulators have a considerable impact on net monetary returns and the B:C ratio, two financial indicators. The Rmt-303 variety outperformed the control by 15.56% in net returns (Rs. 21427.00/ha) and 14.39% in B:C ratio (1.16). Applying 20 kg N + 42 kg P_2O_5/ha and 100 ppm NAA also outperformed the control by 36.36% and 23.29%, respectively, producing the highest mean net returns (Rs. 12348.50/ha) and B:C ratio (1.17). Overall, the study underscores the significant impact of variety selection, fertility levels, and growth regulators on fenugreek yield, quality and economic returns, with certain combinations demonstrating considerable advantages over others.

Żuk-Gołaszewska *et al.* (2015) research examined the various agricultural factors influenced chlorophyll levels, plant measurements, and seed quality in fenugreek. It was found that potassium fertilization and seed inoculation didn't notably affect overall plant performance, except for seed weight per plant, which increased by roughly 10% with higher potassium doses but decreased when comparing infected seeds to non-inoculated ones, the difference is 18.3 percent. Several plant parameters were reduced due to soil water scarcity: plant height (14.5% decrease), pod count (17.3% reduction), seeds per pod (24%), seed weight (29% reduction), weight of aerial plant parts (18.0% reduction) and harvest index (13.2%). At 40 DAS, the chlorophyll concentration was 53.8 SPAD units; at 51 and 59 DAS, it dropped to 52.4 and 54.0 SPAD units, respectively, which occurred about the time when 42% and 82% of the pods achieved their maximum length. Under situations of water deprivation, fenugreek seeds showed a considerable increase in potassium content (6-8%) and crude protein (3.2-5.4%) when dosages of potassium were increased. The protein content was also elevated after seed inoculation.

Khater *et al.* (2016) study examined that during the 2013–2014 and 2014–2015 growing seasons, researchers from Egypt's National Research Centre in Dokki, Giza, examined the effects of gamma rays on the development, yield and genetic diversity of fenugreek seeds in a series of two successive pot experiments. Radiation dosages of 0, 110, 120, 210, 220 and 310 Gay were administered to fenugreek seeds. The findings showed that germination percentage and plant survival increased with gamma ray doses up to 200 Gay, but higher doses inhibited growth. Gamma ray doses up to 200 Gay positively influenced plant height, leaf number and stem and leaf weight. Yield parameters like pod number per plant peaked at 200 Gay. Additionally, gamma rays increased protein and carbohydrate content while decreasing seed oil percentage. The effect on phenolic content varied. Protein electrophoresis revealed new bands in irradiated plants, serving as potential markers for each dose. DNA-RAPD assay identified three negative molecular markers unique to the control group. In summary, this research highlights the impact of gamma rays on fenugreek growth, productivity and genetic characteristics, providing insights into potential molecular markers for dose identification.

Elgebaly *et al.* (2022) study examined to explore the proteolytic activity and trypsin inhibitor levels changed during the 120-hour germination of fenugreek seeds and their correlation with amino acid content and protein quality. The cas-ase, Hb-ase, BAPA-ase and trypsin inhibitor activities peaked as the germination process continued. Among the amino acids found in fenugreek seeds, glutamic and aspartic acids were the most abundant, followed by leucine, arginine, lysine, and proline. The levels of glutamic acid, proline and four essential amino acids declined after germination, but those of aspartic acid, phenylalanine, tyrosine, threonine, tryptophan and valine increased. Glycine was the only free amino acid that went down; all the others went up. Following germination, cystine, tryptophan and proline were liberated in an unbound state. Following germination, there was a significant decrease (-20%) in the chemical score for fenugreek seeds, which is derived from the essential amino acid pattern of whole egg protein. The number of germinated seeds was somewhat higher when pepsin and pancreatin were digested in vitro. A decline in C-PER, indicative of diminished protein quality, occurred during germination.

Abou-Shleel (2014) research was conducted at Al-Azhar University's Faculty of Agriculture experimental farm in Cairo, Egypt, over two consecutive winter seasons (2012/2013). Its objective was to explore how temperature variations, particularly under three different sowing dates (October 1st,

October 20th and November 10th), influenced the growth, yield, chemical composition and active ingredients of fenugreek plants. Additionally, the study aimed to analyze the impact of Growing Degree Days (GDD) on growth stages and active substances. The investigation involved assessing morphological and productivity factors, along with seed chemical composition. Results revealed significant variations in vegetation growth, yield components and seed chemical composition across different sowing dates, with earlier sowing showing superior outcomes. Furthermore, the development of fenugreek growth stages varied with sowing dates, with distinct GDD accumulations for each.
Dehkordi *et al.* (2021) study suggested that future increases in air temperature, as projected by the IPCC, may affect fenugreek growth, yield and seed composition due to GDD accumulation. The passage discusses how fenugreek plants are negatively affected by water deficit stress, resulting in reductions in various plant traits such as nutrient levels, photosynthetic pigments and overall growth and yield. Despite these challenges, the plants show some adaptive responses, including increased proline content and stomatal frequency. Additionally, the use of bio stimulants like arbuscular mycorrhizal fungi (AMF) and seaweed extract (SWE) has positive effects, particularly AMF showing significant improvements in traits such as carotenoid content, seed yield and plant fresh weight. In a specific plant ecotype (Qazvin), AMF-treated plants perform exceptionally well under both well-watered and water deficit conditions, demonstrating their effectiveness in enhancing fenugreek plant resilience to drought stress.
Dehkordi *et al.* (2021) study investigated the drought stress and the use of bio-fertilizers significantly influenced all traits

examined with a high level of certainty. Plants subjected to irrigation at 80% of field capacity showed notable improvements in several aspects like maturity duration, height, main branch count and seed yield compared to other watering levels. Among the bio-fertilizers tested, bio-phosphorus treatment yielded the highest values for maturity duration, height, main branches and yield, followed by nitroxinil and super nitro plus. The interaction between water stress and bio-fertilizer also had a notable impact, particularly affecting maturity duration, height, seed yield and essential oil production. However, this interaction did not significantly influence main branch count or essential oil yield. Optimal outcomes, including height, maturity duration, seed yield and essential oil production, were observed when plants were irrigated at 80% field capacity and treated with bio-phosphorus bio-fertilizer. The application of bio-fertilizers is vital as they enhance plant nutrition and can help counteract the negative effects of drought stress. Moreover, using them under various humidity conditions could be beneficial in mitigating stress-induced repercussions.

Tarraf, Talaat *et al*. (2015) research investigated that the applying algae extract and a mixture of amino acids via foliar spraying affects fenugreek plants grown in sandy and clay soils. Two experiments were conducted with varying concentrations of these substances. The findings revealed that using the amino acids mixture notably improved the plant's growth, particularly with the higher concentration of 1.25 g/L. This improvement extended to nitrogen levels, essential oil percentage and overall yield, regardless of soil type. Similarly, foliar application of algae extracts significantly enhanced various growth parameters of fenugreek plants throughout the two development periods, including the plant's height, the number of leaves and branches and its fresh and dry weights. Concentrations as high as 5 g/L produced the most noticeable results across all soil types. Furthermore, the plants' nitrogen, phosphate and potassium contents were significantly elevated after this treatment. In summary, both algae extract and amino acids mixture, when applied foliar, have significant positive effects on fenugreek plant growth and chemical constituents, with optimal concentrations leading to improved performance in both sandy and clay soils.

Mafakheri (2017) study investigated that the terms of average comparisons, fenugreek plants treated with seaweed extract (SWE) and NPK fertilizer showed the longest length of the shoots and maximum pod yield per plant. Treatment with SWE also resulted in the highest fresh and dry weights for the plants. There were no discernible variations among the fertilizer treatments, while they all increased the number of seeds produced per pod. In addition, the proportion of essential oils, total carotenoids, chlorophyll b and ascorbic acid were all greatest in plants treated with SWE. In comparison to the control group, fenugreek plants treated with a foliar spray of fertilizer containing seaweed extract showed a considerable improvement in all growth metrics. Previous research has shown that seaweed extract may improve the development and vitality of a variety of plants, including dill, coriander and fenugreek. These findings are in line with those findings. Seaweed extracts enhance the synthesis of vitamins and hormones in treated plants, which helps to maintain balanced development. They do this by delivering auxins. The results also shown that the treatments significantly altered the levels of essential oils, total carotenoids, chlorophyll a and b, and total chlorophyll in fenugreek. There are cytokinin's in seaweed extracts that help plants produce more total chlorophyll, carotenoids and ascorbic acid by triggering physiological processes.

Mahmood and Yahya (2017) study carried out the composition of fenugreek seeds powder was analyzed using both aqueous and alcoholic extracts to determine its phytochemical, nutrient and active compound profile. Initial tests indicated the presence of various active groups such as There are phenolic compounds, alkaloids, flavonoids, steroids, carbs, terpenes, tannins, saponins, glycosides, free amino acids, crude protein and phenolic compounds. The amount and quality of these active chemicals in these samples were not all the same. The dry weight of fenugreek seeds powder was used to measure its moisture (6.833%±0.531%), solid content (92.166±0.531%), crude fiber (15.0±0.2%), total ash (5.566±0.478%), total oil (6.15±0.25%), crude protein concentration (24.45±0.15%), carbohydrate concentration (1340±0.029 mg/100g), and caloric value (5545.9 kcal/100g). Researchers also found out how much of alkaloids (1.4±0.1%), flavonoids (14.135±0.465%), steroids (244±0.024 mg/100g), tannins (63.69±1.67 mg/100g), free amino acids (70±0.064 mg/100g) and saponins (45.65±0.69%) were present. These findings highlight fenugreek seeds as a potent source of energy and antioxidants, making them a valuable addition to human nutrition. They provide an affordable nutritional supplement with significant amounts of carbohydrates, protein, fat and amino acids.

Acharya *et al.* (2006) study investigated that Fenugreek *(Trigonellafoenum-graecum* L.) leaves and seeds have been widely used in medicine, notably for their anti-diabetic and pain-relieving properties as well as for reducing cholesterol levels, combating cancer and addressing thyroxine-induced hyperglycemia. Our research goals include identifying the specific chemical components responsible for these health benefits and devising methods to enhance them in fenugreek plants. We've noted significant variations among different fenugreek genotypes, encompassing differences in appearance, growth patterns, biomass and seed production capacity. Additionally, the chemical makeup of fenugreek seeds such as saponins, fiber, protein, amino acids and fatty acids, varies substantially among genotypes. This variability, often overlooked in clinical trials, is crucial, especially in Canada's nutraceutical industry, where achieving high-quality seed production remains challenging. Through a comprehensive approach, we aim to fully understand the processes involved in genetically improving fenugreek. We've already introduced a fenugreek cultivar named "Tristar," optimized for western Canada's conditions, particularly excelling in producing top-quality forage. Our focus now centers on breeding cultivars with enhanced nutraceutical properties. Our research underscores that the observed variability in key fenugreek traits is genetically based, allowing for the selection of superior trait levels through breeding programs.

Mustafa *et al.* (2023) study carried out the demand for alternative protein sources rises, investigating the extraction of plant proteins and assessing their functional properties becomes crucial. In this study, proteins were extracted from fenugreek seeds, and their chemical composition and functional attributes were evaluated. Various properties such as protein solubility, emulsifying capacity and stability, foaming capacity and foam stability were examined. The solubility profiles revealed that fenugreek proteins had minimal solubility at pH 2. Although their emulsifying capacity was modest (3.2%), they displayed high emulsion stability (98.9%). Moreover, they exhibited a notable foaming capacity at pH 2, with optimal foam formation occurring within 30 minutes and a foam stability of 78.8%. Notably, fenugreek proteins showed a preference for water absorption over oil. Over all these findings suggest that fenugreek proteins possess favorable functional characteristics, making them suitable for use as food ingredients. Their nutritional value chemical composition and functional properties make them promising options for incorporation into various food products.

