A DISSERTATION ON

EFFECT OF FOLIAR APPLICATION OF PLANT BASED BIO-STIMULANTS ON GROWTH AND YIELD OF WHEAT (*Triticum aestivum* L.)

SUBMITTED TO THE

DEPARTMENT OF BIOSCIENCES

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MASTER OF SCIENCE IN MICROBIOLOGY

SUBMITTED BY

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M.Sc. Microbiology (IV SEMESTER)

DEPARTMENT OF BIOSCIENCE

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Division of Crop Production and Protection

CSIR- Central Institute of Medicinal and Product

CSIR-CIMAP

केन्द्रीय औषधीय एवं सगंध पौधा संस्थान

CSIR-CENTRAL INSTITUTE OF MEDICINAL AND AROMATIC PLANTS

Human Resource Development Program

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This certificate is issued to the candidate by **CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow** on successful completion of her M.Sc. dissertation work.

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The institute wishes the candidate success in her future endeavours.

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TO WHOM IF MAY CONCERN

This is to certify that **Ms. Mansi Upadhyay** a student of **M.Sc. Microbiology** Life Science (2nd year/4th semester), Integral University Lucknow, has completed her four-month dissertation work entitled **"**evaluate **"Effect of foliar application of plant-based bio-stimulants on growth and yield of wheat (*Triticum aestivum* L.)"** successfully. She has completed this work from CSIR-CIMAP (Central Institute of Medicinal and Aromatic Plants) LUCKNOW under the guidance of **Dr. Priyanka Suryavanshi.** The dissertation was a compulsory part of her M.Sc. Microbiology degree.

I wish her good luck and bright future.

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DECLARATION

I, **MANSI UPADHYAY**, a student of the "M.Sc. Microbiology" session: 2020-2022, Department of Bioscience, Integral University Lucknow, declare that I am solely responsible for all the work presented in the thesis titled "**EFFECT OF FOLIAR APPLICATION OF PLANT BASED BIO-STIMULANTS ON GROWTH AND YIELD OF WHEAT(*Triticum aestivum* L.)",** which is being submitted to Integral University, Lucknow, Uttar Pradesh, India for partial fulfilment for the award of the degree of Master of Science in Microbiology (2022), has been carried out by me under the supervision of **Dr. Priyanka Suryavanshi**, scientist , Crop Production and Protection Division, CSIR-CIMAP, Lucknow, U.P., India. I further declare that I take responsibility for the accuracy of this dissertation report.

Mansi Upadhyay

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Firstly, I bow my head with utmost reverence before the almighty whose eternal blessings has enabled me to accomplish this Nobel effort.

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List of Abbreviation

EC	Electrical conductivity
SOC	Soil organic carbon
REDOX	Reduction –oxidation
Ppm	Parts per million
TTC	Triphenyl tetrazolium chloride
TPF	Triphenyl formazan
MUB	Modified universal buffer
SMBC	Soil microbial biomass carbon
FAS	Ferrous ammonium sulphate
DHA	Dehydrogenase activity
Rpm	Round per minute
P	Phosphorous
K	Potassium
Zn	Zinc
%	Percent
Etc	Et cetera
Ml	Millilitre
Nm	Nanometre
Min	Minute
µl	Microliter
Mm	Millimetre
NaoH	Sodium hydroxide
Fig	Figure
DAS	Days after sowing

TABEL OF CONTENT

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the world's most significant food crops and a member of the Poaceae family, which includes key cereal crops like maize, wheat, and rice. Wheat is the most plentiful sources of energy and proteins for the global population, and increasing output is critical for food security (Chhokar et.al.,2006). Wheat is the most important crop in the world in terms of cropping area (FAOSTAT, 2017). This grain is mostly utilised in the food industry.

In terms of cultivation, wheat is the second most widely grown cereal grain after maize, and occupies about 17% of the world's cropped land and contributes 35% of the staple food (Pingali et.al.,1999). The worldwide trade volume exceeds that of all other crops combined. The total global wheat output in 2020 was 760 million tonnes. China, India, and Russia are the world's three greatest individual wheat producers, accounting for over 41% of global wheat output. The United States is the world's fourth-largest wheat producer. If the European Union were classified as a single entity, it would produce more wheat than any other country saves China.

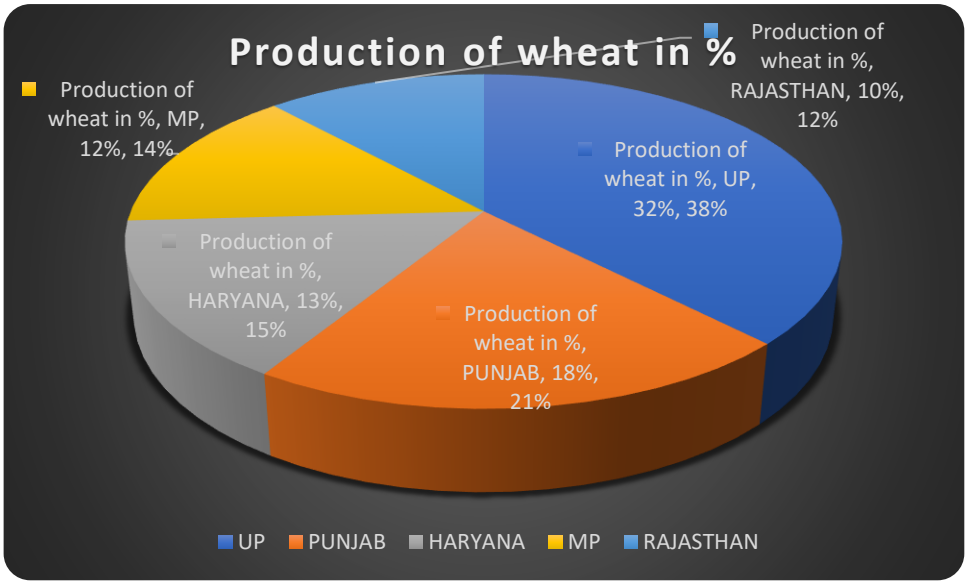
Wheat producing countries in terms of production in tonnes in the order of their production are China with 134,254,000 tonnes of wheat production

per annum, whereas India’s net production of wheat is 107,590,000 tonnes, Russia is the third largest producing countries in terms of wheat production with 85,896,326 tonnes, United States produces 49,690,680 tonnes, Canada produces 35,183,000 tonnes, France produces 30,144,110 tonnes, Pakistan produces 25,247,511 tonnes, Ukraine produces total 24,912,350 tonnes.

Together, Russia and Ukraine account for roughly 30% of world wheat commerce. Because of, Russia's military invasion of Ukraine in 2022 caused global wheat prices rising, putting the country's production capacity at risk, and several nations suspending or terminating trade connections with Russia. Russia is also a key source of fertiliser, which is essential for increasing crop yields, compounding the problems for farmers.

India is 2nd largest wheat producer and shares 13.5% of total wheat production. Uttar Pradesh, Punjab, Madhya Pradesh, Haryana, and Rajasthan, the country's five biggest wheat-growing states, accounted for roughly 86.0 percent of total production. Uttar Pradesh has the greatest production of wheat, as it is located at the highly fertile river basin of the Ganga’s. The state is account for 300.010 lakh net tons of India’s total wheat production. It is followed by Punjab with produces 164.720 lakh net tons of wheat. Haryana comes third in row with 116.30 lakh net tons.

MP accounted for 10.71 percent (3.10 million ha) of the country's land area and 11.10 percent (10.46 million t) of grain output, with an average productivity of 76.271lakh tons (Commission for Agricultural Costs and Prices, 2019) (Shivran et.al). Lastly, Rajasthan occupies 5th position with 72.145 lakh ton. The yield of wheat is low in Himachal Pradesh, and Jammu, and Kashmir as it is grown under rain-fed conditions. The pie chart represents the annual production of wheat state-wise in percentage.



State-wise share in production of wheat.

Wheat requires a chilly temperature throughout the early stages of its development. When the average temperature is 200 degrees Celsius, sowing wheat provides a significant benefit. Sowing at a cooler day temperature causes germination to take longer and shortens the growth season. Early in the season, warm temperatures are detrimental for tillering. Overabundance of rain during the season leads to a high prevalence of rust.

Warm, dry weather is ideal for homogeneous grain ripening around the time of maturity. Areas that receive winter rains but are rain-free in April and May have a significant advantage in wheat production.

Wheat is regarded as the most important cereal owing to the presence of protein with unique chemical and physical characteristics in its grain. Wheat includes protein, minerals (P, Mg, Fe, Cu, and Zn), and vitamins (thiamine, riboflavin, niacin, and vitamin E) in addition to carbs. Wheat proteins, on the other hand, are low in important amino acids like lysine and threonine (Adsule and Kadam et.al., 1986).

Table-1.1. Nutritional composition of wheat flour (Kulkarni et.al., 2012)

Parameter	Amount (%)
Moisture (%)	12.67± 0.025
Protein (%)	10.55± 0.032
Fat (%)	0.94 ±0.006
Total carbohydrate (%)	74.88 ±0.508
Crude fibre (%)	0.36 ±0.010
Calcium(mg/100g)	18 ± 0.506
Iron (mg/100g)	2.1± 0.032
Phosphorus(mg/100g)	107 ±0.150
Ash (%)	0.94±0.010

Refined wheat flour contains pentosan in amount of 1-3%. The pentosans, which are made up of arabinoxylans and arabinogalactans, are the most important non-starch polysaccharides in wheat flour (Neukom et.al., 1973). Between 0.5 and 0.8 percent of wheat flour is made up of the water-soluble component of pentosans. The structure of the pentosan components has been the subject of several studies and is widely understood. Arabinoxylans are made up of linear xylans with single L-arabinose side residues, whereas arabinogalactans are made up of a highly branched galactan chain backbone with single L-arabinose residues and low molecular weight peptides (Amado and Neukom 1985). (Ikhtiar et.al., 2007).