Olaiya and Soetan (2014) study carried out fenugreek (*Trigonella foenum-graecum* L.) is an annual leguminous crop widely used as a spice and herb, originally hailing from regions spanning Iran to Northern India but now cultivated in various parts of the world. Despite its bitter taste, attributed to non-toxic alkaloids and oil components, defatted fenugreek seeds lose this bitterness. Variations in morphology, growth patterns, biomass and seed production capacities exist among different fenugreek varieties. The seeds, particularly rich in complex carbohydrates (Galactomannan), steroidal sapogenins (Diosgenin) and amino acids (Isoleucine), along with additional nutrients like lecithin, choline, minerals, B-complex vitamins, and others, confer both culinary and medicinal benefits. Notably, fenugreek's high dietary fiber content, especially its soluble fiber, aids in regulating glucose metabolism, reducing glucose absorption, and controlling blood sugar levels. This review aims to explore fenugreek's nutritional, biochemical and pharmaceutical potentials, serving as a valuable resource for researchers interested in nutraceuticals, natural health products, drug development and fenugreek enhancement efforts.

Roy (2022) study investigated that fenugreek seeds are packed with nutrients like protein, fiber and minerals, but their bitter taste limits their culinary use. To address this, a study aimed to create wheat-fenugreek-based bread by adding fenugreek seed powder to wheat flour at different levels (5%, 7.5%, and 10%). The goal was to assess its taste and analyze its nutritional content, bioactive compounds, antioxidant capacity, and amino acids. Results showed protein, fat, fiber, ash and carbohydrate contents varied within ranges. The breads had higher levels of bioactive compounds like flavonoids and phenolics compared to the control, though anthocyanin content decreased. Antioxidant capacity significantly increased. The breads also contained essential and non-essential amino acids, with lysine and glycine absent in the control but present in the supplemented bread, particularly in the 7.5% version, which was the most preferred in sensory tests.

Feyzi *et al.* (2017) study looked at how acetone, chloroform, hexane, diethyl ether, and surface and thermal characteristics of fenugreek protein isolate were affected, as well as its functioning. The research showed that all of the fenugreek protein isolates had different secondary structures, depending on the solvent employed and that this was validated by using Fourier-transform infrared (FTIR) analysis. Concerning the coagulated protein %, interfacial tension, emulsifying capabilities, surface hydrophobicity, thermal denaturation temperature (Td) and SDS-PAGE profiles in fenugreek protein isolates, hexane and diethyl ether were the solvents that exhibited the most comparable effects. Because of its exceptional foaming capabilities, fenugreek protein isolates produced from ethanol-defatted wheat have the lowest surface tensions. In contrast, the fenugreek protein isolate with the greatest Td and enthalpy change (ΔH) was obtained by acetone-defatted fenugreek flour. Based on the polarity of the defatting solvents, the amino acid composition analysis showed that fenugreek protein isolates from hexane-defatted fenugreek flours had the largest quantities of hydrophobic amino acids, whereas fenugreek protein isolates from acetone-defatted fenugreek flours had the highest levels of charged hydrophilic amino acids. Additionally, the lowest thermal stability in fenugreek protein isolate was seen in chloroform, as validated by thermographic analysis and coagulated protein %.

Singh *et al.* (2015) Fenugreek (*Trigonella foenum-graecum* L.) growth and quality were examined in relation to zinc (Zn) levels, one of many plant growth regulators. Compared to both untreated controls and plants treated with thiourea, plants given foliar applications of triacontanol and NAA showed significantly improved growth characteristics and nitrogen (N), phosphorous (P) and zinc (Zn) absorption. In addition, the control group had lower quantities of nitrogen, phosphorus and zinc in the straw and seeds, whereas the group that used growth regulators had higher protein content in the seeds. In addition, compared to rates of 0 and 2 kg Zn/ha, the growth and quality attributes were much improved when 5 kg Zn/ha was applied.

Behairy *et al.* (2012) study investigated the treating fenugreek (*Trigonella foenum-graecum* L.) seeds with ascorbic acid led to improved germination, longer seedling shoots and higher chlorophyll levels when exposed to salt stress compared to untreated seeds. Salt-stressed seedlings from untreated seeds exhibited increased factors such as root length, amount of proline, dry and fresh weight and catalase activity (CAT). On the other hand, ascorbic acid treatment of seedlings reduced the effect of salt stress on CAT activity. While treating with ascorbic acid reversed the salt stress-induced decrease in ascorbate oxidase (AO), it seemed that the two factors interacted to reduce proline dehydrogenase activity. To make fenugreek seeds more resistant to salt stress, ascorbic acid was applied to them. This increased their antioxidant activity. Serum ascorbic acid therapy and salt stress both altered seedling metabolism, as seen by changes in salt- and ascorbic acid-linked peroxidase (Prix) and esterase (Est) isozymes, respectively. Because of these alterations, ascorbic acid-treated seedlings were probably more robust and able to survive salt stress.

Legesse *et al.* (2020) study investigated that the species had the greatest impact on the chemical compositions of grains, however variations in composition can also exist amongst varieties. Compared to the teff types, the oat cultivars' chemical composition was almost same, if not superior in certain areas. It is possible to supply mixes that are appropriate for creating injera and other related teff products, in addition to fenugreek, which is a significant of protein and amino acids. It is necessary to conduct more research on how agronomic practices and food processing can alter the biochemical contents of grains.

Chapter 3: Materials and Methods

3.1 Experimental site and Design

A pot experiment was conducted at semi-arid climatological environment of Faisalabad at experimental sector of PARS, University of Agriculture, Faisalabad, Pakistan. Faisalabad is also famous because of textile industry so it is also known as Manchester of Pakistan. Faisalabad (formerly Lyallpur) was also one of British India's first planned Cities and it has since grown into a cosmopolitan metropolis. The Chenab River Delta plain is home to Pakistan's third largest metropolis, which has a population of approximately 2.5 million people. There is little separation of home and industrial trash dumps in Faisalabad. Groundwater is polluted from a variety of sources and its salt level is rising by the day. Many industries discharge their trash straight into sewer system. Toxic metals (Cu, Pb, Ni, Cd and Zn) entered in human bodies by taking contaminated food, by metals than those vegetables which are growing by using ground waste.

Fenugreek seeds of one variety were collected from AARI (Ayub Agriculture Research Institute, Faisalabad). Planting began on 9-10-2023. Factorial arrangement was used to create a CRD experiment. The experimental investigation was carried out in plastic pots. This particular pot measured 12 inches in length and 24 inches in height. The experiment was performed by using 18 standard plastic pots. Each pot was filled with 7kg soil sprout. When plants germinate, only 8-10 plants were left after thinning. Following parameters were recorded. Crop was irrigated according to their needs.

3.1.1 Chemicals

Cadmium, amino acid (proline) acetone, distilled water,

3.1.2 Equipment's

Measuring cylinders, beakers, round bottom flask, test tubes, tubes rack, measuring scale, Spectrophotometer, mortar and pestle, weight balance.

3.2 Methodology

3.2.1 Treatments

<u>Factor A. Amino acid application</u>

C1= 12kg/hac

C2=20kg/hac

<u>Factor B. Cadmium application</u>

C1= control

C2=15ppm

C3=30ppm

3.2.2 Crop husbandry

The manual process of seed sowing was carried out in pots and the application of fertilizer was coincided with seed germination. All other factors such as irrigation and plant protection, were consistently applied across all treatments. Plant growth was systematically monitored throughout the season at regular intervals.

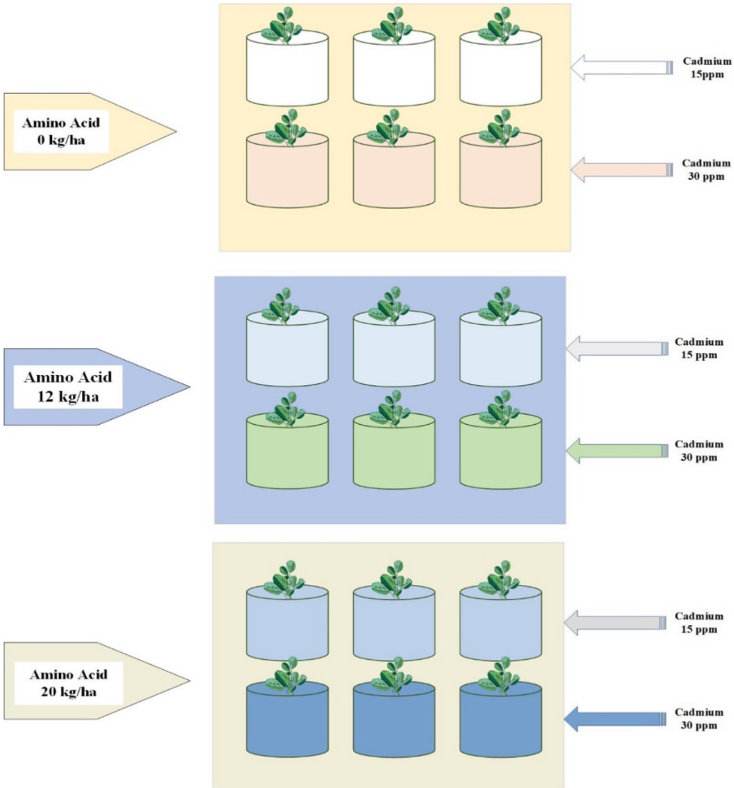

Fig 3.1: Experiment layout of fenugreek

3.3 Observations

During the experiment data was collected on the following parameters

3.3.1 Root Length

One or two plants was uprooted from each replication then length was measured by using a measuring scale and their average were calculated.

3.3.2 Shoot Length

One plant was uprooted and shoot length was measured by using a measuring scale and their mean was calculated.

3.3.3 Root Fresh Weight

One plant from each replication was uprooted carefully and roots were separated from each plant. Roots were washed out, cleaned and their fresh weight was measured by using weighting balance.

3.3.4 Shoot Fresh Weight

One plant was collected from each replication and cleaned. Then fresh shoot weight of each plant.

3.3.5 Dry Root Weight

Plant shoots was kept out door for two days and later on they were put in oven for ten days. Electrical weight balance was used for measuring weight. An average was taken for three plants in each pot and then all replicates for further analysis.

3.4 Yield parameters

3.4.1 Number of flowers on plant[1]

The number of flowers per plant can vary depending on factor like cultivar, growing condition, and climate. On average, a fenugreek plant can produce around 10- 20 flower cluster, with each cluster containing 5-10 individual flowers.

3.4.2 Economic or agricultural yield

The volume or weight per unit area of only those plant parts that have marketable value. Economic yield was calculated after fenugreek harvests and the weight was calculated on electrical weight balance. Then the mean was calculated and recorded.

3.4.3 Biological yield

The total dry matter produced per plant or per unit area. It includes all of the leaf, stem, seeds and root dry matter produced by the plant. After the final round of fenugreek harvest, the plants were uprooted and tied together to weight on weighting balance, then the weight was calculated by adding economic yield to total plant weight, mean was calculated and recorded.

3.4.4 Harvest index

The overall amount of dry matter that a plant produces in relation to its area. It encompasses the plant's whole dry matter output, including leaves, stems, seeds and roots. After the final round of fenugreek harvest, the plants were uprooted and tied together to weight on weighting balance, then the weight was calculated by adding economic yield to total plant weight, mean was calculated and recorded. The harvest index is calculated by dividing the pounds of seeds by the total pounds of above-ground biomass.

Harvest index = economic yield ÷ biological yield × 100

3.5 Physiological parameters

3.5.1 Photosynthetic Pigments

The following method was used to determine the physiological attributes. Chlorophyll content of one plant from each replication was measured by using Davis (1976) and Arnon (1949) method. One fresh leaf from each replication was collected and 0.5 g of leaf was grinded into mortal and pestles. Afterwards, the extract was mixed with 5 ml of 80% acetone and let to sit overnight at 10°C. Centrifuged at 14,000 rpm for 5 minutes the next day was the procedure for the extract. Spectrophotometer readings were taken at 480 nm, 645 nm and 663 nm for carotenoids, chlorophyll b and chlorophyll a, respectively, according to the formula shown below.

$$\text{Carotenoids} = [4.16(\text{OD}480)-0.89(\text{OD}663)] \times V/1000 \times W$$

$$\text{Chl. a} = [12.7\ (\text{A}663)-2.69(\text{A}645)]\ {*}V/1000{*}W$$

$$\text{Chl. b} = [22.9\ (\text{A}645-4.68(\text{A}663)]\ {*}V/1000{*}W$$

$$\text{Total Chl.} = [20.2\,(OD645) - 8.02(OD663)] \times V/1000 \times W$$

While A describes absorbance value (nm), V as volume (ml), W indicates weight (g).

3.5.2 Statistical Analysis

The data analysis for all parameters were conducted through the COSTAT and "Minitab" software, using three replicates in a completely randomized design (CRD). At a 5% probability level, the treatment means were evaluated using ANOVA and Tukey's (HSD) test (Simoncini et al.,2020).

Chapter 4: Analysis & Results

4.1 Growth Parameters

4.1.1 Root Length

The dataset shown in Table 4.2 presents information about the response of fenugreek cultivars to different levels of cadmium stress, together with the application of Amino acid (proline) by using solution methods. The experimental findings demonstrated that the application of different concentrations of amino acid (proline) to exposure the cadmium-induced stress, significantly influenced die growth of root length of fenugreek (Abdelhameed *et al.*, 2021). The results of the data analysis revealed that fenugreek had a statistically significant decrease in root length under the conditions of cadmium-induced stress. The aforementioned observations were conducted in an environment characterized by cadmium stress, with a concentration of 15ppm and 30ppm while the Amino acid concentration of 0ml.The maximum root length seen in the fenugreek experiment conducted where the amino acid (proline) concentration was 12ml. The root length shown a decrease as the level of cadmium stress increased. Nevertheless, it has been shown that the administration of amino acid (proline) to the foliage effectively mitigates the adverse effects caused by cadmium-induced stress (Liu *et al.*, 2021). The use of amino acid (proline) has been shown to be a pivotal growth regulator in mitigating the adverse effects of heavy metal stress on fenugreek plants. Results accordance with those of that amino acid show beneficial effect to growth and development in fenugreek under the stressful condition. Results suggest that amino acid (proline) play vital role in growth and development when plant subjected into heavy metal stress.

Table 4.1: Comparison of mean of Root length of fenugreek on different levels of amino acid (proline) under cadmium stress.

	15ppmCd	30ppmCd	Mean
0Kg AA	3.8	2.9	3.35
12Kg AA	5.1	4.1	4.6
20Kg AA	6.4	4.8	5.6
Mean	5.1	3.93	

Table 4.2: Statistical analysis of data for Root length effected by Amino Acid (proline) under Cadmium stress.

SOV	DF	Adj	MS	F	P
AMINO ACID	2	20.8933	10.4467	160.52	0.000***
CADMIUM	1	2.9606	2.9606	45.49	0.000***
Error	14	0.9111	0.0651		
Lack-of-Fit	2	0.0178	0.0089	0.12	0.888 ns
Pure Error	12	0.8933	0.0744		
Total	17	24.7650			

*, *= significant at 0.05,0.01 and 0.001 level respectively

ns=non-significant

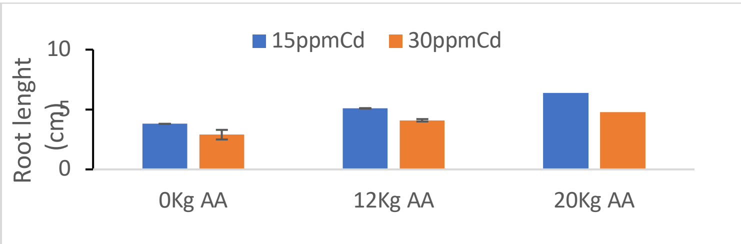

Figure 4.1: Effect of different concentrations of Amino acid (proline) application on root length of fenugreek under cadmium stress.

4.1.2 Shoot Length

ANOVA, exposed that plant length was significantly influence by cadmium stress. Amino acid (proline) enhanced the shoot length. For shoot length, the data presented highly significant difference in stress treatments and amino acid ($P<0.05$), but non-significant ($P>0.05$) collaboration between amino acid, cultivars and cadmium stress factors in table etc. Overall results showed that maximum shoot height was observed under controlled conditions. And minimum shoot height was observed when cadmium stress was applied. Amino acid (proline) enhanced shoot length under cadmium stress as given in Table 4.4. Application of cadmium stress cause significant reduction in shoot length. Decrease shoot length was greater under stress application (15ppm,30ppm) as compared to controlled condition (Suman *et al.*, 2017). Maximum shoot length was detected in fenugreek when plants treated with amino acid (proline) under controlled condition. Cultivar fenugreek more affected with cadmium stress. Exogenous treatments of amino acid (proline) enhanced the shoot length under different stress level. The study exposed that amino acid has maximum effects on height of shoot under cadmium stress levels. The results reported by (Zhu *et al.*, 2018) also explained that cadmium stress affects the growth and shoot length which finally reduced plant growth and shoot length. Our outcomes are in accordance with those of that amino acid (proline) was essential to enhanced plant growth and shoot length of some crop species under heavy metal stress.

Table 4.3: Comparison of mean of shoot length of fenugreek on different levels of amino acid (proline) under cadmium stress.

	15ppCd	**30ppmCd**	**Mean**
0Kg AA	20.3	14.2	3.05
12Kg AA	31.4	26.7	2.35
20Kg AA	48.2	37.8	5.2
Mean	9.93	8.02	

Table 4.4: Statistical analysis of data for Root length effected by Amino Acid (proline) under Cadmium stress.

SOV	DF	SS	MS	F	P
AMINO ACID	2	20.8933	10.4467	160.52	0.000***
CADMIUM	1	2.9606	2.9606	45.49	0.000***
Error	14	0.9111	0.0651		
Lack-of-Fit	2	0.0178	0.0089	0.12	0.888 ns
Pure Error	12	0.8933	0.0744		
Total	17	24.7650			

*, *= significant at 0.05,0.01 and 0.001 level respectively ns=non-significant

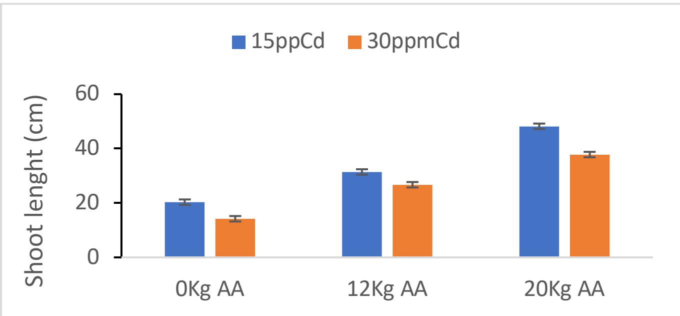

Figure 4.2: Effect of different concentrations of Amino acid (proline) application on shoot length of fenugreek under cadmium stress.

4.1.3 Root Fresh Weight

Table 4.6 shows the different levels of cadmium stress effect on fenugreek when amino acid (proline) is applied into soil it showed that the amount of amino acid and how it works with cadmium stress has a vital effect on the total length of fenugreek plant (Bashri and Prasad, 2016). Under cadmium stress, the results showed that the root length was shorter when

(15ppm,30ppmCd) and amino acid (proline) was applied 0ml concentration. The longest root length was found when amino acid (proline) was applied 20ml on (15ppm and 30ppm) cadmium. As the amount of cadmium stress went up, the root length of plant got shorter. However, the effects of cadmium were mitigated by applying amino acid into the soil on fenugreek plants in the order 12ml and 20ml. As a result, amino acid was a key growth factor for fenugreek plants that helped it deal with heavy metal stress. Reported cadmium stress decrease the fresh weight of root in fenugreek plans. Cadmium more toxic as compare to other heavy metals due to its solubility. According to fresh weight of root decrease due to present of different level of cadmium in soil and effect the overall performance fenugreek plants (Mukherjee, 2017). According to fenugreek plants have more capability to compete with heavy metal stress as compare to other plants. Show the maximum root length under the cadmium stress. Observed that the soil application of amino acid mitigates the negative effects of cadmium stress and boost the mechanism of plants. According to amino acid (proline) mitigate the maximum harmful impacts of cadmium in fenugreek plants and increase the root performance under the stressful condition. Revealed that amino acid (proline) plant growth regulator mitigates the negative impacts of heavy metal stress. Suggested that the soil application of amino acid (proline) play a vital role under the cadmium Suggested m stress and boost the overall mechanism of plant (Zhu *et al.*, 2018).

Table 4.5: Comparison of mean of root fresh weight of fenugreek on different levels of amino acid (proline) under cadmium stress.

	15ppmCd	**30ppmCd**	**Mean**
0Kg AA	3.8	2.9	3.35

12Kg AA	5.1	4.1	4.6
20Kg AA	6.4	4.8	5.6
Mean	5.1	3.933	

Table 4.6: Statistical analysis of data for root fresh weight effected by Amino Acid (proline) under Cadmium stress.

SOV	DF	SS	MS	F	P
AMINO ACID	2	787.19	393.594	130.47	0.000***
CADMIUM	1	66.93	66.932	22.19	0.000***
Error	14	42.23	3.017		
Lack-of-Fit	2	12.06	6.031	2.40	0.133 ns
Pure Error	12	30.17	2.514		
Total	17	896.35			

*, *= significant at 0.05,0.01 and 0.001 level respectively ns=non-significant

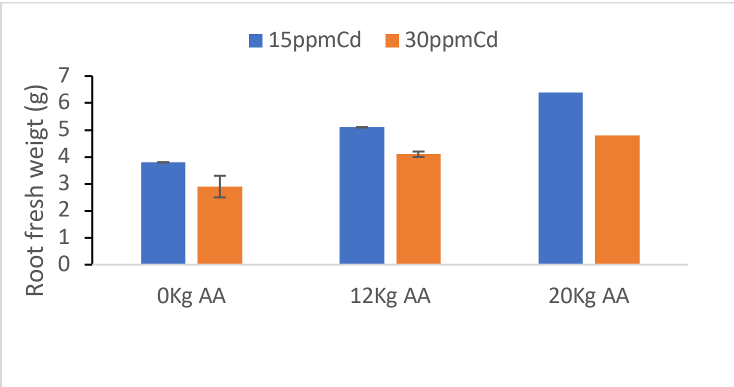

Figure 4.3: Effect of different concentrations of Amino acid (proline) application on root fresh weight of fenugreek under cadmium stress.

4.1.4 Shoot Fresh Weight

Table 4.8 shows the different levels of cadmium have non significantly affect the fresh weight of shoot in fenugreek plant. When amino acid (proline) applied in to the soil. It showed that the amount of amino acid and how it works with a cadmium stress has a vital effect on the total length of fenugreek plant.

Under cadmium stress, the results showed that the fenugreek weight of shoot was decreased. As the amount of cadmium stress went up, the fresh weight of fenugreek. However, the effects of cadmium were mitigated by applying amino acid in to the soil of fenugreek plant in the order (12ml and 20ml). As a result, amino acid (proline) was the key growth factor for fenugreek that helped it deal with heavy metal stress. The results reported by different level of cadmium stress effect the shoot fresh weight in fenugreek plant. Results of his study suggested that as increase the level of cadmium decrease the shoot fresh weight in plants. Reported that shoot fresh weight badly effected by cadmium stress. According to cadmium is more toxic as compare to other heavy metal due to its solubility. Suggest that Amino acid (proline) is a vital plant organic compound for proper growth and development of plants under heavy metal stress. Revealed that amino acid (proline) increases the shoot fresh weight under the stressful conditions. Observed amino acid (proline) important plant growth regulator that enhance the shoot fresh weight and yield under the different level of cadmium stress. Reported that different level of amino acid mitigates the harmful impacts of cadmium stress in fenugreek plant and boost the metabolism in results of these impacts the overall yield production increase by mitigating the negative effects of cadmium. Suggest that amino acid most crucial plant organic compound in heavy metal stress for boosting the plant growth and development.