After Green revolution there are drastic change in the production of wheat. To meet this increasing demand use of chemical fertilizer and herbicide increased. Though chemical fertilizers increase crop production; their overuse has hardened the soil, decreased fertility, strengthened

pesticides, polluted air and water, and released greenhouse gases, thereby bringing hazards to human health and environment as well. It has already been proved how chemical fertilizers pose serious challenges to the balanced and sustainable growth. Herbicide usage is one of the reasons that leads to a reduction in soil microbial activity. Herbicides are used to boost agricultural productivity by increasing crop quality and yield. The concentrations of compounds that do not reach the target species, on the other hand, are of concern because of their possible environmental effect (Kucharski et al. 2009). Herbicides that enter the soil can have an impact on soil microbial population size and activity. Modern herbicides have great biological activity and selectivity, but their misuse and overuse can have negative consequences for the ecosystem. (Baćmaga et.al., 2015).

The most widely accepted and comprehensive definition of a biostimulant is provided by Du Jardin, who defines a plant biostimulant as "any substance or microorganism that, when applied to plants, regardless of its nutritional content, can improve nutrition efficiency, as well as abiotic stress tolerance and quality traits" (Jardin et.al.,2015). Humic compounds, complex organic materials, helpful chemical components, inorganic ions, seaweed extracts, chitin, and chitosan derivatives, anti-transpirant and free amino acids, and other N-containing substances with microbe were considered a possible ninth group by Du Jardin (Jardin et.al.,2015). The use of bio-stimulators and growth regulators is becoming increasingly crucial in current cereal crop growing methods. Biostimulants contribute to better seed germination and induce biological activity of plants. These products are also safe for the environment and contribute to sustainable, high-output low-input crop productions. Their application helps to reduce the amount of chemicals used in agriculture and plant protection (Atanas et.al 2013). The purpose of this study is to verify the effect of various plant based biostimulants on the growth and yield of wheat (variety HD-2967).

2.AIMS AND OBJECTIVE

This research was aimed to evaluate **"Effect of foliar application of plant based bio-stimulants on growth and yield of wheat (*Triticum aestivum* L.)"**with following objectives.

1- Comparative performance of foliar application of plant-based bio-stimulants on crop growth and yield of wheat.

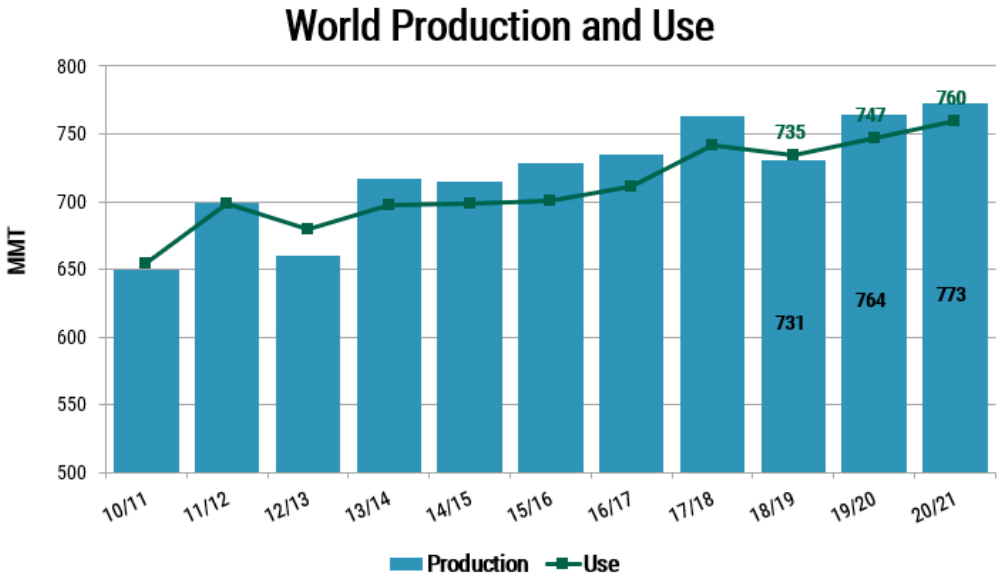
2- Effect of foliar application of plant-based bio-stimulants on physiological attributes of wheat.

3- Effect of foliar application of plant-based bio-stimulants on soil enzymatic activity of wheat.

3.Review of Literature

Wheat belongs to the family Poaceae, it is taken from Greek word poawhich means grass. Poaceae contain monocotyledon flowering plants also called grasses. Grass-dominated plant groups make up around a quarter of the world's vegetation. There are 9500 species in 668 genera. In terms of total quantity of species, they are in the top five flowering plant families, but they are undoubtedly the most prolific and significant family in the world's flora. They can be found on all continents, in a variety of environments ranging from sand to freshwater and marine, and at all except the highest elevations.

The Poaceae family is found all over the planet. Agricultural grains (essential food and alchoholic beverage sources) such as barley , maize , oats, rice, rye, wheat , and several others, as well as crucial fodder and grazing plants, make up the grasses. Many environments, such as grasslands and savannas, benefit from the presence of members of the family.



Graph-2.a Worldwide production and consumption of wheat (Year wise).

The majority of farmed wheat varieties belong to one of three Triticum species. Triticum aestivum L. (bread wheat) is hexaploid (2n=42), Triticum durum Desf is tetraploid (2n=28), Triticum dicoccum Schrank is diploid (2n=14), and Triticum monococcum is diploid (2n=14). Aestivum is the most significant wheat species on the planet, accounting for over 90% of all wheat planted. Triticum durum, or macaroni wheat, is the second most important species. The Triticum monococcum species is ranked third in terms of usefulness and cultivated area. Wheat is an upright tillering bunch grass. In a whorl, the leaves are rolled. The upper side of the leaf blades has a smooth base and a rough tip. The entire lower side is completely flat. The leaf sheath is circular and divided with overlapping borders, and it can be hairy or smooth. Auricles are tiny and hairy, with a thick leaf collar. Ligule seems tattered and rounded. It is possible that hairs will be present. The roots of wheat are fibrous.Seeds range in hue from light tan to dark brown. The front has ribs, but the back is smooth. Wheat is characterized by large genome size (approximately 17000 Mb).

Wheat variety HD 2967 is a double dwarf cultivar with a height of 101 cm on average was employed in the experiment. Sowing takes place mostly between 15 and 30 November. It takes 155-160 days to mature and yields 38-40 q/h on average.It has amber-colored grains that are moderately bold, hard, and glossy.It has moderate yellow rust resistance, moderate brown rust resistance, and is less sensitive to Karnal bunt and loose smut diseases. This variety was released in North Western Plains Zone (NWPZ) comprising of Punjab, Haryana, Delhi, Rajasthan (Except Kota and Udaipur Divisions),Western UP (Except Jhansi Division), Parts of J & K (Kathua district), parts of HP (Una district & Paonta Valley) and Uttrakhand (Tarai region)

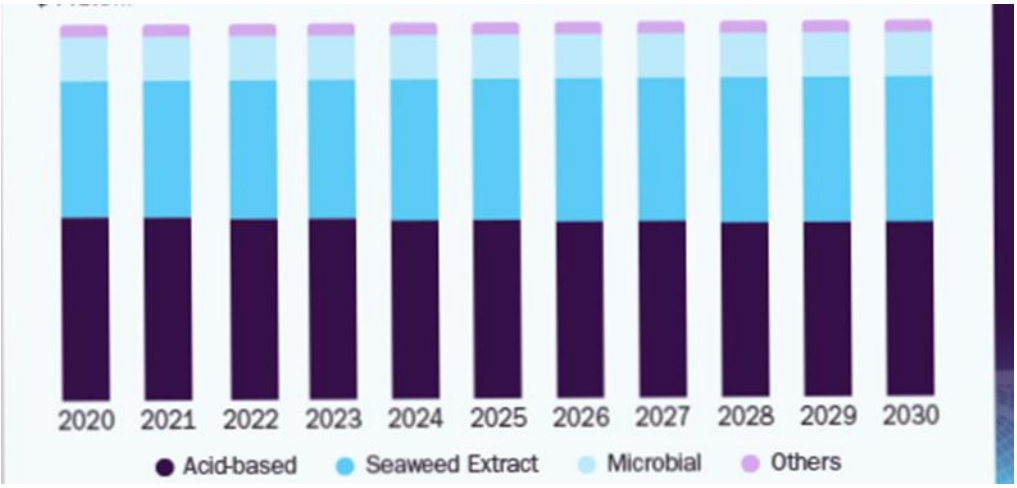
Graph- 2.b Indian wheat has been making bigger inroads into global markets in 2020-21.

As the world's population grows, the agricultural industry will face greater pressure to boost production, yield, and throughput. As we reach the limitations of staple crop genetic potential, as well as the shrinking quantity of arable land, increasing agricultural output requires more production with less resources. This is commonly accomplished by using chemical fertilisers and/or pesticides to alleviate the aforementioned causes as well as biotic and abiotic pressures (Alix et.al.,2018). However, indiscriminate use of agrochemicals has long-term environmental implications. Herbicide usage is one of the reasons that leads to a reduction in soil biological activity. Herbicides are used to boost agricultural productivity by increasing crop quality and yield. The amounts of chemicals that do not reach the target organisms, on the other hand, are of concern because of their possible environmental effect. (Kucharski et al., 2009.)

Sustainable agriculture entails maximising agricultural resources while also maintaining environmental quality and preserving natural resources (Chauhan et al., 2006; Tripathi et al., 2006; Pandey et al., 2007). Various compounds classed as plant growth stimulants are employed in modern agriculture in addition to fungicides, herbicides, and insecticides (Michalak et.al., 2013) (Calvo et.al., 2014). This relatively new class of chemicals is used to increase agricultural output and quality, particularly under unfavourable climatic circumstances for plants growth (Jardin et.al., 2015)

(Radkowski et.al., 2013). .Regardless of the measure used (sales, treated hectares, or number of users), the usage of biostimulants in agriculture has gradually increased over the previous decade, increasing by 10% or more each year (Jardin et.al., 2015).