Table 4.7: Comparison of mean of shoot fresh weight of fenugreek on different levels of amino acid (proline) under cadmium stress.

	15ppmCd	**30ppmCd**	**Mean**
0Kg AA	20.3	14.2	3.05
12Kg AA	31.4	26.7	2.35

20Kg AA	48.2	37.8	5.2
Mean	9.93	8.02	

Table 4.8 Statistical analysis of data for shoot fresh weight effected by Amino Acid (proline) under Cadmium stress.

SOV	DF	SS	MS	F	P
AMINO ACID	2	11.9452	5.97261	56.88	0.000***
CADMIUM	1	0.0128	0.01280	0.12	0.732 ns
Error	14	1.4701	0.10501		
Lack-of-Fit	2	0.0489	0.02445	0.21	0.816 ns
Pure Error	12	1.4212	0.11843		
Total	17	13.4281			

*, *= significant at 0.05,0.01 and 0.001 level respectively ns=non-significant

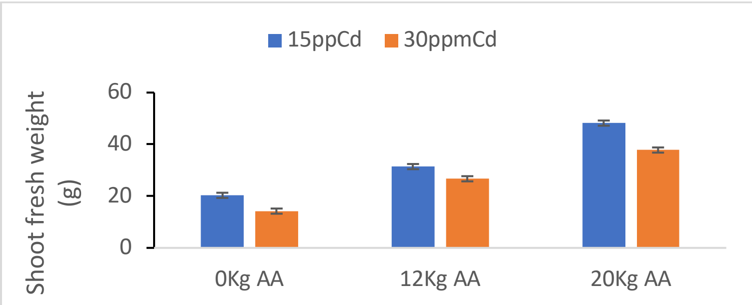

Figure 4.4: Effect of different concentrations of Amino acid (proline) application on shoot fresh weight of fenugreek under cadmium stress.

4.1.5 Root Dry Weight

Table 4.10 shows the different levels of cadmium stress effect the fenugreek plant when Amino acid (proline) is applied into the soil. It showed that the amount of Amino acid (proline) and how it works with cadmium stress has a vital effect on the root dry weight of fenugreek plant. The highest dry compared were found in cadmium stress was not applied and the lowest fresh weight were found the cadmium highest applied. Where cadmium stress was 30ppm and amino acid (proline) was 0ml. The highest root dry weight that was seen in fenugreek where the amino acid (proline) level was 20ml. As the amount of cadmium stress went up, the root dry weight of plant got lower (Zamani *et al.*, 2020). However, the effects of cadmium were mitigated by applying amino acid into the soil of fenugreek applied the various concentration of amino acid in the order 12ml and 20ml. As a result, amino acid was a key growth factor for fenugreek that helped it deal with heavy metal stress.

Table 4.9: Comparison of mean of root dry weight of fenugreek on different levels of amino acid (proline) under cadmium stress.

	15ppm Cd	**30ppm**	**Mean**
0Kg AA	0.03	0.02	0.025
12Kg AA	0.04	0.03	0.035
20Kg AA	0.05	0.04	0.045
Mean	0.04	0.03	

Table 4.10 Statistical analysis of data for root dry weight effected by Amino Acid (proline) under Cadmium stress.

SOV	**DF**	**SS**	**MS**	**F**	**P**
AMINO ACID	2	0.18136	0.09068	3.55	0.057*
CADMIUM	1	0.08027	0.08027	3.14	0.098 ns
Error	14	0.35762	0.02554		
Lack-of-Fit	2	0.14001	0.07000	3.86	0.051*
Pure Error	12	0.21761	0.01813		
Total	17	0.61925			

*, *= significant at 0.05,0.01 and 0.001 level respectively ns=non-significant

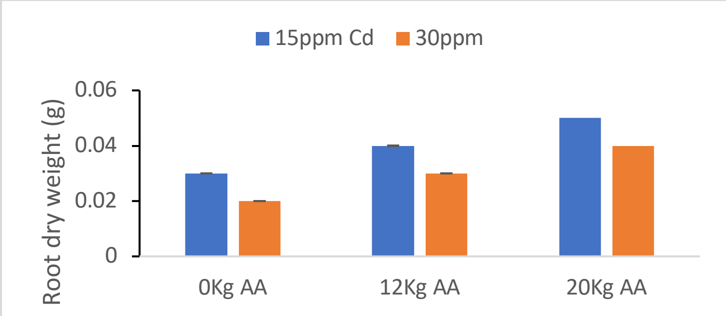

Figure 4.5: Effect of different concentrations of Amino acid (proline) application on root dry weight of fenugreek under cadmium stress.

4.1.6 Shoot Dry Weight

Table 4.12 shows the different levels of cadmium stress effect on fenugreek plant. When amino acid (proline) is applied into the soil. It showed that the amount of amino acid and how it works with cadmium stress has a vital impact in the shoot dry weight of fenugreek plant. Under cadmium. stress, the results showed that the highest shoot dry weight when cadmium applied 30ppm. Where cadmium stress was 30ppm and amino acid (proline) was 0ml the highest shoot dry weight that was seen in fenugreek plant. Where the amino acid (proline) level was 20ml. As the amount of Cd toxicity went up, the dry weight of plant got lower. However, the effects of cadmium were mitigated by applying amino acid (proline) into soil the order (0ml, 12ml, 20ml). As a result, amino acid was a key growth factor of fenugreek that helped it deal with heavy metal stress. The research conducted by (FAIZY *et al.*, 2022) revealed that cadmium has a detrimental effect in the overall performance of fenugreek plant as well as on the shoot dry weight of the fenugreek. The research conducted by (Aqeel *et al.*, 2022) reveals that the presence of cadmium ions disrupts the metabolic processes of fenugreek via their interaction with the essential components of the fenugreek. Find out by (Almutairi *et al.*, 2023) amino acid (proline) a plant organic compound, has a role in mitigating the negative impacts of cadmium stress on plants. It creates a favorable environment for the development of fenugreek. Amino acid (proline) is a naturally occurring organic compound present in several plant species.

Table 4.11 Comparison of mean of shoot dry weight of fenugreek on different levels of amino acid (proline) under cadmium stress.

	15ppmCd	30ppmCd	Mean
0Kg AA	1.12	0.68	0.22
12Kg AA	1.81	1.12	0.345
20Kg AA	2.21	1.36	0.425
Mean	0.395	0.24	

Table 4.12 Statistical analysis of data for shoot dry weight effected by Amino Acid (proline) under Cadmium stress**.**

SOV	DF	SS	MS	F	P
AMINO ACID	2	1158.02	579.008	212.24	0.000***
CADMIUM	1	100.21	100.206	36.73	0.000***
Error	14	38.19	2.728		
Lack-of-Fit	2	5.37	2.687	0.98	0.403 ns
Pure Error	12	32.82	2.735		
Total	17	1296.41			

*, *= significant at 0.05,0.01 and 0.001 level respectively
ns=non-significant

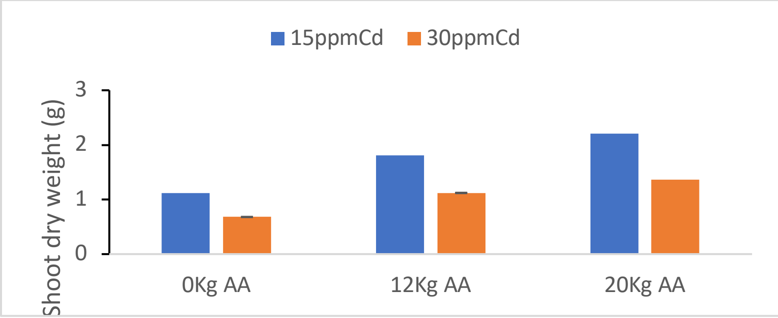

Figure 4.6: Effect of different concentrations of Amino acid (proline) application on shoot dry weight of fenugreek under cadmium stress**.**

4.1.7 Number of flowers plant^{-1}

Table 4.14 presents the impact of varying degrees of cadmium-induced stress on fenugreek plant with the application of amino acid (proline) to the flowers. The results demonstrated that the concentration of amino acid and its interaction with cadmium-induced stress significantly influenced the number of flowers plant^{-1} in fenugreek cultivars (Mahmud *et al.*, 2020).The experimental findings that under the conditions of stress, no of flowers plant^{-1} in fenugreek was seen to be significantly greater in comparison to 15ppm and 30ppm cadmium stress. The fenugreek cultivar exhibited the maximum number of flowers plant^{-1} whereas the 30ppm cadmium stress is applied. Cultivar had the minimum number of flowers plant^{-1} under the conditions of 30ppm cadmium stress and 0ml amino acid. The maximum number of flowers plant^{-1} in fenugreek when the amino acid (proline) concentration was 20ml. As the level of cadmium-induced stress increased, there was a corresponding decrease in the number of flowers per plant of each pot. Nevertheless, the impact of cadmium was alleviated by administering amino acid to the foliage of fenugreek, with the concentrations of (0, 12, 20ml) and being arranged in ascending sequence. Consequently, amino acid (proline) emerged as pivotal growth regulator for fenugreek aiding in its adaptation to the adverse effects of heavy metal stress (Ould said *et al.*, 2021).

Table 4.13 Comparison of mean of Number of flowers plant^{-1} of fenugreek on different levels of amino acid (proline) under cadmium stress.

	15ppmCd	30ppmCd	Mean
0Kg AA	20.7	18.5	1.1
12Kg AA	23.7	17.3	3.2
20Kg AA	34.5	28.4	3.0
Mean	5.4	4.6	

Table 4.14 Statistical analysis of data for Number of flowers plant^{-1} effected by Amino Acid (proline) under Cadmium stress.

SOV	DF	SS	MS	F	P
AMINO ACID	2	1715.89	857.947	219.86	0.000***
CADMIUM	1	183.87	183.872	47.12	0.000***
Error	14	54.63	3.902		
Lack-of-Fit	2	0.35	0.177	0.04	0.962 ns
Pure Error	12	54.28	4.523		
Total	17	1954.40			

*, *= significant at 0.05,0.01 and 0.001 level respectively ns=non-significant

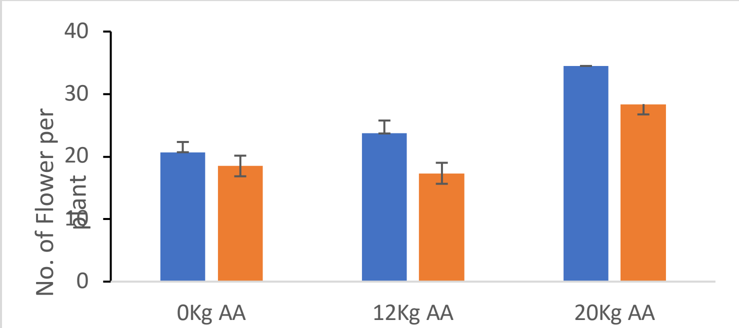

Figure 4.7: Effect of different concentrations of Amino acid (proline) application on Number of flowers plant^{-1} of fenugreek under cadmium stress**.**

4.1.8 Economic yield

Data indicated that the parameter 100 seed weight showed a positive variation by different levels of amino acids (proline). The experimental outcomes clearly showed a gradual enhance in 100 weights of seed with increasing levels of amino acid (proline) (Sadak *et al.*, 2023). Data regarding 100 seed weight are given in Figure (4.8). Significant maximum seed weight (31.83g) was recorded in the pot where amino acid (proline) was applied 12 kg/ha and it was followed (34.83) by the treatment where amino acid (proline) was applied 20kg/ha. Significantly minimum 100 seed weight (18.85) where amino acid (proline) was applied 0 kg/ha and it was followed by treatment where amino acid (proline) was applied 20 kg/ha" (34.83).