Not only in terms of productivity, biostimulants are notable with regards to economic values as well. According to European Biostimulant Industry Council [EBIC] market range of biostimulants range about 1.5 billion to 2.0 billion USD in the year 2022 with the annual growth nearly 10-12% [25]. Comparing the biostimulants market worldwide, it was worth USD 2.79 billion in 2021, and it is expected to increase at a CAGR of 10.4 percent from 2022 to 2030. This is due to rising soil degradation, stricter limitations on the use of chemicals in agriculture, and the need for sustainable and environmentally friendly alternatives to promote the agriculture sector's development and production. Biostimulants are compounds or microorganisms that are applied to plants in order to improve their features and production.EU regulation plant biostimulants as a plant compound that stimulate plant nutrition processes with sole aim of improving one or more features of plant or plant rhizosphere like nutrients efficiency, abiotic stress, quality traits etc(Regulation (EU) 2019). Summarizing all the points, a biostimulants is a product and/or microorganism mixture, supporting the plant in terms of nutrient efficiency and tolerance to stress (Halpern et.al; 2015) (Oosten et.al;2017).As the world's population grows, so does demand for the product, as people become more aware of the negative, long-term impacts of artificial chemicals used in plant development. As a result, there is an increasing demand for organic and natural products. The graph shows the incresing demand of biostimulant over coming years with respect to the different types of biostimulants like acid based, microbial, sea weed extracts and others.



Graph- 2.c Shows the increasing demand of bio-stimulant over coming years

Acid-based products dominate the bulk of the regional market, including the humic acid sector, which is expected to increase rapidly. Biostimulants promote microbiota, which enhances plant nutrient uptake. They boost antioxidant activity and minimise plant strain caused by environmental factors and diseases. The hormones help the plant's potency and general health by fueling root growth, cell formation, and overall growth. They include elements that are natural, eco-friendly, and naturally degradable and do not harm the plant environment. As a result, they are seeing a surge in demand for organic farming methods.

Biostimulants are natural growth promoters that boost crop output by increasing nutrient uptake and efficiency, improving biotic and abiotic stress tolerance, and increasing rhizospheric activity (Jardin et al., 2015) .Biostimulants help seeds germinate better and increase plant biological activity. These products are also environmentally friendly and aid in the cultivation of high-output, low-input crops. Their use aids in the reduction of chemical use in agriculture and plant protection.

Biological soil stimulants are a broad category of compounds. These are mostly organic products made up of peptides, amino acids,

carbohydrates, lipids, and other compounds. They function through a variety of processes, including the stimulation of soil microbial activity and the promotion or enhancement of the activities of important soil enzymes (Chen et.al.,2002), all of which work together to keep soil quality in terms of fertility. Bio-stimulants can come from either natural or synthetic sources. Preparations based on free amino acids, seaweed and fruit extracts, effective microorganisms, humic compounds, and chitosan are examples of natural biostimulants (Calvo et al., 2014).

Growth regulators, phenolic compounds, inorganic salts, vital elements, and other chemicals are examples of synthetic biostimulants (Calvo et al., 2014).

2.1 Manufactured biostimulants

In summary to all the reviewed article, biostimulant consistenly enhaced the production of grains. For example- The cycoflow promoted plant growth (up to 48.5 percent taller plants) and fruit production (up to 105.3 percent). Antioxidant levels in leaves and fruits of plants treated with the biostimulant were higher than in non-treated plants. the level of ascorbic acid also incressed after the treatment.

The table summarizes all the research carried out using manufactured biostimulant product on different plant species.

Table-2.2.1 Summary of manufactured product on test plant species

S.No.	TEST PLANT SPECIES	BIOSTIMULANT (Manufactured product)
1	*Solanum lycopersicum L.*	CycoFlow
2	*Coriandrum sativum*	Nitroxin
3	*Moringa oleifera*	Syringic acid, gallic acid
4	*Peganum harmala*	Gallic acid, vanillic acid 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, syringic acid
5	*Salvia officinalis* L.	Caffeic acid, *p*-coumaric acid
6	*T. vulgaris*	Vanillin
7	*Phaseolus vulgaris*	Atonik

8	*Silybum marianum L.*	Chitosan
9	*Spinacia oleracea L.*	Megafol (MEG
10	*Spinacia oleracea L.*	Aminovert (AM)
11	*Spinacia oleracea L.*	Veramin Ca (V)
12	*Spinacia oleracea L.*	Twin Antistress (TA)
13	*Lavandula x intermedia*	FITOSTIM® and FITOSTIM® ALG A
14	*Triticum aestivum L.*	Vigro
15	*Triticum aestivum L.*	Biomin
16	*Triticum aestivum L.*	Humiplus
17	*Triticum aestivum L.*	Humacare
18	*Abelmoschus esculentus*	Kelpak®

2.2Reported research on bio-stimulant -

Scientist also reported various biostimulant used as alternatives for the sustainable agriculture. The most often used biostimulants in agriculture from natural source include seaweed extracts, protein hydrolysates and amino acids, humic acid, fulvic acid, complex organic materials, chitin and chitosan derivatives, microbial inoculants, biochar, and plant extracts. (Glodowska et al., 2016)

Different bio-stimulant categories-

The table summarizes all the research carried out using biostimulant from different reported sources.

Table- 2.2.1 Summarized bio-stimulants reported on of different plant species

S. No.	Plant species	Biostimulants
1	*Lactuca sativa L*	soybean leaf extracts
2	*Lactuca sativa L*	Chinese chive
3	*Abelmoschus esculentus*	PGPR
4	*Satureja hortensis*	Amino acid biopreparation
5	*Origanum majorana L*	Amino acid biopreparation

6	*Malus domestica*	Sea weed extract
7	*Lactuca sativa L*	Soyabean leaf extract
8	*Pelargonium graveolens L.*	Moringa leaf extract (MLE)
9	*Ocimum basilicum L*	pollen grains extract (PGE)
10	*Zea mays*	Humic acid
11	*Olea europaea*	Natural amino acid
12	*Allium tuberosum*	*Chlorella fusca*
13	*Spinacia oleracea L.*	*Chlorella fusca*
14	*Triticum aestivum L.*	Moringa leaf extract (MLE)
15	*Helianthus annuus L.*	*Mentha× piperita L.*
16	*Triticum turgidum L*	*Matricaria chamomilla L.* (Aqueous extract of different parts)
17	*Triticum aestivum L.*	*Moringa oleifera leaf extract (MLE)*

In this work we focused on foliar application of various medicinal and aromatic plant-based bio-stimulants with different concentrations and their effect on growth and yield character (plant height, tillers canopy); biochemical character (proline, chlorophyll a and b, carotenoids, RLWC) of wheat as well as soil enzyme activity (SMBC, acid phosphatase, alkaline phosphatase, dehydrogenase).

4.MATERIAL AND METHODS

4.1 Experimental design- The experiments was conducted at CSIR-CIMAP (Central institute of medicinal and aromatic plant), Lucknow and various criterions were investigated at Division of Agronomy and Soil science CIMAP, Lucknow, which is situated at 26.8946˚N latitude, 80.9805˚E longitude. The climate of Lucknow is sub-tropical, with average rainfall of990.1mm. The average temperature of the coldest month (January) is 15.0˚C and the warmest month (June) is of 32.9˚. On average July is the wettest month with 20.2 inch (512mm) of precipitation. Lucknow has dry periods in January, February, March, April, November, December. The soil of the experimental site was loamy in texture and slightly alkaline in nature. Wheat variety HD 2967 was used as test crop. The experiment was carried out in pot and replicated thrice. The pot experiment was carried out in a completely randomised design.

Crop	Variety	Date of sowing	Date of harvest
Wheat	HD-2967	1/12/21	6/04/22

4.2 Treatment details- Different categories of bio-stimulant used in the experiment are Sea weed extract, *Andrographis paniculata* (Kalmegh), *Mentha arvensis, Ocimum basiculum, Cymbopogon (Lemon grass), Cymbopogon martinii (Palma rosa), Tegetus minuta, Pelargonium graveoleus*. Foliar application of all the biostimulants were carried out at 30 days (1/01/22) and 60 days of interval (03/03/22)

Treatment details are as follow-

	Treatment	Concentration	
		C1	C2
T0	Control	-	-
T1	Sea weed extract	1%	-
T2	*Andrographis paniculata (Kalmegh)*	3%	5%
T3	*Mentha arvensis (menthol mint)*	0.10%	0.20%
T4	*Ocimum basiculum (Tulsi)*	0.10%	0.20%
T5	*Cymbopogon (Lemon grass)*	0.10%	0.20%
T6	*Cymbopogon martinii (Palma rosa)*	0.10%	0.20%
T7	*Tegetus minuta (wild marigold)*	0.10%	0.20%
T8	*Pelargonium graveoleus (Geranium)*	0.10%	0.20%

SEA WEED

KALMEGH

Aqueous extract

Mentha

Tulsi

Lemon grass

Palmarosa

Wild marigold

Geranium

Essential oils

Image representation of the material used in the study

4.3 Collection of soil samples- Soil samples were collected at both the pre-planting stage as well as after harvest. The initial nutrient content of soil is mentioned in the following table-

Chemical properties of initial stage soil-

EC (µs/cm)	PH
25.97	7.67

N (Kg ha^{-1})	P (Kg ha^{-1})	OC (%)	K (Kg ha^{-1})
257.072	54.438	3.448	77.347

The data was recorded on the following parameters-

4.4 Phyisco- chemical analysis of soil –

- **Electrical conductivity or EC –** Electrical conductivity is the amount of soluble salts in a sample it is measured through conductivity meter

  Principle- Salt ions, like metal, allow the electrical current to pass through them. Hence the electrical conductivity of the soil water system rises with increase in the content of soluble salts in the soil.
- **pH-** pH is a measure of hydrogen ion activity in soil. It is defined as the negative logarithm of hydrogen ion.

  Principle- pH indicates whether the soil is acidic, neutral, or alkaline in reaction since crop growth suffers much more in both very low strongly acidic as well as very high pH alkaline conditions appropriate reclamation measure become essential.

Procedure- (For EC and pH)

1-weigh 10 gm of soil that is dried and sieved.

2-Place the 10 gm of soil in a beaker of 50 ml and add 25ml of distilled water to it.

3-Stir the soil in distilled water with glass rod for about 5min

4-let it stand for about half an hour

5-Take the reading through pH meter and conductivity meter

- **Soil organic carbon (SOC)-** The term soil organic carbon refers to the **C**ooccurring in the soil.

  Principle-(Walkley and black 1934) in this method, organic carbon is oxidized with the chromic acid (Potassium dichromate +sulphuric acid). The unconsumed potassium dichromate is back titrated against ferrous sulphate or ammonium sulphate (Redox titration).

  Reagents -1N Potassium dichromate, 0.5N Ferrous sulphate +Concentrated Sulfuric acid, Diphenylamine indicator, 85% Orthophosphoric acid

  Procedure-

  1-Take 1gm of (0.2mm) soil sample in 500ml dry conical flask.

  2-Add 10 ml of potassium dichromate and 20 ml of Conc Sulphuric acid

  3-Add 200ml of distilled water and10ml of orthophosphoric acid.

  4-Add 1ml of Diphenylamine indicator.

  5-Take 0.5 N Ferrous ammonium sulphate solution in 50 ml burette

  6-titrate the content until green colour appears.

  7- Read titrate value and calculate.

- **Available nitrogen (N)-**It is the amount of mineralized nitrogen available to the plant by the microbial activity.

  Principle- (Alkaline potassium permanganate method) Nitrogen in the soil sample exists in a very complicated bonding structure during digestion a known weight of soil sample in the presence of Sulphuric acid digested under high pressure where complicated structure is broken down to simple structure thereby releasing nitrogen in the form of ammonia radical

Regents – 0.32% $KMnO_4$, 0.1N NaoH, 0.1N Sulphuric acid, 0.1 M oxalic acid ,2.5% NaoH, Mixed indicator.

Procedure-

1-Weigh 10 gm of soil (0.2mm) in 800 ml Kjeldahl flask.

2-Add 100ml $KMnO_4$ and 100ml of 2.5%NaoH

3-Add 10 ml of sulphuric acid and 3-4 drops of mix indicator.

4-Titrate by 0.1N NaoH fill the burette with 0.1N NaoH

5-Titrate till green colour develop and read the titrated value.

- **Available potassium (K)-**

  Principle- The principle underlying is that when a large number of elements when excited in a flame, emits radiation of characteristic wavelength. The excitation causes one of the outer electrons of neutral atom to moves to outer orbit of higher energy level. When the excited atom returns to lower energy level, light at a characteristic wavelength is emitted thus potassium give 404 and 767mμ.

  Reagent – 1N Ammonium acetate

  Stock standard solution: Sodium- NaCl(ppm), potassium-KCl (ppm)

  Procedure-

  1-Take 5gm of soil in 100ml of conical flask

  2-Add 25ml ammonium acetate and shake for 5min

  3-Filter through Whatman No1 filter paper and read the reading by flame photometer.

- **Available phosphorus (P)-**Phosphorous (P) is one of the macronutrients required for the plant growth it regulate the protein synthesis. Therefore, phosphorous important for cell division.

  Principle-Phosphorus is extracted from soil with 0.5M $NaHCo_3$ at a nearly constant pH of 8.5. The phosphate ion in solution treated with ascorbic acid in an acidic medium provides a blue colour complex.

  Reagents- Olsen's reagent (0.5N$NaHCo_3$), solution A (Ammonium molybdate), solution B (Antimony potassium tartrate), 5N Sulphuric acid, Ascorbic acid solution.

Procedure- (Hanway and Heidel)

1-Weigh 5gm of sieved soil.

2-add 50 ml of$NaHCo_3$ and a pinch of activated charcoal

3-Shake for half an hour and then filter with Whatman filter paper No 1

4- Keep the filtrate and transfer 5ml of aliquot in 25 ml volumetric flask and acidify with 5N sulphuric acid.

5-add 200ml of distilled water and then 4ml of ascorbic acid.

6-Take the reading by spectrophotometer at 660 nm wavelength.

4.5 Soil enzyme activity –

- **Soil Dehydrogenase Activity-**. DHA is one of the most adequate and most sensitive bioindicators, relating to soil fertility and quality. Dehydrogenase plays a significant role in the biological oxidation of soil organic matter

  Principle-(Casida et.al1964) Dehydrogenase plays an important role in biological oxidation of soil organic matter by transferring hydrogen from organic substances to inorganic acceptors. Under anaerobic condition 2,3,5 Triphenyl tetrazolium chloride (TTC) act as electron acceptor and converts to pink colour compound Triphenyl formazan (TPF). The Dehydrogenase can be assayed as rate of formation of TPF from TTC.

  Regents- 3% TTC (2,3,4 Triphenyl tetrazolium chloride), 1% glucose, Methanol, standard TPF (Triphenyl formazan).

  Procedure

  1-Take 1gm of fresh soil and place in air tight screw capped test tube of 15ml capacity.

  2-Add 0.2ml of 3% TTC solution in each tube

  3-Add 0.5ml of 1% Glucose solution to each tube shake horizontally all the tubes.

  4-Incubate all the tubes at 28±0.5 ® C for 24 hours.

  5-After incubation add 10ml of methanol to all the tubes shake horizontally and allow to stand for 6 hours.

  6-Filter it after 6 hours and take the absorbance at wavelength of 485nm.

  7- Extrapolate TPF formed from the standard curve drawn.

- **Acid and Alkaline phosphatase test** –Phosphates is an enzyme that catalyses hydrolysis of substrate and belongs to the category hydrolase. It is of 2 types based on pH that is acid phosphatase and alkaline phosphatase.

  Principle-(Tabatabai and Bremmer, 1969; Eivazi and Tabatabai, 1977). In acid and alkaline phosphates test, p- nitrophenyl phosphate acts as a substrate for both acid and alkaline phosphatase on hydrolysis by either of the enzyme it yields p –Nitrophenol.

  Reagents- Toluene, Modified universal buffer (MUB), 0.025M p-Nitro phenyl phosphate, 0.5M Calcium chloride solution, 0.5M Sodium hydroxide solution, Standard p-Nitrophenol solution.

  Procedure-

1- For each soil take 2 sets of 1gm fresh soil in vials, out of this one set will be used as control.
2- Add 0.2ml (200µl) toluene
3- Add 4ml of MUB (pH 6.5 or 11) to all the vials
4- Add 1ml of p- nitro phenyl phosphate solution in only one set of samples
5- Swirl all the vials for few seconds cape them place in incubator for 1 hour
6- Add 1ml of 0.5 M $CaCl_2$
7- Add 4ml of 0.5 NaoH
8- Swirl all the vials for few seconds
9- Add 1ml of p- nitro phenyl phosphate to the control set sample
10- Filter all the suspension quickly through Whatman filter paper No 2
11- Measure the yellow colour intensity @ 440 nm.

- **Soil microbial biomass carbon (SMBC)-** Soil microbial biomass carbon is the living component of soil organic matter.

  Principle- In the fumigation method, a direct measurement of carbon and other nutrients contained in microbial biomass is carried out. Overnight fumigation of chloroform is done to kill the organisms in the soil samples.

Reagents- Distilled chloroform, Conc. sulphuric acid, 0.5M potassium sulphate, 0.2Npotassium dichromate, Orthophosphoric acid, 0.005 N ferrous ammonium sulphate (FAS), ferroin indicator

Procedure-

1- Take fresh soil pass through 2mm sieve, don't dry analyse the soil same day.
2- Take 3 sets of 10gm of soil for each sample. Keep one set in oven at 100®c for 24 hours
3- Weigh the dry soil and calculate the moisture content of the soil
4- Keep one set in 50 ml beaker for fumigation, pack the remaining one set and keep in refrigerator
5- Keep 20ml of distilled chloroform in 100ml beaker. Place some glass beads in it
6- Place all the soil and the chloroform filled beaker in the vacuum desiccator
7- Switch on the vacuum pump until the chloroform boils for 5miN, put the desiccator in dark for 24 hours
8- After 24 hours release the vacuum and take out the beaker containing distilled chloroform
9- Transfer both fumigated and unfumigated soil in 150ml conical flask
10- Add 25ml of 0.5M potassium sulphate
11- Shake for half an hour
12- Filter with Whatman filter paper no -1
13- Transfer 10ml filtrate in 250ml conical flask
14- Add 2ml 0.2N potassium dichromate
15- Add 10ml of conc. sulphuric acid and 5ml orthophosphoric acid
16- Keep it for half an hour
17- Add 200ml of distilled water and transfer it to 500ml of conical flask
18- Titrate against0.005 ferrous ammonium sulphate until the green colour develops.

4.6 Physiological parameters of wheat

- **Proline** –

Principle

The buildup of proline in higher plants is a symptom of a physiological disturbance produced by stress. Plants with high levels of free proline are more susceptible to biotic and abiotic stressors. The determination of proline ranges is a helpful test for revealing physiological condition and assessing increased plant stress resistance. There are three ways for determining the amount of free proline in a sample.

Reagents-

Glacial acetic acid, sulphosalicyclic acid, ninhydrin, toulene

Procedure-

1. By homogenizing 0.5 g of plant material in 10 ml of 3% aqueous sulphosalicyclic acid, the extract was made.
2. The homogenate is filtered through Whatman No. 2 filter paper.
3. 2 ml of filtrate is taken in a test tube and 2 ml of glacial acetic acid and 2 ml acid-ninhydrin are added in a sequence.
4. The mixture is heated it in the boiling water-bath for 1 h.
5. The reaction is stopped by placing the tube in ice-bath.
6. 4 ml toluene is added to the reaction mixture and stir well for 20-30 sec.
7. The toluene layer is separated and warm to room temperature.
8. The red colour intensity is measured at 520 nm.
9. A series of standard with pure proline in a similar way is made and prepare a standard curve. 10. Thus the amount of proline in the test sample is determined from the standard curve.