Table 4.15: Comparison of mean of economic yield of fenugreek on different levels of amino acid (proline) under cadmium stress.

	15ppmCd	30ppmCd	Mean
0Kg AA	0.12	0.1	0.01
12Kg AA	0.21	0.24	0.015
20Kg AA	0.27	0.24	0.015
Mean	0.053	0.062	

Table 4.16 Statistical analysis of data for economic yield effected by Amino Acid (proline) under Cadmium stress.

SOV	DF	SS	MS	F	P
AMINO ACID	2	1890.94	945.471	219.12	0.000***
CADMIUM	1	153.83	153.826	35.65	0.000***
Error	14	60.41	4.315		
Lack-of-Fit	2	5.48	2.738	0.60	0.565 ns
Pure Error	12	54.93	4.578		
Total	17	2105.17			

*, *= significant at 0.05,0.01 and 0.001 level respectively ns=non-significant

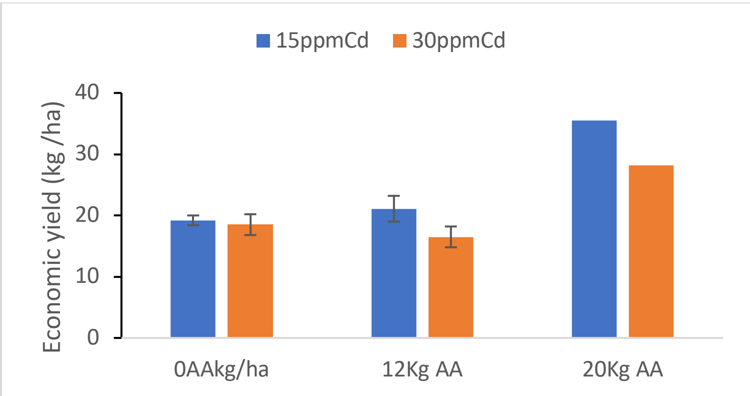

Figure 4.8: Effect of different concentrations of Amino acid (proline) application on economic yield of fenugreek under cadmium stress.

4.1.9 Biological yield

The results of recent research demonstrated that using different levels of amino acid (proline) organic compound promotes biological yield $kgha^{-1}$ in fenugreek plant. Significant maximum biological yield was recorded in the treatment where amino acid (proline) by minimum biological yield (70kg/ha) in where amino acid (proline) was applied 12kg. and maximum biological yield 90kg/ha in were amino acid (proline) applied 20 kg.

Different levels of amino acid organic compound had an impact on the biological yield of fenugreek plants. Amino acid (proline) is crucial for development of plant and plays a have a valuable in enhancing the overall productivity of fenugreek plants (Abdelhameed *et al.*, 2021). Higher amino acid (proline) levels can promote increased vegetative growth, leading to higher biomass production and potentially resulting in a greater biological yield. However, it's essential to strike a balance, as excessive amino acid (proline) can divert resources towards vegetative growth rather than seed production, potentially affecting the overall yield. Similarly, amino acid is also important for plant health and metabolism. In conclusion, providing appropriate levels of amino acid (proline) organic compound can significantly contribute to their overall productivity (Alagawany *et al.*, 2021). The increase in excess production of biological yield due to the effects of amino acid has already been studied by (Kumar and Samadder, 2017) on (*Trigonella foenum graecum* L.) more commonly known as fenugreek, at the Crop Research Centre of Sardar Vallabhbhai Patel University of Agriculture and Technology in Meerut, Uttar Pradesh by using three amino acid (proline) levels (0ml, 12ml, 20ml).

Table 4.17 Comparison of mean of biological yield of fenugreek on different levels of amino acid (proline) under cadmium stress.

	15ppmCd	30ppmCd	Mean
0AAkg /ha	1.7	1.21	0.245
12Kg AA	3.04	2.3	0.37
20Kg AA	4.54	3.8	0.37
Mean	0.964	0.908	

Table 4.18 Statistical analysis of data for biological yield effected by Amino Acid (proline) under Cadmium stress.

SOV	DF	SS	MS	F	P
AMINO ACID	2	24.5494	12.2747	92.06	0.000***
CADMIUM	1	1.3723	1.3723	10.29	0.006**
Error	14	1.8666	0.1333		
Lack-of-Fit	2	0.0591	0.0295	0.20	0.825 ns
Pure Error	12	1.8075	0.1506		
Total	17	27.7883			

*, *= significant at 0.05,0.01 and 0.001 level respectively ns=non-significant

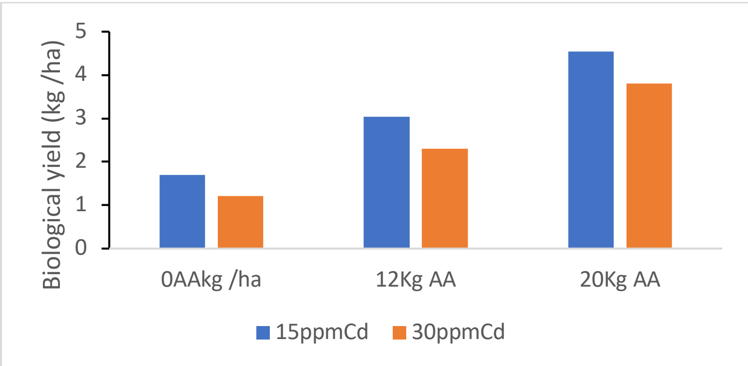

Figure 4.9: Effect of different concentrations of Amino acid (proline) application on biological yield of fenugreek under cadmium stress.

4.1.10 Harvest Index

An indicator of plant output, the harvest index is used in seed production is the seed yield divided by the total biomass of the surface area. The analysis of variance clearly shows that there is a significant disparity. The results of the analysis of variance clearly show that fenugreek and cadmium metal are significantly different. Filler herb and Cadmium metal means. Concerning to this experiment, maximum harvest index (97.15) was found in fenugreek, which differed significantly harvest index (97.15) at 20ml of amino acid (proline) under 30ppm of Cd stress. In addition, the minimum harvest index (61.8) was investigated at 12ml of amino acid (proline) under 15ppm of cadmium stress (Azmat and Moin, 2017). To conclude that our finding is statistically significant, we need a p-value lower than 0.05. The outcome was not be considered significant if the p-value exceeds 0.05. Fenugreek and cadmium interaction showed non-significant result, if probability values are greater than 0.05, then it means that al treatment means are the same and there is no divergence between them.

Table 4.19: Comparison of mean of harvest index of fenugreek on different levels of amino acid (proline) under cadmium stress**.**

	15ppmCd	**30ppmCd**	**Mean**
0Kg AA	46.6	52.7	49.65
12Kg AA	66.4	59.4	62.9
20Kg AA	121	73.3	97.15
Mean	78	61.8	

Table 4.20: Statistical analysis of data for harvest index effected by Amino Acid (proline) under Cadmium stress**.**

SOV	**DF**	**SS**	**MS**	**F**	**P**
AMINO ACID	2	2264.25	1132.13	226.53	0.000***
CADMIUM	1	212.18	212.18	42.46	0.000***
Error	14	69.97	5.00		
Lack-of-Fit	2	1.65	0.83	0.15	0.866 ns
Pure Error	12	68.31	5.69		
Total	17	2546.40			

*, *= significant at 0.05,0.01 and 0.001 level respectively
ns=non-significant

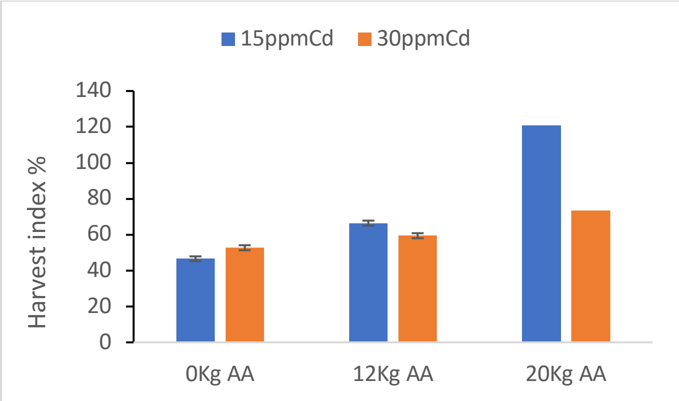

Figure 4.10 Effect of different concentrations of Amino acid (proline) application on biological yield of fenugreek under cadmium stress.

4.2 Quality parameters

4.2.1 Chlorophyll a content of leaves

In all oxygen-evolving photosynthetic organism, including higher green algae, the most prevalent and dominant type of chlorophylls is found most of the absorption occurs between 400 nanometers and 450 and 700, nanometers in the electromagnetic spectrum. It is evident from the analysis of variance that there is a significant difference between fenugreek and Cadmium metal means. Concerning to this experiment, maximum Chl a (0.053) was found in fenugreek with Chl a 0.014) at 30ppm of Cd (Azmat and Moin, 2017). In addition, the minimum Chl a (0.044) was investigated at 15ppm of cadmium. If the p-value is less than 0.05, it indicates that our result is Significant. If the p-value is greater than 0.05, is non-significant. The fenugreek and Cadmium interaction showed non-significant result, it means that all treatment means are there is no divergence between them

Table 4.21: Comparison of mean of Chlorophyll a of fenugreek on different levels of amino acid (proline) under cadmium stress.

	15ppmCd	30ppmCd	Mean
0Kg AA	0.1	0.1	0
12Kg AA	0.21	0.16	0.025
20Kg AA	0.23	0.23	0
Mean	0.053	0.044	

Table 4.22 Statistical analysis of data for chlorophyll a effected by Amino Acid (proline) under Cadmium stress.

SOV	DF	SS	MS	F	P
AMINO ACID	2	0.27840	0.13920	1.07	0.369 ns
CADMIUM	1	0.09202	0.09202	0.71	0.414 ns
Error	14	1.82012	0.13001		
Lack-of-Fit	2	0.28094	0.14047	1.10	0.366 ns
Pure Error	12	1.53917	0.12826		
Total	17	2.19054			

,= significant at 0.05,0.01 and 0.001 level respectively ns=non-significant

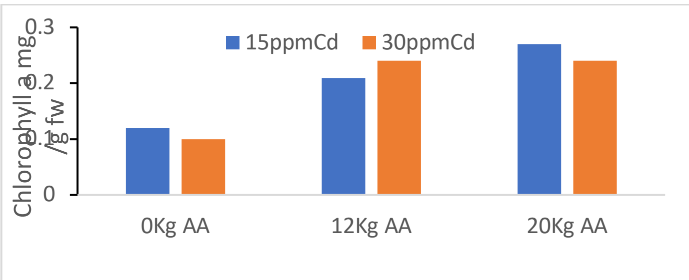

Figure 4.11: Effect of different concentrations of Amino acid (proline) application on Chlorophyll a of fenugreek under cadmium stress.

4.2.2 Chlorophyll b contents of leaves

In all oxygen-evolving photosynthetic organism, including higher green algae, the most prevalent and dominant type of chlorophylls is found most of the absorption occurs between 650 nanometers and 665, nanometers in the electromagnetic spectrum. It is evident from the analysis of variance that there is a significant difference between fenugreek and Cadmium metal means. Concerning to this experiment, maximum Chl b (0.062) was found in fenugreek at 30ppm of Cd. In addition, the minimum Chl b (0.053) was investigated at 15ppm of cadmium (Hare et al., 2017). If the p-value is less than 0.05, it indicates that our result is Significant. If the p-value is greater than 0.05, is non-significant. The fenugreek and Cadmium interaction showed non-significant result, it means that all treatment means are there is no divergence between them.

Table 4.23: Comparison of mean of Chlorophyll b of fenugreek on different levels of amino acid (proline) under cadmium stress.