- **Chlorophyll –**

Reagents- 80% acetone, ninhydrin, spectrophotometer

Protocol-

1- Weigh 0.25gm of leaf sample.
2- Transfer the sample in pestle mortar.
3- Add 80% acetone
4- Crush the leaves

5- Transfer leaves to centrifuge tube and make upto the volume (15ml) using 80% acetone.
6- Centrifuge at 7500rpm at 4˚C for 10 mins.
7- Take the readings at 663nm, 645nm for chlorophyll and at 410nm and 510nm for carotenoid.

- **RLWC-**

PRINCIPLE- In terms of the physiological consequences of cellular water shortage, relative water content (RWC) is probably the most accurate metric of plant water status. In dealing with water transport in the soil-plant-atmosphere continuum, water potential as a measure of the energy state of plant water is useful.

PROCEDURE-

1. Leave samples were collected from branches of mature plants.

2. Leaves were always collected from the mid-section. And leaves were immediately removed from the stem with the help of tweezers. Then a sharp razor blade was used to recut the leaf base.
3. Leaves were then immediately weighed (fresh mass, FM).

4. In order to obtain the turgid mass (TM), leaves were floated (except where noted) in distilled water inside a closed petri dish. During the imbibition period, leaf samples were weighed periodically, after gently wiping the water from the leaf surface with tissue paper.
5. At the end of the imbibition period, leaf samples were placed in a pre-heated oven (Catsky, 1974a; Turner, 1981), at 8O˚C, for 48 h, in order to obtain the dry mass (DM).

4.7 Data collected – Data were collected from 3 pots of each treatment to evaluate the growth, biomass, and yield of wheat. The data was collected at first as well as at final harvest –

1- Plant height– Plant height was measured in centimeter from the base of the plant to tip of the main stem, the mean height was worked out and expressed in cm.

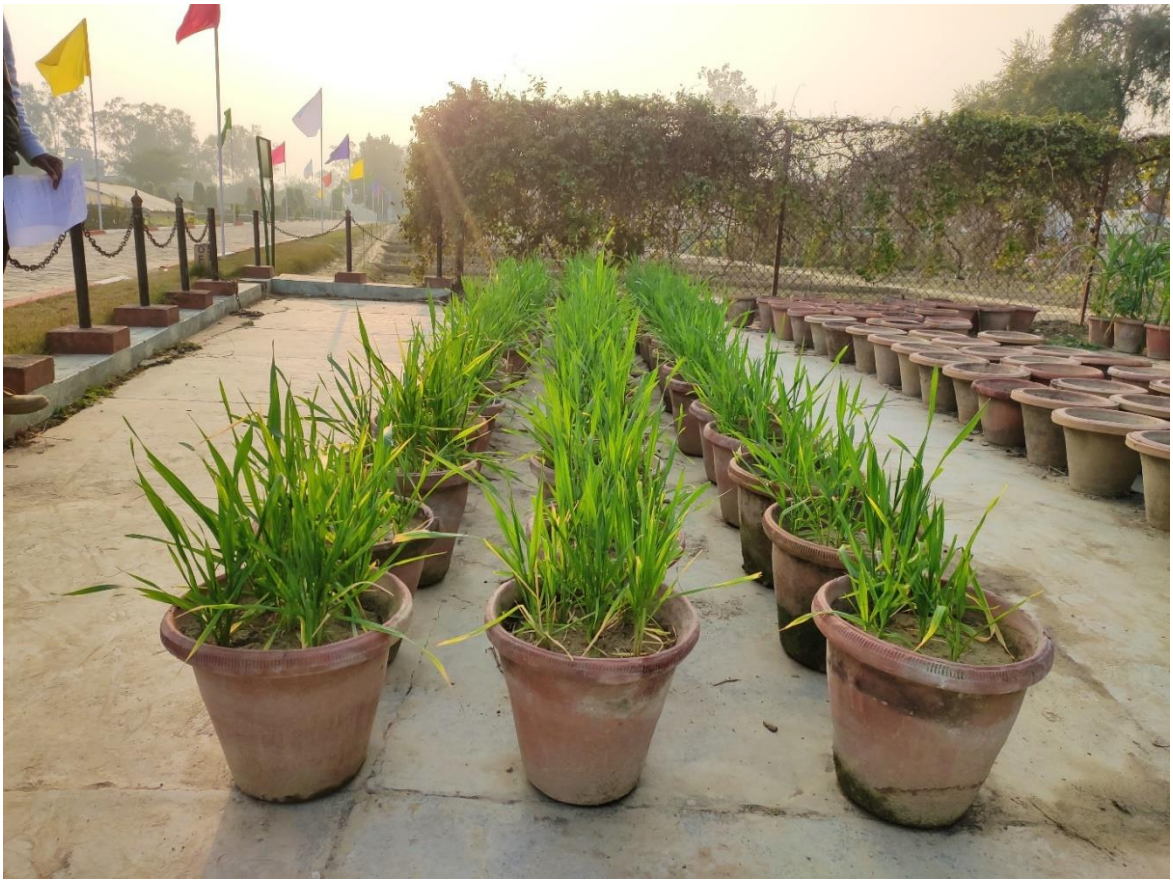
2- Canopy- Canopy of plant was measured in each pot and means value were calculated.

3- Number of tillers per plant- Tillers are additional stems that develop off of the main shoot of the plant. Tiller density was evaluated by counting the number of tillers in each pot. The number of tillers were counted and averaged.

4- Number of grains per spike- Wheat grain number per spike is determined by the combination of number of spikelet per spike and the number of grains per spikelet. Randomly selected five spikes were taken from each pot and threshed manually. The number of grains were counted and averaged for number of grains per spike.

5- Length of spike- five spikes were selected at random from each pot and their length excluding awns was measured and then averaged values were calculated. The average length was expressed in cm.

6- Bundle weight- The total produce was weighed in bundles after harvesting from each pot and expressed in g pot^{-1}.



Experimental site

Foliar application of biostimulant

INSTRUMENT USED

INCUBATOR

WEIGHING BALANCE

PH METER

DESSICATOR

SPECTROPHOTOMETER

HOT AIR OVEN

Vortex

Water bath

Centrifuge

5.Results

The results obtained in the present investigation entitled “Effect of foliar application of plant-based bio-stimulant on growth and yield of wheat (*Ttiticum aestivum*L.*)*” has been presented and discussed here.

5.1 Effect of foliar application of bio-stimulant on Plant growth and yield parameters- Table 1 shows plant height, numbers of tillers, canopy after 60 DAS of foliar spray. The maximum height was showed in treatment **T5C1**(49.067) and **T7C1**(49.067) followed by **T4C2** (47.733) and **T7C2**(48.200) while the minimum height was observed in treatment **T8C2** (40.267) and **T3C1** (42.067). Number of tillers per plant ranges from 4.467 to 3.533. **T7C1** (4.467) and **T5C1** (4.067) showed maximum tillers, minimum tillers were observed in treatment **T2C2** (3.522) and **T6C2** (3.566). The tretment **T7C1** (56.667) showed maximum canopy, while **T5C1, T2C2, T0** showed similar trends (53.333). The lowest canopy was observed in treatment **T8C2** and **T4C1** (46.667).

Table 1. Effect of foliar application of plant-based Bio-stimulants on growth characters of wheat at 60DAS.

TREATMENT	Plant height (cm)	No. of tillers per plant	Canopy (cm)
T0	42.6	3.867	53.333
T1	42.133	3.867	50
T2C1	43.933	3.933	50
T2C2	44	3.533	53.333
T3C1	42.067	3.733	48.333
T3C2	44.4	3.933	51.667
T4C1	43.8	3.8	46.667
T4C2	47.733	3.867	50
T5C1	49.067	4.067	53.333
T5C2	47.4	3.8	50
T6C1	46.333	3.8	50
T6C2	46.8	3.566	48.333
T7C1	49.067	4.467	56.667
T7C2	48.2	3.8	48.333
T8C1	43.467	3.8	51.667
T8C2	40.267	3.733	46.667

Plant height of wheat during harvesting stage (Table 2.)ranges between 78.00cm – 61.733 cm. The maximum height was obseved in treatment **T7C1** and **T5C1**, that is (78.00cm). while the lowest height was observed in treatment **T8C2** (61.733) and **T1** (67.667).

The maximum number of tillers per plant was observed in treatment **T7C1**(7.600) followed by **T5C1** (7.533), while lowest number of tillers was obseved in T1 (4.876) followed by T4C1 (5.400).

Canopy ranges between 63.333cm- 55.00 cm, whereas treatment **T7C1**(63.333) show maximum canopy while treatment **T5C1, T5C2** also showed similar trend, that is 63.333 cm. The lowest canopy was observed in treatment **T3C1** (55.000cm) followed by **T8C2** and **T1**; (56.667cm).

Table 2. Effect of foliar application of plant- based Bio-stimulants on growth characters of wheat at harvest.

TREATMENT	Plant height (cm)	No. of tillers per plant	Canopy (cm)
T0	68.667	5.733	59.333
T1	67.667	4.867	56.667
T2C1	69.867	5.8	58.333
T2C2	68.867	5.8	61.667
T3C1	69.733	5.733	55
T3C2	69	6.133	58.333
T4C1	69.133	5.4	58.333
T4C2	76.467	6	61.667
T5C1	78	7.533	63.333
T5C2	76.533	6.2	63.333
T6C1	77.067	6.2	61.667
T6C2	75.733	6.933	58.333
T7C1	78	7.6	63.333
T7C2	75.6	5.667	58.333
T8C1	69.533	5.733	59.333
T8C2	61.733	6.6	56.667

Table-3 shows effect of biostimulant on yield parameters of wheat; bundle weight, number of grains per spike and spike length. Treatment T7C1 (*Tagetes minuta*) shows maximum yield in terms of bundle weight, number of grains per spike, and spike length followed by treatment T5C1 (*Cymbopogon flexuosus).*

While the lowest bundle weight was observed in treatment T1followed by **T2C1**. Number of grains per spike, lowest yield was observed in treatment

T2C2, followed by **T2C1.** Spike length ranges between 8.600cm - 5.467cm, while the treatment **T0** (control) shows minimum spike length. Results revealed that the application of the biostimulant was the most effective in improving total biomass yield of wheat and the treatment **T7C1** with concentration 0.1% afound significantly superior in improving bundle weight, number of grains per spike, and spike length.

Table 3. Effect of foliar application of plant-based Bio-stimulants on yield of wheat.

TREATMENT	Bundle weight (g pot^{-1})	No. of grains/spike	Spike length (cm)
T0	47.477	38.667	5.467
T1	30.203	48.222	7.033
T2C1	40.607	35.889	7.167
T2C2	56.847	29.667	7.667
T3C1	51.68	39.556	8
T3C2	55.243	36.333	7.033
T4C1	54.007	40.556	6.6
T4C2	56.35	42.444	8
T5C1	71.36	48.889	8.5
T5C2	67.783	46.889	7
T6C1	64.533	39.667	7.933
T6C2	65.677	46.778	8.233
T7C1	76.293	50.556	8.6
T7C2	54.177	42.333	8
T8C1	59.56	43.444	7.433
T8C2	49.493	44.667	8.267

5.2 Effect of foliar application of bio-stimulant on plant physiological parameters-

One of the most important factors affecting crop development and output is relative chlorophyll concentration, and chlorophyll is an important indicator that represents the stress and health of vegetation. Table-4 estimates the effect of Chlorophyll (a,b) and carotenoids content in the leaves of wheat. Treatment **T7C1** shows maximum total chlorophyll, chlorophyll a, and carotenoids, it was likely to be the positive effect of biostimulant *Tagetes minuta*,while treatment **T1** shows highest content of chlorophyll b. It was observed that treatment **T2C2** estimate to have lowest content of chlorophyll.

Table 4. Effect of foliar application of Biostimulants on total chlorophyll, chlorophyll a, chlorophyll b and carotenoids content in leaves of wheat.

TREATMENT	Total Chlorophyll (mg g^{-1} FW)	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
T0	1.355	1.031	0.324	4.472
T1	2.261	1.678	0.584	8.636
T2C1	1.288	0.98	0.308	4.386
T2C2	1.134	0.846	0.289	3.826
T3C1	1.461	1.108	0.353	5.03
T3C2	1.432	1.074	0.359	4.799
T4C1	1.551	1.165	0.386	5.122
T4C2	1.732	1.273	0.46	5.638
T5C1	1.98	1.403	0.577	6.349
T5C2	1.198	0.848	0.351	3.786
T6C1	1.295	0.82	0.475	4.446
T6C2	1.662	1.186	0.476	5.378
T7C1	2.086	1.547	0.539	6.63
T7C2	1.624	1.191	0.433	5.212
T8C1	1.457	1.071	0.387	4.613
T8C2	1.864	1.388	0.477	5.805

Water stress is one of the most common constraints in crops, and it has a negative impact on photosynthesis and plant primary output. The biostmulant had major influence on wheat at given water stress level. The maximum RLWC content was obseved in treatment **T7C1** with 85.320%, followed by **T5C1** (80.688%).

The buildup of proline in higher plants is a sign of a disrupted physiological state, which is triggered by biotic and abiotic factors. When plants are exposed to drought, salinity, cold, or diseases, their proline content rises. Table-5 indicate estimated diposition of proline content in leaves of wheat. It was observed that the proline content in wheat was less in treatment **T7C1** (0.030). The results obtained in our experiments show that an increase in the amount of proline content in control **T0**(0.205).

Table 5. Effect of foliar application of Biostimulants on Relative water content (RLWC) and proline content in leaves of wheat.

TREATMENT	RLWC (%)	PROLINE (µ mole g-1 FW)
T0	75.864	0.205
T1	78.135	0.066
T2C1	72.924	0.073
T2C2	74.415	0.13
T3C1	76.741	0.13
T3C2	78.796	0.11
T4C1	72.634	0.114
T4C2	68.07	0.106
T5C1	80.688	0.079
T5C2	77.915	0.132
T6C1	80.551	0.111
T6C2	67.061	0.149
T7C1	85.32	0.03
T7C2	77.949	0.117
T8C1	76.524	0.082
T8C2	72.632	0.14

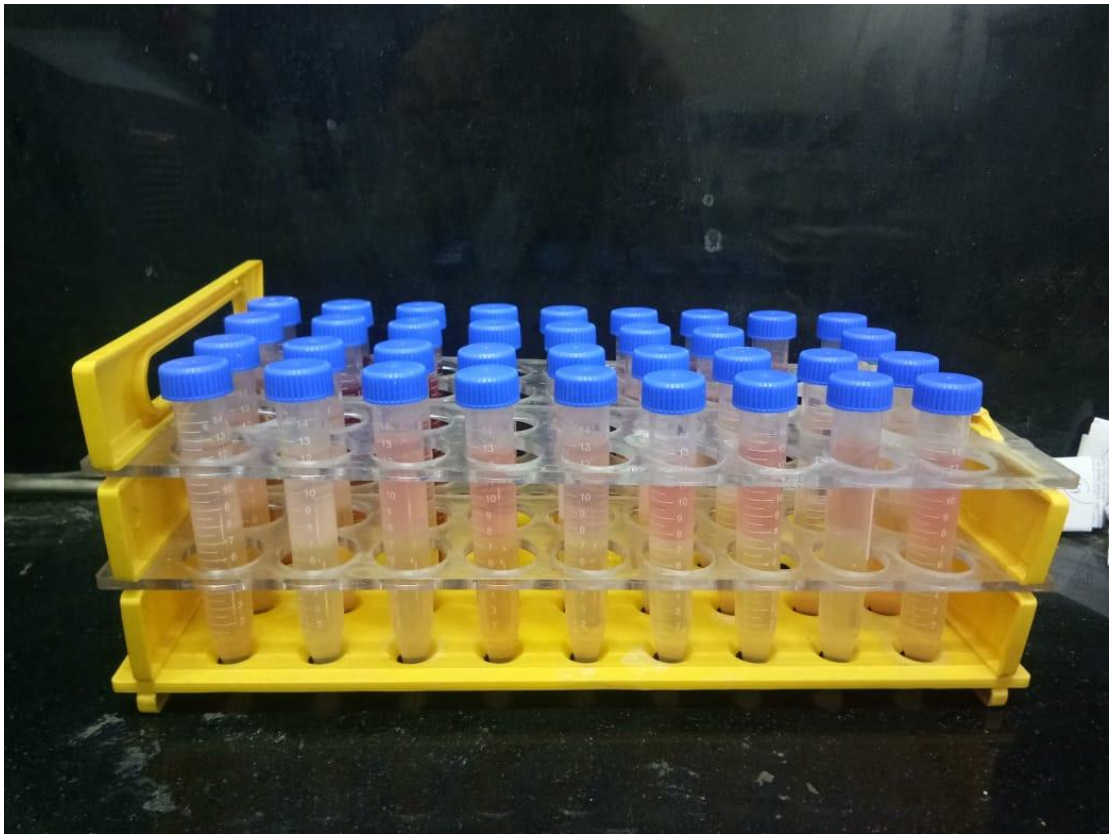


Image showing proline test result

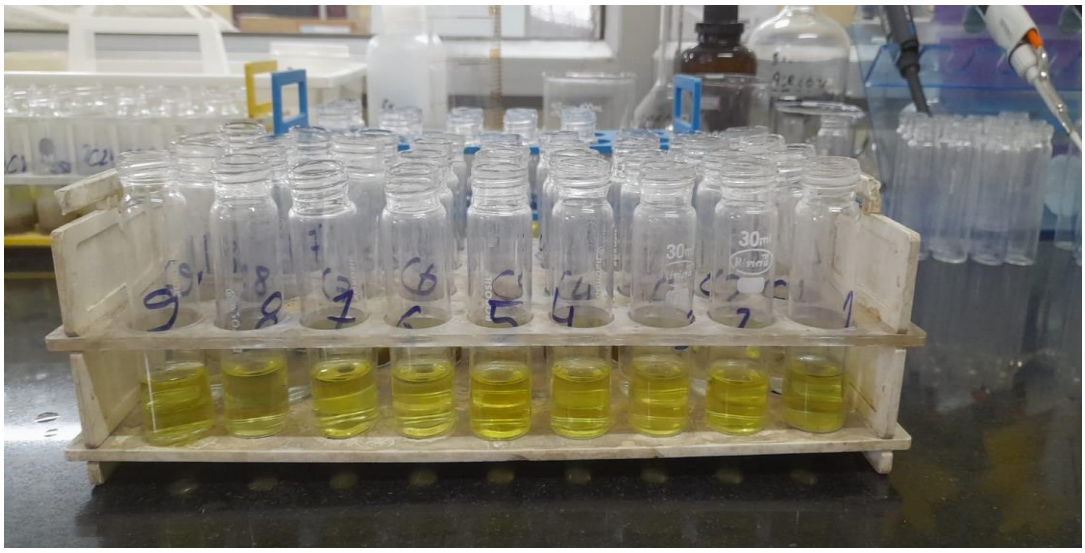
5.3 Effect of foliar application of bio-stimulant on soil enzyme activity-

Acidic phosphatase activity of different biostimulant onwheat crop are represented in table:6. The maximum acidic phosphatase activity are influenced by the treatments was recorded in treatment **T7C1** which is followed by treatment **T5C1**. Whereas the minimum activity was observed in treatment **T1** followed by **T4C1**. The acidic phosphatase activity was recorded much lower than that of alkaline activity irrespective of the treatment which may be due to alkaline reaction of the soil. The maximum alkaline phosphatase activity by the microbes was observed in treatment **T7C1**(305.036) which is followed by treatment T8C1 and the munimum was observed in **T6C1**.