	15ppmCd	30ppmCd	Mean
0Kg AA	0.12	0.1	0.01
12Kg AA	0.21	0.24	0.015
20Kg AA	0.27	0.24	0.015
Mean	0.05	0.06	

Table 4.24 Statistical analysis of data for Chlorophyll b effected by Amino Acid (proline) under Cadmium stress**.**

SOV	DF	SS	MS	F	P
AMINO ACID	2	0.008584	0.004292	0.41	0.673 ns
CADMIUM	1	0.059628	0.059628	5.67	0.032**
Error	14	0.147244	0.010517		
Lack-of-Fit	2	0.070259	0.035129	5.48	0.020**
Pure Error	12	0.076985	0.006415		

Total	17	0.215456			

*, *= significant at 0.05,0.01 and 0.001 level respectively
ns=non-significant

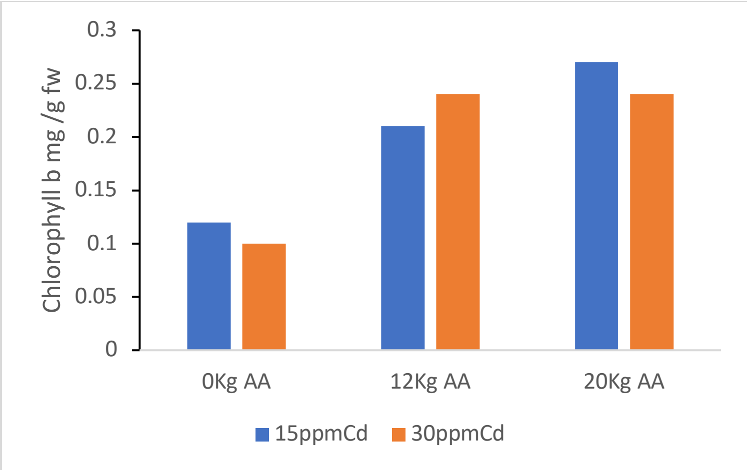

Figure 4.12: Effect of different concentrations of Amino acid (proline) application on Chlorophyll b of fenugreek under cadmium stress.

4.2.3 Carotenoid content of leaves

Pigment found in many plants that gives color to flowers, fruits and seeds photosynthesis as part of the light-harvesting complicated and photo-oxidation prevention are two of their functions is evident from statistical variance that there is a reasonable variation fenugreek and Cadmium metal means. Concerning to this experiment, maximum carotenoids (0.06) was found in fenugreek which significantly differed with carotenoids (0.06) at 30ppm of Cd (Missaoui et al., 2017). In addition, the minimum carotenoids (0.05) were investigated at 15ppm. If the p-value is less than 0.05, it indicates that our result is Significant. If the p-value is greater than 0.05, our result is non-significant. The varieties and cadmium interaction showed non-significant result, if probability values are greater than 0.05, then it means that all treatment means are the same and there is no divergence between them.

Table 4.25: Comparison of mean of Carotenoid of fenugreek on different levels of amino acid (proline) under cadmium stress.

	15ppmCd	30ppmCd	mean
0Kg AA	0.1	0.1	0.1
12Kg AA	0.2	0.2	0.1
20Kg AA	0.3	0.3	0.2
Mean	0.0	0.0	

Table 4.26 Statistical analysis of data for Carotenoid effected by Amino Acid (proline) under Cadmium stress**.**

SOV	DF	SS	MS	F	P
AMINO ACID	2	0.39096	0.195480	24.66	0.000***
CADMIUM	1	0.01796	0.017964	2.27	0.154 ns
Error	14	0.11099	0.007928		
Lack-of-Fit	2	0.02265	0.011325	1.54	0.254 ns
Pure Error	12	0.08834	0.007361		
Total	17	0.51991			

*, *= significant at 0.05,0.01 and 0.001 level respectively ns=non-significant

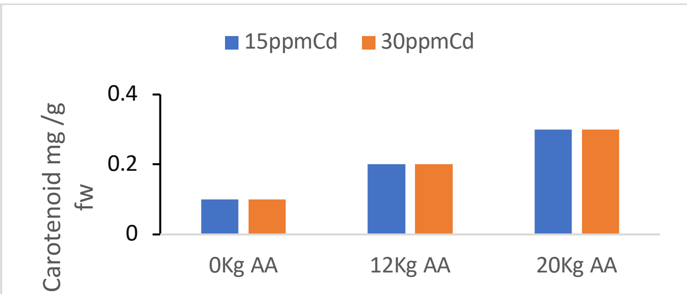

Figure 4.13: Effect of different concentrations of Amino acid (proline) application on Carotenoid of fenugreek under cadmium stress.

Chapter 5: Discussion & Summary

This trial's goal was to determine whether amino acid (proline) might mitigate the negative effects of Cd on fenugreek plant. High level of Cd caused plants to develop more slowly. As cadmium stress rises, seed germination rates decrease. Cadmium has a major impact on seed germination. Reducing the production of agricultural and horticultural crops in response to diverse environmental difficulties is a substantial economic loss (Aslam, Okal and Waseem 2023). Hence, inducing cadmium stress tolerance in plants has been a major focus of study for many decades. For this reason, it is crucial and required to develop strategies to generate stress tolerance in plants against harsh climatic circumstances

One of the major metabolic processes that plants partake in that is very sensitive to environmental perturbations is photosynthesis (Dinakar, Djilianov and Bartels 2012). It is examined that Plants exhibit toxic symptoms as a result of diminished photosynthetic activity and the breakdown of chlorophyll pigments caused by the presence of heavy metals, such as Cd. All the crops showed a minor rise in seed yield, which was likely caused by some beneficial but poorly understood effects of Cd on plant growth at low concentrations. As opposed to this, soil-applied Amino acid (proline) modestly enhanced biomass yields, which most likely happened because Cd toxicity was reduced. In this Amino acid (proline) deficient soil, Amino acid (proline) application (20 kg /ha) also increased crop yield (Iqbal, Jones et al. 2020). Soil-applied Amino acid inhibited the toxic effects of Cd in plant tissues, enabling the plant to deal with its toxicity and promoting plant growth.

Low Cd application levels (15ppm) were observed to have a synergistic effect with Amino acid (proline) accumulation, but larger Cd application (30ppm) had the opposite effect (Al Waeli 2015). Also showed that Amino acid accumulation increased at low Cd levels and decreased at high Cd levels. They showed that Amino acid accumulation in plant roots was greatly reduced by Cd stress, and that the antagonistic interaction between Amino acid (proline) and Cd may be a beneficial tool for the safe production of crops with low Cd content.

In addition to the morphological improvements, AA (proline) treatment also positively influenced the physiological attributes of fenugreek plants under cadmium stress. The study observed that AA (proline) application led to increased chlorophyll content, enhanced photosynthetic efficiency and improved water use efficiency. These physiological changes indicated that Amino acid (proline) treatment helped to maintain the plant's photosynthetic capacity and optimize its water utilization, which are crucial for plant growth and survival (Rahman, Mostofa et al. 2021).

Overall, the study demonstrated that Amino acid (proline) treatment had a significant impact on fenugreek plants under cadmium stress. AA (proline) application alleviated the detrimental effects of cadmium stress by promoting morphological growth, enhancing biomass production and improving physiological attributes related to photosynthesis and water use efficiency. These findings suggest that AA (proline) treatment could be a potential strategy to increase the tolerance of fenugreek plants within cadmium stress and improve their overall performance in contaminated environments.

Amino acid may prevent Cd from loading into phloem. This restricted Cd loading in the phloem may lessen the buildup of Cd in plant seeds. The competition between Amino acid (proline) and Cadmium at different levels during uptake at the surface of roots and transport within plants may be the cause of the influence of amino acid on Cd uptake and translocation in plants.

Through the enhanced uptake of vital nutrients by plants under Cd stress, exogenous amino acid (proline) supplies may improve plants' ability to withstand Cd stress. For instance, compared to control treatments, soil applications of Amino acid+ Cadmium enhanced the levels of important elements (such as Fe, Mn, copper (Cu), calcium (Ca) and magnesium (Mg)) in fenugreek plant (Rizwan, Ali et al. 2017).

Amino acid (proline) may protect the plants from Cd stress by restoring the structures under Cd stress. Excess metal induced structural abnormalities in plants and disrupted subcellular metal distribution in plants. Plant tolerance to Cd stress may be greatly improved by Amino acid-mediated structural changes in Cd-stressed plants. By altering the Cd fractions in various plant tissues, Amino acid (proline) may protect the plant (Yang, Zhu et al. 2018).

In summary, the study demonstrated that Amino acid (proline) treatment had positive impacts in fenugreek plants treater with cadmium toxicity, amino acid (proline) application mitigated the toxic impacts of cadmium stress on plant growth, including plant height, root length and biomass accumulation. Moreover, amino acid (proline) treatment improved physiological attributes by maintaining higher chlorophyll content, enhancing photosynthetic efficiency and strengthening antioxidant mechanism in fenugreek plants exposed to cadmium stress. These findings suggested that amino acid could be a potential tool for ameliorating cadmium-induced stress in fenugreek plant and potentially other plants as well.

Under cadmium stress, fenugreek (*Trigonella foenum graecum* L.) was studied to see how amino acid (proline) affected its morphology and physiology. Heavy metal cadmium has a deleterious effect on plant development and growth. The purpose of this research was to determine whether fenugreek plants might be protected from the harmful effects of cadmium stress by using proline therapy.

In addition to the morphological improvements, AA (proline) treatment also positively influenced the physiological attributes of fenugreek plants under cadmium stress. The study observed that AA (proline) application led to increased chlorophyll content, enhanced photosynthetic efficiency, and improved water use efficiency. These physiological changes indicated that AA (proline) treatment helped to maintain the plant's photosynthetic capacity and optimize its water utilization, which are crucial for plant growth and survival. Overall, the study demonstrated that Amino acid (proline) treatment had a significant impact on fenugreek plants under cadmium stress. AA application alleviated the detrimental effects of cadmium stress by promoting morphological growth, enhancing biomass production, and improving physiological attributes related to photosynthesis and water use efficiency. These findings suggest that AA (proline) treatment could be a potential strategy to increase the tolerance of fenugreek plants within cadmium stress and improve their overall performance in contaminated environments.

REFERENCES

Abd-Elmoniem, A.-B. and N. El-Tayeh. 2017. Heavy metal and phosphorus contents of jew's mallow (*Corchorus olitorius* L.) plants grown on soil amended with phosphate, bio and organic fertilizers. International Journal of Plant & Soil Science 14:1-19.

Abdel hameed, R.E., A.A.H. Abdel Latef and R.S. Shehata. 2021. Physiological responses of salinized fenugreek (*Trigonellafoenum-graecum* L.) plants to foliar application of salicylic acid. Plants 10:657.

Abou-Shleel, S. 2014. Effect of air temperature on growth, yield and active ingredients of fenugreek (T*rigonellafoenum-graecum*). Nat Sci 12:50-54.

Acharya, S., A. Srichamroen, S. Basu, B. Ooraikul and T. Basu. 2006. Improvement in the nutraceutical properties of fenugreek (*Trigonellafoenum-graecum* L.). Songkla nakarin J. Sci. Technol 28:1-9.

Al-Shammari, M.Z.F. 2020. A study of the effect of NPK nanofertilizer and traditional NPK fertilizers and brassinolide spray on the ratio of some amino acids in the seeds of Fenugreek *Trigonellafoenum-graecum* L. EurAsian Journal of BioSciences 14:7759-7766.s

Alagawany, M., S.S. Elnesr, M.R. Farag, R. Tiwari, M.I. Yatoo, K. Karthik, I. Michalak and K. Dhama. 2021. Nutritional significance of amino acids, vitamins and minerals as nutraceuticals in poultry production and health–a comprehensive review. Veterinary Quarterly 41:1-29.

Albasha, M.O. and A.E.-S. Azab. 2014. Effect of cadmium on the liver and amelioration by aqueous extracts of fenugreek seeds, rosemary, and cinnamon in Guinea pigs: histological and biochemical study. Cell Biology 12:7-17.

Alhussain, A. 2010. EFFECT OF SOAKING AND GERMINATION OF FENUGREEK (*Trigonellafoenum-graecum* L.) SEEDS: CHANGES IN UPON CHEMICAL AND AMINO ACID COMPOSITION, ANTINUTRIENTS, PROTEIN DIGESTIBILITY AND MINERALS EXTRACTABILITY. Egyptian Journal of Agricultural Sciences 61:263-273.

Aljuhaimi, F., Ş. Şimşek, M.M. Özcan, K. Ghafoor and E.E. Babiker. 2018. Effect of location on chemical properties, amino acid and fatty acid compositions of fenugreek (*Trigonellafoenum-graecum* L.) seed and oils. Journal of food processing and preservation 42:e13569.

Alle, V., A. Osvalde, M. Vikmane and U. Kondratovics. 2019. The effect of cadmium and lead pollution on growth and physiological parameters of field beans (*Vicia faba*).

Almutairi, K.F., K. Górnik, R.M. Awad, A. Ayoub, H.S. Abada and W.F. Mosa. 2023. Influence of Selenium, Titanium, and Silicon Nanoparticles on the Growth, Yield, and Fruit Quality of Mango under Drought Conditions. Horticulturae 9:1231.

Altuntaş, E., E. Özgöz and Ö.F. Taşer. 2005. Some physical properties of fenugreek (*Trigonellafoenum-graceum* L.) seeds. Journal of food engineering 71:37-43.

Andresen, E. and H. Küpper. 2013. Cadmium toxicity in plants. Cadmium: from toxicity to essentiality:395-413.

Aqeel, U., R. Parwez, T. Aftab, M.M.A. Khan and M. Naeem. 2022. Exogenous calcium repairs damage caused by nickel toxicity in fenugreek (*Trigonellafoenum-graecum* L.) by strengthening its antioxidant defense system and other functional attributes. South African Journal of Botany 150:153-160.

Atlaw, T.K., J.Y. Kumar and N. Satheesh. 2018. Effect of germination on nutritional composition and functional properties of Fenugreek (*Trigonellafoenum-graecum* Linn) Seed Flour. International Journal of Nutrition and Food Sciences 7:110-115.

Azmat, R. and S. Moin. 2017. The monitoring of Cu contaminated water through potato peel charcoal and impact on enzymatic functions of plants. Journal of environmental management 203:98-105.

Bairwa, R. and M. Kaushik. 2010. Response of fenugreek (*Trigonellafoenum-graecum* L.) varieties to fertility levels and growth regulators on productivity, profitability and quality. Journal of Progressive Agriculture 1:65-67.

Bashri, G. and S.M. Prasad. 2016. Exogenous IAA differentially affects growth, oxidative stress and antioxidants system in

Cd stressed *Trigonellafoenum-graecum* L. seedlings: Toxicity alleviation by up-regulation of ascorbate-glutathione cycle. Ecotoxicology and environmental safety 132:329-338.

Behairy, R.T., M. El-Danasoury and L. Craker. 2012. Impact of ascorbic acid on seed germination, seedling growth, and enzyme activity of salt-stressed fenugreek. Journal of Medicinally Active Plants 1:106-113.

Bhardwaj, P., A.K. Chaturvedi and P. Prasad. 2009. Effect of enhanced lead and cadmium in soil on physiological and biochemical attributes of *Phaseolus vulgaris* L. Nature and science 7:63-75.

Çamlýca, M. and G. Yaldýz. 2019. Characterization of morphological and yield variation of fenugreek (*Trigonellafoenum-graecum* L.) genotypes. Legume Research-An International Journal 42:500-504.

Ciftci, O.N., R. Przybylski, M. Rudzinska and S. Acharya. 2011. Characterization of fenugreek (*Trigonellafoenum-graecum*) seed lipids. Journal of the American Oil Chemists' Society 88:1603-1610.

Dehkordi, R.A., S.R. Roghani, S. Mafakheri and B. Asghari. 2021. Effect of biostimulants on morpho-physiological traits of various ecotypes of fenugreek (*Trigonellafoenum-graecum* L.) under water deficit stress. Scientia horticulturae 283:110077.

El-Mahdy, A.R. and L.A. El-Sebaiy. 1985. Proteolytic activity, amino acid composition and protein quality of germinating fenugreek seeds (*Trigonella foenum graecum* L.). Food Chemistry 18:19-33.

El Rasafi, T., A. Oukarroum, A. Haddioui, H. Song, E.E. Kwon, N. Bolan, F.M. Tack, A. Sebastian, M. Prasad and J. Rinklebe. 2022. Cadmium stress in plants: A critical review of the effects, mechanisms, and tolerance strategies. Critical Reviews in Environmental Science and Technology 52:675-726.

Elgebaly, A., E. Sadek, N. Taha and A.F. Abou Hadid. 2022. Effect of Salinity on Seed Germination, Growth and Amino Acid Content in Fenugreek (*Trigonellafaenum-graecum* L.)

Sprouts. Arab Universities Journal of Agricultural Sciences 30:1-9.

ESPANANY, A., S. FALLAH and A. TADAYYON. 2015. The effect of halopriming and salicylic acid on the germination of fenugreek (*Trigonellafoenum-graecum*) under different cadmium concentrations. Notulae Scientia Biologicae 7:322-329.

FAIZY, H.S., B.G. SAHI, H.S. MAHDI and H.M.S. HAMID. 2022. THE IMPACT OF HEAVY METALS (CADMIUM & NICKEL) ON SEED GERMINATION, PHOTOTHYNSISES PIGMENTS AND VEGETATIVE GROWTH OF FENUGREEK (*Trigonellafoenum-Graecum* L.) PLANT. Journal of Duhok University 25:88-100.

Feyzi, S., M. Varidi, F. Zare and M.J. Varidi. 2015. Fenugreek (*Trigonellafoenum graecum*) seed protein isolate: extraction optimization, amino acid composition, thermo and functional properties. Journal of the Science of Food and Agriculture 95:3165-3176.

Feyzi, S., M. Varidi, F. Zare and M.J. Varidi. 2017. A comparison of chemical, structural and functional properties of fenugreek (*Trigonellafoenum graecum*) protein isolates produced using different defatting solvents. International journal of biological macromolecules 105:27-35.

Feyzi, S., M. Varidi, F. Zare and M.J. Varidi. 2018. Effect of drying methods on the structure, thermo and functional properties of fenugreek (*Trigonellafoenum graecum*) protein isolate. Journal of the Science of Food and Agriculture 98:1880-1888.

Galdón, B.R., D.R. Mesa, E.R. Rodríguez and C.D. Romero. 2010. Amino acid content in traditional potato cultivars from the Canary Islands. Journal of food composition and analysis 23:148-153.

Gill, S.S., N.A. Khan and N. Tuteja. 2011. Differential cadmium stress tolerance in five Indian mustard (*Brassica juncea* L.) cultivars: an evaluation of the role of antioxidant machinery. Plant signaling & behavior 6:293-300.

Gladysh, N.S., A.S. Bogdanova, M.A. Kovalev, G.S. Krasnov, V.V. Volodin, A.I. Shuvalova, N.V. Ivanov, M.I. Popchenko, A.D. Samoilova and A.N. Polyakova. 2023. Culturable Bacterial

Endophytes of Wild White Poplar (*Populus alba* L.) Roots: A First Insight into Their Plant Growth-Stimulating and Bioaugmentation Potential. Biology 12:1519.

Gressler, V., M.T. Fujii, A.P. Martins, P. Colepicolo, J. Mancini-Filho and E. Pinto. 2011. Biochemical composition of two red seaweed species grown on the Brazilian coast. Journal of the Science of Food and Agriculture 91:1687-1692.

Gressler, V., N.S. Yokoya, M.T. Fujii, P. Colepicolo, J. Mancini Filho, R.P. Torres and E. Pinto. 2010. Lipid, fatty acid, protein, amino acid and ash contents in four Brazilian red algae species. Food chemistry 120:585-590.

Haider, F.U., C. Liqun, J.A. Coulter, S.A. Cheema, J. Wu, R. Zhang, M. Wenjun and M. Farooq. 2021. Cadmium toxicity in plants: Impacts and remediation strategies. Ecotoxicology and Environmental Safety 211:111887.

Hare, V., P. Chowdhary and V.S. Baghel. 2017. Influence of bacterial strains on *Oryza sativa* grown under arsenic tainted soil: accumulation and detoxification response. Plant Physiology and Biochemistry 119:93-102.

Hooda, S. and S. Jood. 2005. Effect of fenugreek flour blending on physical, organoleptic and chemical characteristics of wheat bread. Nutrition & Food Science 35:229-242.

Huang, D., X. Gong, Y. Liu, G. Zeng, C. Lai, H. Bashir, L. Zhou, D. Wang, P. Xu and M. Cheng. 2017. Effects of calcium at toxic concentrations of cadmium in plants. Planta 245:863-873.

Hunt, E., S. Gattolin, H.J. Newbury, J.S. Bale, H.-M. Tseng, D.A. Barrett and J. Pritchard. 2010. A mutation in amino acid permease AAP6 reduces the amino acid content of the Arabidopsis sieve elements but leaves aphid herbivores unaffected. Journal of experimental botany 61:55-64.

Ivanova, P., V. Chalova, L. Koleva and I. Pishtiyski. 2013. Amino acid composition and solubility of proteins isolated from sunflower meal produced in Bulgaria. International Food Research Journal 20:2995-3000.

Izadi, F., M. Ghabooli, M. Rostami and Z. Movahedi. 2021. Evaluation of fungus *Piriformospora indica* effects on some morphophysiological traits of radish under heavy metal stress. Journal of Agricultural Science and Sustainable Production 31:117-129.

Javad, S., A. Shah, M. Ramzan, R. Sardar, T. Javed, A. Al-Huqail, H. Ali, O. Chaudhry, N. Yasin and S. Ahmed. 2022. Hydrogen sulphide alleviates cadmium stress in *Trigonellafoenum-graecum* by modulating antioxidant enzymes and polyamine content. Plant Biology 24:618-626.

Jiang, W., L. Xu, Y. Liu, W. Su, J. Yan and D. Xu. 2022. Effect of biochar on the growth, photosynthesis, antioxidant system and cadmium content of *Mentha piperita* 'Chocolate'and *Mentha spicata* in cadmium-contaminated soil. Agronomy 12:2737.

Karmakar, N., A. Chakravarty, P.K. B and opadhyay and P.K. Das. 2014. Response of fenugreek (*Trigonellafoenum-graecum* L.) seedlings under moisture and heavy metal stress with special reference to antioxidant system. African Journal of Biotechnology 13

Khater, M., M. El-Awadi, M. Elashtokhy, Y. Abdel-Baky and M. Shalaby. 2016. Physiological and molecular changes in fenugreek (*Trigonellafoenum-graecum* L.) as a response to gamma rays. Int. J. PharmTech Res 9:306-316.

khudhiar AL-Ibraheemi, A.H. and S.A.-H.S. AL-Ajeel. 2024. EFFECT OF SPRAYING NANO-AMINO ACIDS AND IRON ON SOME YIELD CHARACTERISTICS AND THE CHEMICAL AND MEDICINAL CONTENT OF TWO CULTIVARES OF FENUGREEK PLANT, *TRIGONELLAFOENUM-GRAECUM* L. Chelonian Research Foundation 19:294-308.

Kıralan, M., A. Yorulmaz, E. Çalıkoğlu and A. Bayrak. 2017. Fatty acid and sterol composition of fenugreek seed (*Trigonellafoenum-graecum* L.).

Kumar, A. and S.R. Samadder. 2017. A review on technological options of waste to energy for effective management of municipal solid waste. Waste Management 69:407-422.

Kumar, S. and A. Sharma. 2019. Cadmium toxicity: effects on human reproduction and fertility. Reviews on environmental health 34:327-338.