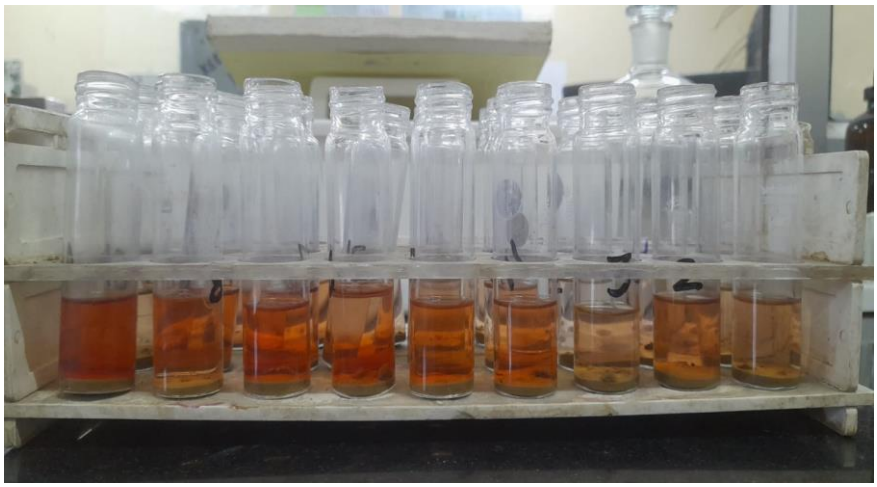
DHA determination is the method which is developed by Casida et.al.,(1964). Dehydrogenase is an enzyme found in all the living microorganisms. These enzymes are used to assess the metabolic health of soil microorganisms. The complete range of oxidative activity of soil microflora (Liang et.al. 2014). The DHAin treatment **T5C1** was significantly higher, followed by treatment **T7C1.** However minimum dehydrogenase activity wasobserved in treatment **T2C2** followed by **T0** (control)

Table 6. Effect of foliar application of plant based Biostimulants on soil microbial enzymes in post - harvest soil

TREATMENT	Acid Phosphatase (µgPNP /gm/hr)	Alkaline phosphatase (µgPNP /gm/hr)	Dehydrogenase (µg TPF $h^{-1}g^{-1}$ soil)
T0	130.01	194.66	151.55
T1	95.992	191.37	314.24
T2C1	144.3	201.65	261.25
T2C2	142.75	204.32	113.61
T3C1	131.96	200.72	178.53
T3C2	144.09	194.96	352.96
T4C1	116.44	230.42	257.54
T4C2	122.92	260.95	196.13
T5C1	157.45	279.45	562
T5C2	149.23	243.37	166.22
T6C1	125.28	179.86	263.4
T6C2	153.34	257.04	211.19
T7C1	172.35	305.04	386.79
T7C2	150.46	247.59	278.85
T8C1	132.07	292.81	297.04
T8C2	145.84	225.39	337.71



Alkaline phosphatase test result.



Dehydrogenase test result

Microbial biomass carbon(MBC) in soil sample showed a significant changes by the foliar application of different biostimulent at post harvest stage of crop and their interactions are shown in table-7. The treatment **T5C1** showed maximum soil microbial biomass which is followed by **T7C1**. Lowest microbial biomass was observed in treatment **T3C1**, followed by **T5C2**.

Table 7. Effect of foliar application of plant based Biostimulants on soil microbial biomass carbon (SMBC) in post -harvest soil.

TREATMENT	SMBC (µg/g)
T0	469.044
T1	426.652
T2C1	391.31
T2C2	427.927
T3C1	233.128
T3C2	521.022
T4C1	400.206
T4C2	419.056
T5C1	665.69
T5C2	239.855
T6C1	276.663
T6C2	404.334
T7C1	529.204
T7C2	325.745
T8C1	506.938
T8C2	440.841

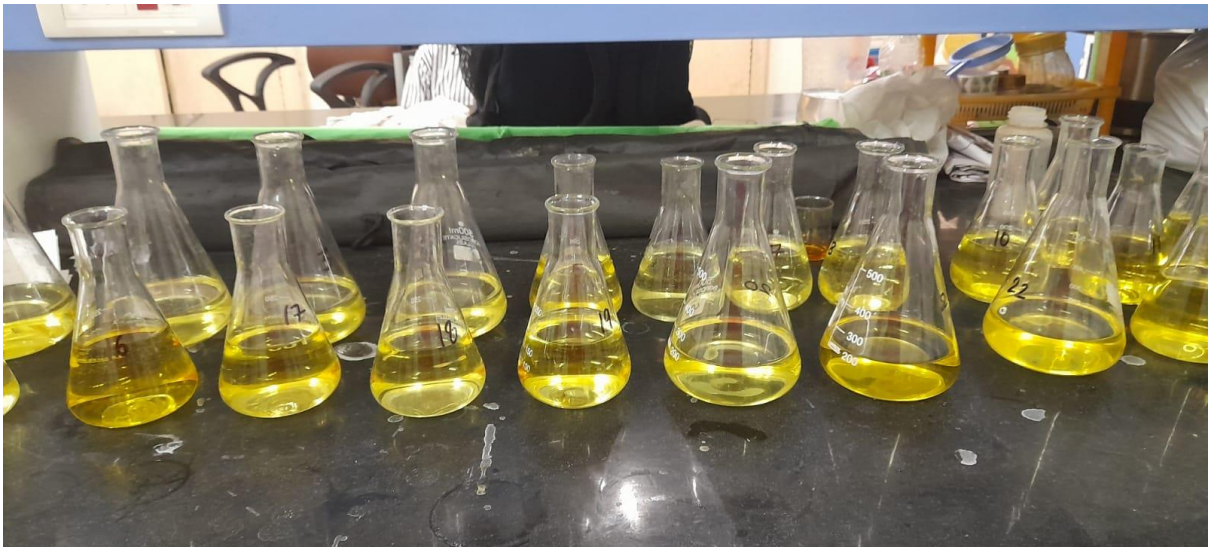


Image showing SMBC test result

6.Discussion

This study shows that Wheat (*Triticum aestivum* L.) on treated with different type of biostimulant named Sea weed extract, Kalmegh, Mentha, Tulsi, Palmarosa, wild marigold, geranium was evaluated. It was observed that treatment T7 (wild marigold) has positive impact on wheat. The higher growth characters (Table 1,2), yield parameters (Table-3), chlorophyll content (Table-4), relative water content (Table-5), soil microbial enzymes (Table-6), soil microbial biomass (Table- 7) were demonstrated in in the majority of plant treated with *Tagetes minuta* at concentration 0.2%compared to non-treated plants and other treatments. Probably the presence of different molecules in the essential oil. A total of 28 compounds were identified representing 74.2% of total oil composition. Major components of the essential oil were (Z)-ocimenone (15.9%), (E)-ocimenone (34.8%), (Z)-beta-ocimene (8.3%), limonene (2.3%), (Z)-tagetone (1.8%), dihydrotagetone (1.4%) and an unidentified dimethylvinylketone derivative (20.6%) Other than T7, plant treated lemon grass at concentration 0.2% show positive impact on different parameters of wheat crop. Here, we conclude that the effective ingredients of lemon grass have beneficial role in improving plant metabolism and growth. Also on the other hand, *Tagetes minuta* at concentration 0.2% does not show as much as positive response when correlated with concentration 0.1%. *Cymbopogon flexuosus*showed similar observation, when compared to 0.1% concentration. Hence response of winter wheat to the foliar application of *Tagetes minuta and Cymbopogon flexuosus* depended on the dose and developmental stage of plant during application. Plant treated with other biostimulant has both positive and negative aspects on the wheat crop. This result could be due to a combination of multiple effects.

7.Conclusion

Bio-stimulants have been shown to boost plant growth and development, increase nutrient uptake, yield, and water content, and improve the nutritional value and quality of their produce in studies involving a range of plants. In this study, we have evaluated over 8 bio-stimulants, that range from geranium to sea weed extract. We conclude that bio-stimulant, *Tagetes minuta* has positive impact on wheat crop in terms of:

- Crop growth characteristics (plant height, number of tillers and canopy).
- Yield attributes (Above ground dry matter accumulation, number of grains per spike and spike length).
- Soil microbial enzymes (alkaline phosphatase, acid phosphatase and dehydrogenase activity).

8. Bibliography

Al Majathoub, M., 2004. Effect of biostimulants on production of wheat (Triticum aestivum L.). *Mediterranean Rainfed Agriculture: Strategies for Sustainability, CIHEAM, Zaragoza*, pp.147-150.

Ali NA, Sharopov FS, Al-Kaf AG, Hill GM, Arnold N, Al-Sokari SS, Setzer WN, Wessjohann L. Composition of essential oil from Tagetes minuta and its cytotoxic, antioxidant and antimicrobial activities. Nat Prod Commun. 2014 Feb;9(2):265-8. PMID: 24689306.

Alix, A. and Capri, E., 2018. Modern agriculture in Europe and the role of pesticides. In *Advances in Chemical Pollution, Environmental Management and Protection* (Vol. 2, pp. 1-22). Elsevier.

Almadi, L., Paoletti, A., Cinosi, N., Daher, E., Rosati, A., Di Vaio, C. and Famiani, F., 2020. A biostimulant based on protein hydrolysates promotes the growth of young olive trees. *Agriculture*, *10*(12), p.618.

Arif, Y., Bajguz, A. and Hayat, S., 2022. Moringa oleifera Extract as a Natural Plant Biostimulant. *Journal of Plant Growth Regulation*, pp.1-16.

Baćmaga, M., Borowik, A., Kucharski, J., Tomkiel, M. and Wyszkowska, J., 2015. Microbial and enzymatic activity of soil contaminated with a mixture of diflufenican+ mesosulfuron-methyl+ iodosulfuron-methyl-sodium. *Environmental Science and Pollution Research*, *22*(1), pp.643-656.

Baćmaga, M., Kucharski, J., Wyszkowska, J., Borowik, A. and Tomkiel, M., 2014. Responses of microorganisms and enzymes to soil contamination with metazachlor. *Environmental earth sciences*, *72*(7), pp.2251-2262.

Calvo, P., Nelson, L. and Kloepper, J.W., 2014. Agricultural uses of plant biostimulants. *Plant and soil*, *383*(1), pp.3-41.

Calvo, P., Nelson, L. and Kloepper, J.W., 2014. Agricultural uses of plant biostimulants. *Plant and soil*, *383*(1), pp.3-41.