Legesse, S., F. Lemessa, P. Wolf and B. Eichler-Löbermann. 2020. Oat (*Avena sativa* L.) supplemented with fenugreek (*Trigonellafoenum-graecum* L.) as a potential alternative for teff [Eragrostis tef (Zucc.) Trotter] for human nutrition in

Ethiopia. Communications in Soil Science and Plant Analysis 51:2846-2857.
Lin, Y.-F. and M.G. Aarts. 2012. The molecular mechanism of zinc and cadmium stress response in plants. Cellular and molecular life sciences 69:3187-3206.
Lindi, A.M., S. Falah, M. Sadeghnezhad and M. Ghorbani. 2024. Optimization of fenugreek seed mucilage extraction for the synthesis of a novel bio-nano composite for efficient removal of cadmium ions from aqueous environments. International Journal of Biological Macromolecules 261:129882.
Liu, J., L. Gai and H. Zong. 2021. Foliage application of chitosan alleviates the adverse effects of cadmium stress in wheat seedlings (*Triticum aestivum* L.). Plant Physiology and Biochemistry 164:115-121.
Mafakheri, S. 2017. Effect of some organic and chemical fertilizers on morphological and biochemical factors of fenugreek (T*rigonellafoenum-graecum* L.). Plant Productions 40:27-40.
Mahmood, N. and K. Yahya. 2017. Nutrient and phytochemical of fenugreek (*Trigonellafoenum graecum*) seeds. International Journal of Sciences: Basic and Applied Research 36:203-213.
Mahmud, J.A., M. Borhannuddin Bhuyan, K. Nahar, K. Parvin and M. Hasanuzzaman. 2020. Response and tolerance of Fabaceae plants to metal/metalloid toxicity. The Plant Family Fabaceae: Biology and Physiological Responses to Environmental Stresses:435-482.
Majeed, R.G. 2020. Effect of organic acid, amino acids and nano-fertilizer on growth, yield and nitrate concentration of lettuce plant under two farming systems. Research on Crops 21:124-128.
Missaoui, T., M. Smiri, H. Chmingui and A. Hafiane. 2017. Effects of nanosized titanium dioxide on the photosynthetic metabolism of fenugreek (*Trigonellafoenum-graecum* L.). Comptes rendus biologies 340:499-511.
Mnafgui, W., H. Hajlaoui, V. Rizzo, G. Muratore and A. Elleuch. 2022. Priming with EDTA, IAA and Fe alleviates Pb toxicity in *Trigonellafoneum graecum* L. growth: Phytochemicals

and secondary metabolites. Journal of Biotechnology 356:42-50.

Mukherjee, M. 2017. Lead and cadmium toxicity on seedling growth and metabolism of *Trigonellafoenum-graecum* L. International Journal of Science and Research 6:1685-1689.

Mustafa, S.A., R. Al-Masry, A. Abu Aita and A. Ibrahim. 2023. FENUGREEK SEEDPROTEIN ISOLATE: AMINOACID COMPOSITION AND FUNCTIONALPROPERTIES. Zagazig Journal of Agricultural Research 50:191-200.

Naeem, M.A., A. Shabbir, M. Amjad, G. Abbas, M. Imran, B. Murtaza, M. Tahir and A. Ahmad. 2020. Acid treated biochar enhances cadmium tolerance by restricting its uptake and improving physio-chemical attributes in quinoa (*Chenopodium quino*a Willd.). Ecotoxicology and Environmental Safety 191:110218.

Naser, W. and O. Mheidi. 2021. Fenugreek Performance Affected by Foliar Application of Gibberellin and Arginine Acids. In: IOP Conference Series: Earth and Environmental Science. p 012059.

Nazar, R., N. Iqbal, A. Masood, M.I.R. Khan, S. Syeed and N.A. Khan. 2012. Cadmium toxicity in plants and role of mineral nutrients in its alleviation.

Niknam, R., H. Kiani, Z.E. Mousavi and M. Mousavi. 2021. Extraction, detection, and characterization of various chemical components of *Trigonellafoenum-graecum* L.(fenugreek) known as a valuable seed in agriculture. Fenugreek: Biology and Applications:189-217.

Nordberg, G.F., A. Bernard, G.L. Diamond, J.H. Duffus, P. Illing, M. Nordberg, I.A. Bergdahl, T. Jin and S. Skerfving. 2018. Risk assessment of effects of cadmium on human health (IUPAC Technical Report). Pure and Applied Chemistry 90:755-808.

Okutu Jackson, B. and O. Enebrayi. 2022. N. ameliorative effect of *allium sativum* and *justicia carnea* extracts co-administration on acute cadmium chloride-induced changes on liver function parameters of albino rats. World J. Pharm. Life Sci 4:11-24.

Olaiya, C.O. and K.O. Soetan. 2014. A review of the health benefits of fenugreek (*Trigonellafoenum-graecum* L.): Nutritional, Biochemical and pharmaceutical perspectives. Am. J. Soc. Issues Humanit 4:3-12.

Ould said, C., K. Boulahia, M.A. Eid, M.M. Rady, R. Djebbar and O. Abrous-Belbachir. 2021. Exogenously used proline offers potent antioxidative and osmoprotective strategies to re-balance growth and physio-biochemical attributes in herbicide-stressed *Trigonellafoenum-graecum*. Journal of Soil Science and Plant Nutrition 21:3254-3268.

Parida, B., I. Chhibba and V. Nayyar. 2003. Influence of nickel-contaminated soils on fenugreek (*Trigonella corniculata* L.) growth and mineral composition. Scientia horticulturae 98:113-119.

Pérez-Santaescolástica, C., J. Carballo, E. Fulladosa, J.V. Garcia-Perez, J. Benedito and J. Lorenzo. 2018. Effect of proteolysis index level on instrumental adhesiveness, free amino acids content and volatile compounds profile of dry-cured ham. Food Research International 107:559-566.

PHYTOCHEMICALS, E.O.P.O. 2012. Addis Ababa Institute of Technology (AAiT) School of Graduate Studies Department of Chemical Engineering, Addis Ababa University.

Riaz, H., F. Feroz, A. Akram, A. Ahmad and T. Iqbal. Biomonitoring of cadmium accumulation in plants treated with cattle manure to enhance morph-physiological attributes of Spinach (*Spinacia oleracea* L.) and fenugreek (*Trigonellafoenum gracum* L.).

Rizwan, M., S. Ali, T. Abbas, M.Z.u. Rehman and M.I. Al-Wabel. 2018. Residual impact of biochar on cadmium uptake by rice (*Oryza sativa* L.) grown in Cd-contaminated soil. Arabian Journal of Geosciences 11:1-8.

Roy, P. 2022. Nutritional attributes, bioactive components, antioxidant activity and amino acid profile of fenugreek seed powder supplemented bread, Chattogram Veterinary & Animal| Sciences University.

Sadak, M., B. Bakry, T. Abdel-Razik and R. Hanafy. 2023. Amino acids foliar application for maximizing growth, productivity and quality of peanut grown under sandy soil. Brazilian Journal of Biology 83:e256338.

Sharangi, A.B., P.H. Bhutia, A.C. Raj and M. Sreenivas. 2018. Underexploited spice crops: present status, agrotechnology, and future research directions. Apple Academic Press.

Sheikh, Z., M. Amin, N. Khan, M.N. Khan, S.K. Sami, S.B. Khan, I. Hafeez, S.A. Khan, E.M. Bakhsh and C.K. Cheng. 2021. Potential application of *Allium Cepa* seeds as a novel biosorbent for efficient biosorption of heavy metals ions from aqueous solution. Chemosphere 279:130545.

Shirai, N., M. Terayama and H. Takeda. 2002. Effect of season on the fatty acid composition and free amino acid content of the sardine Sardinops melanostictus. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 131:387-393.

Singh, A., S. Singh, A. Mahawar and T. Yadav. 2015. Influence of different plant growth regulators and zinc levels on growth and quality aspects of fenugreek (*Trigonellafoenum-graecum* L.) under semi-arid conditions.

Suman, M., P.D. Sangma, D.R. Meghawal and O.P. Sahu. 2017. Effect of plant growth regulators on fruit crops. Journal of Pharmacognosy and Phytochemistry 6:331-337.

T Abbas, W., M. MN Authman, D. A Darwish, A. M Kenawy, I. MK Abumourad and T. B Ibrahim. 2019. Cadmium toxicity-induced oxidative stress and genotoxic effects on Nile tilapia (*Oreochromis niloticus* L.) fish: The protective role of fenugreek (*Trigonellafoenum-graecum*) seeds. Egyptian Journal of Aquatic Biology and Fisheries 23:193-215.

Tarraf, S.A., I.M. Talaat, A.E.-K.B. El-Sayed and L.K. Balbaa. 2015. Influence of foliar application of algae extract and amino acids mixture on fenugreek plants in sandy and clay soils. Nusantara Bioscience 7

Yang, L., L. Chen, K. Zheng, Y.-J. Ma, R.-X. He, M.A. Arowolo, Y.-J. Zhou, D.-F. Xiao and J.-H. He. 2022. Effects of fenugreek seed extracts on growth performance and intestinal health of broilers. Poultry Science 101:101939.

Younis, U., S.A. Malik, M.F. Qayyum, M.H.R. Shah, A.N. Shahzad and S. Mahmood. 2015. Biochar affects growth and biochemical activities of fenugreek (*Trigonella corniculata*) in cadmium polluted soil. Journal of Applied Botany and Food Quality 88

Younis, U., S.A. Malik, M. Rizwan, M.F. Qayyum, Y.S. Ok, M.H.R. Shah, R.A. Rehman and N. Ahmad. 2016. Biochar enhances the cadmium tolerance in spinach (*Spinacia oleracea*) through modification of Cd uptake and physiological and biochemical attributes. Environmental Science and Pollution Research 23:21385-21394.

Zamani, Z., H. Amiri and A. Ismaili. 2020. Improving drought stress tolerance in fenugreek (*Trigonellafoenum-graecum*) by exogenous melatonin. Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology 154:643-655.

Zayneb, C., K. Bassem, K. Zeineb, C.D. Grubb, D. Noured dine, M. Hafedh and E. Amine. 2015. Physiological responses of fenugreek seedlings and plants treated with cadmium. Environmental Science and Pollution Research 22:10679-10689.

Zhu, G., H. Xiao, Q. Guo, Z. Zhang, J. Zhao and D. Yang. 2018. Effects of cadmium stress on growth and amino acid metabolism in two Compositae plants. Ecotoxicology and Environmental Safety 158:300-308.

Żuk-Gołaszewska, K., J. Wierzbowska and T. Bieńkowski. 2015. Effect of potassium fertilization, *Rhizobium* inoculation and water deficit on the yield and quality of fenugreek seeds.

Al Waeli, A. K. M. (2015). "Investigation of the effect of Cd, Se and Zn supplementation in broiler feed on productive and biochemical parameters."

Aslam, M. M., et al. (2023). "Cadmium toxicity impacts plant growth and plant remediation strategies." Plant growth regulation 99(3): 397-412.

Dinakar, C., et al. (2012). "Photosynthesis in desiccation tolerant plants: energy metabolism and antioxidative stress defense." Plant Science 182: 29-41

Iqbal, S., et al. (2020). "Suppression of amino acid and oligopeptide mineralization by organic manure addition in a semiarid environment." Land Degradation & Development 31(15): 1915-1925.

Rahman, M., et al. (2021). "Acetic acid improves drought acclimation in soybean: an integrative response of photosynthesis, osmoregulation, mineral uptake and antioxidant defense." Physiologia plantarum 172(2): 334-350.

Rizwan, M., et al. (2017). "A critical review on effects, tolerance mechanisms and management of cadmium in vegetables." Chemosphere 182: 90-105.

Yang, L. P., et al. (2018). "Effect of Cd on growth, physiological response, Cd subcellular distribution and chemical forms of Koelreuteria paniculata." Ecotoxicology and Environmental Safety 160: 10-18.