Chen, S.K., Subler, S. and Edwards, C.A., 2002. Effects of agricultural biostimulants on soil microbial activity and nitrogen dynamics. *Applied Soil Ecology*, *19*(3), pp.249-259.

Chhokar, R.S., Sharma, R.K., Chauhan, D.S. and Mongia, A.D., 2006. Evaluation of herbicides against Phalaris minor in wheat in north-western Indian plains. *Weed Research*, *46*(1), pp.40-49.

Cycoń, M., Piotrowska-Seget, Z. and Kozdrój, J., 2010. Responses of indigenous microorganisms to a fungicidal mixture of mancozeb and dimethomorph added to sandy soils. *International Biodeterioration & Biodegradation*, *64*(4), pp.316-323.

Darzi, M.T., Shirkhodaei, M. and Haj Seyed Hadi, M.R., 2015. Effects of Vermicompost and Nitrogen Fixing Bacteria on Seed Yield, Yield Components of Seed and Essential Oil Content of Coriander (Coriandrum sativum). *Journal of Medicinal plants and By-product*, *4*(1), pp.103-109.

Du Jardin, P., 2015. Plant biostimulants: Definition, concept, main categories and regulation. *Scientia horticulturae*, *196*, pp.3-14.
Du Jardin, P., 2015. Plant biostimulants: Definition, concept, main categories and regulation. *Scientia horticulturae*, *196*, pp.3-14.
Du Jardin, P., 2015. Plant biostimulants: Definition, concept, main categories and regulation. *Scientia horticulturae*, *196*, pp.3-14.
Elbouzidi, A., Bencheikh, N., Seddoqi, S., Bouhrim, M., Bouramdane, Y. and Addi, M., 2021. Investigation of the Allelopathic Effect of Matricaria chamomilla L. Parts' Aqueous Extracts on Germination and Seedling Growth of Two Moroccan Varieties of Durum Wheat. International Journal of Agronomy, 2021.

European Biostimulant Industry Council [EBIC] Economic Overview of the European Biostimulants Market. https://biostimulants.eu/highlights/economic-overview-of-the-european-biostimulants-market/.

Fenlon, K.A., Andreou, K., Jones, K.C. and Semple, K.T., 2011. The formation of bound residues of diazinon in four UK soils: implications for risk assessment. *Environmental Pollution*, *159*(3), pp.776-781.

Francesca, S., Arena, C., Hay Mele, B., Schettini, C., Ambrosino, P., Barone, A. and Rigano, M.M., 2020. The use of a plant-based biostimulant improves plant performances and fruit quality in tomato plants grown at elevated temperatures. *Agronomy*, *10*(3), p.363.

Głodowska, M., Husk, B., Schwinghamer, T. and Smith, D., 2016. Biochar is a growth-promoting alternative to peat moss for the inoculation of corn with a pseudomonad. *Agronomy for sustainable development*, *36*(1), pp.1-10.

H

Halpern, M., Bar-Tal, A., Ofek, M., Minz, D., Muller, T. and Yermiyahu, U., 2015. The use of biostimulants for enhancing nutrient uptake. *Advances in agronomy*, *130*, pp.141-174.

Henry G. Brockman, Ross F. Brennan & Andrew van Burgel (2020) The impact of phytohormone concentration in Moringaoleifera leaf extract on wheat yield and components of yield, Journal of Plant Nutrition, 43:3, 396-406

Ikhtiar, K. and Alam, Z., 2007. Nutritional composition of Pakistani wheat varieties. *Journal of Zhejiang University Science B*, *8*(8), pp.555-559.

Jang, S.J. and Kuk, Y.I., 2021. Effects of Biostimulants on Primary and Secondary Substance Contents in Lettuce Plants. *Sustainability*, *13*(5), p.2441.
Jang, S.J. and Kuk, Y.I., 2021. Effects of Biostimulants on Primary and Secondary Substance Contents in Lettuce Plants. *Sustainability*, *13*(5), p.2441.
Khan, S., Basra, S.M.A., Nawaz, M., Hussain, I. and Foidl, N., 2020. Combined application of moringa leaf extract and chemical growth-promoters enhances the plant growth and productivity of wheat crop (Triticum aestivum L.). South African Journal of Botany, 129, pp.74-81.

Kim, M.J., Shim, C.K., Kim, Y.K., Ko, B.G., Park, J.H., Hwang, S.G. and Kim, B.H., 2018. Effect of biostimulator Chlorella fusca on improving growth and qualities of chinese chives and spinach in organic farm. *The plant pathology journal*, *34*(6), p.567.
Kim, M.J., Shim, C.K., Kim, Y.K., Ko, B.G., Park, J.H., Hwang, S.G. and Kim, B.H., 2018. Effect of biostimulator Chlorella fusca on improving growth and qualities of chinese chives and spinach in organic farm. *The plant pathology journal*, *34*(6), p.567.
Kocira, A., Kocira, S., Świeca, M., Złotek, U., Jakubczyk, A. and Kapela, K., 2017. Effect of foliar application of a nitrophenolate–based biostimulant on the yield and quality of two bean cultivars. *Scientia Horticulturae*, *214*, pp.76-82.
Köksal, E., Bursal, E., Gülçin, İ., Korkmaz, M., Çağlayan, C., Gören, A.C. and Alwasel, S.H., 2017. Antioxidant activity and polyphenol content of Turkish thyme (Thymus vulgaris) monitored by liquid chromatography and tandem mass spectrometry. *International Journal of Food Properties*, *20*(3), pp.514-525.
Kucharski, M., Sadowski, J. and Wujek, B., 2009. Influence of herbicide application system on lenacil residues in soil and roots of sugar beet. *Progress in Plant Protection*, *49*(4), pp.1868-1874.
Kulkarni, S.S., Desai, A.D., Ranveer, R.C. and Sahoo, A.K., 2012. Development of nutrient rich noodles by supplementation with malted ragi flour. *International Food Research Journal*, *19*(1), p.309.
Majkowska-Gadomska, J., Jadwisieńczak, K., Francke, A. and Kaliniewicz, Z., 2022. Effect of Biostimulants on the Yield and Quality of Selected Herbs. *Applied Sciences*, *12*(3), p.1500.
Makhaye, G., Aremu, A.O., Gerrano, A.S., Tesfay, S., Du Plooy, C.P. and Amoo, S.O., 2021. Biopriming with seaweed extract and microbial-based commercial biostimulants influences seed germination of five Abelmoschus esculentus genotypes. *Plants*, *10*(7), p.1327.
Manguro, L.O.A. and Lemmen, P., 2007. Phenolics of Moringa oleifera leaves. *Natural Product Research*, *21*(1), pp.56-68.
Michalak, I. and Chojnacka, K., 2013. Use of extract from Baltic seaweeds produced by chemical hydrolysis in plant cultivation. *Przemysl Chemiczny*, *92*(6), pp.1046-1049.
Othibeng, K., Nephali, L., Ramabulana, A.T., Steenkamp, P., Petras, D., Kang, K.B., Opperman, H., Huyser, J. and Tugizimana, F., 2021. A metabolic choreography of maize plants treated with a humic substance-based biostimulant under normal and starved conditions. *Metabolites*, *11*(6), p.403.
Pingali, P.L., 1999. CIMMYT 1998-99 World wheat facts and trends. Global wheat research in a changing World: Challenges and Achievements.
Radkowski, A. and Radkowska, I., 2013. Effect of foliar application of growth biostimulant on quality and nutritive value of meadow sward. *Ecological Chemistry and Engineering. A*, *20*(10).

Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 Laying Down Rules on the Making Available on the Market of EU Fertilising Products and Amending Regulations (EC)
Safikhan, S., Khoshbakht, K., Chaichi, M.R., Amini, A. and Motesharezadeh, B., 2018. Role of chitosan on the growth, physiological parameters and enzymatic activity of milk thistle (Silybum marianum (L.) Gaertn.) in a pot experiment. *Journal of Applied Research on Medicinal and Aromatic Plants*, *10*, pp.49-58.
Sevov, A. and Delibaltova, V., 2013. Effect of biostimulant fertigrain on bread wheat (Triticum aestivum) productivity elements and grain yield. *Scientific Papers. Series A. Agronomy*, *56*, pp.353-356.
Shan, B., Cai, Y.Z., Sun, M. and Corke, H., 2005. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *Journal of agricultural and food chemistry*, *53*(20), pp.7749-7759.
Shivran, A.C., Nutrient Uptake, Quality and Yield of Wheat as Influenced by Different Herbicides.
Skrzypek, E., Repka, P., Stachurska-Swakon, A., Barabasz-Krasny, B. and Mozdzen, K., 2015. Allelopathic effect of aqueous extracts from the leaves of peppermint (Mentha piperita L.) on selected physiological processes of common sunflower (Helianthus annuus L.). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *43*(2), pp.335-342.
Sodaeizadeh, H., Rafieiolhossaini, M., Havlík, J. and Van Damme, P., 2009. Allelopathic activity of different plant parts of Peganum harmala L. and identification of their growth inhibitors substances. *Plant Growth Regulation*, *59*(3), pp.227-236.
Soppelsa, S., Kelderer, M., Testolin, R., Zanotelli, D. and Andreotti, C., 2020. Effect of biostimulants on apple quality at harvest and after storage. *Agronomy*, *10*(8), p.1214.
Taha, R.S., Alharby, H.F., Bamagoos, A.A., Medani, R.A. and Rady, M.M., 2020. Elevating tolerance of drought stress in Ocimum basilicum using pollen grains extract; a natural biostimulant by regulation of plant performance and antioxidant defense system. *South African Journal of Botany*, *128*, pp.42-53.
Truzzi, E., Benvenuti, S., Bertelli, D., Francia, E. and Ronga, D., 2021. Effects of biostimulants on the chemical composition of essential oil and hydrosol of lavandin (Lavandula x intermedia emeric ex loisel.) cultivated in tuscan-emilian apennines. *Molecules*, *26*(20), p.6157.
Van Oosten, M.J., Pepe, O., De Pascale, S., Silletti, S. and Maggio, A., 2017. The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. *Chemical and Biological Technologies in Agriculture*, *4*(1), pp.1-12.